Estimation of polyphenol intake and the association with cognitive performance in UK women

Hanis Mastura Yahya

Submitted in accordance with the requirements for the degree of

Doctor of Philosophy

The University of Leeds

School of Food Science and Nutrition

Institute of Psychological Sciences

November 2013

The candidate confirms that the work submitted is her own and that appropriate credit has been given where reference has been made to the work of others.

Acknowledgements

I view the journey to complete my PhD degree as a colourful experience that has taught me about myself. Above all, I would never have been able to complete my thesis without blessings and guidance from Allah. My deepest gratitude goes to all my supervisors who without them my PhD journey would have been much more difficult. Firstly, I offer my deepest appreciation to Professor Gary Williamson for the opportunity he gave to work under his supervision. His mentorship was paramount in providing me with excellent guidance, patience, and detailed and constructive comments which are crucial for my long-term development. To Prof Louise Dye for the patient guidance, persistent support and encouragement which really helped me to have the confidence to carry out my study in the field that I have always been passionate about – Psychology. Finally, to Dr Andrea Day, for her unlimited advice, encouragement and sharing that lifted up my spirit and enthusiasm throughout the ups and downs of the journey. The joint supervision of all my supervisors was a very meaningful experience that I will always remember.

My appreciation extends to my fellow friends in the Food Biochemistry group: Fadhilah, Heidi, Jeab, Sam, Joana, Kerstin, Kayleigh, Chiara, Ebru, Rui, Nicolai, Damon, Reyna, Yuanlu, and Abdurrahman who kept me company in the lab and provided invaluable suggestions and motivation throughout the course of the study. The completion of this thesis would have been much more difficult without your friendship, support and encouragement. These thanks are also extended to the fellow friends and staff I have made in the School of Food Science and Nutrition during my time here.

My sincere thanks also go to Fiona, Iria, Amy, Faye, Emily and all my colleagues in the HARU for their help and advice, and with whom it was a pleasure to work alongside. Special thanks to all volunteers as without their willingness and commitment, the polyphenol food intake and cognitive performance study would not have been possible. This project would not be possible without the tireless support from the volunteers.

Above all, my deepest appreciation to my parents, Yahya Mansor and Azizah Ismail, my siblings Hafiz, Nizam and his wife; Siti Sohana, my nieces, Nabila and Nadiah, and the rest of Yahya's family for countless reasons. Thank you for the endless emotional support and prayers throughout the years. I would like express appreciation to my beloved husband, Khusairi for his supports throughout the years. Not forgotten, a very special thanks to my extended families and friends (Khalisah, Mafuzah, Syazwani, Wani, Lily, Raja, Ain, Nassya, Fairuz, Fatimah, Erra, Izan, Khai, Lai Moon and the Wardah's) for the moral support and sacrifices they have made in many during the many years of my pursuit of knowledge.

Finally, the support from Ministry of Higher Education of Malaysia and Universiti Kebangsaan Malaysia is also greatly appreciated. I thank them for awarding me the scholarship that made this work possible.

Abstract

Interest in estimating polyphenol intake and identification of the major dietary sources of polyphenols have risen in tandem with the reporting of certain health benefits of polyphenols, but only a few studies have reported polyphenol intake in the UK population. This thesis, therefore, aims to address this research gap amongst a sample of 246 UK women aged 18-50 years. A food diary was used to estimate food intake, and only ingredients with a polyphenol content of ≥ 1 mg per portion of food were included for estimation of polyphenol intake. Missing data for polyphenol content in some foods was determined by using the Folin-Ciocalteu assay and performing HPLC analysis. The polyphenol intake of the studied population was 1089 ± 814 mg/day. Tea and coffee were the major polyphenol sources while fruit, vegetables and confectionaries were other important sources of polyphenols. Age was the main predictor of flavonoid, phenolic acid and total polyphenol intake, with the increasing age associated with higher consumption. Cognitive performance was assessed using selected cognitive tests in a sub sample of the participants (Diet and Health study). Participants at the lowest quartile of polyphenol intake and who were non-consumers of coffee or tea were younger and have showed better performance on spatial memory assessed from Visual Spatial Learning Test (VSLT) ($p<0.01$). Age was an important predictor for both polyphenol intake and cognitive performance and partly explains the minor contribution of polyphenol intake to the cognitive performance of the studied population. This study has contributed to the understanding of the relationship between polyphenol intake and cognitive performance.

List of Abbreviations

%	Percent
°C	Degree Celsius
µl	Microlitre
µM	Micromolar
µmol	Micromol
BMI	Body mass index (kg/m^2)
Corsi	Corsi Block Tapping Test
CY3RUT	Cyanidin-3-*O*-rutinoside
DH	Diet and Health study
DINE	Dietary Instrument for Nutrition Education
DP3RUT	Delphinidin-3-*O*-rutinoside
g	Gram
GAE	Gallic acid equivalent
HPLC	High performance liquid chromatography
Kcal	Kilocalorie
kg	Kilogram
LWW	Leeds Women's Well-being study
LWW DINE	Leeds Women's Wellbeing DINE
LWW HF	Leeds Women's Well-being High Fibre study
mg	Milligram
ml	Millilitre
NART	National Adult Reading test
ns	Not significant
RNI	Reference Nutrient Intake
USDA	United States Department of Agriculture
s.d	Standard deviation
TOH	Tower of Hanoi
VSLT	Visual Spatial Learning Test
VVLT	Visual Verbal Learning Test
WAIS	Wechsler Adult Intelligence Scale

Publications

1. List of publication in progress (to be submitted):

   (1) The association between habitual polyphenol intake and cognitive performance in women [International Journal of Food Science and Nutrition].

   (2) Estimated polyphenol intake of UK women [European Journal of Nutrition].

   (3) Estimated polyphenol intake in adults – a review [Food and Function].

2. Posters:

   (1) Hanis Mastura Yahya, Andrea Day, Louise Dye, Clare Lawton, Fiona Croden, Gary Williamson. Estimated daily polyphenol intake among overweight women: a preliminary study. 5th International Conference on Polyphenols and Health (ICPH), 17 to 20 October 2011, Sitges, Barcelona, Spain.

Table of Contents

Appendix list

List of Figures

List of Tables

Chapter 1
Dietary Polyphenol Intake and Cognitive Function

1.1 Abstract

Polyphenols are secondary metabolites which are present abundantly in many plant-based food sources such as coffee, tea, cereals, fruit and vegetables. These compounds can be divided into several major groups including flavonoids, phenolic acids and other polyphenols. Recently, research to estimate the polyphenol intake of the population has increased. A number of approaches have been taken to identify habitual polyphenol intake including using food frequency questionnaires, weighed dietary records, and food diaries. Daily food intake has been estimated to provide up to 1 g/day of polyphenol which exceeds the vitamin C intake by ten times (Scalbert et al., 2005). This highlights the potentially important contribution of polyphenol-containing foods as part of the daily diet. In addition, the increasing number of publications which focus on the potential role of polyphenols for health (see Section 1.6), such as reducing the risk of cardiovascular disease, metabolic syndrome and in the improvement of cognitive performance (see Section 1.6) makes the accurate estimation of polyphenol intake of the population vital.

1.2 Polyphenol sub-classes

Phytochemicals are plant bioactive compounds known for their important role in health preservation and disease prevention (Mollet and Rowland, 2002). Polyphenols are phytochemicals, in which the molecules are built from more than one phenol unit (Landete, 2012). These compounds contribute to the organoleptic properties including colour and taste of plant-derived food products (Cheynier, 2005). Polyphenols are divided into several groups (see Figure 1-1) which include flavonoids, phenolic acids, stilbenes, lignans and "other" polyphenols based on the carbon skeleton (Scalbert and Williamson, 2000). Flavonoids display the main feature of having two aromatic and one heterocyclic ring with different numbers of hydroxyls groups attached to each ring. Phenolic acids can be subdivided into two main groups including the benzoic acid and cinnamic acid derivatives. Gallic acid and protocathecuic acid are derived from benzoic acid, while caffeic acid and ferulic acid are derived from cinnamic acid. "Other" polyphenols includes classes such as stilbenes and lignans which are present in varying amounts in a limited number of food sources.

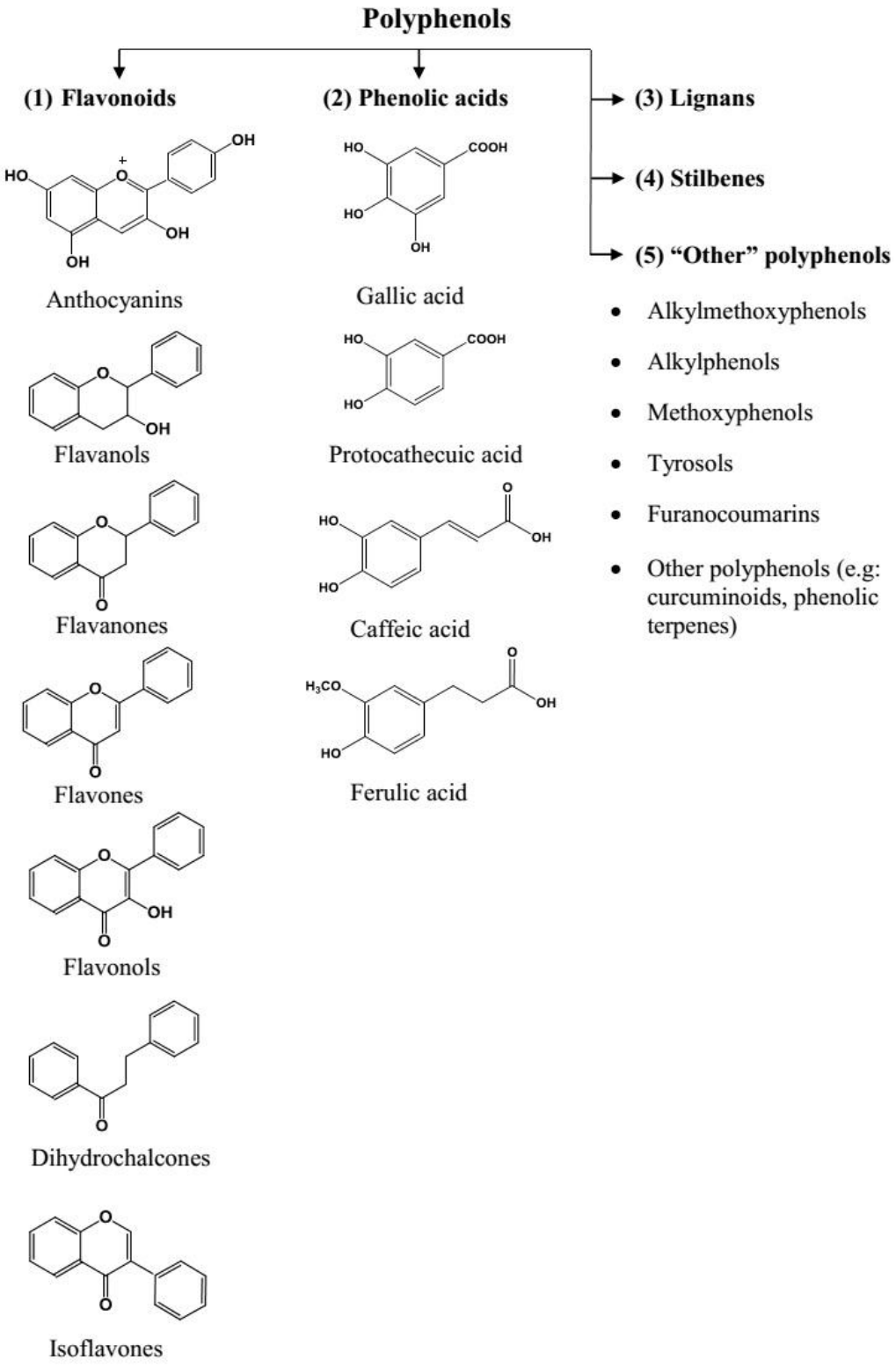


Figure 1-1 Chemical structure of the polyphenols' major classes based on classification from Phenol-Explorer®

1.2.1 Flavonoids

Flavonoids can be subdivided into various sub-classes based on (i) the oxidation of the C-ring, (ii) hydroxylation pattern of ring structure and (iii) replacement in the 3-position (Spencer, 2009b). The seven major sub-classes consist of flavanols, flavonols, flavones, flavanones, anthocyanins, isoflavones and dihydrochalcones. Flavonoids in plants are normally glycosylated with glucose, rhamnose or other sugars with the number of sugars attached varying from one to up to five (Vallejo et al., 2004). The type and degree of glycosylation will affect the absorption of the flavonoid from the intestine (Scalbert and Williamson, 2000).

Table 1-1 summarizes the foods containing the highest amount of each flavonoid sub-class based on the Phenol-Explorer® database, a comprehensive database of the polyphenol content of foods (Neveu et al., 2010b). Most anthocyanins, dihydrochalcones and flavanones are present in fruit and their products. Flavanols, on the other hand, exist in chocolate and various types of beverages. Flavonols and flavones are high in vegetables and herbs respectively, and isoflavonoids are present predominantly in soy products.

Table 1-1 Major food sources for various flavonoid sub-classes

Flavonoid	Food source	Average content*	Ranges^
Anthocyanins	Red raspberry	72	0 – 106
	Strawberry	73	0 – 68
	Blackberry	173	1 – 191
Dihydrochalcones	Apple, dessert, whole	6	0 – 9
	Prune juice	6	2 – 10
	Apple, dessert, puree	9	3 – 6
Flavanols	Green tea	71	0 – 271
	Black tea	73	0 – 68
	Cocoa, powder	512	0 – 330
Flavanones	Lemon, juice from concentrate	46	6 – 38
	Grapefruit, juice from concentrate	51	0 – 64
	Orange blond, juice from concentrate	61	2 – 59
Flavones	Black olives	27	0 – 29
	Artichoke, heads, raw	42	0 – 84
	Mexican oregano, dried	734	23 – 328
Flavonols	Onion, yellow	73	0 – 136
	Capers	131	0 – 1047
	Spinach	119	0 – 113
Isoflavonoids	Soy, yogurt	84	0 – 32
	Soy, tempe	148	0 – 75
	Soybean, roasted (soy nut)	247	0 – 97

*data in (mg/100 ml or mg/100 g) taken from Phenol-Explorer® version 2.0 (Neveu et al., 2010), ^minimum and maximum values

1.2.2 Phenolic acids

Phenolic compounds are present in various edible plants and have the distinctive characteristic of having a benzene ring substituted with at least one hydroxyl group in their structure (Rodríguez et al., 2009). These compounds contribute to a few properties of foods such as aroma, flavour, colour and astringency. Naturally, hydroxycinnamic acid derivatives are present as simple esters with quinic acid or glucose while hydroxybenzoic acid is normally present in the form of glycosides (Mattila and Hellström, 2007). Other phenolic acids include

hydroxyphenylacetic acids, hydroxyphenylpropanoic acids and hydroxyphenylpentanoic acids. Table 1-2 shows the data for the major phenolic acid content in food and beverages taken from Phenol-Explorer®. Herbs, nuts and berries contain the highest amount of phenolic acids, with lower levels found in alcoholic beverages, fruit juices and vegetables.

1.2.3 Other polyphenols

Stilbenes include compound such as resveratrol (see Figure 1-2). This compound is present in wine and originates from the seed and skin of grapes (Fernández-Mar et al., 2012). The typical content of resveratrol in red and white wine are 1.8 and 0.2 to 0.3 g/L respectively (Bertelli and Das, 2009). Apart from wine, stilbenes can also be found in berries and nuts.

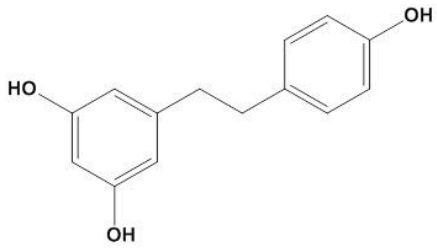


Figure 1-2 Chemical structure of resveratrol

Lignans are polyphenols which are mainly found in high fibre foods. From the Phenol-Explorer® database, there are 30 compounds classified as lignans such as matairesinol, secoisolariciresinol and sesamin. Seeds such as flaxseed and sesame contain a substantial amount of lignans in both raw and oil forms. For example, flaxseed and sesame seed contain 867 mg/100 g and 777 mg/100 g of lignan of fresh weight respectively (Neveu et al., 2010b). Another food source of lignans is vegetables such as radish and asparagus. Two main lignans with substantial

analytical data available include secoisolariciresinol and matairesinol (Blitz et al., 2007). From the Phenol-Explorer® database, secoisolariciresinol presents in high amount in flaxseed (840 mg/100 g) while matairesinol presents in sesame seed (30 mg/100 g).

Table 1-2 Major food sources for various phenolic acid sub-classes

Phenolic acid	Subgroup (s)	Food source (s)	Average content*	Ranges^
Hydroxybenzoic acid	Gallic acid	Red wine	4	0 – 13
		Black tea	5	0 – 15
		Blackberry	5	2 – 9
		Cloves	458	18 – 784
	Ellagic acid[a]	Pomegranate, juice from concentrate	17	0 – 17
		Black raspberry	38	0 – 38
		Blackberry, raw	44	20 – 69
		Raw chestnut	735	271 – 1052
	Benzoic acid	American cranberry	48	0 – 48
Hydroxycinnamic acids	p-Coumaric acid	Dried oregano	6	0 – 12
		Dried date	6	1 – 14
		Green olive	6	0 – 17
	5-Caffeoylquinic acid	Coffee	188	48 – 96
	Caffeic acid	Lingonberry	6	0 – 6
		Common sage, dried	26	11 – 40
		Black chokeberry	141	0 – 141
	Ferulic acid	Chocolate, dark	24	0 – 24
		Dried date	12	6 – 18
		Hard wheat, whole grain flour	72	0 – 72
	Sinapic acid	Cauliflower, raw	4	4 – 5
		Green olive	44	5 – 83

*data in (mg/100 ml or mg/100 g) taken from Phenol-Explorer® version 2.0 (Neveu et al., 2010), [a] Data is taken from the non-hydrolysed form, which may underestimate total levels available in the intestinal tract.

1.3 Determination of polyphenol intake

1.3.1 Measurement of food intake

Dietary assessment plays a very important role in the study of nutrition with the aim of estimating food intake. At the individual level, there are several approaches taken to estimate food intake which include using a food diary of different duration (normally including a weekend day) and 24-hour diet recall. Estimation of portion size of foods consumed is a crucial step in determining energy intake. Food portion size can be misrepresented by participants who failed to estimate or quantify the amount of food or the average food portion size consumed (Poslusna et al., 2009). There is a tendency of over reporting for food perceived as nutritious and underreporting for high energy foods. Difficulties in estimating portion sizes by untrained individuals were identified when participants were asked to estimate the displayed foods and report their food intake (Godwin et al., 2004). In addition, commonly consumed or bought foods such as bread, fruit and beverages in their usual sold sizes were easier to report than foods with no specific usual mass such as cooked meat, poured liquid or vegetables. Moreover, studies have identified that small portion sizes tend to be overestimated and vice versa for large portion sizes (Nelson et al., 1996, Harnack et al., 2004).

The food diary was used when multiple day recording was required. This method normally put a greater emphasis on the portion size of various foods consumed. The respondents are trained on how to record their dietary intake to be as specific as possible, including all the details about the portion sizes, method of food preparation and recipe where relevant. One of the limitations of diet diaries is under reporting because of incomplete recording of foods consumed. Additionally, the

tendency to selectively report food intake can occur and the time-consuming nature of the recording process can lead to under eating (Bathalon et al., 2000). To overcome this problem, food diaries should be reviewed by the researcher to clarify any uncertainties in the recording made by the participants. In addition, the status of food reporting can be determined by the estimation of basal metabolic rate (BMR). The status of food intake reporting can be classified based on the ratio of energy intake to BMR. The Schofield equation can be used to predict BMR by using the weight (in kg) of the individual. This ratio was proposed previously to have three categories - less than 1.14 reflects under reporting, 1.14 to 2.4 as normal reporting and a ratio of more than 2.4 is considered over reporting (Goldberg et al., 1991, Goris et al., 2000).

At the population level, polyphenol intake estimation was performed using several methods including a food frequency questionnaire (FFQ), adaptated from national consumption data and food balance sheets. FFQ method focuses on how frequent selected foods were eaten by the participants. It is the most widely used method of dietary assessment but has several disadvantages including limiting the number and type of foods included in the questionnaires which can cause underestimation not only in specific, but also to total flavonoids intake (Maras et al., 2011). The usage of data from countries' food consumption enables the estimation of food intake for a larger population. For example, a study has estimated flavonoids intake of the UK and Ireland population by using a Food Balance Sheet from the Food and Agriculture Organization (Beking and Vieira, 2011) (see Table 1-3). However, the data are restricted to the amount of foods bought by the population and the foods which are not consumed were not considered in the final estimation, thus not directly implying the actual food consumption.

1.3.2 Estimation of polyphenol content from database

There have been a number of studies which have estimated polyphenol intake in various parts of the world based on intake reports from samples of individuals. Estimations were from established databases available on the polyphenol content of foods such as Phenol-Explorer® and the United States Department of Agriculture (USDA) flavonoid database.

Phenol-Explorer® is a comprehensive database of more than 500 polyphenols contained in more than 400 types of foods set up in an accessible website (Neveu et al., 2010b). It was established in 2010 and has had three major updates since that time (http://www.phenol-explorer.eu/). In order to include data from scientific publications in Phenol-Explorer®, information about food sampling, polyphenol extraction and analytical methods were scrutinized. Only articles which fulfilled the minimum requirements (see http://www.phenol-explorer.eu/methods_used (Neveu et al., 2010a)) were included in the database. The polyphenol content of foods was quantified based on a variety of methods including reversed-phase high performance liquid chromatography (HPLC) as the most common method, acid or alkaline hydrolysis followed by analysis using HPLC, Folin Ciocalteu assay to determine total phenolic content, pH differential (anthocyanins) and normal phase HPLC (proanthocyanidins). Data on polyphenol content of foods might not derive from all five methods mentioned above. Certain food such as oats has only data from the Folin assay while data for black tea derived from HPLC, acid or alkaline hydrolysis followed by analysis using HPLC and the Folin Ciocalteu assay.

The United States Department of Agriculture (USDA) database consists of data on individual flavonoid content of 500 foods. It was established in March 2003

and has had 4 updates since that time with more flavonoid content of foods being included in the most recent update (2011). This database was developed by referring to data from published studies which applied satisfactory analytical procedures including HPLC, capillary zone electrophoresis, and micellar electrokinetic capillary chromatography (USDA, 2011). Methods which produced results for unspecific polyphenols such as radioimmunoassay, pH differential methods, thin layer or paper chromatography or spectrophotometric were not included in the development of the database.

1.4 Polyphenol intake estimation in population studies

Table 1-3 summarizes the studies on polyphenol intake in different countries based on the method used for dietary reporting. The approach taken in these studies varied in the methodology used and the focus on polyphenols or dietary patterns for specific populations. From the table, the FFQ method used in the studies had included up to 200 food items for food intake estimation. A study in the UK has used this method to estimate flavonols intake of the sample studied (Hertog et al., 1997). A large cohort study conducted amongst adults in Greece used this method for total flavonoids estimation which was associated with the adherence to the Mediterranean diet which mainly consists of fruit, vegetables, olive oil and wine (Dilis and Trichopoulou, 2010). A study in China used FFQ in the food intake estimation and has focused on the flavonoid intake from polyphenol-containing fruit, vegetables and nuts (Li et al., 2013). Finally, the other two studies have used FFQ to estimate total polyphenol intake of the studied population (Sohrab et al., 2013, Tresserra-Rimbau et al., 2013).

Table 1-3 Summary of studies assessing the estimated daily polyphenol intake and the major food sources in different countries

Country & authors	Sample size and type	Method(s) for dietary reporting	Major food sources of polyphenols	Database used	Polyphenol(s) studied	Amount consumed (estimated per day)
United Kingdom (Hertog et al., 1997)	Men (N=1900)	56-item food frequency questionnaire (FFQ)	Black tea, onion and apple	Food composition database on flavonol content published in 1992 and 1993	Flavonols	Flavonols: 26 mg/day
Greece (Dilis and Trichopoulou, 2010)	Adults (N=28572)	190-item validated semi quantitative FFQ	Fruits (apples, peaches and grapes), vegetables (parsley onions, greens and tomato) and wine	EPIC-Greece antioxidant database	Total flavonoids	i) Flavanols: 14 mg/day ii) Flavonols: 28 mg/day iii) Flavanones: 27 mg/day iv) Flavones: 7 mg/day v) Anthocyanidins: 10 mg/day vi) Isoflavones: <0.1 mg/day vii) Proanthocyanidins: 75 mg/day viii) Total flavonoids: 92 mg/day (median total intake)
China (Li et al. 2013)	Adults (N=1393) Men: 446, Women: 947	76-item validated quantitative FFQ	Focus on flavonoids in fruit and vegetables consumed by the participants. Among the main contributors are apple, plum, pear Chinese kale and broccoli, citrus fruits, banana and cabbage	Flavonoid content in fruit and vegetables in China published from 2008 to 2010	Total flavonoids	i) Flavonols: 124 mg/day ii) Flavones: 11 mg/day iii) Anthocyanidins: 28 mg/day iv) Isoflavones: 4 mg/day v) Stilbenes: 0.3 mg/day vi) Total flavonoids: 166 mg/day
Iran (Sohrab et al. 2013)	Adults (N=2618) Men: 1162, Women: 1456	168-item validated semi quantitative FFQ	No specific foods mentioned however, a positive significant correlation was found between total polyphenol intake and vegetables, fruits and legumes food group	Phenol-Explorer database	Total polyphenols	Total polyphenol intake of 1780 mg/day
Spain (Tresserra-Rimbau et al., 2013)	Adults (N=7200)	137-item validated quantitative FFQ	Coffee, fruit (oranges and apple), vegetables (potato and spinach) and red wine	Phenol-Explorer database	Total polyphenols	Total polyphenol intake of 820 mg/day

Table 1-3 Continued

Country & authors	Sample size and type	Method(s) for dietary reporting	Major food sources of polyphenols	Database used	Polyphenol(s) studied	Amount consumed (estimated per day)
Australia (Johannot and Somerset, 2006)	Sample of National Nutrition Survey from aged 2 years and over (N=13858)	24-hour dietary recall	Black tea, apple, apricot, grape, wine	United States Department of Agriculture (USDA) flavonoid database	Total flavonoids	i) Flavanols: 423 mg/day ii) Flavonols: 20 mg/day iii) Flavanones: 7 mg/day iv) Flavones: 0.5 mg/day v) Anthocyanidins: 3 mg/day vi) Total flavonoids: 225 mg/day (whole samples) and 454 mg/day (age 19 years and over)
United States (Chun et al., 2007)	Adults (N=8809)	24-hour dietary recall	Black tea, citrus fruit juices, wine and citrus fruits	United States Department of Agriculture (USDA) flavonoid database	Total flavonoids	i) Flavanols: 157 mg/day ii) Flavonols: 13 mg/day iii) Flavanones: 14 mg/day iv) Flavones: 2 mg/day v) Anthocyanidins: 3 mg/day vi) Isoflavones: 1 mg/day vii) Total flavonoids: 190 mg/day
Finland (Ovaskainen et al. 2008)	Adults (N=2007)	48-hours dietary recall and HPLC	Coffee and cereal products	Self-developed database on polyphenol content of selected foods	Total polyphenols	i) Total phenolic content: 653 mg/day ii) Total flavonoids: 209 mg/day iii) Lignans: 0.9 mg/day iv) Total polyphenols: 863 mg/day
France (Perez-Jimenez et al., 2011).	Adults (N=4942) Men: 2596, Women: 2346	24-hour dietary record completed every two months for a period of two years (1995 to 1996)	Non-alcoholic beverages (such as coffee, black tea and orange juice and pummelo juice) and fruits	Phenol-Explorer database	Total polyphenols	i) Total phenolic content: 640 mg/day ii) Total flavonoids: 512 mg/day iii) Other polyphenols: 41 mg/day iv) Total polyphenols: 1193 mg/day
Poland (Zujko et al., 2012).	Adults (N=6661) Men: 3132, Women: 3529	24-hour dietary recall	Tea, coffee, potatoes and apples	Self-developed database on total phenolic content of selected foods	Total polyphenols[a]	Total polyphenol intake of 1172 GAE mg/day (men) & 1031 GAE mg/day (women)

Table 1-3 Continued

Country & authors	Sample size and type	Method(s) for dietary reporting	Major food sources of polyphenols	Database used	Polyphenol(s) studied	Amount consumed (estimated per day)
European countries (EPIC study) (Zamora-Ros et al., 2011a)	Adults (N=36037)	24-hour dietary recall using computerised interview software^	i) Flavonols: leafy vegetables, onion, garlic, tea, wine ii) Flavanones: citrus fruit and citrus fruit juice iii) Flavones: herbal tea, wine, fruits and vegetables	United States Department of Agriculture (USDA) flavonoid database and Phenol-Explorer database^	Specific amount of flavonoids	i) Flavonols: 29 mg/day ii) Flavanones: 35 mg/day iii) Flavones: 5 mg/day iv) Combination of flavonoids (flavonols, flavanones, flavones): (men: 67 mg/day, women: 70 mg/day
European countries (EPIC study) (Zamora-Ros et al., 2011b)	Adults (N=36994)	Same as above^	i) Anthocyanidins: fruits, wine, non-alcoholic beverages and vegetables	Same as above^	Specific amount of flavonoids	Anthocyanidins: (men: 20 mg/day, women: 19 mg/day)
European countries (EPIC study) (Zamora-Ros et al., 2013b)	Adults (N=35628)	Same as above^	Vegetables, fruit (apple and pear), tea, wines, chocolate products, fruit juices	Same as above^	Total flavonoids	Mediterranean countries: 370 mg/day Non-Mediterranean countries: 374 mg/day
Japan (Otaki et al., 2009).	Women (N=516)	24-hour weighed dietary record	Green tea, onion, molokhia (nalta jute) Japanese radish leaves, grapefruit and citrus fruit juices, and tsurumurasaki (malabar spinach) and green pepper	Functional Food Factor Database, Japan	Specific amount of flavonoids	i) Flavanols: 289 mg/day ii) Flavonols: 14 mg/day iii) Flavanones: 7 mg/day iv) Flavones: 3 mg/day v) Isoflavones: 48 mg/day
United Kingdom and Ireland (Beking and Vieira, 2011)	Not available	Data from FAO* Food Balance Sheet for UK and Ireland population	Not available	United States Department of Agriculture (USDA) flavonoid database	Total flavonoids[b]	i) Flavanols: 50 mg/day ii) Flavonols: 32 mg/day iii) Flavanones: 28 mg/day iv) Flavones: 5 mg/day v) Anthocyanidins: 65 mg/day vi) Total flavonoids: 182 mg/day for UK and 177 mg/day for Ireland

*Food and Agriculture Organization, World Health Organization, [a]Value in mg gallic acid equivalent, [b]Value in mg/day/person

The second methodology used for polyphenol intake estimation is dietary recall. In Australia, data from 24-hour dietary recall derived from the National Nutrition Survey was used to estimate the flavonoid intake of a large sample as a representative of the population (Johannot and Somerset, 2006). Black tea, fruits and wine were identified as the major sources of flavonoids. A study in the United States has used the 24-hour dietary recall method to estimate the flavonoid intake as it provided a satisfactory estimate of the flavonoid intake (Chun et al., 2007). Tea was the major flavonoid source of the sample studied. In addition, the same food intake reporting method was adapted in a recent study of Polish adults which reported tea, coffee, potatoes and apples as the main polyphenol food sources of this population (Zujko et al., 2012). In this study, the total polyphenol intake was expressed as total phenolic content (TPC) of the food consumed by the population. The TPC which was estimated from the Folin-Cioclateu assay has yielded a higher value of polyphenol content as compared to HPLC as the commonly used method to estimate specific polyphenol.

Some studies have compared data from food intake with the existing available polyphenol database (see Section 1.3.2). For example, a cohort study of French adults used the Phenol-Explorer® database as the main search engine to assess the population's polyphenol intake. This study used a 24-hour dietary record as the means for food recording every two months over a period of two years (Perez-Jimenez et al., 2011). In this study, non-alcoholic beverages (such as coffee, tea, orange juice and pummelo juice) and fruit were the main contributors to polyphenol intake. A large cohort study involving ten European countries (The European Prospective Investigation into Cancer and Nutrition, EPIC study) applied 24-hour

dietary recall computerised interview software to standardize the procedure across the countries participating (Zamora-Ros et al., 2011a). In this cohort (EPIC), a database was developed and used to establish a database such as USDA and Phenol-Explorer®. Different types of polyphenols and their food sources were estimated in this cohort including flavonols, flavanones and flavones (see Chapter 2, Table 2-1). The major polyphenol food sources reported in this study include fruits, vegetables, tea, fruit juices and wine.

In Finland, a combination of methods was applied to achieve the objective of estimating the polyphenol intake of Finish adults (Ovaskainen et al., 2008). Dietary intake data was assessed from 48-hour dietary recalls and analysed for nutrient composition using Fineli, the national food composition database for Finland. Samples of the main polyphenol food sources were collected and analysed using reversed-phase HPLC. An approximation using recipes and ingredients was applied for mixed dishes. This study found coffee and cereal products to be the main polyphenol food sources. The low polyphenol content of vegetables resulted in the smallest contribution to total polyphenol intake of this food groups in this population.

The weighed dietary record was suggested to be the gold standard in assessing the dietary intake of an individual, which allows a precise quantification of food intake. However, a comparative study between a few dietary estimation methods and weighed food record has reported that the results of food intake estimated from 24-hour recall was well compared to the results from weighed food record (Bingham et al., 1994). This method was applied in a cross-sectional study performed in 516 Japanese women to estimate their intake of flavonoids (Otaki et al., 2009). Green tea, fruit and vegetables were identified as the major polyphenol sources of this sample. Although the method used in this study might increase the

possibility for accurate polyphenol estimation, there are some issues which arise from it. This includes the tendency for the participants to selectively choose to consume the type of foods which are easy to be weighed. In addition, the longer period of recording can cause difficulty for the participants to bring along their weighing scale.

In general, beverages such as coffee, tea, wine and fruit juices are the major contributors to polyphenol food sources in the populations studied. In addition, vegetables such as onion, potato, tomato and leafy vegetables are also important polyphenol food sources. Different methods used for polyphenol estimation has made a direct comparison between studies quite difficult. However, information obtained from these studies is important to facilitate in designing future studies.

1.5 Major contributors to polyphenol intake

1.5.1 Tea and coffee

Tea is derived from the shoot section of the flowering plant *Camellia sinensis*. The three main types of tea consumed worldwide include black, green and oolong with a percentage consumption of 78, 20 and 2 % respectively (Grove and Lambert, 2010). The difference between these three teas is the degree of oxidation (fermentation) involved in processing the fresh leaves. Oxidation, by the endogenous enzyme polyphenol oxidase, is responsible for converting the flavonoids in fresh leaves to theaflavins and thearubugins (Ho and Zhu, 2000). The degree of fermentation will affect the polyphenol content present in different varieties of tea (Song et al., 2012), and has a profound effect on the colour and flavour of the final tea infusion. In addition, the astringency and bitterness of tea is derived from the catechins and caffeine. Green tea processing involves inactivating polyphenol

oxidase soon after plucking via a process of steaming or pan-frying. Thus the main flavonoids in green tea are (-)-epicatechin, (-)-epigallocatechin, (-)-epicatechin-3-*O*-gallate and (-)-epigallocatechin-3-*O*-gallate (EGCG), along with caffeine naturally present in *Camellia sinensis* (Bohn et al., 2012). The total flavonoids in non-fermented green tea consisted of 80 to 90 % catechins and 10 % of flavanols (Deka and Vita, 2011).

The history of the Chinese drinking green tea goes back to around 3000 years ago. Tea was introduced into the UK during the seventeenth century (Hara, 2001). However, due to the long sea voyage the green tea leaves had undergone oxidation, which contained few flavanols, resulting in black tea with the more complex polyphenols such as theaflavins and thearubugins (70 % of flavonols in tea) (Kuhnert, 2010), along with the flavonols glycosides and caffeine.

Coffee, genus *Coffea* and family *Rubiaceae*, consists of more than 90 species. This plant originated in Africa with *Coffea arabica* and *Coffea canephora* the two main types commonly traded around the world (Willson, 1999). Coffee is processed from the seed (bean) and requires separation of coffee beans from the fruit body before it can be further processed (Segall, 2000). Different stages of coffee processing such as bean fermentation, roasting, decaffeination, blending, freeze or spray drying can affect the content of chlorogenic acids (CGA), the main phenolic acid in coffee (Mills et al., 2013). These compounds are lost during the roasting process of the coffee beans, with a medium roast typically resulting in about 60 % decomposition of these compounds (Parlliment, 2000). High temperatures in coffee processing are responsible for the breakdown of sugars through the caramelisation process which produces aromatic oil caffeol which contributes to the typical odour of coffee (Segall, 2000).

CGA present in coffee are phenolic acids esterified to quinic acid. 5-Caffeoyl quinic acid is one of the most widely present chlorogenic acids in coffee (Bohn et al., 2012). The amount of CGA is estimated to range from 20 to 675 mg in a serving of coffee (Stalmach et al., 2006). In a recent study by Mills et al. (2013), various types of pre-ground coffee with different roast grades were used and the CGA content was 27 to 121 mg/ 200 ml of coffee.

Apart from water, tea is the most widely consumed beverage worldwide (Yuan et al., 2011). The daily intake and types of tea consumed are different for different countries. In Asian countries, tea consumption is more prominent than coffee. A study in Singapore identified that most of the population studied consumed black tea (4.4 cups per week) followed by Chinese and green tea (Rebello et al., 2011). An epidemiological study amongst adults in Taiwan has identified that 96 % of the habitual tea drinkers consumed nearly 450 ml of green or oolong tea daily and another 4 % drank black tea (Wu et al., 2003). A large cohort study in Japan found that green tea was the most highly consumed (3 to 5 cups per day) followed by black tea (Mineharu et al., 2011). An inter countries study (Seven Countries Study) reported that tea was the major contributor of flavonoids for both Japan and the Netherland (Hertog et al., 1995). A longitudinal study amongst men in South Wales, UK has reported 5.4 cups of tea intake daily amongst the habitual tea drinkers (94 %) (Hertog et al., 1997). In addition, a study amongst a sub-sample of Scottish adults reported that this population drank more than 5 cups daily (Woodward and Tunstall-Pedoe, 1999).

Coffee drinking is more common in Western countries compared to Asian or Eastern countries. For example, a study in Finland found the majority (45.6%) of their middle aged adults consumed 3 to 5 cups of coffee per day (Eskelinen et al., 2009). A study in the United States has reported a less frequent coffee consumption (43.1%) of 5 or more times per week among the elderly who participated in the study (Arab et al., 2011). Finally, a study amongst Scottish adults has reported a large range of daily coffee consumption (0 to 20 cups) amongst the population studied (Woodward and Tunstall-Pedoe, 1999). Types of coffee consumed by the population were not specified in all the above studies.

1.5.2 Other sources

Apart from coffee and tea, fruit and vegetables are important sources of polyphenols in daily diet. Several studies have used population intake data to assess the major polyphenol food sources for the population studied. A study in Spain estimated the population intake of plant foods from national consumption data from households and institutions based on intake and food purchasing (MAPA, 2001). In this study, vegetables and fruit were found to be the most commonly consumed plant foods in the daily diet with potatoes and oranges being the major source (Saura-Calixto et al., 2007). In another study population data from daily per capita intake of selected fruit and vegetables was used to estimate the polyphenol intake in a Brazilian population. This study proposed that intake of fruit and vegetables facilitate the increasing availability of polyphenols in the daily food consumption of the population (Faller and Fialho, 2009).

A long term cohort study (Seven Countries Study) which determined the flavonoid intake in association with the risk of coronary heart disease and cancer reported that flavonoid intake for the United States, Greece, the former Yugoslavia and Finland found that onions and apples were the main food sources (Hertog et al., 1995). This study, however, only included two subclasses, the flavonols and flavones, in the estimation of flavonoid intake and thus greatly underestimated the potential main flavonoid food sources.

A study amongst Japanese women has estimated the flavonols and flavones intake of the studied population (Arai et al., 2000). In this population, vegetables (onion and molokhia) are the major contributors (72.3%) to the intake of these flavonoids, with tofu and natto the main providers of isoflavones.

Non-alcoholic beverages such as apple, pear and citrus fruit juices are also one of the main polyphenol food sources in certain populations such as in the United States (Chun et al., 2007, Maras et al., 2011) and France (Perez-Jimenez et al., 2011). Other flavonoid food sources for these populations include chocolate and other types of fruit such as peaches, plums and grapes.

Alcoholic beverages like wine are an important flavonoid source in certain population. Data from the EPIC study in Spain has reported wine as the second contributors (21%) to total flavonoid intake after apples (23%) (Zamora-Ros et al., 2010). A study in Greece found that wine was an important contributor to flavanol and proanthocyanidin intake of the population (Dilis and Trichopoulou, 2010). Wine consumption was reported a greater contributor to flavonoid intake in Mediterranean countries (16.7 %) than non-Mediterranean countries (9.8 %) (Zamora-Ros et al., 2013b).

Olives and olive oil, which are widely used in food preparation, are the fourth main polyphenol food source representing 11% of the total intake of the population in Spain (Tresserra-Rimbau et al., 2013).

1.6 Polyphenols and cognitive function

1.6.1 Cognitive functions

Any mental processes or functions which are mediated by the brain can be defined as cognitive functions. Several domains are associated with cognitive function including: executive function or problem solving, memory, attention, perception, psychomotor and language function (Schmitt et al., 2005). Other cognitive skills and abilities include arousal, information processing, speed of movement and accuracy (Bellisle et al., 1998). Assessment of cognitive performance using a range of cognitive tests involved the measurements of speed represented by reaction time and the accuracy of responses given by the participants. Of relevance to this thesis, memory and its divisions and executive function will be discussed.

Memory is one of the most commonly studied cognitive domains in relation to the effects of nutrition. The process of consolidation of information, (converting immediate into long-term memories), occurs in the hippocampus of the brain (Benjafield, 2007). Memory can be differentiated in terms of duration of storage (i.e. short-term and long-term memory) and in terms of processes (i.e. encoding, storage and retrieval). The type of information processed, stored and retrieved by the brain can include visual, spatial, verbal, auditory and procedural (Schmitt et al., 2005). Tulving described memory as procedural memory, perceptual representation system, working memory, semantic memory and episodic memory (Tulving, 1987, Tulving, 1991). (1) Procedural memory is involved with the gaining of multiple skills and

behavioural ability which happens unconsciously for example as in walking and running. (2) Semantic memory specifically refers to memory which enables humans to relate words, concepts and symbols with their properties and associations. (3) Working memory represents the usage of existing or past information in currently active information processing. Working memory consists of three major components, namely central executive, phonological loops, and visuospatial sketchpad. Central executive acts as a control system while the other two are the storage systems for this model (Baddeley, 2003). The phonological loops facilitate in the storing of information in the form of sound and language, while the visuospatial sketchpad facilitate in the processing of visuospatial information which play an important role in visual imagery (Baddeley, 1986, Baddeley, 2007). (4) Episodic memory denotes the process of storage and consciously recalling the past temporally dated experiences of events, episodes or information.

The information retrieval can be in the form of verbal, spatial and visual format. Verbal and visual memory relate to encoding, processing and retrieving information shown verbally or in the form of an image respectively. Spatial memory involves storage and recall of information on the spatial orientation of the environment (de Jager et al., 2013).

Memory tests conducted to study the association between food intake and cognitive function have normally involved short term and working memory. Generally, for word recall of twenty to thirty words the total number of words successfully recalled by the participants are between seven plus and minus two (Dye and Blundell, 2002). A higher number of words successfully recalled can be observed among certain groups such as students who are likely to be more

intelligent. Recognition tests which involve the identification of stimuli that were presented earlier may also be included as a means to assess memory.

Another classification of memory includes declarative or explicit memory and non-declarative or implicit memory (Rendeiro et al., 2012). Declarative memory is defined as the relationship between certain objects or events in their external environment while non-declarative memory is associated with thought, perception, skills and habits. Declarative memory differs from non-declarative memory such that it involves conscious attention while the other can be recollected unconsciously (Squire and Zola, 1996).

Executive functions or problem solving involves processing which is associated with obtaining certain goals including planning, monitoring and regulating, maintenance of goals and cognitive flexibility (McCabe et al., 2010). Executive function is a cognitive control that requires avoiding interference or response from former habit when doing certain actions. The control works by keeping the desired aim in mind and automatically prevents the interference from occurring (Reisberg, 2013).

Recently, interest in assessing the beneficial effect of nutrients on cognitive performance has grown by the increase in the number of publications on this subject. Nutrition may affect cognition throughout the life span. Furthermore, there are several factors which can affect human cognitive performance and need to be considered in the evaluation of the effect of nutritional intake or interventions on cognitive functions. Age is one of the important parameter which can affect cognitive performance. A long term cohort study reported a reduction in performance of all cognitive tests administered over a ten year period, except for vocabulary which was less influenced by age (Singh-Manoux et al., 2012). Other factors include mood,

motivation, attention, sleep, vigilance, effort and intelligence (Westenhoefer et al., 2004). In addition, there are many confounders that can affect cognitive performance and which may or may not have been controlled or considered in the design and analysis of studies of dietary intake and cognitive function. In some studies confounders such as age, education, intelligence and socio-economic status may be included in regression models to identify if the variables moderate or mediate the relationship between cognitive performance and nutrient exposure. For example, a large follow-up birth cohort in Scotland examined the role of flavonoid intake in cognitive decline and included these factors in the assessment (Butchart et al., 2011). Although flavonoid intake was not associated with a reduction in cognitive decline after adjusting for the confounding factors, this finding indicates the importance of controlling factors associated with cognitive performance. This step can prevent misinterpretation of the possible health benefits of certain nutrients to cognitive performance.

1.6.2 Polyphenol intake and cognitive performance

1.6.2.1 Cross-sectional studies

The Mini-Mental State Examination test (MMSE) is often used as the participants' screening tool, especially in a large population study as it measures global cognition of individuals (Folstein et al., 1975). Several studies have used this test for the cognitive assessment (see Table 1-4). A cross-sectional study in Spain used MMSE to measure cognitive capacity and reported better cognitive performance was associated with a higher intake of fruit (Ortega et al., 1997).

Combining several cognitive tests, as well as MMSE, have also been used, for example, with the Hordaland Health Study whereby episodic memory, executive function, semantic memory and visuo-spatial skills were also determined (Nurk et al., 2010). This study reported a dose-dependent association of flavonoid intake to be associated with better cognitive performance in several domains. For example, executive function, global cognition and semantic memory show strong relationships with total vegetable intake, while total fruit intake was associated with visuo-spatial skills, episodic and semantic memory. A study from Spain which used a set of cognitive batteries had identified better cognitive performance amongst participants who consumed more olive oil, coffee, wine and walnuts (Valls-Pedret et al., 2012).

Table 1-4 A summary of cross-sectional studies examining the association between polyphenol intake and cognitive performance

Authors	Study design and setting, sample size, age and gender	Method of dietary assessment	Cognitive tests	Key findings	Comments
Ortega et al. (1997)	Male and female elderly from Spain (N=260) aged 65-90 years	Assessment of food intake using 7 day weighed dietary record	Spanish language version of MMSE, Pfeiffer's Mental Status Questionnaire (PMSQ)	Healthy diet with higher intake of total food, fruit, carbohydrate, thiamine, folate and vitamin C associated with adequate MMSE score (≥ 28 points)	Intake of polyphenol-containing foods from fruit was associated to lower incidence of cognitive deficit. However, no causal-effect relationship can be drawn because the positive result was obtained from a mixed diet contained of several nutrients not just polyphenols.
Kuriyama et al. (2006)	Male and female elderly from Japan (N=1003) aged ≥70 years	Assessment of green tea consumption using self-administered questionnaire	Japanese language version of MMSE	The prevalence of cognitive impairment decreased with increasing consumption of green tea.	No quantification was made based on the volume (ml) of green tea consumed which maybe differed individually thus may affect the association with MMSE results.
Nurk et al. (2009b)	Male and female elderly from Norway (N=2031) aged 70-74 years	Assessment of wine, tea and chocolate consumption using FFQ	Kendrick Object Learning test (KOLT),Trail Making Test, part A (TMT-A), Digit Symbol test (m-DST) Block Design, short form (m-BD), MMSE and Controlled Oral Word Association test (S-task)	• Association between the food intake and cognitive performance are dose dependent. • The strongest effect on cognitive performance was shown in specific intake for wine and chocolate but linear association for tea.	Better cognitive performance was reported from the combinations of several cognitive tests was associated to the intake of specific polyphenol-containing foods. However, food intake estimation from FFQ can cause under or over estimation of polyphenol intake.

Table 1-4 Continued

Authors	Study design and setting, sample size, age and gender	Method of dietary assessment	Cognitive tests	Key findings	Comments
Nurk et al. (2010)	Same participants as Nurk et al. (2009)	Assessment of plant foods intake using FFQ	Same as Nurk et al. (2009)	• Participants in the highest (greater than10th percentile) intake of plant foods intake performed better in cognitive tests. • Associations between the combination of fruit and vegetables intake and cognitive performance are dose dependent (up to 500g/day) and reach plateau for grain products and potato (100 to 150 g/day).	The use of portion size in the form of household measures enables better quantification of the food intake of the population.
Valls-Pedret et al. (2012)	Male and female elderly from Spain (N=447) aged 55 to 80 years	Assessment of Mediterranean foods intake using FFQ and validated by face-to-face interview with trained dietitian	MMSE, Rey Auditory-Verbal Learning Test, Wechsler Memory Scale, Digit span test of the Wechsler Adult Intelligence Scale (WAIS) and Colour Trail Test (part I and II)	Better cognitive performance associated with the intake of specific foods (olive oil, coffee, walnut and wine).	Measurement of urinary polyphenols as biomarker and other measures such as physical activity and apolipoprotein E genotype enabled comparison to be made with many factors associated with cognitive performance.

1.6.2.2 Epidemiological studies

There are several studies which adapted MMSE as an outcome measure to determine cognitive changes of the population studied (see Table 1-5). For example, a study among the elderly in France has used this test as the measure of global mental status (Letenneur et al., 2007). Elderly people with a higher intake of flavonoids have lost fewer MMSE scores as compared to the lowest flavonoid consumers after a 10 year follow up. A large cohort study in the United States has used MMSE to identify the association between coffee and tea consumption with the variation in cognitive performance during nine years of follow-up (Arab et al., 2011). This study reported less cognitive decline with increased consumption of these beverages among women, without a dose-response effect. However, findings from the studies above does not imply direct association of polyphenol intake and cognitive performance as MMSE was used as a measure to screen out an individual with dementia or having mild cognitive impairment. Moreover, this test is unlikely to detect small changes in cognitive performance over a period of time, thus, making it inappropriate to infer the protective effect of polyphenols on cognitive performance. MMSE was reported as showing a ceiling effect in the scores in a follow up study amongst the cognitively normal elderly in Germany (Hensel et al., 2007). This problem was suggested to be due to a practice effect. In addition, MMSE does not have different versions which can be used in a study with repeated measurements or follow up.

Polyphenol-rich foods may improve cognitive function or reduce the risk of cognitive decline. A prospective study among middle-aged adults reported that frequent coffee drinkers had a lower risk of cognitive decline after 21 years of

follow-up (Eskelinen et al., 2009). In addition, frequent tea drinking habits have shown a beneficial effect in reducing the risk of cognitive impairment after two years of follow-up, in a population of elderly Chinese (Ng et al., 2008).

It can be summarized that studies which evaluate the association between polyphenol intake and cognitive performance were mostly performed among the elderly (Ortega et al., 1997, Kuriyama et al., 2006, Nurk et al., 2009b, Nurk et al., 2010, Valls-Pedret et al., 2012). This approach is subject to some weaknesses as the dietary reporting or recording can potentially be affected by cognitive decline as a part of the ageing process. Moreover, no generalization can be made from the results of epidemiological studies because of the chances of sample bias (Lamport et al., 2012). The approach of assessing the intake of polyphenols from the whole diet rather than specific polyphenol compounds is suggested in identifying the possible health benefits for humans. A large cohort study has suggested that looking at overall flavonoid intake rather than its subclass or at flavonoid containing foods can provide a better explanation of the potential effect of flavonoids on health (Devore et al., 2012). This was supported by the increase in the effectiveness of nutrients on health by the synergistic interaction of the complex matrix in a healthy diet (Parletta et al., 2013).

Table 1-5 A summary of epidemiological studies examining the association between polyphenol intake and cognitive performance

Authors	Study design and setting, sample size, age at the beginning of the study and gender	Duration (years)	Polyphenol food sources and method of dietary assessment	Cognitive tests	Key findings	Comments
Kang et al. (2005)	Female nurses from USA (N=15,080) aged between 30 to 55 years	17 years prospective study	Assessment of fruit and vegetable consumption using food frequency questionnaire (FFQ)	Telephone Interview for Cognitive Status (TICS) and East Boston Memory Test category fluency (naming animals)	• No association between total fruit intake and cognitive decline • High vegetable consumption was associated with lower cognitive decline among the elderly.	Dietary intake was self-reported which may be subject to alteration of food intake as a part of ageing process which may be caused changing in food preference. Cognitive decline was assesses only over a period of 2 years.
Letenneur et al. (2007)	Males and females elderly from France (N=1640) aged ≥65 years	10 years prospective study	Assessment of flavonoid intake using FFQ	Mini-Mental State Examination (MMSE), Benton's Visual Retention Test (BVRT) ''Isaacs'' Set Test (IST), Zazzo's cancellation test and Wechsler's Digit Symbol Test	Higher flavonoid intake associated with a better cognitive performance over a 10-year period.	A subsample of detailed dietary survey using 3 day record enables the conversion of food consumed from frequency (FFQ) to quantity for better estimation of flavonoid intake.

Table 1-5 Continued

Authors	Study design and setting, sample size, age at the beginning of the study and gender	Duration (years)	Polyphenol food sources and method of dietary assessment	Cognitive tests	Key findings	Comments
(van Gelder et al., 2007)	Male adults from Finland, Italy and the Netherlands who were born between 1900 to 1920 (N=676)	10 years prospective study	Assessment of coffee consumption using standardized self-administered questionnaire and cross-check with dietary history method	MMSE	Inverse association between numbers of cups of coffee consumed with cognitive decline.	MMSE is not a sensitive test to detect cognitive changes that occur over period of the study. No quantification was made based on the volume (ml) of coffee consumed.
(Ng et al., 2008)	Male and female adults from Singapore (N=1438) aged ≥55 years	2 years prospective study	Assessment of tea consumption using questionnaire on habitual intake of common types of tea	Chinese version of the MMSE	Regular tea consumption associated with lower risk of cognitive impairment and decline.	The approach of excluding participants detected with cognitive impairment at the baseline from MMSE data enables better understanding on the effect of tea drinking to cognitive performance assessed after 2 years.
(Eskelinen et al., 2009)	Male and female elderly from Finland (N=1409) aged 65 to 79 years	21 years prospective study	Assessment of usual dietary intake using FFQ and quantitative measurement of coffee and tea intake at the midlife examination	MMSE	Individual who drank coffee 3 to 5 cups daily was associated with the lowest risk or decrease risk of having dementia or Alzheimer disease.	Self-reported data on coffee and tea consumption might introduce under or over reporting on the drinking habit.

Table 1-5 Continued

Authors	Study design and setting, sample size, age at the beginning of the study and gender	Duration (years)	Polyphenol food sources and method of dietary assessment	Cognitive tests	Key findings	Comments
Arab et al. (2011)	Male and female elderly from USA (N=4809) aged ≥65 years	9 years prospective study	Assessment of usual dietary intake using FFQ	MMSE	Tea or coffee consumption reduce the rate of cognitive decline in women with no dose-response relation was detected.	No quantification was made based on the volume (ml) of coffee consumed.
Kesse-Guyot et al. (2012)	Male and female adults from France (N=12,741) aged between 35 to 60 years	15 years prospective study	Assessment of total and specific polyphenol intake using 24-hour dietary records	RI-48cued recall test, Semantic fluency task, Phonemic fluency task, Forward and Backward Digit Span and Delis-Kaplan Trail Making Test	• High total polyphenol intake was associated with better language and verbal memory but not with executive functioning. • A negative association was found between scores on executive functioning and the intake of some polyphenols.	No baseline data for cognitive performance available and assumption was made that the participants do not experience cognitive impairment at the baseline.

1.6.2.3 Intervention studies

Nutritional interventions were hypothesized as having a potential beneficial effect in slowing the cognitive decline. A recent review by Lamport et al. (2012) has summarized the association of polyphenol intake and cognitive performance from epidemiological and intervention studies. Foods and polyphenols which were used in intervention studies include berry juice, cocoa, soy milk, resveratrol, isoflavone and flavonoid supplements, and intervention studies ranged from acute or from 5 days up to 12 months. The authors summarized that the polyphenol consumption has shown beneficial effects only in a certain cognitive domain and these effects were small. In addition, there was no dose response effect of polyphenol intake on cognition as demonstrated in the intervention studies included in the review. For the purpose of this chapter, only two of these intervention studies will be discussed (Hendrickson and Mattes, 2008, Kennedy et al., 2010) (see Table 1-6). These studies were selected because the acute nature of the study administration. In addition, three recent studies (Cropley et al., 2012, Bookheimer et al., 2013, Pase et al., 2013) which were not included in the review by Lamport et al. (2012) are added to extend the review.

In an acute polyphenol intervention study using grape juice, participants were instructed to avoid energy-containing beverages and avoiding polyphenol containing foods three hours before the start of the study (Hendrickson and Mattes, 2008). This study showed no acute effect of the dietary intervention on memory for the intervention group, as assessed by a word fragment completion task. A study among healthy adults using two doses (250 and 500 mg) of trans-resveratrol instructed the volunteers to fast overnight with only water being allowed to be drunk before the first visit (Kennedy et al., 2010). This study reported an improvement in biological

measures with the increase in cerebral blood flow assessed from the total concentration of haemoglobin. However, cognitive function assessed from Serial Subtraction and Rapid Visual Information Processing was not affected by the intervention.

A recent double-blind, placebo-controlled crossover study with four acute treatments of coffee with various amounts of caffeine (5 to 167 mg in 6 g coffee powder) and chlorogenic acids (224 to 521 mg in 6 g coffee powder) was performed among healthy elderly people (Cropley et al., 2012). Several cognitive tests were administered at baseline and 40 minutes after the consumption of coffee products. A positive behavioural effect of non-caffeine compounds such as chlorogenic acid present in coffee was found amongst the participants such as increased alertness and decreased mental fatigue and headache. However, no treatment effects were found in the results of cognitive performance between treatment groups.

A recent placebo-controlled randomized trial gave 8 ozs of pomegranate juice for 4 weeks to the intervention group (Bookheimer et al., 2013). This study involved 28 elderly subjects with memory complaints screened by using MMSE. Performance in the memory test, as assessed using the Buschke-Fuld selective reminding task, showed a significant improvement compared to the placebo group at the end of the intervention. In addition, increase in functional brain activation during verbal and visual memory tasks was witnessed in the intervention groups as measured by functional magnetic resonance imaging (fMRI). Another current intervention study using a chocolate drink mix with three different dosages was performed among healthy middle-aged participants (Pase et al., 2013). This study did not find any improvement in cognitive function as assessed from a computerized cognitive battery from any dosage given to the participants.

There were some difficulties in determining the association between polyphenols and cognitive function. This includes the variety of the compounds present in foods, and the interaction between various bioactive compounds in polyphenol containing foods (Spencer et al., 2008), which may affect cognitive function. In addition, the application of various cognitive tests within the same cognitive domain or the focus on different cognitive domains has made comparison between studies difficult. In conclusion, there was little evidence of the effects of polyphenol intake on cognitive function and these effects were small and only shown on specific cognitive domain tested in the intervention studies.

Table 1-6 A summary on the effect of polyphenol intake and cognitive performance in intervention studies

Authors	Study design and setting	Study design and polyphenol food sources	Cognitive tests	Key findings	Comments
Hendrickson and Mattes (2008)	Male and female adults from USA (N=35) aged between 18 to 50 years	Acute, double-blind, within-subject and placebo-controlled study using10 ml/kg body weight of a placebo beverage or 100% Concord grape juice containing 2,100 mg/l total phenolics as gallic acid equivalents	Word Fragment Completion task	No acute effect of the dietary intervention on memory for the intervention group	The approach of giving the quantity of the juice based on body weight is a good way to ensure the standardization of the amount of drinks received by all participants in the intervention group.
Kennedy et al. (2010)	Male and female adults from UK (N=22) aged between 21 to 29 years	Acute, randomized, double-blind, placebo-controlled, crossover study. Two doses (250 and 500 mg) of trans-resveratrol and placebo and in counterbalanced order on separate days.	Serial Subtraction, Rapid Visual Information Processing (RVIP)	No acute effect of the dietary intervention on memory for the intervention group	Due to the acute nature of the study, assessment on the bioavailability of the supplements in human body is an essential step to understand the effect of the supplementation to cognitive performance.
(Cropley et al., 2012)	Male and female elderly (N=39) aged between 53 to 79 years	Acute, double-blind, placebo-controlled crossover study with four treatments of coffee with various amount of caffeine and chlorogenic acids, CGA (regular decaffeinated coffee with low CGA, regular decaffeinated coffee with high CGA, caffeinated coffee with low CGA and placebo)	Visual Verbal Learning Test (VVLT), Rapid Visual Information Processing (RVIP), Mismatch negativity (MMN), VVLT verbal delayed recall, VVLT delayed recognition, Emotional face recognition task (EFRT), Inspection time (IT), Stroop Color-Word Test (SCWT)	Positive behavioural effect of non-caffeine compounds such as chlorogenic acid in coffee on behaviour such as increase alertness and decrease mental fatigue and headache. No treatment effect was found in cognitive performance tests between treatment groups.	Short-term wash out period (by at least 1 week) can possibly cause learning effects on the cognitive test administered in the study.

Table 1-6 Continued

Authors	Study design and setting	Study design and polyphenol food sources	Cognitive tests	Key findings	Comments
Bookheimer et al. (2013)	Male and female elderly from USA (N=28) aged ≥60 years	Placebo-controlled randomized trial for 4 weeks involved the consumption of 8 ounces of either pomegranate juice or a flavour-matched placebo drink	Buschke-Fuld selective reminding task, unrelated word-pair associates learning, visual memory task	A significant improvement in memory task was shown in intervention group as compared to the placebo group	Combination of cognitive testing and fMRI enables identification of possible cognitive domain improved from the supplementation.
Pase et al. (2013)	Male and female elderly from USA (N=72) aged 40-65 years	Randomized, double-blind study for 30 days using three different dosage of chocolate drink mix contained 500 mg, 250 mg or 0 mg of polyphenols (placebo).	Immediate Word Recall, Simple Reaction Time, Digit Vigilance, Choice Reaction Time, Tracking, Spatial Working Memory, Numeric Working Memory, Delayed Word Recall, Delayed Word Recognition and Delayed Picture Recognition	Cognitive performance was not affected by the intervention at any doses given.	The cognitive tests administered in the study have covered various cognitive functions. This approach enables better understanding on the potential effect of the intervention on various cognitive domains.

1.6.3 Mechanisms

There are several ways diet can influence brain function which includes neurogenesis (Stangl and Thuret, 2009), facilitating neurotransmitter regulation, affecting membrane fluidity, synaptic transmission and involvement in signal transduction pathways (Gomez-Pinilla, 2008). The metabolic process in the active brain regions is mediated by a brain-derived neurotrophic factor (BDNF), a growth factor which is responsible for the stimulation of synaptic plasticity, enhances learning ability and formation of memory (Gomez-Pinilla and Nguyen, 2012). The hippocampus and hypothalamus are the main brain sites where these factors are present in large amounts and responsible for cognitive functions such as memory (hippocampus) and mood and metabolic control (hypothalamus) (Nawa et al., 1995).

Polyphenols have been linked to several beneficial effects on health but one area gaining interest is cognitive function. Polyphenols have been proposed to possess a neuroprotective effect and to have the ability to improve cognitive performance. There are three main possible sites where polyphenols can have direct action on brain function which include (i) outside the central nervous system (CNS), (ii) inside the CNS and (iii) at the blood brain barrier (Youdim et al., 2004, Ghosh and Scheepens, 2009, Spencer, 2009a).

The blood brain barrier (BBB) creates a block which will allow selected molecules to pass through the blood circulation to the brain. The properties of this barrier include controlling the traffic across the endothelium and control of the composition of brain's extracellular fluid (Youdim et al., 2004). Flavonoid properties such as the charged state and lipophilicity can affect the transportation of the compounds across the BBB by means of transcellular diffusion. In one animal

study, flavanol (-) epicatechin supplementation (100 mg/kg body weight) was given daily to male Wistar rats in three different durations of 1, 5 and 10 days (Abd El Mohsen et al., 2002). This study reported the production of metabolites epicatechin glucuronide and 3'-*O*-methylated epicatechin glucuronide in the plasma and brain tissue of the animal. However, an increase in the polarity of the glucoronides reduced the ability to partition; thus, decreasing the possibility for the compounds to cross the BBB. Another study has administered epicatechins supplemented diet to mice and has tested the spatial learning of the animal by using the Morris water maze (van Praag et al., 2007). This test was used to assess the ability of the test animal to find direction and can be manipulated using certain conditions such as food deprivation. This study has reported that epicatechin metabolites were detected in the brain tissue of the supplemented animal.

Polyphenols were proposed to act upon the modulation of memory and learning by their interaction with several neuron signalling pathways. A review has suggested a few mechanisms associated with this interaction which include (1) direct modulation of the kinases' activity such as mitogen-activated protein kinase (MAPKs) as responses to different stimuli, (2) attachment to enzymes and receptors on the adenosine triphosphate (ATP) sites, (3) maintaining the regulation of Ca^{2+} thus preventing the activation of Ca^{2+}-dependent kinases in neurons, (4) influence the function of phosphatases, enzymes which removes the phosphate group from its substrate by which the role is opposite from kinases, and (5) modulation of signalling cascades associated with the kinases (Spencer, 2009a).

Flavonoids can potentially exert effects on neuroprotection, by which the interaction with a few signalling cascades including MAPK was proposed as the means of action (Kobuchi et al., 1999, Kong et al., 2000). Flavonoids act by

influencing the modification of the expression level of targeted molecules within the pathways and phosphorylation mode (Ebrahimi and Schluesener, 2012). Three main MAPK pathways which are involved in the action of flavonoids in the nervous system include; mitogenic ERK 1/2, JNK and p38 cascades. JNK and ERK 1/2 act oppositely, one initiates neuronal apoptosis while the other mediates neuronal survival and growth.

Polyphenol-containing foods can potentially show beneficial effects to CNS by increasing the cerebral blood flow (CBF). Increase in CBF was suggested to show a promising effect on brain function (Ghosh and Scheepens, 2009). A previous study among sixteen healthy adults evaluated the effect of an acute dose of 450 mg flavanols given on CBF during cognitive tasks (Francis et al., 2006). In this study, CBF was assessed from blood oxygenation level dependent (BOLD) which was measured using functional magnetic resonance imaging (fMRI). An increase in CBF after the acute dose of flavanols given to the participants was reported, suggesting changes in cognitive function including adaptation and effort during the task. Another study gave three single dose treatments of placebo or two doses of epigallocatechhin gallate (EGCG) (135 mg and 270 mg) on separate days to assess the effect of the compound on CBF (Wightman et al., 2012). The changes in CBF during cognitive testing were assessed using near-infrared spectroscopy measured by oxygenated and deoxygenated haemoglobin. This study reported decreases in the concentration of oxygenated haemoglobin and total haemoglobin in the frontal cortex during the task period after the intake of 135 mg of EGCG. Although there was a lack of evidence on the potential effects on cognitive performance, this study has shown the potential beneficial effect of flavanols to brain function by increasing the

CBF. As a conclusion, polyphenols can potentially affect cognitive function through direct or indirect effect on the brain which can be facilitated by several mechanisms.

The dramatic increase of many life-style related diseases such as obesity, metabolic syndrome, diabetes and cardiovascular diseases has initiated many studies to investigate the role of foods in disease prevention and treatment. Polyphenols are suggested to have beneficial effects on a number of health issues. Of relevance to this thesis are the potential mechanisms by which polyphenols may positively affect cognitive function. The association between polyphenol intake and health can be evaluated through several approaches, including short term and long term intervention studies, cell studies, cohort or epidemiological studies, and cross sectional studies.

Obesity, as determined from body mass index (BMI) or waist circumference, was associated with increased risk of many chronic diseases and also poor cognitive performance. Some examples include a cross-sectional study among Canadian adults which reported an increased risk of lower cognitive performance among obese participants specifically on executive function assessed using the Trail Making Test (TMT) (Fergenbaum et al., 2009). In another study of healthy adults aged 20 to 82 years taken from the Brain Resource International Database, an increase in BMI was related to poor cognitive performance specifically in the executive function domain (Gunstad et al., 2007).

As obesity can lead to an increased risk of chronic disease and poor cognitive health, then weight reduction and weight management may help to improve long-term health. Polyphenols have potential beneficial effects on weight regulation. For example, in a study of overweight participants a reduction of waist circumference and body weight was observed by an average of 4.5 % and 4.6 % respectively after a

three month intervention of green tea extract given in the form of capsules (Chantre and Lairon, 2002). However, no control group or placebo arm was included in the study, so it is not possible to estimate whether this effect was significantly better than chance.

A review of short term effects of tea catechin consumption reported mixed results on energy expenditure (Westerterp-Plantenga, 2010). Some studies found an increase in energy expenditure of 4 to 4.6% measured 24-hours after treatment with EGCG (doses between 90 to 94 mg) in combination with caffeine (doses between 50 to 100 mg) (Dulloo et al., 1999, Rudelle et al., 2007). A non-significant result was reported by another study which showed an increase of 2.3% in energy expenditure after treatment with green tea (Gregersen et al., 2009). A long term study (12 weeks) among healthy Japanese men given either a low dose of catechins (77.7 mg) or a high dose (592.9 mg) of tea catechins, showed an increase in energy expenditure in the high dose group from 51 kcal to 90 kcal by week 12 (Harada et al., 2005).

A study using National Health and Nutrition Examination Survey (NHANES) data in the United States examined the association between frequency of tea and coffee consumption with the use of additions to the beverages including milk, cream, sugar or honey, and artificial sweetener in relation to BMI and abdominal obesity (Bouchard et al., 2010). The data suggested that frequency of tea consumption was associated with a lower BMI and waist circumference in men, while coffee showed no association with obesity. Unexpectedly, tea and coffee consumption with the addition of artificial sweeteners was associated with higher BMI.

Fruit and vegetables which are high in fibre and polyphenols have been studied in association with weight reduction. Davis et al. (2006) observed that normal weight individuals consumed more fibre and fruit than their age and height-

matched overweight or obese counterparts. This finding suggested that fibre intake as one of the polyphenol sources was inversely associated with body fat.

A study performed in Brazil which assessed habitual diet at baseline and after six months reported that an increased intake of fruit, vegetables and dietary fibre was associated with significant weight loss in overweight adults (Sartorelli et al., 2008).

A review of the potential association between fruit intake and body weight identified an inverse association in some of the intervention, prospective observational and cross-sectional studies (Alinia, 2009). Three intervention studies were included in this review with durations ranging from 8 to 12 weeks (Rodriguez et al., 2005, Fujioka et al., 2006, de Oliveira et al., 2008). Overall, the incorporation of high fruit intake in the diet significantly reduced body weight by 1 to 6 kg. However, no generalization can be made from the positive results reported in the review as the approach taken in these intervention studies was different in each case. In addition, the sample in each study differed in their dietary patterns which, in turn, could affect overall energy intake.

In conclusion, high BMI is associated with lower cognitive performance. The intake of polyphenol-containing foods such as tea, fruit and vegetables was associated with a reduction in weight, thus may potentially reduce the risk of chronic disease.

Diabetes is a condition of impaired glucose tolerance (IGT) and has been associated with a reduction in cognitive performance. A review by Lamport et al. (2009) suggested that IGT and poor glucose tolerance was related specifically to impairment in verbal memory. The authors suggested that IGT impaired transport of glucose across the blood brain barrier, thus affected the uptake of glucose by the brain. Reduced brain glucose is associated with poor cognitive performance.

Previous cross-sectional studies have reported cognitive decline amongst patients with type 2 diabetes (Stewart and Liolitsa, 1999, Awad et al., 2004). Compared to the control group (without diabetes), the decline was specifically shown in learning and memory skills and executive function.

Coffee, a polyphenol-rich beverage, has been associated with a lower risk of diabetes. For example, the EPIC study reported that coffee consumption as assessed from FFQ, showed an inverse association with type 2 diabetes risk (HR: 0.77 for caffeinated coffee and HR: 0.70 for decaffeinated coffee) (Floegel et al., 2012). In a sub-sample of rural Japanese adults, a lower rate of diabetes mellitus among men consuming coffee was reported after adjusting for age (Iwai et al., 2002). Another study in Japan has found a modest decrease in risk of diabetes among a cohort of community dwelling individuals who consumed coffee (Oba et al., 2010).

An inverse association was found between coffee consumption and inflammatory markers including tumour necrosis factor-α (TNF α) receptor II and C-reactive protein (CRP) among diabetic patients who participated in a cohort study performed in the United States (Williams et al., 2008a). In addition, regular coffee consumption was also associated with a reduction in both insulin resistance and the incidence of type 2 diabetes.

Polyphenols from fruit have also been associated with a reduction in risk of type 2 diabetes. A cohort study conducted in Finland found a strong inverse association of diabetes risk with berries and apples (Knekt et al., 2002). Another large prospective cohort study on American adults reported a lower risk of type 2 diabetes among participants with higher intakes of anthocyanins and specifically from blueberries, apple and pears (Wedick et al., 2012). As a conclusion, polyphenol food sources such coffee and fruit are associated with a lower risk of diabetes.

The metabolic syndrome (MetS) is a term associated with a combination of risk factors connected to cardiovascular disease (CVD) (Kahn et al., 2005). The risk factors include: (1) abdominal obesity measured by waist circumference, (2) hypertriglyceridemia, (3) low level of high density lipoprotein cholesterol (HDL), (4) high blood pressure and (5) high fasting blood glucose (NCEP, 2001). MetS is associated with poorer cognitive performance as found by a lower score in global cognition test, verbal learning and semantic memory with an increased number of MetS criteria fulfilled by the participants (Gatto et al., 2008).

An initiative was made to determine the long term association of several health factors associated with cardiovascular health and cognitive performance (Reis et al., 2013). These factors were defined by the American Heart Association which include blood pressure, physical activity, healthy diet, healthy weight, cholesterol level, smoking status and blood glucose (Go et al., 2013). This study has prospectively followed the participants with the initial age of 18 to 30 years for 7 to 25 years. Authors suggested that health and behavioural factors at a younger age may contribute to cardiovascular health, thus in turn related to better cognitive performance at a later stage of life. A longitudinal study conducted in London has found the same trend of poorer cognitive performance among individuals with long standing CVD (Singh-Manoux et al., 2008). This study suggested the importance of preventive measures at an early age to reduce the risk of CVD, as failure to do so lead to impairment in cognitive function in old age.

Diet is a modifiable factor for reducing the risk of CVD, and increasing evidence suggests polyphenols can make an important contribution to a healthy heart. A recent study in Iran examined the association between dietary polyphenols and metabolic syndrome among a sub sample of the adult population (Sohrab et al.,

2013). This study assessed measurements associated with metabolic syndrome including anthropometric, blood pressure, fasting blood glucose, triglycerides and HDL cholesterol. High consumption of flavonoids was associated with lower incidence of metabolic syndrome. Another study in China found an association between higher anthocyanidin intake and increased HDL cholesterol in females, and higher total flavonoids were associated with lower plasma triglycerides (Li et al., 2013).

Polyphenols may have a protective effect in other CVD risk factor such as hypertension. A cohort study has proposed a reduction in risk of hypertension in participants in the highest quintile of anthocyanin intake (Cassidy et al., 2011). An intervention was performed among participants with metabolic syndrome by which a daily dose of 50 gram freeze-dried blueberries was given for 8 weeks while the control groups consumed the same volume of fluid intake. The supplementation which was high in anthocyanins showed a significant decrease in systolic and diastolic blood pressure as compared with the control group (Basu et al., 2010). Another study among a group of adults at high risk of CVD reported a significant increase in HDL cholesterol and reduction in low density lipoprotein (LDL) after four weeks intervention using a combination of cocoa powder and skimmed milk (Khan et al., 2012). A randomized, placebo-controlled, double-blind crossover study over 8 weeks in diabetic patients used polyphenol-rich chocolate as the active product and chocolate with cocoa butter and without cocoa solids as placebo. A significant improvement in HDL cholesterol level and reduction in the ratio of LDL to HDL was reported after the intervention (Mellor et al., 2010). Furthermore, a meta-analysis showed a dose-dependent reduction in blood cholesterol after short-term (8 to 12 weeks) supplementation of cocoa products in participants with

cardiovascular risk (Jia et al., 2010). However, no positive effects on HDL or LDL following cocoa polyphenols have been shown in healthy participants.

In conclusion, higher polyphenol intake was negatively associated with the criteria of MetS, thus it may reduce the risk of CVD incidence. The summary on the potential indirect effect of polyphenol to health is shown in Figure 1-3. Intake of polyphenol-containing foods can potentially reduce the risk of several chronic diseases which are associated with poorer cognitive performance.

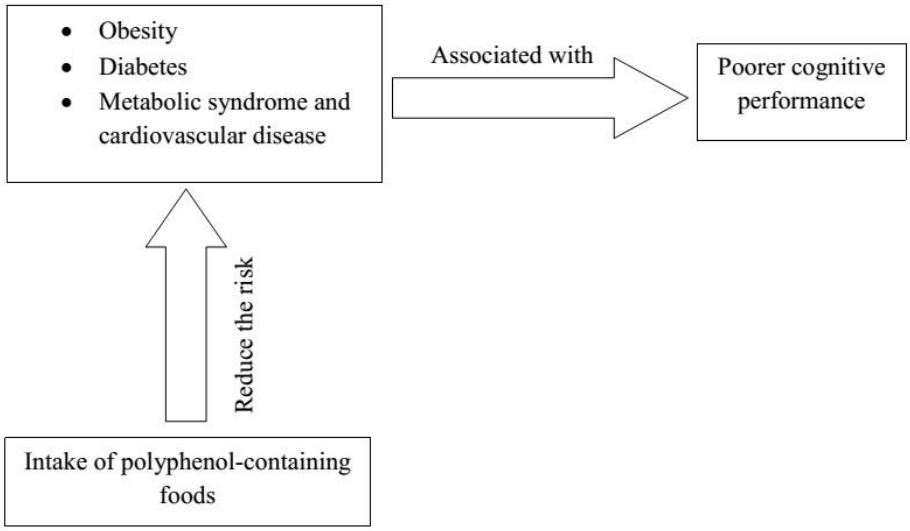


Figure 1-3 Summary of potential indirect effect of polyphenol on health

1.7 Summary of the relationship between polyphenol intake and cognitive performance

This chapter has summarized previous studies which focused on polyphenol intake in association with cognitive performance. Cross-sectional studies have reported that intake of specific polyphenol-containing foods generally produces better results in cognitive tests. Although no causal-effect conclusion can be made,

the cross-sectional studies can give an insight into the major polyphenol food sources of the population studied. Several prospective studies have reported positive associations between polyphenol intake and the reducing risk of cognitive decline or better cognitive performance. However, the variation of dietary intake throughout the length of the study can affect the polyphenol intake of the population studied, thus this needs to be evaluated carefully. Intervention studies using polyphenol-containing foods have reported mixed results in the effectiveness of the intervention in improving cognitive performance or reducing cognitive decline.

1.8 Aims and objectives

Only a few studies have reported the likely polyphenol intake of the UK population. There is limited research on the association of polyphenols and cognitive performance on the UK population. Taken together, this thesis aims to address some of the gaps in research by assessing both the polyphenol intake and the cognitive performance, using selected cognitive tests, on the UK female population. The hypothesis is that a high consumption of polyphenol containing foods will be associated with better cognitive performance amongst the study population. The objectives of this thesis are:

1. To estimate the polyphenol intake and major contributing polyphenol food or beverage sources in a sub sample of UK women.
2. To determine the total phenolic content and specific flavonoid content of selected foods and beverages using the Folin-Ciocalteu assay and HPLC respectively.
3. To examine the association between polyphenol intake and cognitive performance in a sample of UK women.

Chapter 2
Habitual polyphenol intake of UK women

2.1 Abstract

In this study an estimation of polyphenol intake was performed among two sub samples of UK women one overweight and/or obese and normal weight. The polyphenol intake of the studied population was 1089 ± 814 mg/day. Tea and coffee were the major polyphenol sources of the studied population. Apart from these beverages, fruit and vegetables and confectionaries were amongst the important sources of polyphenol. This study has moved a step in analysing a few quantitative variables for their association with polyphenol intake. A predictive equation was derived from the variables with significant association with polyphenol intake. Age was consistently associated with total polyphenol intake and two major polyphenol groups; flavonoids and phenolic acids. Coffee and tea drinking habits increased concomitantly with increasing age and can be associated to this finding. Employment status can directly affect the food choices, whereby students have shown a lower macronutrient intake as compared to the participants who are employed. The socio-economic status had an impact on the food selection of the studied population. The limitation of this study is that the existence of two distinct groups of population from Leeds Women's Wellbeing Study (LWW) and Diet and Health Study (DH) with different age range. Finally, the information obtained on the polyphenol intake by the

participants has shown the important to promote healthy eating amongst all age groups to enrich the polyphenol food source in the daily diet.

2.2 Introduction

The vast developments in agriculture and food processing have shifted the dietary pattern of the world's population by changing their food sources, selection, and preferences. A wide range of foods are now available and affordable to people in the Western world. Polyphenol-containing foods have been associated with some health benefits (see Chapter 1, Section 1-6); hence, interest in estimating polyphenol intake and dietary sources of polyphenols has grown. Different sources of information can be used to estimate polyphenol intake either at a population level for example by using Food Balance Sheet or based on samples of individuals who report their food intake. The analysis of these food intake reports relies on the availability of comprehensive databases which provide details of the polyphenol content of foods.

The Food Balance Sheet represents a country's food supply and utilisation (Food and Agricultural Organization, 2013) i.e. the food that is purchased in the country but not necessarily consumed. An assessment of flavonoid intake of the population of the United Kingdom and Ireland was performed based on data from the Food and Agricultural Organization's (FAO) Food Balance Sheet (Beking and Vieira, 2011). The results were presented in grams per capita per day. The flavonoid intake of the population estimated from five flavonoids (anthocyanins, flavanols, flavanones, flavones and flavonols) were 182.2 and 176.8 mg/day/person for the UK and Ireland respectively. Unfortunately this study did not estimate intake of other

polyphenols and estimation was based on raw food purchases rather than actual consumption.

Two polyphenol databases that are widely used in the estimation of polyphenol intake and satisfactorily fulfilled most of the criteria of an ideal food composition database are United States Department of Agriculture (USDA) database and Phenol-Explorer®. USDA has developed a few databases on the phytochemical content of food. The latest database is on the flavonoids content of selected foods where the data was collated from refereed journals and unpublished works (USDA, 2011). Phenol-Explorer® database contains data on polyphenol content in foods and was assembled and critically evaluated from original data (Neveu et al., 2010b). Methods used to quantify polyphenol content in foods in order to be included in the database were discussed in Chapter 1. For the estimation of polyphenol intake reported in this chapter, data from HPLC was selected as the first preference. Data from the Folin Ciocalteu assay was only used when HPLC data was not available. Morever, analysis of total phenolic content using Folin Ciocalteu assay is subjected to interference such as ascorbic acid (see Chapter 3 for further explanations). Further details on these databases are explained in Chapter 1.

There have been a few studies performed which aimed to estimate the polyphenol intake and their association to health benefits. A recent study identified the association between daily flavonoids and stilbenes intake and lipid profiles amongst Chinese adults (Li et al., 2013). Emphasis in this study was on fruit, vegetables and nuts which are commonly consumed by the respective populations. A study of Iranian adults examined dietary polyphenol intake using Phenol-Explorer® and the associated health benefits (Sohrab et al., 2013). Another study performed in Mexico specifically measured the contribution of beverages to the intake of

polyphenols in obese women using 24-hour food recall (Hervert-Hernandez and Goni, 2011). The methods used to estimate polyphenol intake and a summary of the intakes estimated in various populations is shown in Table 1-3 (Chapter 1).

The recent, large European Prospective Investigation into Cancer and Nutrition (EPIC) study (Zamora-Ros et al., 2011a, 2011b) estimated intake of particular flavonoids including flavonols, flavanones and flavones, phytoestrogens and anthocyanins in ten European countries using 24-hour dietary recall methods. The objective of the EPIC study was to discover food sources of selected polyphenols and in addition to assess confounders related to intake such as socio-demographic, anthropometric and lifestyle factors. Of particular relevance to the data presented in this chapter are the studies which assessed polyphenol intake in the UK.

As summarized in Table 2-1, a UK health conscious sample consumed higher amounts of certain flavonoid subclasses as compared to the general population. The health-conscious group consists of vegetarians, vegans and other health-conscious individuals (Riboli et al., 2002). However, no comparison can be made for total flavonoid intake because no data are available for this health conscious sample. In the EPIC study, tea and fruit were the major flavonoid contributors for the UK sample (Zamora-Ros et al., 2013b).

Table 2-1 Flavonoids intake of UK general and health-conscious samples in the EPIC study

Flavonoid groups	UK general population sample (N=403) (mg/day)	UK health-conscious sample (N=113) (mg/day)
Phytoestrogens[1]	4	21
Anthocyanidins[2]	22	28
Flavonols, flavones and flavanones[3]	98	131
Total flavonoids[4]	502	Not available

[1]Zamora-Ros et al. (2012), [2]Zamora-Ros et al. (2011b), [3]Zamora-Ros et al. (2011a), [4]Zamora-Ros et al. (2013b).

The studies presented in this chapter focus on the habitual polyphenol intake of women in the UK. Women generally undertake most of the food-associated tasks in the family, which includes purchasing groceries, cooking and serving food to other family members. This highlights the important role of women in food selection and determining foods consumed within the family (Johnson et al., 2011). In addition, women reportedly perceived themselves to be more conscious about food, more likely to read nutritional labels, practise healthy eating and be more knowledgeable about health and nutrition as compared to men (Oakes and Slotterback, 2001).

2.2.1 Study objectives

Previous studies performed among UK samples have mainly focused on flavonoid intake and have not included other polyphenol groups in the estimation of polyphenol intake. However, it is important to estimate the intake of other polyphenols group for a comprehensive approximation of total polyphenol intake of the population studied. In addition, too little attention has been paid to assess polyphenol consumption from the daily diet of the UK population especially from processed foods. Furthermore, the quantitative variables associate to the polyphenol intake is also important to be identified for a future promotion of healthy food intake.

These variables can be used as justifications in the development of future research. Therefore, this study intended to fill these research gaps.

The objectives of this study are:

- To estimate the polyphenol intake in a sub sample of UK women.
- To identify the main polyphenol food or beverage sources consumed by UK women.
- To identify quantitative variables associated with polyphenol intake in UK women.

2.3 Methodology

2.3.1 Study design

This study employed a cross sectional design where the habitual polyphenol intake was assessed using food diaries. The diaries were collected from two different studies (see Section 2.3.2).

2.3.2 Study samples

There are two groups of participants included in the study. These include participants from the Leeds Women's Wellbeing Study (LWW) (NHS ethics reference number: 10/H1305/6) and the Diet and Health Study (DH: study code D) (Ref No: 12-0020). Both studies were conducted in the Human Appetite Research Unit (HARU) at the Institute of Psychological Sciences, University of Leeds. Data taken from this study (LWW) were only from socio-demographic information and 7 day food diaries collected during the screening phase of the study. Women who took part in this 12 week intervention study were low fibre consumers (LF: study code R) whereas those excluded from the intervention but included in the current study were

high fibre consumers (HF: study code S). Low and high fibre consumers defined as fibre intake less than 15 and more than 15 g/day respectively. The intervention was focused on facilitating weight loss through two approaches; healthy eating advice alone or healthy eating with extra advice to increase fibre intake to a minimum of 25 g/day. The selection of participants into the LWW intervention was made based on a combination of three parameters; low scores results for the Dietary Instrument for Nutrition Education (DINE) for fibre (Roe et al., 1994), an adapted measure, the Leeds Women's Wellbeing DINE (LWW DINE) and fibre intake score all based on data from a 7 day food diary. The DINE score for fibre is categorized as low fibre intake (less than 30), medium fibre intake (30-40) and high fibre intake (more than 40). The LWW DINE and fibre intake score were estimated from fibre-containing foods consumed by the participants and were presented as gram of fibre intake per day. Hence, those with a fibre intake of more than 15 g per day were excluded from the intervention. Figure 2-1 shows the source of participants for the estimation of polyphenol intake of the samples. All of these participants were included as the diary data was collected prior to the LWW study intervention, thus, represents typical diets of women. Data of food intake from the Diet and Health study (DH) was obtained from dietary reporting by using a 3 day food diary. Inclusion criteria for this study include women aged 18 to 50 years, not pregnant, non-smoker, having normal body mass index and above ($\geq$18.5 kg/m^2) and having English as the first language. Further details of this study will be described in Chapter 4.

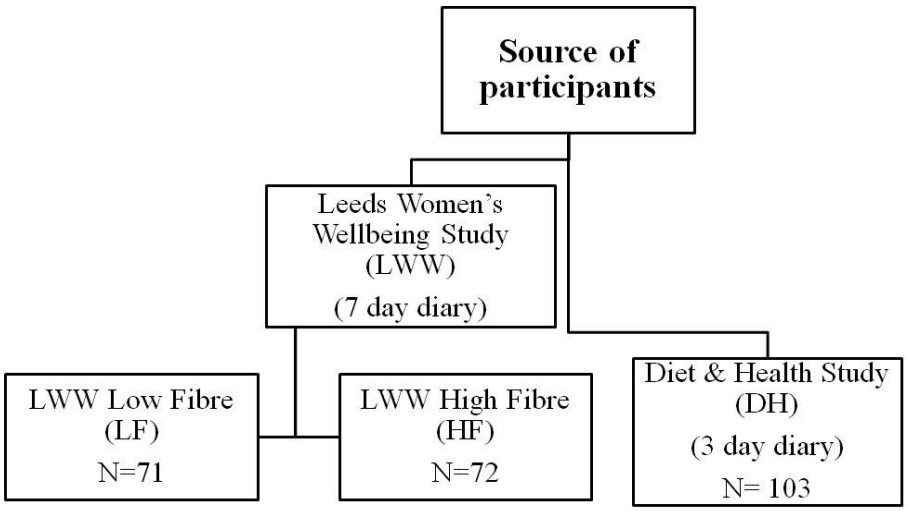


Figure 2-1 Source of participants for the habitual polyphenol intake study and type of food diary used to estimate polyphenol intake (LF: low fibre consumers, HF: high fibre consumers)

2.3.3 Measurement of habitual food intake

In this study, daily diaries were selected as the methodology to assess habitual food intake. Food intake was assessed using a self-completed food diary. 7 day food diaries were collected from LWW participants during the screening phase. For the DH study, a 3 day food diary in which all food consumed for 2 weekdays and 1 weekend day was recorded. Participants from both studies were asked to fill in the diary and bring the completed diary to their next visit at HARU. They were encouraged to record their food intake by using household measures and to include the food packaging within the diary where possible. The participants were informed about how to fill in the food diary and were shown examples of good dietary recording from other participants. In the DH study, a figure showing usual household measures was given to all participants to assist them in estimating their food intake. This figure was adapted from a food atlas developed by the Food Standard Agency (Nelson et al., 2002).

2.3.4 Estimation of nutrients intake

Food intake data from the food diaries were analysed using WinDiets® software. This software comprised of two food databases; namely UK Food Tables 2008 and USA Food Tables 2008. Food intake was assessed for all food consumed during the day which include; breakfast, morning snack, lunch, afternoon snack, evening meal and evening snack. The data were inputted in gram (g) of foods consumed by the participants. The weight of each food referred to the UK food portion size available in the database. To facilitate the approximation of portion size, the latest food portion guideline book for selected UK foods was used in the study (Cheyette and Balolia, 2010).

The macronutrient content of processed food i.e. carbohydrate, protein, fat and dietary fibre was sourced from the packaging provided by participants. Alternatively, some of the values were obtained by browsing supermarkets' websites such as Tesco, ASDA and Sainsbury's. The kilocalories of foods were calculated using conversion factor; 3.75, 4 and 9 for carbohydrate, protein and fat, respectively. Values for fibre-containing foods such as fruit and vegetables were extracted in WinDiets® using USA Food Tables 2008 because UK data did not follow the Association of Analytical Communities (AOAC) method to estimate fibre content of foods. Participants have reported coffee and tea consumption using household measures such as a cup or a mug and some in a specific amount (millilitre). Data from one cup was assumed as equal to 190 ml while 260 ml was used for one mug. These portions were suggested from the WinDiets® software and were used in data entering. The output for food intake of each day was exported into Microsoft Excel® for further analysis.

2.3.5 Estimation of polyphenol intake

2.3.5.1 Estimation from databases

A list of foods consumed by the participants was developed for reference purposes (see Appendix 12). It contained foods which include raw or processed foods, brand and participant study code. This list was used to assess the polyphenol-containing ingredients which were present in the food products. It also enabled a standardization process whenever the same food products were consumed by other participants. Processes involved in the estimation of samples' polyphenol intake are shown in Figure 2-2. Foods which do not contain any polyphenols such as meat-based products were omitted from the analysis of polyphenols. Ingredients of processed foods such as supermarket brand pasta meals were checked for polyphenol-containing ingredients. Foods that contained more than 1 mg per portion of any polyphenol were identified using the Phenol-Explorer® database when possible, and in combination with the USDA database on selected flavonoids to enable examination of the polyphenol content of as many foods as possible. The cut off used for foods to be included in the polyphenol estimation was based on a previous study which referred to foods that contributed less than 1 mg/day as minor contributors to polyphenol intake (Perez-Jimenez et al., 2011). Thus, foods that contained less than 1 mg of polyphenols as consumed in a usual portion were removed from the analysis.

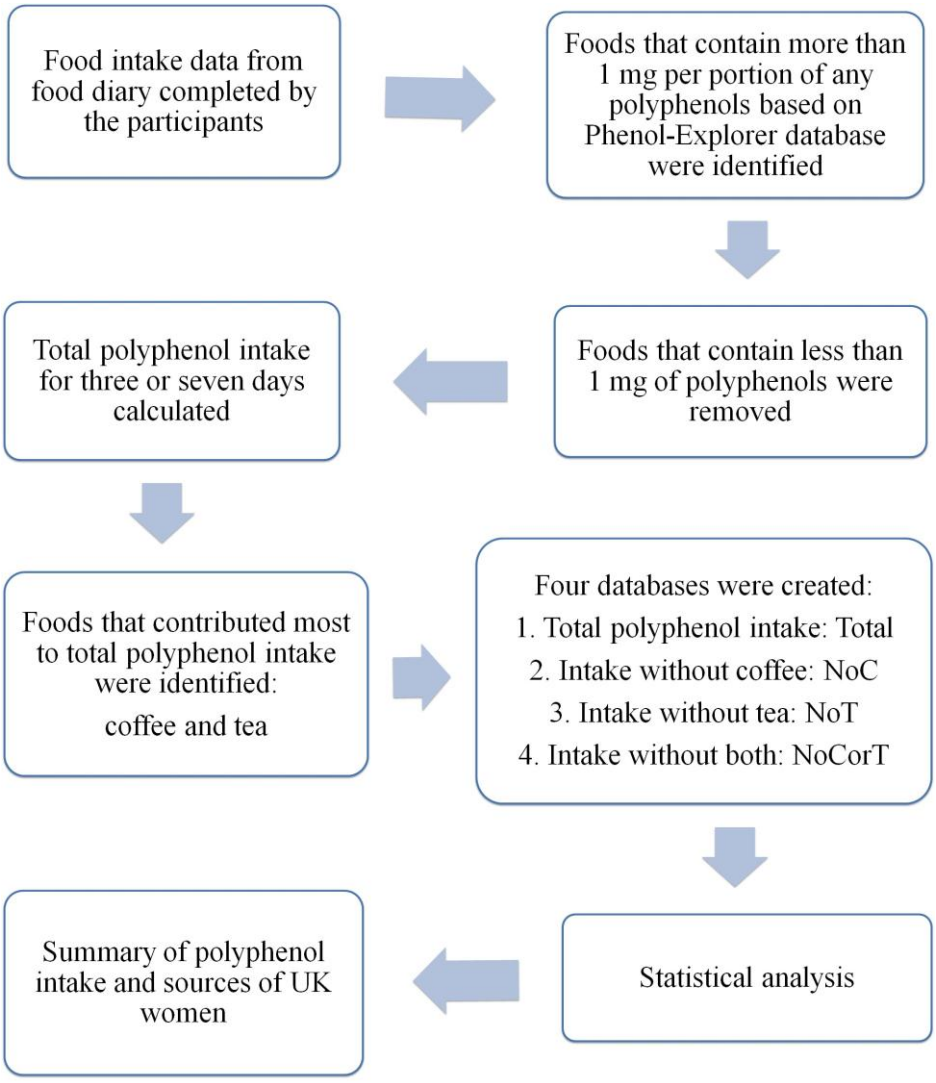


Figure 2-2 Flow chart of food diary analysis to estimate polyphenol intake

Table 2-2 shows the list of foods for which the USDA database was used for the estimation of flavonoids content of food consumed by the participants. Data for thearubugins from the USDA database was added to the existing data in Phenol-Explorer® because this compound is a major contributor to the flavanol content of tea (Kuhnert, 2010). Data for all other foods were taken from Phenol-Explorer® as it is more comprehensive database than USDA and comprised of various polyphenol contents of foods, not only flavonoids.

Table 2-2 List of foods for which the USDA database was used to estimate flavonoid intake

No.	Foods
1.	Okra
2.	Onions, spring or scallions (includes tops and bulb)
3.	Sweet potato
4.	Cranberry juice
5.	Beans, snap, green, cooked, boiled, drained, without salt
6.	Cabbage, chinese (pak-choi)
7.	Sour cherry, juice concentrate
8.	Blackberry, juice concentrate
9.	Blackcurrant juice
10.	Strawberry juice
11.	Tangerines, mandarin oranges

Using these two databases, total polyphenol intake for three or seven days was calculated and foods that contributed most to total polyphenol intake were identified. Intake was then analysed by number of diary days to find the estimated daily polyphenol intake. Figure 2-3 shows the polyphenol compounds included in the estimation of the samples' polyphenol intake. The 20 polyphenols in Figure 2-3 were selected on the basis that these compounds are commonly present in foods consumed by the sample studied. All compounds listed below were derived from HPLC methods in both databases. "Other" polyphenols do not belong to a specific class as defined within Phenol-Explorer®. Examples of compounds within this category include catechol, phlorin and pyrogallol.

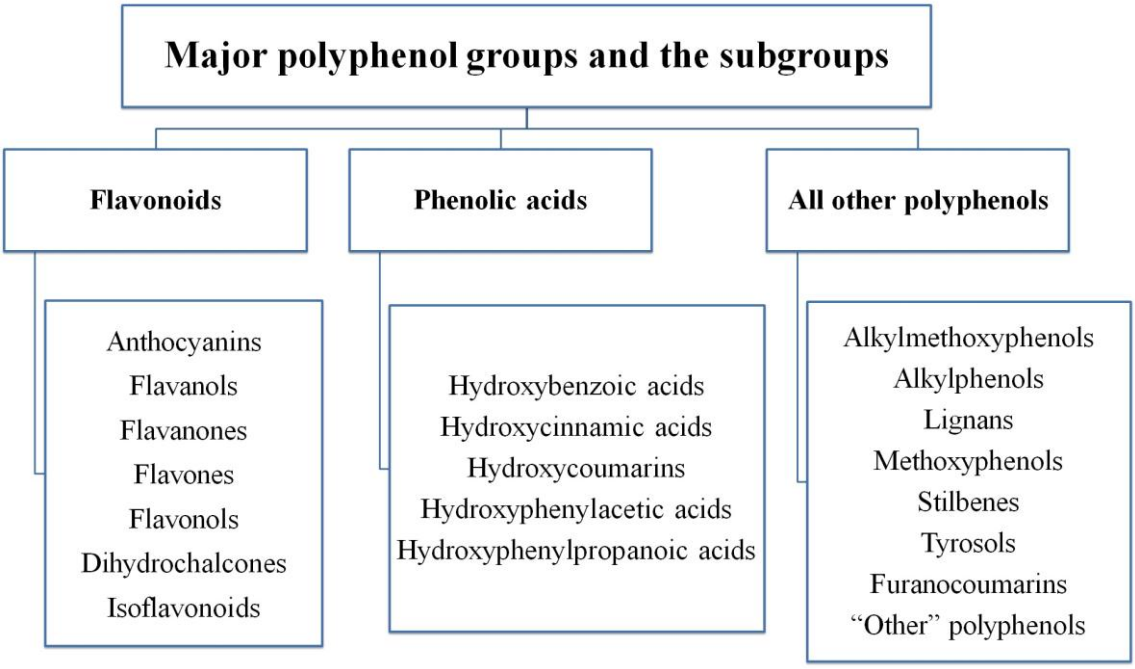


Figure 2-3 Compounds included in the analysis of polyphenol intake

2.3.5.2 Estimation from substitution of similar polyphenol content

Missing data from fruits, such as citrus fruits and sultanas, were estimated based on tangerine and raisin data from USDA and Phenol-Explorer® respectively (see Table 2-3). For other food groups which are mainly comprised of processed foods, the estimation was made according to the percentages of ingredients in the food products. Only ingredients with a polyphenol content of ≥ 1 mg per serving were included in the calculation of polyphenol intake.

Table 2-3 Strategy used to estimate polyphenol content in selected foods*

Food groups	Foods	Strategy used to estimate polyphenol content
Fruits	Satsuma	Referred to tangerine data from USDA and raisin data in Phenol-Explorer®
	Clementine	
	Mandarin	
	Sultana	
Cereals, wheat and seed	Cereal bar (fruit)	Referred to percentage of ingredients from product labelling
	Muesli (mixed cereals)	
	Oat flapjack	
	Cereal bar	
	Hummus	
	Mustard, whole grain	
Fruit based products	Fruit smoothies	Referred to percentage of ingredients from product labelling
	Mixed fruit juices	
	Fruit yoghurts	
	Fruit concentrates	
Soup and sauces	Instant soups	Referred to percentage of ingredients from product labelling
	Tomato ketchup	
	Apple sauce	
	Curry sauces	
	Pasta sauces	
Beverages	Earl Grey tea	Use data from black tea
	Red tea	
	Herbal tea	Use data from camomile tea
	Fruit tea	

*Data not directly available in Phenol-Explorer® and USDA.

Table 2-4 shows the list of foods where the polyphenol intake was made based on total phenolic content by using Folin Ciocalteau method. However, for the purpose of estimation of the polyphenol, these foods were not included in the summary of the polyphenol intake.

Table 2-4 Foods reported as total phenolic content in Phenol-Explorer®

Food (s)	Total phenolic content (in GAE mg/100 g or mg/100 ml)*
Squash	34.2
Pineapple juice	35.8
Turnip root	54.5
Pine nut	58.2
Honeydew	59.3
Green beets	61.1
Garlic	87
Fig	95.7
Cantaloupe	109.6
Peas	118.7
Whole oat	183.4
Tangerine	192
Ginger root	204.7
Fresh basil	231.8
Brazil nuts	244
Coriander seed	357.4
Fresh peppermint	980.4
Black pepper	1000
Adzuki beans	8970

2.3.6 Statistical analysis

Statistical analyses were performed using the Statistical Package for Social Science (SPSS, version 19). All data were examined for outliers using boxplots and the normality assumptions were checked for each inferential analysis. Data from continuous variables are presented as mean ± standard deviation. Percentages are used for categorical variables. Macronutrients and polyphenol intake data were combined from LWW and DH samples to provide a better estimation and greater representativity of the polyphenol intake of UK women. The total number of participants was 246 women. The results are presented in two perspectives; namely comparison between study groups to identify differences between 3 days and 7 days

food recording and between beverage consumption groups. Chi-square test was used to identify the association between two categorical variables. Independent t-test and analysis of variance (ANOVA) test were used to test differences of polyphenol intakes by study groups and beverage consumption groups. Data which are not normally distributed were analyzed using non-parametric tests to determine the differences between groups; namely Mann-Whitney U-test for two groups and Kruskall-Wallis test for more than two groups. To identify the association between variables and outcome, Spearman's Rank coefficient correlation was performed because most of the data were not normally distributed. Partial correlation was used to control the potential effect of other confounding variable which allow a better understanding on the association between two variables of interest (Pallant, 2007). In this study, polyphenol intake is the dependent variable. Multiple linear regression analysis was conducted to predict factors which were associated to polyphenol intake of the studied samples. *p* values of <0.05 or <0.01 were considered as statistically significant.

2.4 Results

2.4.1 Participant characteristics based on study groups

Table 2-5 presents the characteristics of participants based on study populations either between two study groups (LWW and DH) or between three studies (LWW-LF, LWW-HF and DH). From the data, there was a significant difference in age between the two study groups [$t\ (244) = 10.294$; $p<0.01$] and between the three study groups [$F\ (2, 243) = 54.641$, $p<0.01$]. Bonferroni corrected post hoc tests showed significant differences between LWW-LF and DH ($p<0.01$) and between LWW-HF and DH ($p<0.01$), however, there was no significant

difference between LWW-LF and LWW-HF. The same pattern was observed for body mass index between the two study groups [t (244) = 12.107; $p<0.01$] and between the three study groups [F (2, 243) = 83.919, $p<0.01$] with the significant differences observed between LWW and HF participants ($p<0.01$). LWW participants were older and heavier than DH participants. This difference was to be expected because LWW study participants were recruited specifically because they were overweight and obese and intended to participate in a weight loss intervention and increased weight is associated with increasing age.

More of the DH participants performed regular exercise ($\chi^2 = 4.58$; df = 1; $p<0.05$) and were students ($\chi^2 = 62.42$; df = 3; $p<0.01$) than the LWW participants. The regularity of performing exercise was solely based on participants' self-assessment and was not measured by any tool. Participants were asked to choose from two choices; whether they perform regular exercise or not, therefore, there is potential for participants to over report this variable. A significant difference in smoking status was expected because being a smoker was an exclusion criterion for the DH study.

In this chapter, basal metabolic rate (BMR) was calculated using the Schofield prediction equations (Schofield, 1985). The BMR value was used to determine the status of dietary recording of the participants by dividing energy intake with BMR (EI/BMR). The EI/BMR is categorized as under reporter (<1.14), normal reporter (1.14 to 2.4) and over reporter (>2.4) (Goldberg et al., 1991). There was a significant difference between the three study groups in the value of EI/BMR ($\chi^2 = 31.28$; df = 2; $p<0.01$). Interestingly, both LWW-HF and DH participants were

categorized as normal reporters, whereas LWW-LF participants were under reporters with the mean of EI/BMR less than 1.14.

2.4.2 Daily intake of selected nutrients based on study groups

Mean daily intake of selected nutrients by the study groups is shown in Table 2-6. The majority of the nutrients, including energy, protein, carbohydrate, fat and alcohol showed highly significant differences between the two study groups ($p<0.01$) with LWW participants have higher intake than DH participants. The difference found in energy intake between the two studies can partly be explained by the variety of foods consumed by LWW participants as compared to DH. In addition, most of the DH participants were students (65%) which might contribute to food availability and resources to buy foods. Overall, nutrient intakes of all macronutrients, except for carbohydrate, fat and dietary fibre exceeded the recommendation of the Reference Nutrient Intake (RNI). In addition, the percentages of the RNI for all macronutrients of DH participants are lower than LWW participants, except for carbohydrate and vitamin C. These differences can partly be explained by the lower intake of vitamin C by LWW-LF participants which leads to a lower overall mean of vitamin C intake of LWW participants. When a comparison was made between LWW-HF and DH, it was apparent that participants from LWW-HF have higher vitamin C intake than DH.

Fibre intake (in grams) as estimated from LWW DINE shows a significant difference between the two study groups [$U = 4842.5$; $Z= -4.58$, $p<0.01$] with DH participants have higher intake of fibre. Finally, score for fibre based on DINE showed no significant difference between the two study groups. From the ANOVA test, all macronutrients, DINE score and fibre intake estimated from LWW DINE showed significant difference between the three study groups (see Appendix 13).

However, only dietary fibre and fibre intake estimated from LWW DINE show significant difference between the study pairs as indicated from Bonferroni corrected post hoc tests. Dietary fibre intake shows highly significant difference between LWW-LF and LWW-HF ($p<0.01$); between LWW-LF and DH ($p<0.01$) and LWW-HF and DH ($p<0.01$).

Table 2-5 Participant characteristics between study groups*

	LWW (LF) (N=71)	LWW (HF) (N=72)	LWW (total) (N=143)	DH (N=103)	Whole sample (N=246)
Age (years)[1,2]	35.8 (10.1)	38.7 (8.5)	37.2 (9.4)	25.0 (9.0)	32.1 (11.0)
Weight (kg) [1,2]	85.1 (12.0)	84.2 (10.8)	84.6 (11.3)	66.5 (14.1)	77.1 (15.4)
Height (cm)	164.5 (6.6)	166.2 (6.4)	165.4 (6.6)	164.4 (6.1)	164.9 (6.4)
Body mass index (kg/m^2)[1,2]	31.3 (3.2)	30.4 (3.0)	30.8 (3.1)	24.5 (4.6)	28.2 (4.9)
Basal metabolic rate, BMR (kcal)^[3]	1626 (200)	1569 (138)	1597 (173)	1434 (154)	1529 (183)
Ratio of energy intake to BMR[1]	1.1 (0.3)	1.4 (0.3)	1.3 (0.3)	1.2 (0.3)	1.2 (0.3)
Category of employment[4]					
Employed (%)	63.4	75.0	69.2	24.3	50.4
Student (%)	22.5	15.3	18.9	65.0	38.2
Doing regular exercise (%)[5]	63.4	66.7	65.0	77.7	70.3
Units of alcohol consumed per week (unit)	5.1 (5.9)	5.9 (7.0)	5.5 (6.5)	5.1 (6.1)	5.3 (6.3)
Volume of beverages consumed (ml/day)	coffee: 207.9 (266.9) tea: 345.1 (381.0)	coffee: 220.20 (240.0) tea: 416.6 (413.2)	coffee: 214.0 (252.8) tea: 381.1 (397.7)	coffee: 87.0 (197.4) tea: 255.2 (333.1)	coffee: 160.8 (239.2) tea: 328.4 (376.5)
Non-smoker (%)[4]	67.6	68.1	67.8	100.0	81.3
Vegetarian (%)	2.8	13.9	8.4	12.6	10.2
Regular breakfast consumer (%)	74.6	86.1	80.4	87.4	83.3

*Data presented as mean (standard deviation) or percentage, BMR: Basal metabolic rate was calculated using the Schofield prediction equations, LWW: Leeds Women Wellbeing, LWW-LF: Leeds Women Wellbeing Low Fibre, LWW-HF: Leeds Women Wellbeing High Fibre, DH: Diet and Health.LWW: Leeds Women's Wellbeing Study, DH: Diet and Health Study. ^One unit of pure alcohol is equal to 10 ml or 8 grams, [1]$p<0.01$ ANOVA test, [2]$p<0.01$ independent t-test, [3]$p<0.01$ Kruskall-Wallis test, [4]$p<0.01$ Chi-square test, [5]$p<0.05$ Chi-square test.

Table 2-6 Selected nutrients intake in different study samples*

	LWW (LF) (N=71)	LWW (HF) (N=72)	LWW (total) (N=143)[ł]	% RNI^ LWW (total)	DH (N=103)[δ]	% RNI DH	Whole sample (N=246)	% RNI Whole sample
Energy (kcal)[1,2]	1797 (353)	2158 (426)	1976 (431)	101.9	1718 (430)	88.6	1868 (448)	96.3
Protein (g) [1,2]	74.2 (15.3)	80.2 (18.0)	77.2 (16.9)	171.6	68.4 (21.5)	151.9	73.5 (19.4)	163.4
Carbohydrate (g)[~ 1,2]	209.3 (48.0)	271.5 (54.6)	240.6 (60.0)	45.7	219.0 (60.6)	47.8	231.5 (61.1)	46.5
Fat (g)[§ 1,2]	68.0 (19.0)	80.5 (24.3)	74.3 (22.6)	33.8	63.4 (21.6)	33.2	69.7 (22.8)	33.6
Alcohol (g)[ς1,2]	10.0 (12.9)	11.0 (10.8)	10.5 (11.8)	0.5	5.4 (11.9)	0.3	8.4 (12.1)	0.4
Dietary fibre (g)[2]	14.1 (2.2)	26.5 (14.1)	20.3 (11.8)	84.6	19.8 (7.3)	82.3	20.1 (10.2)	83.7
Vitamin C (mg) [2]	46.6 (32.7)	92.4 (60.8)	69.7 (53.9)	174.3	82.2 (61.5)	205.5	74.9 (57.4)	187.3
DINE DFscore[2]	26.4 (10.4)	34.0 (8.6)	30.2 (10.3)	-	31.5 (10.8)	-	30.8 (10.5)	-
LWW DINE (g)[1,2]	10.5 (3.5)	13.2 (3.0)	11.9 (3.5)	-	15.4 (6.0)	-	13.4 (5.0)	-

*Data presented as mean (standard deviation), [1]p<0.01 Mann-Whitney test, [2]p<0.01 Kruskall-Wallis test. ^% RNI: Percentage from the Reference Nutrient Intake, [~]recommended at 50 % of food energy, [§]recommended at 35 % of food energy, [ς]should be less than 5% of total energy, [ł]food recorded for 7 days, [δ]food recorded for 3 days, DINE: Dietary Instrument for Nutrition Education for dietary fibre (DF), LWW DINE: Leeds Women Well-being DINE, LWW: Leeds Women's Wellbeing Study, DH: Diet and Health Study.

As mentioned previously, status of dietary reporting was assessed by calculating the ratio of energy intake to BMR. Table 2-7 displays the pattern of dietary reporting across three BMI categories. Results from Chi-square showed no significant difference (χ^2 = 2.78; df = 2; ns) in dietary reporting between BMI categories. Further analysis was performed between the three studies to identify the distribution in dietary reporting within the study groups (see Table 2-7). From the analysis, there is a significant difference between the three study groups in dietary reporting (χ^2 = 22.73; df = 2; p<0.01) by which more participants in the DH (54.9 %) and LWW-HF (80.6 %) study were normal reporters as compared to LWW-LF.

Table 2-7 Classification of dietary reporting based on body mass index (BMI) categories and different studies [number (%)]*

BMI categories	**Under reporter**	**Normal reporter**
Normal (N=67)	28 (41.8)	39 (58.2)
Overweight (N=84)	29 (34.5)	55 (65.5)
Obese (N=94)	44 (46.8)	50 (53.2)
Studies[1]	**Under reporter**	**Normal reporter**
LWW-LF (N=71)	41(57.7)	30 (42.3)
LWW-HF (N=72)	14 (19.4)	58 (80.6)
LWW total (N=143)	55 (38.5)	88 (61.5)
DH (N=102)	46 (45.1)	56 (54.9)
Whole sample (N=245)	101 (41.2)	144 (58.8)

*Dietary reporting was calculated from ratio of energy intake to basal metabolic rate calculated from Schofield equation, BMI: normal (18.5 to 24.9 kg/m^2), overweight (25.0 to 29.9 kg/m$^{2)}$, obese (> 30.0 kg/m^2), LWW: Leeds Women Wellbeing, LWW-HF: Leeds Women Wellbeing High Fibre, DH: Diet and Health, [1]p<0.01 Chi-square test.

2.4.3 Polyphenol food sources

Table 2-8 displays the major sources of polyphenols consumed by the population studied. Coffee and tea became the major beverage consumed by the participants. There were various types of tea consumed by the participants including black, green, camomile, and fruit tea. Onion and carrot became the most important vegetable food source for the population studied. Finally, foods such as baked bean and cooking sauce were identified as frequently consumed processed foods. The determination of polyphenol food sources relies on two aspects. Firstly, whether the foods are high in polyphenol content, so even if a small amount is consumed the contribution to polyphenol intake is significant. Secondly, some foods are consumed in large quantities; however, because of their low polyphenol content, their contribution to total polyphenol intake is not significant. An example of the first situation is spinach and onion which have high polyphenol content, while the second is pineapple and cabbage which have low polyphenol content. Conversely, coffee and tea fulfil both aspects whereby these beverages are consumed in high amount (millilitre) and have high polyphenol content. In summary, the most vital aspects to determine the polyphenol source in population is the quantity of foods consumed and the polyphenols content of the food products.

Table 2-8 Major polyphenol food sources of the studied population

Food groups	Foods
Beverages	Tea, coffee, hot chocolate, fruit concentrates
Vegetables	Onion, potato, tomato, lettuce, carrot
Fruits	Banana, apple
Others	Milk chocolate
Processed foods	Baked beans, hummus, cooking sauce, soups (various type and method of preparation)

2.4.4 Daily intake of polyphenols based on study groups

A comparison was made for a specific polyphenol intake between the LWW and the DH study (see Table 2-9). Overall, the intake of polyphenols for LWW was higher than for women in DH except for dihydrochalcones and lignans. The differences might be due to the higher coffee and tea consumption and the diversity of food sources consumed by the LWW participants. The daily intake of all major polyphenol groups was significantly different between the two studies ($p<0.01$), whereby LWW participants' intakes were higher. Moreover, the daily total polyphenol intake also showed a significant different between the two studies [$U = 4724$; $Z = -4.80$, $p<0.01$] with the mean intake of 1292.0 ± 843.5 and 807.6 ± 680.0 mg/day for LWW and DH participants respectively.

Participants were further categorized into high (> 1 g/day) and low polyphenol intake (< 1 g/day). This category was based on the suggestion that total polyphenol intake can be up to 1 g/day for individuals with daily consumption of several serving of fruit and vegetables (Manach et al., 2004). There is a significant difference between the two study groups ($\chi^2 = 10.60$; $df = 1$; $p<0.01$), by which the LWW participants have higher percentage of participants who consumed more than 1 g of polyphenol daily (56 %) than DH (35 %).

Table 2-9 Daily polyphenol intake of the population based on different study populations in mg per day*

	LWW (N=143)	DH (N=103)	Whole sample (N=246)	Foods with highest content of the compounds^
Anthocyanins[1]	19.9 (24.3)	18.6 (34.0)	19.1 (28.6)	Black elderberry, blackberry, blackcurrant
Flavanols[1]	588.9 (600.0)	391.5 (480.2)	505.8 (559.1)	Cocoa powder, dark chocolate, plum juice
Flavanones[1]	24.8 (31.6)	16.8 (25.8)	21.4 (29.3)	Oregano (herb), peppermint (herb), orange juice
Flavones[1]	3.1 (4.4)	2.4 (3.8)	2.8 (4.1)	Artichoke, sage (herb), peppermint (herb)
Flavonols	60.7 (39.5)	55.2 (42.5)	58.3 (40.7)	Spinach, capers (spices), black chokeberry
Dihydrochalcones	1.5 (2.2)	2.4 (3.1)	1.9 (2.7)	Apple, plum juice, apple puree
Isoflavonoids	3.2 (12.7)	1.2 (5.0)	2.4 (10.3)	Soybean, tofu, soy paste (cheonggukang)
Total flavonoids[1]	700.7 (638.4)	487.9 (525.1)	611.6 (601.8)	
Hydroxybenzoic acids[1]	66.8 (64.2)	45.8 (53.3)	58.0 (60.6)	Chestnut, blackberry, black raspberry
Hydroxycinnamic acids[1]	477.9 (516.6)	235.7 (416.9)	376.3 (491.2)	Plum, coffee, black chokeberry
Hydroxycoumarins[1]	0.2 (0.3)	0.1 (0.3)	0.2 (0.3)	Chinese cinnamon, white wine, beer
Hydroxyphenylacetic acids[1]	0.2 (0.3)	0.1 (0.2)	0.1 (0.2)	Green olive, black olive, red wine
Hydroxyphenylpropanoic acids	0.1 (0.2)	0.1 (0.1)	0.1 (0.2)	Green olive, black olive
Total phenolic acids[1]	544.8 (516.0)	281.7 (415.7)	434.6 (493.1)	

*Data presented as mean (standard deviation) in mg/day, ^Data taken from Phenol-Explorer® version 2.0 (Neveu et al., 2010), [1]$p<0.01$ with Mann-Whitney test, [2]$p<0.05$ with Mann-Whitney test, LWW: Leeds Women Wellbeing, LWW-HF: Leeds Women Wellbeing High Fibre, DH: Diet and Health

Table 2-9 Continued

	LWW (N=143)	**DH (N=103)**	**Whole sample (N=246)**	**Foods with highest content of the compounds^**
Alkylmethoxyphenols[1]	2.2 (2.7)	0.9 (2.2)	1.7 (2.5)	Rape seed oil, coffee, beer
Alkylphenols	27.4 (36.1)	24.3 (31.1)	26.1 (33.9)	Cereal bran, rye bread, whole grain flour
Lignans	4.6 (9.2)	6.5 (18.4)	5.4 (13.8)	Flaxseed, sesame seed, olive oil
Methoxyphenols[1]	0.3 (0.4)	0.1 (0.3)	0.2 (0.4)	Sesame seed oil, coffee
Stilbenes[2]	0.8 (1.2)	0.5 (1.2)	0.7 (1.2)	Muscandine grape wine, lingonberry, European cranberry
Tyrosols[1]	7.4 (18.1)	3.8 (8.8)	5.9 (15.0)	Black olive, green olive, olive oil
Furanocoumarins	0.1 (0.1)	0.1 (0.1)	0.1 (0.1)	Dried parsley, fresh parsley, celery stalks
"Other" polyphenols[1]	3.8 (3.3)	2.0 (3.1)	3.1 (3.3)	Coffee, pummelo juice, grapefruit juice
Total all other polyphenols[2]	46.5 (44.0)	38.1 (40.5)	43.0 (42.7)	
Total polyphenols[1ζ]	1292.0 (843.5)	807.6 (680.0)	1089.2 (813.7)	

*Data presented as mean (standard deviation) in mg/day, ^Data taken from Phenol-Explorer® version 2.0 (Neveu et al., 2010), [1]$p<0.01$ with Mann-Whitney test, [2]$p<0.05$ with Mann-Whitney test, LWW: Leeds Women Wellbeing, LWW-HF: Leeds Women Wellbeing High Fibre, DH: Diet and Health, [ζ]The addition of total flavonoids, total phenolic acids and total other polyphenols.

Further analysis was performed to compare the total polyphenol intake based on the major polyphenol groups between under and normal reporters (see Table 2-10). Result shows that underreporting in food intake does contribute to the differences in two major polyphenol groups namely flavonoids [U = 5852; Z = -2.60, $p<0.01$] and other polyphenols [U = 5999; Z = -2.33, $p<0.05$]. In addition, daily total polyphenols showed a significant difference between the two dietary reporting categories [U = 5877; Z = -2.56, $p<0.05$]. The intake of major polyphenol groups was higher amongst normal reporters.

Table 2-10 Daily polyphenol intake categorized by dietary reporting based on main polyphenol groups in mg per day

	Under reporter (N=101)	**Normal reporter (N=144)**
Total flavonoids[1]	541.17 (637.35)	664.03 (577.16)
Total phenolic acids	375.38 (436.07)	478.91 (527.37)
Total other polyphenols[2]	35.64 (35.77)	48.39 (46.31)
Total polyphenols*[2]	952.19 (797.43)	1191.32 (814.87)

[1]$p<0.01$ with Mann-Whitney test, [2]$p<0.05$ with Mann-Whitney test. *Data presented as mean (standard deviation). Total polyphenols defined as the addition of total flavonoids, total phenolic acids and total others polyphenols. Categories of dietary reporting based on the ration of basal metabolic rate to energy intake include under reporter (<1.14), normal reporter (1.14 to 2.4) and over reporter (>2.4).

2.4.5 Participant characteristics based on beverage consumption groups

From the diary analysis, it was evident that coffee and tea were the major sources of polyphenol intake in these women and, therefore, major contributors to total polyphenol intake of the studied samples. In order to compare the amount and type of total polyphenol intakes with and without the contribution of coffee and tea, the whole dataset was partitioned into four subsets for analysis. These subsets were:

1. Total polyphenol intake: Total
2. Intake without coffee: NoC
3. Intake without tea: NoT
4. Intake without coffee or tea: NoCorT

Table 2-11 displays the distribution of beverage consumers. It is apparent that most of the participants consumed both coffee and tea (39%) followed by consumed tea only (32.5%). In addition, more of LWW participants were consumers of both coffee and tea while more of DH participants were tea consumers. There is a significant difference between three studies (χ^2 = 38.83; df = 6; $p<0.01$) and between LWW and DH study (χ^2 = 36.31; df = 3; $p<0.01$) in the consumption of beverages.

Table 2-11 Grouping of participants from studies based on beverage consumption groups*[1]

	LWW (LF) (N=71)	LWW (HF) (N=72)	LWW (total) (N=143)	DH (N=103)	Whole sample (N=246)
Coffee only	10 (14.1)	8 (11.1)	18 (12.6)	11 (10.7)	29 (11.8)
Tea only	17 (23.9)	20 (27.8)	37 (25.9)	43 (41.7)	80 (32.5)
Consume both coffee and tea	35 (49.3)	41 (56.9)	76 (53.1)	20 (19.4)	96 (39.0)
Non-consumer of coffee or tea	9 (12.7)	3 (4.2)	12 (8.4)	29 (28.2)	41 (16.7)

*Data presented as number (%), [1]$p<0.01$ Chi-square test

Table 2-12 shows the demographic data of the population based on the beverage consumption groups. There is a significant difference for age [$F (3, 242) = 20.69$, $p<0.01$] between the four groups. Bonferroni corrected post hoc tests showed significant differences between non-consumers of coffee or tea with all three other groups (consumers of both coffee and tea: $p<0.01$, coffee only: $p<0.01$ and tea only: $p<0.05$) in age, with the non-consumers of coffee or tea are younger. Furthermore, a significant difference in age was also found between consumers of both coffee and tea and tea consumers ($p<0.01$) with tea consumers are younger.

BMI differed significantly between beverage consumption groups [$F (3, 242) = 6.08$, $p<0.01$] specifically between consumers of both coffee and tea with two other groups (non-consumers of coffee or tea: $p<0.01$ and tea only: $p<0.05$). Consumers of both coffee and tea have higher BMI than the two other groups. There is a tendency of participants with higher BMI and older age to consume both beverages and younger participants to not consume either coffee or tea.

There were more employed participants consuming both coffee and tea while the majority of those consuming neither drink were students (χ^2 = 30.89; df = 9; $p<0.01$). Results from ratio of energy intake to basal metabolic rate showed that participants from all beverage consumption groups were categorized as normal reporters which are different from previous finding reported in this chapter when comparison was made between the study groups (see section 2.4.1). This difference might due to a better distribution of participants in term of age (in year) when the categorization was made based on beverage consumption groups.

There was a significant difference in the volume of the drinks consumed daily [coffee: (χ^2 = 207.94; df = 3; $p<0.01$), tea: (χ^2 = 153.44; df = 3; $p<0.01$)] between the four study populations. The disparity is expected because the comparison was made with the non-consumers of coffee or tea. No significant difference was found in performing regular exercise (χ^2 = 5.39; df = 3; ns), consumed regular breakfast (χ^2 = 5.22; df = 3; ns) and being vegetarian (χ^2 = 1.69; df = 3; ns).

Table 2-12 Participant characteristics between beverage consumption groups*

	Coffee only (N=29)	Tea only (N=80)	Consume both coffee and tea (N=96)	Non-consumer of coffee or tea (N=41)
Age (years)[1]	33.8 (10.4)	29.5 (10.4)	37.3 (10.2)	23.8 (7.4)
Body mass index (kg/m^2)[1]	28.6 (5.0)	27.4 (5.4)	29.6 (4.1)	26.1 (4.8)
Basal metabolic rate, BMR (kcal)*	1544 (137)	1530 (200)	1538 (169)	1497 (212)
Ratio energy intake to BMR	1.2 (0.4)	1.2 (0.3)	1.3 (0.3)	1.2 (0.3)
Category of employment[3]				
Employed (%)	58.6	46.2	62.5	24.4
Student (%)	34.5	41.2	25.0	65.9
Doing regular exercise (yes) (%)	65.5	67.5	67.7	85.4
Units of alcohol consumed per week (unit)^	3.5 (4.3)	4.9 (5.6)	6.4 (7.8)	4.98 (4.21)
Volume of beverages consumed (ml/day)[2]	coffee: 434.3 (317.5)	tea: 467.4 (365.2)	coffee: 280.9 (217.9) tea: 452.0 (378.8)	-
Vegetarian (yes) (%)	6.9	11.2	8.3	14.6
Regular breakfast consumer (yes) (%)	75.9	85.0	83.3	85.4

*Data presented as mean (standard deviation) or percentage, BMR: Basal metabolic rate was calculated using the Schofield prediction equations, ^One unit of pure alcohol is equal to 10 ml or 8 grams, [1]$p<0.01$ ANOVA test, [2]$p<0.01$ Kruskall-Wallis test, [3]$p<0.01$ Chi-square test.

2.4.6 Daily intake of selected nutrients based on beverage consumption groups

Table 2-13 displays the distribution of BMI based on beverage consumption groups. There is a significant difference between study groups in BMI categories (χ^2 = 30.10; df = 6; $p<0.01$) with more tea consumers categorized as having normal BMI and more participants who consumed both coffee and tea having a BMI more than 30 kg/m^2 (obese).

Table 2-13 Relation of body mass index (BMI) to beverage consumption*

BMI categories	Normal	Overweight	Obese
Coffee consumer (N=29)	7 (24.1)	10 (34.5)	12 (41.4)
Tea consumer (N=80)	30 (37.5)	23 (28.7)	27 (33.8)
Consume both coffee and tea (N=96)	10 (10.4)	40 (41.7)	46 (47.9)
Non-consumer of both drinks (N=41)	21 (51.2)	11 (26.8)	9 (22.0)

*Data presented as number (%). [1]$p<0.01$ with Chi-square test

Table 2-14 shows the daily nutrient intake based on beverage consumption groups. There was only a significant difference in nutrient intake for energy (χ^2 = 9.0; df = 3; $p<0.05$), protein (χ^2 = 17.93; df = 3; $p<0.01$) and alcohol (χ^2 = 20.22; df = 3; $p<0.01$) intake. Further Mann-Whitney test showed significant differences in daily energy intake between non-consumers of coffee or tea and consumers of both coffee and tea ($p<0.05$) and between consumers of both coffee and tea and tea consumer ($p<0.01$). Consumers of both coffee and tea have the higher intake of energy than the other two groups.

Protein intake was significantly difference between consumers of both coffee and tea with three other groups (non-consumers of coffee or tea: $p<0.01$, coffee consumers: $p<0.05$ and tea consumers: $p<0.01$). Consumers of both coffee and tea have the higher intake of protein than the other three groups.

Daily alcohol intake was significantly different between non-consumers of coffee or tea with three other groups (consumers of both coffee and tea: $p<0.01$, coffee consumers: $p<0.01$ and tea consumers: $p<0.01$). Alcohol intake was also significantly different between consumer of both coffee and tea and tea consumer ($p<0.05$). Non-consumers of coffee or tea have the lower intake of alcohol than the other three groups.

DINE DF score showed slight differences ($\chi^2 = 7.95$; $df = 3$; $p<0.05$) between beverage consumption groups. The different was significantly different between non-consumers of coffee or tea and consumers of both coffee and tea ($p<0.01$). Non-consumers of coffee or tea have the lower intake of fibre than consumers of both coffee and tea. The lack of significant differences in other variables can be partly explained by the distribution of participants from various age, BMI and employment status in the beverage consumption groups.

Table 2-14 Selected nutrients intake in different beverage consumption groups*

	Coffee only (N=29)	% RNI^	Tea only (N=80)	% RNI	Consume coffee and tea (N=96)	% RNI	Non-consumer of coffee and tea (N=41)	% RNI
Energy (kcal)[1]	1845 (525)	95.1	1797 (426)	92.6	1965 (430)	101.3	1797 (500)	92.6
Protein (g)[2]	69.6 (20.8)	154.6	70.8 (19.9)	157.3	79.4 (18.3)	176.4	67.9 (17.4)	150.9
Carbohydrate (g)[~]	226.5 (66.2)	46.0	222.8 (54.5)	46.5	238.0 (60.4)	45.4	237.0 (70.3)	49.5
Fat (g)[§]	69.4 (26.2)	33.9	67.1 (23.1)	33.6	73.8 (21.8)	33.8	65.6 (21.2)	32.9
Alcohol (g) [ς2]	10.1 (12.1)	4.9	8.1 (12.7)	4.0	10.4 (12.7)	4.8	3.1 (7.3)	1.6
Dietary fibre (g)	18.7 (9.4)	77.9	19.7 (12.1)	81.9	20.8 (9.2)	86.5	20.3 (8.7)	84.5
Vitamin C (mg)	74.0 (56.2)	123.4	72.8 (54.0)	121.4	77.4 (52.9)	128.9	73.9 (74.5)	123.2
DINE DF score[1]	28.6 (9.9)	-	31.7 (12.1)	-	32.1 (9.0)	-	27.2 (9.8)	-
LWW DINE (g)	12.6 (5.9)	-	14.1 (5.6)	-	13.3 (4.3)	-	12.7 (4.5)	-

*Data presented as mean (standard deviation) or percentage. [1]$p<0.05$ Kruskall-Wallis test, [2]$p<0.01$ Kruskall-Wallis test. ^% RNI: Percentage from the Reference Nutrient Intake, [~]recommended at 50 % of food energy, [§]recommended at 35 % of food energy, [ς]should be less than 5% of total energy; DINE: Dietary Instrument for Nutrition Education for dietary fibre (DF); LWW DINE: Leeds Women Well-being DINE.

2.4.7 Daily intake of polyphenol with and without the contribution from coffee and tea

In order to identify the contribution of polyphenol sources other than coffee and tea, a comparison was made between total polyphenol intake (Total dataset) and intake excluding coffee and tea (NoCorT dataset) (see Table 2-15). The average intakes of total polyphenols were 1089 ± 814 and 213 ± 129 mg/day for Total and NoCorT dataset respectively. From the data, clearly some polyphenols are only present in coffee or tea, or in fruit and vegetables, but others are present in all these foods for example the flavonols. The percentages of NoCorT to Total dataset were calculated to identify the contribution of coffee and tea polyphenols to polyphenol intake. 100 % indicates that all polyphenols are derived from fruit and vegetable sources, whereas 0 % shows that the beverages provide all the polyphenols in these categories. Alkylmethoxyphenols and flavanols which mainly contained in coffee and tea are among polyphenol with the highest contribution to the Total dataset. This is evident by the low percentages of difference found in alkylmethoxyphenols (5.9 %) and flavonols (6.5 %) between the two datasets. Other polyphenols which also contributed to Total dataset include hydroxybenzoic acids (13.9 %) and hydroxycinnamic acids (14.7 %). The differences between Total and NoCorT datasets in total flavonoids and total phenolic acids are expected because the majority of the compounds are present in coffee and tea. Finally, the total polyphenol intake after the addition of total flavonoids, total phenolic acids and total all other polyphenols were five times greater for the Total dataset as compared to NoCorT dataset.

Table 2-15 Impact of coffee and tea to total polyphenol intake based on Total and No Coffee and Tea (NoCorT) dataset in mg per day*

	Total polyphenol intake[a] [mean (SD)]	**Total polyphenol intake excluding coffee and tea intake[b] [mean (SD)]**	**Percentage of NoCorT to All (%)**
Anthocyanins	19.1 (28.6)	19.1 (28.6)	100
Flavanols	505.8 (559.1)	32.8 (35.8)	6.5
Flavanones	21.4 (29.3)	21.4 (29.3)	100
Flavones	2.8 (4.1)	2.8 (4.1)	100
Flavonols	58.3 (40.7)	29.7 (22.5)	50.9
Dihydrochalcones	1.9 (2.7)	1.9 (2.7)	100
Isoflavonoids	2.4 (10.3)	2.4 (10.3)	100
Total flavonoids	611.6 (601.8)	110.3 (79.6)	18
Hydroxybenzoic acids	58.0 (60.6)	8.1 (11.3)	13.9
Hydroxycinnamic acids	376.3 (491.2)	55.3 (51.0)	14.7
Hydroxycoumarins	0.2 (0.3)	0.2 (0.3)	100
Hydroxyphenylacetic acids	0.1 (0.2)	0.1 (0.2)	100
Hydroxyphenylpropanoic acids	0.1 (0.2)	0.1 (0.2)	100
Total phenolic acids	434.6 (493.1)	63.8 (54.5)	14.7
Alkylmethoxyphenols	1.7 (2.5)	0.1 (0.2)	5.9
Alkylphenols	26.1 (33.9)	25.7 (33.9)	98.5
Lignans	5.4 (13.8)	5.4 (13.8)	100
Methoxyphenols	0.2 (0.4)	0.1 (0.1)	50
Stilbenes	0.7 (1.2)	0.7 (1.2)	100
Tyrosols	5.9 (15.1)	5.9 (15.1)	100
Furanocoumarin	0.1 (0.1)	0.1 (0.1)	100
"Other" polyphenols	3.1 (3.3)	1.5 (2.5)	48.9
Total all other polyphenols	43.0 (42.7)	39.2 (42.0)	91.1
Total polyphenol	1088.7 (813.6)	212.7 (129.4)	19.5

*Data presented as mean (standard deviation) in mg/day or percentage, [a]Data from Total dataset, [b]Data from NoCorT dataset

Percentage contribution of each polyphenol was calculated for each beverage consumption group (see Figure 2-4). The contribution of hydroxycinnamic acids to total polyphenol intake of coffee consumers was higher (86%) than flavanols for tea consumers (71%). The disparity may be caused by the higher polyphenol content in coffee as compared to tea, although tea was consumed by more participants with higher daily consumption. Similar to coffee consumers, hydroxycinnamic acids is the major polyphenols for non-consumers of coffee or tea (30%) too, although this time derived only from fruit and vegetables. Interestingly, there were no big differences in the contribution of flavanols and hydroxycinnamic acids for the consumers of coffee and tea with percentages of 45% and 39% respectively. Alkylphenols and lignans are two main sources of all other polyphenols. Alkylphenols are commonly present in wheat products such as wholemeal bread and pasta while lignans are found in olive-based products and cereals. The contributions of these compounds are highest in non-consumers of both drinks (14.4%).

A comparison was made between beverage consumption groups in the daily polyphenol intake, where grouping was made based on high (more 1 g/day) or low (less than 1 g/day) polyphenol intake. There was a significant difference between the four groups (χ^2 = 61.26; df = 3; $p<0.01$). There are more of tea consumers with intake of less than 1 g polyphenol per day (36.2 %) while more consumers of both coffee and tea consumed more than 1 g polyphenol daily (59.5 %).

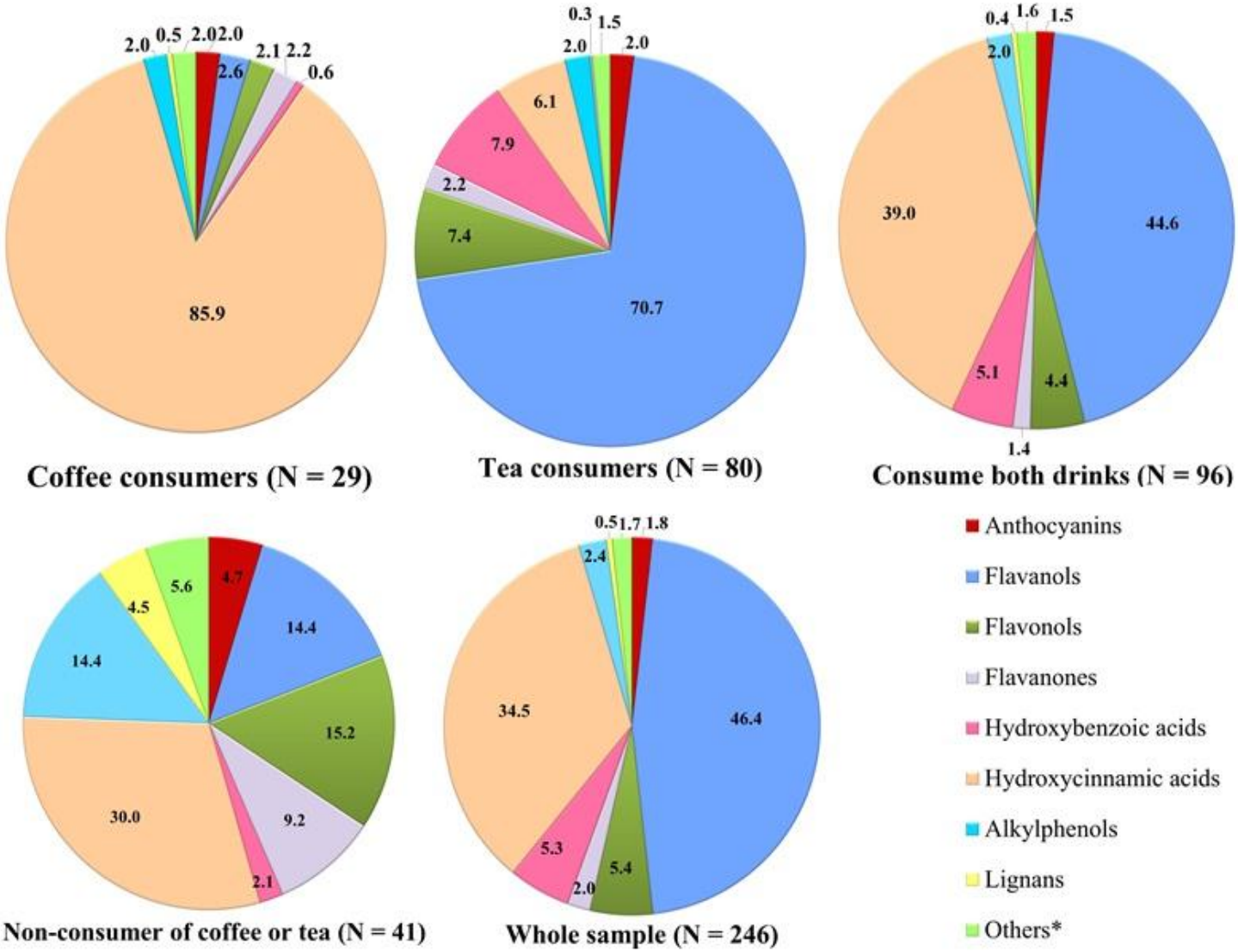


Figure 2-4 Contribution of polyphenols compound to daily polyphenol intake (%) from Total dataset

(*Others include flavones, dihydrochalcones, isoflavonoids, alkylmethoxyphenols, methoxyphenols, stilbenes, tyrosols, other polyphenols)

2.4.8 Comparison between daily polyphenol intake from Total dataset and No Coffee or Tea dataset

Figure 2-5 compares daily polyphenol intake between the Total and the NoCorT datasets, presented according to beverage consumption groups. The polyphenol intake for Total and NoCorT dataset are shown on the left and right axis respectively. For example, polyphenol intake for Total and NoCorT dataset for participant (1) shown in coffee consumers (graph A) are 2492.6 and 69.1 mg/day respectively. It can be deduced that coffee is the major polyphenol food source for the participant because of the reduction up to 36 times in polyphenol intake after the contribution of coffee was eliminated in the estimation. The other example is shown for participant (2) on the same graph. There is only a small difference between the two datasets with the value of 251.5 and 232.8 mg/day from the Total and the NoCorT dataset respectively.

Overall, daily polyphenol intake from all beverage groups except for non-consumers of both drinks are reduced after polyphenol content from coffee and tea were excluded. Interestingly, there is a participant who had high value of polyphenol intake for both datasets. Participant (3) with the second highest value in NoCorT dataset (shown in Figure 2-5, graph A) had the highest total polyphenol in Total dataset. Specifically, polyphenol intake for this participant was contributed by high consumption of fruits and vegetable in her daily diet.

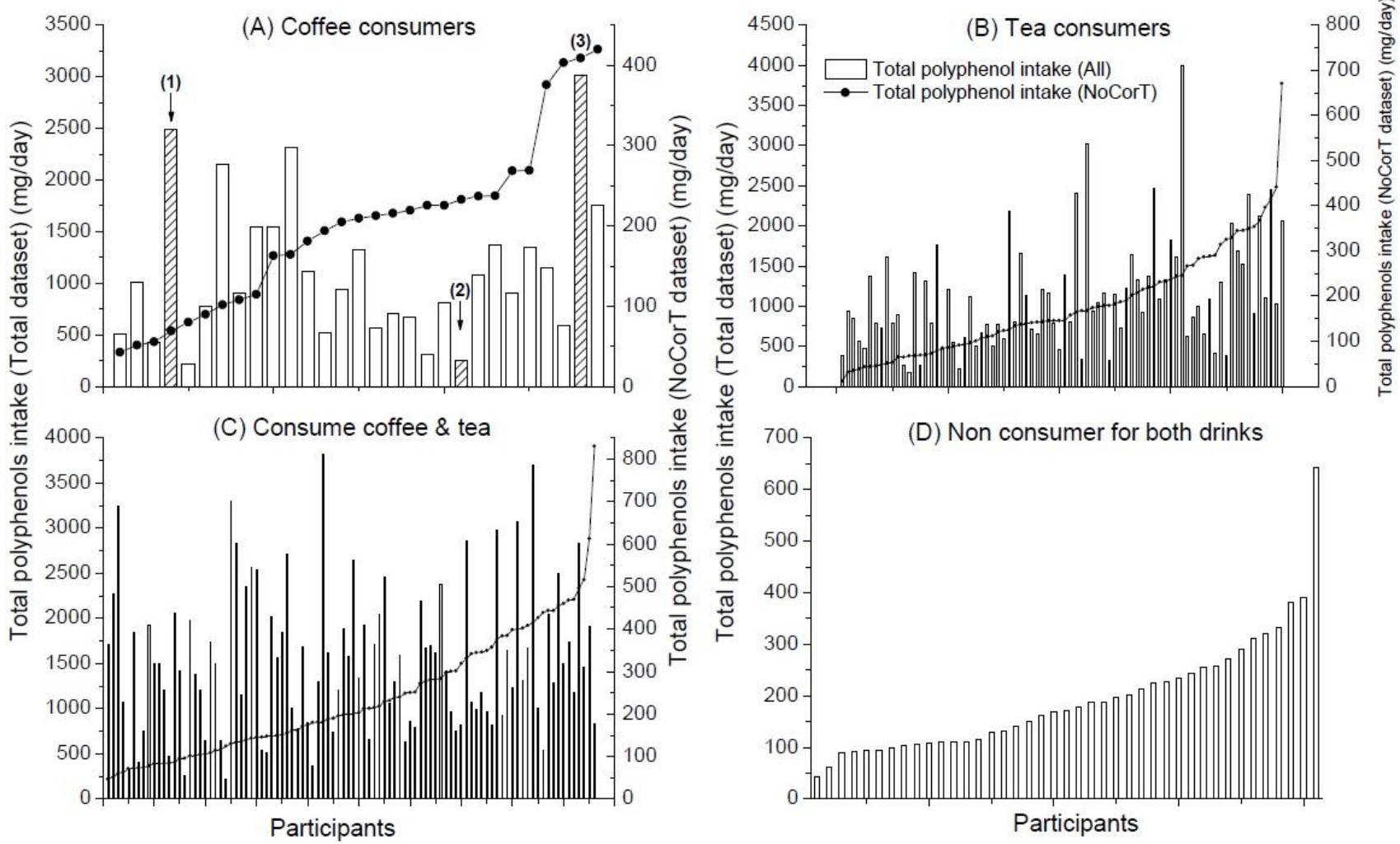


Figure 2-5 Comparison of total polyphenol intake per day (mg/day) between Total and No Coffee and Tea (NoCorT) dataset [A: Coffee consumer (N=29), B: Tea consumer (N=80), C: Consume coffee and tea (N=96), D: Non-consumer of coffee or tea (N=41)]

*(1), (2), (3) – individuals discussed in text Section 2.4.8

2.5 Variables associated with polyphenol intake

Table 2-16 displays the correlations between continuous variables associated with the polyphenol intake of the participants. The correlation analysis was performed using the Total and NoCorT dataset. Partial correlation analysis was performed controlling for daily energy intake (kcal). For the Total dataset, demographic variables that were significantly correlated ($p<0.01$) with daily total polyphenol intake were age, BMI and ratio of energy intake to BMR. The correlation coefficient (R value) between total polyphenol intake in the NoCorT dataset and age was lower than in the Total dataset. Interestingly, the R values are higher for the ratio energy intake to BMR, LWW DINE and DINE DF score in NoCorT as compared to Total dataset. The differences in fibre intake from these two datasets as assessed by LWW DINE and DINE DF can partly be explained by the contribution of fibre-containing foods (i.e. fruit and vegetables) to total polyphenol intake.

Daily volume of coffee and tea consumed were highly correlated ($p<0.01$) with total polyphenol intake with an R value of 0.561 and 0.719 respectively. The same association was found between the frequency of coffee and tea consumption and total polyphenol intake. This finding is expected because frequent coffee and tea drinking can contribute to a higher polyphenol intake.

No strong correlation was found between the variables and polyphenol intake from NoCorT dataset except for carbohydrate, dietary fibre and vitamin C. This finding is expected because other polyphenol food sources such as fruit and vegetables have high content of these nutrients. Variables with high correlation coefficient values were selected for further analysis by using multiple linear regression to predict factors associated to polyphenol intake.

Table 2-16 Correlation between variables associated to daily total polyphenol intake (Total and NoCorT dataset) with and without adjusting for daily energy intake (R value, correlation coefficient value)

	Total polyphenol intake per day (Total dataset)	
Variables	**R value**	**R value adjusted for daily energy intake**
Age	0.622**	0.589**
BMI	0.249**	0.181**
Ratio of energy intake to BMR	0.232**	NS
LWW DINE	0.130*	NS
DINE Dietary fibre score	0.219**	0.149*
Volume of coffee consumed per day	0.561**	0.537**
Volume of tea consumed per day	0.719**	0.771**
Frequency of coffee consumption per day	0.556**	0.522**
Frequency of tea consumption per day	0.702**	0.735**
Energy intake per day (kcal)	0.237**	-
Protein intake per day	0.304**	0.175**
Carbohydrate intake per day	-	-0.147*
Fat intake per day	0.218**	NS
Alcohol intake per day	0.330**	0.146*
Dietary fibre intake per day	0.160*	NS
	Total polyphenol intake per day (NoCorT dataset)	
Variables	**R value**	**R value adjusted for daily energy intake**
Age	0.161*	NS
Ratio energy intake to BMR	0.352**	NS
LWW DINE	0.289**	0.260**
DINE Dietary fibre score	0.324**	0.234**
Energy intake per day (kcal)	0.352**	-
Protein intake per day	0.164*	NS
Carbohydrate intake per day	0.401**	NS
Fat intake per day	0.156*	-0.196**
Alcohol intake per day	0.315**	0.185**
Dietary fibre intake per day	0.583**	0.348**
Vitamin C intake per day	0.497**	0.425**

**Correlation is significant at the 0.01 level (2-tailed), *Correlation is significant at the 0.05 level (2-tailed) Spearman's Rank coefficient correlation. NS: not significant. DINE: Dietary Instrument for Nutrition Education for dietary fibre (DF), LWW DINE: Leeds Women Well-being DINE.

Table 2-17 displays the correlations between variables associated with both flavonoid and phenolic acid intake of the participants. The correlation analysis was performed using the Total dataset. For both polyphenol groups, demographic variables that were significantly correlated ($p<0.01$) with daily total polyphenol intake were age, BMI and ratio of energy intake to BMR. The correlation coefficient between both polyphenol groups and age are almost the same. The association between BMI and flavonoids was diminished, while the association between BMI and phenolic acids was reduced after controlling for the energy intake. The intake of macronutrients was associated with phenolic acids intake, with some of the associations being reduced or diminished after adjusting for the energy intake. Interestingly, carbohydrate had negative association with phenolic acids intake only after controlling for energy intake. In addition, association between the intake of phenolic acids with protein and alcohol intake have shown a reduction in the R value and the significance level after controlling for energy intake. As expected, frequency of tea and coffee consumption were highly correlated with intake of flavonoids and phenolic acids.

Table 2-17 Correlation between variables associated to daily total flavonoid and phenolic acid intake (Total dataset) with and without adjusting for daily energy intake (R value, correlation coefficient value)

Variables	Total flavonoid intake per day (Total dataset)	
	R value	**R value adjusted for daily energy intake**
Age	0.445**	0.431**
BMI	0.131*	NS
Ratio energy intake to BMR	0.139*	NS
LWW DINE	0.176**	0.174**
DINE Dietary fibre score	0.196**	0.181**
Frequency of tea consumption per day	0.947**	0.947**
Energy intake per day (kcal)	0.127*	NS
Protein intake per day	0.166*	NS
Dietary fibre intake per day	0.132*	NS

Variables	Total phenolic acid intake per day (Total dataset)	
	R value	**R value adjusted for daily energy intake**
Age	0.448**	0.421**
BMI	0.203**	0.164*
Ratio EI to BMR	0.198**	NS
Frequency of coffee consumption per day	0.962**	0.960**
Energy intake per day (kcal)	0.221**	NS
Protein intake per day	0.265**	0.158*
Fat intake per day	0.186**	NS
Alcohol intake per day	0.174**	0.130*
Carbohydrate intake per day	-	-0.141*

**Correlation is significant at the 0.01 level (2-tailed), *Correlation is significant at the 0.05 level (2-tailed) Spearman's Rank coefficient correlation. NS: not significant. DINE: Dietary Instrument for Nutrition Education for dietary fibre (DF), LWW DINE: Leeds Women Well-being DINE

2.6 Prediction of polyphenol intake of UK women

Variables which were associated with daily polyphenol intake from the Total dataset were selected for regression analysis to predict the factors associated with polyphenol intake (see Table 2-16). Using the backward method, a few models were developed; however, because of multicollinearity these models were revised. In the regression analysis, multicollinearity is said to be present when two independent variables are highly correlated to each other (Kachigan, 1991). The examples of multicollinearity include the volume and frequency of coffee and tea consumption daily.

Table 2-18 Dependent variables to predict polyphenol intake from Total dataset

Predictor variables	**Beta**	**Pearson r**	**p value**
Dependent variables to predict polyphenol intake from Total dataset			
Age (years)	0.496	0.601	p <0.01
Body mass index (kg/m^2)	-0.126	0.230	p = 0.020
Protein intake per day (g)	0.110	0.292	p = 0.022
Volume of coffee consumed per day (ml)	0.364	0.561	p <0.01
Dependent variables to predict polyphenol intake from NoCorT dataset			
Fat intake per day (g)	0.115	0.227	p = 0.029
Alcohol intake per day (g)	0.250	0.314	p <0.01
Dietary fibre intake per day (g)	0.266	0.442	p <0.01
Vitamin C intake per day (mg)	0.393	0.495	p <0.01
Dependent variables to predict flavonoids intake from Total dataset			
Age (years)	0.058	0.418	p = 0.013
Dietary fibre intake per day (g)	0.078	0.172	p <0.01
Frequency of tea consumed daily	0.909	0.940	p <0.01
Dependent variables to predict phenolic acids intake from Total dataset			
Age (years)	0.115	0.240	p <0.01
Alcohol intake per day (g)	0.074	0.219	p <0.01
Frequency of coffee consumed daily	0.899	0.942	p <0.01

Table 2-18 shows variables associated to total polyphenol, flavonoids and phenolic acids. For the Total dataset, three outliers from participants with the highest polyphenol intake from were excluded, thus, leaving 243 cases for analysis. The final model retained four of the eight initial predictors. The model was statistically significant [$F(4, 238) = 60.78$, $p<0.01$], and accounted for approximately 50.5 % (R value = 0.505) of the variance in total polyphenol intake in the Total dataset. Total polyphenol intake was primarily predicted by older age and higher volume of coffee consumed daily, and to a lesser extent by lower BMI and increased in daily protein intake. Information obtained from the analysis was used to derive a predictive equation for total polyphenol intake as shown below:

Total polyphenol intake = 2.289 (constant) + 0.496(age, years) – 0.126*(BMI) + 0.110*(protein intake per day, g) + 0.364*(volume of coffee consumed per day)*

Further regression analysis was performed to predict variables which were associated with daily polyphenol intake from the NoCorT. Five outliers from participants with the highest polyphenol intake were excluded before the analysis. The final model retained four of the nine initial predictors. The model was statistically significant [$F(5, 234) = 36.30$, $p<0.01$], and accounted for approximately 43.7 % (R value = 0.437) of the variance in total polyphenol intake in the NoCorT dataset. Total polyphenol intake without the contribution from coffee and tea was primarily predicted by an increase in fat, alcohol, dietary fibre and vitamin C intake per day. The predictive equation for total polyphenol intake from NoCorT dataset using the significant predictors is shown below:

Total polyphenol intake = 1.158 (constant) + 0.565(fat intake per day, g) + 2.290*(alcohol intake per day, g) + 2.947*(dietary fibre intake per day, g) + 0.804*(vitamin C intake per day, mg)*

The same regression analysis was performed on the NoC and NoT dataset. By using the backward method and excluding outliers from the analysis, the problems of multicollinearity still remain. This situation was indicated by the value of variance inflation factor (VIF) of more than 10 (Pallant, 2007). The multicollinearity was found between frequency and volume of coffee and tea consumed daily for NoC and NoT datasets respectively. Therefore, no predictive equation can be accepted to predict the total polyphenol intake in both datasets.

The regression analysis was performed to predict factors associated to flavonoids intake from the Total dataset. After removing the outliers, the final model retained three of the eight initial predictors. The model was statistically significant [$F(3, 233) = 643.78$, $p<0.01$], and accounted for approximately 89.2 % (R value = 0.892) of the variance in flavonoids intake. The flavonoid intake can be predicted by higher frequency of tea consumed, increase in dietary fibre intake and to a lesser extent by increase of age. The predictive equation for flavonoid intake is shown below:

Flavonoid intake = -43.13 (constant) + 2.65(age, years) + 3.76*(dietary fibre intake per day, g) + 370.20*(frequency of tea consumed daily)*

Phenolic acid intake was predicted by using three variables. The model was statistically significant [F (4, 233) = 548.23, $p<0.01$], and accounted for approximately 90.4 % (R value = 0.904) of the variance in phenolic acid intake. The phenolic acid intake can be predicted by more frequent of coffee consumed, increase of age and to a lesser extent by increase in daily alcohol intake. The predictive equation for phenolic acid intake is shown below:

Phenolic acid intake = 77.29 (constant) + 4.09(age, years) + 2.37*(alcohol intake per day, g) + 464.75*(frequency of coffee consumed daily)*

2.7 Discussion

The work presented in Chapter 2 describes the estimation of polyphenol intake of UK women from food diary recording and predicted the variables associated to the intake. In this study, coffee and tea were identified as the major polyphenol food sources. Fruits, vegetables, milk chocolate and processed foods prepared with polyphenol-containing ingredients were the important source of polyphenol intake.

Generally, the intake of total polyphenols was higher amongst LWW participants and the consumers of both coffee and tea. DH participants had 37.5 % lower total polyphenols intake than the LWW participants. In addition, 56 % of LWW participants had achieved the intake of polyphenol more than 1g/day as compared to DH with 36 %. Both LWW participants and the consumers of both coffee and tea were older, heavier as assessed from BMI and had the highest energy intake than the other comparison groups. The intake of total polyphenols from the Total dataset was five times higher than the intake from the NoCorT dataset.

With respect to food intake recording, overall there are more normal reporters (58.8 %) than under reporters for the whole sample. The higher number of under reporters in DH study was because they are younger, and may possibly practise certain dietary restriction for weight maintenance. A previous study among university students has suggested that trying to lose weight become the norm among women and the effort to lose weight were higher amongst students with low body weight (Wardle et al., 2006).

Comparison was made in the polyphenol intake between under and normal reporter, and no significant difference was found between these groups (see Table 2-10). This finding can partly be explained by the perception that coffee and tea drinking are not considered as unhealthy habits, therefore, participants have reported the consumption honestly. Furthermore, flavonoids and phenolic acids which are widely present in fruit and vegetables would be less likely to be under reported by the participants because these foods are categorized as healthy foods. Moreover, participants from DH study were informed about the objectives of the study and the major sources of polyphenols were briefly explained in the participant information sheet (refer to Appendix 2), thus become one of the limitations of this study. Knowing the purpose of a study can lead participants into making socially desirable responses. In relation to food intake, this is often reflected by over reporting of foods perceived to be healthy and underreporting of foods perceived to be unhealthy. A previous study which reported that the studied participants believed that the consumption of foods perceived as ‘good’ in larger quantity will promote less weight gain than consuming small amount of foods perceived as ‘bad’ (Oakes, 2005). Surreptitious recording of food intake or disguising the purpose of the study can be applied so that emphasis is drawn away from the particular food groups under study.

From the regression analysis, age was the major predictor of polyphenol intake (see Table 2-18). Interestingly, age was not a predictor after excluding tea and coffee consumption as shown in the prediction of total polyphenol from the NoCorT dataset. It can be inferred that increase of age was associated with a prominent tea and coffee drinking habit among older the participants. The finding reported in this chapter is consistent with a cohort study which assessed the flavonoid intake in association to risk of breast cancer (Zamora-Ros et al., 2013a). Results from the cohort suggested that women with the highest total flavonoid intake were older and had a lower BMI. However, in this chapter BMI appeared as a weak predictor of total polyphenol intake in the regression analysis.

Alcohol intake was a predictor for total polyphenol intake from the NoCorT dataset and in the prediction of phenolic acid intake from Total dataset. Interestingly, no difference was found between the study groups in the unit of alcohol consumed per week (see Table 2-5), by which this information was solely based on participants' self-assessment and has the tendency of underestimation. A previous review on the alcohol drinking habit amongst UK undergraduate students has suggested that there was a tendency of underestimating the alcohol intake when the estimation was based from size of UK standards drinks (Gill, 2002). To confirm this hypothesis, a conversion was made from the questionnaire of how many units of alcohol were consumed weekly (refer to Appendix 4) to grams by multiplying the value by 8 (BDA, 2012) and the results then divided by seven or three to determine the daily alcohol intake. These data were then compared to the daily alcohol intake (in gram) assessed by food diary. The correlation (R value) between the two datasets is near to 0.3 ($R = 0.295$, $p<0.01$) which indicated a weak relationship, suggesting that the alcohol intake which was self-reported from the questionnaire was not

reflecting the common amount consumed by the participants. Further analysis has showed that LWW participants have the tendency to under estimate their alcohol intake in unit from the questionnaire while DH participants showed the opposite. The contribution of alcohol intake in the prediction of phenolic acids intake can partly be explained by the substantial amount of phenolic acids presents in alcoholic drinks such as beer (1.2 to 2.6 mg/100 ml) and wines (value range from 6.2 to 17.2 mg/100 ml).

In this chapter, it is apparent that there are more participants than who consumed both coffee and tea (39 %) and consumed tea only (32.5 %). The average of coffee and tea consumed by the studied samples were 160 ± 239 and 328 ± 377 ml/day respectively. A higher daily tea consumption (814 ± 450 ml/day) was reported from a longitudinal study among UK men in South Wales (Hertog et al., 1997). The men were older and were mainly working class in the industrial town, whereby tea would be routine part of their daily lives thus they became a very different group to average population and to the population in our study. From our data in this chapter, consumers of both coffee and tea were shown to drink more tea than coffee in terms volume consumed daily. A similar inverse association was reported in a study among Scottish adults, whereby a high coffee consumers tend to consumed less tea and vice versa (Woodward and Tunstall-Pedoe, 1999).

The determination of major polyphenol food sources can be made by assessing the amount of polyphenols present in food and the quantity of food consumed (Cieślik et al., 2006). In this chapter, the major polyphenol food sources consumed by the studied population included tea, coffee, potatoes and apples which are similar to those reported from previous studies (Perez-Jimenez et al., 2011, Zujko

et al., 2012). A study from Australia also identified black and green tea as the major flavonoid food sources along with wine, apples and oranges (Somerset and Johannot, 2008). A recent study has estimated the total flavonoid intake amongst the non-Mediterranean countries in Europe including Germany, the Netherlands, UK, Sweden and Norway (Zamora-Ros et al., 2013b). This study reported two major contributors to flavonoid intake of the non-Mediterranean countries namely tea and fruits, with UK population showing the highest intake of total flavonoids (average of 549 mg/d in men and 502 mg/d in women). Tea was also become the major contributor for flavonoids intake in our study.

Correlation analyses were performed between several quantitative variables and total polyphenol intake from all foods from the Total dataset and also when excluding coffee and tea (i.e. the NoCorT dataset) (see Section 2.5). Daily intake of dietary fibre and vitamin C are included in the predictive equation for the intake of polyphenol not from coffee and tea (see Table 2-18). This finding suggests that the consumption of polyphenol-containing foods which high in dietary fibre and vitamin C can contribute to the total polyphenol intake not derived from coffee and tea. An implication of this is the possibility that the health promotion to increase the serving size of fruit and vegetables can also include the point that these two food sources are also significant contributors to polyphenol intake.

2.7.1 Polyphenol intake in comparison to the intake from other countries

Table 2-19 shows the comparison in polyphenol intake between countries and the intake reported in this chapter. The flavonoid intake reported in this chapter (612 mg/day) is higher than the results reported from other studies (see Table 2-19). Studies performed in China and Greece which has used FFQ in the estimation of flavonoids reported lower value of total flavonoids as compared to the intake reported in this chapter (Dilis and Trichopoulou, 2010, Li et al., 2013). The contribution of flavanols to total flavonoid intake (82.7%) in this chapter are similar to the United States (83.5%) (Chun et al., 2007) with tea as the major food source for these populations. In comparison, the study in China (Li et al., 2013) had only assessed flavonoid intake from fruit, vegetables and nuts which might account for the low value in the flavonoid intake.

There are two studies that have previously estimated the flavonoid intake of the UK population. Beking and Vieira (2011) estimated the flavonoid intake using food balance sheets. The authors claimed this approach has the advantage of normal distribution of the data despite of over-representation of tea drinkers in the UK population. The lower flavonoid intake estimated from this study as compared to data from this chapter might be associated with the limited number of food items (105 foods) from specific food groups which were included in the estimation. In addition, only fresh plant-based foods were counted in while polyphenols contained in processed foods such ready-made pasta sauces were not added.

Zamora-Ros et al. (2013b) reported a slightly lower flavonoid intake (501.7 mg/day) in the UK general population compared to data from this chapter (616.6 mg/day). Flavanols were the major flavonoid class for both of these two studies, with the contribution of 82.7% (current study) and 75% (EPIC-UK) respectively to the total flavonoid intake. The EPIC study has not included thearubigins, the major group of compounds found in tea in the estimation of flavanols, thus can lead to an underestimation of flavanol intake. In this chapter, data for thearubigins content in tea was obtained from USDA database (USDA, 2011).

The total polyphenol intake as reported from other studies ranges from 800 to 1200 mg/day. The value of total polyphenols estimated in this chapter by summating 20 polyphenols is within a reasonable range when compared to the other studies. The main polyphenol food sources for the studies with total polyphenol intake above 1 g per day are beverages such as coffee, tea and fruit juices as reported by study from France and Poland (Perez-Jimenez et al., 2011, Zujko et al., 2012). The other polyphenol food sources include fruit, vegetables, legumes and cereal products. The disparity between all these studies in the estimation of total polyphenols can partly be explained by the different number of polyphenols included in the estimation in work for this thesis more polyphenol (20 compounds) were used in the estimation of total polyphenols. The different database used to estimate polyphenol intake also can contribute to the differences in total polyphenol estimation between countries.

Table 2-19 Polyphenol intake in different countries as comparison to the value obtained in the chapter

Country(s) & authors	Method(s) for dietary reporting	Polyphenol studied (number of compounds), database used	Amount consumed (per day)	Amount consumed in this chapter (per day)
United Kingdom and Ireland (Beking and Vieira, 2011)	Data from FAO* Food Balance Sheet for UK and Ireland population	Total flavonoids (5), USDA	182 mg/day for UK and 177 mg/day for Ireland	612 mg/day (estimated from 7 flavonoids)
European countries (EPIC study) (Zamora-Ros et al., 2013b)	24-hour dietary recall using computerised interview software^	Total flavonoids (NA), USDA and Phenol-Explorer®	Mediterranean countries: 370 mg/day Non- Mediterranean countries: 374 mg/day	
Australia (Johannot and Somerset, 2006)	Face-to-face 24-hour recall food frequency questionnaire (FFQ)	Total flavonoids (5), USDA	225 mg/day of flavonoids for the whole population, 454 mg/day of flavonoids for population age 19 years and over	
United States (Chun et al., 2007)	24-hour dietary recall	Total flavonoids (6), USDA	190 mg/day	
Greece (Dilis and Trichopoulou, 2010)	190-item validated semi quantitative FFQ	Total flavonoids (7), self-developed database	92 mg/day[1]	
China (Li et al. 2013)	76-item validated quantitative FFQ	Total flavonoids (5), self-developed database	166 mg/day	
Finland (Ovaskainen et al. 2008)	48-hours dietary recall & HPLC	Total polyphenols (NA), self-developed database	863 mg/day	1089 mg/day
France (Perez-Jimenez et al., 2011).	24-hour dietary record as the means for food recording every two months between the periods of two years	Total polyphenols (NA), Phenol-Explorer®	1193 mg/day	
Poland (Zujko et al., 2012).	24-hour recall method	Total polyphenols[2] (NA), self-developed database	men: 1172 mg/day, women: 1031 mg/day	
Iran (Sohrab et al. 2013)	168-item validated semi quantitative FFQ	Total polyphenols (NA), Phenol-Explorer®	1780 mg/day	
Spain (Tresserra-Rimbau et al., 2013)	137-item validated quantitative FFQ	Total polyphenols (NA), Phenol-Explorer®	820 mg/day	

[1]Median total intake of flavonoids, [2]Estimated from total phenolic content of foods using Folin Ciocalteu assay, *Food and Agriculture Organization, World Health Organization, NA: the total number compounds was not available

2.8 Conclusions

The study in this chapter intended to estimate the total polyphenol intake in a population of UK women. Based predominantly on Phenol-Explorer® data, the estimation of polyphenol intake from food diary analysis was 1089 mg/day. Flavonoids and phenolic acids contributed 55.3% and 39.9% respectively to the total polyphenol intake of the samples. Specifically, flavanols which are predominant in tea, and hydroxycinnamic acids, predominant in coffee, are the highest in polyphenol classes consumed by the population. This finding is expected due to the high representation of tea and coffee drinkers in the study (see Table 2-11), with only 16.7% of the population non-consumers of both drinks. After the exclusion of coffee and tea from the estimation of polyphenol intake (NoCorT dataset), the main polyphenols which contributed to total intake were flavanols, hydroxycinnamic acids and flavonols, derived from fruit, vegetables and chocolates (see Table 2-8).

Furthermore, the data in this chapter were analysed to predict factors associated with polyphenol intake in the UK women studied. From the regression analysis, age was the major predictor of polyphenol intake and to the two major polyphenol groups; flavonoids and phenolic acids. A higher socio-economic status, shown by employment and the accessibility to buy foods, may also affect the food choices of the studied sample.

Chapter 3
Polyphenols Content in Food

3.1 Abstract

In this study an estimation of polyphenol content was performed in selected foods. These foods were commonly consumed by the participants (see Chapter 2) with no data on polyphenol content present in Phenol-Explorer® or USDA polyphenol database. In the screening phase, Folin-Ciocalteu assay was used to estimate total polyphenol content in these foods. Blackcurrant concentrates were selected for further analysis on specific anthocyanins and ascorbic acid content by using high performance liquid chromatography (HPLC). The adjusted values were used for the association of polyphenol intake and cognitive performance (see Chapter 4). The intake blackcurrant concentrates has played a vital contribution to daily anthocyanin intake of the participants. However, the current study was limited by only assessing the contribution of blackcurrant concentrates consumption to daily total anthocyanins and total polyphenol intake. This highlights the need for further analysis on other processed foods which were commonly consumed by the population for a better estimation of the polyphenol intake.

3.2 Introduction

In recent years, there has been an increasing interest in determining the polyphenol content of processed foods. There have been a few studies carried out in the United Kingdom that have analysed some processed foods such as commercial fruit and vegetable juices and oat-based breakfast cereals (Mullen et al., 2007, Ryan et al., 2011, Wootton-Beard et al., 2011). However, far too little attention has been paid to analysing processed foods based on foods that are commonly consumed by the UK population.

In Chapter 2, an estimation of the polyphenol intake of the study population was determined from the food intake reported using a 3 day food diary. The polyphenol content of the food was assessed using two main databases namely: Phenol-Explorer® (Neveu et al., 2010b) and USDA (USDA, 2011). However, there is missing data for the polyphenol content of some foods consumed by the study sample, but for which no data is present in these two databases (see Table 3-1). Folin-Ciocalteu assay was used in order to screen foods with a potentially high phenolic content that could then be assessed more specifically using HPLC analysis.

Table 3-1 Commonly consumed foods[ξ], without polyphenol composition data in Phenol-Explorer®

No.	Food groups	Foods
1.	Fruits	Satsuma*
		Clementine*
		Mandarin*
		Sultana*
2.	Cereals, wheat and seed	Cereal bar (fruit)~
		Muesli (mixed cereals)~
		Oat flapjack~
		Cereal bar~
		Hummus~
		Mustard, whole grain*
3.	Fruit based products	Fruit smoothies*
		Mixed fruit juices*
		Fruit concentrates*^
		Fruit yoghurts~
4.	Soup and sauces	Instant soups~
		Tomato ketchup~
		Apple sauce~
		Curry sauces~
		Pasta sauces*

[ξ]Commonly consumed foods based on analysis of food diaries in Chapter 2, *Foods analysed by Folin-Ciocalteu assay in this chapter; ^ Foods analysed by HPLC in this chapter, ~Foods which were not analysed.

The Folin-Ciocalteu assay was widely used to measure the total phenolic content in food products and supplements. It was suggested as the standardized method for routine quality control in food analysis (Prior et al., 2005). The principle of this assay is based on the oxidation, or reduction reaction, from a few compounds in foods including phenols, other non-phenolic reducing agents and possibly metal chelators (Medina, 2011).

Further analysis using high performance liquid chromatography (HPLC) was performed on selected blackcurrant concentrates. Fruit concentrates were selected because 36% of the participants consumed them as part of their habitual diet. Two anthocyanins; cyanidin-3-*O*-rutinoside (CY3RUT) and delphinidin-3-*O*-rutinoside (DP3RUT) were selected for quantification by HPLC. These compounds were selected based on results from previous studies and are well known as the major anthocyanins present in blackcurrants (Matsumoto et al., 2001, Bermúdez-Soto and Tomás-Barberán, 2004).

This study is therefore intended to address missing data from some commonly consumed processed foods to improve the estimation of polyphenol intake of the study population.

3.2.1 Study objectives

The objectives of this study were:

- To determine the total phenolic content of selected foods and beverages using the Folin-Ciocalteu assay.
- To identify some specific compounds of selected foods and beverages using HPLC.

3.3 Materials and Methods

3.3.1 Materials

Caffeic acid, catechin, gallic acid, sucrose, fructose, glucose and sodium metabisulfite were purchased from Sigma-Aldrich (Poole, Dorset, UK). Ascorbic acid, myricitrin, cyanidin-3-*O*-rutinoside and delphinidin-3-*O*-rutinoside were

purchased from Extrasynthese (Genay, France). The terms ascorbic acid and vitamin C are used interchangeably.

HPLC grade ethanol, methanol and acetonitrile were purchased from Fisher Scientific (Loughborough, Leicestershire, UK). Folin-Ciocalteu phenol reagent, formic acid and sodium carbonate were purchased from Sigma-Aldrich (Poole, Dorset, UK). Distilled water was used for the Folin-Ciocalteu assay while Milli-Q purified water was used for HPLC analysis. The water source was from Merck Millipore lab water systems (Massachusetts, USA).

All food samples were purchased from local commercial outlets. Citrus fruits (mandarins, clementines and satsumas), clementine juice, pineapple juices, mustards, marmalades, Britvic J2O mixed fruit juices, Morrison's brand mixed fruit juices and Sun Exotic Tropical fruit juice were purchased from Morrisons, Leeds, UK. Sultanas, Innocent's fruit smoothies, Ribena juice drinks and Robinson's fruit concentrate samples were purchased from ASDA, Leeds, UK. The Co-operative's cranberry, blackberry and raspberry smoothie was bought from Leeds University Union convenience shop. Orange squash samples were bought from Sainsbury's, Leeds and Waitrose, Leeds, UK. Supermarket own brand's blackcurrant and apple and blackcurrant concentrate samples were bought from a few stores (ASDA, Morrisons, Marks & Spencer and Tesco) around Leeds, UK. All foods were bought between November 2011 to March 2012. Fresh samples were weighed (40 g) while other foods were aliquoted (2 ml) and stored at -20°C for a maximum of one month prior to analysis.

3.3.2 Methods

3.3.2.1 Food sample preparation

Food samples were extracted using 80% methanol at different ratios depending on the nature of food sample. Fresh food was extracted at 1:12 (v:v); fruit juice with bits and thick fruit juices and smoothies were extracted at 1:6 (v:v) and (1:6 v:v); and finally, pasta sauce samples were homogenized at a 1:1 ratio and then further extracted with 15 ml of solvent, centrifuged and the pellet extracted twice more with 15 ml of solvent. The food samples were extracted using different methods based on the structure and texture of the food: (i) fruit juice concentrates and fruit juices without bits were extracted with methanol (80 %, 1:1, v:v); (ii) samples were centrifuged (3000 G, 15 minutes, 4 °C) using a centrifuge (Centrifuge 5810R, Eppendorff, Hamburg, Germany) and the pellet extracted 3 times to ensure maximum extraction; (iii) thick texture or solid foods were homogenised first using a food homogeniser for 1 minute 30 seconds to 2 minutes and if further refinement was needed, then a fine blade food homogeniser (POLYTRON 1600E) was used to refine the food products. For each original sample two extractions were performed. For each extraction, it was then divided into two replicates; therefore there were four replicates in total for each original sample. In this study, Heidi Lai has contributed to the preparation and sample analysis for some of the Folin-Ciocalteu assay and data analysis using HPLC.

3.3.2.2 Folin-Ciocalteu assay

The Folin-Ciocalteu assay was adapted from a method described by Singleton and Rossi (1965) and Singleton et al. (1999). The mechanism involves electron transfer in alkaline medium from phenolic compounds to phospomolybdic or phototungstic acid complexes in the Folin-Ciocalteu reagent. This reaction forms blue complexes and the optical density can be determined using a spectrophotometer (CECIL CE7200, Cambridge, United Kingdom) at 765 nm (Singleton and Rossi, 1965). The supernatant from the sample extraction (1 ml) was added to 5 ml of freshly prepared Folin–Ciocalteu reagent (1:10, v:v with water). The mixture was vortexed (Vortex Genie-2, New York, USA) and then mixed with 4 ml of Na_2Co_3 solution (75 g/L, 0.71 nmol/L) within 3 to 8 minutes after the addition of Folin–Ciocalteu reagent. The samples were incubated in a water bath set at 26°C for 2 h, and then the absorbance of the mixture was read at 765 nm using distilled water used as the blank.

Gallic acid is typically used as the reference compound due to its availability, price, reproducibility and solubility. Gallic acid (0.1 g) was weighed and dissolved in 100 ml of distilled water to make a stock solution at a concentration of 1 mg/ml. Gallic acid (0 to 200 µg/ml) was prepared in distilled water and the same Folin assay procedure was followed. Results for samples are expressed as mg of gallic acid equivalent (GAE) per 100 ml or per serving size of samples.

A trial was performed to compare gallic acid as reference with caffeic acid, catechin, and two major anthocyanins in blackcurrant concentrate namely cyanidin-3-*O*-rutinoside (CY3RUT) and delphinidin-3-*O*-rutinoside (DP3RUT). The samples

for the standard curve ranged from 0 to 200 µg/ml and the assay procedure was performed in the same way as for gallic acid.

The Folin assay can be affected by certain interferences such as ascorbic acid, fructose, protein and some inorganic substances (Box, 1983, Prior et al., 2005). Compounds that are commonly present or are added to processed foods were selected for testing for interference. These compounds included: ascorbic acid, fructose, sodium metabisulfite (added as a preservative in food samples in food processing), glucose and sucrose. All compounds were tested at standard concentrations from 0 to 300 µg/ml and assessed in the Folin assay.

3.3.2.3 Vitamin C correction methods

Vitamin C, present in fruit and vegetables and derived products, interferes with the Folin assay and therefore needs to be accounted for. Food samples were extracted as in Section 3.3.2.1, and then a 1 ml extract was added to 4 ml Folin reagent and 4 ml distilled water (instead of Na_2Co_3) to be measured in the Folin assay (Perla et al., 2012).

3.3.2.4 Validation of Folin-Ciocalteu assay

Pineapple juice was used as a positive control to compare with data of total phenolic content in pineapple juice recorded in Phenol-Explorer®. Two types of pineapple juice (Morrisons and Tropicana brand) were used for this purpose and samples were prepared in duplicate from the same batch. 2 ml of each pineapple juice was added to 2 ml of 80 % methanol. This mixture was centrifuged (3000 G, 15 minutes, 4 °C) and the pellet extracted 3 times with methanol to ensure maximum extraction. Samples were prepared in double duplicate similar to food sample

preparation and yielded four replicates and followed the same Folin assay procedure described in Section 3.3.2.2.

The accuracy of measurement for total phenolic content using the Folin assay was determined. This was done by spiking a blackcurrant concentrate sample (Tesco's Blackcurrant High Juice 50 %: BHJ) with a known concentration (100 µg/ml) of ascorbic acid and gallic acid. The preparation of stock solution of ascorbic acid and gallic acid followed the same procedure as described in Section 3.3.2.2. The Folin assay was performed to five different groups in the validation experiment: (1) assay for gallic acid standard curves (from 0 to 150 µg/ml); (2) assay for known concentration of ascorbic acid and gallic acid (100 µg/ml); (3) assay for food sample (BHJ) by mixing 50 µL undiluted concentrate with 950 µL 80% methanol; (4) assay for the mixture of 72 µL of undiluted BHJ, 160 µL of standard from stock solution of gallic acid (end concentration of 100 µg/ml) and top up the balance to 1600 µL with 80% methanol and (5) assay for the mixture similar to (4) but using 160 µL of stock from stock solution of ascorbic acid (end concentration of 100 µg/ml). Vitamin C correction assay was performed to (2), (3), (4) and (5). Samples were prepared in duplicate from the same batch and assessed in the Folin assay and corrected vitamin C assay as mentioned above.

3.3.2.5 HPLC-DAD analyses

In this study, selected fruit concentrates with high total phenolic content were selected for HPLC analysis. Reversed-phase HPLC with a photo-diode array detector was used to analyse these samples. A simple dilution step using 80% methanol was applied prior to analysis, and samples were then filtered through a 0.2 µm polytetrafluorethylene (PTFE) membrane filter (Chromacol Hertfordshire, United

Kingdom). The system used was an Agilent 1200 Series HPLC consisting of a solvent degassing unit, binary pump, auto-sampler, thermostatic column oven and diode array detector. The column used was an Agilent Zorbax Eclipse XDB C-18 (4.6 x 50 mm and 1.8 µm internal diameter). Solvent A used was Milli-Q purified water with 0.1% formic acid and solvent B was acetonitrile with 0.1 % formic acid. The solvents were placed in the sonicator bath 30 mins. prior to analysis. The solvent flow rate and column temperature were fixed at 0.5 ml/min and 30°C respectively. A 33-min gradient was used, starting from 5% solvent B (0-5 min), increasing to 10% solvent B (5-20 min), 40% solvent B (20-25 min), 90% solvent B (25-29 min) and finally 5% solvent B (29-33 min). In this analysis, myricitrin-3-*O*-rhamnoside, also known as myricitrin (MY3RNS), was chosen as the internal standard. Chromatograms were recorded at 350 and 520 nm for myricitrin and anthocyanins respectively (see Figure 3-1).

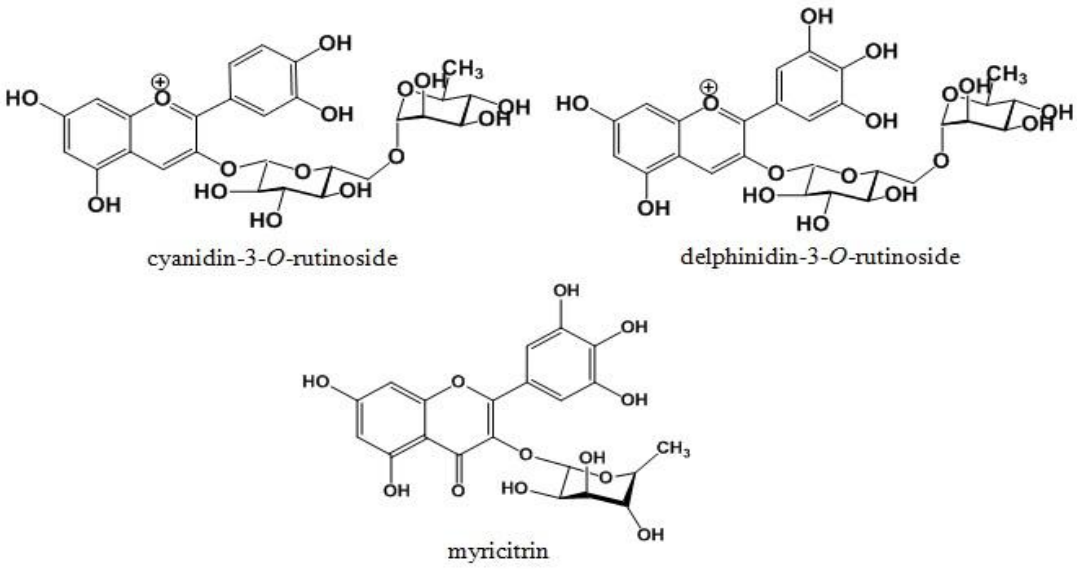


Figure 3-1 Chemical structures of two main anthocyanins in blackcurrant (cyanidin-3-*O*-rutinoside and delphinidin-3-*O*-rutinoside) and myricitrin used as an internal standard

The same HPLC system was used to determine ascorbic acid with some modifications. A µBondapak C-18 column (3.9 x 300 mm, Water, UK) was used. The column used for the analysis was from Waters (µBondapak C-18 3.9 x 300 mm). Solvent A was 0.2% formic acid in Milli-Q purified water while solvent B was 0.2% formic acid in acetonitrile with a solvent flow rate of 1 mL/min. Chromatograms were recorded at 245 nm.

3.3.3 Statistical analysis

Samples were prepared in duplicate for the validation analysis and the results are presented as mean. All results from the Folin assay and HPLC analysis are presented in mean ± standard deviation.

3.4 Results and Discussion

3.4.1 Folin assay

3.4.1.1 Validation and interferences in the Folin assay

Table 3-2 shows the total phenolic content in two pineapple juices as compared to data in Phenol-Explorer®. It can be seen that the total phenolic content obtained from sample 2 is fairly close to the one from the database. However, the difference in values from the database was expected because of various factors such as the different processing of juice, the variety of pineapple used and the different methods applied in respective studies. Nevertheless, the similarity in this result confirmed that the method used was valid and thus could be applied throughout the study.

Table 3-2 Comparison of total phenolic content of pineapple juice between Phenol-Explorer and Folin assay adapted in the study*

	Phenol-Explorer®	Pineapple juice 1 (Morrisons) (n = 2)	Pineapple juice 2 (Tropicana) (n = 2)
Total polyphenol content (GAE mg/100 ml)	35.8	16.3 ± 0.1	30.9 ± 0.1

*Samples were prepared in duplicate from the same batch.

Ascorbic acid reacts in the Folin assay, as demonstrated by the similarity in standard curves shown for gallic acid and ascorbic acid in Figure 3-2 (a and b). However, sodium carbonate (Na_2CO_3) is essential for ascorbic acid to react, thus leading to a much weaker response when this alkaline medium was replaced by distilled water (d compared to b).

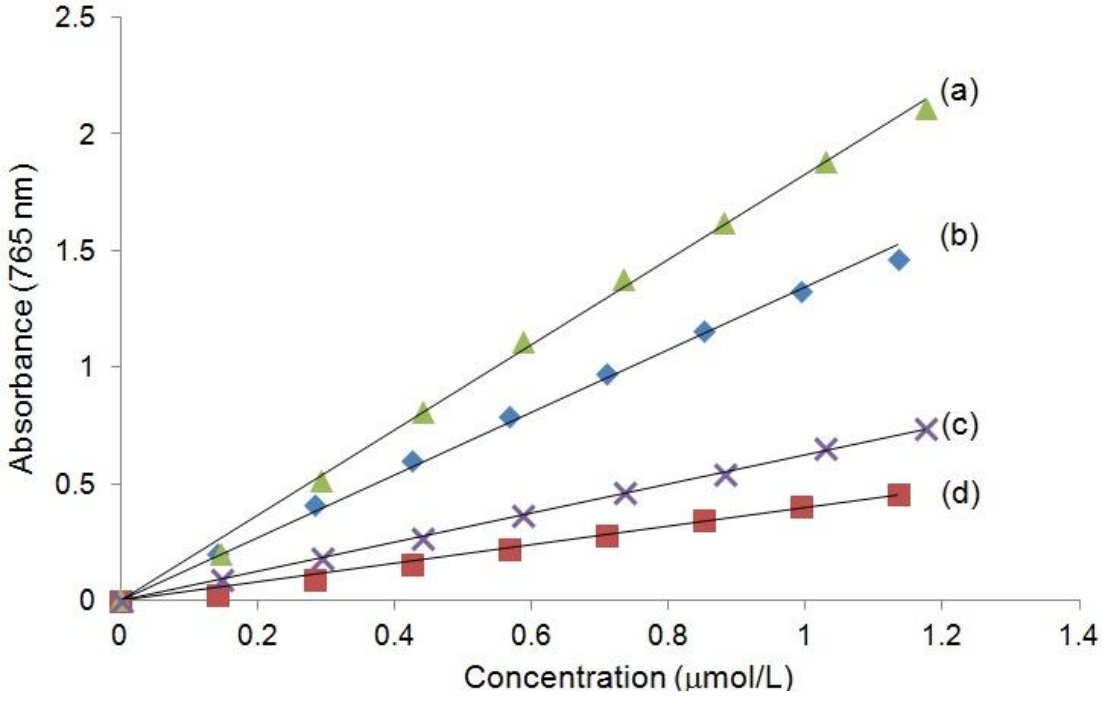


Figure 3-2 Response in the Folin assay to various compounds [gallic acid (▲), ascorbic acid (◆), corrected gallic acid, without Na_2CO_3 (×) and corrected ascorbic acid, without Na_2CO_3 (■)]

Table 3-3 represents the results for the spiking trial using known concentrations of gallic and ascorbic acid. This trial was performed to ensure that the absorbance from the assay comes from either the sample or the known standards spiked to the sample. Theoretically, the absorbance and total phenolic content of the mixture of BHJ and known concentration of standards should be higher than absorbance and total phenolic content of BHJ alone.

Table 3-3 Spiking trial with sample (BHJ) added with known concentration (100 µg/ml) of ascorbic acid and gallic acid

Folin-Ciocalteu assay (1)		
Samples	Total phenolic content [GAE (µg/ml)]	Total phenolic content after subtraction from mixture [GAE µg/ml)]
BHJ*	135.3	(i) BHJ without ascorbic acid: 120.4[#]
Ascorbic acid (100 µg/ml)[a]	70.2	(ii) BHJ without gallic acid: 114.8[^]
Gallic acid (100 µg/ml)[b]	125.7	
BHJ + Ascorbic acid (100 µg/ml)[c]	190.6	
BHJ + Gallic acid (100 µg/ml)[d]	240.5	
Vitamin C correction assay (2)		
Samples	Total phenolic content [GAE (µg/ml)]	Total phenolic content after subtraction from mixture [GAE µg/ml)]
BHJ	29.6	(i) BHJ without ascorbic acid: 23.4[~]
Ascorbic acid (100 µg/ml)[e]	22.2	(ii) BHJ without gallic acid: 20.7[ζ]
Gallic acid (100 µg/ml)[f]	50.8	
BHJ + Ascorbic acid (100 µg/ml)[g]	45.6	
BHJ + Gallic acid (100 µg/ml)[h]	71.5	

*Tesco's Blackcurrant High Juice 50%, [#]Value c minus a, ^Value d minus b, [~]Value g minus e, [ζ]Value h minus f

Percentage differences of total phenolic content of BHJ only and the total phenolic content of BHJ after subtraction from the mixture were calculated for both Folin and vitamin C correction assays. Results for the Folin assay (1) yielded 11.1%

and 15.2% difference from the BHJ without spiking for ascorbic acid and gallic acid respectively. On the other hand, 20.9% and 29.8% differences were shown for ascorbic acid and gallic acid in the vitamin C correction assay (2). The percentage difference obtained in this trial is satisfactory and this suggested that the assays were able to show an increment in absorbance with the addition of compound with a known concentration.

Figure 3-3 displays the standard curves for gallic acid, caffeic acid and catechin, CY3RUT and DP3RUT. From the figure, it shows that gallic acid and catechin were able to read the absorbance up to 2 and would make a good reference compounds. Catechin was not selected as the reference for the Folin assay because it is light sensitive (Sigma-Aldrich, 2013) and more expensive than gallic acid. CY3RUT and DP3RUT also gave excellent response in the Folin assay, but these are deemed not suitable as these compounds are expensive. Caffeic acid was weak and serves no advantage over gallic acid.

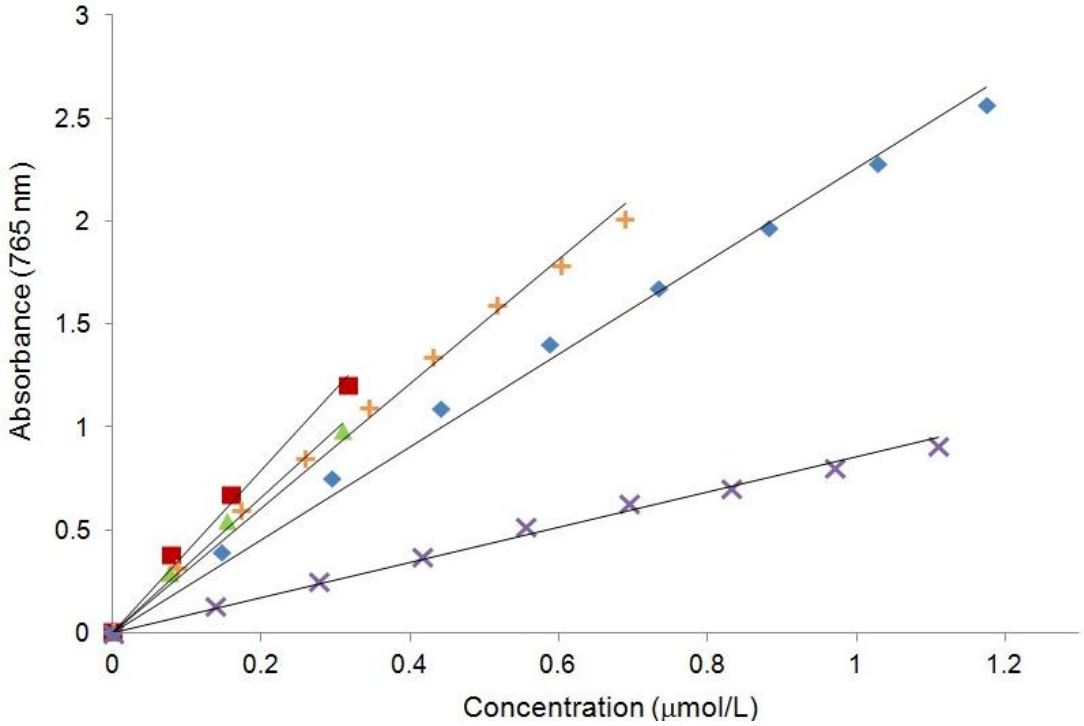


Figure 3-3 Response in the Folin assay to various compounds [cyanidin-3-*O*-rutinoside (■), delphinidin-3-*O*-rutinoside (▲), catechin (+), gallic acid (◆), caffeic acid (×)]

Result from the Folin assay, which was performed on several interfering compounds, is shown in Figure 3-4. It can be summarized that the absorbance for these compounds is very low and did not follow a linear increment as the concentration of these compounds increased.

None of the compounds tested reacted in the Folin assay, even at high concentrations. It can be deduced that these compounds will not significantly affect the assay of food products containing these compounds. In summary, only ascorbic acid was therefore corrected in further Folin assay tests.

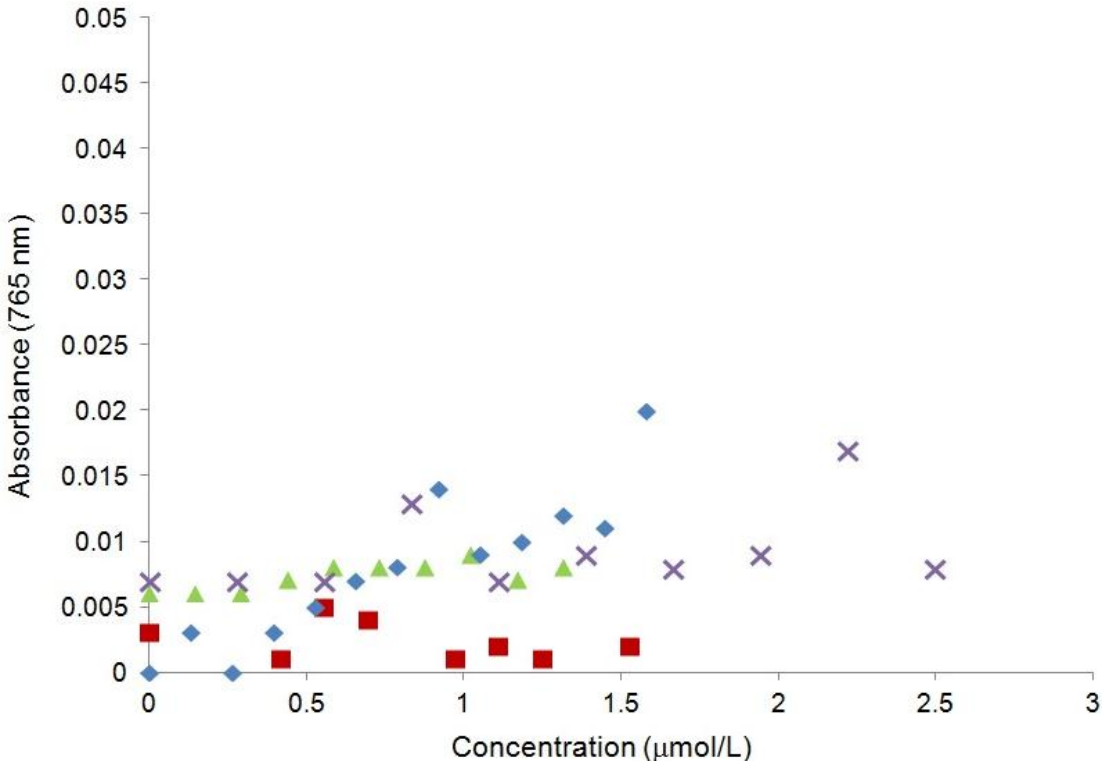


Figure 3-4 Response in the Folin assay to various compounds [sucrose (▲), sodium metabisulfite (◆) and fructose (■), glucose (×)]

Table 3-4 displays the relative response factor (RRF) of several compounds which were tested in the Folin assay (see Figure 3-3 and Figure 3-4). RRF is the ratio of response factor of individual compounds to the standard which was gallic acid. RRF was calculated by dividing the gradient value (m value) from equation of standard curve of these compounds by the m value of gallic acid. From the table, it shows anthocyanins and catechin have high RRF while other compounds have a lower value with the potential interfering compounds having a negligible effect.

Table 3-4 Relative response factors of several compounds

Compounds	Relative response factor
Fructose	0.0007
Sucrose	0.001
Glucose	0.001
Sodium metabisulfite	0.006
Caffeic acid	0.38
Ascorbic acid	0.50
Catechin	1.34
Delphinidin-3-*O*-rutinoside	1.47
Cyanidin-3-*O*-rutinoside	1.74

3.4.1.2 Analysis of foods by the Folin assay

(a) Sultanas, citrus fruits, mustards and marmalades

Figure 3-5 shows total phenolic content for mustards (A), marmalades (B), citrus fruits (C) and sultanas (D). There were three processed mustards in the form of a condiment and one mustard seed sample analysed in the current study. From the results, the total phenolic content of the mustard seed was higher than processed mustard when compared on a GAE mg/100 g basis, but little difference was found between samples on a serving size basis (average value of 41.6 ± 2.4 GAE mg/serving size). Not much difference was found in the total phenolic content between different mustard varieties (wholegrain, Dijon and English mustards).

Two samples of marmalades were analysed in the current study. The average total phenolic content for marmalade was 95.2 ± 5.3 mg/100g or 14.3 ± 7.1 mg/serving size but a large variation was observed between the samples. A previous study found a lower total phenolic content (43.7 mg GAE/100g) of orange jam prepared in the laboratory (Rababah et al., 2011). A recent study compared the edible

part and the peel of sour oranges extracted using high pressure extraction using a syringe pump (Wang et al., 2011). The total phenolic content reported for edible fruit is 53.9 GAE/100g while the peel is 112.2 mg GAE/100g. In this thesis the total phenolic content of marmalade with orange peel (Morrison's Extra Thick Orange Marmalade) is double that of marmalade without peel. This finding is supported by higher total phenolic content found in the orange peel as reported by Wang et al. (2011).

Three different sources of sultanas and citrus fruits were analysed in the current study. Sultanas showed no major difference between samples with a mean value of 200.5 ± 23.8 mg/100 g or 50.1 ± 6.0 mg/serving size. In comparison, a higher total phenolic content for raisins (1065.0 ± 1.6 GAE mg/100g) was obtained by a previous study in the United States (Wu et al., 2004). Furthermore, a recent study on sultana samples also reported higher total phenolic content (363.5 ± 4.4 GAE mg/100g) (Kountouri et al., 2013). One possible reason for these discrepancies is the different varieties of grapes used in sultana production.

The results for citrus fruits showed no big difference in total phenolic content between samples. The mean value of citrus fruits was 106.2 ± 3.6 mg/100g or 84.9 ± 2.9 mg/serving size. A study in Iran reported a value of 55.8 GAE mg/100ml for satsuma juice (C. *unshiu* M.) (Hashempour et al., 2013). Another study reported a value of 80 GAE mg/100ml mandarin juice (C. *reticulata*) (80 GAE mg/100ml) (Rekha et al., 2012). The lower value of citrus juice as compared to results from fresh fruits is expected and may be due to processing of the food product.

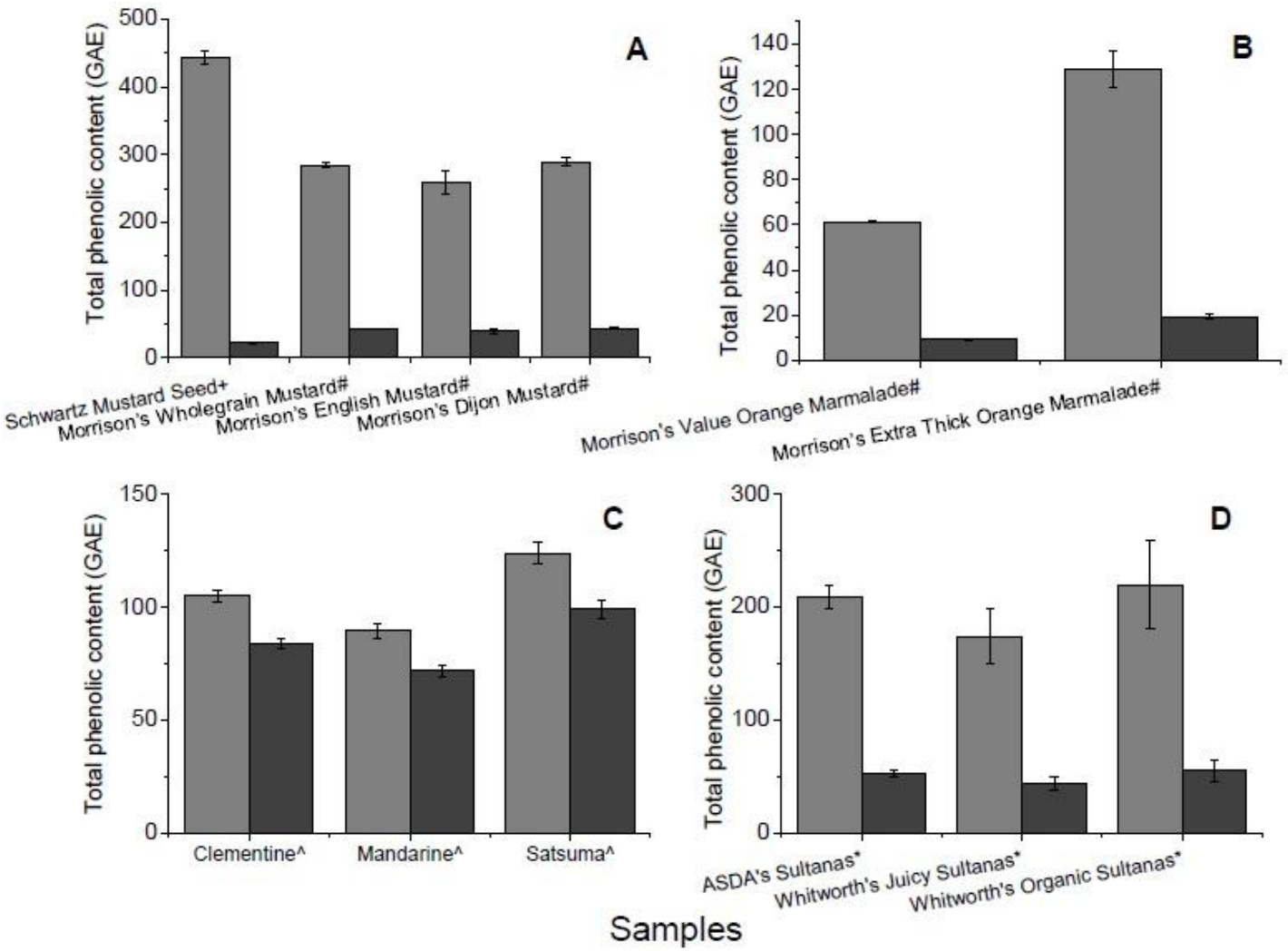


Figure 3-5 Total phenolic content in mustards (A), marmalades (B), citrus fruits (C) and sultanas (D) measured by Folin assay [GAE mg/100 g (□) and GAE mg/serving size (■)]

Serving size: 5 g (+), 15 g (#), 80 g (^), 25 g (*)

(b) Fruit smoothies

Figure 3-6 displays results from the Folin assay for fruit smoothie samples. Innocent Strawberry and Banana had the highest GAE value (208.9 ± 3.4 mg/serving size) whilst Innocent Orange, Mango and Pineapple had the lowest (95.0 ± 1.2 mg/serving size). In the current study, no comparison can be made between eight samples because they differed significantly in the mixture and the percentage of fruits used in the production.

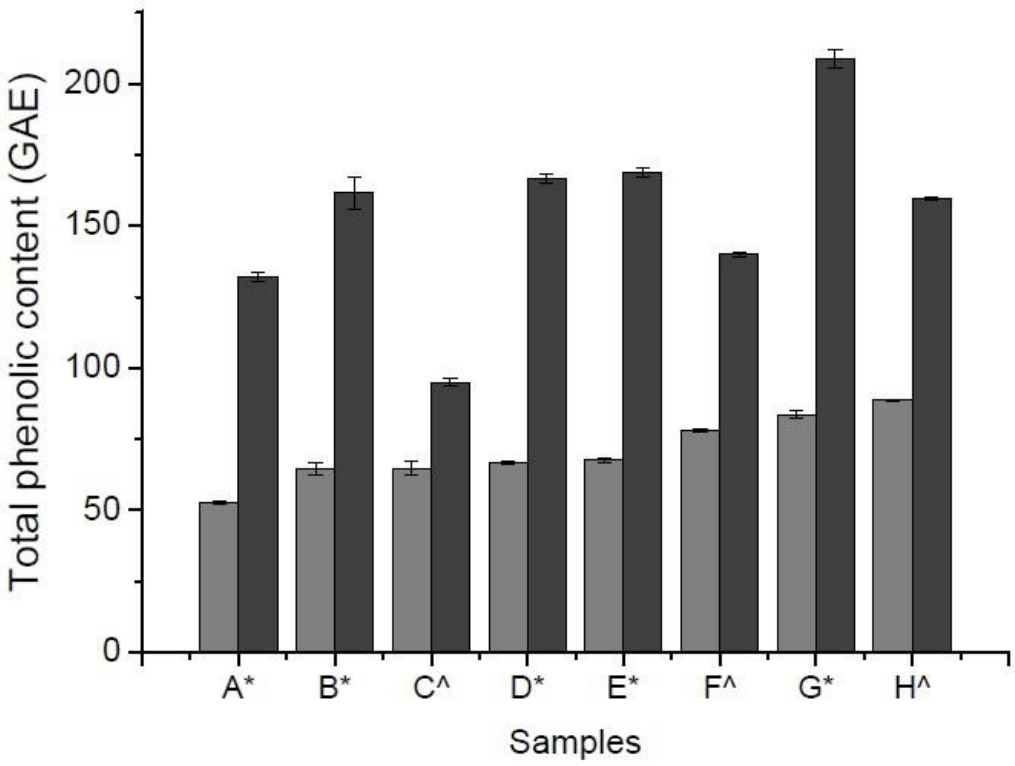


Figure 3-6 Total phenolic content in fruit smoothies measured by the Folin assay [GAE mg/100 g (▫) and GAE mg/serving size (■)]

Serving size: 200 ml (*), 180 ml (^); Samples: A: Innocent Pineapple, Banana & Coconut*, B: Innocent Mango & Passion fruit*, C: Innocent Orange, Mango & Pineapple^, D: Innocent Kiwi, Apple & Lime*, E: Co-operative Cranberry, Blackberry & Raspberry*, F: Innocent Apple & Blackcurrant^, G: Innocent Strawberry & Banana*, H: Innocent Strawberry, Blackberry & Raspberry^

(c) Fruit juices and orange concentrates

Figure 3-7 shows the range of total phenolics for 16 different mixed fruit juices. Tropicana Pomegranate, Grape and Apple juice (P) had the highest total phenolic content (179.6 ± 2.4 GAE mg/serving size), while ASDA's Apple & Blackberry juice drink (A) had the lowest (47.6 ± 0.7 GAE mg/serving size). Overall, it can be proposed that juice drinks have a lower GAE value (less than 100 mg/serving size) as compared to fruit juices.

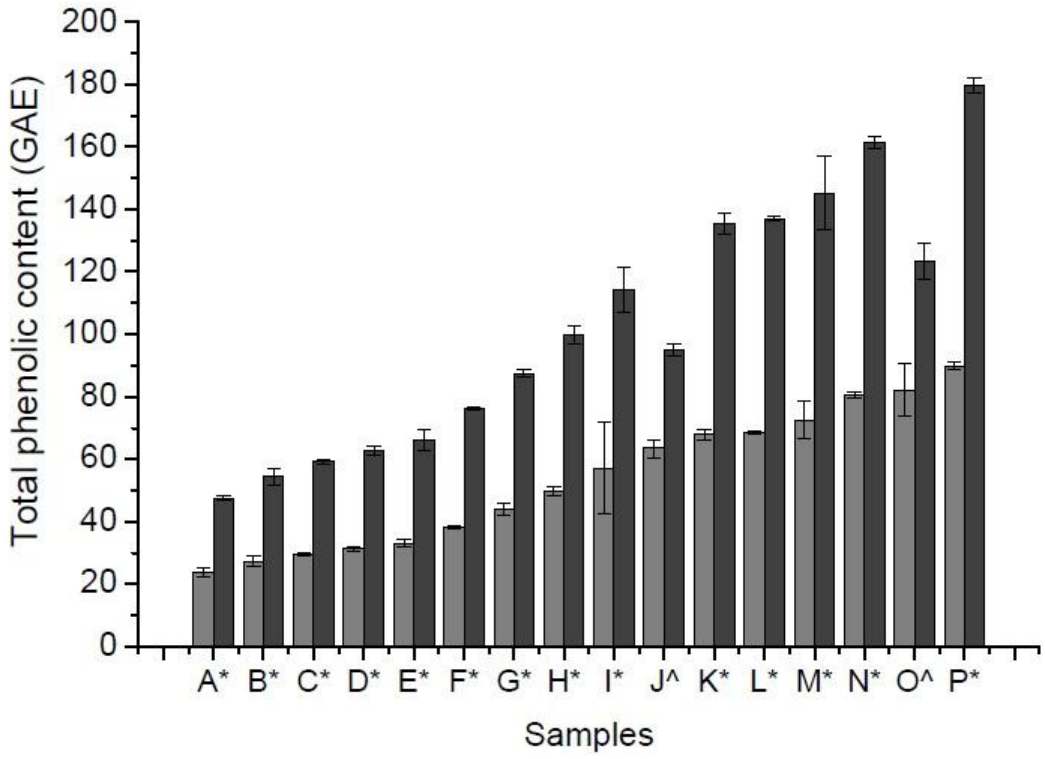


Figure 3-7 Total phenolic content in mixed fruit juices measured by the Folin assay [GAE mg/100 g ( ) and GAE mg/serving size ( )]

JD: juice drink; Serving size: 200 ml (*), 150 ml (^); Samples: A: ASDA's Apple & Blackberry JD, B: J2O Apple & Mango, C: J2O Apple & Raspberry*, D: Sun Exotic Tropical Fruit*, E: J2O Orange & Passion fruit*, F: ASDA's Grape, Apple & Raspberry JD*, G: ASDA's Citrus JD*, H: J2O Apple & Blackberry*, I: ASDA's Forest Fruits JD*, J: Innocent Tropical Juice^, K: Tropicana Ruby Breakfast*, L: Morrison's Tropical Juice*, M: Tropicana Mango, Peach & Papaya*, N: Morrison's Orange & Cranberry*, O: Innocent Apple & Raspberry Juice^, P: Tropicana Pomegranate, Grape & Apple*

Figure 3-8 displays the total phenolic content of 5 orange concentrates after vitamin C correction measured by the Folin assay. The graphs represent total phenolic content before (the whole bar) and after the correction of vitamin C (light grey area). There is a trend in increment of vitamin C content as the total phenolic content increases. Orange high juice cordials have higher total phenolic content and vitamin C than ordinary cordial or squash.

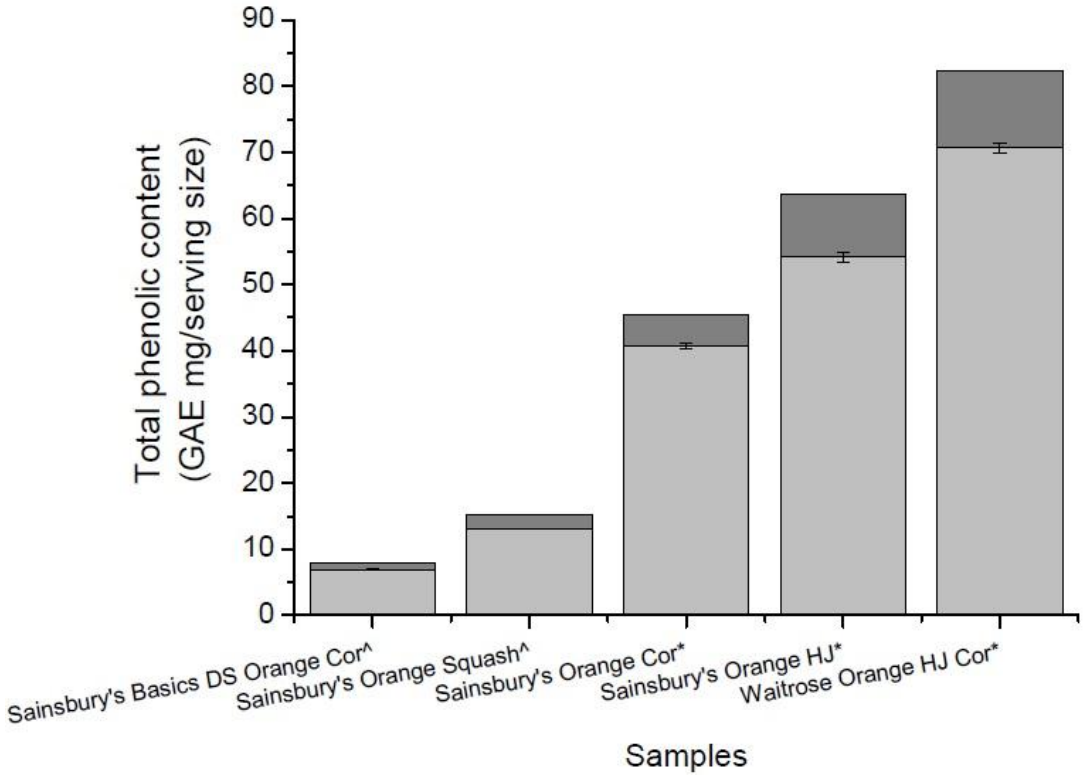


Figure 3-8 Total phenolic level of orange concentrates [total phenolic content after correction for vitamin C content (▫) and estimated vitamin C level from the Folin assay (■)]

Cor: Cordial, HJ: High juice, DS: Double strength, Serving size: 1 to 4 dilution (*), 1 to 9 dilution (^)

(d) Tomato based pasta sauces

Nineteen tomato based pasta sauces were assessed using the Folin assay. The main ingredients are always tomato and onion (see Table 3-5) with a mean of 65% and 8% respectively, although for some samples, no percentage was shown on the label for onion. Sauces may contain other ingredients such as herbs, peppers, beef, olive oil and red wine.

Table 3-5 Main ingredients of tomato based pasta sauce (by percentage, %)

Samples	Code	Total tomato~ (%)	Onion (%)
Dolmio Italian Chilli Microwaveable	A	88	NA
Morrisons Bolognese*	B	13	10
Dolmio Tomato & Basil Microwaveable	C	89	NA
Dolmio Meatball Bolognese[#]	D	87	NA
Morrisons Tomato Pasta Sauce	E	75	NA
Marks & Spencer Bolognese	F	63	NA
Morrisons Tomato Pasta Sauce (long shelf life)	G	67	2
Dolmio Minced Beef Bolognese	H	67	NA
Sacla Bolognese	I	63	NA
Seeds of Change Bolognese	J	92	NA
Dolmio Smoked Bacon & Tomato Stir in Sauce	K	68	12
Dolmio Sweet Pepper	L	52	8
Loyd Grossman Bolognese*	M	44	NA
Dolmio Oven-roasted Vegetable Stir in Sauce	N	67	7
Napolina Bolognese^	O	47	8
Dolmio Slow-roasted Garlic & Tomato Stir in Sauce	P	70	NA
Dolmio Sun-dried Tomato Stir in Sauce (Light)	Q	72	8
Dolmio Sun-dried Tomato Stir in Sauce	R	66	9
Meenas Bolognese*	S	45	NA

~total tomato refers to addition of tomato and tomato puree contained in the sample, *contains red wine, ^ contains red wine vinegar, [#]contains meat, NA: not available on the label

The results for the total phenolic content for all tomato-based pasta sauces are shown in Figure 3-9. Dolmio Italian Chili (A) has the lowest total phenolic content (43 GAE mg/100 g) while Meenas Bolognese (S) sauce has the highest (86 GAE mg/100 g). Interestingly, although the total tomato content of Dolmio Italian Chilli (A) is third highest, it does not affect the total phenolic content of the samples. To confirm, a correlation analysis was performed between total tomato and onion content and total phenolic content of the samples. No correlation was found between total phenolic content and both total tomato ($R = -0.248$, $p>0.05$, $N=19$) and onion content ($R = 0.176$, $p>0.05$, $N=8$). No clear pattern of increment in GAE value with reduction in total tomato content in pasta sauce. In addition, increase in total phenolic content was not associated with percentage of onion content of the sauces.

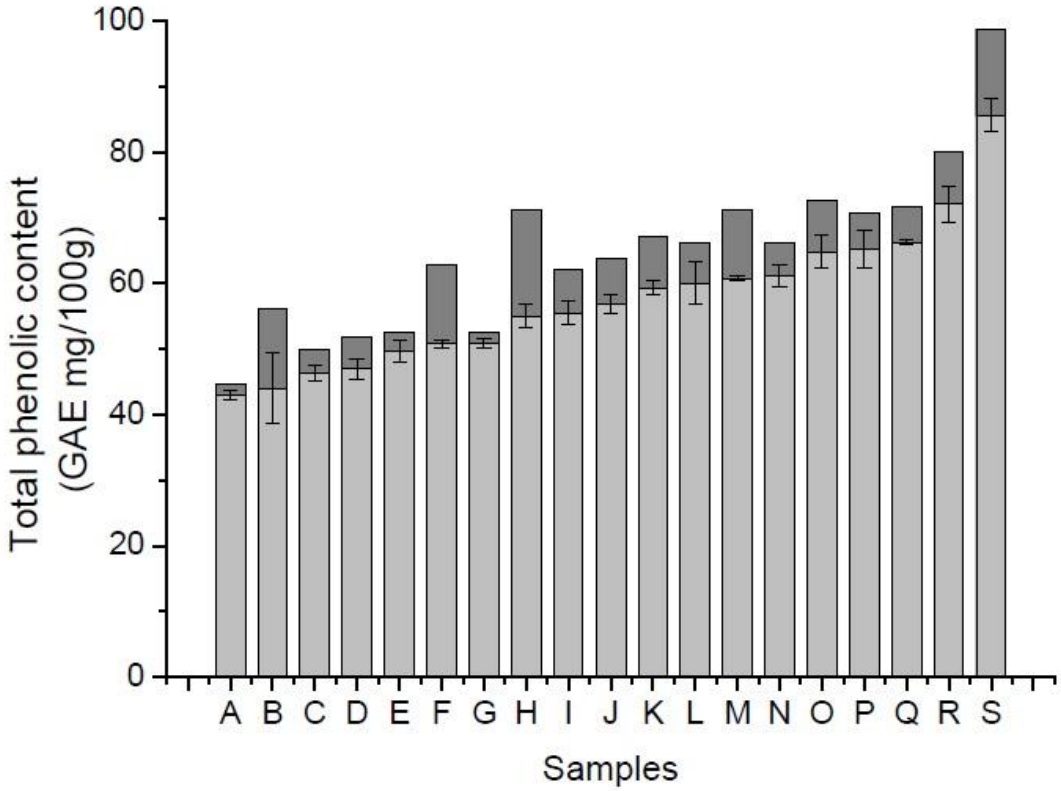


Figure 3-9 Total phenolic content of tomato based pasta sauces measured by the Folin assay [total phenolic content after correction for vitamin C content (▫) and estimated vitamin C level from Folin assay (■)]

(d) Blackcurrant concentrates

Blackcurrant (*Ribes nigrum* L.) is a type of currant with a very dark purple colour known for its high content of anthocyanins and vitamin C (Hollands et al., 2008). This crop is commonly processed into juice concentrate, cordial, jellies, frozen fruits and other food products. Blackcurrant concentrate was consumed by 14 % of the participants in the current study based on the food diary analysis (see Chapter 2). Thirty blackcurrant concentrate drinks were purchased and assessed for their total phenolic content. The results are presented as total phenolic content per serving size of 50 ml of neat concentrate diluted to 250 ml of water (1 to 4 ratio of concentrate to water).

Figure 3-10 (A) displays the total phenolic content for blackcurrant concentrates with a serving suggestion of dilution at a ratio of 1 to 4. Mixed concentrates tended to have a lower total phenolic content as compared to blackcurrant-only concentrates. In addition, the mixed concentrates had a lower estimated vitamin C content. ASDA Blackcurrant Juice Drink No added sugar (A16) had the highest vitamin C content (39 GAE mg/serving size). The mean of total phenolic content and estimated vitamin C content in these samples are 83 GAE mg/serving size and 21 GAE mg/serving size respectively. Marks & Spencer British Blackcurrant Cordial (A17) had the highest total phenolic content (144 GAE mg/serving size). Morrisons Apple Blackcurrant Cordial (A1) had the lowest total phenolic content and the vitamin C content contributed to the total phenolic content of the sample. This finding might be due to the high amount of ascorbic acid purposely added to the product by the manufacturer.

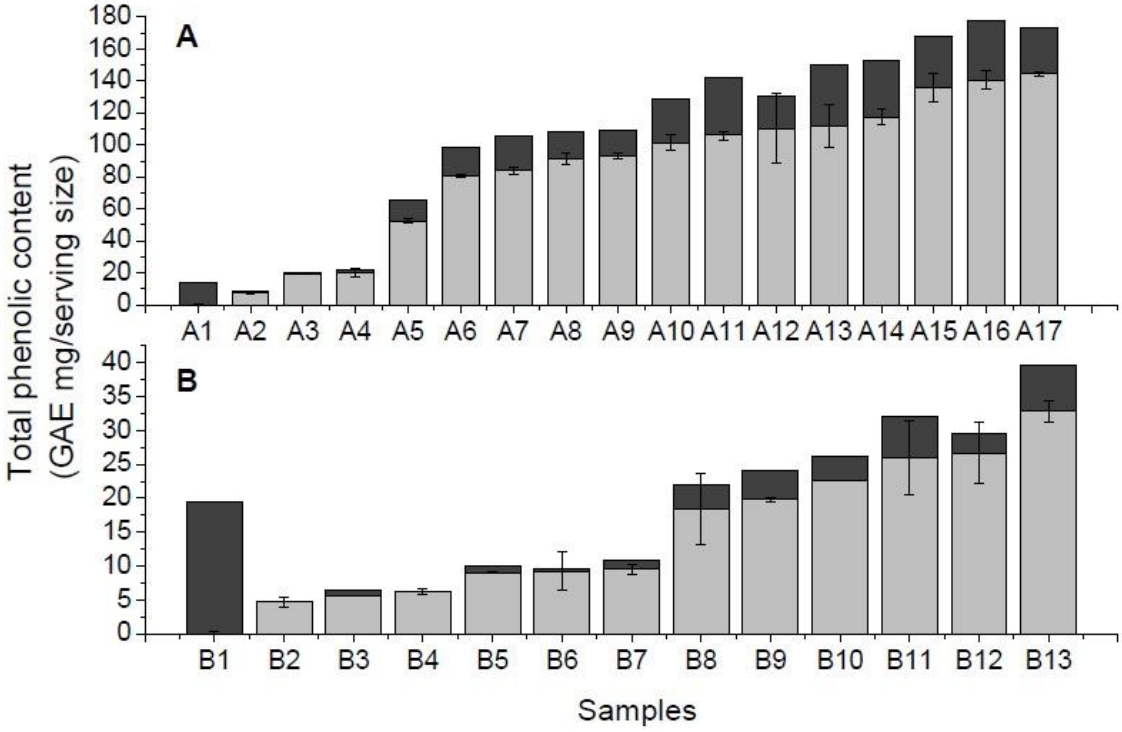


Figure 3-10 Total phenolic level of undiluted blackcurrant concentrates (A) with 1 to 4 dilution and (B) with 1 to 9 dilution [total phenolic content after correction for vitamin C content (▫) and estimated vitamin C level from Folin assay (■)]

Con: Concentrate, Cor: Cordial, JD: Juice drink, HJ: High juice, NS: No added sugar, DS: Double strength, BC: Blackcurrant, serving size is 50 ml of neat concentrate diluted to 250 ml of water

Samples (A): A1: Morrisons Apple BC Cor NS, A2: Schweppes BC Cor, A3: Robinson's Apple BC NS, A4: Robinson's Apple BC, A5: ASDA HJ Apple BC NS, A6: Marks & Spencer BC HJ NS, A7: Morrisons BC HJ, A8: Sainsbury's BC HJ, A9: M&S BC HJ NS, A10: Ribena Con, A11: Ribena Plus Immunity Support, A12: Tesco BC HJ, A13: ASDA BC JD, A14: Tesco BC Cor, A15: Ribena NS, A16: ASDA BC JD NS, A17: Marks & Spencer British BC Cord

Samples (B): B1: Morrisons DS Apple BC Cor NS, B2: ASDA Smart Price Apple BC DS NS, B3: Sainsbury's Basics DS Apple BC Cor, B4: Tesco Value Apple BC DS NS, B5: Sainsbury's Apple BC Cor, B6: Tesco Apple BC DS, B7: M&S Apple BC Cor NS, B8: ASDA Apple BC DS NS, B9: ASDA Apple BC DS, B10: Sainsbury's BC Cor, B11: Tesco BC Squash DS, B12: Tesco Apple BC DS NS, B13: ASDA BC DS NS

Total phenolic content for blackcurrant concentrates with a serving suggestion of dilution at a ratio of 1 to 9 is shown in Figure 3-10 (B), as most of the concentrates in this group are categorized as “double strength”. The mean of total phenolic content and estimated vitamin C content in these samples is 15 GAE mg/serving size and 4 GAE mg/serving size respectively. The sample with the highest total phenolic content is ASDA Blackcurrant Double Strength added sugar Concentrate (B13) (33 GAE mg/serving size). The same value was obtained for total phenolic content and estimated vitamin C content for Morrison’s Apple and Blackcurrant Double Strength added sugar Concentrate (B1). It can be deduced that the phenolic content in this sample came mainly from interfere from vitamin C.

Association between total phenolic content (GAE mg/serving size) and vitamin C (GAE mg/serving size) obtained from all blackcurrant concentrates is presented in Figure 3-11. The correlation analysis was performed with (A: Correlation 1, N=30) and without outliers (B: Correlation 2, N=28). In Correlation 2 (B), two outliers with very low total phenolic content (A1 and B1) are removed from the analysis. The coefficient correlation (r value) was improved from 0.887 ($p<0.01$) to 0.951 ($p<0.01$) between these two variables when the outliers were removed from the analysis. The result shows that there is a directly proportional association between total phenolic content and vitamin C content of the concentrates.

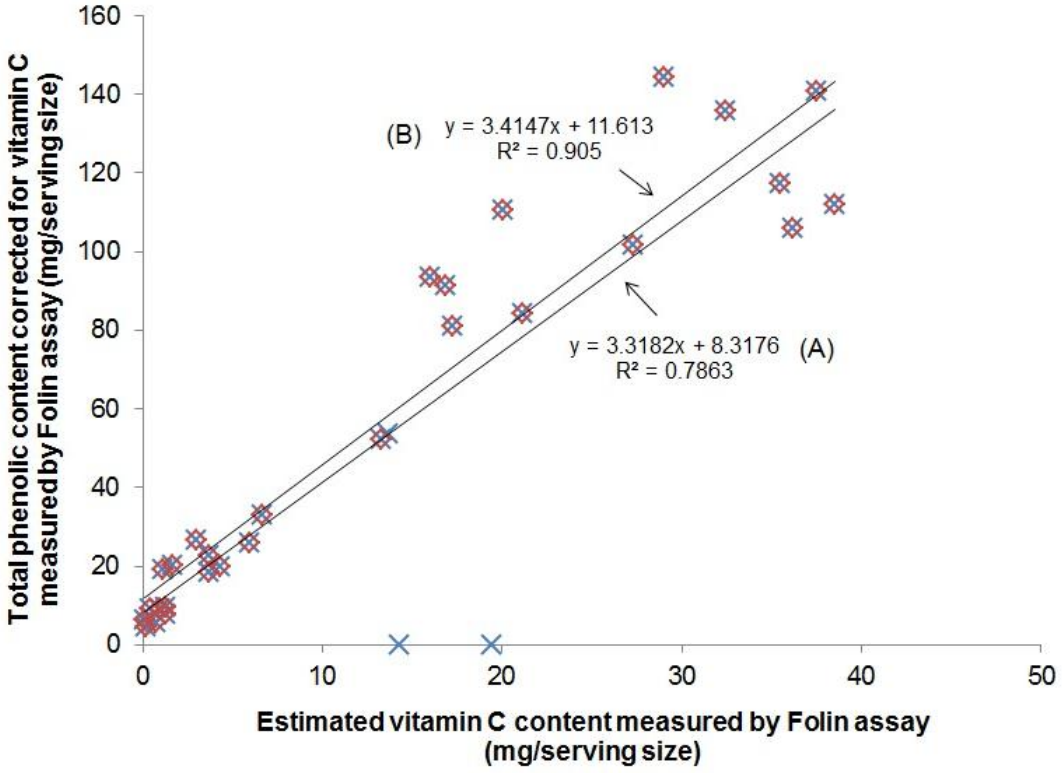


Figure 3-11 Association between corrected total phenolic content with estimated vitamin C level measured by the Folin assay of blackcurrant concentrates [A: correlation 1 with outliers, N=30 (×) and B: correlation 2 without outliers, N=27 (◇)]

3.4.1.3 Discussion of Folin data

Vitamin C naturally presents in foods, reacts with the Folin reagent and therefore is a source of interference in determining total phenolic content. This was demonstrated in the marked reduction in total phenolic content of commercially available fruit juice samples after correction of vitamin C content (Borges et al., 2010). Because most samples selected for the Folin-Ciocalteu assay in the current study contained a substantial amount of vitamin C, correction for this compound was performed to remove the interference.

There is a wide range in total phenolic content of selected foods and beverages analysed in this study, ranging from 53.7 to 277.2 GAE mg/100g with fresh foods have higher total phenolic content than processed foods (see Table 3-6). It is probable that food processing can affect total phenolic content. This is in accordance with a recent study on grape juice concentrate which suggested that processing can lead to degradation in anthocyanins, thus, modifying total phenolic content (Capanoglu et al., 2013). Blackcurrant concentrates were selected for further analysis by using HPLC (Section 3.3.2.5) based on food diary analysis (Chapter 2); from the 36% of participants who consumed fruit concentrates 39% consumed blackcurrant concentrates as a part of their habitual diet.

Table 3-6 Summary of total phenolic content of selected food samples

Sample (number of samples)	Mean total phenolic content (GAE mg/100 ml)	Mean total phenolic content (GAE mg/serving size)
Mixed fruit juices (N=16)	53.7 ± 2.8	102.8 ± 3.0
Fruit smoothies (N=8)	70.8 ± 1.0	154.1 ± 2.1
Orange concentrates (N=5)	74.2 ± 0.8	37.1 ± 0.4
Marmalades (N=2)	95.2 ± 4.5	14.3 ± 0.7
Citrus fruits (N=3)	106.2 ± 3.6	84.9 ± 2.9
Blackcurrant concentrates (N=30)	120.0 ± 8.2	53.6 ± 3.3
Sultanas (N=3)	200.5 ± 24.5	50.1 ± 6.1
Mustards (N=3)	277.2 ± 8.9	41.6 ± 1.3

There are certain difficulties in making a comparison of total phenolic content values between studies. Certain studies presented the total phenolic content as GAE/mg of dry weight, thus making results incomparable. The different types of reference used in the Folin assay other than gallic acid, has led to a variation in the amount of detected phenols in foods, thus, a standardization step is indispensable (Prior et al., 2005).

A comparison was made between total phenolic content of blackcurrant concentrates obtained in this study and total phenolic content of raw blackcurrant as reported from Phenol-Explorer (820.6 GAE mg/100 g of fresh weight) (see Table 3-7). The percentage of total phenolic content obtained from the current analysis accounted for an average of 20.2% from total phenolic content of raw fruit. This finding is as expected since processing can lead to a reduction in total phenolic content.

Table 3-7 Total phenolic content of blackcurrant concentrate compared to total phenolic content of raw blackcurrant reported from Phenol-Explorer

Blackcurrant concentrates* [brand and number of sample(s)]	**Mean total phenolic after correction for vitamin C (assay) (GAE mg/100 ml)[1]**	**% of total phenolic content [1] to total phenolic content of raw blackcurrant[2]**
Sainsbury (N=2)	136.6	16.6
Ribena (N=3)	229	27.9
Marks & Spencer (N=3)	212.7	25.9
ASDA (N=3)	212.1	25.8
Tesco (N=3)	186.6	22.7
Schweppes (N=1)	15.3	1.9
Morrisons (N=1)	168.2	20.5
Blackcurrant concentrates (all) (N=16)	186	22.7
Mean ± SD	165.8 ± 73.3	20.2 ± 8.9

*Blackcurrant only concentrates
[1]After correction of vitamin C interference
[2]Total phenolic content of fresh fruits (blackcurrants) from Phenol-Explorer: 820.6 GAE mg/100 g of fresh weight

3.4.2 HPLC analysis of blackcurrant concentrates and discussion

Selected blackcurrant concentrates with high total phenolic content were analysed by HPLC to quantify the two major anthocyanins, namely cyanidin-3-*O*-rutinoside (CY3RUT) and delphinidin-3-*O*-rutinoside (DP3RUT). An example HPLC chromatogram for Ribena No Added Sugar (RNAS) is shown in Figure 3-12. Chromatogram 1 represents myricitrin as internal standard. Both CY3RUT and DP3RUT (Chromatogram 2) were identified based on their retention time and UV spectra compared to authentic standards. All blackcurrant samples contained both anthocyanins.

Total anthocyanins for the selected blackcurrant samples ranged from 9 to 71 mg/100 ml sample with mean a value of 25 mg/100ml of undiluted concentrate (see Figure 3-13). Previous findings have identified two main anthocyanidins (or the aglycones) in blackcurrant, cyanidin and delphinidin, while key anthocyanins are the 3-rutinoside and 3-glucoside (Hollands et al., 2008). Profiling of polyphenol content in blackcurrant juice concentrate has shown delphinidin-3-rutinoside as an explicit characteristic of blackcurrant fruit (Obón et al., 2011).

Two main anthocyanins analysed in this study, CY3RUT and DP3RUT have a range from 3.1 to 21.1 and 6.0 to 49.4 mg/100 ml of undiluted concentrates respectively. These values are low when compared to a recent study which reported values of 34.9 and 35.0 mg/100 ml for CY3RUT and DP3RUT respectively (Torronen et al., 2012). One possible reason for this discrepancy is that the juice prepared in the study by Torronen et al. was prepared by diluting the juice concentrate at 1:10 dilution.

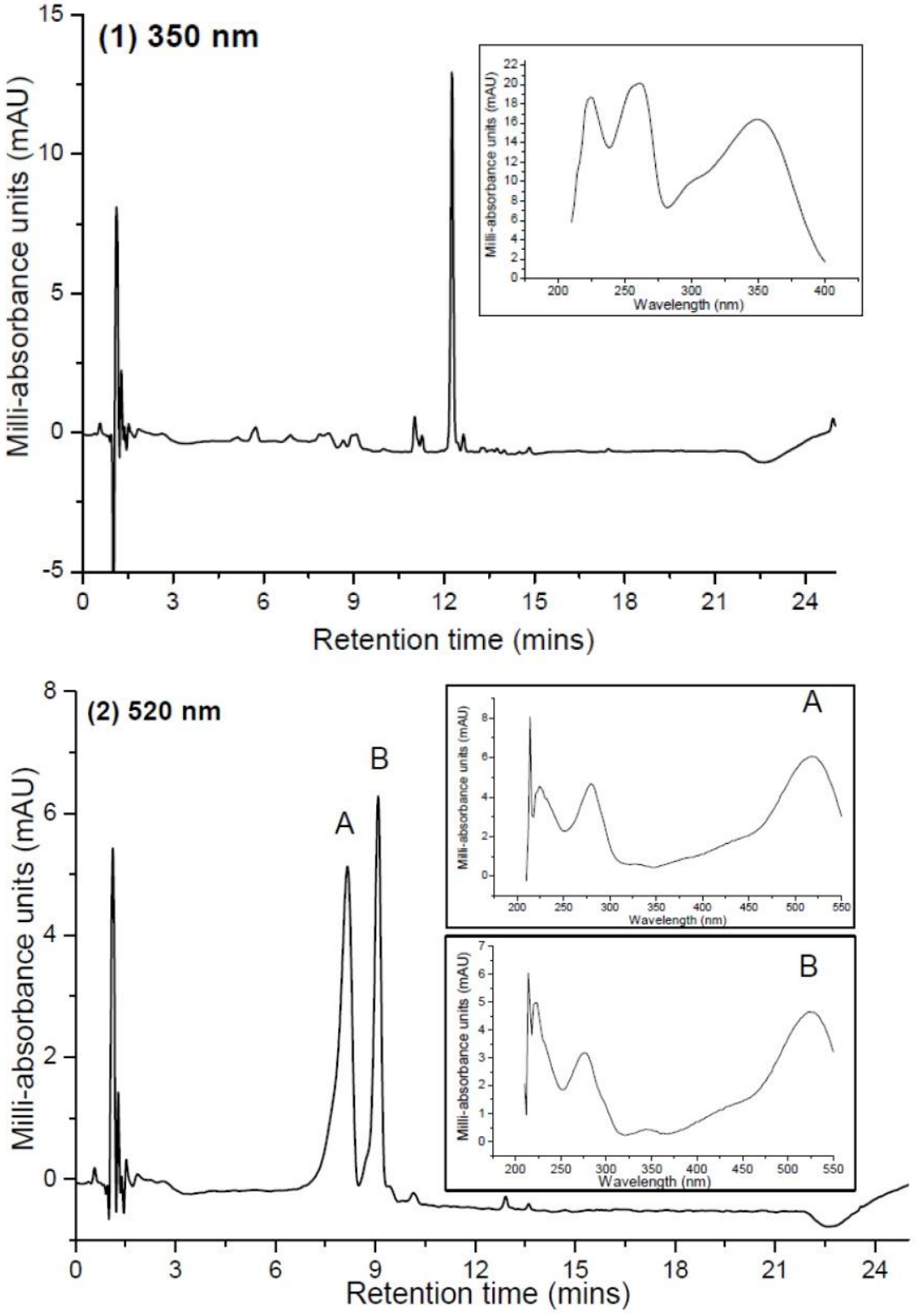


Figure 3-12 Representative HPLC chromatogram of (1) myricitrin and (2) anthocyanins [A: cyanidin-3-*O*- rutinoside and B: delphinidin-3-*O*-rutinoside] in Ribena No Added Sugar. Inserts show the UV-absorption spectra of the main peaks from corresponding chromatograms.

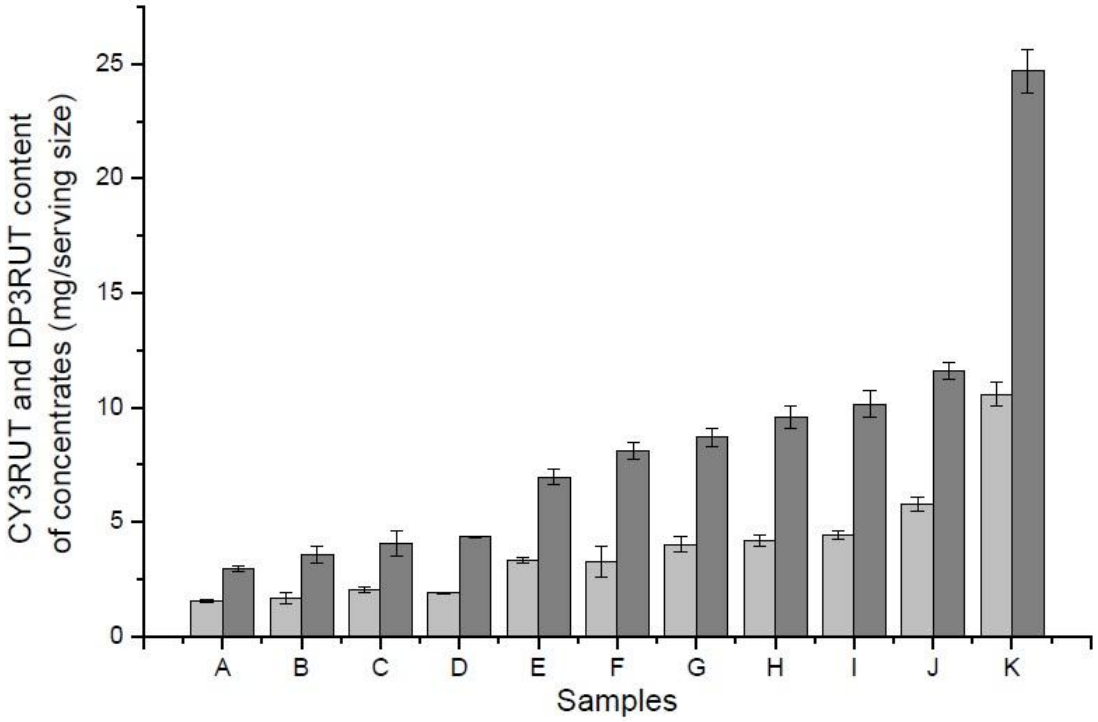


Figure 3-13 Two major anthocyanins in blackcurrant drinks [cyanidin-3-*O*-rutinoside (■) and delphinidin-3-*O*-rutinoside (■)] measured by HPLC

Con: Concentrate, Cor: Cordial, JD: Juice Drink, HJ: High Juice, NS: No Added Sugar, serving size is 50 ml of neat concentrate diluted to 250 ml of water

Samples: A: Ribena Plus, B: ASDA JD NS , C: Tesco Cor , D: ASDA JD ,
E: Ribena Con , F: Marks & Spencer Cor, G: Tesco HJ, H: Marks & Spencer HJ NS,
I: Ribena Con NS, J: Morrisons HJ, K: Marks & Spencer HJ

A recent study that examined polyphenol content in commercial blackcurrant juice products from four European countries including Finland, Germany, Poland and the United Kingdom identified three major anthocyanins present in all samples analysed (Mattila et al., 2011). The compounds found include the main one, delphinidin-3-*O*-rutinoside, followed by cyanidin-3-*O*-rutinoside and a lower amount of cyanidin-3-glucoside. Authors reported that products from the UK (N=3) have low anthocyanins content as compared to other countries with a mean value from three samples of 7.5 mg/250 ml.

In our study, cyanidin-3-glucosides were not determined in the blackcurrant concentrates, although it is possible that the shoulder on peak A (Figure 3-12, 2) is cyanidin-3-glucosides, no quantification was performed. The average total anthocyanin obtained from the current study is 62.5 mg/250 ml, 800% higher than the value obtained by Mattila et al. (2011). The possible reason for the large difference between these studies is that the current studies had analysed various types of blackcurrant concentrate (N = 11) such as high juice, cordial and juice drink, and a wide range of values were found, with four samples at similar values to the ones reported by Mattila et al. (2011).

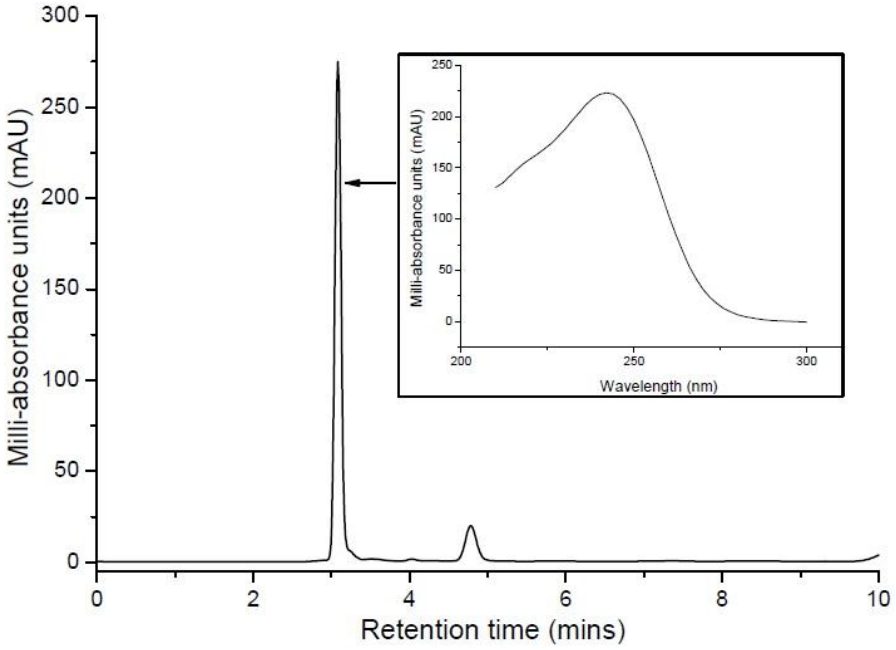


Figure 3-14 Representative HPLC chromatogram of vitamin C in Ribena No Added Sugar (at 245 nm). Inserts show the UV-absorption spectra of ascorbic acid.

Figure 3-14 shows an example of a chromatogram from the RNAS blackcurrant concentrate analysed for vitamin C. The vitamin C content of the blackcurrant concentrates samples showed a large variation ranging from 40 mg to 517 mg/ serving size of undiluted concentrates (see Figure 3-15). The mean value of vitamin C of the samples is 261 mg/serving size. Based on the results, a serving of all blackcurrant concentrates are able to fulfil the Reference Nutrient Intake (RNI) for vitamin C, which is 40 mg (Department of Health, 1991). The company that produced Ribena concentrate has claimed a serving of the concentrate (250 ml) specifically Ribena Plus (sample G) can fulfil the recommended amounts of vitamin C (GlaxoSmithKline, 2012b).

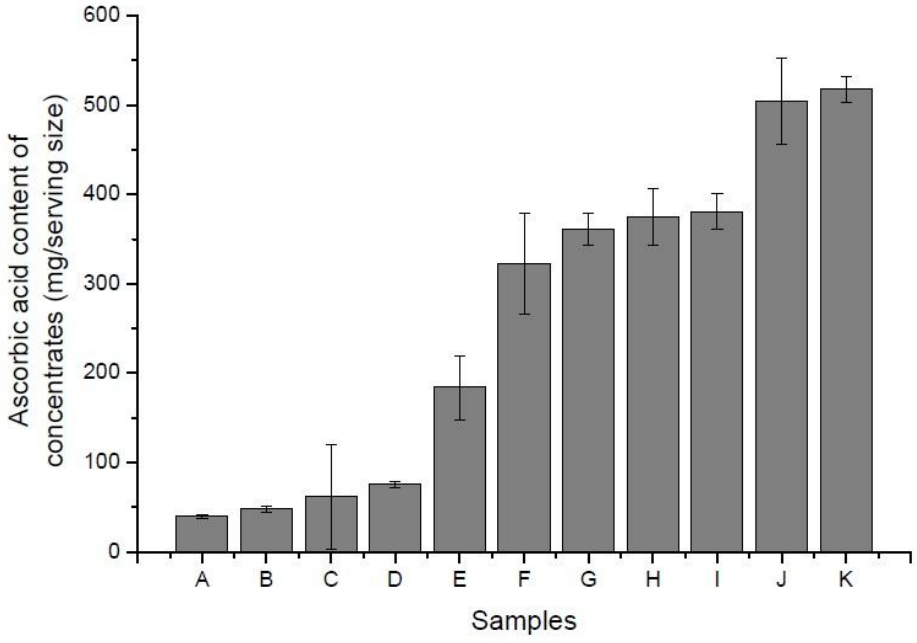


Figure 3-15 Ascorbic acid content of selected blackcurrant concentrate samples measured by HPLC

Con: Concentrate, Cor: Cordial, JD: Juice Drink, HJ: High Juice, NS: No Added Sugar, serving size is 50 ml of neat concentrate diluted to 250 ml of water

Samples: A: Marks & Spencer HJ, B: Marks & Spencer HJ NS, C: Marks & Spencer Cor, D: Morrisons HJ, E: Tesco HJ, F: Ribena Con NS, G: Ribena Plus, H: Ribena Con, I: ASDA JD NS, J: ASDA JD, K: Tesco Cor

The association between total anthocyanins (CY3RUT and DP3RUT) and ascorbic acid is displayed in Figure 3-16. The correlation analysis was performed with (A: Correlation 1) and without outliers (B: Correlation 2). In Correlation 2, an outlier with high total anthocyanins (Marks & Spencer cordial) was removed from the analysis. This sample contained 50% blackcurrant juice (not from concentrate) as the ingredient. Inverse association was shown between total anthocyanins (CY3RUT and DP3RUT) and ascorbic acid with an increase in correlation coefficient from -0.685 ($p<0.05$, N=11) to -0.754 ($p<0.05$, N=10). The inverse association is supported by the finding of low vitamin C content of the blackcurrant concentrate sample with the highest (Marks & Spencer high juice) and second highest (Morrisons high juice) anthocyanins content (see Figure 3-13).

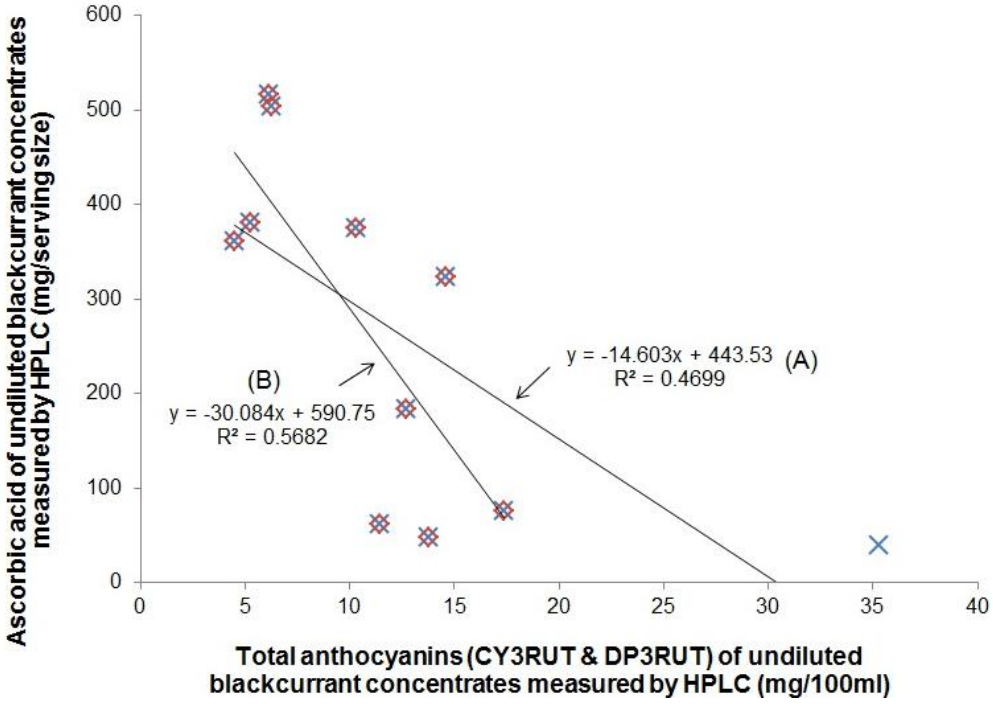


Figure 3-16 Association between ascorbic acid with total anthocyanins (CY3RUT and DP3RUT) content of selected blackcurrant concentrates measured by HPLC [correlation 1 with outliers, N=11 (×) and correlation 1 without outliers, N=10 (◇)]

3.4.2.1 Summary

The vitamin C content of selected blackcurrant concentrates and the percentage RNI provided per serving is shown in Table 3-8. Consumption of a standard serving of all eleven blackcurrant concentrates would achieve more than the daily vitamin C recommendations (40 mg) for the UK. These findings should be interpreted carefully because the consumption of standard serving of blackcurrant drinks can contribute to a very high vitamin C intake, thus might lead to several adverse effect to human urinary excretion including renal stones, oxaluria and renal tubular disease (Food Standard Agency, 2003). In addition, high intake of vitamin C at 2 to 6 g per day can leads to gastrointestinal disturbances or diarrhoea (Anderson et al., 1997, Johnston, 1999). However, high dose of vitamin C given intravenous in complimentary medicine has shown to be safe to human (Padayatty et al., 2010) and reducing the intake of the vitamin can reversed the potential side effects of high dose (Naidu, 2003).

Table 3-8 Vitamin C content of selected blackcurrant samples*[1] compared to daily vitamin C intake as recommended by Reference Nutrient Intake[2]

Blackcurrant concentrates	Vitamin C (mg/serving size)	Vitamin C (% from RNI for vitamin C*)
Marks & Spencer high juice	39.7	99.3
Marks & Spencer high juice no added sugar	48.0	120.0
Marks & Spencer cordial	61.7	154.3
Morrisons high juice	75.6	189.0
Tesco high juice	184.2	460.5
Ribena Concentrate no added sugar	323.0	807.5
Ribena Plus	360.6	901.5
Ribena Concentrate	374.8	937.0
ASDA juice drink no added sugar	381.0	952.5
ASDA juice drink	504.4	1261.0
Tesco cordial	517.3	1293.3
Mean (N=11)	260.9	652.3

*Vitamin C was measured by HPLC, [1]Vitamin C presented as per serving size (dilution in a ratio of 1 part of concentrate to 4 part of water), [2]Reference Nutrient Intake for vitamin C is 40 mg per day

3.5 Overall Discussion

3.5.1 Contribution of blackcurrant concentrates to vitamin C intake to women's cohort

The food diary analysis in Chapter 2 found that blackcurrant concentrates, including the Ribena brand, were consumed by many of the subjects. However, the vitamin C content of the concentrates was not included in the estimation of total vitamin C intake. The vitamin C content for blackcurrant concentrates consumed per day was calculated from the HPLC data by using the mean value of 261 mg/serving size (see Table 3-8). A specific analysis was performed among high Ribena

consumers to identify the contribution of the consumption of blackcurrant concentrate to vitamin C intake (see Table 3-9). The suggestion can be made that the estimation of vitamin C was improved after addition of data on blackcurrant drinkers.

Table 3-9 Vitamin C intake of Ribena's consumers estimated from food diary and after improved data from HPLC analysis

Participants' code[1]	Intake of Ribena concentrate (times/day)	Total volume consumed per day (ml)	Vitamin C intake (mg per day)[2]	Vitamin C of concentrates (mg)[3]	Total vitamin C intake per day (mg)[4]
D140	1	190.0	75.4	39.7	115.1
D004	2	576.7	75.5	120.4	195.9
R079	1.9	211.0	10.5	44.0	54.5
S228	1.7	794.3	52.6	165.8	218.4

[1]Code D: DH: Diet and Health, Code R: LWW: Leeds Women Wellbeing, Code S: Leeds Women Wellbeing High Fibre, [2]Estimated from food diary, [3]Estimated from HPLC, [4]After addition of value from food diary estimation and HPLC.

3.5.2 Contribution of blackcurrant concentrates to polyphenol intake

Table 3-10 shows the total anthocyanins quantified from HPLC as compared to weight of raw blackcurrant fruit in a serving of blackcurrant concentrate. A comparison was made with the weight of a portion size of blueberries (40 g) as reported from a recent portion size guide book (Cheyette and Balolia, 2010). Data from Phenol-Explorer® reported that the total anthocyanin of raw blackcurrant is 237 mg/serving size. When this value is compared to the value of other anthocyanin-rich foods (see Table 3-10), the total anthocyanins of these foods can be considered low, thus show the importance of blackcurrant as the high anthocyanin food source.

From Table 3-10, a serving of blackcurrant concentrate can provide the average of 7 % of a portion of raw blackcurrant. Although the value is small, the consumption of more than one serving of the blackcurrant drinks can increase the anthocyanins intake. In this study, most of the women consumed more than one serving of fruit concentrate in a day. It can be suggested that blackcurrant concentrates can provide a sufficient amount of anthocyanin source in human diet. Moreover, blackcurrant was not normally consumed raw, so consumption as drink can become a good alternative of anthocyanins source.

A comparison was made on the estimation of total anthocyanins of blackcurrant concentrates based on food diary analysis and HPLC. The anthocyanins value of the blackcurrant concentrates was based on the assumption that a serving of the drink contained 5 % of blackcurrant juice (GlaxoSmithKline, 2012a). Data for blackcurrant juice was taken from USDA database. In a serving size of 250 ml, the total anthocyanin estimated from food diaries is 3.1 mg. On the other hand, the average of total anthocyanins from HPLC analysis in a serving of blackcurrant drinks is 12.5 mg (see Table 3-10). This value is four times higher than the estimated amount from the food diary. This finding shows that the total anthocyanins estimated from food diaries are lower than the estimation from HPLC analysis, thus show the important to correct the anthocyanin intake of the blackcurrant drink’s consumers.

Table 3-10 Total anthocyanins quantified from HPLC as compared to the weight of raw blackcurrant fruit (g) in a serving of blackcurrant concentrate

Blackcurrant concentrates (N=11)	Total anthocyanins from HPLC (mg/serving size)[1]	Weight of raw blackcurrants based on total anthocyanins[2] from Phenol-Explorer[3] (g)	Percentage of portion size (%)[a]	Total anthocyanins of other anthocyanin-rich foods (mg/serving size)[4]
Ribena Plus	4.5	1.0	2.5	Highbush blueberry: 54[a]
ASDA juice drink no added sugar	5.3	1.1	2.8	Blackberry, raw: 69[a]
Tesco cordial	6.1	1.3	3.3	Sweet cherry: 69[a]
ASDA juice drink	6.3	1.4	3.5	Red wine: 39[b]
Ribena concentrate	10.3	2.2	5.5	Strawberry: 29[a]
Marks & Spencer cordial	11.4	2.4	6.0	
Tesco high juice	12.7	2.7	6.8	
Marks & Spencer high juice no added sugar	13.8	3.0	7.5	
Ribena concentrate no added sugar	14.6	3.1	7.8	
Morrison high juice	17.4	3.7	9.3	
Marks & Spencer high juice	35.3	7.6	19.0	
Mean ± SD	12.5 ± 8.7	2.7 ± 1.9	6.7 ± 4.7	

[1]Serving size of 50 ml of neat concentrate diluted to 250 ml of water, [2]Total anthocyanins (CY3RUT and DP3RUT) reported in Phenol-Explorer® is 465.7 mg/100 g of raw blackcurrant, [3]Weight of raw blackcurrant in 50 ml of undiluted concentrates, [4]Data taken from HPLC analysis using Phenol-Explorer®, [a]Assuming portion size is 40 g (as estimated for blueberries), [b]Serving size is 175 ml

Specific analysis was performed among high Ribena consumers to identify the contribution of the consumption of blackcurrant concentrate to both anthocyanin and polyphenol intake (see Table 3-11). The average contribution of total anthocyanins from blackcurrant concentrate consumed by these participants to daily total anthocyanins is 75%. The comparison was made with daily polyphenol intake from No Coffee or Tea (NoCorT) dataset (see Chapter 2) where the contribution of tea and coffee as a major polyphenol source to total polyphenol was excluded in the estimation of polyphenol intake. The percentage contribution to daily total polyphenol intake ranges from 2.9 to 30.7%. The suggestion can be made that the consumption of fruit concentrates can make an important contribution to daily total anthocyanin but not to total polyphenol intake. A possible explanation for this might be that other anthocyanin food sources consumed by these participants are limited to white wine and olives. The only participant who acquired anthocyanins from different sources such as fruits is participant D004. This participant consumed substantial amount of strawberries, blueberries and cranberries which contributed to 77% of daily total anthocyanin intake.

Table 3-11 Contribution of Ribena consumption to daily anthocyanins and total polyphenol intake estimated from food diary analysis

Participants' code (see Chapter 2)	Total anthocyanins of concentrates (mg/day)[1]	Daily total anthocyanins intake (mg/day)[1]	Daily total polyphenol intake (mg/day)[1]	% contribution of total anthocyanins[2] to daily total anthocyanins intake (from food diary)	% contribution of total anthocyanins[2] to daily total polyphenol intake[3]
D140	7.1	7.2	245.9	99.5	2.9
D004	21.7	105.5	409.0	20.6	5.3
S228	29.3	30.1	142.2	97.5	20.6
R079	39.6	47.8	128.7	82.9	30.7

[1]Estimated from food diary analysis, [2]Value obtained from blackcurrant concentrate intake, [3]Estimated from No Coffee or Tea (NoCorT) dataset which excluded the contribution of tea and coffee to total polyphenol intake.

3.6 Conclusions

The results from the Folin assay show that the total phenolic content is higher in fresh foods, such as citrus fruit and mustard seeds, compared to processed foods, such as fruit smoothies, mixed fruit juices, fruit concentrates and tomato based pasta sauces. Most of the processed samples were added with ascorbic acid purposely by the manufacturers. A specific analysis on anthocyanin intake was performed among high blackcurrant consumers and a higher value was derived from the food diary estimation as compared to updated results based on analysis by HPLC analysis. Therefore, there was a need for adjustment of estimated vitamin C and anthocyanin intake specifically consumers of blackcurrant concentrates. The new adjusted data are used for the next chapter on the association of polyphenol intake and cognitive performance (see Chapter 4). The current study was limited by only assessing the contribution of blackcurrant concentrates consumption to daily total anthocyanins and total polyphenol intake, but this highlights the need for further analysis on other processed foods (such as more in the list in Table 3-1) for a better estimation of the population's polyphenol intake in any population group.

Chapter 4
The Association between Habitual Polyphenol Intake and Cognitive Performance in Women

4.1 Abstract

In this study, the association between polyphenol intake estimated from food diary and cognitive performance assessed from several cognitive tests has been analysed. It was only results for spatial memory from VSLT test showed a significant difference between polyphenol consumption quartiles and beverage consumption groups. No other differences associated with polyphenol intake were found for other cognitive domains. In general, there is a trend for increases in test scores and decreased in mean reaction time as polyphenol intake decreased. Polyphenol intake was determined by age, by which coffee and tea drinking habits were prominent amongst the older participants. No generalization can be made about the polyphenol intake of UK women because this study participated by mainly younger adults (aged 20 to 30 years old). In summary, better performance on spatial memory was shown by non-consumers of coffee or tea and the very low polyphenol consumption quartiles, thus the hypothesis for this study is rejected. The minor contribution of coffee and tea as the two major polyphenol sources to cognitive performance can partly be explained by the age factor.

4.2 Introduction

Although there have been a number of randomised controlled intervention studies to examine the acute or medium term effects of polyphenols on cognitive function (see Chapter 1 – Section 1.7.2.3), these do not tell us very much about the impact of regular consumption of polyphenols on cognitive function. Studies on the effects of polyphenol consumption on cognitive performance are usually acute or short-term interventions (less than 12 weeks) with the dose of polyphenol compared to a placebo treatment. For example intervention studies have included examination of the effects of cocoa flavanols (Scholey et al., 2010, Pase et al., 2013), resveratrol (Kennedy et al., 2010) and soy isoflavones (File et al., 2001, Islam et al., 2008). Some of these studies have reported improvements (Scholey et al., 2010, File et al., 2001, Islam et al., 2008) and some have shown no significant effect on cognitive performance following the intervention (Kennedy et al., 2010, Pase et al., 2013).

There have been limited epidemiological studies assessing intake of polyphenols and the associations with cognitive performance (see review in Section 1.7.2.2). These studies tend to consider whether there is evidence of less cognitive decline in individuals who have a higher intake of either total polyphenols, specific polyphenols, or certain polyphenol-rich foods (e.g. total fruit and vegetables; coffee and tea). Some of the studies report a lower risk of cognitive decline with high intake of polyphenol-containing foods (Letenneur et al., 2007, Kesse-Guyot et al., 2012). However, the positive finding from one study (Kesse-Guyot et al., 2012) was questionable because as baseline cognitive performance data was not available, this could not be taken into account in the analysis of subsequent cognitive performance of the participants. Longitudinal studies which involve elderly participants are open

to flaws of interpretation because as people age, the quality and reliability of the recording of food intake performed by this population may also be questionable due to declines in memory which can affect their ability to properly recall or record their food intake. Letenneur et al. (2007) has addressed this issue and has suggested that these flaws could be due to changes in dietary reporting or dietary habits of the elderly througout the study period.

There is still a lack of studies amongst non-elderly individuals which focus on the association between polyphenols consumed in the habitual diet and their cognitive performance. A study performed amongst nurses recruited at the age of 30 to 55 years old estimated flavonoid intake and measured cognitive performance at baseline and after 4 year follow-up (Devore et al., 2012). This study reported a slower cognitive decline amongst individuals with higher total flavonoid intake. The limitation of this study is that only flavonoids were estimated, while other polyphenol groups such as phenolic acids were not included. Therefore, the current study sought to consider the association with a larger range of polyphenol groups by performing a cross-sectional study among adult women with the majority aged 18 to 30 years. This study employed a battery comprising several cognitive tests which reflect specific cognitive domains. This approach enables identification of any possible association between the different cognitive domains and polyphenol intake which might shed light on the possible mechanisms of action of polyphenols on cognitive function.

Results from Chapter 2 indicated that coffee and tea are the major polyphenol food sources for the women studied in this investigation. To date, several studies have found an inverse association between the high consumption of these beverages and cognitive decline (van Gelder et al., 2007, Ng et al., 2008, Eskelinen

et al., 2009) (see Table 1-5). This chapter aims to examine whether total polyphenols, or polyphenols specifically from coffee and tea (as the major contributors to polyphenol intake), are associated with better cognition and in which cognitive domains. In addition, the results of cognitive tests were also compared between groups formed on the basis of their absolute polyphenol consumption.

4.2.1 Study hypothesis and objectives

The hypothesis of this study is individuals with higher polyphenol intake will have better cognitive performance as tested in several cognitive tests.

The objectives of this study are:

- To examine the relationship between polyphenol intake and cognitive performance and to determine for which cognitive domains relationships are present.
- To identify demographic variables associated with polyphenol consumption and cognitive performance of UK women.

4.3 Method

4.3.1 Participants

4.3.1.1 Recruitment

Personal emails provided in relation to a previous intervention study (Leeds Women's Wellbeing study) for which some of these women volunteered, were used for recruitment if the women had consented to be contacted about future studies. In addition, posters were placed at several locations around university and local area to promote participation from other women. Furthermore, emails were sent via university distribution lists to staff across the university. The study advertisements

asked for women interested in participating in a brief cognitive test battery and food intake assessment study.

Characteristics of the study participants have been described previously (see Chapter 2, Section 2.3.2). The inclusion criteria were that participants were pre-menopausal women aged 18 to 50, in general good health and with a body mass index (BMI) above 18.5 kg/m^2. The study was conducted in the Human Appetite Research Unit (HARU) in the Institute of Psychological Sciences, University of Leeds. The exclusion criteria for the study were smokers, being pregnant or lactating and experiencing or having passed menopause. In total, 103 participants participated in the study.

4.3.2 Study design

This study employed a cross sectional design in healthy adult females in which the extent to which performance on cognitive functions across range of domains could be predicted by habitual polyphenol intake measured using a 3 day food diary (see Chapter 2) was assessed. The primary end point for this study is the polyphenol intake estimated from 3 day food diary and cognitive performance assessed by several cognitive tests.

4.3.3 Ethical Approval

Ethical approval was obtained from the Institute of Psychological Sciences Research Ethics Committee, University of Leeds (Ref No: 12-0020, see Appendix 1). Information sheets were emailed to all participants to provide details of the study (see Appendix 2) and a screening visit to the HARU provided potential participants with an opportunity to ask questions about the study procedure and what they were

requested to do if they agreed to participate in the study. Participants were asked to provide their informed consent form before the commencement of the study (see Appendix 3). Upon completion of the study visit, participants received a £10 Love to Shop Voucher to thank them for the time they invested in the study.

4.3.4 Measures

4.3.4.1 Assessment of dietary intake

The Dietary Instrument for Nutrition Education questionnaire (DINE; see Appendix 5) (Roe et al., 1994) was used to assess participants' dietary fibre intake in their usual diet which was one criterion for recruitment into the Leeds Women's Wellbeing study (see Chapter 2). This questionnaire lists 24 foods or food groups which have been pre-scored according to the relative amount of fibre contained in an average portion of each of the foods on the questionnaire. The frequency of intake reported by the participants were weighted and summed to give a score which reflected low, medium or high fibre intake.

The adapted Leeds Women's Wellbeing DINE (LWW-DINE; see Appendix 6) was used to evaluate participants' fibre intake in grams according to the same foods as listed in the original DINE. Three major food groups were assessed in the questionnaire i.e. breads and rolls, breakfast cereals and fruits and vegetables. The fibre intake in grams was calculated according to the frequency, portion size and the fibre content of the food consumed.

Participants' food intake was estimated from 3 day food diaries, in which they were asked to record all food consumed for 2 weekdays and 1 weekend day. Data from the food diary were analysed using WinDiets® software for the

macronutrient and micronutrient intake. Further descriptions on the food intake analysis and the estimation of polyphenol intake from established databases are provided in Chapter 2 (see Section 2.3).

Food analysis using HPLC was performed on several blackcurrant concentrates because the drink was frequently consumed by the studied participants. Adjustment for the polyphenol content of blackcurrant concentrates reported in the food diaries, was made according to the results of HPLC (see Chapter 3, Section 3.3.2). Intake data for anthocyanins and vitamin C from blackcurrant concentrate consumed and reported in the food diaries were corrected according to the HPLC-adjusted content (see

Table 4-1). No significant difference was found in anthocyanin intake after correction [z = -0.82, ns]. However, vitamin C intake was significantly different after applying the correction to the intake data [z = -7.32, p <0.01)]. The updated data was used for the statistical analysis presented in this chapter.

Table 4-1 Comparison of daily vitamin C and anthocyanin intake before and after correction using HPLC

Compounds	Before correction - HPLC data	After correction - HPLC data
Vitamin C[1]	82.2 (61.5)	84.9 (65.1)
Anthocyanins	18.1 (34.0)	18.6 (33.8)

*Data presented as mean (standard deviation) in mg/day, [1]p<0.01 Wilcoxon signed-ranks test.

4.3.4.2 Estimation of intelligence

The following measures were used to estimate the intelligence quotient (IQ) of the participants:

(i) National Adult Reading test (NART)

The National Adult Reading test (Nelson, 1982) is a test to estimate premorbid general intelligence. The NART is simple test to administer and to perform. The person taking the test was asked to look at and pronounce, aloud, 50 words. None of the words fully follows the usual rules of English grapheme-phoneme correspondence (e.g., gaoled, heir, facade). Participants' responses were recorded on audiotape to permit verification of scoring.

(ii) Wechsler Adult Intelligence Scale (WAIS)

The Wechsler Adult Intelligence Scale (WAIS) (Wechsler, 1981) is a measure of general cognitive ability. In this study, two subscales of WAIS were used, namely vocabulary and matrix reasoning. This short version was selected because it can be administered relatively rapid and efficiently. In the vocabulary subscale, the participants were asked to define a series of orally and visually presented words. In the matrix reasoning subscale, participants were presented with a series of incomplete grid picture sequences and then were asked to indicate which pictures were required to complete the sequences from five possible responses. Vocabulary and matrix reasoning subtests were categorized as verbal and visual performance subscales respectively in the scoring. This test took 15 to 30 minutes to complete. Participants' responses were recorded verbatim to permit verification of

scoring. Finally, the raw scores were converted into scaled scores according to participants' age groups as described in the WAIS manual (Wechsler, 1999).

4.3.4.3 Cognitive performance

Table 4-2 shows the cognitive battery and the associated cognitive domains according to the order of test administration. The battery took approximately 40 minutes to administer. Computerised versions of Visual Verbal Learning Test (VVLT), Corsi, Tower of Hanoi and VVLT Recognition Test were performed using E-Prime® software. The Visual Spatial Learning Test (VSLT) was administered in a paper format. For two of the tests namely the VSLT and VVLT, immediate and delayed versions were administered in this study.

Table 4-2 Summary of cognitive tests used in this study, the associated cognitive domain and the test duration

Cognitive test	Cognitive domain	Test duration (minutes)
1. Visual Spatial Learning Test (VSLT)	Spatial memory	6
2. Visual Verbal Learning Test (VVLT)	Verbal memory	12
3. Corsi Block Tapping Test (Computerised version)	Spatial working memory	4
4. Tower of Hanoi (TOH)	Problem solving (executive function)	5
5. Delayed Visual Spatial Learning Test	Spatial memory	3
6. Delayed Visual Verbal Learning Test	Delayed verbal memory	3
7. VVLT Recognition Test	Delayed auditory and verbal memory	3
Total time for testing		36

(a) Visual Verbal Learning Test (VVLT)

(i) Immediate

The VVLT is a visual analogue of the Rey Auditory-Verbal Learning Test (RAVLT) (Rey, 1964) which measures verbal learning and memory. Figure 4-1

shows a screenshot of a stimulus word to be remembered. Participants were presented with three trials of 16 words (List A) in a random sequence in the middle of a computer screen at the rate of one word every 2 seconds. An interference word list (List B) was presented after the three trials of list A (Trial A1, A2 and A3). At the end of each trial, participants were instructed to verbally recall as many of the words as possible in 60 seconds using a digital voice recorder. The outcome variable was the number of words correctly recalled.

Figure 4-1 Screenshot of VVLT

(ii) Delayed

In the delayed version of the VVLT, participants were asked to verbally recall as many words as possible from List A, approximately 30 minutes prior. There was a time limit of one minute for the recall. The outcome variable was the number of words correctly recalled.

(iii) VVLT Recognition

This test consisted of stimuli with a total of 48 words: 32 words (16 from List A and 16 from List B) from the initial VVLT presentation, and a further 16 new distractor words (List C). The words were presented in a random order visually or

aurally via the computer. Words presented visually remained on the screen for 3 seconds. Participants were required to respond to the stimuli by pressing the 1, 2, 3 keys on the computer number keypad, corresponding to word lists A, B, or C. Outcome variables of this test were number correct and reaction time for correct responses only.

(b) Visual Spatial Learning Test (VSLT)

(i) Immediate

The Visual Spatial Learning Test (VSLT) (Malec et al., 1991) is a test of visuospatial memory and learning. The test comprises a 6 x 4 grid and 15 different nonsense black and white designs that are very difficult to verbalise. Participants were shown all 15 designs and the empty grid. Thereafter, participants were shown seven of the designs which were placed on the grid by the researcher. They were required to remember the designs and their positions and were permitted to study these on the grid for 10 seconds after which they were removed by the researcher. The participants were required to select the seven target designs from amongst the original 15 and place them in the correct position on the grid. The process was repeated twice with the same designs and positions. Therefore each test administration involved three identical trials. Figure 4-2 shows the 15 designs and empty grid board used for this test.

There were three outcome variables; (i) number of correctly identified target designs per trial (maximum 7), (ii) number of correctly identified target positions per trial (maximum 7) (iii) number of correct targets designs placed in the correct positions per trial (maximum 7).

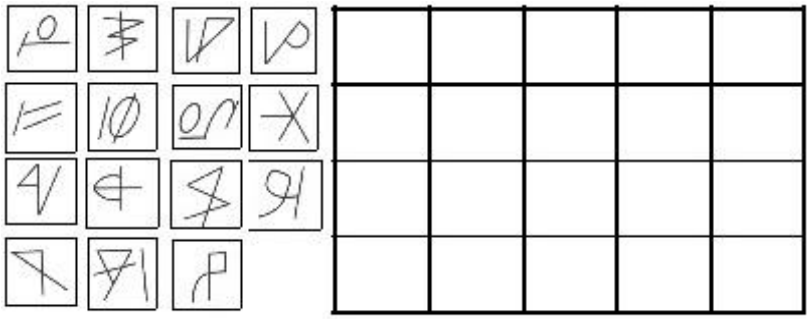


Figure 4-2 VSLT pattern cards and empty grid board

(ii) Delayed

In the delayed version of the VSLT, participants were asked to select the seven target designs and place them in the correct position on the grid according to the designs and positions shown during the immediate recall approximately 25 minutes prior. Participants were given 10 seconds to complete the task.

(c) Corsi Block Tapping Test (computerised version)

The Corsi Block Tapping Test (Corsi, 1972, Milner, 1971) is a test to examine spatial memory. Nine white blocks were presented on the screen. Figure 4-3 shows a screenshot of the Corsi test layout. During the test, the blocks changed colour from white to red and then back to white again at a speed of one second per block. The number of blocks that changed colour in any one sequences ranged from two to nine. Participants were asked to remember the order in which the white blocks turn to red. They were then asked to click on the blocks in the order in which the blocks turned red and were told to do this as quickly as possible. Outcome variables were the number of sequences correctly recalled, and accuracy and reaction time for each box selected in the sequence.

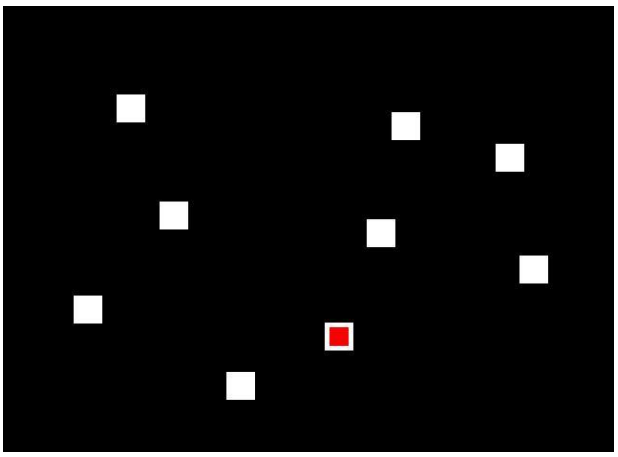


Figure 4-3 Screenshot of Corsi

(d) Tower of Hanoi (TOH)

The Tower of Hanoi (Simon, 1975) is a test of planning ability. Figure 4-4 shows an example of a screenshot for TOH. The test consisted of a visual representation of three rods upon which four discs of different size and colour were placed. The number of the target formation of discs on the rods was shown on top of the starting formation. The participant's task was to move the disk around the starting formation rods to match the pre-determined target formation in the fewest possible moves. There was only one correct sequence of moves for each trial and this was the fewest number of moves required to match the target formation. If the participants made any wrong moves, the screen showed a message saying "this was not the correct sequence please try again", and subsequently the screen refreshed to the original starting formation. Participants were forced to apply the fewest number of moves, which should encourage problem solving rather than guessing. There was one rule for this test: a disk could not be placed on a disk which was larger than itself, such a move was not recorded as an error as the computer would not allow it. A disk was moved by clicking on the disk and then clicking on the desired

rod, therefore dragging was not required. The number of moves required to complete a trial was shown on the screen which was updated after each move, hence the participant was not required to retain in working memory of the number of moves they had made. There was no time limit and therefore each trial had to be completed before the test concluded. There were ten trials per test administration, consisting of two trials for each of the five levels of 4,5,6,7 or 8 moves. There were two outcome variables: (i) the number of errors made, and (ii) the time to complete each trial (i.e. time taken to solve the problem).

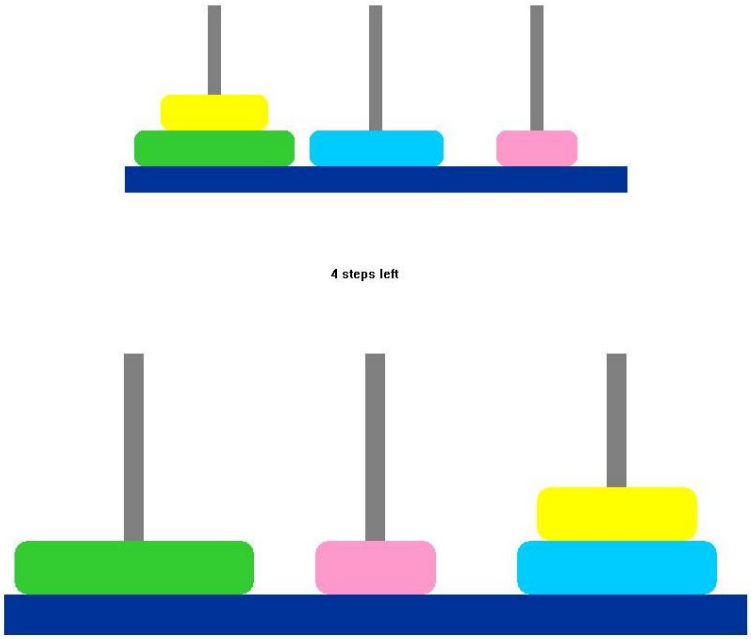


Figure 4-4 Screenshot

4.3.5 Procedure

The study followed a standard operating procedure which comprised two visits to the HARU. The flow of the visits is shown in Figure 4-5 below. Participants who expressed an interest in the study were provided with the participant information sheet (PIS) and were asked to read this at their leisure. If, after reading the PIS, they

were interested in participating in the study, an arrangement was made for their first visit. After providing their informed consent, participants' height was measured using a free standing height measuring unit (Seca, Leicester Height Measure, Birmingham, Ltd) with participants barefoot. Body weight was measured without shoes on a calibrated electronic weighing scale to the nearest 0.1 kg (MSP200P, Adam Equipment Co. Ltd.). Body mass index was calculated from the weight and height measures. The first visit also included a practise session of the cognitive test battery in order to minimise practise or potential learning effects. The participants were given a 3 day food diary which the researcher explained how to complete and asked them to return this during the second visit.

WAIS and NART were administered during the second visit. Thereafter, the cognitive test battery was administered with the order of tests completed as shown in Table 4-2 above. Participants were then asked to complete the cognitive test evaluation questionnaire. The participants' habitual polyphenol food intake was estimated from a 3 day food diary which required them to list their entire food and beverage intake for 2 weekdays and one weekend day. This was returned to the researcher when the participant attended for the test day visit. Details regarding the methods used to estimate polyphenol intake from the food diary were described in Chapter 2 (see Section 2.3.5).

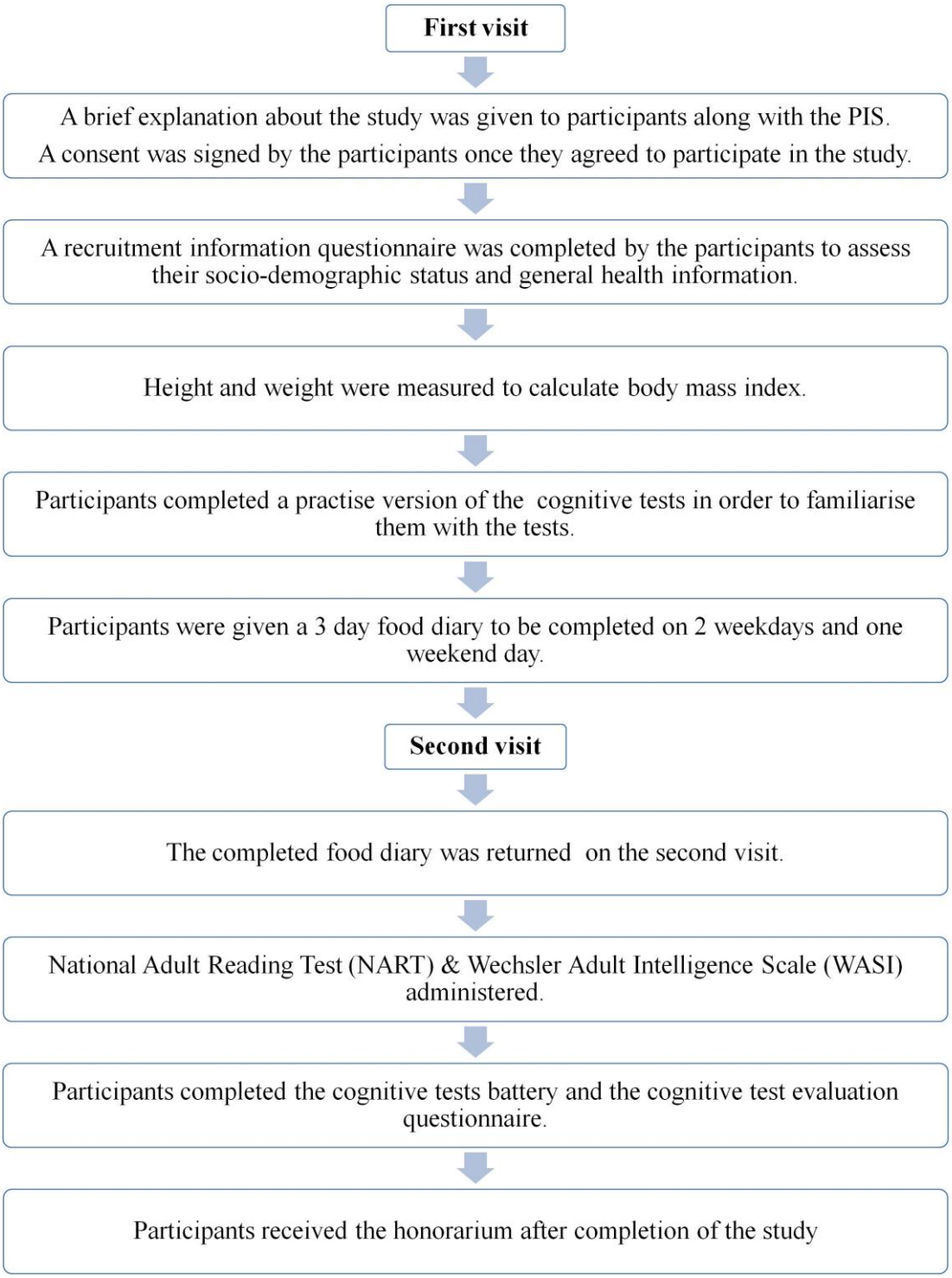


Figure 4-5 Flow chart of study visits

4.3.6 Statistical analysis

Food diary data (from WinDiets®, see Chapter 2), scored questionnaires (DINE and LWW-DINE), socio-demographic data, VVLT and VSLT scores and other outcome variables from computerized cognitive tests administered using E-Prime® software were transferred into Microsoft Excel. All data were analysed using Statistical Package for Social Science (SPSS, version 20). These data were examined for outliers and assumptions checked for each inferential analysis by using boxplots.

The polyphenol data was used to group participants in two ways; (1) according to type of beverage consumed such that participants were grouped according to their consumption of polyphenol containing beverages (coffee consumers, tea consumers, consumers of both coffee and tea, and non-consumers of coffee or tea) and (2) according to the percentile of total polyphenol intake into which each participant fell. Four groups were formed – up to the 25th percentile (very low consumers), from the 26th to the 50th percentile (low consumers), from the 51st to the 75th percentile (moderate consumers) and above the 76th percentile (high consumers). Demographic, food intake data, questionnaires and cognitive data for each of the polyphenol consumption quartiles formed using both of the approaches above were compared.

The four polyphenol intake groups formed on the basis of beverage consumption or intake in milligram (mg) were compared for each cognitive dependent variable using either ANOVA with group as a between subjects factor and trial as a within subjects factor where appropriate. One way between groups ANOVA or the non-parametric Kruskall-Wallis when the data were not normally distributed was employed to examine differences in demographic and questionnaire data.

Bonferroni corrected post hoc tests were performed to identify differences between each of the beverage consumption groups or intake groups when ANOVA was used. Whenever Kruskall-Wallis test was used for data which was not normally distributed, Mann-Whitney U tests were performed to identify which groups were statistically different from each other (Pallant, 2007). Correlations between variables were determined using either Pearson's Product Moment correlation coefficients for parametric, interval data or Spearman's Rank correlation coefficient for non-parametric ordinal data.

In this study, cognitive performance outcome measures (e.g. number of correct responses, reaction times, number of errors) from the various cognitive tests administered are considered the dependent or outcome variables. Multiple linear regression analysis was conducted to predict cognitive outcomes according to socio-demographic factors and absolute polyphenol intake of the studied samples. In all analyses p values of <0.05 or <0.01 were considered statistically significant.

4.4 Results

The results are presented in three sections including (a) characteristics of the whole sample, (b) comparisons according to polyphenol consumption quartiles for all measures (see Section 4.3.4), and (c) comparisons according to beverage consumption groups for all measures. Finally, results of multiple linear regressions to predict cognitive performance from polyphenol intake of the whole sample, controlling for various demographic characteristics is presented.

(a) Characteristics of the whole sample

The participant characteristics for the whole sample (N = 103) are shown in Table 2-5 (see Section 2.4.1). Overall, participants in this study were young adults with mean age of 25 ± 9 years old and a normal BMI (24.5 ± 4.6 kg/m^2). Fibre consumption assessed by LWW-DINE showed that the participants' average fibre intake in g was about 15 g, which is below the RNI of 24g/day. The score obtained from the DINE measure of dietary fibre intake classified the study group as medium fibre consumers (see Table 2-6, Section 2.4.1). The result from DINE might overestimate the fibre intake and might not indicate the actual intake of fibre. The majority of the participants were students (65 %) and a high percentage of participants (77.7 %) reported that they practise regular exercise indicated by answering yes in the recruitment information questionnaire (RIQ) (See Appendix 4). In addition, the majority of the participants (87.4 %) reported that they were regular breakfast consumers whereby they were asked if they usually consume breakfast in the RIQ. Table 4-3 displays the distribution of participants according to beverage consumption groups. It is clear that the distribution is dominated by tea consumers (41.7 %) followed by non-consumers of coffee or tea (28.2 %), consumers of both coffee and tea (19.4 %) with the smallest proportion of participants being coffee consumers only (10.7 %).

Table 4-3 Distribution of participants according to consumption of tea or coffee and daily polyphenol intake

Beverage consumption	Number (%)
Coffee consumers	11 (10.7)
Tea consumers	43 (41.7)
Consume both coffee and tea	20 (19.4)
Non-consumers of coffee or tea	29 (28.2)

(b) Comparison according to polyphenol consumption quartiles

The intake of polyphenols for each quartile of the sample is shown in Table 4-4. The average intake of polyphenols ranged from 150 mg/day in the very low (bottom quartile) polyphenol consumers to 1700 mg/day in the high polyphenol (uppermost quartile) consumers. There was a very large variation in polyphenol intake particularly amongst the high polyphenol consumers.

Table 4-4 Mean (s.d) daily polyphenol intake of the participants according to quartile (mg/day)

Polyphenol consumption quartiles	Mean (s.d) (mg/day)	Minimum (mg/day)	Maximum (mg/day)
Lowest quartile (<25%) (N=25)	152.2 (58.6)	43.2	242.5
Low quartile (26-50%) (N=26)	388.7 (115.0)	242.7	594.6
Moderate quartile (51-75%) (N=26)	891.1 (173.1)	629.0	1178.8
Highest quartile (>75%) (N=26)	1772.1 (500.5)	1206.5	3000

4.4.1 Participant characteristics according to the polyphenol consumption quartiles

Table 4-5 displays the demographic characteristics of the participants according to the polyphenol consumption quartiles. A Kruskall-Wallis test showed a statistically significant difference across polyphenol consumption quartiles in age [H (3) = 34.6; $p<0.01$)], body mass index [H (3) = 10.3; $p<0.05$)] and basal metabolic rate [H (3) = 8.50; $p<0.05$)]. There was a significant difference in employment status between the polyphenol consumption quartiles [H (9) = 41.9; $p<0.01$)] with the majority (68 %) of high polyphenol consumers employed whilst most of the participants with polyphenol intake less than the 50^{th} percentile were students.

There were no significant differences between polyphenol consumption quartiles in relation to frequency of regular exercise, units of alcohol consumed per week, being vegetarian or regular breakfast consumption. Finally, there were significant differences between the polyphenol consumption quartiles in terms of the amount (in ml) of coffee (χ^2 = 22.1; df = 3; $p<0.01$) and tea (χ^2 = 52.7; df = 3; $p<0.01$) consumed daily. Beverages consumed increased in a linear fashion from low to high polyphenol consumption.

Table 4-5 Participant characteristics according to quartiles of daily polyphenol consumption

	Q1: Very low consumers (N=25)	Q2: Low consumers (N=26)	Q3: Moderate consumers (N=26)	Q4: High consumers (N=26)	P value
Age (years)	21.0 (5.7)	22.1 (6.0))	22.5 (6.1)	34.2 (10.5)	<0.01˜
Body mass index (kg/m^2)	23.6 (3.1)	24.4 (3.8)	22.7 (3.2)	27.4 (6.2)	<0.05˜
Basal metabolic rate, BMR (kcal)^	1417 (136)	1456 (168)	1369 (111)	1493 (167)	<0.05˜
Ratio energy intake to BMR	1.19 (0.32)	1.17 (0.33)	1.27 (0.27)	1.19 (0.31)	0.586
Category of employment:					<0.01$^{\zeta}$
Employed (%)	4.0	8.0	20.0	68.0	
Student (%)	34.3	31.3	25.4	9.0	
Doing regular exercise (yes) (%)	26.2	23.8	23.8	26.2	ns
Units of alcohol consumed per week (unit)^	5.7 (4.5)	4.0 (4.3)	6.7 (9.1)	3.9 (4.9)	ns
Volume of beverages consumed	coffee: 2.6 (12.9)	coffee: 23.0 (50.5)	coffee: 92.3 (140.5)	coffee: 227.0 (323.6)	<0.01˜
(ml/day)	tea: 6.6 (22.7)	tea: 103.0 (120.5)	tea: 291.1 (176.1)	tea: 608.4 (436.8)	<0.01˜
Vegetarian (yes) (%)	15.4	30.8	30.8	23.1	ns
Regular breakfast consumer (yes) (%)	24.4	22.2	25.6	27.8	ns

*Data presented as mean (standard deviation), ^BMR: Basal metabolic rate was calculated using the Schofield prediction equations, (%): percentage, ˜Kruskall-Wallis test, $^{\zeta}$ p<0.01 Chi-square ($\chi^{2)}$ test, ns: not significant

4.4.2 Estimation of intelligence across polyphenol consumption quartiles

There was a significant difference between polyphenol consumption quartiles in NART score [F (3, 99) = 7.92, $p<0.01$] (see Figure 4-6). Bonferroni corrected post hoc tests showed significantly lower NART scores were found in the very low polyphenol consumers than in the low polyphenol consumers ($p<0.05$), moderate polyphenol consumers ($p<0.05$) and high polyphenol consumers ($p<0.01$) such that very low polyphenol consumers scored significantly lower than all other three quartiles.

A significant difference between polyphenol consumption quartiles was found in the WAIS vocabulary subtest [F (3, 99) = 4.38, $p<0.01$] (see Figure 4-6). These differences were found between very low polyphenol consumers and two other quartiles namely moderate polyphenol consumers ($p<0.05$) and high polyphenol consumers ($p<0.01$) WAIS. Very low polyphenol consumers scored significantly lower than the other two quartiles. No significant difference between the polyphenol consumption quartiles was found in WAIS matrix reasoning subscale [F (3, 99) = 2.42, ns].

The WAIS total score represents the sum of scores from the vocabulary and matrix reasoning subtests. The whole sample scored above 100 for the WAIS total score, indicating that the sample as a whole was generally of above average IQ. There was a significant difference between polyphenol consumption quartiles for the WAIS total score [F (3, 99) = 6.07, $p<0.001$]. Bonferroni corrected post hoc tests showed significantly difference in WAIS total score between very low polyphenol consumers with two other quartiles namely moderate polyphenol consumers

($p<0.05$) and high polyphenol consumers ($p<0.001$). Very low polyphenol consumers scored significantly lower than the other two quartiles.

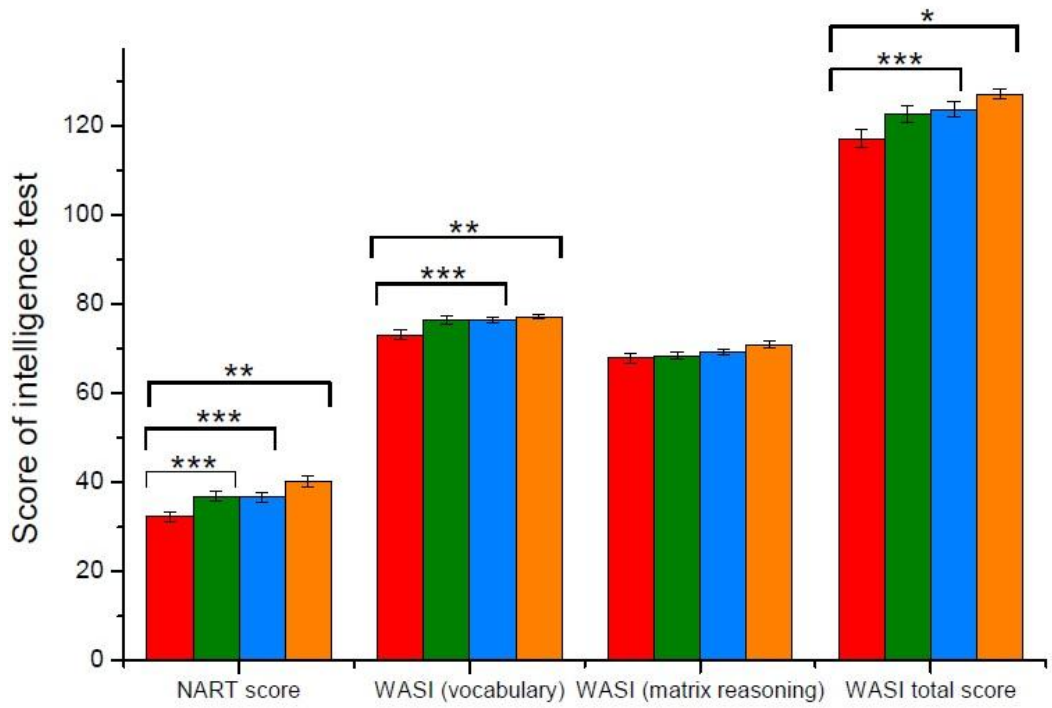


Figure 4-6 Score for intelligence tests according to polyphenol consumption quartiles [(■) Q1: very low consumers, (■) Q2: low consumers, (■) Q3: moderate consumers, (■) Q4: high consumers] (mean ± SE)

4.4.3 Daily nutrient intake across the polyphenol consumption quartiles

Table 4-6 represents the daily intake of major nutrients according to the polyphenol consumption quartiles. The daily energy intakes of all the groups were lower than the recommended intake (1940 kcal) (Department of Health, 1991). Protein and vitamin C intake exceeded their respective RNI levels. There were significant differences in alcohol intake between the groups [$H(3) = 9.47$; $p<0.05$). Mann-Whitney test showed a significant difference between low and high

polyphenol consumers ($p<0.01$) in the alcohol intake with higher intake in high polyphenol consumers. Vitamin C intake also showed significant differences between groups [$H (3) = 13.43$; $p<0.01$) with the intake of low and moderate polyphenol consumers higher than the high polyphenol consumers. Further Mann-Whitney test showed that there were significant differences in Vitamin C intake between very low and low ($p<0.001$), very low and moderate ($p<0.01$) and very low and high ($p<0.05$) polyphenol consumers. Very low polyphenol consumers had lower vitamin C intake than the other three groups. Carbohydrate and dietary fibre are the nutrients which came closest to fulfilling the RNI recommendations.

There were significant differences between polyphenol consumption quartiles in fibre intake assessed from DINE [$H (3) = 18.01$; $p<0.01$] and LWW DINE [$H (3) = 10.37$; $p<0.05$). Further Mann-Whitney test showed that there were significant differences in DINE between high polyphenol consumers and the three other quartiles namely very low ($p<0.01$), low ($p<0.01$) and medium ($p<0.05$) polyphenol consumers. High polyphenol consumers obtained the highest DINE score of all groups. Fibre intake assessed from LWW DINE showed significant differences between high polyphenol consumers and the very low ($p<0.01$) and low ($p<0.05$) polyphenol consumers, with high polyphenol consumers having the highest fibre intake according to this measure.

Table 4-6 Daily nutrient intake and fibre intake classification in each quartile of polyphenol consumption and percentage of RNI achieved in each group*

	Q1: Very low consumers (N=25)	^% RNI	Q2: Low consumers (N=26)	% RNI	Q3: Moderate consumers (N=26)	% RNI	Q4: High consumers (N=26)	% RNI
Energy (kcal)	1666 (437)	85.9	1700 (480)	87.6	1738 (376)	89.6	1769 (439)	91.2
Protein (g)	66.5 (19.0)	147.8	65.0 (23.8)	144.4	70.0 (22.5)	155.6	72.1 (21.1)	160.2
Carbohydrate (g) ˜	216.7 (68.5)	48.8	225.6 (73.8)	49.8	221.0 (51.9)	47.7	212.7 (47.3)	45.1
Fat (g)§	60.7 (21.3)	32.8	61.5 (22.5)	32.6	61.8 (21.1)	32.0	69.3 (21.5)	35.3
Alcohol (g) ς,2,3	3.0 (6.3)	1.6	2.8 (11.5)	1.5	8.0 (13.7)	4.1	7.9 (14.0)	4.0
Dietary fibre (g)	17.7 (5.3)	73.8	20.6 (8.7)	85.8	21.1 (8.6)	87.9	19.6 (5.7)	81.7
Vitamin C (mg)[1,3,4]	45.5 (28.2)	113.8	99.3 (73.4)	248.3	100.0 (74.1)	250.0	79.3 (57.9)	198.3
LWW DINE (g)[2,3,4]	13.1 (4.2)	-	14.4 (6.6)	-	15.6 (5.6)	-	18.4 (6.1)	-
DINE DF score[1,3,4]	26.4 (8.1)	-	30.9 (13.4)	-	31.2 (10.5)	-	37.3 (8.2)	-

*Data presented as mean (standard deviation), [1]$p<0.01$, [2]$p<0.05$ Kruskall-Wallis test, [3]$p<0.01$, [4]$p<0.05$ Mann-Whitney test, ^% RNI: Percentage from the Reference Nutrient Intake, ˜recommended at 50 % of food energy, §recommended at 35 % of food energy, ςshould be less than 5% of total energy, DINE: Dietary Instrument for Nutrition Education for dietary fibre (DF), LWW DINE: Leeds Women Well-being DINE.

4.4.4 Daily polyphenol intake across the polyphenol consumption quartiles

All the polyphenol classes in the Table 4-7 follow a linear pattern increasing with each quartile group such that not only does total polyphenol intake increase across the groups which we would expect because the groups were formed on this basis but also this is due to a linear increase across the groups of each major polyphenol class. The participants were split into four groups according to their total daily polyphenol consumption and as expected the quartile groups formed differed in terms of total polyphenol consumption in a linear manner (see 4.3.6) [H (3) = 95.63; df = 3; $p<0.01$]. Nevertheless, it is possible that there are differences in consumption of specific polyphenols which do not follow this linear pattern on which the groups were based and hence it may be illuminating to examine differences between the groups in terms of specific polyphenols.

There was a significant difference in polyphenol intake of each quartile for two of the major polyphenol groups. A Kruskall-Wallis test revealed a significant difference between the polyphenol consumption quartiles in daily intake of flavonoids [H (3) = 60.37; $p<0.01$) and phenolic acids [H (3) = 66.38; $p<0.01$]. Mann-Whitney tests showed that the lowest quartile group had significantly lower intake of flavonoids and, phenolic acids than the other polyphenol consumption quartiles. No significant difference was found in terms of intake of all other polyphenols [H (3) = 6.07; ns].

Table 4-7 Polyphenol intake of major polyphenol groups (mg per day)*

	Q1: Very low consumers (N=25)	Q2: Low consumers (N=26)	Q3: Moderate consumers (N=26)	Q4: High consumers (N=26)	p value
Flavonoids[1]	65.1 (51.6)	250.4 (138.7)	541.4 (260.1)	1077.2 (659.4)	<0.01
Phenolic acids[1]	58.3 (38.4)	103.9 (98.6)	307.0 (276.8)	648.8 (627.4)	<0.01
All other polyphenols	28.8 (31.6)	34.4 (44.7)	42.7 (49.0)	46.2 (33.7)	0.108
Total polyphenols[1]	152.2 (58.6)	388.7 (115.0)	891.1 (173.1)	1772.1 (500.5)	<0.01

*Data presented as mean (standard deviation), [1]p<0.01 Kruskall-Wallis test.

4.4.5 Cognitive performance according to polyphenol consumption quartiles

4.4.5.1 Visual Verbal Learning Test

(i) Rate of learning (word List A)

Rate of learning over the three trials presented for word List A is shown in Figure 4-7. For this analysis 'Trial' was added as an additional within subjects factor with 3 levels (Trials A1, A2, and A3, see Section 4.2.4.3 a). Therefore, a 3x4 (Trial* polyphenol consumption quartiles) ANOVA was calculated. Age, NART score, and WAIS total score were added as separate covariates due to significant intercorrelations between these characteristics (see Appendix 13).

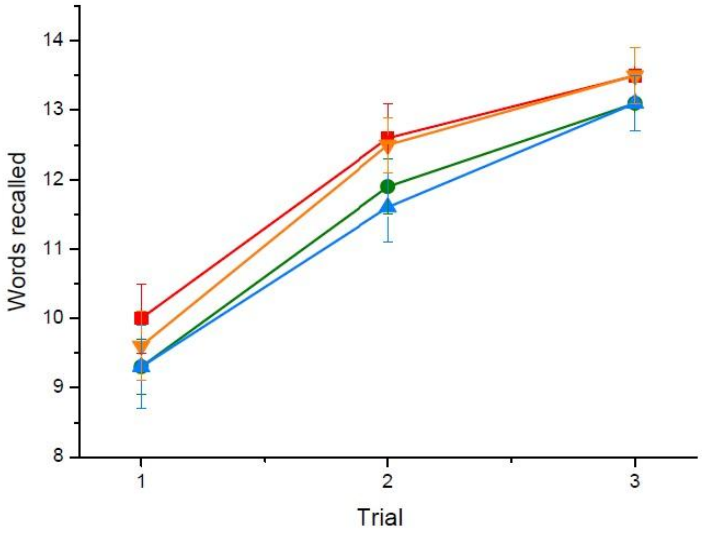


Figure 4-7 Rate of learning according to polyphenol consumption quartiles [(■) Q1: very low consumers, (●) Q2: low consumers, (▲) Q3: moderate consumers, Q4: (▼) high consumers] (mean ± SE)

Rate of learning increased significantly from Trial 1 to Trial 3 such that the main effect of trial was significant [F (2, 198) = 192.63, p<0.001]. The Trial*polyphenol consumption quartiles interaction showed no difference in the number of words recalled at each trial according to polyphenol consumption quartiles [F (6, 198) = 0.33, ns] suggesting that the groups did not differ in their memory capacity or rate of learning. An interaction between Trial and the covariates was only found for age [F (2, 196) = 20.60, p<0.001], while other covariates showed no significant interactions with Trial [largest F (1, 98) = 1.73, ns)] or polyphenol consumption quartiles [largest F (3, 98) = 1.21, ns)] (see Appendix 13).

(ii) New learning (recall of List B)

No significant difference was found between polyphenol consumption quartiles in the new learning which implies the recall of words from the interference list (List B) [F (3, 99) = 0.83, ns]. None of the covariates were significant [largest F

(1, 95) = 3.32, ns)] and none had significant interactions with polyphenol consumption quartiles [largest F (3, 95) = 2.25, ns)] (see Appendix 13).

(iii) Retroactive interference (Trial A3-A4)

Retroactive interference refers to the effect of new learning on the recall of old previously learned material. Retroactive interference is calculated by subtracting the number of words recalled on Trial A3 from those recalled on Trial A4. The examination of the effect of the interference is possible because the new word list (List B) was presented after Trial A3. If the value from subtraction is less than '0', there is an increment in the number of words recalled from List A, thus show that List B has not interfere the recall of List A. On the other hand, value bigger than '0' indicated the reduction in number of words recalled from List A and finally, no changes in number of words recalled is shown through the '0' value.

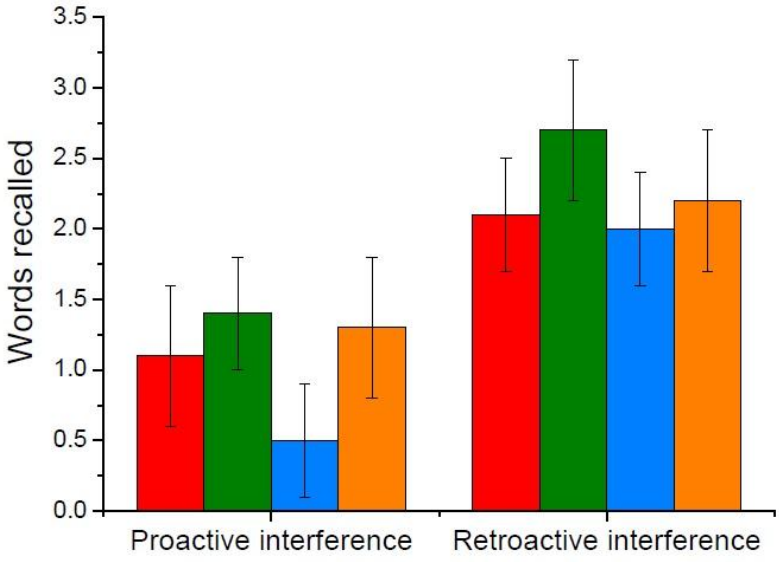


Figure 4-8 Number of interference according to polyphenol consumption quartiles [(■) Q1: very low consumers, (■) Q2: low consumers, (■) Q3: moderate consumers, (■) Q4: high consumers] (mean ± SE)

No significant difference in retroactive interference was found between the polyphenol consumption quartiles [F (3, 99) = 0.55, ns] (see Figure 4-8 above). There was an interaction between polyphenol consumption quartiles and age [F (3, 95) = 5.53, $p<0.01$] (see Appendix 15). No clear pattern is apparent from the figure because of the uneven distribution of participants in terms of age within polyphenol consumption quartiles. However, from the figure a suggestion can be made that List B has interfered with the recall of words from List A in all quartiles since recall is lower than that for list A (refer to figure for rate of learning). None of the covariates interacted significantly with Trial [largest F (1, 95) = 1.55, ns)] (see Appendix 13).

(iv) Proactive interference (Trial A1-B1)

Proactive interference occurs when the remembrance of previously learned material hinders the ability to learn new information. The interference was calculated as the difference between recall of Trial A1 (i.e. the first time List A was shown) and recall of List B (interference list, which was shown only once after Trial A3). List A has proactively interfered the ability to learn new material if the recall of List B is poorer than that of Trial A1. A greater degree of proactive interference indicates poorer learning of new material. No significant difference in proactive interference was found between the polyphenol consumption quartiles [F (3, 99) = 0.73, ns] (see Figure 4-8 above). None of the covariates were significant [largest F (1, 95) = 0.53, ns)] and none had significant interactions with polyphenol consumption quartiles [largest F (3, 95) = 2.49, ns)] (see Appendix 13).

(v) Delayed recall

Delayed recall is the delayed recall of List A about 30 minutes after the test was first administered. No significant difference was found between the polyphenol consumption quartiles [F (3, 99) = 0.93, ns]. None of the covariates were significant [largest F (1, 95) = 3.29, ns)] and none had significant interactions with polyphenol consumption quartiles [largest F (3, 95) = 2.44, ns)] (see Appendix 13).

(vi) Word recognition accuracy

In the recognition test, participants were asked to recognise which words originated from List A and which were from B shown in VVLT. List C consisted of new words which had not been seen previously and which were presented in this test as a distractor list. In one analysis, 'Word list' was the within subjects factor with three different word lists (List A, B and C) and in a further analysis modes of presentation was included as a within subject factor with two modes of word presentation (visual and aural). Therefore, a 3x4 (Word list*polyphenol consumption quartiles) and a 2x4 (Modes of presentation*polyphenol consumption quartiles) ANOVA was performed. Table 4-8 displays the number of correctly recognised words from different word lists and different method of word list presentations. There was a tendency for those in the lowest quartile of polyphenol intake to recognise fewer words.

Table 4-8 Number of words correctly recognised from three different word lists and different modes of presentation*

Word list	Very low consumers (N=25)	Low consumers (N=26)	Moderate consumers (N=26)	High consumers (N=26)
List A (seen 3 times)	11.6 (4.8)	12.0 (3.8)	12.4 (4.0)	12.5 (3.7)
List B (seen once)	9.8 (4.7)	11.2 (3.5)	11.0 (4.1)	11.3 (3.5)
List C (new list)	11.8 (5.1)	12.2 (3.5)	12.1 (4.0)	12.7 (3.7)
Words presented visually	16.9 (7.1)	18.1 (4.4)	17.7 (5.6)	18.6 (5.0)
Words presented aurally	16.3 (6.9)	17.4 (4.9)	17.7 (5.9)	17.8 (4.8)
Overall correctly identified words	33.2 (13.8)	35.5 (8.9)	35.4 (11.2)	36.4 (9.5)

*Data presented as mean (standard deviation)

There were significant main effects of Word List [$F(2, 198) = 13.36$, $p<0.001$] and Modes of presentation [$F(1, 99) = 4.81$, $p<0.05$]. Fewer words from List B and fewer words presented aurally were correctly recognised by the participants. There was no main effect of polyphenol consumption quartiles in the Word list [$F(6, 198) = 0.37$, ns] and Modes of presentation [$F(3, 99) = 0.44$, ns]. None of the covariates interacted significantly with Word list [largest $F(1, 98) = 2.37$, ns)] or polyphenol consumption quartiles [largest $F(3, 98) = 0.23$, ns)] in the 3x4 ANOVA analysis. WAIS total score was the only predictor for the accuracy in the number of words correctly recalled in different modes of presentation [$F(1, 95) = 4.51$, $p<0.05$] in 2x4 ANOVA analysis. Higher WAIS total score was associated with better performance in the number of words correctly recognised by the participants. Finally, none of the covariates were significantly interacted with polyphenol consumption quartiles [largest $F(3, 95) = 1.28$, ns)] for the 2x4 ANOVA analysis (see Appendix 13).

(vii) Word recognition reaction time

Table 4-9 displays the time taken (in milliseconds) to correctly recognise the words from the three different lists. For this analysis, 'Word list' and 'Modes of presentation' were included as two within subject factors in the analysis. Therefore, a 3x4 (Word list*polyphenol consumption quartiles) and a 2x4 (Modes of presentation*polyphenol consumption quartiles) ANOVA was performed.

Table 4-9 Mean reaction time to correctly recognise from three different word lists and different modes of presentations (millisecond)*

Word list	Very low consumers (N=25)	Low consumers (N=26)	Moderate consumers (N=26)	High consumers (N=26)
List A	1225.1 (487.4)	1387.0 (344.1)	1279.2 (413.5)	1388.9 (323.8)
List B	1318.3 (537.2)	1433.0 (328.4)	1368.2 (444.1)	1493.8 (336.9)
List C	1356.2 (535.4)	1519.6 (348.2)	1430.0 (471.6)	1546.0 (358.9)
Words presented visually	1049.0 (414.5)	1183.6 (290.0)	116.9 (372.7)	1221.8 (293.2)
Words presented aurally	1548.5 (594.0)	1709.4 (380.0)	1592.9 (491.6)	1726.5 (376.2)
Overall correctly identified words	1293.3 (497.5)	1439.1 (316.5)	1355.3 (425.9)	1469.2 (323.2)

*Data presented as mean (standard deviation)

There were significant main effects of Word List [$F(2, 198) = 23.70$, $p<0.001$] and Modes of presentation [$F(1, 99) = 607.45$, $p<0.001$]. A longer time was taken to recall correctly words from List C and words presented aurally. There was no significant interaction was found between Word List and polyphenol consumption quartiles [$F(6, 198) = 0.24$, ns]. None of the covariates interacted significantly with Word List [largest $F(1, 98) = 3.20$, ns)] or polyphenol consumption quartiles [largest $F(3, 98) = 2.47$, ns)] in the 3x4 ANOVA analysis (see

Appendix 13). A significant interaction between Modes of presentation and covariates was found for age [F (1, 98) = 45.69, p<0.001] and NART score [F (1, 98) = 10.57, p<0.001] in the 2x4 ANOVA analysis (see Appendix 16). Participants who are younger and who obtained higher NART scores showed better performance with less time taken to correctly recognise the words.

4.4.5.2 Visual Spatial Learning Test (VSLT)

VSLT immediate recall is the data obtained during the immediate recall of the test when all seven designs and positions were introduced to the participants over trials. For this analysis 'Trial' was added as an additional within subjects factor with 3 levels (Trials A1, A2, and A3, see Section 1.2.4.3.1). Therefore, a 3x4 (Trial* polyphenol consumption quartiles) ANOVA was calculated. Age, NART score, WAIS total score were added separately as covariates.

(i) Correctly identified designs (immediate)

The main effect of trial was significant [F (2, 98) = 76.78; p<0.001] as shown in Figure 4-9 (a) and reflects the increase in correctly identified designs with each successive trial. There is no main effect of polyphenol consumption quartiles in the number of correctly identified designs [F (6, 198) = 0.59, ns].

A significant interaction was found between Trial and age [F (2, 196) = 4.68, p<0.05] and NART score [F (2, 196) = 4.99, p<0.05] (see Appendix 17 and Appendix 18). Participants who were younger and who obtained higher NART scores showed better performance in the number of correctly identified designs. None of the covariates were significant [[largest F (1, 98) = 1.90, ns)] and there was

no interaction with polyphenol consumption quartiles [largest F (3, 98) = 1.24, ns)] (see Appendix 13).

(ii) Correctly identified positions (immediate)

There was a significant main effect of trial [F (2, 98) = 59.39, $p<0.01$] as shown in Figure 4-9 (b) which reflects the increase in correctly identified positions with each successive trial. There is no main effect of polyphenol consumption quartiles in the number of correctly identified positions [F (6, 198) = 0.81; ns].

There was a significant interaction of Trial with age [F (2, 196) = 5.54, $p<0.01$] (see Appendix 19). Age [F (1, 98) = 4.95, $p<0.05$] and WAIS total score [F (1, 98) = 4.15, $p<0.05$] were significant covariates and predicted performance on this VSLT measure. Participants who were younger and who obtained higher WAIS scores showed better performance on this VSLT measure. None of the covariates interacted significantly with polyphenol consumption quartiles [largest F (3, 98) = 1.52, ns)] (see Appendix 13).

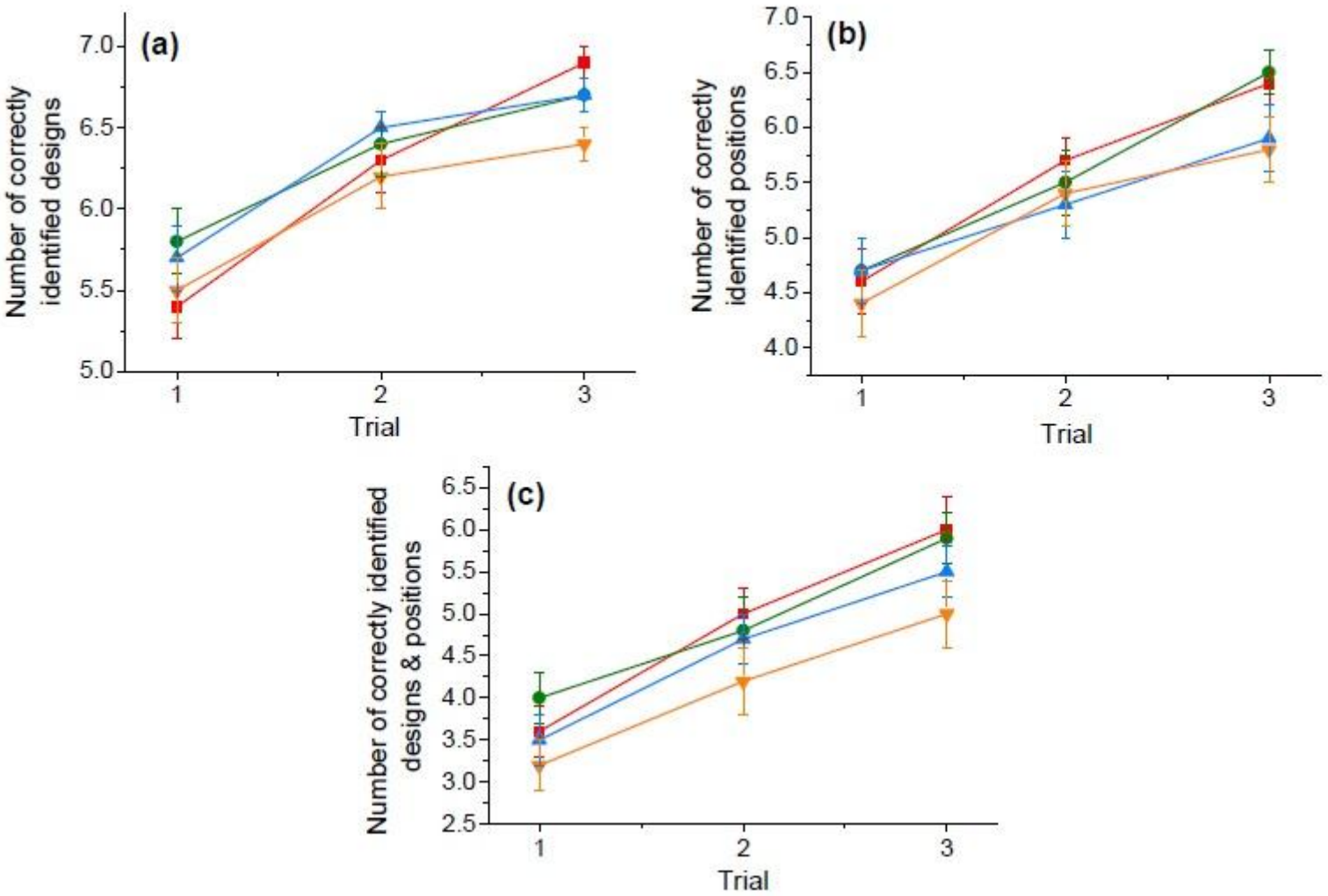


Figure 4-9 Number of correctly identified designs (a), position (b) and designs and positions (c) according to polyphenol consumption quartiles [(■) Q1: very low consumers, (●) Q2: low consumers, (▲) Q3: moderate consumers, (▼) Q4: high consumers] (mean ± SE)

(iii) Correctly identified target designs placed in the correct positions (immediate)

The main effect of Trial was significant [F (2, 98) = 115.08, p<0.01] which is shown in Figure 4-9 (c) and reflects the increase in correctly identified designs placed in the correct positions with each successive trial. There was no main effect of polyphenol consumption quartiles in the number of correctly identified designs placed in the correct positions [F (6, 196) = 0.65; ns], however, from Figure 4-9 (c), high consumers of polyphenol seem to have a poorer rate of learning as compared to the other polyphenol consumption quartiles. There was a significant interaction of Trial with age [F (2, 196) = 13.38, p<0.001] and NART score [F (2, 196) = 3.24, p<0.05] (see Appendix 20 and Appendix 21). Participants who were younger and who obtained higher NART scores showed better performance on this VSLT measure. None of the covariates were significant with Trial [largest F (1, 98) = 2.55, ns)] or polyphenol consumption quartiles [largest F (3, 98) = 2.00, ns)] (see Appendix 13).

(iv) Total immediate recall per trial

Total immediate recall was calculated by the addition of correctly identified designs, correctly identified positions and correctly identified designs and positions for all three trials. The sum of these three variables will yield a maximum score of 63 (21 score per trial). Total number of correct responses was not significantly different between polyphenol consumption quartiles [F (3, 99) = 1.24, ns] (see Figure 4-10). None of the covariates were significant [largest F (1, 95) = 2.07, ns)] and none had

significant interactions with polyphenol consumption quartiles [largest F (3, 95) = 2.56, ns)] (see Appendix 13).

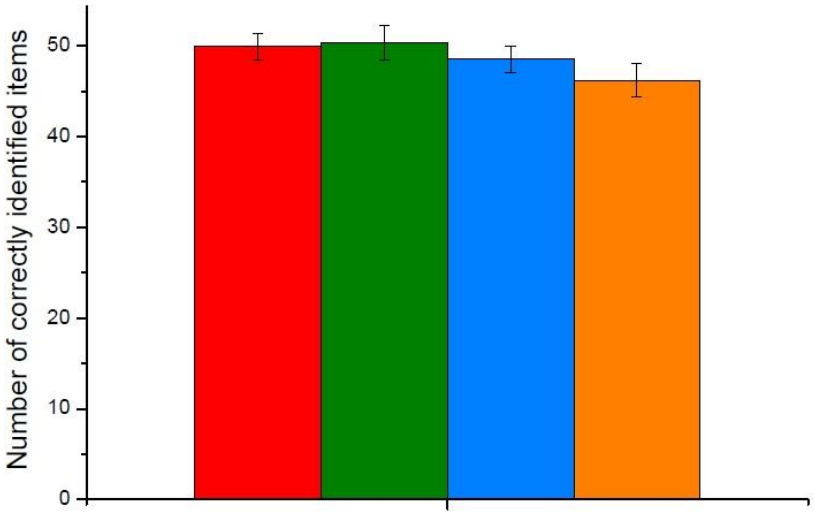


Figure 4-10 VSLT immediate total recall average per trial according to polyphenol consumption quartiles [(■) Q1: very low consumers, (■) Q2: low consumers, (■) Q3: moderate consumers, (■) Q4: high consumers] (mean ± SE)

(v) Correctly identified designs (delayed)

Delayed recall represents the recall of designs and positions about 25 minutes after the test was first administered. There was no significant difference in correctly identified designs in delayed recall between polyphenol consumption quartiles [F (3, 99) = 0.60, ns] (see Figure 4-11, first section). None of the covariates were significant [largest F (1, 98) = 2.02, ns)] or interacted with polyphenol consumption quartiles [largest F (3, 98) = 0.67, ns)] (see Appendix 13).

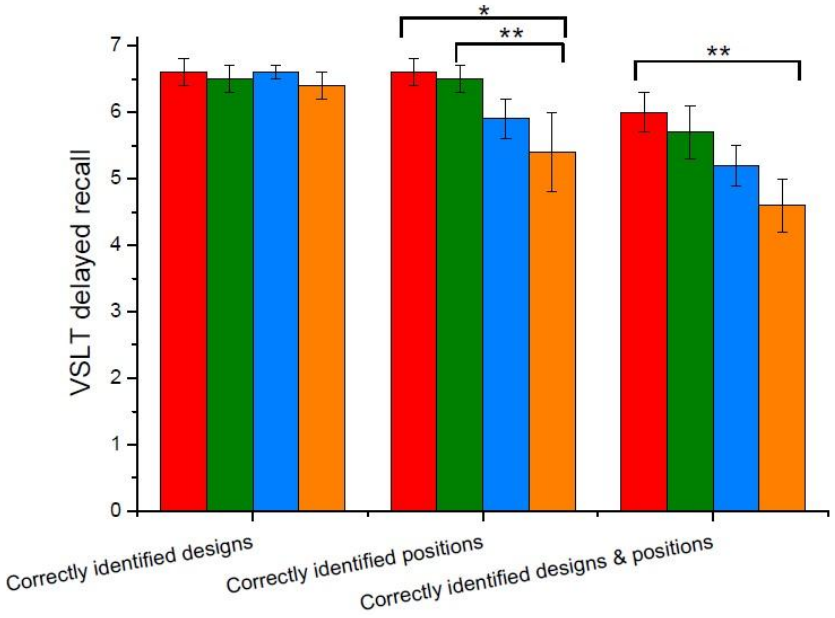


Figure 4-11 Number of correctly identified designs and positions in the delayed recall according to polyphenol consumption quartiles [(■) Q1: very low consumers, (■) Q2: low consumers, (■) Q3: moderate consumers, (■) Q4: high consumers] (mean ± SE)

(vi) Correctly identified positions (delayed)

There was a significant difference between polyphenol consumption quartiles in correctly identified positions [F (3, 99) = 5.06, p<0.01] (see Figure 4-11, second section). Bonferroni corrected post hoc tests showed significant differences between high consumers and two other groups namely very low (p<0.01) and low consumers (p<0.05). High polyphenol consumers performed worse than the other two groups. Age was a significant covariate [F (1, 95) = 5.31, p<0.05] with younger participants showing better performance on this VSLT measure (see Appendix 22). None of the covariates interacted significantly with polyphenol consumption quartiles [largest F (3, 95) = 0.76, ns)] (see Appendix 13).

(vii) Correctly identified target designs placed in the correct positions (delayed)

There was a significant difference between polyphenol consumption quartiles in the number of correctly identified designs placed in the correct positions [F (3, 99) = 4.13, $p<0.05$] (see Figure 4-11, third section). Bonferroni corrected post hoc tests showed significant differences between very low and high consumers ($p<0.05$). Very low polyphenol consumers performed better than the high polyphenol consumers. None of the covariates were significant [largest F (1, 95) = 2.15, ns)] and there were no interactions of covariates with polyphenol consumption quartiles [largest F (3, 95) = 1.06, ns)] (see Appendix 13).

(viii) Total delayed recall

There was a significant difference between the polyphenol consumption quartiles for Total delayed recall [F (3, 99) = 4.05, $p<0.01$] (see Figure 4-12). Bonferroni corrected post hoc tests showed significant differences between very low and high ($p<0.05$) and between low and high ($p<0.05$) polyphenol consumers for total delayed recall. High polyphenol consumers performed worse than the other two groups. None of the covariates were significant [largest F (1, 95) = 3.02, ns)] and these did not interact with polyphenol consumption quartiles [largest F (3, 95) = 1.14, ns)] (see Appendix 13).

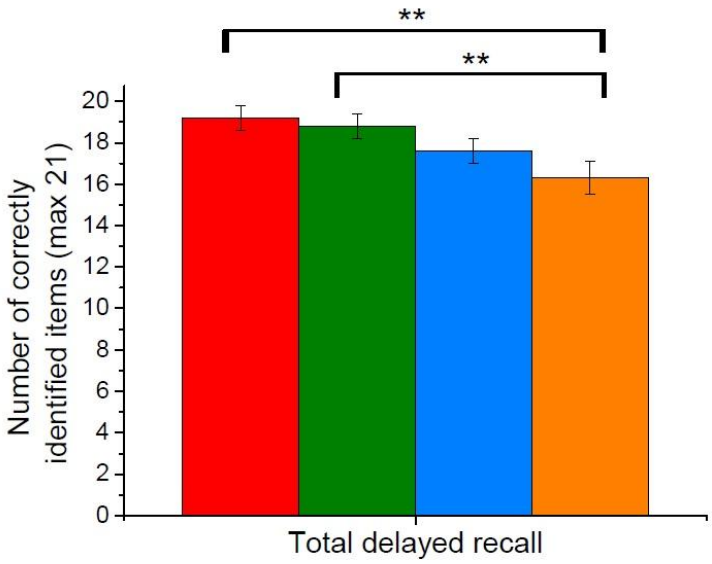


Figure 4-12 Number of total correctly identified item in delayed recall [(■) Q1: very low consumers, (■) Q2: low consumers, (■) Q3: moderate consumers, (■) Q4: high consumers] (mean ± SE)

4.4.5.3 Corsi Block Tapping Test

Two outcome variables were assessed from the Corsi block tapping test, namely accuracy and reaction time. A total of 88 responses were administered per test administration. There were eight levels of test (consisting of 2 to 9 blocks) and each level was repeated twice, giving a total of 16 trials per test session. For the analysis, number of correct response and reaction time used to correctly complete the sequence by participants in different polyphenol consumption quartiles was analysed using ANOVA models. Age, NART score, and WAIS total score were included as separate covariates. In addition, 'Level' was added as an additional within subjects' factor with 8 levels (Level 2 to Level 9). Therefore, an 8x4 (Level* polyphenol consumption quartiles) ANOVA was performed. Age, NART score, and WAIS total score were added separately as covariates due to significant intercorrelations between these characteristics.

(i) Correct responses - accuracy

No significant difference was found between the polyphenol consumption quartiles in the number of completed sequences [F (3, 99) = 0.79, ns]. The mean number of completed sequences was 66.2 ± 10.0 with minimum and maximum values of 35 and 86 respectively. There was a significant interaction between the number of correct responses and WAIS total score [F (1, 95) = 7.48, $p<0.001$] (see Appendix 23). Participants with high WAIS total scores have performed better on this measure. None of the covariates interacted significantly with polyphenol consumption quartiles [largest F (3, 95) = 2.36, ns)] (see Appendix 13).

(ii) Correct responses - reaction time

No significant difference was found between polyphenol consumption quartiles in the time taken to correctly complete the sequence on the Corsi test [F (3, 99) = 1.34, ns]. Age was the only significant covariate [F (1, 95) = 10.96, $p<0.001$], such that reaction time increased with higher age (see Appendix 24). None of the covariates interacted significantly with polyphenol consumption quartiles [largest F (3, 95) = 1.08, ns)] (see Appendix 13).

(iii) Percentage correct per level

The percentage of correct responses per level was calculated by dividing the number of correct response by the total possible responses and multiplying by 100. For example there were two trials at each level, so for level 2 there were 4 responses, for level 3 there were two trials each with 3 responses so 6 responses in total and so on. The main effect of level was significant [F (7, 693) = 101.07, $p<0.001$] such that the percentage correct per level decreased from the lower to the higher level in non-

linear pattern (see Figure 4-13). There was no main effect of polyphenol consumption quartiles on the percentage of correct responses [F (21, 693) = 1.11; ns]. No significant interaction was found between Level and polyphenol consumption quartiles [largest F (21, 686) = 1.45, ns)]. Age F (7, 686) = 6.88, p<0.001], NART score F (7, 686) = 4.92, p<0.001] and WAIS total score F (7, 686) = 3.80, p<0.001] all interacted significantly with Level (see Appendix 25). Younger age, higher NART and higher WAIS total scores were associated with better performance i.e. a higher percentage of correct responses. WAIS total score is the only significant covariate [F (1, 98) = 8.59, p<0.01] and none of the covariates showed significant interactions with polyphenol consumption quartiles [largest F (3, 98) = 2.04, ns)] (see Appendix 13).

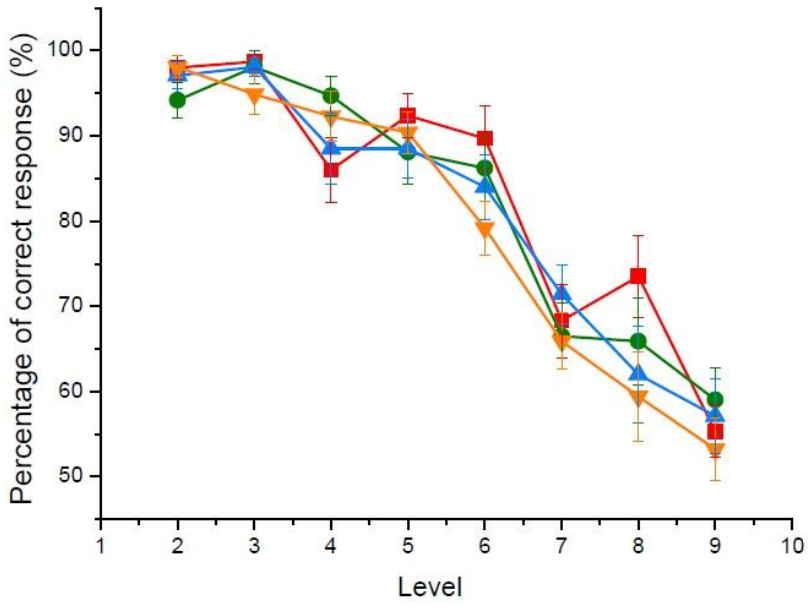


Figure 4-13 Percentage of correct response in each level (%) according to polyphenol consumption quartiles [(■) Q1: very low consumers, (●) Q2: low consumers, (▲) Q3: moderate consumers, (▼) Q4: high consumers] (mean ± SE)

(iv) The effect of crossing - accuracy

A crossing effect in Corsi occurs when the path between successive red to be remembered trials crosses over. Trials in which crossed paths occur are more difficult for the participant to recall. No significant difference between polyphenol consumption quartiles was found for responses with [F (3, 99) = 0.48, ns] and without crossing [F (3, 99) = 0.88, ns] (see Table 4-10).

Table 4-10 Number of correct responses without and with crossing effect according to quartiles of daily polyphenol consumption (mean ± SE)

Number of correct responses	Very low consumers (N=25)	Low consumers (N=26)	Moderate consumers (N=26)	High consumers (N=26)
Without crossing	42.2 (1.0)	41.7 (1.4)	41.7 (1.4)	39.7 (1.0)
With crossing	25.8 (1.2)	25.2 (0.9)	24.3 (1.0)	24.2 (1.3)

With crossing, WAIS total score was the only significant covariate [F (1, 95) = 7.94, $p<0.01$] (see Appendix 26). None of the covariates showed significant interactions with polyphenol consumption quartile on trials with crossing [largest F (3, 95) = 1.91, ns)]. Age showed a significant interaction with polyphenol consumption quartile on trials without crossing [F (3, 95) = 3.17, $p<0.05$]. Younger participants in different polyphenol consumption quartile showed better performance in the numbers of correct responses without crossing (see Appendix 27). None of the covariates showed significant interactions with number of correct responses without crossing [largest F (1, 95) = 3.57, ns)] (see Appendix 13).

(v) The effect of crossing – reaction time

No significant difference between polyphenol consumption quartiles was found in the time taken to correctly complete the sequence with [F (3, 99) = 1.00, ns] and without crossing [F (3, 99) = 1.47, ns] (see Table 4-11).

Table 4-11 Reaction time for correct responses without and with crossing effect according to quartiles of daily polyphenol consumption (mean ± SE)

Reaction time for correct responses	Very low consumers (N=25)	Low consumers (N=26)	Moderate consumers (N=26)	High consumers (N=26)
Without crossing	926.0 (23.4)	957.4 (33.2)	941.6 (26.5)	1012.8 (38.6)
With crossing	914.8 (26.3)	976.6 (34.4)	948.9 (28.5)	996.2 (47.1)

With crossing, none of the covariates were significant [largest F (1, 95) = 3.59, ns)] and there were no interactions with polyphenol consumption quartiles [largest F (3, 95) = 1.32, ns)] (see Appendix 13). Age was a significant covariate [F (1, 95) = 14.58, $p<0.001$] on trials without crossing (see Appendix 28). None of the covariates showed significant interactions with polyphenol consumption quartiles for trials with crossing [largest F (3, 95) = 1.32, ns)] and without crossing [largest F (3, 95) = 1.10, ns)] (see Appendix 13).

4.4.5.4 Tower of Hanoi

This test consisted of 5 levels (Levels 4 to Level 8), corresponding to the numbers of moves required for each level. For the analysis, 'Level' was added as an additional within subjects' factor. Therefore a 5x4 (Level* polyphenol consumption quartiles) ANOVA was calculated. The main effect of Level was significant for the

number of errors made for all levels [F (4, 396) = 14.22, p<0.001], and indicates an increase in errors occurred with increasing difficulty level (see Figure 4-14).

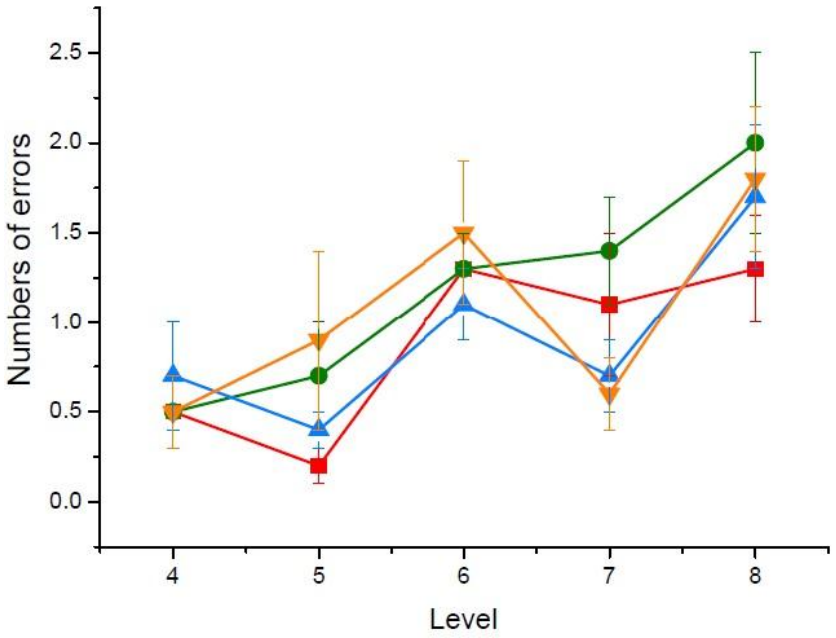


Figure 4-14 TOH mean error per level according to polyphenol consumption quartiles [(■) Q1: very low consumers, (●) Q2: low consumers, (▲) Q3: moderate consumers, (▼) Q4: high consumers] (mean ± SE)

There was no main effect of polyphenol consumption quartiles on the numbers of errors made for all levels [F (12, 396) = 0.93; ns]. In addition, there was no interaction between Level and polyphenol consumption quartiles [largest F (12, 326) = 0.80; ns]. None of the covariates were significant [largest F (1, 98) = 2.00, ns)] and there were no interactions of covariates with polyphenol consumption quartiles [largest F (3, 98) = 0.96, ns)] (see Appendix 13).

4.4.6 Subjective evaluation of cognitive performance according to polyphenol consumption quartiles

Figure 4-15 displays the participants' subjective evaluation of their cognitive performance reported after completing the cognitive test battery (see Appendix 11). Visual analogue scales were scored in millimetres for six variables. Generally, participants' ratings indicated that they exerted high effort and concentration during the test administration but time pressure was not a concern for the participants during the test. ANOVA test were performed to compare polyphenol consumption quartiles on each of the visual analogue rating scales. There were no significant differences in visual analogue rating scales scores between the polyphenol consumption quartiles (see Appendix 13).

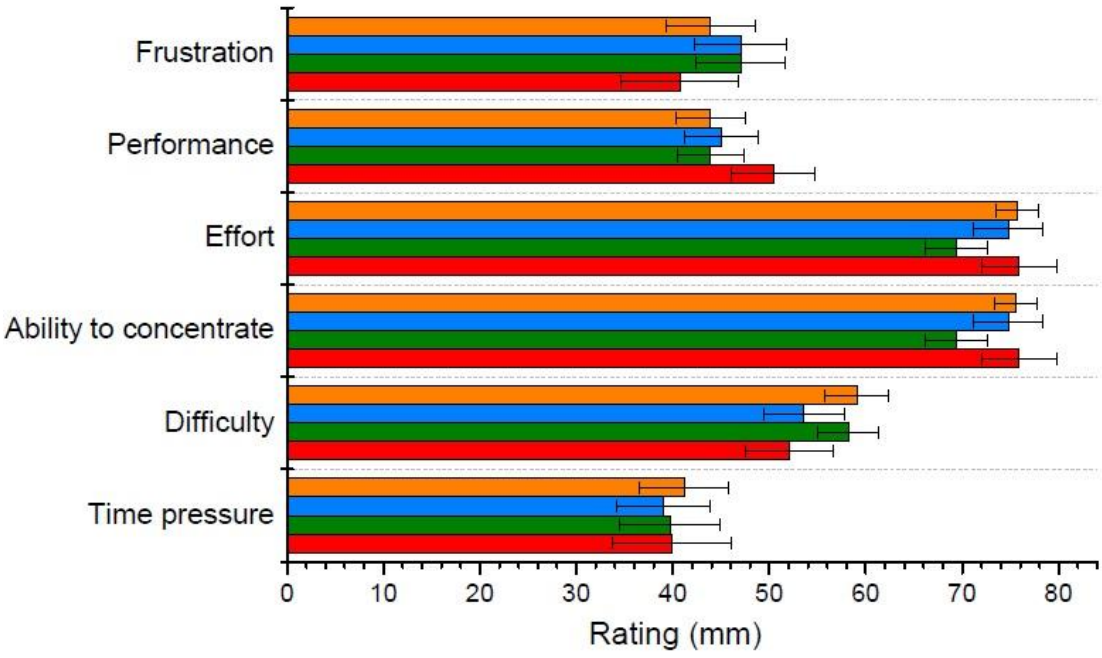


Figure 4-15 Subjective evaluation of cognitive performance [(■) Q1: very low consumers, (■) Q2: low consumers, (■) Q3: moderate consumers, (■) Q4: high consumers] (mean ± SE)

(c) Comparison according to beverage consumption groups

4.4.7 Participant characteristics according to beverage consumption groups

Table 4-12 displays the demographic characteristics of the participants group according to their consumption of polyphenol containing beverages. This table shows socio-demographic data including age, employment status and eating habits such as breakfast consumption, alcohol intake and dietary practises such as being vegetarian. A Kruskall-Wallis test showed a statistically significant difference across the four beverage consumption groups in age ($\chi^2 = 17.35$; df = 3; $p<0.01$), volume of coffee consumed daily ($\chi^2 = 97.97$; df = 3; $p<0.01$) and volume of tea consumed daily ($\chi^2 = 77.25$; df = 3; $p<0.01$). Further Mann-Whitney tests showed significant differences in age between non-consumers of coffee or tea and the other two groups namely consumers of both coffee and tea ($p<0.01$) and coffee consumers ($p<0.05$). Non-consumers of coffee or tea were younger than the other two groups. In terms of volume of beverage consumed daily, the consumers of both coffee and tea consumed less tea than the tea consumers but a higher volume of coffee than coffee consumers. No significant differences were found in other demographic data.

Table 4-12 Participant characteristics according to beverage consumption groups*

	Coffee consumers (N=11)	**Tea consumers (N=43)**	**Consumers of coffee and tea (N=20)**	**Non-consumers coffee or tea (N=29)**	**p value**
Age (years)[1]	25.6 (8.6)	23.1 (6.4)	32.8 (11.9)	22.3 (7.4)	0.001~
Body mass index (kg/m^2)	24.1 (3.3)	23.7 (4.1)	27.1 (6.6)	23.9 (3.5)	0.190
Basal metabolic rate, BMR (kcal)	1482 (166)	1426 (145)	1461 (185)	1409 (136)	0.559
Ratio energy intake to BMR	1.3 (0.3)	1.2 (0.3)	1.2 (0.3)	1.2 (0.4)	0.967
Category of employment:					0.055
Employed (%)	36.4	20.9	45.0	10.3	
Student (%)	63.6	65.1	40.0	82.8	
Doing regular exercise (%)	72.7	74.4	80.0	82.8	0.822
Units of alcohol consumed per week (unit)^	2.3 (2.2)	4.9 (5.2)	6.8 (9.9)	5.2 (4.4)	0.292
Volume of beverages consumed (ml/day)[1]	coffee: 407.0 (379.6)	tea: 408.9 (328.3)	coffee: 224.3 (159.7) tea: 435.3 (364.5)	-	0.001~ 0.001~
Vegetarian (%)	9.1	9.3	15.0	17.2	0.749
Regular breakfast consumer (%)	81.8	86.0	95.0	86.2	0.690

*Data presented as mean (standard deviation) or percentage, BMR: Basal metabolic rate was calculated using the Schofield prediction equations, ^One unit of pure alcohol is equal to 10 ml or 8 grams, ˜p<0.01 Kruskall-Wallis test.

4.4.8 Estimation of intelligence across beverage consumption groups

There was a significant difference between beverage consumption groups in NART score [$F(3, 99) = 4.20$, $p<0.01$] (see Figure 4-16). Bonferroni corrected post hoc tests showed significant difference between consumers of both coffee and tea and non-consumers of coffee or tea ($p<0.05$) in NART score. Lower scores were obtained by non-consumers of coffee or tea.

Significant differences were found between the beverage consumption groups in the WAIS vocabulary subscale [$F(3, 99) = 4.05$, $p<0.01$] (see Figure 4-16). Bonferroni corrected post hoc tests showed significant differences between non-consumers of coffee or tea and coffee consumers ($p<0.05$). The non-consumers of coffee or tea scored lower than coffee consumers. No significant difference between the beverage consumption groups was found in WAIS matrix reasoning subscale [$F(3, 99) = 1.32$, ns].

WAIS total score showed significant differences between the beverage consumption groups [$F(3, 99) = 4.54$, $p<0.01$]. Bonferroni corrected post hoc tests showed significant differences between non-consumers of coffee or tea and consumers of both coffee and tea ($p<0.05$). The non-consumers of coffee or tea scored lower than consumers of both coffee and tea.

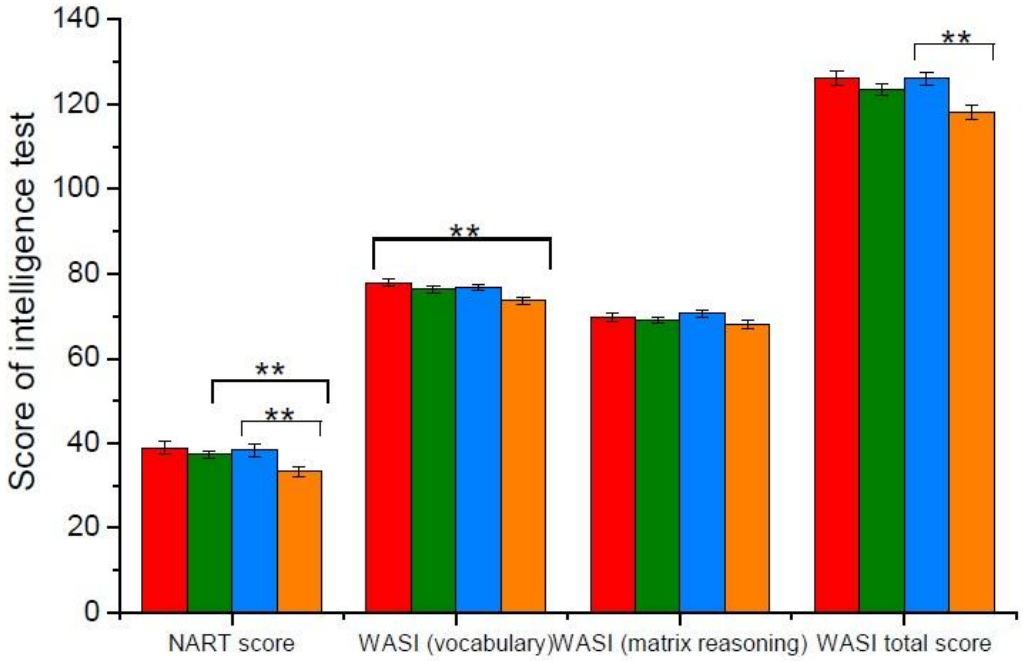


Figure 4-16 Score for intelligence tests according to beverage consumption groups [(■) coffee consumers, (■) tea consumers, (■) consumers of coffee and tea, (■) non-consumers of coffee or tea] (mean ± SE)

4.4.9 Daily nutrient intake differences between beverage consumption groups

Table 4-13 represents the daily intake of major nutrients according to beverage consumption groups. Protein and vitamin C intake exceeded their respective RNI levels. Carbohydrate and dietary fibre are the nutrients which came next closest to fulfilling the RNI recommendations.

There were significant differences between beverage consumption groups in fibre intake assessed from DINE [$H(3) = 10.36$; $p<0.05$]. Further Mann-Whitney test showed significant differences in DINE between non-consumers of coffee or tea and the other two groups including consumers of both coffee and tea ($p<0.01$) and tea consumers ($p<0.01$). The non-consumers of coffee or tea had lower scores on DINE as compared to the other two groups. Interestingly, data from the food diary showed

that the non-consumers of coffee or tea met 90 % of the recommended intake for dietary fibre, however, this group had the lowest DINE score and were categorized as low fibre intake (score less than 30). A Spearman's correlation coefficient between fibre intake assessed from food diary and DINE score was computed to confirm this finding. A significant but weak association ($R = 0.29$, $p<0.01$) was found between DINE DF and daily dietary fibre intake based on the diary. This finding can partly be explained by a possible over reporting by the non-consumers of coffee or tea in fibre containing foods as assessed using the food diary. No significant difference was found between the beverage consumption groups in fibre intake assessed from LWW DINE [$H(3) = 4.43$; ns] and alcohol intake [$H(3) = 3.52$; ns].

Table 4-13 Daily nutrient intake in each beverage consumption groups and percentage of RNI achieved in each group*

	Coffee consumers (N=11)	^% RNI	Tea consumers (N=43)	% RNI	Consumers of coffee and tea (N=20)	% RNI	Non-consumers of coffee or tea (N=29)	% RNI
Energy (kcal)	1841 (433)	95.0	1672 (413)	86.2	1712 (404)	88.3	1746 (478)	90.0
Protein (g)	68.6 (24.1)	152.4	68.8 (23.1)	152.9	69.2 (20.7)	153.8	67.0 (19.8)	148.8
Carbohydrate (g)~	227.3 (53.1)	46.3	210.0 (51.7)	47.1	212.7 (53.7)	46.6	233.6 (77.4)	50.2
Fat (g)[§]	68.7 (19.7)	33.6	62.2 (23.6)	33.5	64.1 (19.3)	33.7	62.5 (21.3)	32.3
Alcohol (g)[ς]	10.4 (17.3)	5.1	5.3 (11.7)	2.9	7.4 (14.7)	3.9	2.4 (5.9)	1.2
Dietary fibre (g)	18.9 (5.9)	78.8	18.9 (6.2)	78.8	19.5 (6.6)	81.3	21.6 (9.4)	90.0
Vitamin C (mg)	98.0 (78.9)	245.0	80.4 (48.9)	201.0	99.3 (61.3)	248.3	76.9 (82.1)	192.3
LWW DINE (g)	16.0 (7.3)	-	16.1 (6.3)	-	16.7 (5.9)	-	13.3 (4.6)	-
DINE DF score[1]	31.9 (5.7)	-	33.8 (12.4)	-	34.1 (9.1)	-	26.1 (9.2)	-

*Data presented as mean (standard deviation), [1]p<0.01 Mann-Whitney test, [2]p<0.01 Kruskall-Wallis test. ^% RNI: Percentage from the Reference Nutrient Intake, ~recommended at 50 % of food energy, [§]recommended at 35 % of food energy, [ς]should be less than 5% of total energy, [ι]food recorded for 7 days, [δ]food recorded for 3 days, DINE: Dietary Instrument for Nutrition Education for dietary fibre (DF), LWW DINE: Leeds Women Well-being DINE, LWW: Leeds Women's Wellbeing Study, DH: Diet and Health Study.

4.4.10 Daily polyphenol intake across beverage consumption groups

Table 4-14 displays the polyphenol intake of major polyphenol groups. A Kruskall-Wallis test showed a significant difference between beverage consumption groups in daily flavonoids [H (3) = 63.75; p<0.01], phenolic acids [H (3) = 55.40; p<0.01], all other polyphenols [H (3) = 13.10; p<0.01] and total polyphenols intake [H (3) = 56.51; p<0.01]. It is clear that the non-consumers of coffee or tea have the lowest intake of all major polyphenol groups except for all other polyphenols as compared to the other three groups. The intake of all other polyphenols was highest amongst the consumers of both coffee and tea followed by coffee consumers; the non-consumers of coffee or tea and finally the lowest was tea consumers. Flavonoids and phenolic acids become the major contributors for tea consumers and coffee consumers respectively. Flavonoids intake was higher than phenolic acids for consumers of both coffee and tea. This finding is coincides with a higher volume of tea consumed by the participants in this group (see Table 4-12).

Table 4-14 Polyphenol intake of major polyphenol groups (mg per day)*

	Coffee consumers (N=11)	**Tea consumers (N=43)**	**Consumers of coffee and tea (N=20)**	**Non-consumers of coffee or tea (N=29)**
Flavonoids[1]	100.1 (78.0)	724.4 (491.1)	773.5 (625.4)	86.2 (73.2)
Phenolic acids[1]	896.4 (832.0)	131.3 (89.3)	573.9 (338.8)	69.9 (42.0)
All other polyphenols[1]	47.3 (35.3)	25.20 (29.0)	51.1 (39.7)	44.8 (52.4)
Total polyphenol[1]	1043.7 (860.8)	880.9 (577.6)	1398.5 (596.8)	201.0 (120.1)

*Data presented as mean (standard deviation), p<0.01 difference between beverage consumption groups with Kruskall-Wallis test

4.4.11 Cognitive performance according to beverage consumption groups

4.4.11.1 Visual Verbal Learning Test

(i) Rate of learning (word List A)

Rate of learning for word List A according to beverage consumption groups is shown in Figure 4-17. For this analysis 'Trial' was added as an additional within subjects factor with 3 levels (Trials A1, A2, and A3, see Section 4.2.4.3 a). Therefore, a 3x4 (Trial* beverage consumption groups) ANOVA was calculated. Age, NART score, and WAIS total score were added as separate covariates due to significant intercorrelations between these characteristics.

Rate of learning increased significantly from Trial 1 to Trial 3 such that the main effect of Trial was significant [$F(2, 98) = 160.78$, $p<0.01$]. There was no main effect of beverage consumption groups in the rate of learning [$F(6, 98) = 1.05$, ns]. No interaction was found between Trial*beverage consumption groups [$F(6, 196) = 1.05$, ns] suggesting that the groups did not differ in their memory capacity or rate of learning. None of the covariates significantly interacted with Trial [largest $F(1, 98) = 1.30$, ns)] or beverage consumption groups [largest $F(3, 98) = 0.77$, ns)] (see Appendix 14).

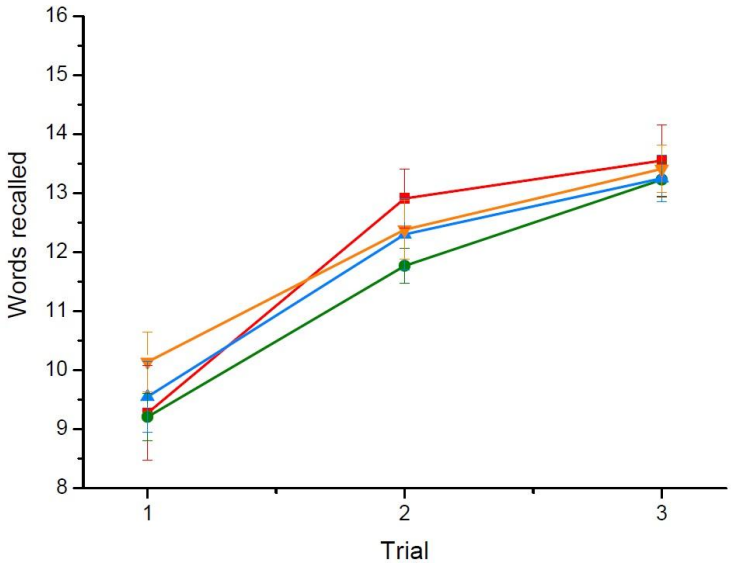


Figure 4-17 Rate of learning according to beverage consumption groups [(■) coffee consumers, (●) tea consumers, (▲) consumers of coffee and tea, (▼) non-consumers of coffee or tea] (mean ± SE)

(ii) New learning (recall of List B)

No significant difference was found between beverage consumption groups in the new learning which implies the recall of words from interference list (List B) [F (3, 99) = 0.40, ns]. None of the covariates were significant [largest F (1, 95) = 2.21, ns)] and none had significant interactions with beverage consumption groups [largest F (3, 95) = 1.38, ns)] (see Appendix 14).

(iii) Retroactive interference (Trial A3-A4)

No significant difference was found between the beverage consumption groups in retroactive interference [F (3, 99) = 1.43, ns] (see Figure 4-18). None of the covariates were significant [largest F (1, 95) = 0.14, ns)] and none had significant interactions with beverage consumption groups [largest F (3, 95) = 2.17, ns)] (see Appendix 14).

(iv) Proactive interference (Trial A1-B1)

No significant difference in proactive interference was found between the beverage consumption groups [F (3, 99) = 0.13, ns] (see Figure 4-18). None of the covariates were significant [largest F (1, 95) = 1.38, ns)] and none had significant interactions with beverage consumption groups [largest F (3, 95) = 2.12, ns)] (see Appendix 14).

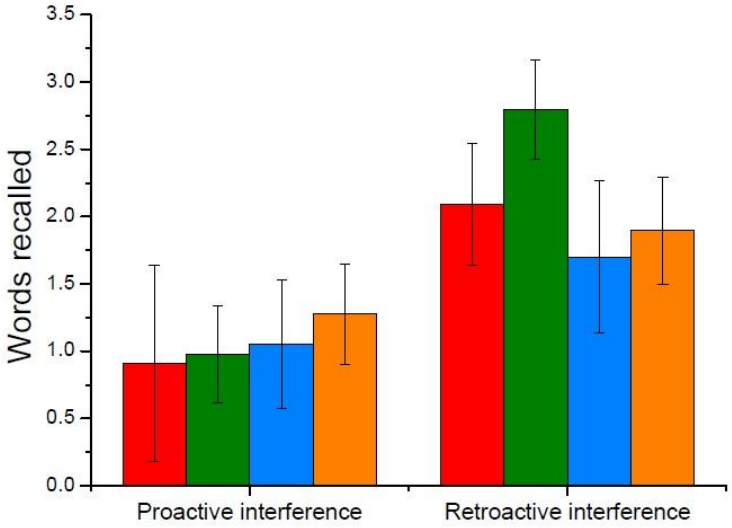


Figure 4-18 Number of interference according to beverage consumption groups [(■) coffee consumers, (■) tea consumers, (■) consumers of coffee and tea, (■) non-consumers of coffee or tea] (mean ± SE)

(v) Delayed recall

Delayed recall is the recall of List A in approximately 30 minutes after the test first administered. No significant difference [F (3, 99) = 0.31, ns] was found between beverage consumption groups in delayed recall. None of the covariates were significant predictors of words recalled [largest F (1, 95) = 1.10, ns)] and none had significant interactions with beverage consumption groups [largest F (3, 95) = 2.00, ns)] (see Appendix 14).

(vi) Word recognition accuracy

For this analysis, 'Word list' and 'Modes of presentation' were selected as two within subject factors. There are three different word (List A, B and C) with two method of word presentations (visual and aural). Therefore, a 3x4 (Word list* beverage consumption groups) and a 2x4 (Modes of presentation*beverage consumption groups) ANOVA was calculated. Table 4-15 displays the number of correctly recognised words from different word lists and different modes of word list presentations.

Table 4-15 Number of correctly recognised words from three different word lists and different modes of presentations*

Word list	Coffee consumers (N=11)	Tea consumers (N=43)	Consumers of coffee and tea (N=20)	Non-consumers of coffee or tea (N=29)
List A (seen 3 times)	12.3 (3.1)	11.7 (4.1)	12.9 (3.6)	12.1 (4.6)
List B (seen once)	12.2 (2.0)	10.9 (4.0)	11.1 (3.7)	10.1 (4.6)
List C (new list)	13.5 (1.7)	11.7 (4.4)	12.2 (3.5)	12.5 (4.5)
Words presented visually	19.3 (1.9)	17.4 (5.8)	18.5 (4.9)	17.5 (6.6)
Words presented aurally	18.6 (3.4)	16.9 (5.8)	17.6 (5.1)	17.2 (6.4)
Overall correctly identified words	37.9 (4.8)	34.3 (11.3)	36.1 (9.7)	34.7 (12.8)

*Data presented as mean (standard deviation)

There were significant main effects of Word List [$F(2, 198) = 10.51$, $p<0.001$] and Modes of presentation [$F(1, 99) = 4.39$, $p<0.05$]. Fewer words from List B and fewer words presented aurally were correctly recognised by the participants. There was no main effect of beverage consumption groups in the Word list [$F(6, 198) = 1.56$, ns] and Modes of presentation [$F(3, 99) = 0.22$, ns]. None of

the covariates were significant with Word list [largest F (1, 98) = 3.06, ns)] and none had significant interactions with beverage consumption groups [largest F (3, 98) = 0.31, ns)] in the 3x4 ANOVA analysis. Finally, none of the covariates were significant [largest F (3, 98) = 3.06, ns)] and none had significant interactions with beverage consumption groups [largest F (3, 98) = 0.31, ns)] for the 2x3 ANOVA analysis (see Appendix 14).

(vii) Word recognition reaction time

Table 4-16 displays the time taken (in milliseconds) to correctly recognise the words from the three different lists. For this analysis, 'Word list' and 'Method of presentation' were selected as two within subject factors. Therefore, a 3x4 (Word list* beverage consumption groups) and a 2x4 (Modes of presentation* beverage consumption groups) ANOVA were performed.

Table 4-16 Mean reaction time to correctly recognise words from three different word lists and different modes of word list presentation (millisecond)*

Word list	Coffee consumers (N=11)	Tea consumers (N=43)	Consumer coffee and tea (N=20)	Non-consumers of coffee or tea (N=29)
List A (seen 3 times)	1403.2 (168.9)	1328.3 (410.3)	1338.2 (343.0)	1276.5 (474.5)
List B (seen once)	1454.1 (148.0)	1430.7 (428.7)	1454.3 (394.2)	1311.3 (485.2)
List C (new list)	1506.0 (182.0)	1491.0 (449.0)	1525.7 (409.6)	1365.6 (495.4)
Words presented visually	1172.7 (144.2)	1164.6 (352.3)	1193.7 (337.0)	1067.4 (399.3)
Words presented aurally	1746.2 (158.5)	1663.5 (485.2)	1679.4 (411.9)	1556.4 (551.0)
Overall correctly identified words	1454.1 (129.8)	1409.3 (409.0)	1430.6 (359.7)	1309.7 (468.6)

There were significant main effects of Word List [F (2, 198) = 17.38, p<0.001] and Modes of presentation [F (1, 99) = 498.91, p<0.001]. A longer time was taken to recall correctly words from List C and words presented aurally. There was no main effect of beverage consumption groups in the Word list [F (6, 198) = 0.58, ns] and Modes of presentation [F (3, 99) = 0.53, ns]. None of the covariates interacted significantly with Word List [largest F (1, 98) = 3.56, ns)] or beverage consumption groups [largest F (3, 98) = 0.43, ns)] in the 3x4 ANOVA analysis (see Appendix 14). An interaction between Modes of presentation and covariates was found for age [F (1, 98) = 53.03, p<0.001] and NART score [F (1, 98) = 13.22, p<0.001] in the 2x4 ANOVA analysis (see Appendix 16). Participants who were younger and who obtained higher NART scores showed better performance with less time taken to correctly recognise the words.

4.4.11.2 Visual Spatial Learning Test (VSLT)

VSLT immediate recall is the data obtained during the immediate recall of the test when all seven designs and positions were introduced to the participants three times. For this analysis 'Trial' was added as an additional within subjects factor with 3 levels (Trials A1, A2, and A3, see Section 1.2.4.3.1). Therefore, a 3x4 (Trial* polyphenol consumption quartiles) ANOVA was calculated. Age, NART score, WAIS for verbal subscale and WAIS total score were added as separate covariates.

(i) Correctly identified designs (immediate)

The main effect of Trial was significant [F (2, 98) = 51.29; p<0.01] (see Figure 4-19, a) which reflects the increase in correctly identified designs with each sequential trial. This indicated that the rate of learning was significantly increased with each repeated trial. There was no main effect of beverage consumption groups on the number of correctly identified designs [F (6, 198) = 0.96; ns]. An interaction was found between the Trial and age [F (2, 198) = 5.84, p<0.01] and NART score [F (2, 196) = 5.73, p<0.01] (see Appendix 17 and 18). Participants who were younger and who obtained higher NART scores showed better performance in terms of correctly identified designs. None of the covariates were significant [largest F (3, 98) = 2.85, ns)] and none had significant interactions with beverage consumption groups [largest F (3, 98) = 1.30, ns)] (see Appendix 14).

(ii) Correctly identified positions (immediate)

Figure 4-19 (b) shows the correctly identified positions in the immediate recall. The main effect of Trial was significant [F (2, 198) = 41.90, p<0.001] which reflects the increase in correctly identified positions with each successive trial. There was no main effect of beverage consumption groups on the number of correctly identified positions [F (6, 198) = 0.31; ns]. There was a significant interaction of Trial with age [[F (2, 198) = 5.05, p<0.05] (see Appendix 19) with younger participants showing better performance on this VSLT measure. Age [F (1, 98) = 4.16, p<0.05] was a significant covariate and predicted performance on this VSLT measure. None of the covariates interacted significantly with beverage consumption groups [largest F (3, 98) = 1.23, ns)] (see Appendix 14).

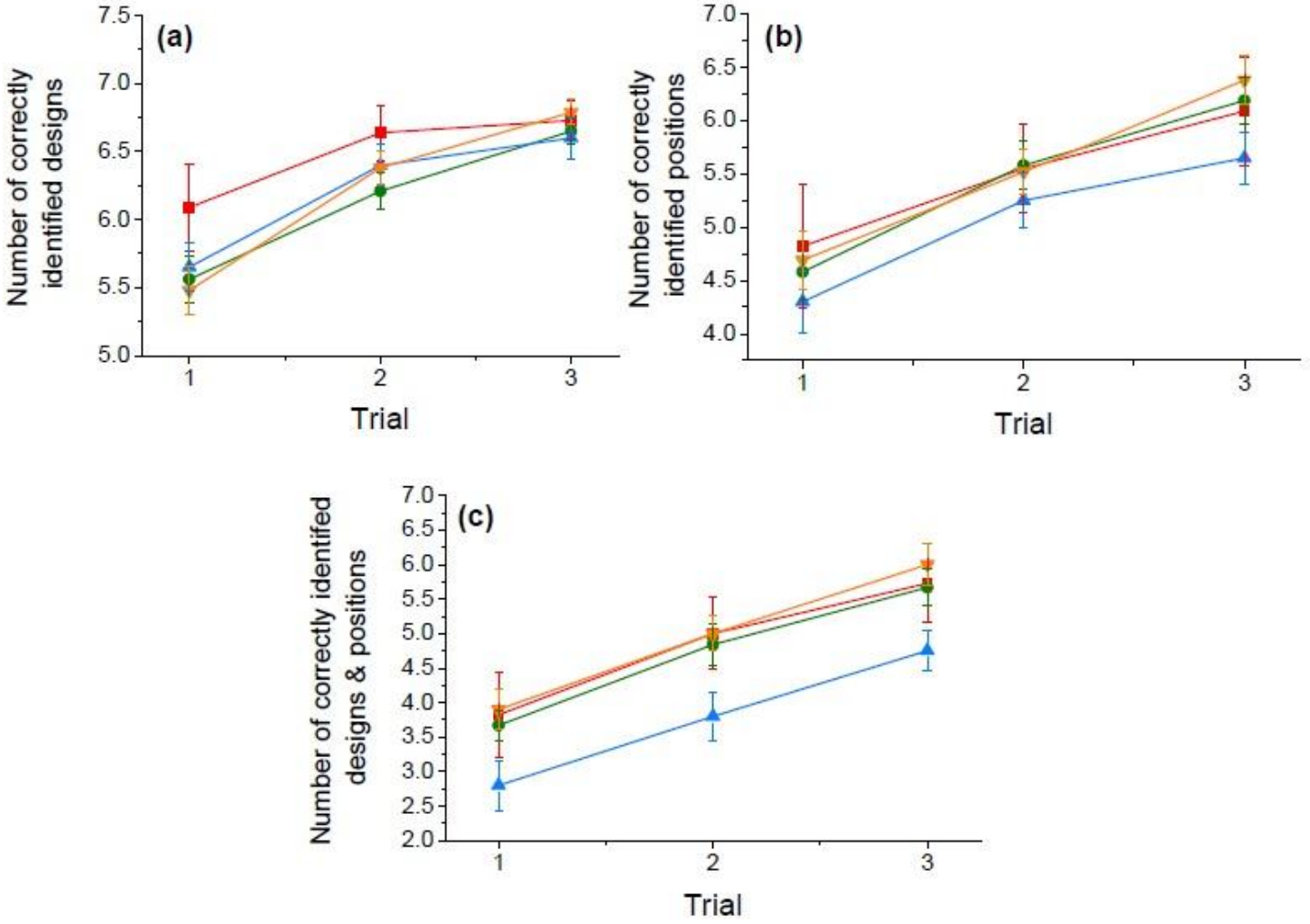


Figure 4-19 Number of correctly identified designs (a), position (b) and designs and positions (c) according to beverage consumption groups [(■) coffee consumers, (●) tea consumers, (▲) consumers of coffee and tea, (▼) non-consumers of coffee or tea] (mean ± SE)

(iii) Correctly identified target designs placed in the correct positions (immediate)

Figure 4-19 (c) shows the correctly identified designs placed in the correct positions in the immediate recall. The main effect of Trial was significant [$F(2, 98) = 86.61$, $p<0.01$] which indicates the increase in correctly identified designs placed in the correct positions with each successive trial. There was no main effect of beverage consumption groups in the number of correctly identified designs placed in the correct positions [$F(6, 196) = 0.10$; ns]. There was a significant interaction of Trial with age [$F(2, 196) = 15.29$, $p<0.001$] and NART score [$F(2, 196) = 4.57$, $p<0.05$] (see Appendix 19 and 20). Participants who were younger and who obtained higher NART scores showed better performance on this VSLT measure. There was a significant interaction of beverage consumption groups with NART [$F(3, 98) = 3.11$, $p<0.05$] and WAIS total score [$F(3, 98) = 3.36$, $p<0.05$] (see Appendix 29). Participants with higher NART and WAIS total score in all beverage consumption groups showed better performance on this VSLT measure. None of the covariates were significant with Trial [largest $F(1, 98) = 2.09$, ns)] (see Appendix 14).

(iv) Total immediate recall per trial

Total immediate recall was calculated by the addition of correctly identified designs, correctly identified positions and correctly identified designs and positions for all three trials. The sum of these three variables yields a maximum score of 63 (21 score per trial). The total number of correct responses was not significantly different between beverage consumption groups [$F(3, 99) = 1.56$, ns] (see Figure 4-20). An interaction between total immediate recall and covariates was only found

in age [F (1, 95) = 8.06, p<0.01] (see Appendix 30). There was a significant interaction of beverage consumption groups with age [F (3, 95) = 2.97, p<0.05] (see Appendix 31). In these interactions, younger participants in all beverage consumption groups showed better performance on this VSLT measure.

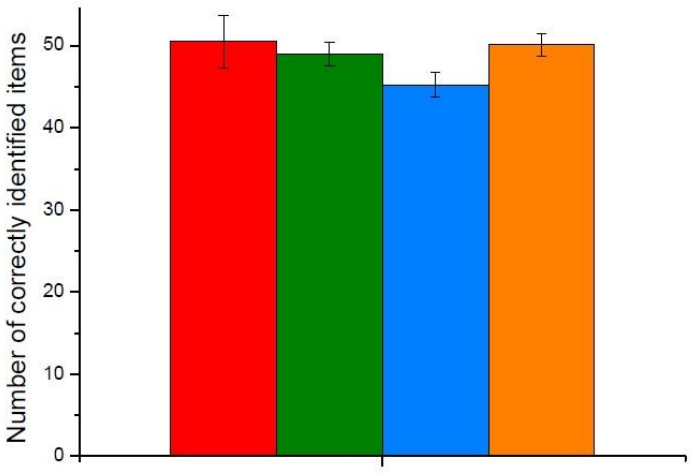


Figure 4-20 VSLT total immediate recall average per trial according to beverage consumption groups [(■) coffee consumers, (●) tea consumers, (▲) consumers of coffee and tea, (▼) non-consumers of coffee or tea] (mean ± SE)

(v) Correctly identified designs (delayed)

There was no significant difference in correctly identified designs in delayed recall between beverage consumption groups [F (3, 99) = 0.57; ns] (see Figure 4-21, first section). None of the covariates were significant [largest F (1, 95) = 2.87, ns)] or interacted with beverage consumption groups [largest F (3, 95) = 1.56, ns)] (see Appendix 14).

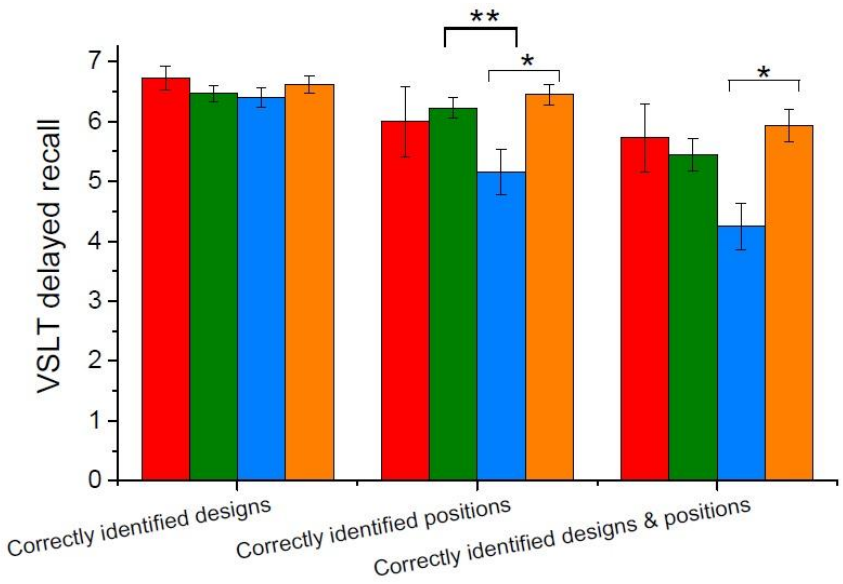


Figure 4-21 Number of correctly identified designs and positions in the delayed recall according to beverage consumption groups [(■) coffee consumers, (■) tea consumers, (■) consumers of coffee and tea, (■) non-consumers of coffee or tea] (mean ± SE)

(vi) Correctly identified positions (delayed)

There was a significant difference between beverage consumption groups in correctly identified positions [F (3, 99) = 4.29; p<0.01] (see Figure 4-21, second section). Bonferroni corrected post hoc tests showed significant differences between consumers of both coffee and tea with the other two groups namely non-consumers coffee or tea and (p<0.01) and tea consumers (p<0.05) in number of correctly identified positions. The non-consumers of coffee or tea performed better than the other two groups. Age was a significant covariate [F (1, 95) = 18.20, p<0.001] with younger participants showing better performance on this VSLT measure (see Appendix 22). There was a significant interaction of beverage consumption groups with age [F (3, 95) = 3.59, p<0.05] (see Appendix 32). Younger participants in all beverage consumption groups showed better performance on this VSLT measure.

(vii) Correctly identified target designs placed in the correct positions (delayed)

There was a significant difference between beverage consumption groups in the number of correctly identified designs and positions in delayed recall [F (3, 99) = 4.13, $p<0.01$] (see Figure 4-21, third section). Bonferroni corrected post hoc tests showed significant differences between non-consumers coffee or tea and consumers of both coffee and tea ($p<0.01$) in number of correctly identified positions in delayed recall. The non-consumers of coffee or tea performed better than the consumers of both coffee and tea. Age was a significant covariate [F (1, 95) = 8.26, $p<0.01$] with younger participants showing better performance on this VSLT measure (see Appendix 33). None of the covariates significantly interacted with beverage consumption groups [largest F (3, 95) = 2.62, ns)] (see Appendix 14).

(viii) Total delayed recall

There was a significant difference between the beverage consumption groups for Total delayed recall [F (3, 99) = 3.83, $p<0.05$] (see Figure 4-22). Bonferroni corrected post hoc tests showed significant differences between non-consumers coffee or tea and consumers of both coffee and tea ($p<0.01$) consumers for total delayed recall. The non-consumers of coffee or tea performed better than the consumers of both coffee and tea. Age was a significant covariate F (1, 95) = 12.87, $p<0.001$]. There was a significant interaction of beverage consumption groups with age [F (3, 95) = 3.47, $p<0.05$] (see Appendix 34), with younger participants showing better performance on this VSLT measure.

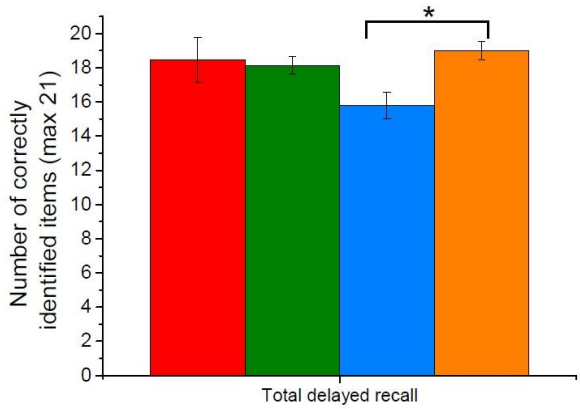


Figure 4-22 VSLT total delayed recall according to beverage consumption groups [(■) coffee consumers, (■) tea consumers, (■) consumers of coffee and tea, (■) non-consumers of coffee or tea] (mean ± SE)

4.4.11.3 Corsi Block Tapping Test

For the analysis, ANOVA test was performed to analyse the number of correct response and reaction time used to correctly complete the sequence by participants in different beverage consumption groups. 'Level' was added as an additional within subjects' factor with 8 levels (Level 2 to Level 9). Therefore, a 8x4 (Level*beverage consumption groups) ANOVA was calculated. Age, NART score, and WAIS total score were added as separate covariates due to significant intercorrelations between these characteristics.

(i) Correct responses - accuracy

No significant difference was found between the beverage consumption groups in the number of completed sequences [$F(3, 99) = 2.14$, ns]. Two covariates were significant - age [$F(1, 95) = 6.98$, $p<0.01$] and WAIS total score [$F(1, 95) = 8.12$, $p<0.01$]. There was a significant interaction between beverage consumption groups and age [$F(3, 95) = 3.00$, $p<0.05$] (see Appendix 35), with younger

participants in all beverage consumption groups showing a higher number of correct responses.

(ii) Correct response – reaction time

No significant difference was found between beverage consumption groups in the time taken to correctly complete the sequence for Corsi test [F (3, 99) = 1.21, ns]. Age was a significant covariate such that reaction time increased with higher age [F (1, 95) = 36.89, $p<0.01$] (see Appendix 24). There was a significant interaction of beverage consumption groups with age [F (3, 95) = 3.01, $p<0.05$] and WAIS total score [F (3, 95) = 4.47, $p<0.01$] (see Appendix 36). Participants who were younger and who obtained higher WAIS total scores showed better performance on this cognitive measure.

(iii) Percentage correct per level

The main effect of level was significant [F (7, 693) = 79.65, $p<0.001$] such that the percentage correct per level was decreased from the lower to the higher level in a non-linear manner (see Figure 4-23). A significant difference was found between beverage consumption groups in the percentage of correct level [F (21, 693) = 1.70, $p<0.05$]. Bonferroni corrected post hoc tests showed significant differences at level 8 between consumers of coffee and tea and two other groups namely non-consumers of coffee or tea ($p<0.01$) and consumers of tea ($p<0.05$). Consumers of coffee and tea had lower percentage of correct response than the other two groups. All the covariates including age F (7, 686) = 8.01, $p<0.001$], NART score F (7, 686) = 4.97, $p<0.001$] and WAIS total score F (7, 686) = 3.84, $p<0.001$] interacted significantly with Level (see Appendix 25). Younger age, higher NART and higher WAIS total

score were associated with better performance in terms of the percentage of correct responses. There was an interaction between Level*beverage consumption groups and WAIS total score [F (21, 686) = 2.02, p<0.01] (see Appendix 37). Participants with higher WAIS total score showed higher percentage of correct response across the levels and in relation to the beverage consumption groups.

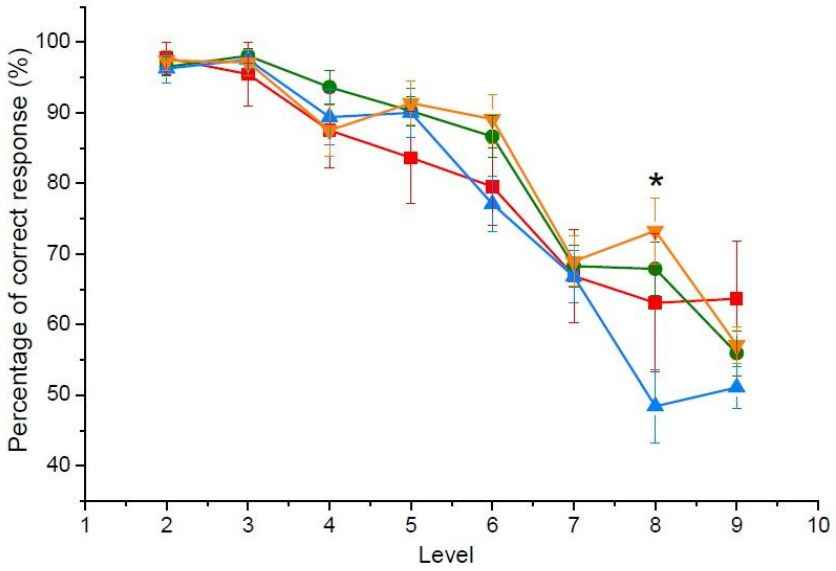


Figure 4-23 Percentage of correct response in each level (%) according to beverage consumption groups [(■) coffee consumers, (●) tea consumers, (▲) consumers of coffee and tea, (▼) non-consumers of coffee or tea] (mean ± SE)

(iv) The effect of crossing - accuracy

A crossing effect in Corsi represents crossing in the appearance of red blocks in the test administration. Table 4-17 displays the number of correct responses with and without crossing effect for all beverage consumption groups. It is clear that the numbers of correct responses are higher without crossing. No significant difference between beverage consumption groups was found in the correct response with [F (3, 99) = 1.06, ns] and without crossing [F (3, 99) = 2.49, ns].

Table 4-17 Number of correct responses without and with crossing effect according to beverage consumption groups (mean ± SE)

Number of correct responses	Coffee consumers (N=11)	Tea consumers (N=43)	Consumers of coffee and tea (N=20)	Non-consumers of coffee or tea (N=29)
Without crossing	40.8 (2.3)	42.4 (0.9)	38.2 (1.0)	42.1 (1.1)
With crossing	24.6 (2.6)	24.8 (0.7)	23.3 (1.1)	26.1 (1.0)

With crossing, age [$F(1, 95) = 6.43$, $p<0.05$] and WAIS total score [$F(1, 95) = 7.43$, $p<0.01$] were significant covariates with number of correct response (see Appendix 38). Participants who were younger and who obtained higher WAIS total scores showed better performance on this cognitive measure. Age also had a significant interaction with beverage consumption groups [$F(3, 95) = 3.79$, $p<0.05$] in the sequence shown with crossing (see Appendix 39). Younger participants in all beverage consumption groups showed better performance on this Corsi measure. Without crossing, WAIS total score showed a significant interaction with the number of correct responses [$F(1, 95) = 4.53$, $p<0.05$] (see Appendix 40) and beverage consumption groups [$F(3, 95) = 3.03$, $p<0.05$] (see Appendix 41). Participants with higher WAIS total scores showed better performance in this Corsi measure in all beverage consumption groups.

(v) The effect of crossing – reaction time

Table 4-18 displays the reaction time for correct responses with and without crossing effect for all beverage consumption groups. It is clear that the times taken for correct responses are longer without crossing. No significant difference between polyphenol consumption quartiles was found in the time taken to correctly complete the sequence with [$F(3, 99) = 1.32$, ns] and without crossing [$F(3, 99) = 0.97$, ns].

Table 4-18 Reaction time for correct responses without and with crossing effect according to beverage consumption groups (mean ± SE)

Reaction time for correct responses	Coffee consumers (N=11)	Tea consumers (N=43)	Consumers of coffee and tea (N=20)	Non-consumers of coffee or tea (N=29)
Without crossing	1034.3 (78.7)	955.2 (23.5)	957.1 (34.7)	940.2 (21.3)
With crossing	1053.1 (104.0)	935.6 (20.2)	970.6 (38.1)	951.9 (28.0)

With crossing, age [F (1, 95) = 20.61, p<0.001] and WAIS total score [F (1, 95) = 7.06, p<0.01] showed significant interactions with time taken for correct responses (see Appendix 42). Participants who were younger and who obtained higher WAIS total scores showed better performance on this cognitive measure. Significant interactions were also found between these covariates and beverage consumption groups (age [F (3, 95) = 3.72, p<0.05] and WAIS total score [F (3, 95) = 5.09, p<0.01]) (see Appendix 43). Without crossing, two interactions were found namely between age and number of correct responses [F (1, 95) = 41.70, p<0.001] (see Appendix 44) and WAIS total score and beverage consumption groups [F (3, 95) = 3.31, p<0.05] (see Appendix 45). Younger participants and participants who obtained higher WAIS total scores showed better performance on this cognitive measure.

4.4.11.4 Tower of Hanoi

This test consisted of 5 levels (Levels 4 to Level 8), corresponding to the numbers of moves required for each level. For the analysis, 'Level' was added as an additional within subjects' factor. Therefore a 5x4 (Level*beverage consumption groups) ANOVA was calculated. The main effect of Level was significant for the number of errors over all levels [F (4, 396) = 9.97, p<0.001] and indicates that an

increase in errors occurred with increasing difficulty level (see Figure 4-24). There was no main effect of beverage consumption groups in the number of errors made over different levels [F (12, 396) = 0.74, ns]. None of the covariates were significant with Level [largest F (1, 98) = 2.16, ns)] or beverage consumption groups [largest F (3, 98) = 0.89, ns)] (see Appendix 14).

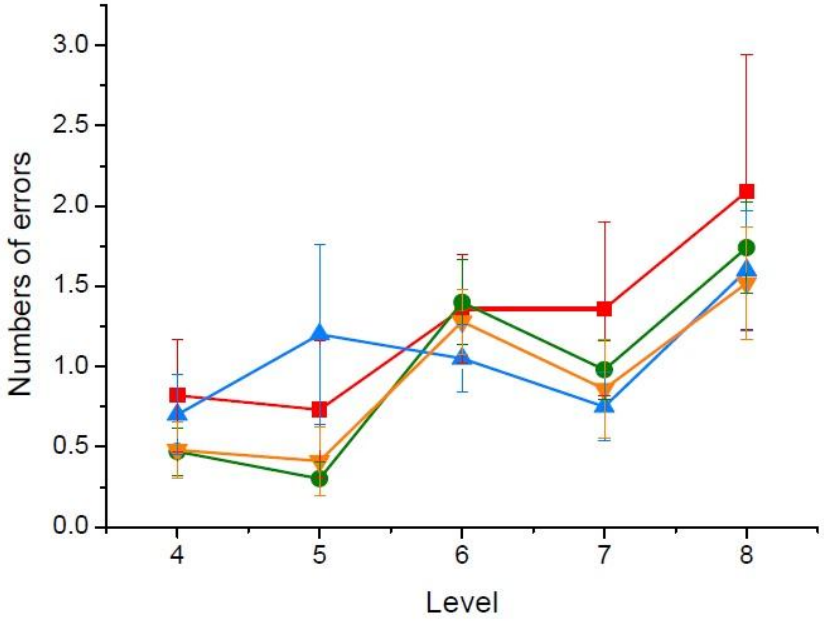


Figure 4-24 TOH mean error per level according to polyphenol consumption quartiles [(■) coffee consumers, (●) tea consumers, (▲) consumers of coffee and tea, (▼) non-consumers of coffee or tea] (mean ± SE)

4.4.12 Subjective evaluation of cognitive performance according to beverage consumption groups

Figure 4-25 displays the participants' subjective evaluation of their cognitive performance reported after completing the cognitive test battery (see Appendix 11). Generally, participants' ratings indicated that they exerted high effort and concentration during the test administration but time pressure was not a concern for

the participants during the test. ANOVAs were performed to compare polyphenol consumption quartiles on each of the visual analogue rating scales. There were no significant differences in visual analogue rating scales between the beverage consumption groups (see Appendix 14).

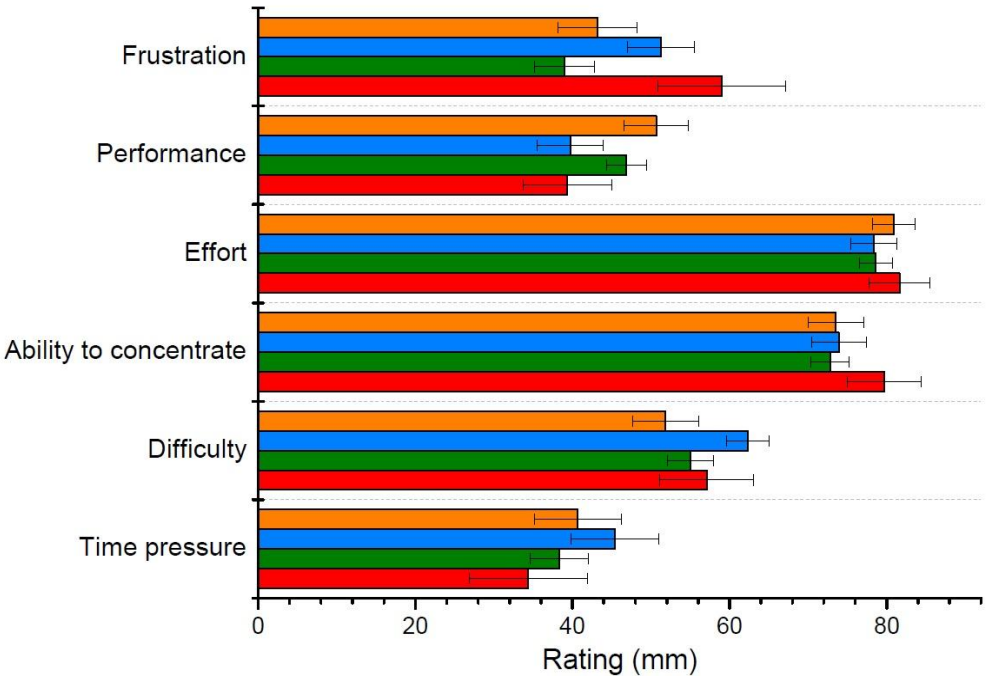


Figure 4-25 Subjective evaluation of cognitive performance [(■) coffee consumers, (■) tea consumers, (■) consumers of coffee and tea, (■) non-consumers of coffee or tea] (mean ± SE)

4.4.13 Summary of cognitive findings

Table 4-19 summarises the results of the cognitive tests analyses performed in this study according to two approaches of data presentation namely; polyphenol consumption quartiles and beverage consumption groups (see Section 4.2.6). Only results from the cognitive tests which have shown significant differences between quartiles and/or beverage groups are reported in the table below.

Table 4-19 Summary of findings from cognitive tests performed in the study

Cognitive domain	Cognitive test	Outcome variables	Different between beverage consumption groups	Different between polyphenol consumption quartiles (percentile)
Verbal memory immediate	VVLT	Rate of learning		
		New learning		
		Proactive interference		
		Retroactive interference		
	VVLT Recognition	Accuracy		
		Reaction time		
Verbal memory delayed		Delayed recall		
Spatial memory immediate	VSLT	Correctly identified designs		
		Correctly identified positions		
		Correctly identified target designs placed in the correct positions		
		Total immediate recall		
	Corsi	Correct responses (overall)		
		Reaction time (overall)		
		Correct responses (crossed trial)		
		Reaction time (crossed trial)		
		Correct responses (uncrossed trial)		
		Reaction time (uncrossed trial)		
Spatial memory delayed	VSLT	Correctly identified designs		
		Correctly identified positions	** [NCCT > T > C > CCT]	**[VL > L > M > H]
		Correctly identified target designs placed in the correct positions	**[NCCT > C > T > CCT]	*[VL > L > M > H]
		Total delayed recall	**[NCCT > C > T > CCT]	**[VL > L > M > H]
Executive function	TOH	Mean sum errors		
		Mean sum think time (overall)		

*p<0.05, **p<0.01, C: coffee consumers, T: tea consumers, CCT: consumers of coffee and tea, NCCT: non-consumers of coffee or tea, VL: very low, L: low, M: moderate, H: high,

There are several cognitive measurements which showed the same effects for both approaches to categorisation of polyphenol consumption. For example, significant differences between polyphenol quartiles or beverage consumption groups was found on the VSLT including correctly identified positions in delayed recall, correctly identified designs placed in correct positions in delayed recall and total delayed recall. Participants with very low polyphenol intake and participants who were non-consumers of coffee or tea performed better on these cognitive measures.

In the analyses of covariates, age and intelligence frequently predicted performance on the cognitive tests. From the analyses performed according to polyphenol consumption quartiles, age was the main predictor of cognitive performance. Both age and WAIS total score were the main predictors of cognitive performance in the analyses performed according to beverage consumption groups. The findings suggest that younger participants and participants with higher WAIS total scores showed better cognitive performance. For example, results from the Corsi test in terms of the number of correct responses (without crossing effect) showed that younger participants in all polyphenol consumption quartiles have scored higher than their older counterparts (see Appendix 27). The same pattern was found in the result presented based on beverage consumption groups for Corsi number of correct responses (with crossing effect), such that younger participants in all beverage consumption groups performed better (see Appendix 39). In terms of intelligence, those participants in all beverage consumption groups with higher WAIS total scores took less time to make correct responses on the Corsi test (see Appendix 36).

Table 4-20 displays the distribution of the participants based on both polyphenol consumption quartiles and beverage consumption groups. From the table, consumers of both coffee and tea were mostly categorized as the high polyphenol consumers (60 %) while the majority (72.4 %) of the non-consumers of coffee or tea were categorized as the low polyphenol consumers. These finding coincides with the fact that coffee and tea are the major polyphenol food sources of the studied population and the regular intake of these beverages contributed majorly to high polyphenol intake. The high polyphenol consumers and the consumers of both coffee and tea were older than the other groups (see Table 4-5 and Table 4-12), which might be the possible explanation for the poor performance of these groups on the cognitive tests administered.

Table 4-20 Participants' distribution according to polyphenol consumption quartiles and beverage consumption groups [number (%)]

	Very low polyphenol consumers	Low polyphenol consumers	Moderate polyphenol consumers	High polyphenol consumers	Total (number)
Non-consumers of coffee or tea (N=29)	21 (72.4)	7 (24.1)	1 (3.5)	0 (0)	29
Consumers of coffee and tea (N=20)	0 (0)	1 (5.0)	7 (35.0)	12 (60.0)	20
Coffee consumers (N=11)	1 (9.0)	4 (36.4)	3 (27.3)	3 (27.3)	11
Tea consumers (N=43)	3 (7.0)	14 (32.6)	15 (34.9)	11 (25.5)	43
Total (number)	25	26	26	26	

4.4.14 Variables associated with polyphenol intake

Correlational analysis was performed between outcome variables from each cognitive test (see Table 4-21) and demographic data, dietary variables and polyphenol intake. Only significant correlations are reported in this chapter (see Table 4-22). Furthermore, separate correlation analysis between cognitive outcomes and demographic data, dietary variables and polyphenol intake was performed controlling for energy intake was also executed. However, because not much difference in the proportion of variance accounted for (R^2) was found between the two analyses, R^2 after controlling for energy intake is not reported in this chapter. A positive correlation implies that there is an increase in cognitive performance scores proportionate with an increase in polyphenol intake. On the other hand, a negative correlation suggests a decrease in cognitive test scores with increases in polyphenol intake.

Table 4-21 Selected outcome variables for each cognitive test

Cognitive test (s)	Outcome variables
Visual Verbal Learning Test	-Total number of words correctly recalled in delayed recall
VVLT Recognition Test	-Reaction time for correct response for words presented visually -Reaction time for correct response for words presented using aurally -Total number of correctly recognised words Reaction time for all correct responses
Visual Spatial Learning Test	-Number of correctly identified designs and positions in delayed recall
Corsi Block Tapping Test	-Total number of correct responses Reaction time for all correct responses

Results from the correlation analysis shows inverse associations between age and BMI with the outcome variable from VSLT ($p<0.01$) and Corsi total number of correct responses ($p<0.01$) respectively. These findings suggest that older participants with higher BMI performed worse on these cognitive measures. A positive association was found between Corsi reaction time for all correct responses and two other variables namely age ($p<0.05$) and BMI ($p<0.01$).

Results from WAIS vocabulary subscale were positively correlated with reaction time on the VVLT recognition test, which suggests that higher scores on the vocabulary subscale are associated with a longer time taken to correctly recognise words from the different lists. The WAIS matrix reasoning subscale showed positive associations with total number of words recall on VVLT (delayed) and the number of correct response for words presented visually on VVLT Recognition. These findings suggest better recall in words presented visually amongst participants with higher scores on the matrix reasoning subscale. Performance on the Corsi test was positively associated with NART score, such that participants with higher NART scores took longer to make correct responses.

Nutrients and food intake were also associated with several cognitive tests. For example dietary fibre intake assessed from DINE and volume of coffee consumed daily was negatively associated with performance on the VSLT measure. This finding suggests that high fibre and high coffee consumption were associated with poorer performance on this measure. The negative finding between VSLT and volume of coffee consumed daily is consistent with the inverse association between VSLT and daily intake of total phenolic acid and total polyphenols. Corsi task performance was positively associated with LWW DINE but negatively associated

with daily coffee consumed. These findings are contradictory to the findings from VSLT.

Unit of alcohol intake daily was negatively associated with VVLT recognition, such that longer times were taken to correctly recognise words aurally and overall time taken for correct responses was longer amongst participants with lower alcohol intake. Daily total flavonoid intake and volume of tea consumed showed positive associations with VVLT recognition. These findings are to be expected because tea is one of the major flavonoid food sources. A previous cross-over study performed amongst adults using several types of tea reported an association between tea consumption and increase in alertness and performance assessed from a set of cognitive battery (Hindmarch et al., 1998). Previous study reported that frequent tea drinking habits has imposed a beneficial effect in reducing the risk of cognitive impairment in a sample of Chinese elderly after two years follow-up (Ng et al., 2008). In addition, a recent review by Jäger and Saaby (2011) has suggested that an *in vivo* study which used selected flavonoids such as epigallocatechin, a type of flavanol found in tea has the ability to pass through the BBB and potentially exhibit effects on brain function.

Table 4-22 Independent associations of cognitive test score with several variables by correlation analysis (R^2 value^)

Cognitive tests (outcome variables)	Independent variables	R^2 value
VVLT (total number of words recalled in delayed recall)[a]	WAIS (matrix reasoning)	0.038*
VVLT Recognition (number of correct response for words presented visually)[b]	WAIS (matrix reasoning)	0.041**
VVLT Recognition (reaction time for correct response for words presented visually)[b]	Volume of tea consumed per day	0.045**
	Total flavonoids intake per day	0.046**
VVLT Recognition (reaction time for correct response for words presented aurally)[b]	Unit of alcohol per day	-0.075*
	WAIS (vocabulary)	0.058**
	Carbohydrate intake per day	0.040**
VVLT Recognition (reaction time for all correct responses)[b]	Unit of alcohol per day	-0.066*
	Volume of tea consumed per day	0.045**
	WAIS (vocabulary)	0.039**
	Total flavonoids intake per day	0.040**
VSLT (number of correctly identified designs and position in delayed recall)[a]	Age	-0.096*
	DINE Dietary fibre score	-0.043**
	Volume of coffee consumed per day	-0.097*
	Total phenolic acid intake per day	-0.117*
	Total polyphenol intake per day	-0.100*
Corsi (total number of correct responses)[b]	BMI	-0.066*
	LWW DINE	0.057**
	Volume of coffee consumed per day	-0.062**
Corsi (reaction time for all correct responses)[b]	Age	0.056**
	BMI	0.075*
	NART score	0.045**

^Proportion of variance accounted for, *Correlation is significant at the 0.01 level (2-tailed), **Correlation is significant at the 0.05 level (2-tailed), [a]Pearson coefficient correlation, [b]Spearman rho coefficient correlation, [c]Corrected vitamin C from HPLC analysis (refer Table 4-1).

4.4.14.1 Prediction of cognitive performance of UK women

Selected independent variables which were associated with cognitive test scores were selected for regression analysis to predict the factors associated with cognitive performance (see Table 4-23). Performance on VSLT (delayed recall) was predicted by daily total phenolic acid intake and age. The model was statistically significant [F (2,100) = 8.720, $p<0.01$], and accounted for approximately 13.1 % (R^2 value = 0.131) of the variance in the number of correctly identified designs and positions.

The Corsi test has two outcomes which include number of correct response and reaction time for correct responses. The first model significantly [F (3, 99) = 8.760, $p<0.01$] predicted the number of correct responses with BMI, LWW DINE and frequency of coffee consumed daily included as the predictor variables. This model explained approximately 18.6 % (R^2 value = 0.186) of the variance in the outcome variable. The second model for time taken to make correct moves included only age in the prediction [F (1, 101) = 23.213, $p<0.01$]. This model accounted for 17.9 % (R^2 value = 0.179) of the variance in the reaction time for the test.

Reaction time to recognise words in VVLT recognition test was predicted by two variables including unit of alcohol intake daily and WAIS (vocabulary). This model explained approximately 17 % (R^2 value = 0.170) of the variance in the outcome variable.

Table 4-23 Independent associations of cognitive test scores with food intake by multiple linear regression

Cognitive domain	Cognitive test	Independent variables	Regression coefficient (95 % confidence interval)	Model summary (adjusted R^2 value)	B*	P value
Spatial memory	VSLT (correctly identified designs and positions-delayed recall)	Age	-0.039 (-0.080 to 0.001)	0.131	-0.200	0.053
		Total phenolic acid intake per day	-0.001 (-0.002 to 0.000)		-0.254	0.015
	Corsi (total number of correct responses)	BMI	-0.392 (-0.786 to 0.003)	0.186	-0.180	0.052
		LWW DINE score	0.418 (0.119 to 0.717)		0.249	0.007
		Volume of coffee consumed per day	-0.015 (-0.024 to -0.006)		-0.297	0.002
	Corsi (reaction time for correct responses)	Age	7.365 (4.332 to 10.397)	0.179	0.432	<0.001
Delayed auditory and verbal memory	VVLT Recognition (reaction time for all correct responses)	Unit of alcohol per day	-157.206 (-240.103 to -74.308)	0.170	-0.342	<0.001
		WAIS (vocabulary)	19.454 (3.577 to 35.332)		0.221	0.017

*Standardized regression coefficient

In the predictive models shown in Table 4-23, polyphenol intake was not included in the final model because of the multicollinearity between the compound present in either coffee or tea and the volume of drinks consumed. For example, multicollinearity was found for VVLT recognition outcome between frequency and volume of tea with total flavonoids intake. The predictive model for the Corsi test showed multicollinearity between volume and frequency of coffee consumed daily. The only model which included daily total phenolic acid intake in the prediction was for VSLT.

Overall, the weak predictive power of the models reflected by small value of adjusted R square was partly due to the small sample size of the studied population. The adjusted R square values were presented in this chapter because in small samples, the usage of R square value can lead to overestimation of the true value of the variance accounted for in the population (Tabachnick and Fidell, 2007).

4.5 Discussion

This chapter has included results of tests of cognitive performance according to polyphenol consumption quartiles and beverage consumption groups. There were significant differences in age and measures of intelligence (NART and WAIS total score) between both the polyphenol consumption quartiles and beverage consumption groups. These differences necessitated controlling for these variables by including them as covariates in the analysis performed on each outcome variable of the cognitive tests. From the findings, age and WAIS total score are suggested as the predictors of the performance specifically for the tests associated with spatial memory (VSLT and Corsi).

Cognitive tests focusing on the spatial cognitive domain (Corsi and VSLT) tended to demonstrate higher scores in the younger participants (see Section 4.4.13). This finding can be partly be explained by the short duration of the administration of these test. Increased age was associated with a reduction in the number of correct responses and an increase in reaction time. Participants with highest polyphenol intake obtained the lowest scores in VSLT for correctly identified designs and locations. In addition, the total number of recalled items in both immediate and delayed recall was also lower amongst participants in the highest quartile of polyphenol consumption. From all the cognitive tests administered, Corsi was the test which showed greatest sensitivity to the effects of polyphenols. This is supported by the interaction found between polyphenol consumption quartiles and one of the outcome measures of Corsi (see Appendix 27). However, age remained a significant predictor in the performance of this test, such that younger participants performed better than the older participants in all polyphenol consumption quartiles. From this finding, future studies on the association between polyphenol consumption and cognitive performance might usefully employ the Corsi test as one of the cognitive measures. A recent systematic review by Lamport et al. (2012) suggested that spatial memory appeared to be most positively affected by polyphenol intake according to results from both intervention and epidemiological studies. However, our study has found a contradictory result by which individuals with lower polyphenol intake have performed better in Corsi test. This finding was confounded by the age of participants, where younger participants with lower polyphenol intake have better spatial memory performance assessed from Corsi test.

VVLT requires a long term attention span although repetition of the same word list a few times could cause boredom. This test requires motivation and patience to memorize the word list. The only independent variable associated with the number of words recalled in immediate and delayed recall is WAIS (matrix reasoning) however, the association can be considered as weak.

The Tower of Hanoi test showed no significant difference between participants in different polyphenol consumption quartiles or beverage consumption groups. This finding can partly be explained by the fact that no time limit was applied for the test and the participants were allowed to take their own time to solve the problems. This finding coincides with response given on the subjective evaluation of cognitive performance such that participants indicated that time pressure was not a concern for them during the test (see Section 4.4.6 and Section 4.4.12). Less time pressure as compared to other tests administered allowed the participants to make the correct moves in order to arrange the disks as shown by the target formation.

Age of the participants was one of the most important determinants in the results of all cognitive tests. It is suggested that the decline in cognitive performance amongst healthy educated adults can possibly start at an early age during adulthood before the age of 60 years old (Salthouse, 2009). Increases in age can also be associated with an increase in intelligence and knowledge associated with verbal material and larger vocabulary. Figure 4-26 displays the distribution of IQ test according to age. There is no clear pattern on the distribution; however, higher NART (Figure 4-26, A) and WAIS (total score) (Figure 4-26, D) score tend to be elevated in individuals above 30 years old. The present finding seem to be consistent with other research which found highest scores amongst middle-aged adults on the

verbal test in the WAIS whereby the non-verbal test scores were highest score amongst younger adults aged 16 to 34 years old (Ardila, 2007). Another longitudinal study has also reported that verbal ability assessed by several tests including WAIS increased over time (Larsen et al., 2008). A contradictory finding was reported by Crawford et al. (1988) in relation to NART score, whereby in this study, age was suggested to have little or no effect on the score of a wider age range (17 to 88 years) of the adult population.

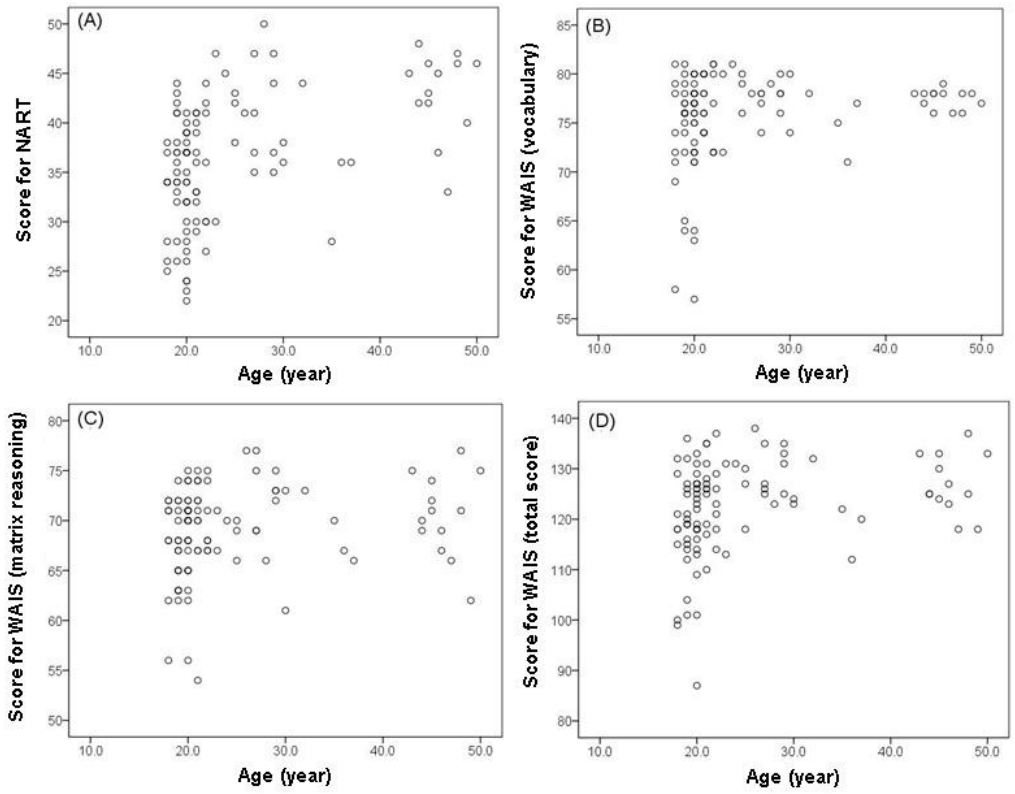


Figure 4-26 Distribution of score for IQ tests according to age of the participants

The current study was limited by a small sample size with the majority of the participants being younger adults. There was also a selection bias, such that the majority of the participants in the study were students and well-educated. Although

age was a significant predictor of cognitive performance, older participants obtained relatively high scores on the intelligence measures in line with the majority of the younger participants. This finding suggests that the participants in this study might not be the appropriate sample to examine the effect of different polyphenol intakes on cognitive performance. A study which includes a cognitively challenged sample such as the elderly might potentially be better able to demonstrate an effect of polyphenol intake on cognitive performance. This is corroborated with findings from previous cross-sectional studies amongst elderly participants in whom a high intake of selected flavonoid containing foods was associated with better cognitive performance in a dose-dependent manner (Nurk et al., 2009a, Nurk et al., 2010).

An analysis was performed to identify the age distribution of polyphenol intake in Total and NoCorT dataset Polyphenol intake from Total dataset. Figure 4-27(A) shows a trend for increased intake with increases in age. This is supported by a significant difference in age of the participants, whereby consumers of both drinks were generally older than other beverage consumption groups ($\chi^2 = 17.35$; df = 3; $p<0.01$). Polyphenol intakes of ≥ 1 g/day were found amongst participants aged 30 years and above. After the contribution of coffee and tea was removed from the polyphenol intake estimation (NoCorT dataset), age of the participants did not remain the main determinant of intake (see Figure 4-27, B). It can be deduced that polyphenol intakes are higher amongst older participants, and which were mainly contributed by coffee and tea drinking habits. A study amongst Australians reported a trend for increased flavonoid intake as age increased (Johannot and Somerset, 2006). The authors suggested that the difference shown in the flavonoid intake among adults was primarily accounted for by an increase in tea drinking habits with incremental age.

Increased age can also be associated with higher total energy, thus contributing to higher total polyphenol intake. In the current study, energy intake was highest amongst participants in the highest quartile of polyphenol consumption; although no significant difference was found between the quartiles (see Table 4-6). This finding is supported by a previous study performed amongst adults in the Netherlands which reported higher total energy intake amongst high flavonoids consumers (Hughes et al., 2008).

In this study, the polyphenol intake of the participants who were non-consumers of coffee or tea was mainly contributed by foods including fruits and vegetables (see Section 2.4.3). For example, onion and potato intake in various forms were widely consumed by the participants in this group. In addition fruit such as apples and bananas were consumed regularly by participants in this group. A previous study among healthy adults in Germany reported that participants who have fulfilled the recommended daily intake of fruit and vegetables obtained better cognitive scores as compared to participants with lower intake of these foods (Polidori et al., 2009).

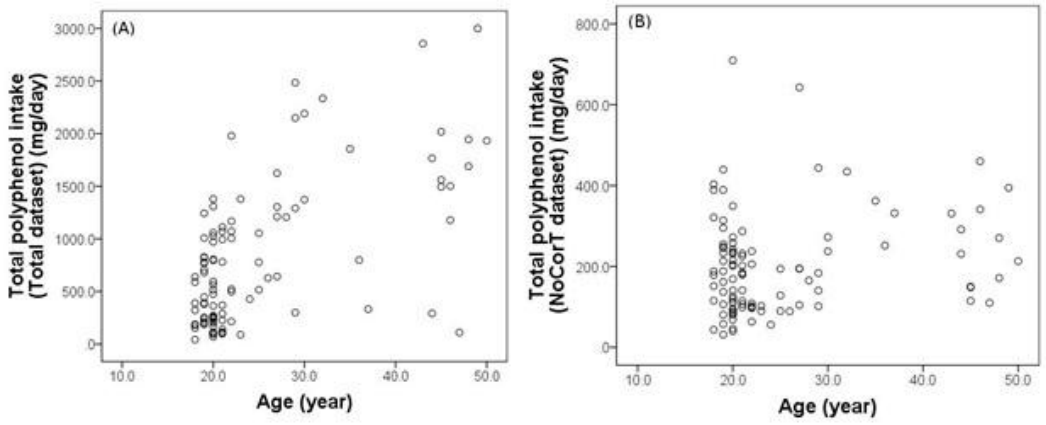


Figure 4-27 Distribution of polyphenol intake [from Total (A) and NoCorT (B) dataset] according to age of the participants

4.6 Conclusion

In this study, the association between polyphenol intake and cognitive performance assessed from several cognitive tests has been analysed. It can be summarized that only results for spatial memory from VSLT test showed a significant difference between polyphenol consumption quartiles and beverage consumption groups. Other domains (refer Table 4-2) did not show any differences associated with polyphenol intake. Overall, there is a trend for increases in test scores and decreased reaction time as polyphenol intake decreased. Age was an important determinant of polyphenol intake and this factor related to coffee and tea drinking habits amongst the older participants. No generalization can be made about the polyphenol intake of UK women in general as this study consisted mainly of younger adults (aged 20 to 30 years old). A more evenly spread age range of participants is warranted for a better understanding of the possible association of polyphenol intake with cognitive performance. In summary, better performance on spatial memory was shown by non-consumers of coffee or tea and the very low polyphenol consumption quartiles, thus the hypothesis for this study is rejected. The insignificant contribution of two major polyphenol sources namely coffee and tea to cognitive performance can partly be explained by the age factor. This finding explains in part the finding that the contribution of polyphenol intake to the cognitive performance assessed in this study is minor.

Chapter 5
General Discussion

5.1 Summary of thesis findings

The main aim of this thesis was to estimate the polyphenol intake (Chapter 2) and identify any association with cognitive performance in a subsample of UK women (Chapter 4). Polyphenol analysis performed in the current study covered selected items which were commonly consumed by the participants from the food diary analysis for which data was lacking (Chapter 3). A summary of polyphenol intake in the UK women studied in this thesis is shown in Table 5-1.

Table 5-1 Summary of polyphenol intake from Total and NoCorT dataset*

	Mean (S.D)	Median	Percentile	
			25	75
Total dataset				
Total flavonoids	611.2 (602.0)	431.5	128.8	886.6
Total phenolic acids	434.6 (493.1)	213.3	84.7	652.6
Total other polyphenols	43.0 (42.7)	28.8	13.5	59.7
Total polyphenols	1088.7 (813.6)	940.8	388.6	1598.7
NoCorT dataset				
Total flavonoids	109.8 (79.6)	90.0	52.2	148.7
Total phenolic acids	63.7 (54.5)	54.7	35.8	75.5
Total other polyphenols	39.2 (42.0)	24.9	9.3	56.1
Total polyphenols	212.7 (129.4)	188.4	110.8	276.7

*Results presented for all studies samples (N = 246), S.D: standard deviation, NoCorT: no coffee or tea dataset.

The key findings of the thesis are summarized below:

1. The average total polyphenol intake estimated from 20 polyphenols present in commonly consumed foods exceeded 1 g per day.
2. The major polyphenol food sources of the women studied in this thesis are tea and coffee, thus women not consuming tea or coffee have much lower average polyphenol intake (192 mg/day).
3. Age is the main factor predicting flavonoid, phenolic acid and total polyphenol intake.
4. The total phenolic content of processed foods, as assessed by the Folin-Ciocalteau method is lower than in fresh foods.
5. Participants in the lowest quartile of polyphenol intake and who were non-consumers of coffee or tea were younger and showed better performance on spatial memory assessed by the Visual Spatial Learning Test (VSLT).
6. Age of the participants was one of the most important determinants of the results of cognitive tests, whereby younger participants showed better cognitive performance as compared to the older participants.
7. Daily total phenolic acid intake was the only independent variable associated with polyphenol intake which was included in the model to predict spatial memory performance.

5.2 Estimation of polyphenol intake

The total polyphenol intake of the women gave an average of 1089 mg/day (see Table 5-1), similar to the finding from a previous study in France of 1193 mg/day (Perez-Jimenez et al., 2011). In the current study, coffee and tea were the major polyphenol food sources consumed by the participants, with nearly 40% of the whole sample drinking both tea and coffee, followed by 33% who consumed tea only. Flavanols were the highest flavonoid subclass to be consumed, while hydroxycinnamic acid was the most highly consumed phenolic acid. The contribution of these two compounds to the total polyphenol intake in the NoCorT dataset remained with the addition of flavonols because these compounds are widely present in various food sources such as fruit, vegetables and chocolates.

Total phenolic content of selected foods was determined by using the Folin-Ciocalteau assay. Further HPLC analysis was performed on blackcurrant concentrates to quantify the anthocyanins and vitamin C content of this product since it was consumed by a significant proportion of the women in the study. The new data generated was compared to the original assumptions made to calculate the data in Chapter 2, and no significant difference was found.

5.3 Polyphenol intake and cognitive performance

Several cognitive tests were performed to assess the association between polyphenol intake and cognitive performance in the women. The VSLT test was the only test used that showed a significant difference between high and low polyphenol intake. VSLT assesses the spatial memory, during which the prefrontal cortex in the brain area is activated (Thorp et al., 2009). Our study has found a negative association between polyphenol intake and spatial memory performance, whereby

individuals with lower polyphenol intake have performed better in this cognitive domain. However, there were several animal studies have found a better spatial memory performance with polyphenol supplementation. For example, a blueberry supplementation study (500 mg/day of blueberries for 12 weeks) among three different groups of rat (young, old and old-supplemented) compared spatial working memory by using a cross-maze alternation task (Williams et al., 2008b). Results showed that more correct choices were made by the old-supplemented group than the old group, although the number of correct choices made was lower than for the young group. In addition, less time was taken to make correct choices by the supplemented group as compared to their age matched counterparts. This finding was supported by the finding of increased levels of brain-derived neurotrophic factor (BDNF) in the hippocampus of the supplemented rats. An increase in neuronal activity has also been shown to promote the secretion of this neurotrophin (Balaratnasingam and Janca, 2012).

A recent study performed in aged rats administered blueberry and pure flavonoids in equal amounts present in the blueberry diet to the animals for 6 weeks (Rendeiro et al., 2013). This study found an improvement in the performance of spatial memory as measured by the cross-maze alternation task in the intervention groups over the study period. Analysis of BDNF performed on the brains of the rats found an increased level of this protein in animals which received the interventions. This finding suggested that flavanols and anthocyanins consumed at a dietary level as present in blueberries may have a positive effect on spatial memory mediated by the neurotrophin BDNF. Although no direct extrapolation can be made to humans based on the findings from the animal studies, new information was obtained about the possible mechanisms of the effect of polyphenols on cognitive performance.

In order to demonstrate a beneficial effect on humans, polyphenols are required to to pass through the brain. The Blood Brain Barrier (BBB), which is formed by the brain capillary endothelial cells, plays a role as a physical and metabolic barrier to selectively allow transport of compounds into the brain (de Boer and Gaillard, 2007). A review by Jäger and Saaby (2011) has suggested that an *in vivo* study which used selected flavonoids such as epigallocatechin, (a type of flavanol) and several flavanones have the ability to pass through the BBB and exhibit effects on brain function. On the contrary, an *in vitro* study using several metabolites of hydroxycinnamic acids produced after coffee consumption displayed low permeability to the brain (Lardeau and Poquet, 2013). However, authors have suggested that the indirect effect of these compounds on the brain by protecting the BBB is an important role to be highlighted. Taken together, polyphenols show a promising potential benefit relevant to human cognitive performance but more studies are needed to provide more definitive evidence and elucidate the exact mechanism.

5.4 Implications of the findings

The findings of this study have a number of important implications which may help in future practice. Firstly, we have determined the polyphenol intake of a sub-sample of UK women. These findings enhance our understanding of the major polyphenol food sources consumed by the participants. In addition, the findings from this study suggest that age is an important factor associated with polyphenol intake. There is, therefore, a definite need to promote the intake of polyphenol from food sources other than coffee and tea to younger women and to make them aware of other polyphenol-containing food sources widely available in the market.

Another important practical implication is that this study has combined data of food intake from food diaries and food analysis in the estimation of polyphenol intake. Although only data for blackcurrant concentrate consumers was improved after HPLC analysis, this approach can be proposed as an essential step for better polyphenol intake estimation.

We have performed a pilot study assessing the possible association of polyphenol food intake and cognitive performance. In our study, there is no evidence of any significant positive relationship between polyphenol intake and cognitive performance. However, there is a trend of increasing polyphenol intake especially from coffee and tea amongst older participants. Age has become a confounder in the regression analysis performed to determine quantitative variable associated with cognitive function. A suggestion can be made that polyphenol sources other than coffee and tea could offer potential benefits for cognitive performance. However, the positive impact will depend on the age of the participants. This is supported by a better performance in spatial memory shown by participants who were younger and were non-consumers of coffee or tea. The findings from the current study contribute new information about the association of polyphenol intake and cognitive performance.

5.5 General strengths and limitations

The current study has made a better estimation of total polyphenol intake in UK women as compared to previous studies performed among the UK population (Beking and Vieira, 2011, Zamora-Ros et al., 2011a, 2011b, 2013b). Primarily, the improved polyphenol intake estimation is a result of including more polyphenol classes in the estimation which includes phenolic acids and other minor polyphenols.

Furthermore Zamora-Ros et al. (2011a, 2011b, 2013) used a 24-hour recall, whereas Beking and Vieira (2011) was based on Food Balance Sheet, methods which are less accurate and less likely to obtain a comprehensive assessment of the variety of polyphenols consumed than the diary methods employed in this thesis. The current thesis used data collected from food diaries (3 or 7 day), increasing the detail on food and polyphenol servings and accuracy of estimation.

In addition, the inclusion of thearubigins in the estimation of flavanols was demonstrated to be an important approach for a better estimation of polyphenol content in tea. The importance of this compound was reported by the EPIC study which focused on thearubigin intake in several European countries (Zamora-Ros et al., 2013c). This study has reported that the UK general population were the highest tea consumers, with 48 % of total flavonoids being contributed by thearubigins.

Tests of various cognitive domains including verbal memory, spatial memory and problem solving administered to the participants in the current thesis provided an insight into the possible association of polyphenol intake and cognitive performance. The selection of these domains was based on the findings reported by Lamport et al. (2012). The authors suggested that the selection of cognitive tests included in the study must be based on specific brain regions targeted by specific polyphenols. In addition, verbal, spatial and executive function (or problem solving) domains were evident to potentially have an effect on human cognitive function and were considered relevant to be included in the study.

The current study was limited by a small sample size of participants recruited for the study (N = 246 for diary analysis, N = 103 for cognitive study). The large range in age of the participants (18 – 50 years) might also contribute to the identification of this variable as the one of the predicting factors of polyphenol

intake. There was a selection bias in the recruitment for the cognitive study as most of the participants were students and well-educated despite significant attempts at recruitment of women from across the Leeds region. In addition, being health-conscious might be a possible motivating factor for the participants to join the study, or might influence the foods consumed by the participants during the dietary recording. Thus, the representativeness of this sample to the general population may not be great. Our study is based on a small group of participants and has found no potential association between polyphenol intake from various sources and better cognitive performance which contradicted with positive effects reported from several studies (Nurk et al., 2009b, Nurk et al., 2010, Polidori et al., 2009).

Food recording can possibly cause some alterations in the habitual food intake of the participants. However, to deal with this possibility, participants were encouraged to bring all food packaging along with them in case they had difficulties in explaining the food portion size. The participants were also advised to be honest about their intake, especially with respect to the intake of foods which might be perceived as "unhealthy" such as chocolate or snacks.

There is a substantial lack of information on the polyphenol content of processed foods. Because of time constraints and the frequency with which these were consumed in the samples studied, blackcurrant concentrates were selected for the full HPLC analysis. Although the specific content of anthocyanins and vitamin C in blackcurrant concentrates did not affect the overall intake estimated in Chapter 2, this approach should be taken in future studies to provide a complete and accurate estimation of the polyphenol content of food intake data.

Results from the cognitive association study reported null findings between polyphenol consumption groups and results from the cognitive test battery except for

Visual Spatial Learning Test (VSLT) test. Participants in the lowest quartile of polyphenol intake showed better cognitive performance as compared to the other groups in the VSLT test ($p<0.01$). Younger participants obtained higher scores on tests from the spatial domain namely Corsi and VSLT. These tests require participants to give responses within a limited time. These finding corroborated those from a previous study which reported a significantly lower score on the Corsi test amongst older adults as compared to younger adults (Anguera et al., 2011). The null finding for the other cognitive tests such as verbal memory can partly be explained by the participants' education level. This finding is supported by a previous study performed in the United States which found that education can potentially benefit the verbal fluency domain and has a smaller influence on processing speed (Zahodne et al., 2011).

5.6 Recommendations for future research

Several suggestions can be made for future research in this field. This includes performing a large population study among various age groups and educational background. This approach will enable a better understanding of the association of food intake and cognitive performance in the context of the whole population. In addition, participants at their middle-age can be recruited to identify the potential positive effect of polyphenol in this age group. A comparison in polyphenol intake can also be performed between persons who are vegans or vegetarians and individuals who consumed diet from mix food sources. Habitual food intake is quite difficult to define, as it may be affected by many factors including the intake of seasonal foods, food availability, and food preference. A

longitudinal study to identify the diversity of polyphenol food sources throughout different seasons is much needed for a better estimation of polyphenol intake.

Kyle and Duthie (2006) made some suggestions to improve the estimation of flavonoids intake of a population which is also relevant to polyphenol estimation. This study developed a flavonoid database based on the foods commonly consumed by the population studied. These suggestions include:

(1) To perform a constant up-date on the flavonoids content using compositional analysis to compensate the diversity of food consumption of the population.

(2) To consider the recipe calculation of composite dishes to increase the accuracy of polyphenol intake estimation.

(3) To consider the proportion of polyphenol-containing ingredients of retail products such as canned or pre-prepared foods in polyphenol estimation.

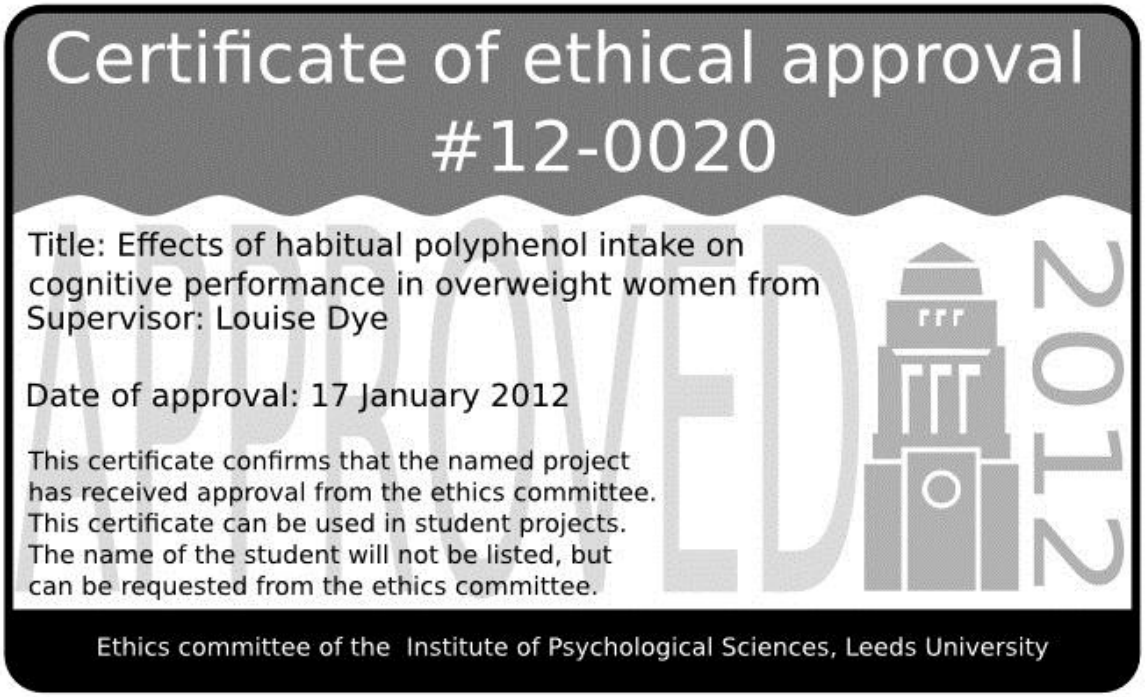
Suggestions (2) and (3) have been considered in the estimation of polyphenols in the current thesis. There is, therefore, a definite need for analysis of various processed foods which are commonly consumed by the population to improve the database. With respect to the current thesis, the possible foods to be analysed include different types of tea such as fruit yoghurts, instant soups and curry sauces. The effect of food processing such as steaming, baking or frying on the polyphenol content of foods can be determined by using retention factors. In the latest version of Phenol-Explorer database, this factor has been included, by which the weight changes as a result from water loss in the foods during processing was considered in the calculation. However, the data for retention factor only available for some of the foods in the database. The addition of retention factors will allow a better estimation of the polyphenol intake of the population.

5.7 Overall conclusions

The results from this study provide a promising approach to be taken in estimating the population's polyphenol intake. The effect of food processing on the polyphenol content of foods should be taken into consideration in subsequent research. The null finding on the effect of polyphenols on cognitive test results, apart from negative association between polyphenol intake and spatial memory, show potential possibility for future investigation using different tests and cognitive domains and different, more varied samples, which has been applied in several studies before. Moreover, future research is warranted in order to develop a better understanding of the association between polyphenol intake and cognitive performance, which in turn could help in the promotion of the consumption of polyphenol-containing foods for better health.

Appendices

Appendix 1 Certificate of ethical approval



Appendix 2 Participant information sheet

UNIVERSITY OF LEEDS

Human Appetite Research Unit
Institute of Psychological Sciences
University of Leeds
Leeds LS2 9JT

Hanis Yahya & Faye Clancy
Email: LWWCOG@leeds.ac.uk

PARTICIPANT INFORMATION SHEET

Effects of habitual polyphenol intake on cognitive performance in women

We would like to invite you to take part in our research study. Before you decide we would like you to understand why the research is being done and what it would involve for you. Please take time to read the following information carefully and discuss it with others if you wish. Please ask us if there is anything that is not clear or if you would like more information. Please take your time to decide whether or not you wish to take part.

What is the purpose of the study?

Polyphenols are produced by plants and are found in tea, coffee, in fruits, vegetables, cocoa, chocolate and cereals. Polyphenols have been associated with better cognitive performance in some studies; however, habitual or usual polyphenol intake has not been investigated and may be of greater importance. Accurate assessment of total polyphenol intake from diet is important in order to determine the role of these compounds in health and wellbeing. This study is looking at the association between habitual polyphenol intake and cognitive performance among women.

The aims of this study are:

1.) To assess habitual polyphenol intake in UK women across range of body mass index (BMI) from normal to obese.
2.) To examine relationship between polyphenol intake and cognitive performance.
3.) To examine the relationship between polyphenol intake and psychological and behavioural characteristics

Why have I been invited?

You are invited to consider participating in this study because;

a) you have already indicated (in your initial contact or recruitment information questionnaire) that you are willing to be contacted about further research or,
b) have expressed an interest to participate in this study by responding to posters, flyers and email advertisements that we have distributed around the University of Leeds campus and the local area.

Do I have to take part?

It is up to you to decide whether or not to take part. If you do decide to take part you will be given this information sheet to keep and be asked to sign a consent form. If you decide to take part you are still free to withdraw at any time, without giving a reason.

What will happen to me if I take part?

You will be asked to come to Human Appetite Research Unit (HARU), University of Leeds on two occasions. During the first visit, you will be required to fill out a recruitment information questionnaire to tell us about yourself and your general health. This first visit will also familiarise you with the cognitive tests of memory and problem solving so that you know what to expect on the actual cognitive test day. This practice session will take about 1 hour. You will be required to read the participant information sheet (PIS) and sign a consent form. During this visit you will be given a food diary to complete where you report your food intake using household measures on 2 weekdays and 1 weekend day. Additionally, you will be asked to complete some questionnaires. The first visit will last for about 1 hour and 30 minutes.

The second visit will need to be within 7 days of the practice session. During this visit you will be required to perform a slightly longer version of the cognitive tests. You will be asked to refrain from eating or drinking (except water) for 2 hours before the test session. This battery will consist of computerised tests of both verbal and spatial memory and problem solving (which you will have already practiced at the first visit). You will also complete the National Adult Reading Test (NART) and the vocabulary and matrix reasoning from the Wechsler Adult Intelligence Scale. This visit will last for about 1 hour and 30 minutes. The 3 day food diary will need to be returned during this visit.

What are the possible disadvantages and risks of taking part?

There are no health risks related to participating in this study.

What are the possible benefits of taking part?

We cannot promise that this study will help you but this research study may provide important new information regarding habitual polyphenol intake and cognitive performance, which in turn could help with improve dietary advice for women.

What if something goes wrong?

If you are harmed by taking part in this research project, there are no special compensation arrangements. If you are harmed due to someone's negligence, then you may have grounds for a legal action but you may have to pay for it. Any complaint about the way you have been dealt with during the study or any possible harm you might suffer will be addressed. If you wish to complain or have any concerns about any aspect of the way you have been approached or treated during the study you should contact the principal investigators (Professor Louise Dye, Professor Gary Williamson or Dr Andrea Day) who will investigate your complaint. If you remain unhappy and wish to complain formally, this can be done through the University complaints procedure.

Will my taking part in the study be kept confidential?

Yes. We will follow ethical and legal practice and all information about you during the study will be handled in confidence. All information that is collected from you will be treated in the strictest of confidence at all times and will only be used for the purposes of this research. All data will be recorded safely using the code that you were given during the recruitment period. The link between your name (and other personal data) and your unique study identity code will be maintained and stored securely in the HARU at the University of Leeds and will only be accessible to the University research team. Anything that you say will be treated in confidence and no names will be mentioned in any reports of the study. Some results from the study will be used towards an educational qualification by members of the research team. Individuals will not be identifiable from any details in reports, presentations or scientific publications based on the results of the study.

What will happen to the results of the research study?

Once all participants have completed the study, the information obtained will need to be collated and analysed before any results are published. If you would also like to know the results of the study, the research team will be able to give this information to you when it becomes available. Remember that your own results are confidential and that your name will not be associated with any information published from this study.

Will I receive anything for taking part?

Upon completion of the study visit you will receive £10 to compensate you for the time that you have invested in the study.

Who is organising and funding the research?

This research is a collaboration between School of Food Science and Nutrition, University of Leeds and Human Appetite Research Unit (HARU), Institute of Psychological Sciences (IPS) University of Leeds. The PhD studentship for Hanis Yahya, who is conducting the study is funded by Ministry of Higher Education Malaysia. Faye Clancy is funded by a Wellcome Undergraduate Research Bursary. Emily Christodoulou is an undergraduate student at IPS, University of Leeds and some of the data will be used for her research dissertation.

Who has reviewed this study?

This study has been reviewed and given a favourable opinion by University of Leeds Research Ethics Committee (Ref No: 12-0020).

Who do I contact for further information?

If you want further information about this study or information regarding this research or if you need extra advice please contact one of the following researchers:

Hanis Yahya & Faye Clancy
Email: LWWCOG@leeds.ac.uk
Mobile: 07741069434
Telephone: 0113 343 2284

This study is supervised by Professor Louise Dye (l.dye@leeds.ac.uk), Dr Andrea Day (a.j.day@leeds.ac.uk) and Professor Gary Williamson.

Finally, thank you for taking the time to read this information.

Appendix 3 Informed consent form

INFORMED CONSENT FORM

Effects of habitual polyphenol intake on cognitive performance in women

		Please initial
1	I confirm that I have read and understood the Participant Information Sheet dated 30th May 2012 (Version 5) for the above study. I have had the opportunity to consider the information, ask questions about the study and have had these answered satisfactorily.	_____
2	I understand that my participation is voluntary and that I am free to withdraw at any time without giving any reason, without my medical care or legal rights being affected. In addition, should I not wish to answer any particular question or questions, I am free to decline.	_____
3	I understand that data collected during the study, may be looked at by individuals from the University research team, collaborators on the research project and the University of Leeds for the purposes of research governance. All data will be anonymised with the exception of the recruitment questionnaires containing personal data. I give permission for these individuals to have access to my data. I understand that my name will not be linked with the research materials, and I will not be identified or identifiable in the report or reports that result from the research.	_____
4	I agree to take part in the above study	_____

_____	_____	_____
Participant's name	Date	Signature
_____	_____	_____
Researcher's name	Date	Signature

30th May 2012 (Version 5)

Appendix 4 Recruitment information questionnaire

RECRUITMENT INFORMATION QUESTIONNAIRE

Date of contact _____ /_____ /_____ Researcher .....

CONTACT INFORMATION

Name .....

Address .....

.....

Contact phone number .....

E-mail .....

Date of Birth ____ / ____ /____ Age .....

GENERAL INFORMATION

Occupation
- Employed Part time ☐
- Employed Full time ☐
- Student ☐
- Unemployed ☐
- Housewife ☐
- Retired ☐
- Other ☐

Has your employment status changed within the last year?.....

If yes, please provide details.....

.....

Do you work night shifts? Yes/No

If yes, how long have you been working night shifts?.....

EDUCATION

How old were you when you finished your full time education? ☐☐ years old

Do you have any of the following qualifications? Tick all applicable

- CSE ☐
- GCE "O" Level ☐
- City & Guilds ☐
- Other ☐ describe ☐
- "A" Level, Highers ☐
- Teaching diploma, HNS ☐
- Degree ☐
- None of these ☐

HEALTH

How would you rate your general health?.....

Do you have or have you had any medical conditions? (i.e. heart condition, asthma, diabetes) ?.....

Are you currently taking any medication or supplements or suffering from any illness?

Yes/No

If yes, please provide the type of medication and how long you have been taking it for.....

.....

Do you have a cardiac pacemaker fitted? Yes/No

Have you started smoking since your last study visit? Yes/No

If yes, how long time ago?.....

PHYSICAL ACTIVITY

In a typical week during the last 12 months, how many hours did you spend on each of the following activities? Put "0" if none

Housework, such as cleaning, washing, cooking, child care		☐☐ hours	☐☐ minutes per week
Do-It-Yourself		☐☐ hours	☐☐ minutes per week
Gardening	In summer	☐☐ hours	☐☐ minutes per week
	In winter	☐☐ hours	☐☐ minutes per week
Walking, including to work, shopping & leisure	In summer	☐☐ hours	☐☐ minutes per week
	In winter	☐☐ hours	☐☐ minutes per week
Cycling, including to work & leisure	In summer	☐☐ hours	☐☐ minutes per week
	In winter	☐☐ hours	☐☐ minutes per week
Other physical exercise, such as keep-fit, aerobics, jogging, tennis, swimming	In summer	☐☐ hours	☐☐ minutes per week
	In winter	☐☐ hours	☐☐ minutes per week

In a normal week, do you do any of these activities vigorously enough to cause sweating or a faster heartbeat? Yes/No

If yes, for how long each week do you do such vigorous physical activity? ☐☐ hours ☐☐ minutes per week

EXERCISE

Do you do regular exercise? Yes / No

If yes, how many times a week do you exercise? One to four ☐

More than four ☐

What type of exercise do you do? .....

How long have you been exercising for?.....

PREGNANCY

Are you currently pregnant? Yes / No

Have you had a baby or have you been pregnant in the last 6 months? Yes/ No

Date of delivery (if applicable) ___ / ___ / ___

Have you breast fed in the last 6 months? Yes / No

MENOPAUSAL SYMPTOMS

Do you think you have reached the menopause (the menopause means not having had a period for 12 months of more)?.....

Are you taking/ have you taken hormone replacement therapy (HRT)?.....

What was the date of your last period? .....

How many periods have you had in the last 12 months?.....

Are you experiencing hot flushes? Yes / No How often?.....

Are you experiencing night sweats? Yes / No How often?.....

DIET

Are you vegetarian? Yes / No If yes are you Vegan? Yes / No

Do you usually consume breakfast? Yes / No

How many times per week do you consume breakfast? .....

What do you normally consume for breakfast?.....

Does it differ at the weekends? Yes / No

Details .....

How many units of alcohol do you usually drink per week? .....

N.B. 1 small (125mls) glass of wine or half a pint of lager or 1 shot of spirits = 1 unit

Has your weight varied within the last 6 months? Yes / No

If yes, by how much? .....

Are you currently on any form of a weight loss diet? Yes / No

Details.....

Have you been on any weight loss diet the last year? Yes/ No

If yes, how long did you follow this diet?.....

Details.....

OTHER INFORMATION

Can we keep this information on file and contact you about future studies? Yes / No

ADDITIONAL NOTES

Appendix 5 Dietary Instrument for Nutrition Education (DINE)

Eating Habits Questionnaire

Purpose

The purpose of this questionnaire is to get an idea of your usual eating habits. For the listed foods, we would like to know how many servings you eat in a typical day or week. A serving is an average portion that would be served at a meal. If you usually eat more than one serving of the food at a time, you should count all the servings you eat.

Instructions

For each food listed, tick the box that describes the number of servings that you usually eat. If you never eat a particular food, tick the box under "None". Do not leave any lines blank.

ID number			
DF score			
TF score			
UF score			

About how many **pieces or slices a day** do you eat of the following types of bread, rolls, or chapattis? (Please tick one box on each line)

	Breads & Rolls	None	Less than 1 a day	1 to 2 a day	3 to 4 a day	5 or more a day
1.	White bread or rolls					
2.	Brown or granary bread or rolls					
3.	Wholemeal bread or rolls					

About how many **servings a week** do you eat of the following types of breakfast cereal or porridge? (Please tick one box on each line)

	Breakfast cereals	None	Less than 1 a week	1 to 2 a week	3 to 5 a week	6 or more a week
4.	Sugared type: Frosties, Coco Pops, Ricicles Sugar Puffs Rice or Corn type: Corn Flakes, Rice Krispies, Special K					
5.	Porridge or Ready Brek Wheat type: Shredded Wheat, Weetabix, Fruit 'n Fibre, Puffed Wheat, Nutri-grain, Start Muesli type: Alpen, Jordan's					
6.	Bran type: All-Bran, Bran Flakes, Sultana Bran					

About how many **servings a week** do you eat of the following foods?
(Please tick one box on each line)

	Vegetable foods	None	Less than 1 a week	1 to 2 a week	3 to 5 a week	6 to 7 a week	8 to 11 a week	12 or more a week
7.	Pasta or rice							
8.	Potatoes							
9.	Peas							
10.	Beans (baked, tinned, or dried) or lentils							
11.	Other vegetables (any type)							
12.	Fruit (fresh, frozen, canned)							

About how many **servings a week** do you eat of the following foods?
(Please tick one box on each line)

		None	Less than 1 a week	1 to 2 a week	3 to 5 a week	6 or more a week
13.	Cheese (any except cottage)					
14.	Beef burgers or sausages					
15.	Beef, pork, or lamb (for vegetarians: nuts)					
16.	Bacon, meat pie, processed meat					
17.	Chicken or turkey					
18.	Fish (NOT fried fish)					
19.	ANY fried food: fried fish, chips, cooked breakfast, samosas					
20.	Cakes, pies, puddings, pastries					
21.	Biscuits, chocolate, or crisps					
		None	Less than 1 a week	1 to 2 a week	3 to 5 a week	6 or more a week

About how much of the following types of milk do you yourself use **in a day**, for example in cereal, tea, or coffee? (Please tick one box on each line)

	Milks	None	Less than a quarter pint	About a quarter pint	About half a pint	1 pint or more
22.	Full cream (silver top) or Channel Islands (gold top)					
23.	Semi-skimmed (red striped top)					
24.	Skimmed (blue checked top)					

About how many **rounded teaspoons a day** do you usually use of the following types of spreads, for example on bread, sandwiches, toast, potatoes, or vegetables?

	Spreads	None	1 a day	2 a day	3 a day	4 a day	5 a day	6 a day	7 or more
25.	**Regular margarine or butter** or **Reduced fat spread** such as sunflower or olive spread, Flora, Vitalite, Clover, Golden Churn, Olivio, Stork, Utterly Butterly, Pure								
26.	**Low fat spread** such as Flora Light, St. Ivel Gold, Half-fat butter, Olivite, Flora Pro-activ, Diet Clover								

What type of fat do you usually use for the following purposes?
(Please tick one box on each line)

		Butter, lard, or dripping	**Solid cooking fat** (White Flora, Cookeen) **Half-fat butter** **Hard margarine** (Stork)	**Soft margarine** (sunflower, soya) **Reduced fat spread** (olive, Flora Buttery, Olivio)	**Vegetable oil** or **Low fat spread** (Flora Light, Olivite, St. Ivel Gold)	**No fat used**
27.	On bread and vegetables					
28.	For frying					
29.	For baking or cooking					

Thank you for completing the Eating Habits Questionnaire.

Please go back and check that you have ticked one box on every line.

Appendix 6 Leeds Women's Wellbeing DINE (LWW-DINE)

A new scoring system has been designed to enable us to use the DINE questionnaire as a basis to get a daily fibre intake estimate in g's using current (AOAC) fibre contents. Use this sheet as an interview guide to estimate the scores to enter into the Excel scoring file.

1. Breads and Rolls

Ask about the types of bread they buy or eat on a regular basis and use the questions below to find out how much bread they eat in an average week.

What types of bread do you regularly buy? (medium or thick sliced?)

_____

Questions to ask	Frequency per week	Type of bread	Number & size of slices	Average per week
How many times a week do you have bread/toast at breakfast?				
How many times a week do you have bread/rolls at lunch?				
How many times a week do you have bread/rolls at dinner?				
How many times a week do you have bread/rolls in between meals?				
How often do you have other types of bread i.e. chapatti, rye, bagels, crispbreads				

Scoring - use the information above to estimate how many slices of each type of bread they consume each week.

High fibre white or soft grain bread is scored as brown/granary. Use portion size 1 for medium bread and 1.5 for thick sliced bread. Multiply frequency by portion size to get total.

Breads & Rolls	Average per week	Portion size	Total
White			
Brown/Granary			
Wholemeal			

2. Breakfast Cereals

Ask about the types of breakfast cereals they buy or eat on a regular basis and use the questions below to find out how much cereal they eat in an average week.

What types of cereals do you regularly buy? _____

Type of cereal	Type of cereal	Frequency per week	Portion size
Cereal eaten at breakfast?			
Cereal eaten at other times of day?			

Scoring - use the information above to estimate how much of each type of cereal they consume each week. Use 1 for average portion size and 1.5 for large portion size. Multiply frequency by portion size to get total (this does not need to be a whole number).

Type of cereal	Frequency per week	Portion size	Total
Low fibre			
Medium fibre			
High fibre			

3. Other foods

Type of food	Frequency per week	Portion size	Total
White pasta			
Wholewheat pasta			
White rice			
Brown rice			
Potatoes			
Peas			
Beans (baked, tinned or dried) or lentils			
Other vegetables (any type)			
Fruit (fresh, frozen or canned)			

Use 1 for average portion size and 1.5 for large portion size. Multiply frequency by portion size to get total.

Appendix 7 VVLT Practice scoring

VVLT Practice Scoring

Subject Number	
Initials	
Visit	
Condition	
Session	

VVLT 1a	1	2	3	4	5	VVLT 1b	1
CLEAR						UNIQUE	
RISE						LORD	
BASE						SEEK	
PROPERTY						TROUBLE	
QUALITY						VALLEY	
SYMBOL						HEART	
TYPE						REALITY	
GLASS						RACE	
FOOT						WEST	
TEXT						VALUE	
AFRAID						WRONG	
COVER						TEST	
WORTH						AMOUNT	
DEEP						DRAW	
PORTION						STUDENT	
PICTURE						FRONT	
TOTAL							

Intrusions

VVLT 1a	1	2	3	4	5	VVLT 1b	1

Appendix 8 VVLT Visit 1 scoring

WLT 1 Scoring

Subject Number	
Initials	
Visit	
Condition	
Session	

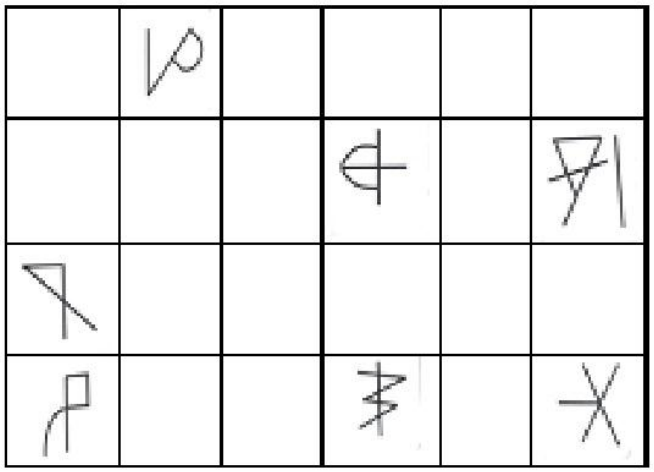
VVLT 1a	1	2	3	4	5	VVLT 1b	1
BOOK						NOSE	
EARTH						METAL	
ROOM						SNOW	
PERSON						BRIDGE	
HOLE						POOL	
COAST						MARCH	
PATIENT						PLATFORM	
LOVE						VOTE	
FAIR						AREA	
BEST						COOL	
SHORT						EVENT	
DISTANCE						RESPECT	
FLOW						LACK	
BRIEF						IDEAL	
CHOOSE						FACTOR	
SESSION						ATTEMPT	
TOTAL							

Intrusions

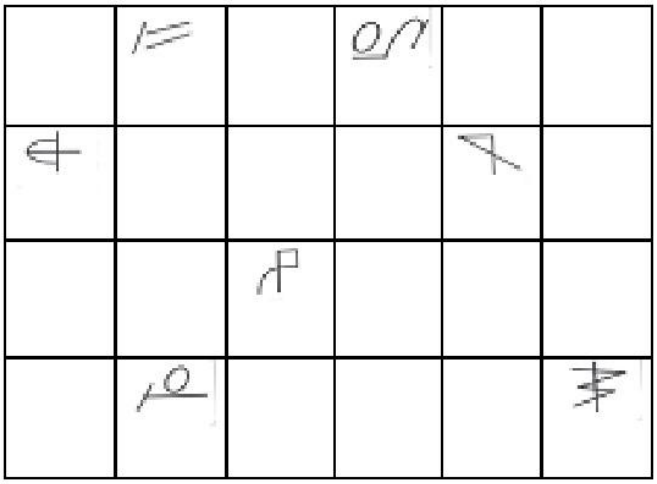
VVLT 1a	1	2	3	4	5	VVLT 1b	1

Appendix 9 VSLT versions

VSLT Practice



VSLT Visit 1



VSLT scoring

	Trial 1	Trial 2	Trial 3	Recall
Correct designs picked				
Incorrect designs picked				
Correct positions picked				
Correct design and position				

Appendix 10 National Adult Reading Test (NART)

WORD	ANSWER	WORD	ANSWER
Chord		Superfluous	
Ache		Simile	
Depot		Banal	
Aisle		Quadruped	
Bouquet		Cellist	
Psalm		Façade	
Capon		Zealot	
Deny		Drachm	
Nausea		Aeon	
Debt		Placebo	
Courteous		Abstemious	
Rarefy		Détente	
Equivocal		Idyll	
Naïve		Puerperal	
Catacomb		Aver	
Gaoled		Gauche	
Thyme		Topiary	
Heir		Leviathan	
Radix		Beatify	
Assignate		Prelate	
Hiatus		Sidereal	
Subtle		Demesne	
Procreate		Syncope	
Gist		Labile	
Gouge		Campanile	
TOTAL		**TOTAL**	
GRAND TOTAL			

20th February 2012-Scoring Sheet: National Adult Reading Test list of words (NART)

Appendix 11 Cognitive test evaluation questionnaire

Cognitive Test Evaluation Questionnaire

Volunteer number: _____ **Date:** _____

1. How much **time pressure** did you feel due to the rate/pace of the tests?

Not very much _____ Very much

2. How **difficult did you find** these tests today?

Not at all difficult _____ Extremely difficult

3. How much **did you** concentrate during these tests?

A small amount _____ A large amount

4. How **hard did you try** in these tests?

Not at all hard _____ Extremely hard

5. How **well** do you think you **performed** in these tests?

Not at all well _____ Extremely well

6. How **frustrating did you find** these tests today?

Not at all frustrating _____ Extremely frustrating

7. Please number the following tests from the battery you have just completed indicating how difficult you found them? (1 = easiest test, 9 = hardest test)

Visual Spatial Learning Test (Pattern memory test) _____

Visual Verbal Learning Test (Word memory test) _____

Corsi block tapping test (red square sequences) _____

Tower of Hanoi (moving disks task) _____

Delayed Visual Spatial Learning Test (Pattern memory test) _____

Delayed Visual Verbal Learning Test (Word memory test) _____

Word Recognition test _____

Version 3 (29/05/12)

Appendix 12 List of foods used in the estimation of polyphenol intake

No.	Food	Brand	Subject(s) code
12.	Jaffa cake	McVities	R006
13.	Karai cooking sauce	Patak's	R006
14.	Chocolate croissant	McVities	R006
15.	Quarter pounder beef burger	Sainsbury	R008
16.	Houmus/humus	Sainsbury	R006
17.	Thin crust pepperoni pizza	Sainsbury	R005
18.	Potato	-	R005
19.	Kit kat chocolate	Nestle	R009
20.	Olive oil, refined		R007
21.	Coleslaw recipe		R002
22.	Fruit & fibre	Kellog's	R002
23.	Wine, white		R003
24.	Coffee, filter		R003
25.	Chocolate mini rolls	Cadbury	R003
26.	Apple raw, with skin		R003
27.	Puttanesca sauce	Lloyds Grossman	R004
28.	Fish pie	ASDA	R004
29.	Cream of tomato soup	ASDA	R004
30.	Best of both bread	Hovis	R004
31.	Tikka masala sauce	Patak's	R005
32.	Classic basil pesto	Sacla	R005
33.	Sultana bran: raisin TLNI for raisin	Sainsbury	R005
34.	Lentil soup	Heinz	R005
35.	Mini choc brownies	Thornton	R006
36.	Vegetable Curry	Estimation from recipe	R006
37.	Rhubarb crumble	Sainsbury	R006
38.	Fruit flapjack	Estimation from recipe	R006
39.	Lemon chicken	Estimation from recipe	R007
40.	Thin & crispy vegetable supreme pizza	Sainsbury	R007
41.	Light strawberry yogurt	Muller	R007
42.	Rice apple	Muller	R007
43.	Garlic		R007
44.	Oatcake	Sainsbury	R008
45.	Peanut butter & choc candy, also used for Hershey`s Nutrageous (same brand)	Reese	R008
46.	Rice strawberry	Muller	R008
47.	Chicken chasseur	Estimation from recipe	R008
48.	Simply delicious muesli	Dorset	R009
49.	Chocolate chip cookies	Sainsbury	R009
50.	Raspberry yogurt	Longley farm	R009
51.	Multiseeded bloomer	Sainsbury	R009
52.	Teacake, fruity	Warburton	R009

53.	Chicken chow mein	Estimation from recipe	R009
54.	Wine, rose		R010
55.	Wine, red		R010
56.	Spinach & ricotta cappeleti	Sainsbury	R010
57.	Swiss style muesli	Sainsbury	R006
58.	Sesame bagel	New York	R010
59.	Chocolate chip bar	Harvest Chewee	R011
60.	Biscuit chocolate	Blue Riband	R011
61.	Caramel cake bars	Cadbury	R011
62.	Instant cappuccino powder	Kenco	R011
63.	Toblerone tinys	Toblerone	R011
64.	Coffee, instant		R007
65.	Lager/beer		R007
66.	Strawberry, raw		R007
67.	Raspberry, raw		R007
68.	Apple juice, concentrate		R007
69.	Lentil, whole, raw		R007
70.	Plain/dark chocolate		R017
71.	Grapes, red/green		R009
72.	Orange juice, from concentrate		R006
73.	Blueberry, raw		R009
74.	Sesame		R010
75.	Tea, Chinese: oolong tea data		R010
76.	Broccoli, raw		R010
77.	Milk chocolate digestive caramel	McVitie's	R010
78.	Blackcurrant		R011
79.	Onion bhajia	Sainsbury	R024
80.	Vegetable samosa	Sainsbury	R020
81.	Baked bean, including five beans	Heinz	R017
82.	Blueberry muffin	Commercial	R018
83.	Cherry Tango		R016
84.	Thin & crispy cheese & tomato pizza		R014
85.	Wispa	Cadbury	R017
86.	Carrot, broccoli & corn (steam vegetable)	Birdseye	R017
87.	Arrabbiata pasta sauce	Sainsbury	R017
88.	Lasagne	Sainsbury	R063
89.	Curry sauce or korma sauce	Patak's	R018
90.	Mixed vegetable stir fry	Sainsbury	R018
91.	Wholemeal muesli	Sainsbury	R018
92.	Cherry yogurt	Muller light	R018
93.	Lasagne	Tesco	R019
94.	Strawberry yogurt	Shape	R019
95.	Fish, chips & peas		R020
96.	Aloo Keema (potato & mince curry)		R020

97.	Mixed nut	Alesto	R020
98.	Balti sauce	Patak's	R021
99.	Fat free strawberry yogurt	Activia Danone	R022
100.	Chicken curry (chilled, froze)	Tesco	R022
101.	Kit Kat chunky	Nestle	R022
102.	Galaxy Minstrel	McVities	R022
103.	Cauliflower cheese	Tesco	R022
104.	Malt loaf	Soreen	R023
105.	Beef burger, original, use for chicken burger as well	Birdseye	R023
106.	Olive spread	Bertolli *if light: 16% olive oil	R004
107.	Green tea, infusion		R014
108.	Olive, black		R004
109.	Olive, green		R014
110.	Tea, black, infusion		R007
111.	Tomato, whole, raw		R016
112.	Cherry, raw		R034
113.	Chickpeas		R018
114.	Lettuce		R019
115.	Walnut		R020
116.	Almond		R020
117.	Plum, raw		R021
118.	Flapjack (oat: 39%)	Sainsbury	D049
119.	Olive oil, extra virgin		R022
120.	Raisin		R023
121.	Chicken balti	Sainsbury	R024
122.	Milk chocolate hobnobs	McVities	R024
123.	Margherita pizza	Sainsbury	R024
124.	Large salad bowl	Sainsbury	R024
125.	Homemade, pure lemon juice, lemonade (data from pure lemon juice)		R024
126.	Deli style coleslaw		R024
127.	Chocolate button, use for Cadbury Freddo as well	Cadbury	R025
128.	Bolognese sauce, original	Dolmio	R025
129.	Milk chocolate digestive	McVitie's	R025
130.	Sultana scone (16% sultana)	Sainsbury	R026
131.	Hob nob, original	McVitie's	R026
132.	Butternut squash soup	Sainsbury	R027
133.	Guacamole (54% avocado puree; 16% avocado)	Deleted	R027
134.	Winter vegetable soup	New Covent Garden	R027
135.	Grapefruit juice, pure		R027
136.	Hoi Sin & Five spice cooking sauce	Sharwood	R027
137.	Nectarine, raw		R027
138.	Galaxy caramel	McVities	R028

139.	Cocoa powder		R028
140.	Pear, raw		R028
141.	Macaroni & cheese, canned	ASDA	R028
142.	Apple slice	Go ahead	R038
143.	Tuna pasta bake	ASDA	R030
144.	Forest fruit yogurt breaks	Go ahead	R031
145.	Tortelloni tomato & mozzarella	ASDA	R031
146.	Teacakes	Tesco	R031
147.	Pepperoni pizza	Tesco	R032
148.	Lemon muffin	Sainsbury	R034
149.	Apple pie	Sainsbury	S023
150.	Chocolate milk drink		R034
151.	Chocolate	Smarties	R034
152.	Fibre plus chocolate milk bar	Kellogg's	R035
153.	Chocolate cake (2% milk chocolate)	Commercial	R035
154.	Triple chocolate chip muffin (milk choc chunks: 8%; plain choc chunks: 3%)	Sainsbury	R035
155.	Choc chip muffin (14% milk choc chunks)	Sainsbury	
156.	Eccles cakes	Tesco	R035
157.	Brunch bar raisin	Cadbury	R035
158.	Cherry bakewell	Sainsbury	R035
159.	Ristorante pizza pollo	Dr. Oetker	R035
160.	Horlick, original		R035
161.	Mini milk choc HobNobs (20%: rolled oat; 24%: milk choc)	McVitie's	R051
162.	Mixed vegetable	Sainsbury	R037
163.	Wafer, filled, chocolate coated		R037
164.	Spaghetti bolognese	Heinz	R037
165.	Pasta & sauce, chicken & mushroom	Batchelor	R037
166.	Spaghetti	Heinz	R036
167.	Original crispbread (data from rye, wholegrain flour)	Ryvita	R038
168.	Rice & wheat bar	Special K	R038
169.	Cranberry, raw		R038
170.	Chicken chasseur mix	Colmans	R037
171.	Apple crumble		R037
172.	Applesauce		R038
173.	Chicory, green		R038
174.	Piccalilli	Tesco	R039
175.	Fruit muesli	Sainsbury	R039; D018 (for larger portion size)
176.	Fruit yoghurt, cherry	Muller corner	R039
177.	Broccoli & stilton soup	Sainsbury	R039
178.	Vegetable pot Indian dhal	Innocent	R039
179.	Twirl	Cadbury	R040
180.	Tomato soup	Weight watcher, Heinz	R041

181.	Vinegar		R041
182.	Tuna and sweetcorn pasta	Tesco	R041
183.	Minestrone soup, dry, mix	ASDA	R042
184.	Mixed sliced pepper: too low	ASDA	R042
185.	Wholenut chocolate bar	Cadbury	R042
186.	Cheese & onion rolls	Sainsbury	R043
187.	Spinach pakora	Refer ingredient from onion bhaji	R043
188.	Ham & mushroom pizza	Sainsbury	R005
189.	Chocolate mousse	Tesco	R044
190.	Chocolate fudge cake	Sainsbury	R045
191.	Tomato & parmesan	Dolmio	R045
192.	Cream of tomato	Heinz	R045
193.	Tomato & roasted garlic sauce	Lloyds Grossman	R045
194.	Chocolate spread	ASDA	R046
195.	Big soup Italian meatball	Heinz	R046
196.	Garlic & olive oil butter dip	Pizza express	R046
197.	Peanut		R046
198.	Bramley apple pie	Mr Kipling	R047
199.	Pepper, green		R047
200.	Apple turnover	Tesco	R048
201.	Bolognese sauce	Sainsbury	R048
202.	Mediterranean tomato soup	Batchelor’s	R049
203.	Garlic & parsley flatbread, baguette	Sainsbury	R049
204.	Really seeded bread	Kingsmill	R050
205.	Chilli con carne	Tesco	R050
206.	Spinach & ricotta sauce	Sainsbury	R050
207.	Wagon wheel biscuit		R051
208.	Chocolate chunk brownies	Tesco	R051
209.	Cornish pastry	Sainsbury	R051
210.	Oat so simple pot golden syrup	Quacker	R052
211.	Light bar, chocolate & orange	Alpen	R052
212.	Bread sauce	Tesco	R052
213.	Cranberry sauce	Tesco	R052
214.	Hot & spicy mixed beans	Tesco	R052
215.	Mug shot pasta snack creamy cheese	Mug shot	R052
216.	Chocolate	Snickers	R052
217.	Pasta salad Italian	ASDA	R053
218.	Mini chocolate croissant	McVitie’s	R053
219.	Rogan josh sauce (lamb, chicken, etc.)	Sainsbury	R053
220.	Spaghetti with sausages	Heinz	R054
221.	Danish malted bread	Weight watcher	R057
222.	Sweetcorn pasta salad	Sainsbury	R057
223.	Twix		R058
224.	Potato gratin	Sainsbury	R059

225.	Chocolate éclair	Sainsbury	R059
226.	Macaroni cheese	Tesco	R060
227.	Bio yogurt strawberry with granola	Muller corner	R017
228.	Yogurt with toffee hoops	Muller corner	R017
229.	Green salad		R034
230.	Minestrone soup	Tesco	D020
231.	Orange marmalade	Tesco	R018
232.	Coriander, fresh		R021
233.	Lamb curry	Recipe from Pataks	R021
234.	Chicken chow mein	Sainsbury	R024
235.	Strawberry yogurt	Ski Nestle	R024
236.	Peach yogurt	Tesco	R027
237.	Jam tart, raspberry	Mr Kipling	R034
238.	Cherry crumble	Adapted from rhubarb crumble (Sainsbury)	R034
239.	Chocolate covered caramel	Tesco	R034
240.	Fruit cake	Sainsbury	R034
241.	Kingsmill 50:50	Kingsmill	R037
242.	Vegetable soup	Tesco	R038
243.	Breakfast biscuit	Belvita	R041
244.	Light juice drink	Ribena	R041
245.	Vegetarian stew	Stewed!	R041
246.	Triffle, fruit	USDA	R042
247.	Blackcurrant squash or concentrate (after dilution)	Ribena (refer to USDA data)	D004
248.	Asparagus soup	Batchelor cup	R049
249.	Turkish delight	Frys	R052
250.	Turkish delight	Tesco	R052
251.	Blueberry jam		S001
252.	Pasta, cooked		R029
253.	Broad bean, cooked with pods		R061
254.	Lemon tart	Sainsbury	R061
255.	Organic fruit & nut mix	Sainsbury	R062
256.	Chocolate fingers	Cadbury`s	R062
257.	Mango		R062
258.	Banana		R062
259.	Cottage Pie (300g)	Quorn	R063
260.	Chicken and cashew nuts	Ken Hom's, Tesco	R063
261.	Pineapple juice		R063
262.	Chicken hot pot, 320g	Weight watchers	R063
263.	Chocolate Fudge Cream Biscuits: TLNI	Fox`s	R015
264.	Thai Coconut Curry Pot	Innocent	R064
265.	Brussels sprouts		R064
266.	Carrot		R044
267.	Turnip		R064

268.	Parsnips		D110
269.	Leek		R064
270.	Rye bread	Kelderman	R064
271.	Cheese & onion slice	Sainsbury`s	R064
272.	Ginger, fresh		R064
273.	Tinned minced beef & onion	Sainsbury`s	R065
274.	Raita dip	Sainsbury`s	R065
275.	Mint, peppermint fresh		R065
276.	Sauce, salsa, ready-to-serve, hot	Tesco	R065
277.	Peas, green		R020
278.	Tomato and Sweet Basil Soup	New Covent Garden	R066
279.	Beet greens, raw		R066
280.	Spanish Potato And Onion Omelette	Tesco	R066
281.	Ciabatta Loaf	Tesco	R067
282.	Sesame snap bar	Cofresh	R067
283.	Chocolate orange bar	Terry`s	R067
284.	Orange, mango and passionfruit juice (some TLNI)	Asda	R067
285.	Sardine, in tomato sauce (16% tomato, canned): TNLI	John West	R068
286.	Peanut chocolate	M&M	R071
287.	Mini chocolate cake	Thornton`s	R068
288.	Chocolate cheesecake	Sainsbury`s	R069
289.	Tartare sauce	Tesco	R070
290.	Capers		R070
291.	Honey nut country crisp	Jordan`s	R071
292.	Stock cubes, vegetable: TLNI	Knorr	R071
293.	Beans with sausages	Heinz	R071
294.	Cider, dry		R071
295.	Orange, mango and pineapple smoothies	Sainsbury`s	R071
296.	Thai red curry	Sainsbury`s	R073
297.	Lemon grass		R073
298.	Almondy tarte	Daim	R073
299.	Leek and potato soup	New Covent Garden	R074
300.	Bentos meatballs in tomato sauce	Fray	R073
301.	Onion rings	Sainsbury`s	R073
302.	Ready to roast butternut squash	Sainsbury`s	R074
303.	Squash, raw		R074
304.	Brown bread, wholemeal		R074
305.	Can Red Wine Cook In Sauce	Homepride	R074
306.	Profiteroles	Sainsbury`s	R074
307.	Chocolate spread	Tesco	R074
308.	Strawberry cheesecake	Sainsbury`s	R074
309.	All butter fruit biscuits	Sainsbury`s	R076
310.	Mint sauce	Colman’s	R077

311.	Peppermint, fresh		R077
312.	Orange Squash	Robinsons	R077
313.	Saag paneer curry	Sainsbury`s	R077
314.	Beetroot		R078
315.	Zucchini		R078
316.	Chicken fajitas	Tesco	R078
317.	Chewy mixed berry bar	Nature Valley	R078
318.	Red berries bar	Jordans Frusli	R078
319.	Mediterranean tomato slim a soup	Batchelor`s	R078
320.	Breakfast cereals, bran (Weetabix, Shreddies, Nestle Curiously Cinnamon, malt wheat)	Use data from bran	R063
321.	Blackberries, raw		R080
322.	Chocolate tiffin	Gu	R080
323.	Fresh fruit salad	Sainsbury`s	S045
324.	Bramley Apple Pies	Tesco	R080
325.	Tomato chutney: TLNI	Tesco	R001
326.	Scotch whisky: substitute for vodka: TLNI		R001
327.	Panini roll: extra virgin olive oil content	Sainsbury's	R001
328.	Tomato ketchup: use data from Heinz (132 g tomato per 100 g ketchup)	Heinz	R019
329.	Mars bar, use this data for Malteser, Crème egg, Cadbury as well, Revels, marzipan Ritters, Milky way, Bounty, Double Decker, Boost	Mars	R002
330.	Okra	USDA data	R002
331.	Applesauce	Sainsbury's	R003
332.	Breakaway biscuit	Nestle	R003
333.	Spinach and ricotta cannelloni	Recipe BBC good food	R004
334.	Basil, fresh		R004
335.	Parsley, fresh		R004
336.	Coffee, latte	Nestle	R004
337.	Soy milk		R004
338.	Granary bread roll		R004
339.	Couscous, cooked (data from hard wheat, semolina)		R004
340.	Citrus Kick Cous Cous	Ainsley Harriot	R014
341.	Onions, spring or scallions (includes tops and bulb), raw	Use USDA data	R004
342.	Oats So Simple apple and blueberry	Quaker	R006
343.	After eight (assumed as 30%)	Nestle	R006
344.	Gooseberry	Raw	R006
345.	Common thyme, dried		R007
346.	Rosemary, dried		R007
347.	Pepper spice, black		R007
348.	J2O Orange and Passionfruit	Britvic	R007

349.	Passionfruit, fresh		R007
350.	Hot and sour chicken soup	Estimation from recipe	R007
351.	Sweet and sour chicken	Estimation from recipe	R007
352.	Lamb kheema	Estimation from recipe	R007
353.	Cinnamon		R007
354.	Clove		R007
355.	Coriander seed		R007
356.	Cumin		R007
357.	Chicken chasseur	Estimation from recipe	R008
358.	Starbucks Cappucino	Use data from Nestle Cappuccino	R009
359.	Pesto pasta salad	From recipe	R009
360.	Scotch whisky (use for spirit 40% volume)		R010
361.	Sesame oil		R010
362.	Shrimp fried rice	Estimation from recipe	R010
363.	Sweet potato: TLNI	USDA data	R010
364.	Pistachio nuts		R011
365.	Belgian Chocolate Instant Hot Chocolate Drink	Options	R011
366.	Green bean, raw		R015
367.	Chocolate Fudge Brownie Ice Cream	From recipe	R015
368.	Courgette		R018
369.	Mango chutney (41% mango)	Sharwood	R018
370.	Cheese layered salad	Tesco	R019
371.	Cranberry Juice: use data from USDA	Ocean Spray	R020
372.	Sardine in tomato sauce (15% of tomato puree)	Sainsbury	
373.	Beans, snap, green, cooked, boiled, drained, without salt	Use USDA data	R061
374.	Celery, raw		R064
375.	Chicken biryani	Sainsbury	R067
376.	Orange, mango and passionfruit juice	Asda	R067
377.	Mini chocolate cake	Thornton	R068
378.	Camomile tea (German camomile)		R071
379.	Raspberry jam		R074
380.	Peppermint tea		R077
381.	Cherry bakewell	Mr Kipling	R021
382.	Twiglets		R022
383.	Soya and Linseed Bread	Burgen	R022
384.	Vegelicious Lentil Moussaka	Tesco	S067 (large portion)
385.	Lamb moussaka	Sainsbury	R027
386.	Orange & Passionfruit juice drink	Drench	R028
387.	Carbonara tortellini pasta	Sainsbury	R032
388.	Strawberry & banana smoothie	Innocent	R034
389.	Beer, ale		R034
390.	Chocolate soya milk	Alpro	R036

391.	Sticky toffee pudding pot	Aunty`s	R042
392.	Artichokes		D001
393.	Basil, dried, ground		D001
394.	Ice lolly	Twister	D002
395.	Spring greens, raw: use USDA data for Cabbage, chinese (pak-choi) , raw		D002
396.	Eclairs, custard-filled with chocolate glaze		D003
397.	Orange, blond (raw)		D004
398.	Sunflower seed		D004
399.	Lemon cheesecake	Sainsbury	D006
400.	Plum tomato & mascarpone soup	New Covent Garden	D006
401.	Strawberries, blackberries & raspberries smoothies	Innocent	D006
402.	Smoky gazpacho dip	Graze	D007
403.	Sandwich pickle (value too low)	Tesco	D007
404.	Ginger, ground		D008
405.	Cabbage, common (for large quantity)		D008
406.	Dates, dried		S018
407.	Turmeric, ground		D008
408.	Pomegranate, raw		D008
409.	Oat And Raisin Cookie	Tesco	D009
410.	Melons, honeydew, raw		D011
411.	Onion And Garlic Pasta Sauce	Tesco	D012
412.	Plantain		D014
413.	Yogurt drink, strawberry	Actimel	D015
414.	Ovaltine powder		D015
415.	Tomato and basil pasta sauce	Napolina	D015
416.	Lemon cake	Tesco	D016
417.	Orange & chocolate light yogurt	Muller	D016
418.	0% Fat Forest Fruits Yogurt	Activia	D017
419.	Tropical squash	Tesco	D017
420.	Lime, pure juice		D017
421.	No added sugar lemon and lime squash	Tesco	D017
422.	Prune		D017
423.	Papaya		D018
424.	Barley malt flour		D018
425.	Apple & Blackcurrant squash	Robinson	D023
426.	Spicy veggie sandwich	Estimation from recipe	D022
427.	Cup a Soup Creamy Broccoli & Cauliflower	Batchelors	D020
428.	Pecan nut		D020
429.	Micro Oats Golden Syrup	Tesco	D020
430.	Beef Savoury Rice	Batchelors	D020
431.	Creamy Vegetable Cup Soup	Campbell`s	D020
432.	Organic Carrot Cake	Respect	D020

433.	Strawberry yogurt	Sainsbury	D021
434.	Wholegrain Strawberry Yogurt	Onken	D021
435.	Sambar mix	MTR	D021
436.	Kiwis, apples & limes smoothies	Innocent	D022
437.	Tomato & Red Pepper Relish: TLNI	Branston	D022
438.	Tomato and mozarella pasta bake	Sainsbury`s	D022
439.	Granola Bars Nut Crunch	Nature Valley	D022
440.	Pear juice		D022
441.	Organic Low Fat Strawberry Yogurt	Rachel`s	D023
442.	Thin & Crispy Sweet Chilli Chicken Pizza	Sainsbury`s	D024
443.	Grapefruit juice, from concentrate		D024
444.	Fruit And Barley Grapefruit	Robinsons	D024
445.	No added sugar orange lemon and pineapple DS squash	Tesco	D024
446.	Dairy Milk Chocolate Fruit And Nut Bar (TLNI)	Cadbury	D025
447.	Original 2 In 1 White Coffee	Nescafé	D025
448.	Spanish Almond Stuffed Olives	Sainsbury's	D026
449.	Spices, oregano, dried (majoram, dried)		D026
450.	Tzatziki Dip	Tesco	D026
451.	Potato Salad	Sainsbury's	D026
452.	Forest Fruits Juice Drink	ASDA	D026
453.	Cherry juice: data from USDA juice concentrate, sour cherry	USDA	D026
454.	Strawberry juice: data from USDA, strawberry juice	USDA	D026
455.	Blackberry juice: data from USDA, Juice concentrate, blackberry	USDA	D026
456.	Tomato and Red Pepper Pasta Sauce	Napolina	D027
457.	Oats So Simple Sweet Cinnamon	Quaker	D027
458.	Belgian Chocolate Brownie With Pecans	Tesco	D033
459.	Oat So Simple Raspberry And Pomegranate Porridge	Quaker	D033
460.	Deep pan BBQ chicken pizza	Sainsbury`s	D033
461.	Finest Strawberries And Cream Tart	Tesco	D033
462.	Ingredient sweet chilli sauce	Tesco	D034/D045
463.	Finest Soy & Sesame Dip for dim sum	Tesco	D034
464.	Tofu		D034
465.	Light Choices 2 Chicken Tomato And Basil	Tesco	D034
466.	Apple & raspberry juice	Innocent	D034
467.	Subs & sandwich	Subway	D034
468.	Mediterranean Bread	Tesco	D037
469.	Reduced Fat Onion & Garlic Dip	ASDA	D037
470.	Dark Chocolate Hobnob	Mcvities	D037
471.	Falafel	Cauldron Foods	D038
472.	Tomato And Pepper Fajita Spice Mix	Old El Paso	D039

473.	Bramley apple & blackberry pie	Aunt Bessie's	D040
474.	Oat So Simple Original Porridge	Quaker	D043
475.	Chocolate Peanut Bar	Slimfast	D043
476.	Oat So Simple Sultana, Raisin, Cranberry & Apple	Quaker	D043
477.	Lilt Regular	Lilt	D044
478.	Chow Mein Stir Fry Sauce	Blue Dragon	D046
479.	Tomato Chargrilled Vegetable Pasta Sauce	Loyd Grossman	D046
480.	Date & Walnut loaf cake	Tesco	D047
481.	Munchies	Nestle	D047
482.	All Butter Pains Au Chocolate	Tesco	D051
483.	Kinder Bueno		D051
484.	Chickpea dhal	Tesco	D052
485.	Soy, mince, meat		D054
486.	Tangerines, mandarine, clementine, Satsuma, (mandarin oranges) , raw	Data from USDA	D054
487.	Seeds, flaxseed		D054
488.	Finesse Milk Chocolate	Nestle Aero	D056
489.	Mango Exotic Juice Drink	Rubicon	D056
490.	Pumpkin, raw		D057
491.	Milk Bubbly Bar	Nestle Aero	D057
492.	Sage, dried		D061
493.	Mint and dark chocolate yoghurt	Muller	D061
494.	Mince Pies	Sainsbury`s	D062
495.	Chocolate Shake	Slimfast	D038
496.	Instant Herbal Beverage with Tea Extracts	Herbalife	D038
497.	Sausage Pasta Bake	Sainsbury`s	D063
498.	Chocolate milk shake	Yazoo	D063
499.	Semolina halwa	Semolina	S001
500.	Pickle, lime, oily	Tesco	S001
501.	Tropical fruit juice	Sainsbury`s	S001
502.	Potato farls	From recipe	S001
503.	Pasta primavera	Sainsbury`s	S001
504.	Carrot and lentil soup	Weight Watchers from Heinz	S003
505.	Banana bread (20% banana)	Respect Organic	S003
506.	Soy sausage		S003
507.	Oreo cookies	Oreo	S003
508.	Orange and mango squash no added sugar, concentrate	Robinsons	S005
509.	Tomato and herb	Pasta Mug Shot	S005
510.	Mixed dried fruits and nuts		S003
511.	Tango orange	Tango	S005
512.	Orange And Pineapple No Added Sugar	Robinsons	S005

513.	Alforno ricotta pasta bake sauce	Loyd Grosman	S005
514.	Chicken hotpot	Sainsbury`s	S005
515.	McFlurry (assumption for ingredients)	McDonalds	S005
516.	Select Orange, mandarin and peach, concentrate	Robinsons	S007
517.	Strawberry Trifle	Sainsbury`s	S007
518.	Apple and mango	J2O	S007
519.	Oats and More Raisin cereal	Nestle	S007
520.	Sun dried tomato pesto	Sacla	S007
521.	Cranberry and praline pecan muesli	Assumption from other brand	S010
522.	Oat and chocolate chip cookies	Cadbury	S010
523.	Lemon sorbet	Tesco	S010
524.	Cherry bakewell tart	Tesco	S010
525.	Banana and pineapple smoothies	Innocent	S010
526.	Pea, spinach and coriander soup	Yorkshire Provender	S010
527.	Smoky bacon pasta sauce	Loyd Grosman	S010
528.	Plain chocolate digestive biscuits	ASDA	S015
529.	Sesame seeds crispbread	Ryvita	S017
530.	Moroccan Couscous salad	Tesco	S017
531.	Cherry, cranberry & blueberry smoothies: no ingredient-assumption	Innocent	S017
532.	Pepper, red		S017
533.	Berry Delight cereal bar	Nakd	S018
534.	Cocoa Delight wholefood cereal bars	Nakd	S018
535.	Double Choca Mocha 8 Sachets	Nescafe	S210
536.	French Onion soup	Baxters	S018
537.	0% Fat Mango And Peach Yogurt	Shape	S020
538.	Cheese and onion quiche	Tesco	S020
539.	Chocolate fondant torte	Gu	S020
540.	Cherry & blueberry soy yogurt	Alpro	S018
541.	Carrot and butterbean soup	Baxters	S020
542.	Fennel, raw		S033
543.	Crunchie ice cream	Cadbury	S033
544.	Ice cream bar, chocolate coated		S033
545.	Carrot and coriander soup	New Covent Garden	S027
546.	Crispy Slices Orange and Sultana	Go Ahead	S027
547.	No Added Sugar Fruit & Barley Apple & Pear	Robinsons	S035
548.	Original Seed Mix/ blend	The Food Doctor	S035
549.	Chocolate Caramel Treat	Slim Fast	S035
550.	Morello Cherry fruit compote	NOM	S035
551.	Yogurt with Belgian Milk Chocolate coated Raisin	NOM	S035
552.	Rhubarb yoghurt: TLNI	Activia	S036
553.	Thai carrot & lemongrass soup (fragrant)	Glorious foods	S036
554.	Redcurrant, raw		S036

555.	Summer fruit mix (fresh))	Tesco	S036
556.	Sweet And Sour Sauce Original	Uncle Bens	S036
557.	Blackberry jam		S038
558.	Soybean, sprout		D131
559.	Salmon & Broccoli Wedge Melt	Weight Watchers® from Heinz	S045
560.	Moroccan Spinach & Chickpea Soup, Be Good To Yourself	Sainsbury`s	S045
561.	0% Yogurts Peach & Passion Fruit	Shape Delights	S045
562.	Tomato And Wild Mushroom Sauce	Loyd Grossman	S047
563.	Onion Chutney	Tesco	S050
564.	Shredded Wheat Honey Nut	Nestle	S056
565.	Original, classic, ice cream	Walls Cornetto	S056
566.	Rosemary, fresh		S058
567.	Orange And Mango Juice	Tropicana	S058
568.	Mandarin Yogurt	Muller Light	S067
569.	Fruit Mousse Strawberry & Strawberry Coulis	Ski	S067
570.	Chicken & vegetable soup	Heinz big soup	S067
571.	Chocolate Raisin Clusters	Cadbury	S071
572.	Weetabix Chocolate	Weetabix	S177
573.	Cashews, blueberries and a yoghurt coating	Eat Natural bar	D137
574.	Milk Chocolate Coated Raisins	Tesco	D137
575.	Blueberry juice, from concentrate		D137
576.	Hobnobs Milk Chocolate Biscuit Flapjacks	McVities	D139
577.	Fudge chocolate	Cadburys	D139
578.	Finest Moroccan Chicken Tagine	Tesco	D140
579.	Goan Tomato & Lentil soup	Glorious	D142
580.	Easy Entertaining Mixed Bean Salad	Tesco	D142
581.	Pineapples, bananas & coconuts smoothies	Innocent	D142
582.	0% Fat Peach Yogurt	Activia	D143
583.	Pea & Mint Risotto Bake	Sainsbury	D145
584.	Super Nutty Granola	Jordans	D145
585.	Cup a soup Vegetable with croutons	Batchelors	D146

Appendix 13 ANOVA table for results based on polyphenol consumption quartiles

ANOVA test between three study groups in macronutrients and dietary fibre intake (Chapter 2)

Energy: [F (2, 243) = 26.41, p<0.01]

Protein: [F (2, 243) = 8.42, p<0.01]

Carbohydrate: [F (2, 243) = 27.12, p<0.01]

Fat: [F (2, 243) = 13.50, p<0.01]

Alcohol: [F (2, 243) = 5.61, p<0.01]

Dietary fibre: [F (2, 243) = 33.90, p<0.01]

Vitamin C: [F (2, 243) = 14.18, p<0.01]

DINE Dietary fibre: [F (2, 243) = 10.54, p<0.01]

LWW DINE: [F (2, 243) = 24.08, p<0.01]

Correlation between the covariates (R value)

Covariates	Age	NART	WAIS total score
Age	1	0.459**	0.259**
NART	0.459**	1	0.666**
WAIS	0.259**	0.666**	1

**p<0.01

(a) Visual Verbal Learning Test

(i) Rate of learning (word List A)

Covariates	Age	NART	WAIS total score
Trial	F (2, 196) = 20.60*	F (2, 196) = 7.46	F (2, 196) = 2.97
Trial*Covariate	F (2, 196) = 2.23	F (2, 196) = 1.26	F (2, 196) = 0.63
Trial*polyphenol consumption quartiles	F (6, 196) = 0.45	F (6, 196) = 0.44	F (6, 196) = 0.45
Covariate	F (1, 98) = 1.73	F (1, 98) = 0.52	F (1, 98) = 1.29
Polyphenol consumption quartiles	F (3, 98) = 1.21	F (3, 98) = 0.90	F (3, 98) = 1.00

*p<0.001

(ii) New learning (recall of List B)

Covariates	Age	NART	WAIS total score
Covariate	F (1, 95) = 0.31	F (1, 95) = 3.32	F (1, 95) = 2.33
Polyphenol consumption quartiles	F (3, 95) = 0.24	F (3, 95) = 2.04	F (3, 95) = 2.25

(iii) Retroactive interference (Trial A3-A4)

Covariates	Age	NART	WAIS total score
Covariate	F (1, 95) = 1.55	F (1, 95) = 1.50	F (1, 95) = 0.52
Polyphenol consumption quartiles	F (3, 95) = 5.53*	F (3, 95) = 2.09	F (3, 95) = 0.18

*p<0.001

(iv) Proactive interference (Trial A1-B1)

Covariates	Age	NART	WAIS total score
Covariate	F (1, 95) = 0.53	F (1, 95) = 0.17	F (1, 95) = 0.29
Polyphenol consumption quartiles	F (3, 95) = 0.58	F (3, 95) = 2.11	F (3, 95) = 2.49

(v) Delayed recall

Covariates	Age	NART	WAIS total score
Covariate	F (1, 95) = 3.29	F (1, 95) = 0.04	F (1, 95) = 1.65
Polyphenol consumption quartiles	F (3, 95) = 2.44	F (3, 95) = 1.65	F (3, 95) = 1.42

(vi) Word recognition – accuracy - word list

Covariates	Age	NART	WAIS total score
Word list	F (2, 196) = 0.97	F (2, 196) = 2.99	F (2, 196) = 0.20
Word list*Covariate	F (2, 196) = 0.30	F (2, 196) = 1.40	F (2, 196) = 0.04
Word list*polyphenol consumption quartiles	F (6, 196) = 0.38	F (6, 196) = 0.24	F (6, 196) = 0.32
Covariate	F (1, 98) = 0.23	F (1, 98) = 1.01	F (1, 98) = 2.37
Polyphenol consumption quartiles	F (3, 98) = 0.23	F (3, 98) = 0.10	F (3, 98) = 0.06

(vii) Word recognition – accuracy - mode

Covariates	Age	NART	WAIS total score
Mode	F (1, 98) = 0.62	F (1, 98) = 0.00	F (1, 98) = 0.65
Mode*Covariate	F (1, 98) = 2.18	F (1, 98) = 0.08	F (1, 98) = 0.91
Mode*polyphenol consumption quartiles	F (3, 98) = 1.45	F (3, 98) = 0.56	F (3, 98) = 0.19
Covariate	F (1, 98) = 0.08	F (1, 98) = 0.38	F (1, 98) = 4.51***
Polyphenol consumption quartiles	F (3, 98) = 0.10	F (3, 98) = 0.75	F (3, 98) = 1.28

***p<0.05

(viii) Word recognition reaction time - word list

Covariates	Age	NART	WAIS total score
Word list	F (2, 196) = 1.84	F (2, 196) = 0.93	F (2, 196) = 0.54
Word list*Covariate	F (2, 196) = 0.43	F (2, 196) = 0.18	F (2, 196) = 0.17
Word list*polyphenol consumption quartiles	F (6, 196) = 0.31	F (6, 196) = 0.27	F (6, 196) = 0.27
Covariate	F (1, 98) = 2.47	F (1, 98) = 1.47	F (1, 98) = 3.20
Polyphenol consumption quartiles	F (3, 98) = 2.47	F (3, 98) = 0.47	F (3, 98) = 0.46

(ix) Word recognition reaction time - mode

Covariates	Age	NART	WAIS total score
Mode	F (1, 98) = 45.69*	F (1, 98) = 10.57**	F (1, 98) = 0.07
Mode*Covariate	F (1, 98) = 0.00	F (1, 98) = 0.29	F (1, 98) = 1.95
Mode*polyphenol consumption quartiles	F (3, 98) = 0.25	F (3, 98) = 0.26	F (3, 98) = 0.36
Covariate	F (1, 98) = 2.51	F (1, 98) = 1.55	F (1, 98) = 3.53
Polyphenol consumption quartiles	F (3, 98) = 0.55	F (3, 98) = 0.48	F (3, 98) = 0.47

*p<0.001, **p<0.01

(b) Visual Spatial Learning Test (VSLT)

(i) Correctly identified designs (immediate)

Covariates	Age	NART	WAIS total score
Trial	F (2, 196) = 4.68***	F (2, 196) = 4.99***	F (2, 196) = 1.70
Trial*Covariate	F (2, 196) = 0.15	F (2, 196) = 1.05	F (2, 196) = 0.73
Trial*polyphenol consumption quartiles	F (6, 196) = 1.43	F (6, 196) = 1.02	F (6, 196) = 1.17
Covariate	F (1, 98) = 0.45	F (1, 98) = 0.70	F (1, 98) = 1.90
Polyphenol consumption quartiles	F (3, 98) = 0.53	F (3, 98) = 1.13	F (3, 98) = 1.24

***p<0.05

(ii) Correctly identified positions (immediate)

Covariates	Age	NART	WAIS total score
Trial	F (2, 196) = 5.54**	F (2, 196) = 2.66	F (2, 196) = 1.43
Trial*Covariate	F (2, 196) = 0.12	F (2, 196) = 0.22	F (2, 196) = 0.55
Trial*polyphenol consumption quartiles	F (6, 196) = 0.80	F (6, 196) = 0.72	F (6, 196) = 0.68
Covariate	F (1, 98) = 4.95***	F (1, 98) = 0.02	F (1, 98) = 4.15***
Polyphenol consumption quartiles	F (3, 98) = 0.37	F (3, 98) = 0.61	F (3, 98) = 1.52

**p<0.01, ***p<0.05

(iii) Correctly identified target designs placed in the correct positions (immediate)

Covariates	Age	NART	WAIS total score
Trial	F (2, 196) = 13.38*	F (2, 196) = 3.24***	F (2, 196) = 0.63
Trial*Covariate	F (2, 196) = 1.05	F (2, 196) = 0.11	F (2, 196) = 0.80
Trial*polyphenol consumption quartiles	F (6, 196) = 0.66	F (6, 196) = 0.56	F (6, 196) = 0.77
Covariate	F (1, 98) = 2.55	F (1, 98) = 1.06	F (1, 98) = 1.47
Polyphenol consumption quartiles	F (3, 98) = 0.33	F (3, 98) = 1.90	F (3, 98) = 2.00

*p<0.001, ***p<0.0

(iv) Total immediate recall per trial

Covariates	Age	NART	WAIS total score
Covariate	F (1, 95) = 2.07	F (1, 95) = 0.72	F (1, 95) = 1.58
Polyphenol consumption quartiles	F (3, 95) = 0.83	F (3, 95) = 2.56	F (3, 95) = 0.66

(v) Correctly identified designs (delayed)

Covariates	Age	NART	WAIS total score
Covariate	F (1, 95) = 0.13	F (1, 95) = 1.33	F (1, 95) = 2.02
Polyphenol consumption quartiles	F (3, 95) = 0.34	F (3, 95) = 0.67	F (3, 95) = 0.63

(vi) Correctly identified positions (delayed)

Covariates	Age	NART	WAIS total score
Covariate	F (1, 95) = 5.31***	F (1, 95) = 0.08	F (1, 95) = 0.81
Polyphenol consumption quartiles	F (3, 95) = 0.23	F (3, 95) = 0.76	F (3, 95) = 0.40

***p<0.05

(vii) Correctly identified target designs placed in the correct positions (delayed)

Covariates	Age	NART	WAIS total score
Covariate	F (1, 95) = 2.51	F (1, 95) = 0.80	F (1, 95) = 0.69
Polyphenol consumption quartiles	F (3, 95) = 0.67	F (3, 95) = 1.06	F (3, 95) = 0.33

(viii) Total delayed recall

Covariates	Age	NART	WAIS total score
Covariate	F (1, 95) = 3.02	F (1, 95) = 0.41	F (1, 95) = 1.28
Polyphenol consumption quartiles	F (3, 95) = 0.54	F (3, 95) = 1.41	F (3, 95) = 0.38

(a) Corsi Block Tapping Test

(i) Correct responses – accuracy

Covariates	Age	NART	WAIS total score
Covariate	F (1, 95) = 2.83	F (1, 95) = 1.22	F (1, 95) = 7.48**
Polyphenol consumption quartiles	F (3, 95) = 1.76	F (3, 95) = 2.36	F (3, 95) = 1.20

**p<0.01

(ii) Correct responses - reaction time

Covariates	Age	NART	WAIS total score
Covariate	F (1, 95) = 10.96	F (1, 95) = 1.31	F (1, 95) = 0.36
Polyphenol consumption quartiles	F (3, 95) = 1.08	F (3, 95) = 0.56	F (3, 95) = 0.73

(iii) The effect of crossing – accuracy – with crossing

Covariates	Age	NART	WAIS total score
Covariate	F (1, 98) = 1.23	F (1, 98) = 1.29	F (1, 98) = 7.94**
Polyphenol consumption quartiles	F (3, 98) = 0.46	F (3, 98) = 1.91	F (3, 98) = 1.31

**p<0.01

(iv) The effect of crossing – accuracy – without crossing

Covariates	Age	NART	WAIS total score
Covariate	F (1, 98) = 3.07	F (1, 98) = 0.57	F (1, 98) = 3.57
Polyphenol consumption quartiles	F (3, 98) = 3.17***	F (3, 98) = 1.59	F (3, 98) = 1.09

***p<0.05

(v) The effect of crossing – reaction time – with crossing

Covariates	Age	NART	WAIS total score
Covariate	F (1, 98) = 3.59	F (1, 98) = 0.02	F (1, 98) = 2.27
Polyphenol consumption quartiles	F (3, 98) = 1.32	F (3, 98) = 0.31	F (3, 98) = 0.56

(vi) The effect of crossing – reaction time – without crossing

Covariates	Age	NART	WAIS total score
Covariate	F (1, 98) = 14.58*	F (1, 98) = 2.61	F (1, 98) = 0.01
Polyphenol consumption quartiles	F (3, 98) = 1.04	F (3, 98) = 0.48	F (3, 98) = 1.01

*p<0.001

(vii) Percentage correct per level

Covariates	Age	NART	WAIS total score
Level	F (7, 686) = 6.88*	F (7, 686) = 4.92*	F (7, 686) = 3.80*
Level*Covariate	F (7, 686) = 2.52***	F (7, 686) = 0.92	F (7, 686) = 1.93
Level*polyphenol consumption quartiles	F (21, 686) = 0.73	F (21, 686) = 0.97	F (21, 686) = 1.45
Covariate	F (1, 98) = 3.29	F (1, 98) = 1.19	F (1, 98) = 8.59
Polyphenol consumption quartiles	F (3, 98) = 0.13	F (3, 98) = 1.00	F (3, 98) = 2.04

*p<0.001, ***p<0.05

(b) Tower of Hanoi

Covariates	Age	NART	WAIS total score
Level	F (4, 392) = 1.33	F (4, 392) = 2.23	F (4, 392) = 1.34
Level*Covariate	F (4, 392) = 1.63	F (4, 392) = 1.35	F (4, 392) = 0.94
Level*polyphenol consumption quartiles	F (12, 392) = 0.77	F (12, 392) = 0.71	F (12, 392) = 0.80
Covariate	F (1, 98) = 1.42	F (1, 98) = 0.58	F (1, 98) = 2.00
Polyphenol consumption quartiles	F (3, 98) = 0.64	F (3, 98) = 0.49	F (3, 98) = 0.96

Subjective evaluation of cognitive performance according to polyphenol consumption quartiles

Visual analogue scales rating	F value
Time pressure	F (3,99) = 0.03, ns
Difficulty	F (3,99) = 0.81, ns
Ability to concentrate	F (3,99) = 0.88, ns
Effort	F (3,99) = 0.78, ns
Performance	F (3,99) = 0.67, ns
Frustration	F (3,99) = 0.36, ns

Appendix 14 ANOVA table for results based on beverage consumption groups

(a) Visual Verbal Learning Test

(i) Rate of learning (word List A)

Covariates	Age	NART	WAIS total score
Trial	F (2, 196) = 20.85*	F (2, 196) = 8.59*	F (2, 196) = 3.86***
Trial*Covariate	F (2, 196) = 1.66	F (2, 196) = 1.11	F (2, 196) = 0.99
Trial*beverage consumption groups	F (6, 196) = 0.97	F (6, 196) = 1.11	F (6, 196) = 1.29
Covariate	F (1, 98) = 0.70	F (1, 98) = 0.60	F (1, 98) = 1.30
Beverage consumption groups	F (3, 98) = 0.63	F (3, 98) = 0.70	F (3, 98) = 0.77

*p<0.001, ***p<0.05

(ii) New learning (recall of List B)

Covariates	Age	NART	WAIS total score
Covariate	F (1, 95) = 1.76	F (1, 95) = 2.21	F (1, 95) = 1.09
Beverage consumption groups	F (3, 95) = 1.38	F (3, 95) = 0.49	F (3, 95) = 0.20

(iii) Retroactive interference (Trial A3-A4)

Covariates	Age	NART	WAIS total score
Covariate	F (1, 95) = 0.14	F (1, 95) = 0.04	F (1, 95) = 0.08
Beverage consumption groups	F (3, 95) = 0.87	F (3, 95) = 1.48	F (3, 95) = 2.17

(iv) Proactive interference (Trial A1-B1)

Covariates	Age	NART	WAIS total score
Covariate	F (1, 95) = 0.02	F (1, 95) = 1.38	F (1, 95) = 0.17
Beverage consumption groups	F (3, 95) = 2.12	F (3, 95) = 0.40	F (3, 95) = 1.69

(v) Delayed recall

Covariates	Age	NART	WAIS total score
Covariate	F (1, 95) = 1.10	F (1, 95) = 0.20	F (1, 95) = 0.96
Beverage consumption groups	F (3, 95) = 2.00	F (3, 95) = 0.82	F (3, 95) = 1.52

(vi) Word recognition – accuracy - word list

Covariates	Age	NART	WAIS total score
Word list	F (2, 196) = 2.21	F (2, 196) = 2.28	F (2, 196) = 0.07
Word list*Covariate	F (2, 196) = 0.98	F (2, 196) = 0.94	F (2, 196) = 0.01
Word list*beverage consumption groups	F (6, 196) = 1.79	F (6, 196) = 1.26	F (6, 196) = 1.48
Covariate	F (1, 98) = 0.40	F (1, 98) = 1.65	F (1, 98) = 3.06
Beverage consumption groups	F (3, 98) = 0.28	F (3, 98) = 0.31	F (3, 98) = 0.28

(vii) Word recognition – accuracy - mode

Covariates	Age	NART	WAIS total score
Mode	F (1, 98) = 0.08	F (1, 98) = 0.01	F (1, 98) = 0.37
Mode*Covariate	F (1, 98) = 1.06	F (1, 98) = 0.08	F (1, 98) = 0.59
Mode*beverage consumption groups	F (3, 98) = 0.05	F (3, 98) = 0.17	F (3, 98) = 0.10
Covariate	F (1, 98) = 0.40	F (1, 98) = 1.65	F (1, 98) = 3.06
Beverage consumption groups	F (3, 98) = 0.28	F (3, 98) = 0.31	F (3, 98) = 0.28

(viii) Word recognition reaction time - word list

Covariates	Age	NART	WAIS total score
Word list	F (2, 196) = 2.10	F (2, 196) = 1.15	F (2, 196) = 0.87
Word list*Covariate	F (2, 196) = 0.21	F (2, 196) = 0.15	F (2, 196) = 0.39
Word list*beverage consumption groups	F (6, 196) = 0.57	F (6, 196) = 0.59	F (6, 196) = 0.68
Covariate	F (1, 98) = 3.52	F (1, 98) = 2.02	F (1, 98) = 3.56
Beverage consumption groups	F (3, 98) = 0.43	F (3, 98) = 0.20	F (3, 98) = 0.13

(ix) Word recognition reaction time - mode

Covariates	Age	NART	WAIS total score
Mode	F (1, 98) = 53.03*	F (1, 98) = 13.22*	F (1, 98) = 0.30
Mode*Covariate	F (1, 98) = 0.01	F (1, 98) = 0.14	F (1, 98) = 1.40
Mode*beverage consumption groups	F (3, 98) = 0.53	F (3, 98) = 0.47	F (3, 98) = 0.45
Covariate	F (1, 98) = 3.55	F (1, 98) = 2.08	F (1, 98) = 3.84
Beverage consumption groups	F (3, 98) = 0.45	F (3, 98) = 0.21	F (3, 98) = 0.13

*p<0.001

(b) Visual Spatial Learning Test (VSLT)

(i) Correctly identified designs (immediate)

Covariates	Age	NART	WAIS total score
Trial	F (2, 196) = 5.84**	F (2, 196) = 5.73**	F (2, 196) = 1.89
Trial*Covariate	F (2, 196) = 0.02	F (2, 196) = 1.31	F (2, 196) = 0.75
Trial*beverage consumption groups	F (6, 196) = 0.91	F (6, 196) = 0.64	F (6, 196) = 0.17
Covariate	F (1, 98) = 2.85	F (1, 98) = 0.20	F (1, 98) = 1.02
Beverage consumption groups	F (3, 98) = 1.30	F (3, 98) = 0.92	F (3, 98) = 0.90

**p<0.01

(ii) Correctly identified positions (immediate)

Covariates	Age	NART	WAIS total score
Trial	F (2, 196) = 5.05***	F (2, 196) = 2.75	F (2, 196) = 1.45
Trial*Covariate	F (2, 196) = 0.01	F (2, 196) = 0.26	F (2, 196) = 0.54
Trial*beverage consumption groups	F (6, 196) = 0.27	F (6, 196) = 0.24	F (6, 196) = 0.19
Covariate	F (1, 98) = 4.16***	F (1, 98) = 0.16	F (1, 98) = 3.33
Beverage consumption groups	F (3, 98) = 0.11	F (3, 98) = 0.64	F (3, 98) = 1.23

***p<0.05

(iii) Correctly identified target designs placed in the correct positions (immediate)

Covariates	Age	NART	WAIS total score
Trial	F (2, 196) = 15.29*	F (2, 196) = 4.57***	F (2, 196) = 0.70
Trial*Covariate	F (2, 196) = 0.97	F (2, 196) = 0.25	F (2, 196) = 0.45
Trial*beverage consumption groups	F (6, 196) = 0.09	F (6, 196) = 0.65	F (6, 196) = 0.10
Covariate	F (1, 98) = 2.09	F (1, 98) = 0.56	F (1, 98) = 1.39
Beverage consumption groups	F (3, 98) = 1.50	F (3, 98) = 3.11***	F (3, 98) = 3.36***

*p<0.001, ***p<0.05

(iv) Total immediate recall per trial

Covariates	Age	NART	WAIS total score
Covariate	F (1, 95) = 8.06**	F (1, 95) = 0.01	F (1, 95) = 0.81
Beverage consumption groups	F (3, 95) = 2.97***	F (3, 95) = 0.74	F (3, 95) = 0.58

**p<0.01, ***p<0.05

(v) Correctly identified designs (delayed)

Covariates	Age	NART	WAIS total score
Covariate	F (1, 95) = 2.87	F (1, 95) = 0.10	F (1, 95) = 1.64
Beverage consumption groups	F (3, 95) = 1.56	F (3, 95) = 0.96	F (3, 95) = 0.99

(vi) Correctly identified positions (delayed)

Covariates	Age	NART	WAIS total score
Covariate	F (1, 95) = 18.20*	F (1, 95) = 0.56	F (1, 95) = 0.34
Beverage consumption groups	F (3, 95) = 3.59***	F (3, 95) = 0.51	F (3, 95) = 0.68

*p<0.001, ***p<0.05

(vii) Correctly identified target designs placed in the correct positions (delayed)

Covariates	Age	NART	WAIS total score
Covariate	F (1, 95) = 8.26**	F (1, 95) = 0.05	F (1, 95) = 0.26
Beverage consumption groups	F (3, 95) = 2.62	F (3, 95) = 1.16	F (3, 95) = 1.12

**p<0.01

(viii) Total delayed recall

Covariates	Age	NART	WAIS total score
Covariate	F (1, 95) = 12.87*	F (1, 95) = 0.10	F (1, 95) = 0.66
Beverage consumption groups	F (3, 95) = 3.47***	F (3, 95) = 1.04	F (3, 95) = 1.19

*p<0.001, ***p<0.05

(a) Corsi Block Tapping Test

(i) Correct responses – accuracy

Covariates	Age	NART	WAIS total score
Covariate	F (1, 95) = 6.98**	F (1, 95) = 0.46	F (1, 95) = 8.12**
Beverage consumption groups	F (3, 95) = 3.00***	F (3, 95) = 0.51	F (3, 95) = 1.97

**p<0.01, ***p<0.05

(ii) Correct responses - reaction time

Covariates	Age	NART	WAIS total score
Covariate	F (1, 95) = 36.89*	F (1, 95) = 1.14	F (1, 95) = 3.77
Beverage consumption groups	F (3, 95) = 3.01***	F (3, 95) = 1.25	F (3, 95) = 4.47

*p<0.001, ***p<0.05

(iii) The effect of crossing – accuracy – with crossing

Covariates	Age	NART	WAIS total score
Covariate	F (1, 95) = 6.43***	F (1, 95) = 0.01	F (1, 95) = 7.43**
Beverage consumption groups	F (3, 95) = 3.79***	F (3, 95) = 1.04	F (3, 95) = 1.01

**p<0.01, ***p<0.05

(iv) The effect of crossing – accuracy – without crossing

Covariates	Age	NART	WAIS total score
Covariate	F (1, 95) = 3.85	F (1, 95) = 1.32	F (1, 95) = 4.53***
Beverage consumption groups	F (3, 95) = 1.17	F (3, 95) = 1.34	F (3, 95) = 3.03***

***p<0.05

(v) The effect of crossing – reaction time – with crossing

Covariates	Age	NART	WAIS total score
Covariate	F (1, 95) = 20.61*	F (1, 95) = 0.10	F (1, 95) = 7.06**
Beverage consumption groups	F (3, 95) = 3.72***	F (3, 95) = 0.95	F (3, 95) = 5.09**

*p<0.01, **p<0.01, ***p<0.05

(vi) The effect of crossing – reaction time – without crossing

Covariates	Age	NART	WAIS total score
Covariate	F (1, 95) = 41.70*	F (1, 95) = 1.95	F (1, 95) = 1.98
Beverage consumption groups	F (3, 95) = 2.28	F (3, 95) = 1.24	F (3, 95) = 3.31***

*p<0.01, ***p<0.05

(vii) Percentage correct per level

Covariates	Age	NART	WAIS total score
Level	F (7, 686) = 8.01*	F (7, 686) = 4.97*	F (7, 686) = 3.84*
Level*Covariate	F (7, 686) = 2.21***	F (7, 686) = 0.96	F (7, 686) = 1.86
Level*beverage consumption groups	F (21, 686) = 1.20	F (21, 686) = 1.57	F (21, 686) = 2.02**
Covariate	F (1, 98) = 1.85	F (1, 98) = 0.91	F (1, 98) = 9.05**
Beverage consumption groups	F (3, 98) = 0.81	F (3, 98) = 2.10	F (3, 98) = 3.43***

*p<0.01, **p<0.01, ***p<0.05

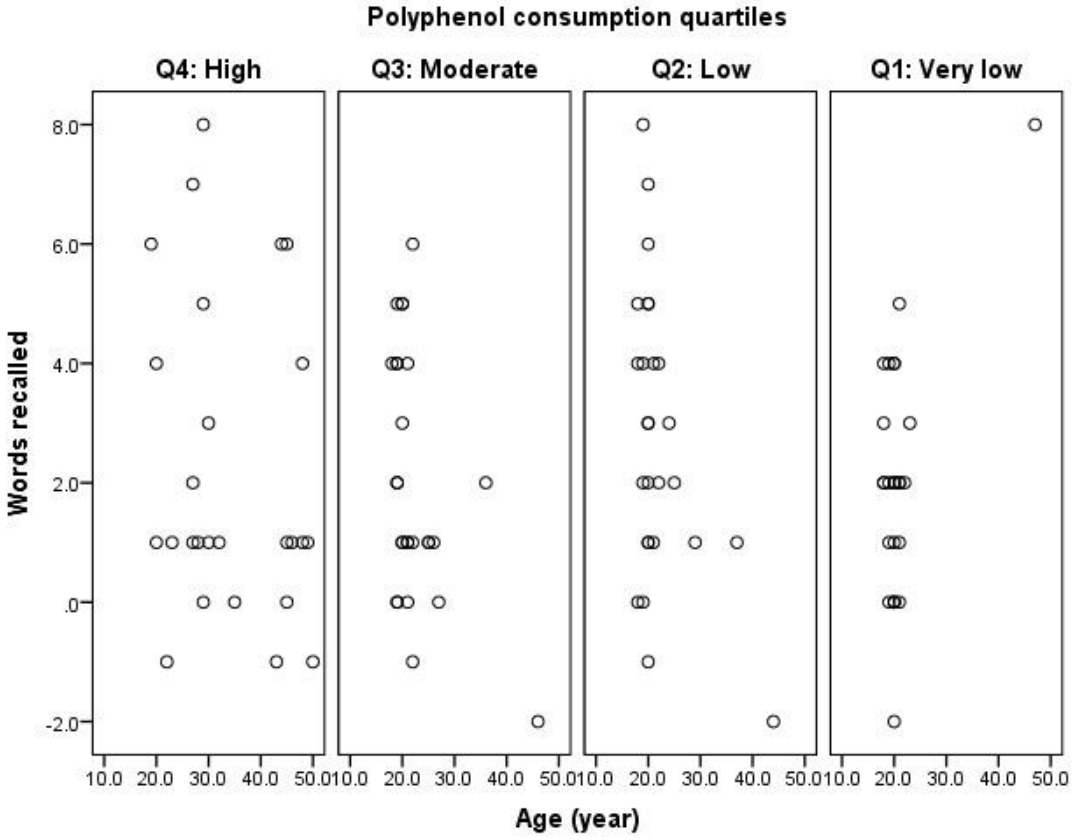
(b) Tower of Hanoi

Covariates	Age	NART	WAIS total score
Level	F (4, 392) = 0.80	F (4, 392) = 2.42	F (4, 392) = 1.30
Level*Covariate	F (4, 392) = 2.06	F (4, 392) = 1.85	F (4, 392) = 1.02
Level*beverage consumption groups	F (12, 392) = 0.73	F (12, 392) = 0.69	F (12, 392) = 0.64
Covariate	F (1, 98) = 1.10	F (1, 98) = 0.58	F (1, 98) = 2.16
Beverage consumption groups	F (3, 98) = 0.41	F (3, 98) = 0.36	F (3, 98) = 0.89

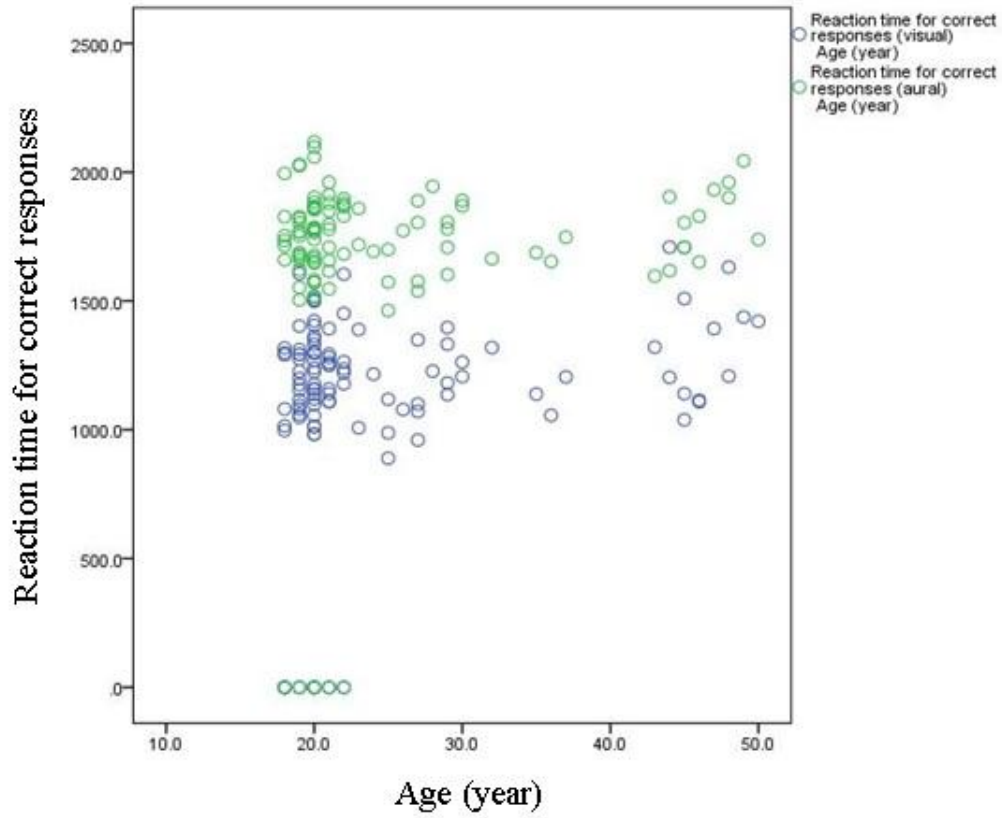
Subjective evaluation of cognitive performance according to beverage consumption groups

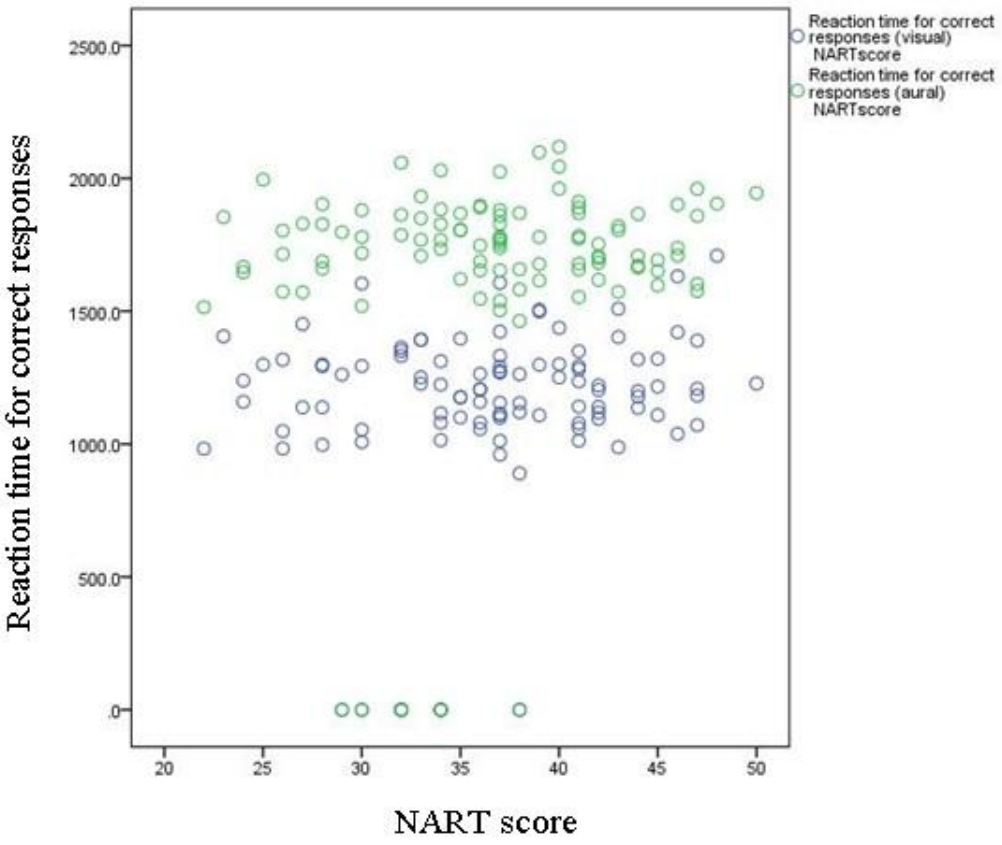
Visual analogue scales rating	F value
Time pressure	F (3,99) = 0.52, ns
Difficulty	F (3,99) = 1.22, ns
Ability to concentrate	F (3,99) = 0.52, ns
Effort	F (3,99) = 0.30, ns
Performance	F (3,99) = 1.80, ns
Frustration	F (3,99) = 2.48, ns

Appendix 15 Number of words recalled on list A after presentation of List B (retroactive interference) according to age and polyphenol consumption quartiles

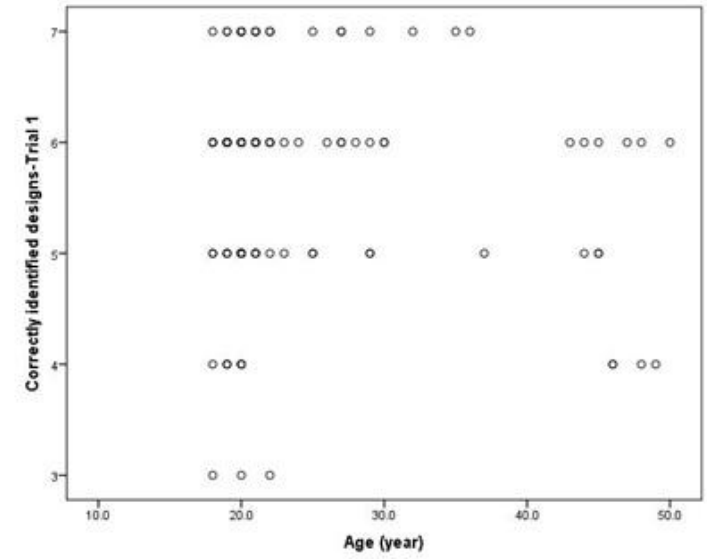


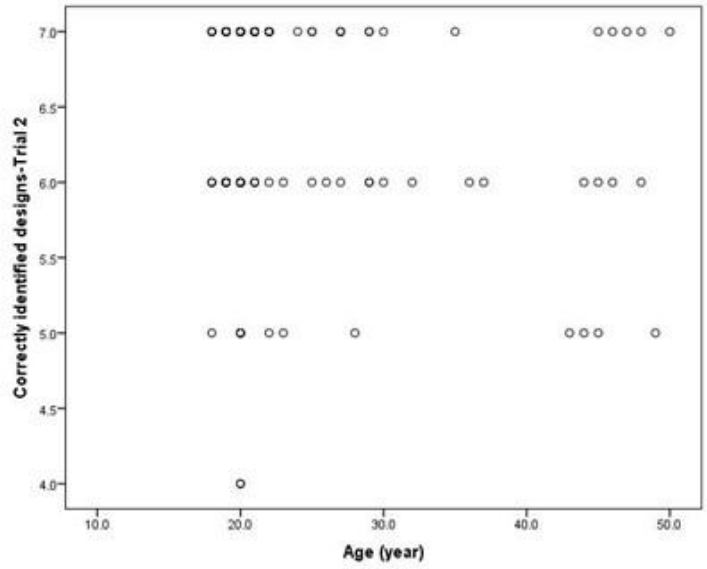
Appendix 16 Interaction between reaction time for correct responses in different modes of presentations with age and NART score

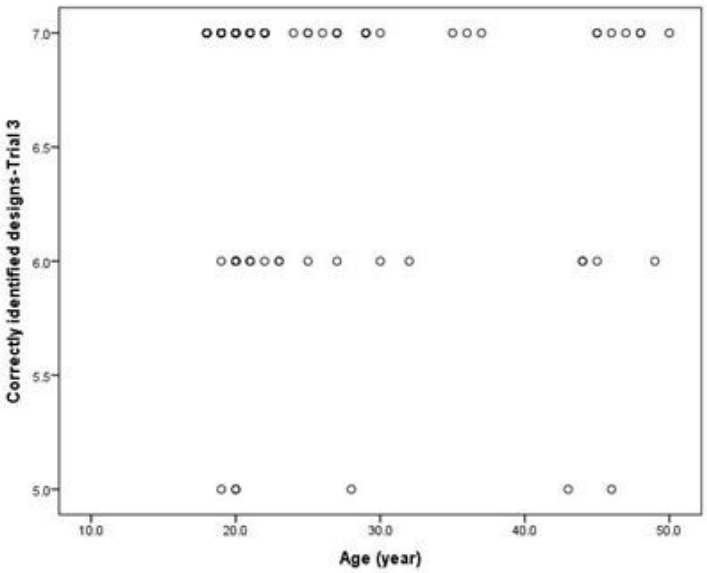




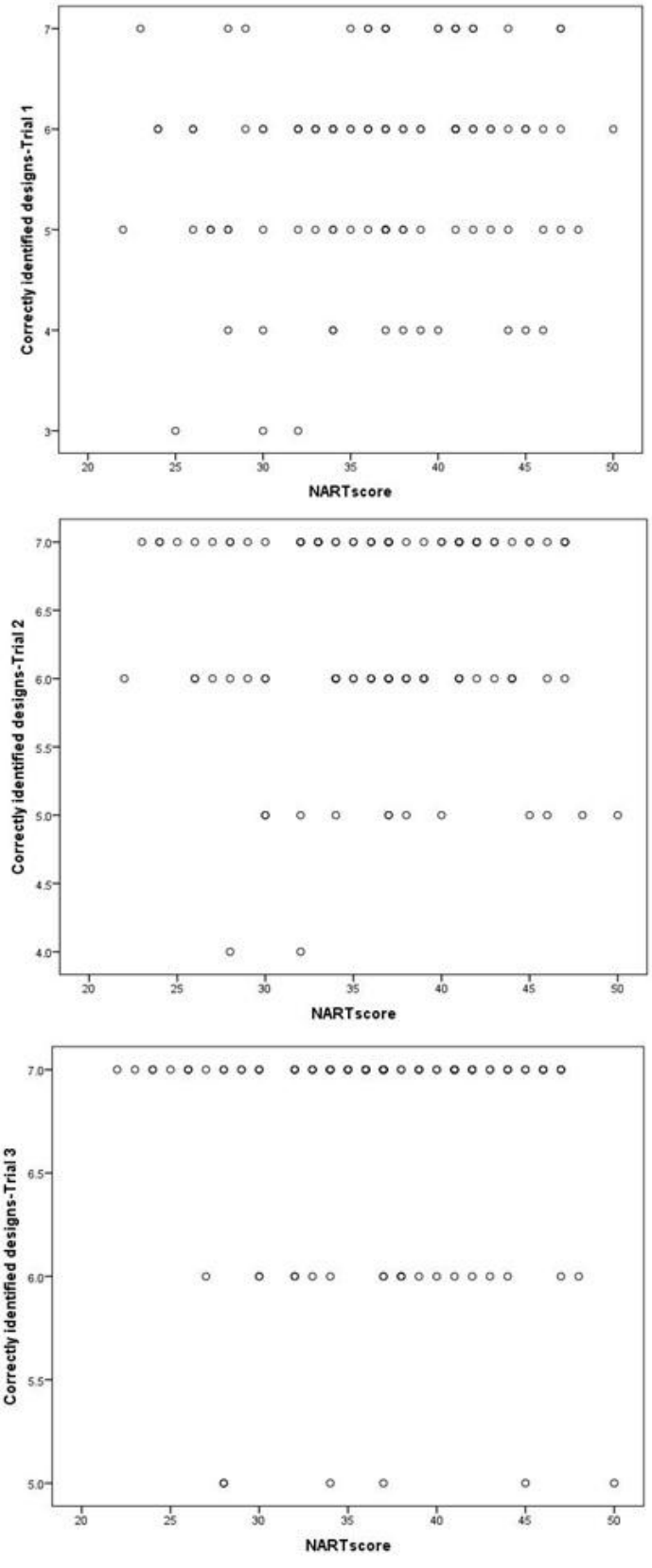
Appendix 17 Interaction between correctly identified designs in different trials (VSLT-immediate) with age



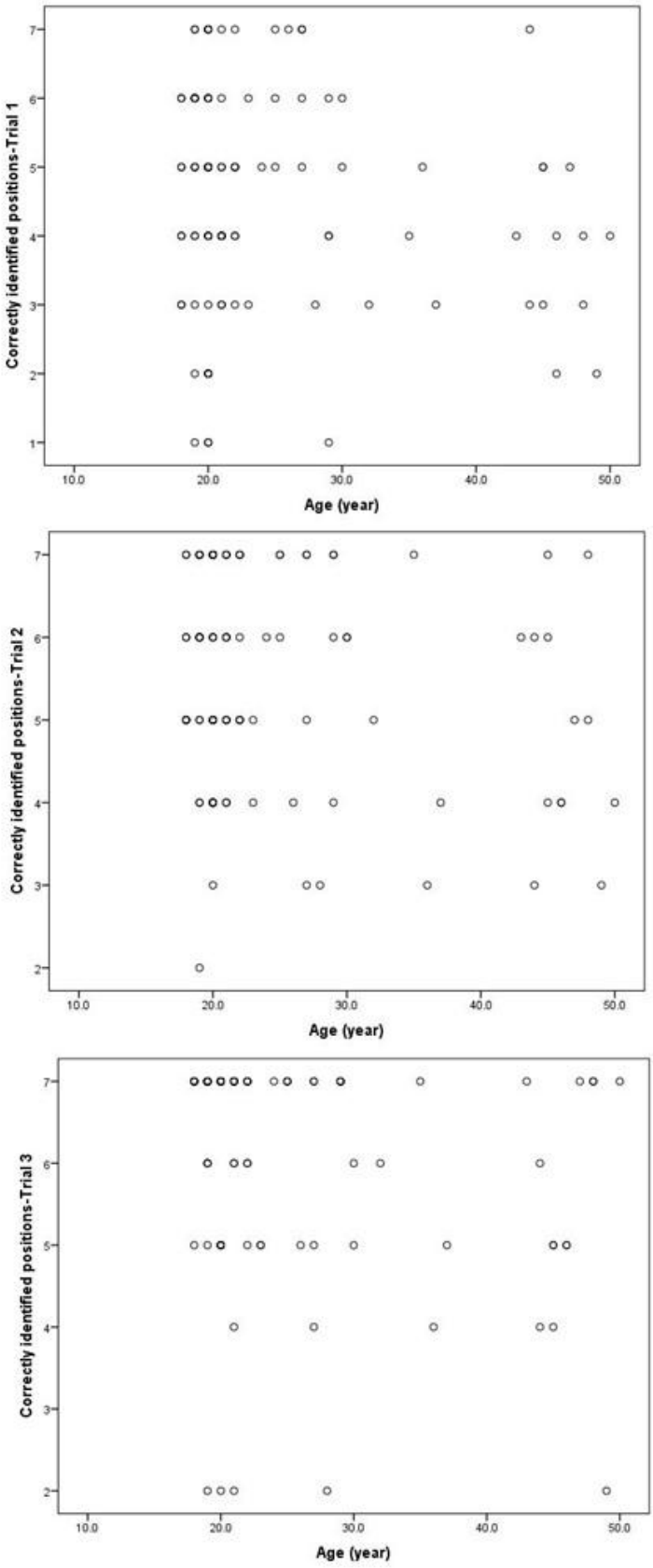




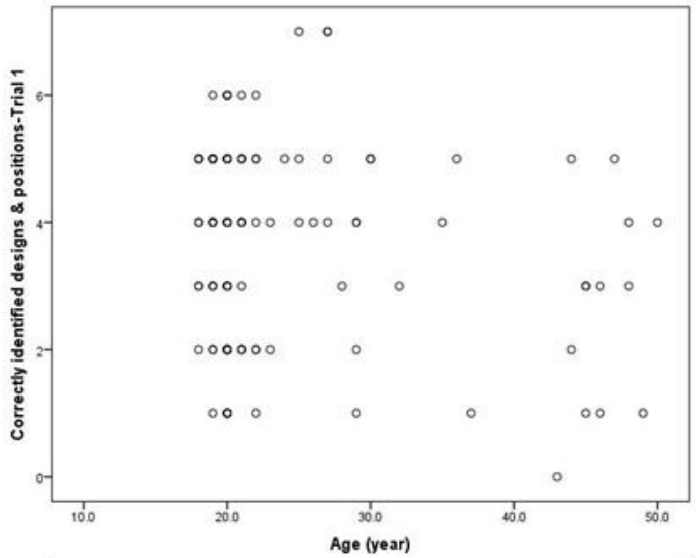
Appendix 18 Interaction between correctly identified designs in different trials (VSLT-immediate) with NART score

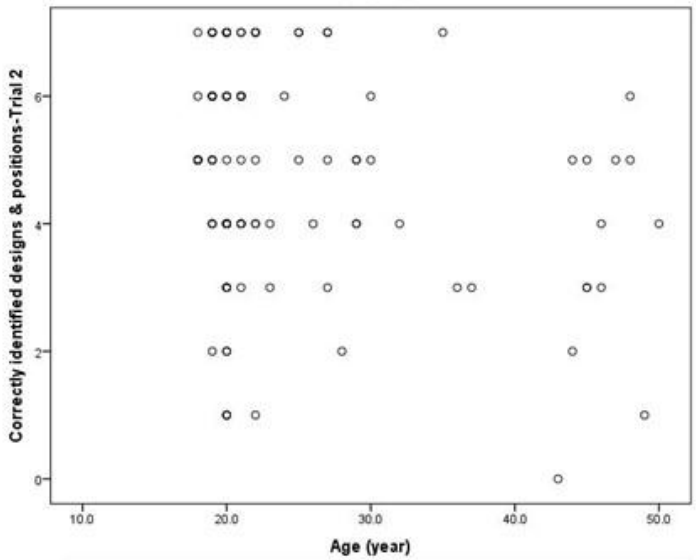


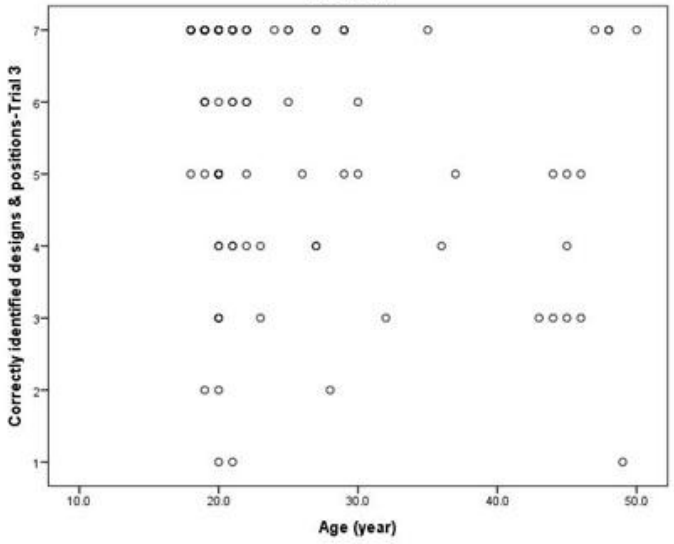
Appendix 19 Interaction between correctly identified positions in different trials (VSLT-immediate) with age



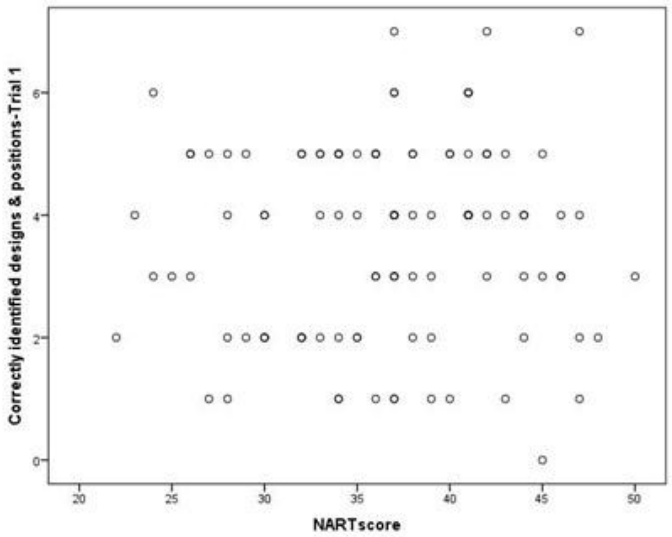
Appendix 20 Interaction between correctly identified designs placed in the correct positions in different trials (VSLT-immediate) with age

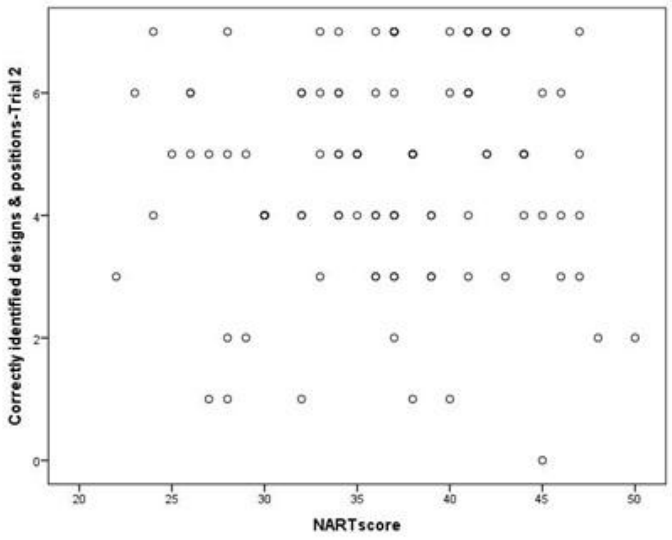


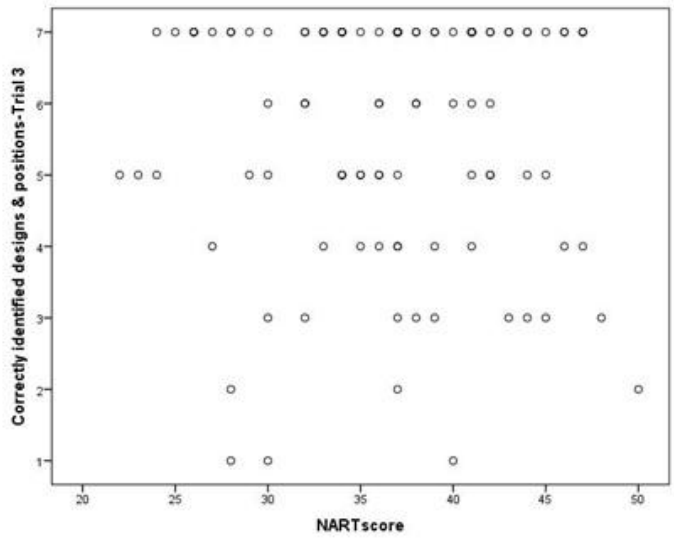




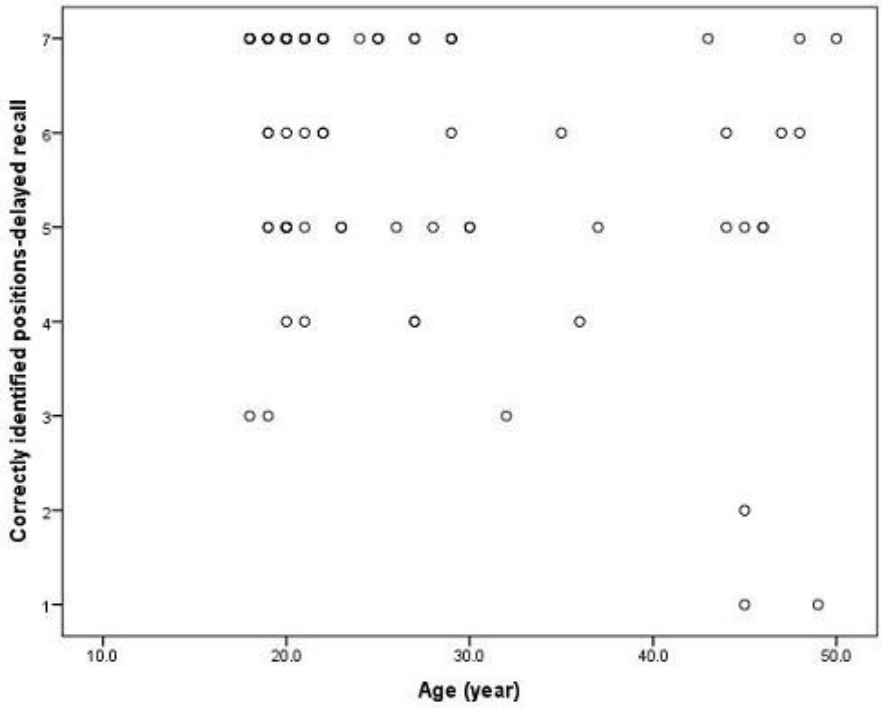
Appendix 21 Interaction between correctly identified designs placed in the correct positions in different trials (VSLT-immediate) with NART score



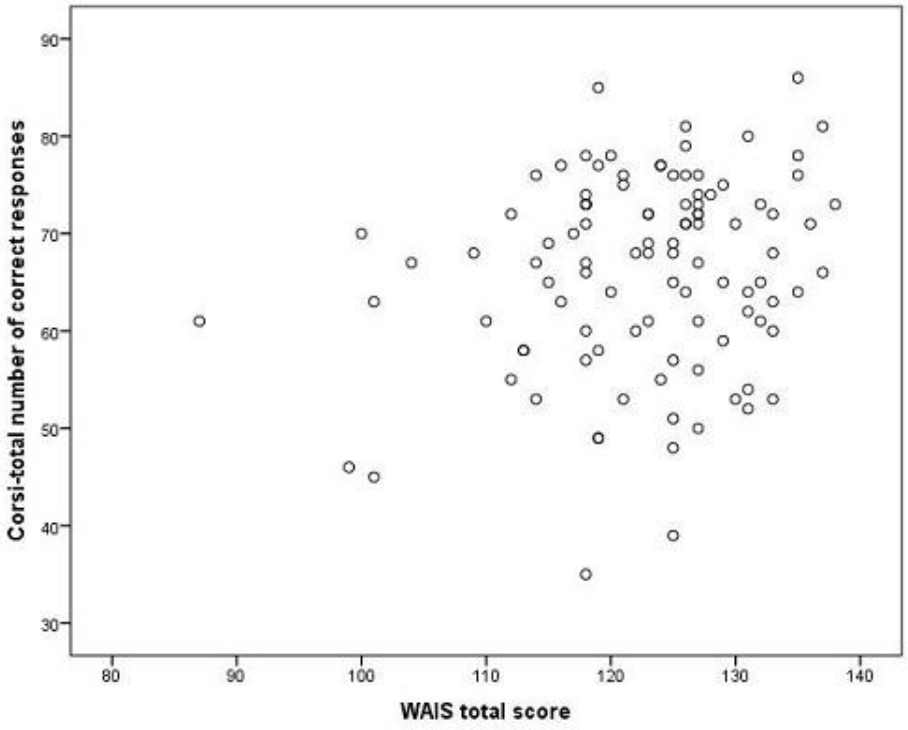




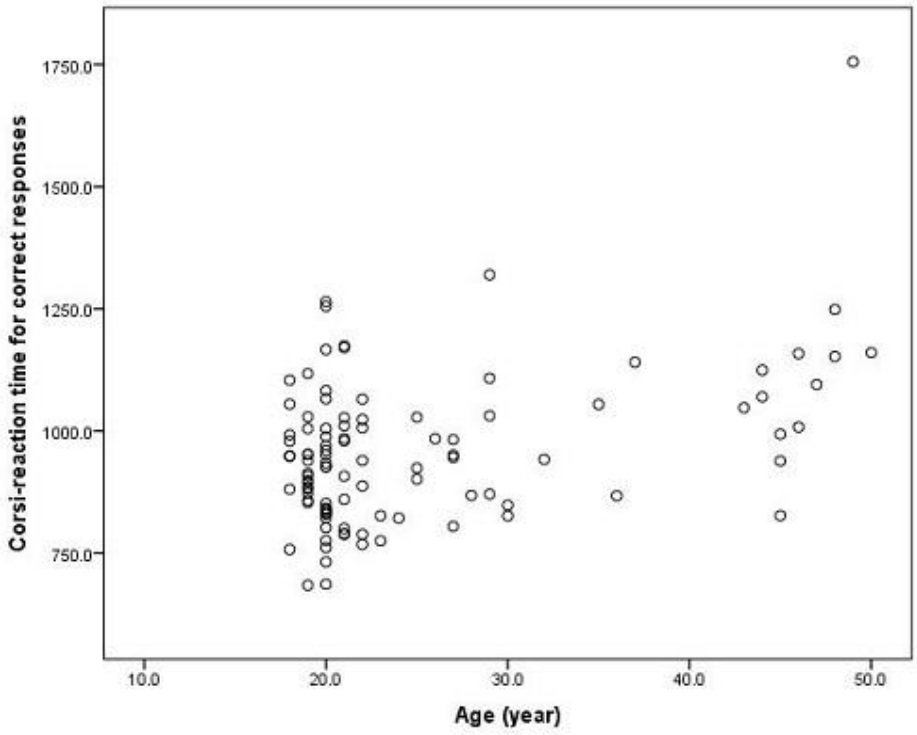
Appendix 22 Interaction between correctly identified positions (VSLT-delayed) with age



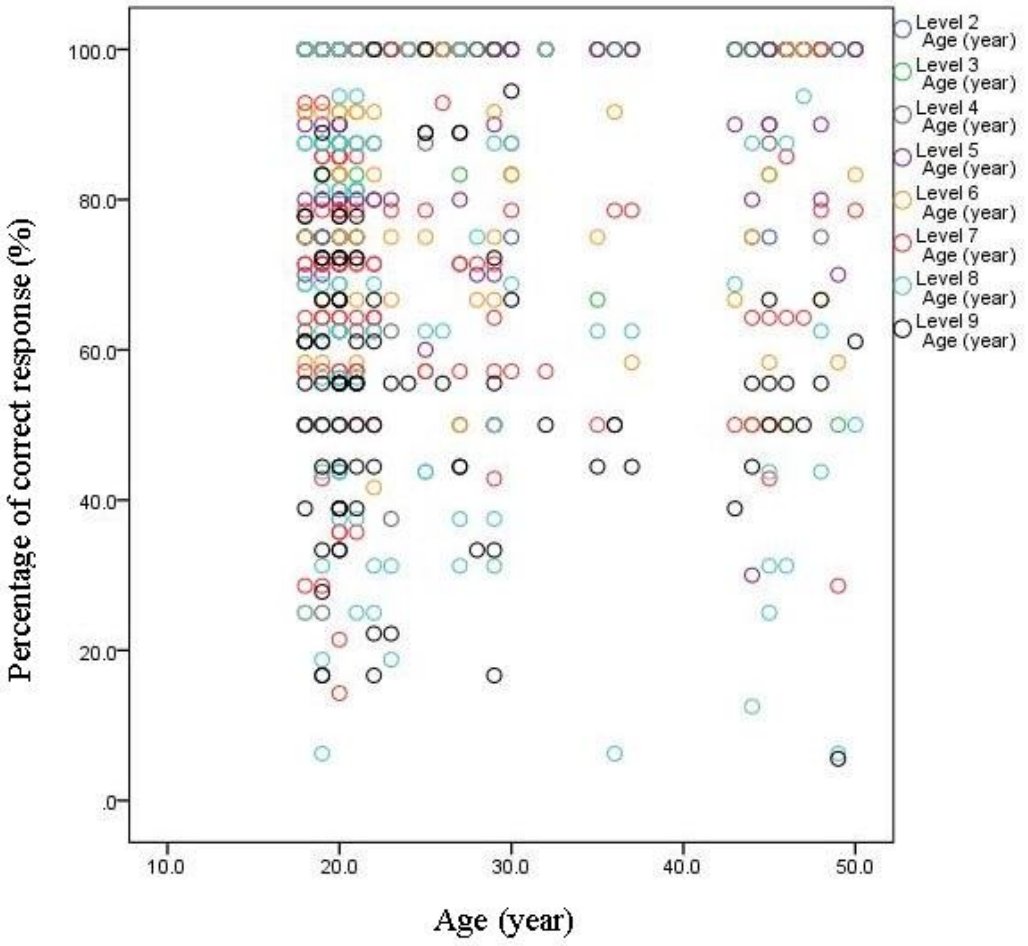
Appendix 23 Interaction between Corsi total numbers of correct responses with WAIS total score

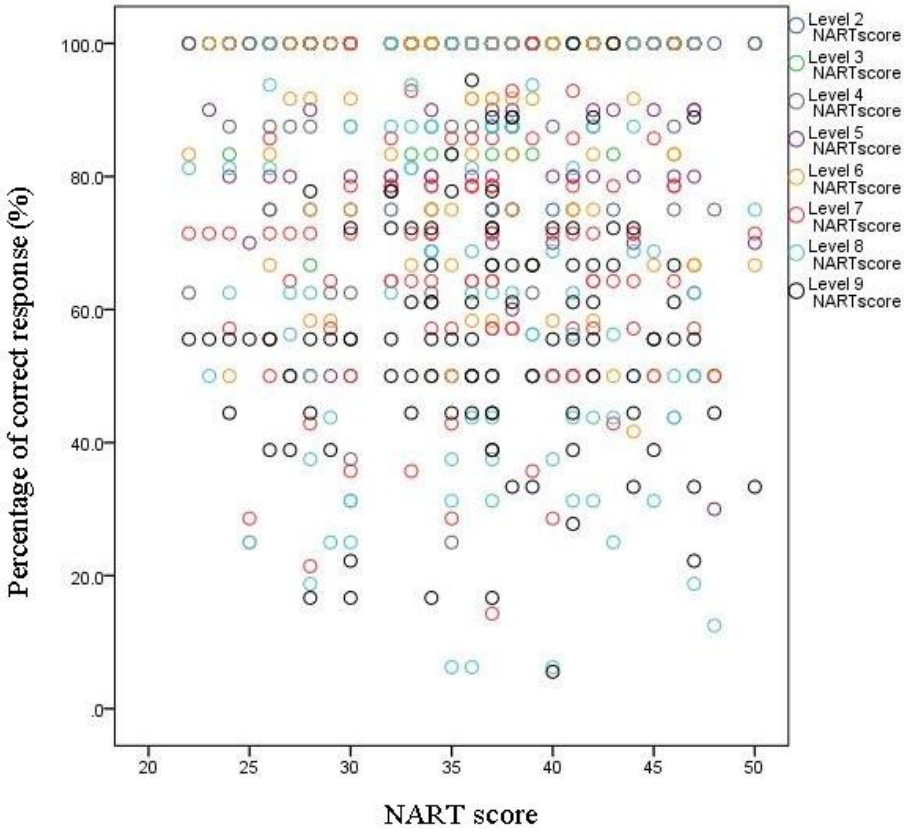


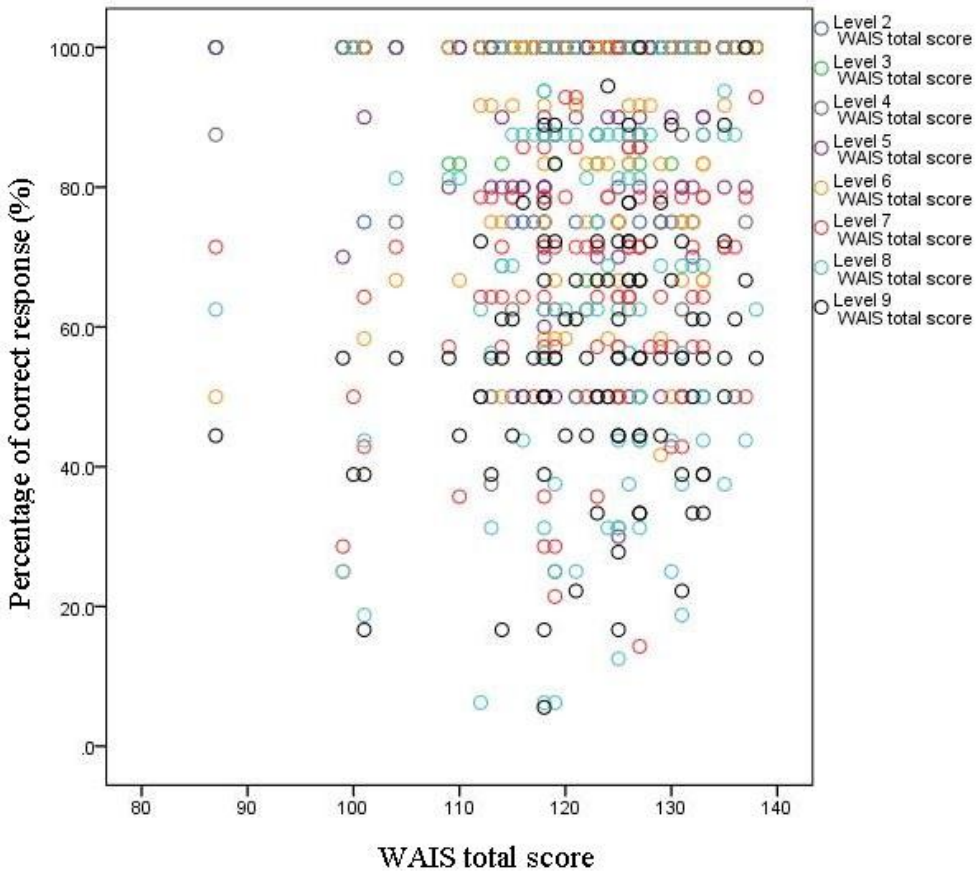
Appendix 24 Interaction between Corsi reaction time for correct responses with age



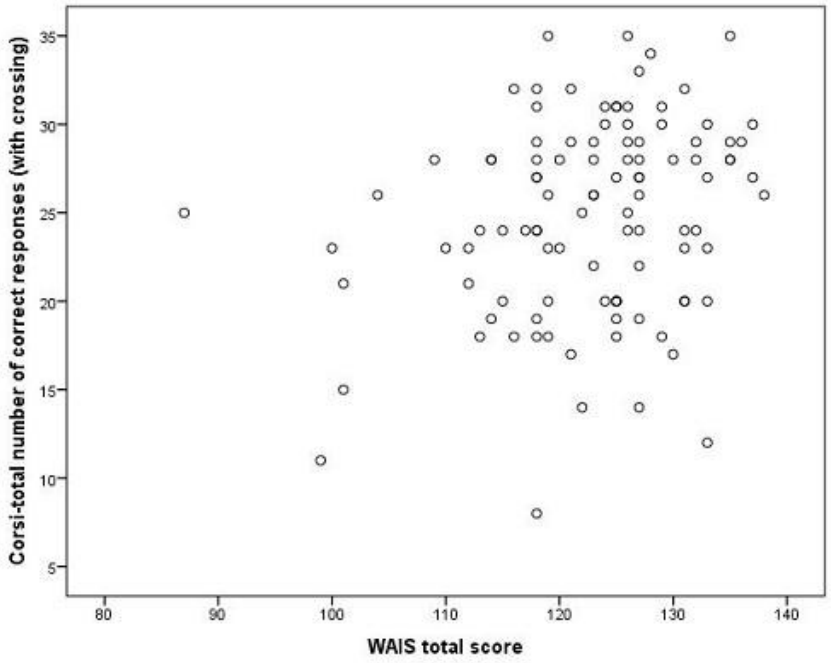
Appendix 25 Interaction between Corsi percentage of correct responses per level with age, NART score and WAIS total score



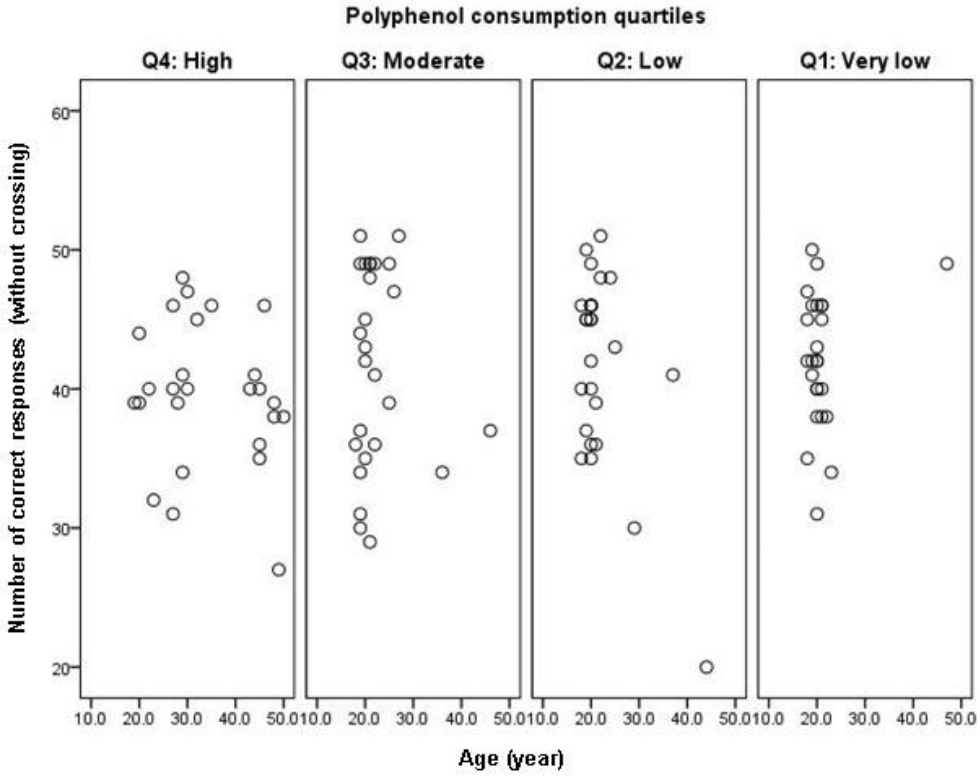

Percentage of correct response (%)
100.0
80.0
60.0
40.0
20.0
.0
20
25
30
35
40
45
50
NART score
Level 2 NARTscore
Level 3 NARTscore
Level 4 NARTscore
Level 5 NARTscore
Level 6 NARTscore
Level 7 NARTscore
Level 8 NARTscore
Level 9 NARTscore


Percentage of correct response (%)
100.0
80.0
60.0
40.0
20.0
.0
80
90
100
110
120
130
140
WAIS total score
Level 2 WAIS total score
Level 3 WAIS total score
Level 4 WAIS total score
Level 5 WAIS total score
Level 6 WAIS total score
Level 7 WAIS total score
Level 8 WAIS total score
Level 9 WAIS total score

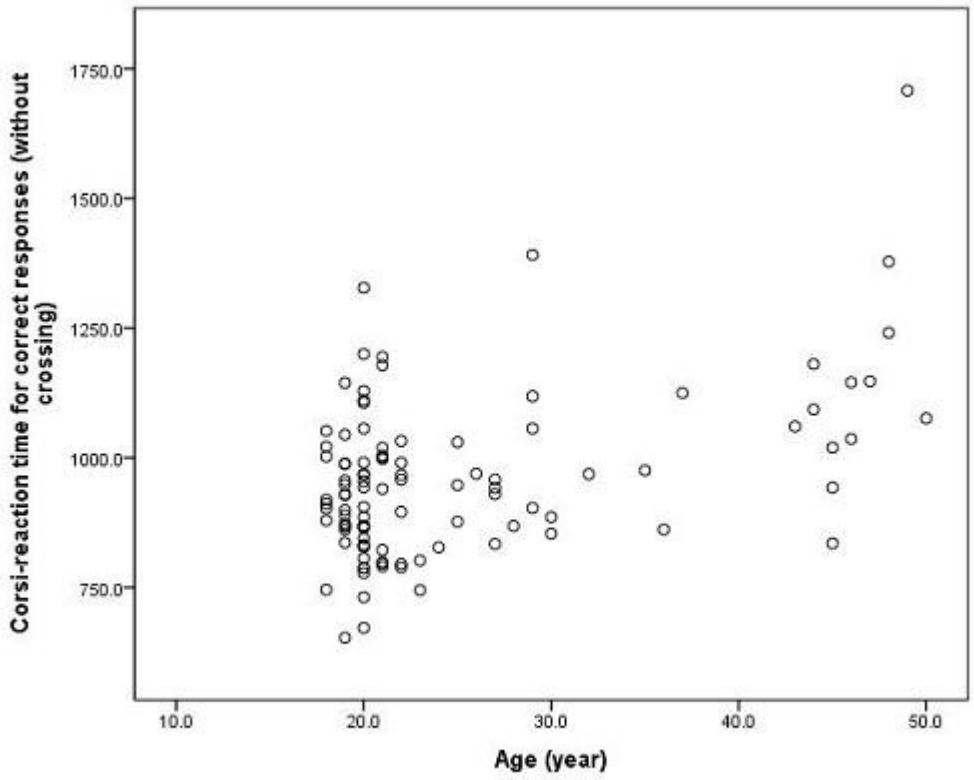
Appendix 26 Interaction between Corsi numbers of correct responses (with crossing) with WAIS total score



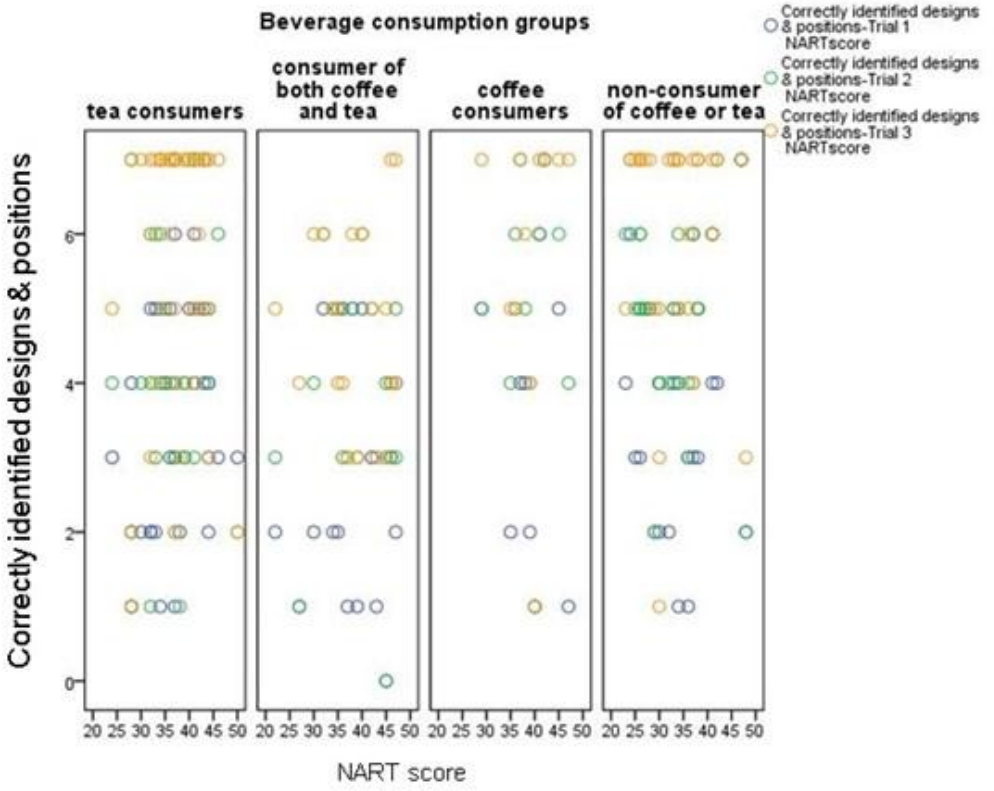
Appendix 27 Interaction between Corsi numbers of correct responses (without crossing) with age

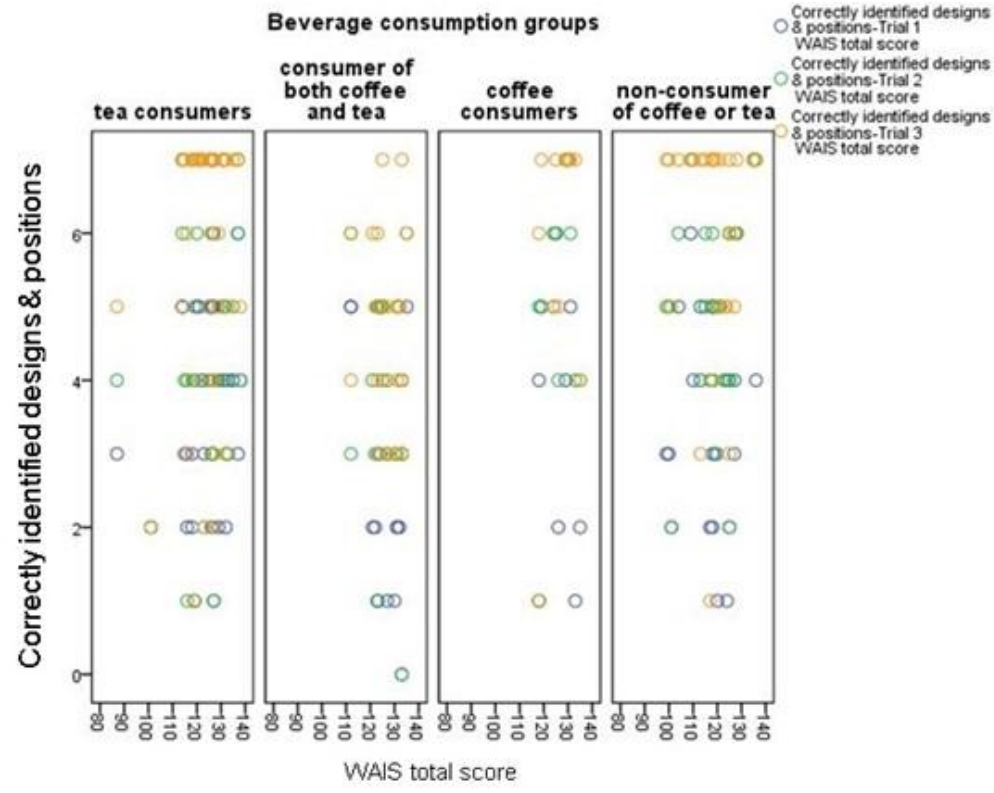


Appendix 28 Interaction between Corsi reaction time for correct responses (without crossing) with age

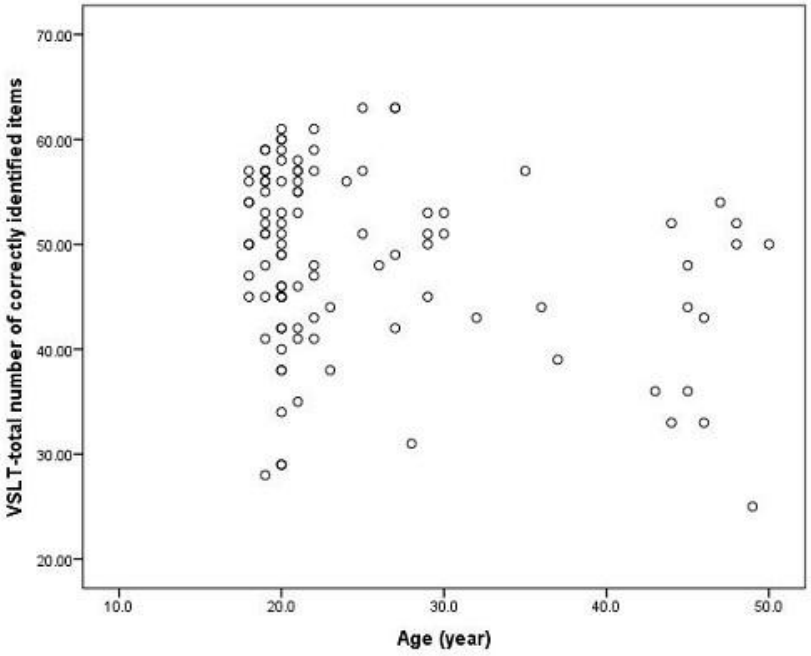


Appendix 29 Interaction between correctly identified designs placed in the correct positions with NART score and WAIS total score in different trials and beverage consumption groups (VSLT-immediate)

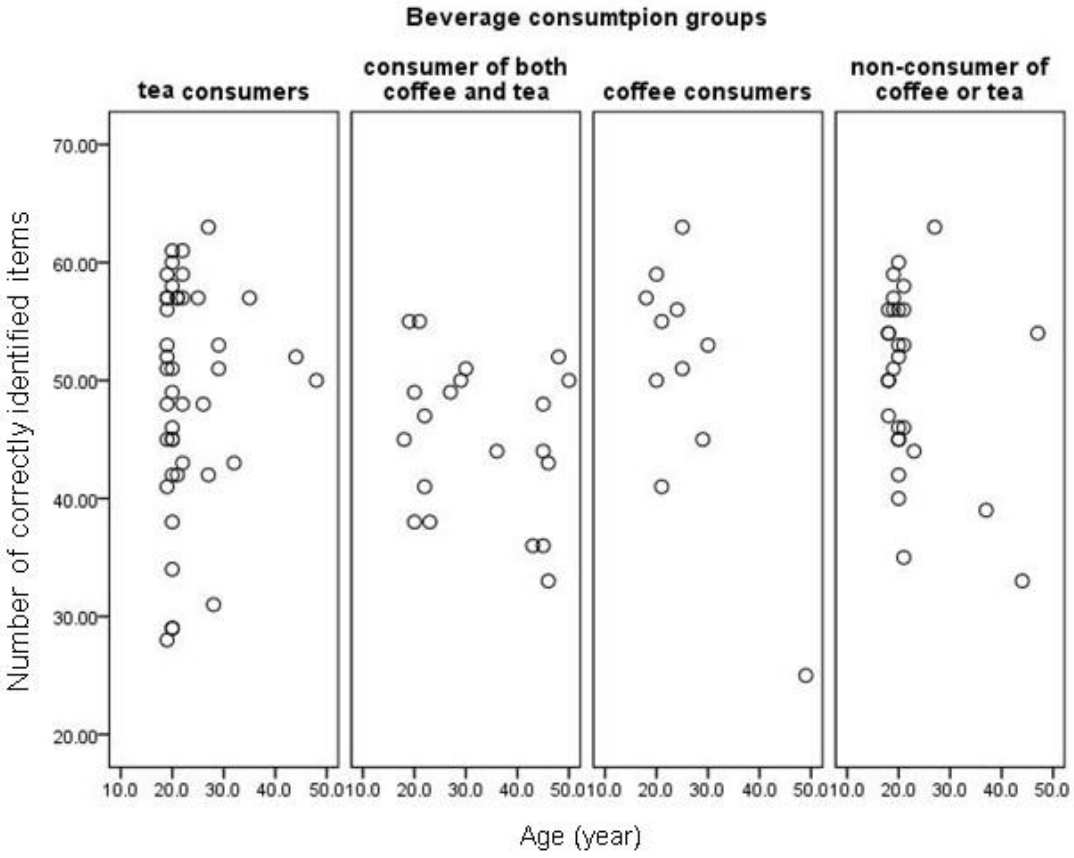




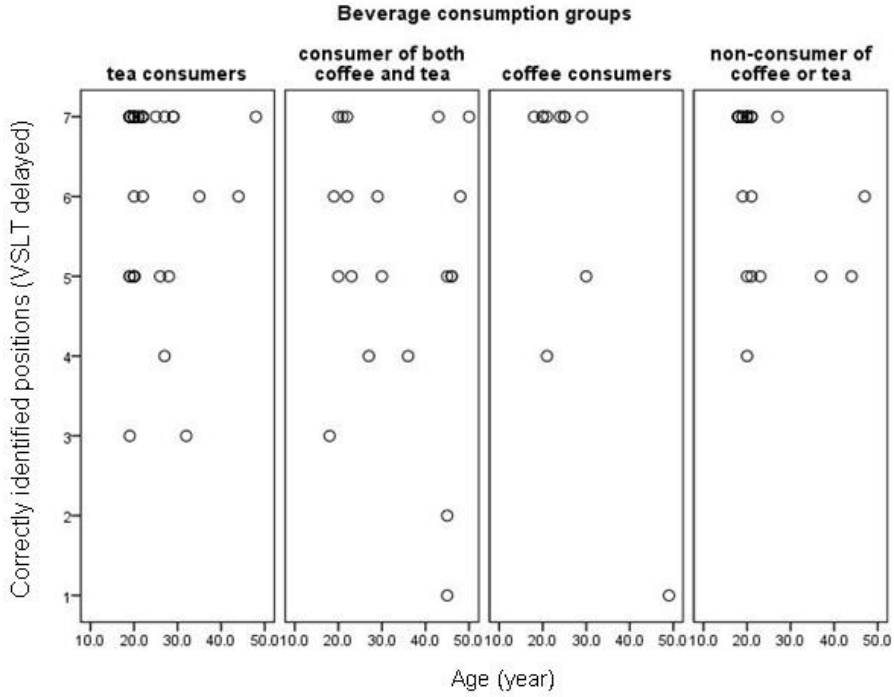
Appendix 30 Interaction between total immediate recall (VSLT) and age



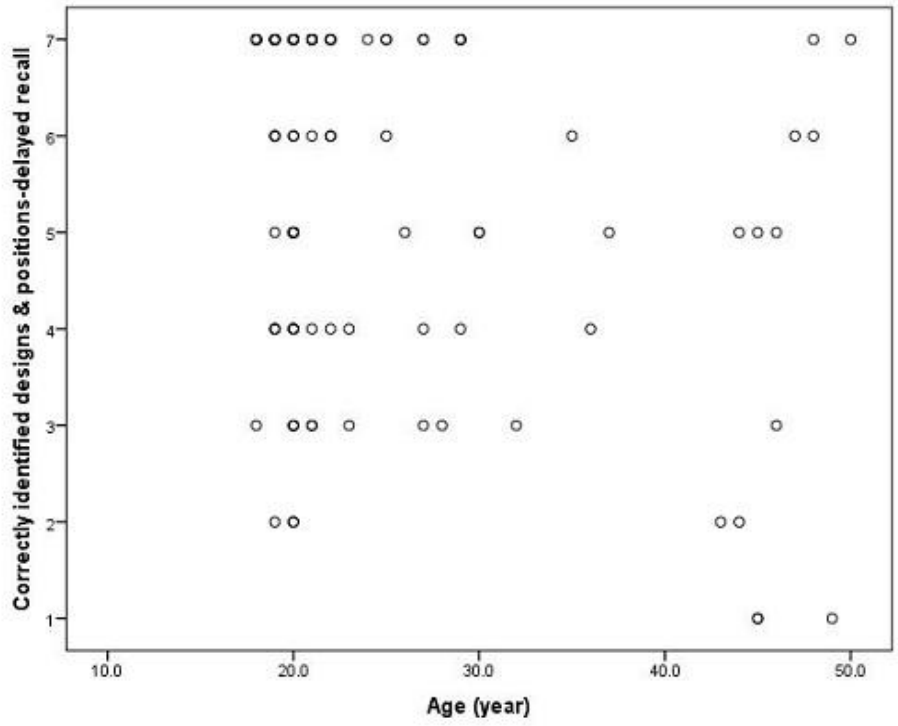
Appendix 31 Interaction between total immediate recall (VSLT) and age in different beverage consumption groups



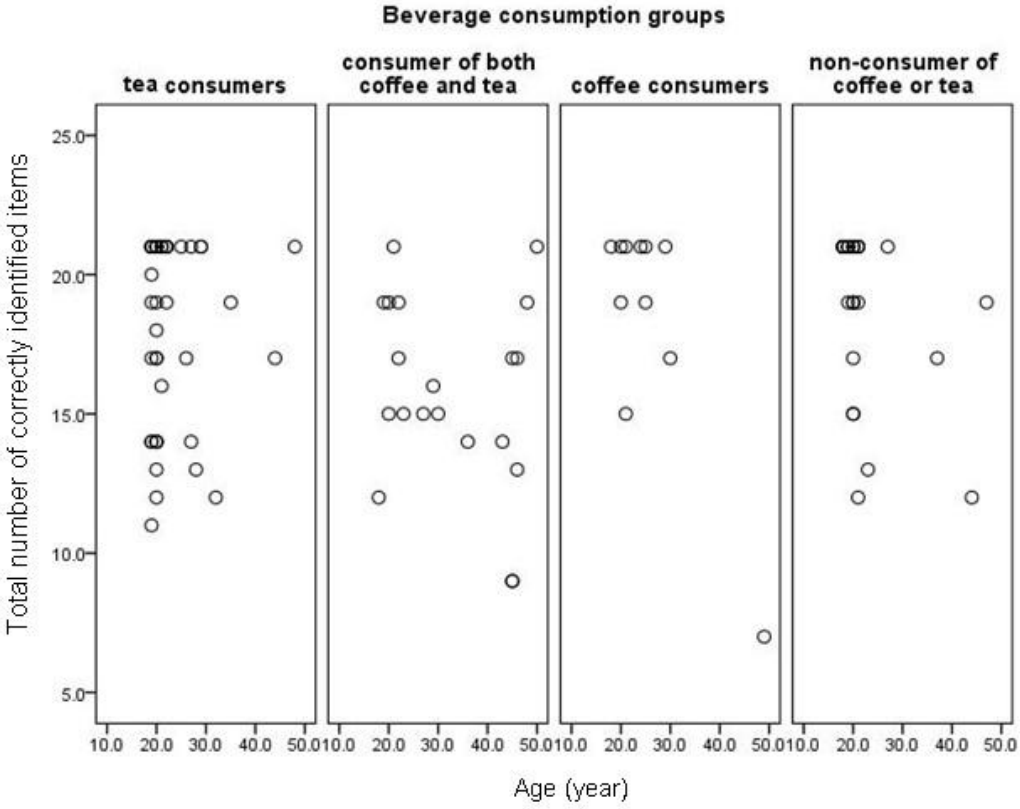
Appendix 32 Interaction between correctly identified positions (VSLT-delayed) and age in different beverage consumption groups



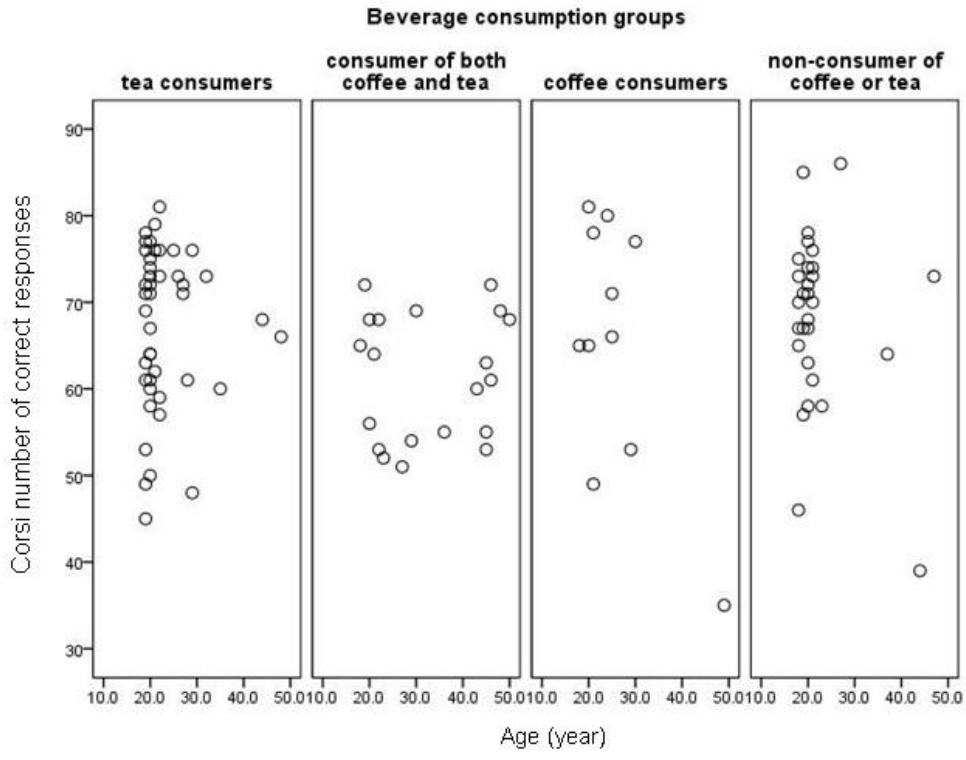
Appendix 33 Interaction between correctly identified designs placed in the correct positions (VSLT-delayed) with age



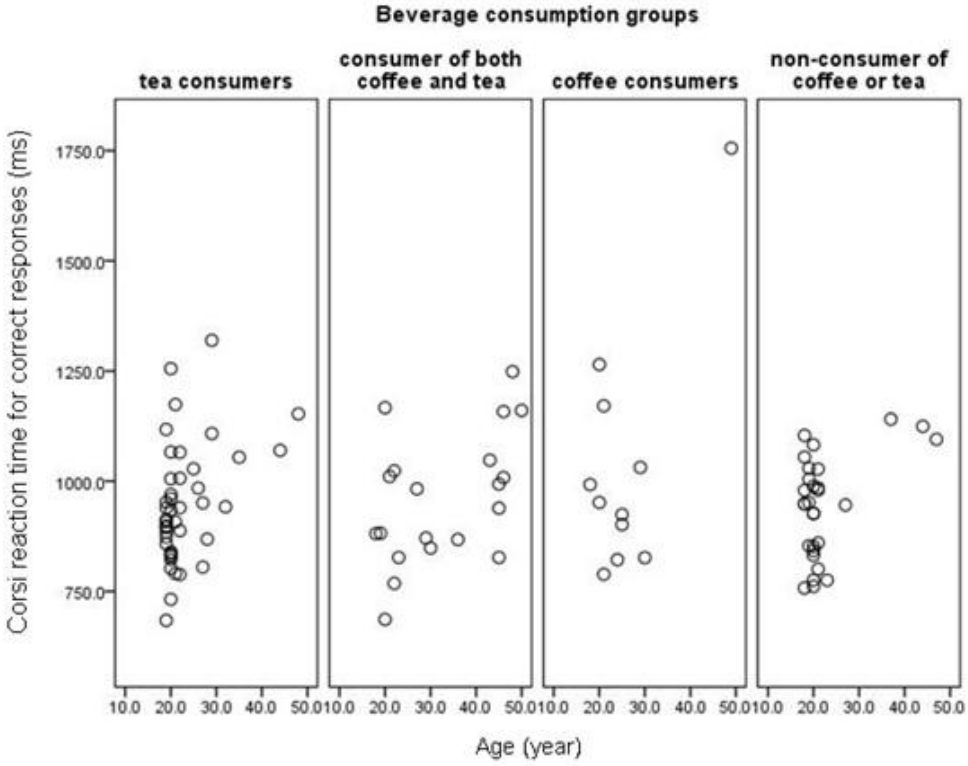
Appendix 34 Interaction between total delayed recall (VSLT-delayed) and age in different beverage consumption groups

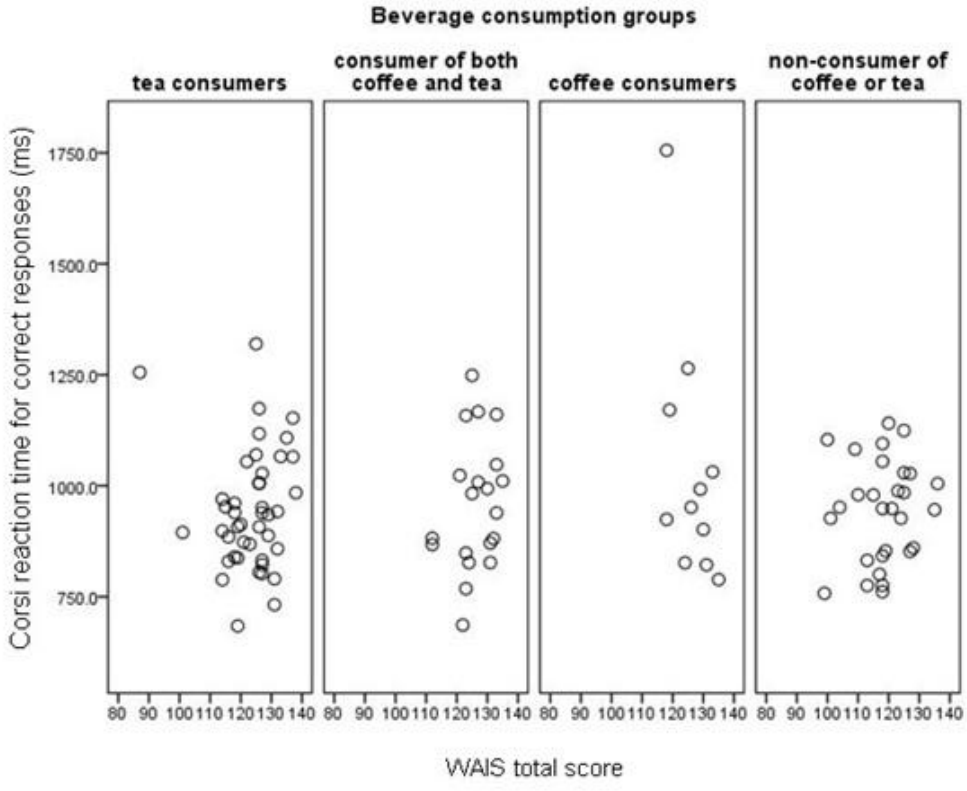


Appendix 35 Interaction between numbers of correct responses (Corsi) and age in different beverage consumption groups

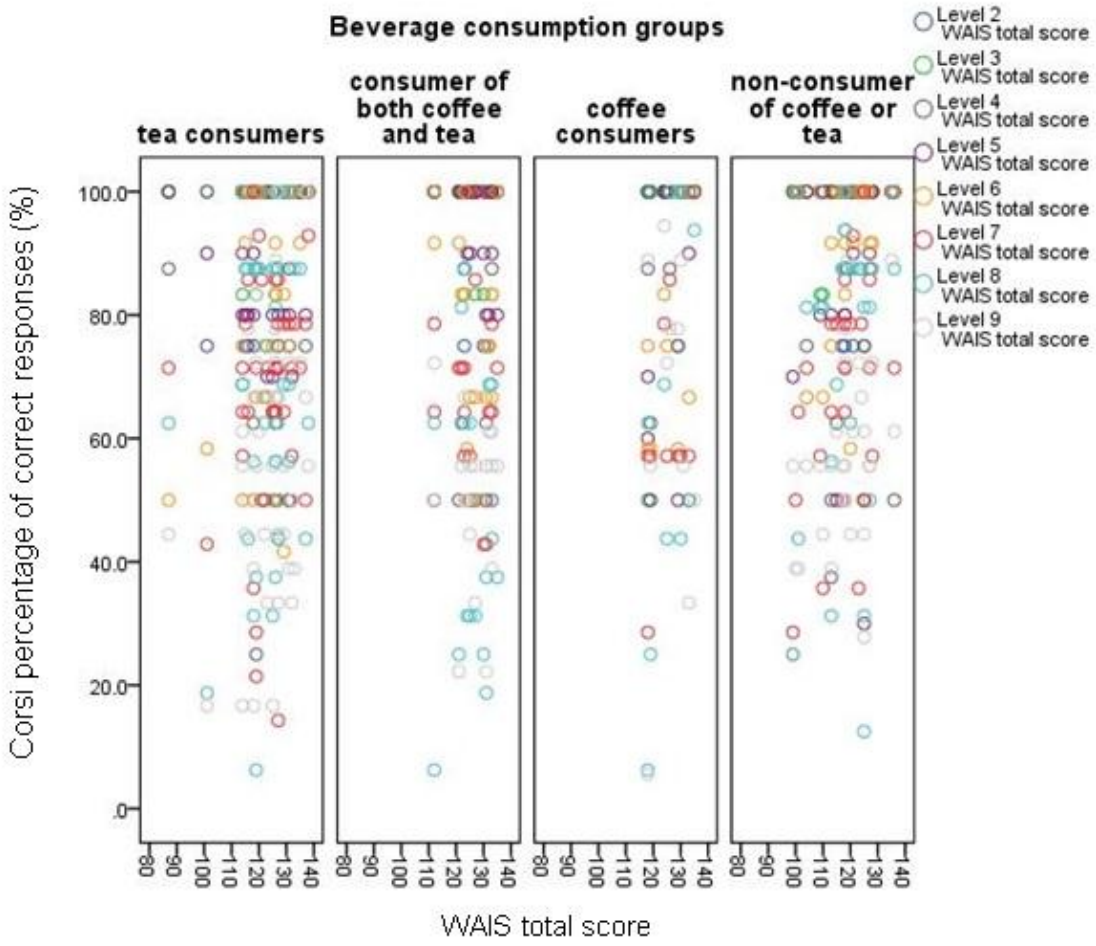


Appendix 36 Interaction between in reaction time for correct responses (Corsi) with age and WAIS total score in different beverage consumption groups

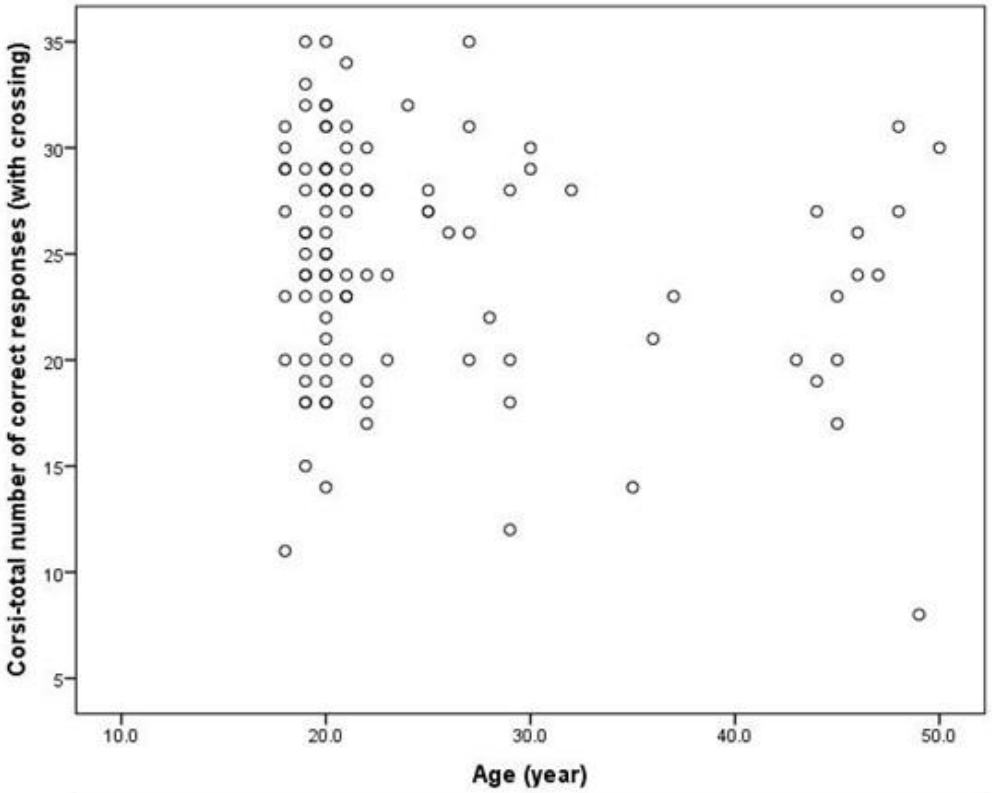


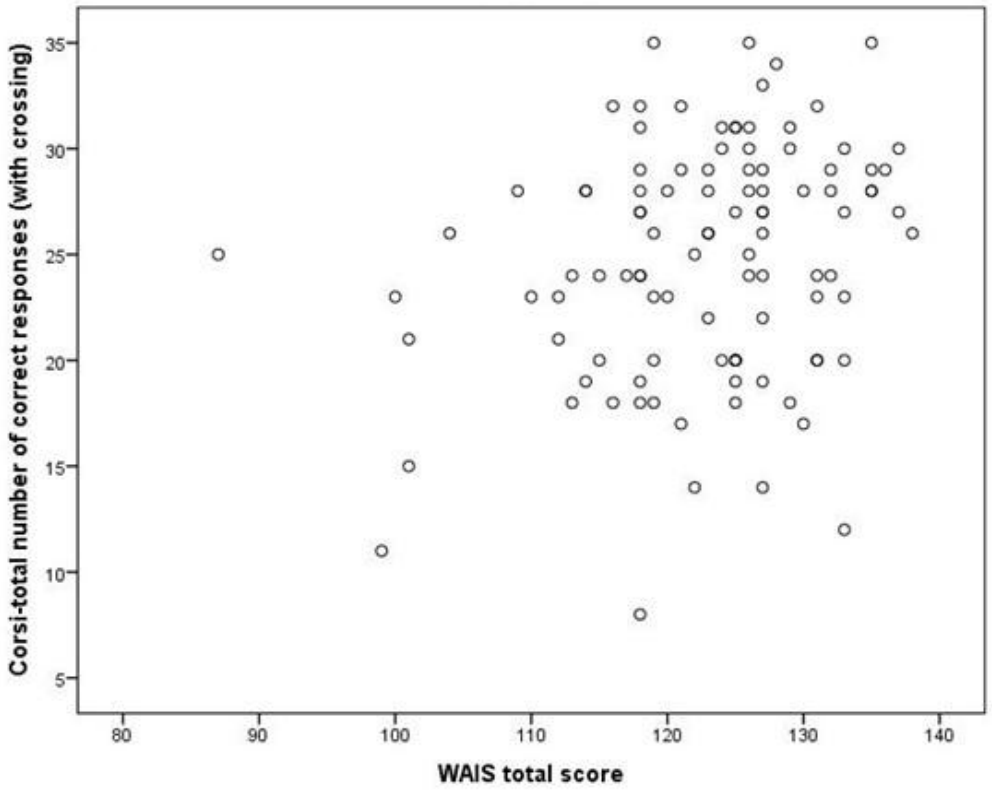


Appendix 37 Interaction between percentage of correct responses for different levels (Corsi) with WAIS total score in different beverage consumption groups

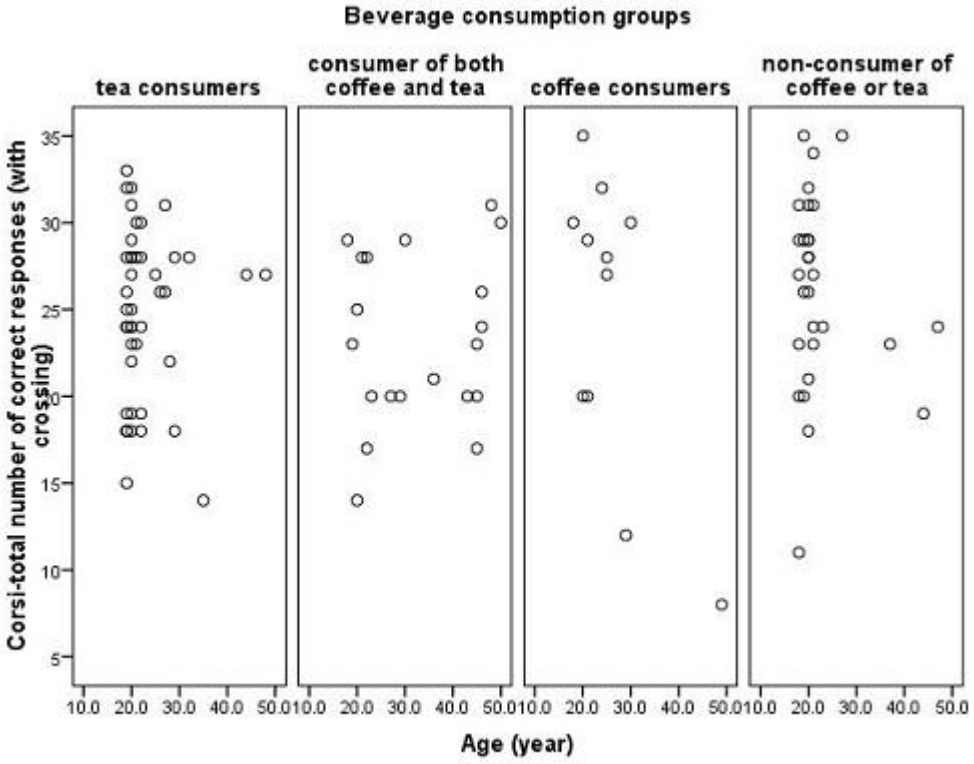


Appendix 38 Interaction between numbers of correct responses with crossing (Corsi) with age and WAIS total score

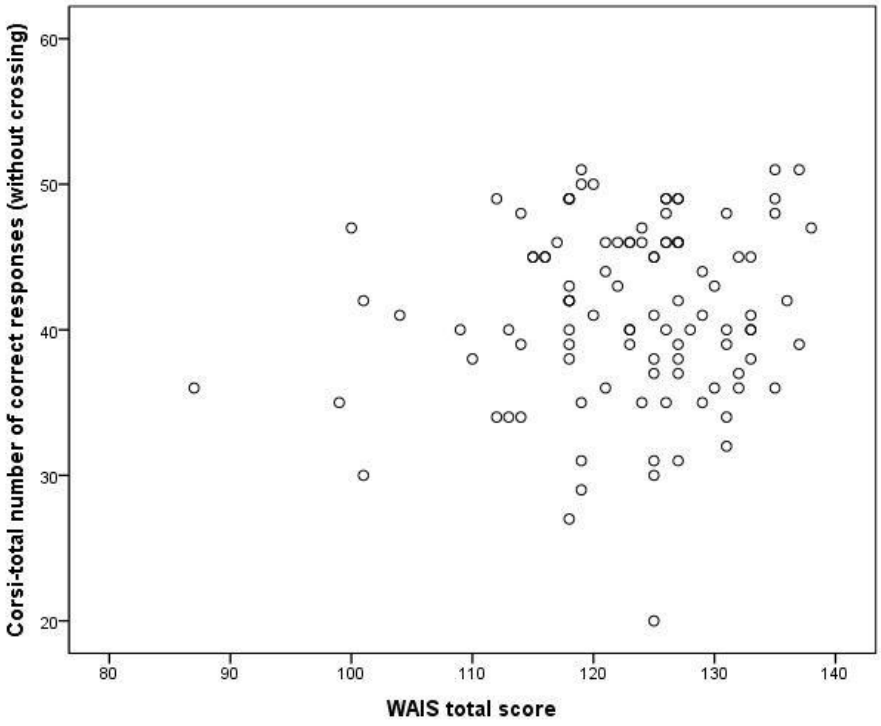




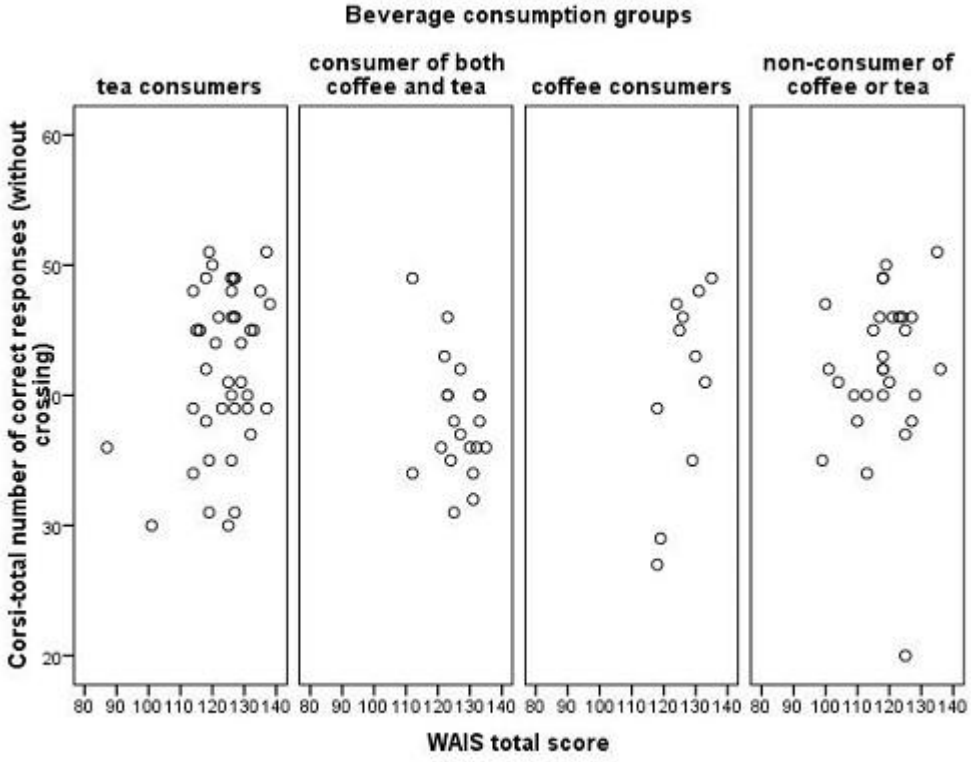
Appendix 39 Interaction between numbers of correct responses with crossing (Corsi) in different beverage consumption groups



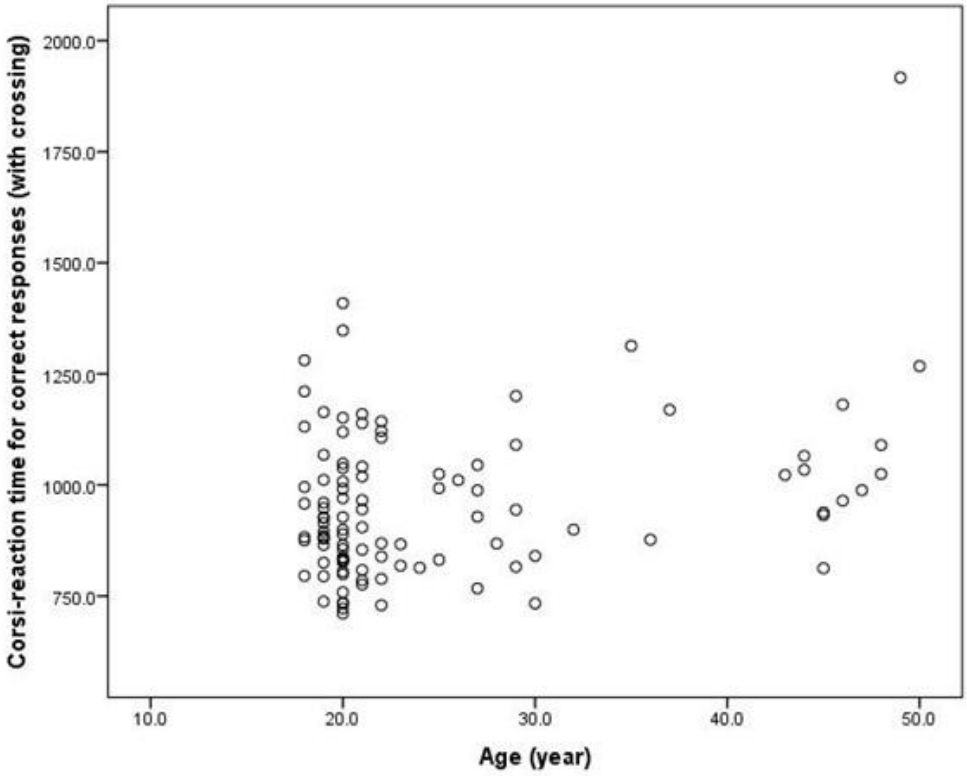
Appendix 40 Interaction between numbers of correct responses without crossing (Corsi) with WAIS total score

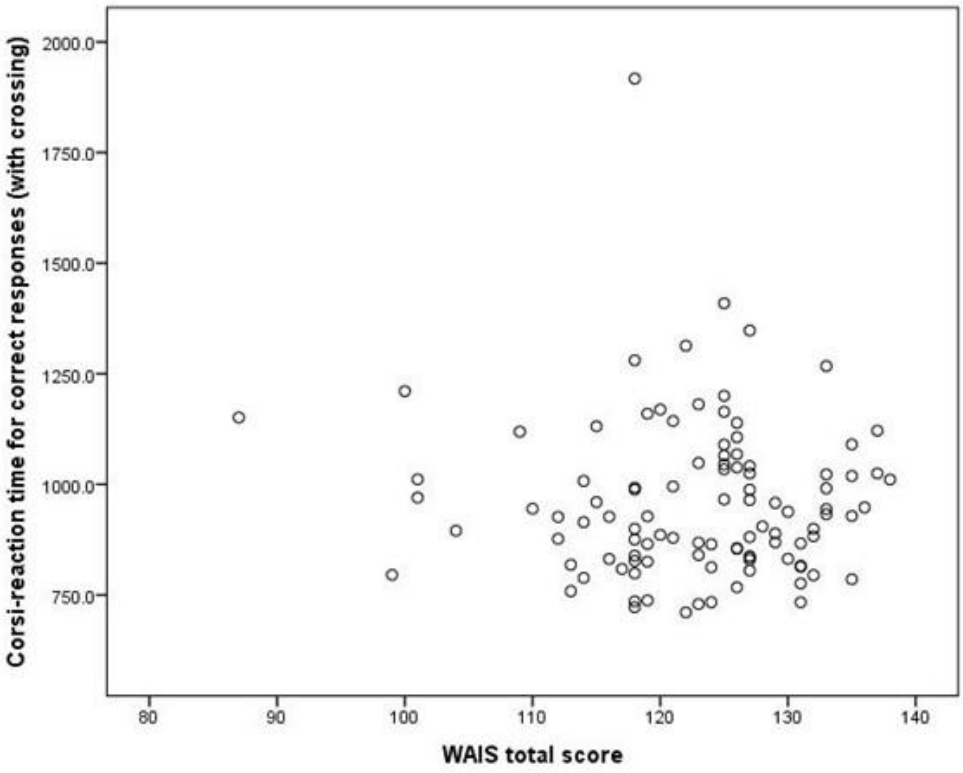


Appendix 41 Interaction between numbers of correct responses without crossing (Corsi) with WAIS total score in different beverage consumption groups

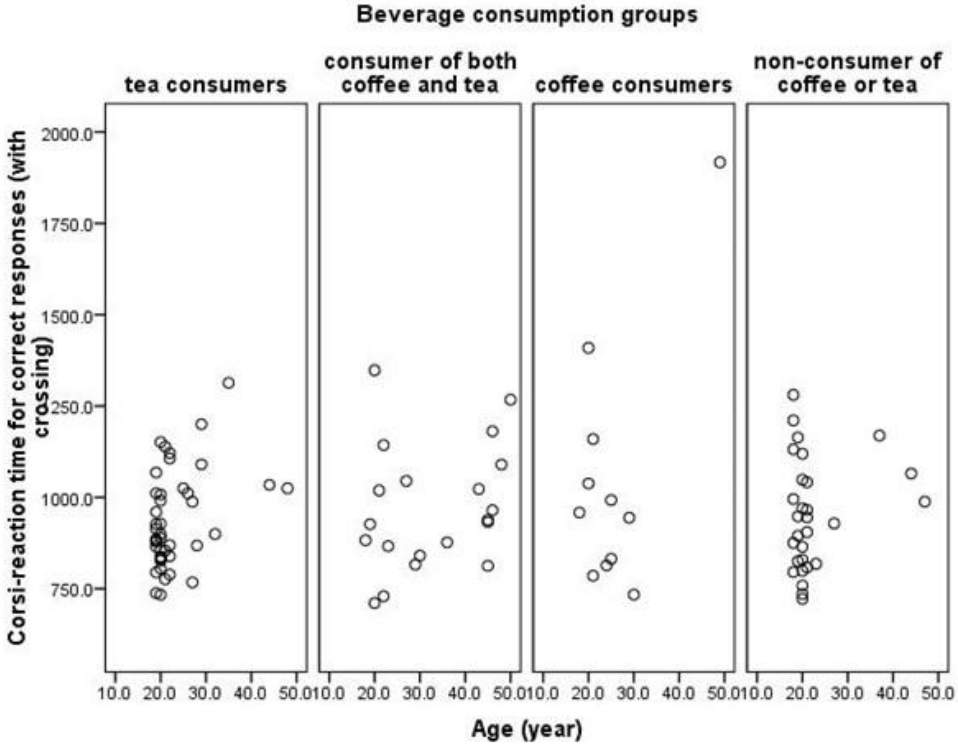


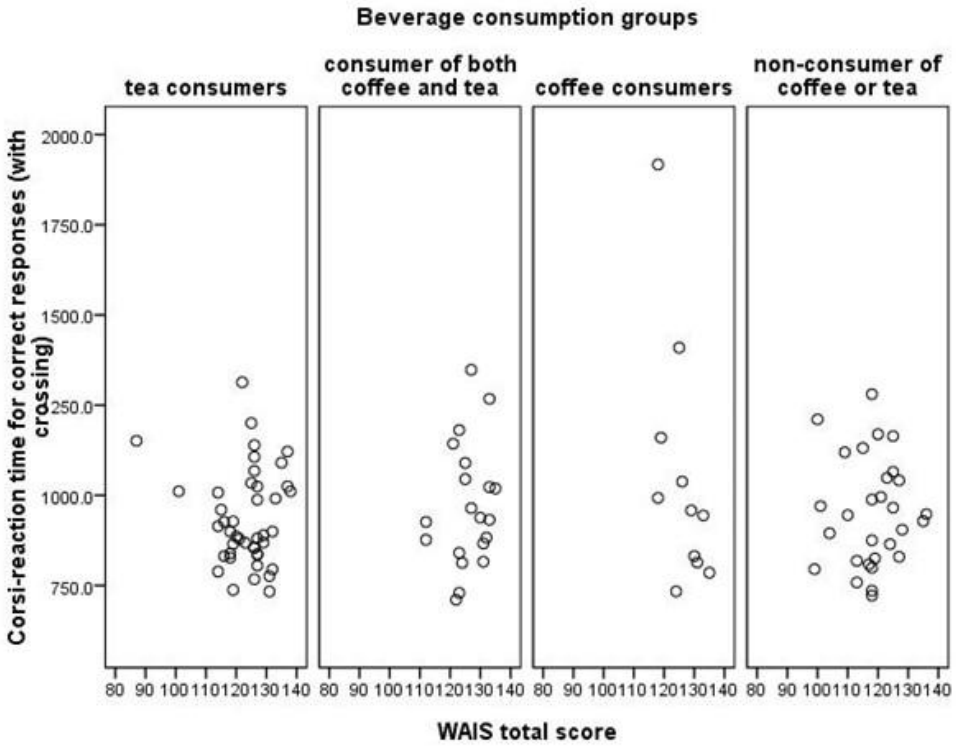
Appendix 42 Interaction between reaction time for correct responses with crossing (Corsi) with age and WAIS total score



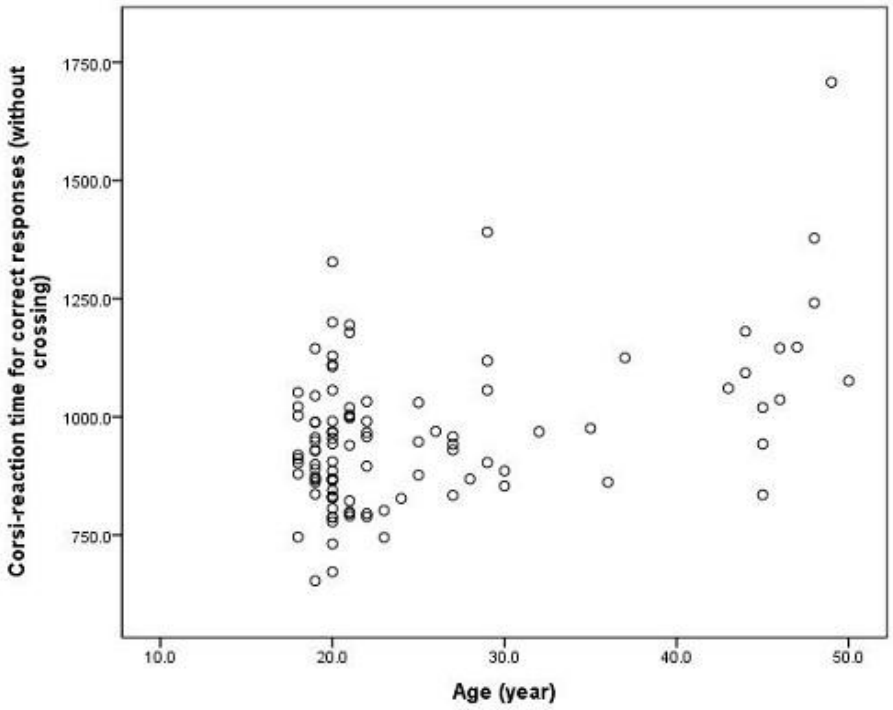


Appendix 43 Interaction between reaction time for correct responses with crossing (Corsi) with age and WAIS total score in different beverage consumption groups

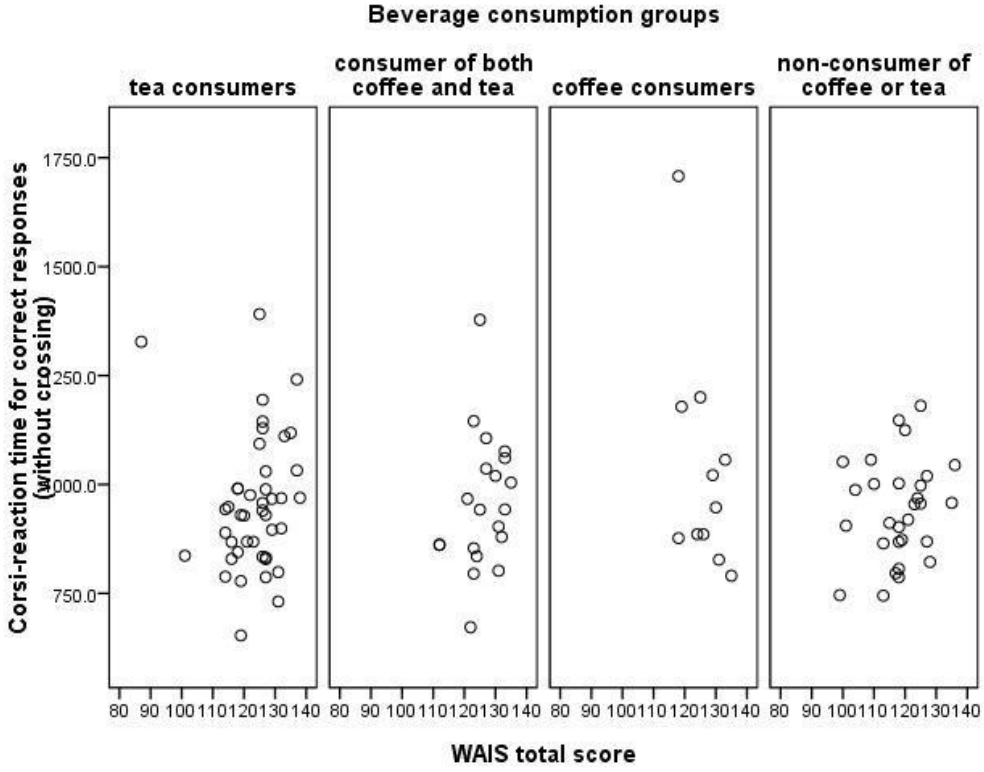




Appendix 44 Interaction between reaction time for correct responses without crossing (Corsi) with age



Appendix 45 Interaction between reaction time for correct responses without crossing (Corsi) with WAIS total score in different beverage consumption groups



References

ABD EL MOHSEN, M. M., KUHNLE, G., RECHNER, A. R., SCHROETER, H., ROSE, S., JENNER, P. & RICE-EVANS, C. A. 2002. Uptake and metabolism of epicatechin and its access to the brain after oral ingestion. *Free Radical Biology and Medicine,* 33**,** 1693-1702.

ALINIA, S., HELS, O. & TETENS, I. 2009. The potential association between fruit intake and body weight – a review. *Obesity Reviews,* 10**,** 639–647.

ANDERSON, D., PHILLIPS, B. J., YU, T. W., EDWARDS, A. J., AYESH, R. & BUTTERWORTH, K. R. 1997. The effects of vitamin C supplementation on biomarkers of oxygen radical generated damage in human volunteers with "low" or "high" cholesterol levels. *Environ Mol Mutagen,* 30**,** 161-74.

ANGUERA, J. A., REUTER-LORENZ, P. A., WILLINGHAM, D. T. & SEIDLER, R. D. 2011. Failure to engage spatial working memory contributes to age-related declines in visuomotor learning. *J Cogn Neurosci,* 23**,** 11-25.

ARAB, L., BIGGS, M. L., O'MEARA, E. S., LONGSTRETH, W. T., CRANE, P. K. & FITZPATRICK, A. L. 2011. Gender differences in tea, coffee, and cognitive decline in the elderly: The cardiovascular health study. *Journal of Alzheimer's Disease,* 27**,** 553-566.

ARAI, Y., WATANABE, S., KIMIRA, M., SHIMOI, K., MOCHIZUKI, R. & KINAE, N. 2000. Dietary intakes of flavonols, flavones and isoflavones by Japanese women and the inverse correlation between quercetin intake and plasma LDL cholesterol concentration. *J Nutr,* 130**,** 2243-50.

ARDILA, A. 2007. Normal aging increases cognitive heterogeneity: analysis of dispersion in WAIS-III scores across age. *Arch Clin Neuropsychol,* 22**,** 1003-11.

AWAD, N., GAGNON, M. & MESSIER, C. 2004. The relationship between impaired glucose tolerance, type 2 diabetes, and cognitive function. *Journal of Clinical and Experimental Neuropsychology,* 26**,** 1044-1080.

BADDELEY, A. 1986. *Working memory,* Oxford, Oxford University Press.

BADDELEY, A. 2003. Working memory: Looking back and looking forward. *Nature Reviews Neuroscience,* 4**,** 829-839.

BADDELEY, A. 2007. *Working memory, thought and action,* Oxford, Oxford University Press.

BALARATNASINGAM, S. & JANCA, A. 2012. Brain Derived Neurotrophic Factor: a novel neurotrophin involved in psychiatric and neurological disorders. *Pharmacol Ther,* 134**,** 116-24.

BASU, A., DU, M., LEYVA, M. J., SANCHEZ, K., BETTS, N. M., WU, M., ASTON, C. E. & LYONS, T. J. 2010. Blueberries decrease cardiovascular risk factors in obese men and women with metabolic syndrome. *J Nutr,* 140**,** 1582-7.

BATHALON, G. P., TUCKER, K. L., HAYS, N. P., VINKEN, A. G., GREENBERG, A. S., MCCRORY, M. A. & ROBERTS, S. B. 2000. Psychological measures of eating behavior and the accuracy of 3 common

dietary assessment methods in healthy postmenopausal women. *Am J Clin Nutr,* 71**,** 739-45.

BDA. 2012. *The British Dietetic Association's Food Factsheet: Alcohol* [Online]. United Kingdom: The Information Standard. Available: http://www.bda.uk.com/foodfacts/Alcohol.pdf [Accessed 17 July 2013].

BEKING, K. & VIEIRA, A. 2011. An assessment of dietary flavonoid intake in the UK and Ireland. *Int J Food Sci Nutr,* 62**,** 17-9.

BELLISLE, F., BLUNDELL, J. E., DYE, L., FANTINO, M., FERN, E., FLETCHER, R. J., LAMBERT, J., ROBERFROID, M., SPECTER, S., WESTENHOFER, J. & WESTERTERP-PLANTENGA, M. S. 1998. Functional food science and behaviour and psychological functions. *Br J Nutr,* 80 Suppl 1**,** S173-93.

BENJAFIELD, J. G. 2007. *Cognition-3rd ed.*, Canada, Oxford University Press.

BERMÚDEZ-SOTO, M. J. & TOMÁS-BARBERÁN, F. A. 2004. Evaluation of commercial red fruit juice concentrates as ingredients for antioxidant functional juices. *European Food Research and Technology,* 219**,** 133-141.

BERTELLI, A. A. & DAS, D. K. 2009. Grapes, wines, resveratrol, and heart health. *J Cardiovasc Pharmacol,* 54**,** 468-76.

BINGHAM, S. A., GILL, C., WELCH, A., DAY, K., CASSIDY, A., KHAW, K. T., SNEYD, M. J., KEY, T. J., ROE, L. & DAY, N. E. 1994. Comparison of dietary assessment methods in nutritional epidemiology: weighed records v. 24 h recalls, food-frequency questionnaires and estimated-diet records. *Br J Nutr,* 72**,** 619-43.

BLITZ, C. L., MURPHY, S. P. & AU, D. L. M. 2007. Adding lignan values to a food composition database. *Journal of Food Composition and Analysis,* 20**,** 99-105.

BOHN, S. K., WARD, N. C., HODGSON, J. M. & CROFT, K. D. 2012. Effects of tea and coffee on cardiovascular disease risk. *Food Funct*.

BOOKHEIMER, S. Y., RENNER, B. A., EKSTROM, A., LI, Z., HENNING, S. M., BROWN, J. A., JONES, M., MOODY, T. & SMALL, G. W. 2013. Pomegranate juice augments memory and FMRI activity in middle-aged and older adults with mild memory complaints. *Evid Based Complement Alternat Med,* 2013**,** 946298.

BORGES, G., MULLEN, W. & CROZIER, A. 2010. Comparison of the polyphenolic composition and antioxidant activity of European commercial fruit juices. *Food Funct,* 1**,** 73-83.

BOUCHARD, D. R., ROSS, R. & JANSSEN, I. 2010. Coffee, tea and their additives: association with BMI and waist circumference. *Obes Facts,* 3**,** 345-52.

BOX, J. D. 1983. Investigation of the Folin-Ciocalteau Phenol reagent for the determination of polyphenolic substances in natural waters. *Water Res.,* 17**,** 511-525.

BUTCHART, C., KYLE, J., MCNEILL, G., CORLEY, J., GOW, A. J., STARR, J. M. & DEARY, I. J. 2011. Flavonoid intake in relation to cognitive function in later life in the Lothian Birth Cohort 1936. *Br J Nutr,* 106**,** 141-8.

CAPANOGLU, E., DE VOS, R. C., HALL, R. D., BOYACIOGLU, D. & BEEKWILDER, J. 2013. Changes in polyphenol content during production of grape juice concentrate. *Food Chem,* 139**,** 521-6.

CASSIDY, A., O'REILLY É.J., KAY, C., SAMPSON, L., FRANZ, M., FORMAN, J. P., CURHAN, G. & RIMM, E. B. 2011. Habitual intake of flavonoid subclassess and incident hypertension in adults. *Am J Clin Nutr,* 93**,** 338-347.

CHANTRE, P. & LAIRON, D. 2002. Recent findings of green tea extract AR25 (Exolise) and its activity for the treatment of obesity. *Phytomedicine,* 9**,** 3-8.

CHEYETTE, C. & BALOLIA, Y. 2010. *Carbs & Cals & Protein & Fat: A visual guide to carbohydrate, protein, fat & calorie counting for healthy eating and weight loss*, London, United Kingdom, Chello Publishing Limited.

CHEYNIER, V. 2005. Polyphenols in foods are more complex than often thought. *Am J Clin Nutr* 81**,** 223S-229S

CHUN, O. K., CHUNG, S. J. & SONG, W. O. 2007. Estimated dietary flavonoid intake and major food sources of U.S. adults. *J Nutr,* 137**,** 1244-52.

CIEŚLIK, E., GRĘDA, A. & ADAMUS. 2006. Contents of polyphenols in fruit and vegetables. *Food Chemistry,* 94**,** 135-142.

CORSI, P. M. 1972. Human memory and the medial temporal region of the brain. *Dissertation Abstracts International,* 34**,** 891B.

CRAWFORD, J. R., STEWART, L. E., GARTHWAITE, P. H., PARKER, D. M. & BESSON, J. A. O. 1988. The relationship between demographic variables and NART performance in normal subjects. *Brit J Clin Psychol,* 27**,** 181-182.

CROPLEY, V., CROFT, R., SILBER, B., NEALE, C., SCHOLEY, A., STOUGH, C. & SCHMITT, J. 2012. Does coffee enriched with chlorogenic acids improve mood and cognition after acute administration in healthy elderly? A pilot study. *Psychopharmacology,* 219**,** 737-749.

DAVIS, J. N., HODGES, V. A. & GILLHAM, M. B. 2006. Normal-weight adults consume more fiber and fruit than their age- and height-matched overweight/obese counterparts. *J Am Diet Assoc,* 106**,** 833-40.

DE BOER, A. G. & GAILLARD, P. J. 2007. Drug targeting to the brain. *Annu Rev Pharmacol Toxicol,* 47**,** 323-55.

DE JAGER, C. A., DYE, L., DE BRUIN, E. A., BUTLER, L., FLETCHER, J., LAMPORT, D. J., LATULIPPE, M. E., SPENCER, J. P. E. & WESNES, K. 2013. Validation of cognitive tests for nutrition studies (in press).

DE OLIVEIRA, M. C., SICHIERI, R. & VENTURIM, M. R. 2008. A low-energydense diet adding fruit reduces weight and energy intake in women. *Appetite,* 51**,** 291–295.

DEKA, A. & VITA, J. A. 2011. Tea and cardiovascular disease. *Pharmacological Research,* 64**,** 136-145.

DEPARTMENT OF HEALTH, D. 1991. *Dietary reference values for food and energy and nutrients for the United Kingdom. Report on heatlh and social subjects no. 41. ,* Norwich, United Kingdom, Her Majesty's Stationery Office.

DEVORE, E. E., KANG, J. H., BRETELER, M. M. B. & GRODSTEIN, F. 2012. Dietary intakes of berries and flavonoids in relation to cognitive decline. *Ann Neurol,* 72**,** 135-143.

DILIS, V. & TRICHOPOULOU, A. 2010. Antioxidant intakes and food sources in Greek adults. *J Nutr,* 140**,** 1274-9.

DULLOO, A. G., DURET, C., ROHRER, D., GIRARDIER, L., MENSI, N., FATHI, M., CHANTRE, P. & VANDERMANDER, J. 1999. Efficacy of a green tea extract rich in catechin polyphenols and caffeine in increasing 24-h energy expenditure and fat oxidation in humans. *Am J Clin Nutr,* 70**,** 1040-5.

DYE, L. & BLUNDELL, J. 2002. Functional foods: psychological and behavioural functions. *Br J Nutr,* 88 Suppl 2**,** S187-211.

EBRAHIMI, A. & SCHLUESENER, H. 2012. Natural polyphenols against neurodegenerative disorders: Potentials and pitfalls. *Ageing Research Reviews,* 11**,** 329-345.

ESKELINEN, M. H., NGANDU, T., TUOMILEHTO, J., SOININEN, H. & KIVIPELTO, M. 2009. Midlife coffee and tea drinking and the risk of late-life dementia: a population-based CAIDE study. *J Alzheimers Dis,* 16**,** 85-91.

FALLER, A. L. & FIALHO, E. 2009. [Polyphenol availability in fruits and vegetables consumed in Brazil]. *Rev Saude Publica,* 43**,** 211-8.

FERGENBAUM, J. H., BRUCE, S., LOU, W., HANLEY, A. J., GREENWOOD, C. & YOUNG, T. K. 2009. Obesity and lowered cognitive performance in a Canadian First Nations population. *Obesity (Silver Spring),* 17**,** 1957-63.

FERNÁNDEZ-MAR, M. I., MATEOS, R., GARCÍA-PARRILLA, M. C., PUERTAS, B. & CANTOS-VILLAR, E. 2012. Bioactive compounds in wine: Resveratrol, hydroxytyrosol and melatonin: A review. *Food Chemistry,* 130**,** 797-813.

FILE, S. E., JARRETT, N., FLUCK, E., DUFFY, R., CASEY, K. & WISEMAN, H. 2001. Eating soya improves human memory. *Psychopharmacology (Berl),* 157**,** 430-6.

FLOEGEL, A., PISCHON, T., BERGMANN, M. M., TEUCHER, B., KAAKS, R. & BOEING, H. 2012. Coffee consumption and risk of chronic disease in the European Prospective Investigation into Cancer and Nutrition (EPIC)-Germany study. *Faseb Journal,* 26.

FOLSTEIN, M. F., FOLSTEIN, S. E. & MCHUGH, P. R. 1975. "Mini-mental state". A practical method for grading the cognitive state of patients for the clinician. *J Psychiatr Res,* 12**,** 189-98.

FOOD AND AGRICULTURAL ORGANIZATION, F. 2013. *Food Balance Sheets* [Online]. Rome, Italy. Available: http://faostat3.fao.org/home/index.html#DOWNLOAD [Accessed 31 May 2013 2013].

FOOD STANDARD AGENCY, F. 2003. *Part 1-water soluble vitamins: risk assessment of vitamin C* [Online]. London, United Kingdom: Food Standard Agency, FSA. Available: http://www.food.gov.uk/multimedia/pdfs/evm_c.pdf [Accessed 24 October 2013].

FRANCIS, S. T., HEAD, K., MORRIS, P. G. & MACDONALD, I. A. 2006. The effect of flavanol-rich cocoa on the fMRI response to a cognitive task in healthy young people. *J Cardiovasc Pharmacol,* 47 Suppl 2**,** S215-20.

FUJIOKA, K., GREENWAY, F., SHEARD, J. & YING, Y. 2006. The effects of grapefruit on weight and insulin resistance: relationship to the metabolic syndrome. *J Med Food,* 9**,** 49-54.

GATTO, N. M., HENDERSON, V. W., JOHN, J. A. S., MCCLEARY, C., HODIS, H. N. & MACK, W. J. 2008. Metabolic syndrome and cognitive function in healthy middle-aged and older adults without diabetes. *Aging Neuropsychology and Cognition,* 15**,** 627-641.

GHOSH, D. & SCHEEPENS, A. 2009. Vascular action of polyphenols. *Mol Nutr Food Res,* 53**,** 322-31.

GILL, J. S. 2002. Reported levels of alcohol consumption and binge drinking within the UK undergraduate student population over the last 25 years. *Alcohol Alcoholism* 37**,** 109-120.

GLAXOSMITHKLINE. 2012a. *Ribena Blackcurrant Squash* [Online]. Available: http://www.ribena.co.uk/products/original/ [Accessed 25 June 2013].

GLAXOSMITHKLINE. 2012b. *Ribena Plus Benefits for you and your family* [Online]. Available: http://www.ribena.co.uk/plus-benefits/ [Accessed 25 June 2013].

GO, A. S., MOZAFFARIAN, D., ROGER, V. L., BENJAMIN, E. J., BERRY, J. D., BORDEN, W. B., BRAVATA, D. M. & DAI, S., ET AL. 2013. Heart disease and stroke statistics—2013 update: a report from the American Heart Association. *Circulation,* 127**,** e6-e245.

GODWIN, S. L., CHAMBERS, E. T. & CLEVELAND, L. 2004. Accuracy of reporting dietary intake using various portion-size aids in-person and via telephone. *J Am Diet Assoc,* 104**,** 585-94.

GOLDBERG, G. R., BLACK, A. E., JEBB, S. A., COLE, T. J., MURGATROYD, P. R., COWARD, W. A. & PRENTICE, A. M. 1991. Critical evaluation of energy intake data using fundamental principles of energy physiology: 1. Derivation of cut-off limits to identify under-recording. *Eur J Clin Nutr,* 45**,** 569–581.

GOMEZ-PINILLA, F. 2008. Brain foods: the effects of nutrients on brain function. *Nat Rev Neurosci,* 9**,** 568-78.

GOMEZ-PINILLA, F. & NGUYEN, T. T. 2012. Natural mood foods: the actions of polyphenols against psychiatric and cognitive disorders. *Nutr Neurosci,* 15**,** 127-33.

GORIS, A. H., WESTERTERP-PLANTENGA, M. S. & WESTERTERP, K. R. 2000. Undereating and underrecording of habitual food intake in obese men: selective underreporting of fat intake. *Am J Clin Nutr,* 71**,** 130-4.

GREGERSEN, N. T., BITZ, C., KROG-MIKKELSEN, I., HELS, O., KOVACS, E. M., RYCROFT, J. A., FRANDSEN, E., MELA, D. J. & ASTRUP, A. 2009. Effect of moderate intakes of different tea catechins and caffeine on acute measures of energy metabolism under sedentary conditions. *Br J Nutr,* 102**,** 1187-94.

GROVE, K. A. & LAMBERT, J. D. 2010. Laboratory, Epidemiological, and Human Intervention Studies Show That Tea (Camellia sinensis) May Be Useful in the Prevention of Obesity. *Journal of Nutrition,* 140**,** 446-453.

GUNSTAD, J., PAUL, R. H., COHEN, R. A., TATE, D. F., SPITZNAGEL, M. B. & GORDON, E. 2007. Elevated body mass index is associated with executive dysfunction in otherwise healthy adults. *Compr Psychiatry,* 48**,** 57-61.

HARA, Y. 2001. *Green Tea: Health Benefits and Applications: Introduction to the History of Tea,* New York, Marcel Dekker, Inc.

HARADA, U., CHIKAMA, A., SAITO, S., TAKASE, H., NAGAO, T. & HASE, T., ET AL. 2005. Effects of long-term ingestion of tea catechins on energy expenditure and dietary fat oxidation in healthy subjects. *J Health Sci,* 51**,** 248-52.

HARNACK, L., STEFFEN, L., ARNETT, D. K., GAO, S. & LUEPKER, R. V. 2004. Accuracy of estimation of large food portions. *J Am Diet Assoc,* 104**,** 804-6.

HASHEMPOUR, A., SHARIFZADEH, K., BAKHSHI, D., FOTUHIGHAZVINI, R., GHASEMNEZHAD, M. & MIGHANI, H. 2013. Variation in total phenolic, ascorbic acid and antioxidant activity of citrus fruit of six species cultivated in north of Iran. *Intl. J. Agric: Res & Rev.,* 3**,** 1-5.

HENDRICKSON, S. & MATTES, R. D. 2008. No acute effects of grape juice on appetite, implicit memory and mood. *Food Nutr Res.* .

HENSEL, A., ANGERMEYER, M. C. & RIEDEL-HELLER, S. G. 2007. Measuring cognitive change in older adults: reliable change indices for the Mini-Mental State Examination. *J Neurol Neurosurg Psychiatry,* 78**,** 1298-303.

HERTOG, M. G., SWEETNAM, P. M., FEHILY, A. M., ELWOOD, P. C. & KROMHOUT, D. 1997. Antioxidant flavonols and ischemic heart disease in a Welsh population of men: the Caerphilly Study. *Am J Clin Nutr,* 65**,** 1489-94.

HERTOG, M. L., KROMHOUT, D., ARAVANIS, C. & ET AL. 1995. FLavonoid intake and long-term risk of coronary heart disease and cancer in the seven countries study. *Archives of Internal Medicine,* 155**,** 381-386.

HERVERT-HERNANDEZ, D. & GONI, I. 2011. Contribution of beverages to the intake of polyphenols and antioxidant capacity in obese women from rural Mexico. *Public Health Nutr***,** 1-7.

HINDMARCH, I., QUINLAN, P. T., MOORE, K. L. & PARKIN, C. 1998. The effects of black tea and other beverages on aspects of cognition and psychomotor performance. *Psychopharmacology,* 139**,** 230-8.

HO, C. T. & ZHU, N. 2000. *Caffeinated Beverages: Health Benefits, Physiological Effects, and Chemistry: The Chemistry of Tea*, Washington, American Chemical Society.

HOLLANDS, W., BRETTA, G. M., RADREAU, P., SAHA, S., TEUCHERA, B., BENNETTA, R. N. & KROON, P. A. 2008. Processing blackcurrants dramatically reduces the content and does not enhance the urinary yield of anthocyanins in human subjects. *Food Chemistry,* 108**,** 869–878.

HUGHES, L. A. E., ARTS, I. C. W., AMBERGEN, T., BRANTS, H. A. M., DAGNELIE, P. C., GOLDBOHM, R. A., VAN DEN BRANDT, P. A. & WEIJENBERG, M. P. 2008. Higher dietary flavone, flavonol, and catechin intakes are associated with less of an increase in BMI over time in women: a longitudinal analysis from the Netherlands Cohort Study. *American Journal of Clinical Nutrition,* 88**,** 1341-1352.

ISLAM, F., SPARKES, C., ROODENRYS, S. & ASTHEIMER, L. 2008. Short-term changes in endogenous estrogen levels and consumption of soy isoflavones affect working and verbal memory in young adult females. *Nutr Neurosci,* 11**,** 251-62.

IWAI, N., OHSHIRO, H., KUROZAWA, Y., HOSODA, T., MORITA, H., FUNAKAWA, K., OKAMOTO, M. & NOSE, T. 2002. Relationship between coffee and green tea consumption and all-cause mortality in a cohort of a rural Japanese population. *J Epidemiol.,* 12**,** 191-8.

JÄGER, A. & SAABY, L. 2011. Flavonoids and the CNS. *Molecules,* 16**,** 1471-1485.

JIA, L., LIU, X., BAI, Y. Y., LI, S. H., SUN, K., HE, C. & HUI, R. 2010. Short-term effect of cocoa product consumption on lipid profile: a meta-analysis of randomized controlled trials. *Am J Clin Nutr,* 92**,** 218-25.

JOHANNOT, L. & SOMERSET, S. M. 2006. Age-related variations in flavonoid intake and sources in the Australian population. *Public Health Nutr,* 9**,** 1045-54.

JOHNSON, C. M., SHARKEY, J. R., DEAN, W. R., ALEX MCINTOSH, W. & KUBENA, K. S. 2011. It's who I am and what we eat. Mothers' food-related identities in family food choice. *Appetite,* 57**,** 220-228.

JOHNSTON, C. S. 1999. Biomarkers for establishing a tolerable upper intake level for vitamin C. *Nutr Rev,* 57**,** 71-7.

KACHIGAN, S. K. 1991. *Multivariate Statistical Analysis. A Conceptual Introduction. 2nd ed.,* New York, Radius Press.

KAHN, R., BUSE, J., FERRANNINI, E. & STERN, M. 2005. The metabolic syndrome: Time for a critical appraisal - Joint statement from the American Diabetes Association and the European Association for the Study of Diabetes. *Diabetes Care,* 28**,** 2289-2304.

KANG, J. H., ASCHERIO, A. & GRODSTEIN, F. 2005. Fruit and vegetable consumption and cognitive decline in aging women. *Ann Neurol,* 57**,** 713-20.

KENNEDY, D. O., WIGHTMAN, E. L., REAY, J. L., LIETZ, G., OKELLO, E. J., WILDE, A. & HASKELL, C. F. 2010. Effects of resveratrol on cerebral blood flow variables and cognitive performance in humans: a double-blind, placebo-controlled, crossover investigation. *Am J Clin Nutr,* 91**,** 1590-7.

KESSE-GUYOT, E., FEZEU, L., ANDREEVA, V. A., TOUVIER, M., SCALBERT, A., HERCBERG, S. & GALAN, P. 2012. Total and specific polyphenol intakes in midlife are associated with cognitive function measured 13 years later. *J Nutr,* 142**,** 76-83.

KHAN, N., MONAGAS, M., ANDRES-LACUEVA, C., CASAS, R., URPÍ-SARDÀ, M., LAMUELA-RAVENTÓS, R. M. & ESTRUCH, R. 2012. Regular consumption of cocoa powder with milk increases HDL cholesterol and reduces oxidized LDL levels in subjects at high-risk of cardiovascular disease. *Nutr Metab Cardiovasc Dis.,* 22**,** 1046-53.

KNEKT, P., KUMPULAINEN, J., JÄRVINEN, R., RISSANEN, H., HELIÖVAARA, M., REUNANEN, A., HAKULINEN, T. & AROMAA, A. 2002. Flavonoid intake and risk of chronic diseases. *Am J Clin Nutr.,* 76**,** 560-8.

KOBUCHI, H., ROY, S., SEN, C. K., NGUYEN, H. G. & PACKER, L. 1999. Quercetin inhibits inducible ICAM-1 expression in human endothelial cells through the JNK pathway. *Am J Physiol,* 277**,** C403-11.

KONG, A. N., YU, R., CHEN, C., MANDLEKAR, S. & PRIMIANO, T. 2000. Signal transduction events elicited by natural products: role of MAPK and caspase pathways in homeostatic response and induction of apoptosis. *Arch Pharm Res,* 23**,** 1-16.

KOUNTOURI, A. M., GIOXARI, A., KARVELA, E., KALIORA, A. C., KARVELAS, M. & KARATHANOS, V. T. 2013. Chemopreventive properties of raisins originating from Greece in colon cancer cells. *Food Funct,* 4**,** 366-72.

KUHNERT, N. 2010. Unraveling the structure of the black tea thearubigins. *Arch Biochem Biophys,* 501**,** 37-51.

KURIYAMA, S., HOZAWA, A., OHMORI, K., SHIMAZU, T., MATSUI, T., EBIHARA, S., AWATA, S., NAGATOMI, R., ARAI, H. & TSUJI, I. 2006.

Green tea consumption and cognitive function: a cross-sectional study from the Tsurugaya Project *Am J Clin Nutr,* 83**,** 355-61.

KYLE, J. A. M. & DUTHIE, G. G. 2006. Flavonoids in foods. *In:* ANDERSEN, O. M. & MARKHAM, K. R. (eds.) *Flavonoids: chemistry, biochemistry and applications.* Florida: Taylor & Francis Group.

LAMPORT, D. J., DYE, L., WIGHTMAN, J. D. & LAWTON, C. L. 2012. The effects of flavonoid and other polyphenol consumption on cognitive performance: A systematic research review of human experimental and epidemiological studies. *Nutrition and Aging,* 1**,** 5-25.

LAMPORT, D. J., LAWTON, C. L., MANSFIELD, M. W. & DYE, L. 2009. Impairments in glucose tolerance can have a negative impact on cognitive function: A systematic research review. *Neurosci Biobehav Rev,* 33**,** 394-413.

LANDETE, J. M. 2012. Updated Knowledge about Polyphenols: Functions, Bioavailability, Metabolism, and Health. *Crit Rev Food Sci Nutr,* 52**,** 936-948.

LARDEAU, A. & POQUET, L. 2013. Phenolic acid metabolites derived from coffee consumption are unlikely to cross the blood-brain barrier. *Journal of Pharmaceutical and Biomedical Analysis,* 76**,** 134-138.

LARSEN, L., HARTMANN, P. & NYBORG, H. 2008. The stability of general intelligence from early adulthood to middle-age. *Intelligence,* 36**,** 29-34.

LETENNEUR, L., PROUST-LIMA, C., LE GOUGE, A., DARTIGUES, J. F. & BARBERGER-GATEAU, P. 2007. Flavonoid intake and cognitive decline over a 10-year period. *American Journal of Epidemiology,* 165**,** 1364-1371.

LI, G., ZHU, Y., ZHANG, Y., LANG, J., CHEN, Y. & LING, W. 2013. Estimated daily flavonoid and stilbene intake from fruits, vegetables, and nuts and associations with lipid profiles in chinese adults. *J Acad Nutr Diet,* 113**,** 786-94.

MALEC, J. F., IVNIK, R. J. & HINKELDEY, N. S. 1991. Visual spatial learning test. *Psychol Assess,* 3**,** 82–88.

MANACH, C., SCALBERT, A., MORAND, C., REMESY, C. & JIMENEZ, L. 2004. Polyphenols: food sources and bioavailability. *American Journal of Clinical Nutrition,* 79**,** 727-747.

MAPA 2001. Ministerio de Agricultura Pesca y Alimentacio´n. La alimentacio´n en Espan˜a. Madrid, Spain: Secretarı´a General de Agricultura y Alimentacio´n.

MARAS, J. E., TALEGAWKAR, S. A., QIAO, N., LYLE, B., FERRUCCI, L. & TUCKER, K. L. 2011. Flavonoid intakes in the Baltimore Longitudinal Study of Aging. *Journal of Food Composition and Analysis,* 24**,** 1103-1109.

MATSUMOTO, H., HANAMURA, S., KAWAKAMI, T., SATO, Y. & HIRAYAMA, M. 2001. Preparative-scale isolation of four anthocyanin components of black currant (Ribes nigrum L.) fruits. *J Agric Food Chem,* 49**,** 1541-5.

MATTILA, P. & HELLSTRÖM, J. 2007. Phenolic acids in potatoes, vegetables, and some of their products. *Journal of Food Composition and Analysis,* 20**,** 152-160.

MATTILA, P. H., HELLSTRÖM, J., MCDOUGALL, G., DOBSON, G., PIHLAVA, J. M., TIIRIKKA, T., STEWART, D. & KARJALAINEN, R. 2011. Polyphenol and vitamin C contents in European commercial blackcurrant juice products. *Food Chemistry,* 127**,** 1216-1223.

MCCABE, D. P., ROEDIGER, H. L., MCDANIEL, M. A., BALOTA, D. A. & HAMBRICK, D. Z. 2010. The relationship between working memory capacity and executive functioning: evidence for a common executive attention construct. *Neuropsychology,* 24**,** 222-43.

MEDINA, M. B. 2011. Simple and Rapid Method for the Analysis of Phenolic Compounds in Beverages and Grains. *Journal of Agricultural and Food Chemistry,* 59**,** 1565-1571.

MELLOR, D. D., SATHYAPALAN, T., KILPATRICK, E. S., BECKETT, S. & ATKIN, S. L. 2010. High-cocoa polyphenol-rich chocolate improves HDL cholesterol in Type 2 diabetes patients. *Diabet Med,* 27**,** 1318-21.

MILLS, C. E., ORUNA-CONCHA, M. J., MOTTRAM, D. S., GIBSON, G. R. & SPENCER, J. P. E. 2013. The effect of processing on chlorogenic acid content of commercially available coffee. *Food Chemistry,* 141**,** 3335-3340.

MILNER, B. 1971. Interhemispheric differences in the localization of psychological processes in man. *British Medical Bulletin.,* 27**,** 272-277.

MINEHARU, Y., KOIZUMI, A., WADA, Y., ISO, H., WATANABE, Y., DATE, C., YAMAMOTO, A., KIKUCHI, S., INABA, Y., TOYOSHIMA, H., KONDO, T. & TAMAKOSHI, A. 2011. Coffee, green tea, black tea and oolong tea consumption and risk of mortality from cardiovascular disease in Japanese men and women. *J Epidemiol Community Health,* 65**,** 230-40.

MOLLET, B. & ROWLAND, I. 2002. Functional foods: at the frontier between food and pharma. *Current Opinion in Biotechnology,* 13**,** 483-485.

MULLEN, W., MARKS, S. C. & CROZIER, A. 2007. Evaluation of phenolic compounds in commercial fruit juices and fruit drinks. *J Agric Food Chem,* 55**,** 3148-57.

NAIDU, K. A. 2003. Vitamin C in human health and disease is still a mystery ? An overview. *Nutr J,* 2**,** 1-10.

NAWA, H., CARNAHAN, J. & GALL, C. 1995. BDNF protein measured by a novel enzyme immunoassay in normal brain and after seizure: partial disagreement with mRNA levels. *Eur J Neurosci,* 7**,** 1527-35.

NCEP 2001. Executive Summary of The Third Report of The National Cholesterol Education Program (NCEP) expert panel on detection, evaluation, and treatment of high blood cholesterol in adults (Adult Treatment Panel III). *JAMA,* 285**,** 2486-2497.

NELSON, H. E. 1982. The National Adult Reading Test (NART): test manual. *NFER-Nelson.*

NELSON, M., ATKINSON, M. & DARBYSHIRE, S. 1996. Food photography II: use of food photographs for estimating portion size and the nutrient content of meals. *Br J Nutr,* 76**,** 31-49.

NELSON, M., ATKINSON, M. & MEYER, J. 2002. Food portion sizes : a photographic atlas. *Food Standards Agency.*

NEVEU, V., PEREZ-JIMENEZ, J., VOS, F., CRESPY, V., DU CHAFFAUT, L., MENNEN, L., KNOX, C., EISNER, R., CRUZ, J., WISHART, D. & SCALBERT, A. 2010a. *http://www.phenol-explorer.eu/methods_used* [Online]. [Accessed 11 July 2013].

NEVEU, V., PEREZ-JIMENEZ, J., VOS, F., CRESPY, V., DU CHAFFAUT, L., MENNEN, L., KNOX, C., EISNER, R., CRUZ, J., WISHART, D. & SCALBERT, A. 2010b. Phenol-Explorer: an online comprehensive database on polyphenol contents in foods. *Database (Oxford),* 2010**,** bap024.

NG, T. P., FENG, L., NITI, M., KUA, E. H. & YAP, K. B. 2008. Tea consumption and cognitive impairment and decline in older Chinese adults. *Am J Clin Nutr,* 88**,** 224-31.

NURK, E., REFSUM, H., DREVON, C. A., TELL, G. S., NYGAARD, H. A., ENGEDAL, K. & SMITH, A. D. 2009a. Intake of Flavonoid-Rich Wine, Tea, and Chocolate by Elderly Men and Women Is Associated with Better Cognitive Test Performance. *Journal of Nutrition,* 139**,** 120-127.

NURK, E., REFSUM, H., DREVON, C. A., TELL, G. S., NYGAARD, H. A., ENGEDAL, K. & SMITH, A. D. 2009b. Intake of flavonoid-rich wine, tea, and chocolate by elderly men and women is associated with better cognitive test performance. *J Nutr,* 139**,** 120-7.

NURK, E., REFSUM, H., DREVON, C. A., TELL, G. S., NYGAARD, H. A., ENGEDAL, K. & SMITH, A. D. 2010. Cognitive performance among the elderly in relation to the intake of plant foods. The Hordaland Health Study. *British Journal of Nutrition,* 104**,** 1190-1201.

OAKES, M. E. 2005. Stereotypical thinking about foods and perceived capacity to promote weight gain. *Appetite,* 44**,** 317-324.

OAKES, M. E. & SLOTTERBACK, C. S. 2001. Gender differences in perceptions of the healthiness of foods. *Psychology & Health* 16**,** 57-65.

OBA, S., NAGATA, C., NAKAMURA, K., FUJII, K., KAWACHI, T., TAKATSUKA, N. & SHIMIZU, H. 2010. Consumption of coffee, green tea, oolong tea, black tea, chocolate snacks and the caffeine content in relation to risk of diabetes in Japanese men and women. *British Journal of Nutrition,* 103**,** 453-459.

OBÓN, J. M., DÍAZ-GARCÍA, M. C. & CASTELLAR, M. R. 2011. Red fruit juice quality and authenticity control by HPLC. *Journal of Food Composition and Analysis,* 24**,** 760–771.

ORTEGA, R. M., REQUEJO, A. M., ANDRES, P., LOPEZ-SOBALER, A. M., QUINTAS, M. E., REDONDO, M. R., NAVIA, B. & RIVAS, T. 1997. Dietary intake and cognitive function in a group of elderly people. *Am J Clin Nutr,* 66**,** 803-9.

OTAKI, N., KIMIRA, M., KATSUMATA, S., UEHARA, M., WATANABE, S. & SUZUKI, K. 2009. Distribution and major sources of flavonoid intakes in the middle-aged Japanese women. *J Clin Biochem Nutr,* 44**,** 231-8.

OVASKAINEN, M. L., TORRONEN, R., KOPONEN, J. M., SINKKO, H., HELLSTROM, J., REINIVUO, H. & MATTILA, P. 2008. Dietary intake and major food sources of polyphenols in Finnish adults. *J Nutr,* 138**,** 562-6.

PADAYATTY, S. J., SUN, A. Y., CHEN, Q., ESPEY, M. G., DRISKO, J. & LEVINE, M. 2010. Vitamin C: intravenous use by complementary and alternative medicine practitioners and adverse effects. *PLoS One,* 5**,** e11414.

PALLANT, J. 2007. *SPSS Survival Manual. A step by Step Guide to Data Analysis using SPSS for Windows. 3rd ed.,* Berkshire, Open University Press. McGraw-Hill Education.

PARLETTA, N., MILTE, C. M. & MEYER, B. J. 2013. Nutritional modulation of cognitive function and mental health. *J Nutr Biochem*.

PARLLIMENT, T. H. 2000. *Caffeinated Beverages: Health Benefits, Physiological Effects, and Chemistry: An Overview of Coffee Roasting.,* Washington, American Chemical Society.

PASE, M. P., SCHOLEY, A. B., PIPINGAS, A., KRAS, M., NOLIDIN, K., GIBBS, A., WESNES, K. & STOUGH, C. 2013. Cocoa polyphenols enhance positive mood states but not cognitive performance: a randomized, placebo-controlled trial. *J Psychopharmacol,* 27**,** 451-8.

PEREZ-JIMENEZ, J., FEZEU, L., TOUVIER, M., ARNAULT, N., MANACH, C., HERCBERG, S., GALAN, P. & SCALBERT, A. 2011. Dietary intake of 337 polyphenols in French adults. *Am J Clin Nutr,* 93**,** 1220-8.

PERLA, V., HOLM, D. G. & JAYANTY, S. S. 2012. Effects of cooking methods on polyphenols, pigments and antioxidant activity in potato tubers. *LWT - Food Science and Technology,* 45**,** 161-171.

POLIDORI, M. C., PRATICO, D., MANGIALASCHE, F., MARIANI, E., AUST, O., ANLASIK, T., MANG, N., PIENTKA, L., STAHL, W., SIES, H., MECOCCI, P. & NELLES, G. 2009. High Fruit and Vegetable Intake is Positively Correlated with Antioxidant Status and Cognitive Performance in Healthy Subjects. *Journal of Alzheimers Disease,* 17**,** 921-927.

POSLUSNA, K., RUPRICH, J., DE VRIES, J. H., JAKUBIKOVA, M. & VAN'T VEER, P. 2009. Misreporting of energy and micronutrient intake estimated by food records and 24 hour recalls, control and adjustment methods in practice. *Br J Nutr,* 101 Suppl 2**,** S73-85.

PRIOR, R. L., WU, X. & SCHAICH, K. 2005. Standardized methods for the determintaion of antioxidant capacity and phenolics in foods and dietary supplements. *J. Agric. Food Chem.,* 53**,** 4290–4302.

RABABAH, T. M., AL-MAHASNEH, M. A., KILANI, I., YANG, W., ALHAMAD, M. N., EREIFEJ, K. & AL-U'DATT, M. 2011. Effect of jam processing and storage on total phenolics, antioxidant activity, and anthocyanins of different fruits. *J Sci Food Agric,* 91**,** 1096-102.

REBELLO, S. A., CHEN, C. H., NAIDOO, N., XU, W., LEE, J., CHIA, K. S., TAI, E. S. & VAN DAM, R. M. 2011. Coffee and tea consumption in relation to inflammation and basal glucose metabolism in a multi-ethnic Asian population: a cross-sectional study. *Nutr J,* 10**,** 61.

REIS, J. P., LORIA, C. M., LAUNER, L. J., SIDNEY, S., LIU, K., JACOBS, D. R. J., ZHU, N., LLOYD-JONES, D. M., HE, K. & YAFFE, K. 2013. Cardiovascular health through young adulthood and cognitive functioning in midlife. *Ann Neurol.,* 73**,** 170-9.

REISBERG, D. 2013. *Cognition: exploring the science of the mind-5th ed.,* New York, W.W. Norton & Company.

REKHA, C., POORNIMA, G., MANASA, M., ABHIPSA, V., PAVITHRA DEVI, J., VIJAY KUMAR, H. T. & PRASHITH KEKUDA, T. R. 2012. Ascorbic acid, total phenol content and antioxidant activity of fresh juices of four ripe and unripe citrus fruits. *Chem Sci Trans.,* 1**,** 303-310.

RENDEIRO, C., GUERREIRO, J. D., WILLIAMS, C. M. & SPENCER, J. P. 2012. Flavonoids as modulators of memory and learning: molecular interactions resulting in behavioural effects. *Proc Nutr Soc,* 71**,** 246-62.

RENDEIRO, C., VAUZOUR, D., RATTRAY, M., WAFFO-TEGUO, P., MERILLON, J. M., BUTLER, L. T., WILLIAMS, C. M. & SPENCER, J. P. 2013. Dietary levels of pure flavonoids improve spatial memory performance and increase hippocampal brain-derived neurotrophic factor. *PLoS One,* 8**,** e63535.

REY, A. 1964. L'Examen Clinique en Psychologie. *Presses Universitaires de France, Paris*.

RIBOLI, E., HUNT, K. J., SLIMANI, N., FERRARI, P., NORAT, T., FAHEY, M., CHARRONDIÈRE, U. R., HÉMON, B., CASAGRANDE, C., VIGNAT, J., OVERVAD, K., TJØNNELAND, A., CLAVEL-CHAPELON, F., THIÉBAUT, A., WAHRENDORF, J., BOEING, H., TRICHOPOULOS, D., TRICHOPOULOU, A., VINEIS, P., PALLI, D., BUENO-DE-MESQUITA, H. B., PEETERS, P. H., LUND, E., ENGESET, D., GONZÁLEZ, C. A., BARRICARTE, A., BERGLUND, G., HALLMANS, G., DAY, N. E., KEY, T. J., KAAKS, R. & SARACCI, R. 2002. European Prospective Investigation into Cancer and Nutrition (EPIC): study populations and data collection. *Public Health Nutr.*, 5**,** 1113-24.

RODRÍGUEZ, H., CURIEL, J. A., LANDETE, J. M., DE LAS RIVAS, B., DE FELIPE, F. L., GÓMEZ-CORDOVÉS, C., MANCHEÑO, J. M. & MUÑOZ, R. 2009. Food phenolics and lactic acid bacteria. *International Journal of Food Microbiology,* 132**,** 79-90.

RODRIGUEZ, M. C., PARRA, M. D., MARQUES-LOPES, I., DE MORENTIN, B. E., GONZALEZ, A. & MARTINEZ, J. A. 2005. Effects of two energy-restricted diets containing different fruit amounts on body weight loss and macronutrient oxidation. *Plant Foods Hum Nutr,* 60**,** 219-24.

ROE, L., STRONG, C., WHITESIDE, C., NEIL, A. & MANT, D. 1994. Dietary assessment in primary care: validity of the DINE method for dietary assessment. *Fam. Pract.*, 11**,** 375 - 381.

RUDELLE, S., FERRUZZI, M. G., CRISTIANI, I., MOULIN, J., MACE, K., ACHESON, K. J. & TAPPY, L. 2007. Effect of a thermogenic beverage on 24-hour energy metabolism in humans. *Obesity (Silver Spring),* 15**,** 349-55.

RYAN, L., THONDRE, P. S. & HENRY, C. J. K. 2011. Oat-based breakfast cereals are a rich source of polyphenols and high in antioxidant potential. *Journal of Food Composition and Analysis,* 24**,** 929-934.

SALTHOUSE, T. A. 2009. When does age-related cognitive decline begin? *Neurobiol Aging,* 30**,** 507-14.

SARTORELLI, D. S., FRANCO, L. J. & CARDOSO, M. A. 2008. High intake of fruits and vegetables predicts weight loss in Brazilian overweight adults. *Nutr Res,* 28**,** 233-8.

SAURA-CALIXTO, F., SERRANO, J. & GONI, I. 2007. Intake and bioaccessibility of total polyphenols in a whole diet. *Food Chemistry,* 101**,** 492-501.

SCALBERT, A., JOHNSON, I. T. & SALTMARSH, M. 2005. Polyphenols: antioxidants and beyond. *American Journal of Clinical Nutrition,* 81**,** 215-217.

SCALBERT, A. & WILLIAMSON, G. 2000. Dietary intake and bioavailability of polyphenols. *J Nutr,* 130**,** 2073S-85S.

SCHMITT, J. A., BENTON, D. & KALLUS, K. W. 2005. General methodological considerations for the assessment of nutritional influences on human cognitive functions. *Eur J Nutr,* 44**,** 459-64.

SCHOFIELD, W. N. 1985. Predicting basal metabolic rate, new standards and review of previous work. *Hum Nutr: Clin Nutr* 39C(Suppl. 1)**,** 5-41.

SCHOLEY, A. B., FRENCH, S. J., MORRIS, P. J., KENNEDY, D. O., MILNE, A. L. & HASKELL, C. F. 2010. Consumption of cocoa flavanols results in acute

improvements in mood and cognitive performance during sustained mental effort. *J Psychopharmacol,* 24**,** 1505-14.

SEGALL, S. 2000. *Caffeinated Beverages: Health Benefits, Physiological Effects, and Chemistry: Comparing coffee and tea.,* Washington, American Chemical Society.

SIGMA-ALDRICH. 2013. *Safety data sheet for catechin* [Online]. Poole, Dorset, UK: Sigma-Aldrich Co. Available: http://www.sigmaaldrich.com/MSDS/MSDS/DisplayMSDSPage.do?country=GB&language=en&productNumber=43412&brand=FLUKA&PageToGoToURL=http%3A%2F%2Fwww.sigmaaldrich.com%2Fcatalog%2Fsearch%3Finterface%3DProduct%2520Name%26term%3D%2528%2B%2529-Catechin%26lang%3Den%26region%3DGB%26focus%3Dproduct%26N%3D220003048%2B219853121%2B219853286%26mode%3Dmode%2520matchpartialmax [Accessed 24 September 2013].

SIMON, H. A. 1975. The functional equivalence of problem solving skills. *Cognitive Psychology.,* 7**,** 268-288.

SINGH-MANOUX, A., KIVIMAKI, M., GLYMOUR, M. M., ELBAZ, A., BERR, C., EBMEIER, K. P., FERRIE, J. E. & DUGRAVOT, A. 2012. Timing of onset of cognitive decline: results from Whitehall II prospective cohort study. *British Medical Journal,* 344.

SINGH-MANOUX, A., SABIA, S., LAJNEF, M., FERRIE, J. E., NABI, H., BRITTON, A. R., MARMOT, M. G. & SHIPLEY, M. J. 2008. History of coronary heart disease and cognitive performance in midlife: the Whitehall II study. *Eur Heart J.* , 29**,** 2100-7.

SINGLETON, V. L., ORTHOFER, R. & LAMUELA-RAVENTOS, R. M. 1999. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. *Methods Enzymol,* 299**,** 152-178.

SINGLETON, V. L. & ROSSI, J. A. 1965. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *Am J Enol Vitic.,* 16**,** 144-158.

SOHRAB, G., HOSSEINPOUR-NIAZI, S., HEJAZI, J., YUZBASHIAN, E., MIRMIRAN, P. & AZIZI, F. 2013. Dietary polyphenols and metabolic syndrome among Iranian adults. *Int J Food Sci Nutr***,** 1–7.

SOMERSET, S. M. & JOHANNOT, L. 2008. Dietary flavonoid sources in Australian adults. *Nutr Cancer,* 60**,** 442-9.

SONG, J., XU, H., LIU, F. & FENG, L. 2012. Tea and cognitive health in late life: current evidence and future directions. *J Nutr Health Aging,* 16**,** 31-4.

SPENCER, J. P. 2009a. Flavonoids and brain health: multiple effects underpinned by common mechanisms. *Genes Nutr,* 4**,** 243-50.

SPENCER, J. P. 2009b. The impact of flavonoids on memory: physiological and molecular considerations. *Chem Soc Rev,* 38**,** 1152-61.

SPENCER, J. P., ABD EL MOHSEN, M. M., MINIHANE, A. M. & MATHERS, J. C. 2008. Biomarkers of the intake of dietary polyphenols: strengths, limitations and application in nutrition research. *Br J Nutr,* 99**,** 12-22.

SQUIRE, L. R. & ZOLA, S. M. 1996. Structure and function of declarative and nondeclarative memory systems. *Proc Natl Acad Sci U S A,* 93**,** 13515-22.

STALMACH, A., MULLEN, W., NAGAI, A. & CROZIER, A. 2006. On-line HPLC analysis of the antioxidant activity of phenolic compounds in

brewed, paper-filtered coffee. *Brazilian Journal of Plant Physiology* 18**,** 253-62.

STANGL, D. & THURET, S. 2009. Impact of diet on adult hippocampal neurogenesis. *Genes Nutr,* 4**,** 271-82.

STEWART, R. & LIOLITSA, D. 1999. Type 2 diabetes mellitus, cognitive impairment and dementia. *Diabetic Medicine,* 16**,** 93-112.

TABACHNICK, B. G. & FIDELL, L. S. 2007. *Using multivariate analysis (5th. ed.),* Boston, Pearson Education.

THORP, A. A., SINN, N., BUCKLEY, J. D., COATES, A. M. & HOWE, P. R. 2009. Soya isoflavone supplementation enhances spatial working memory in men. *Br J Nutr,* 102**,** 1348-54.

TORRONEN, R., MCDOUGALL, G. J., DOBSON, G., STEWART, D., HELLSTROM, J., MATTILA, P., PIHLAVA, J. M., KOSKELA, A. & KARJALAINEN, R. 2012. Fortification of blackcurrant juice with crowberry: Impact on polyphenol composition, urinary phenolic metabolites, and postprandial glycemic response in healthy subjects. *Journal of Functional Foods,* 4**,** 746-756.

TRESSERRA-RIMBAU, A., MEDINA-REMON, A., PEREZ-JIMENEZ, J., MARTINEZ-GONZALEZ, M. A., COVAS, M. I., CORELLA, D., SALAS-SALVADO, J., GOMEZ-GRACIA, E., LAPETRA, J., AROS, F., FIOL, M., ROS, E., SERRA-MAJEM, L., PINTO, X., MUNOZ, M. A., SAEZ, G. T., RUIZ-GUTIERREZ, V., WARNBERG, J., ESTRUCH, R. & LAMUELA-RAVENTOS, R. M. 2013. Dietary intake and major food sources of polyphenols in a Spanish population at high cardiovascular risk: The PREDIMED study. *Nutr Metab Cardiovasc Dis*.

TULVING, E. 1987. Multiple memory systems and consciousness. *Hum Neurobiol,* 6**,** 67-80.

TULVING, E. 1991. *Concepts of human memory,* New York, Oxford University Press.

USDA 2011. United States Department of Agriculture (USDA) Database for the Flavonoid Content of Selected Foods, Release 3.0.

VALLEJO, F., TOMAS-BARBERAN, F. A. & FERRERES, F. 2004. Characterisation of flavonols in broccoli (Brassica oleracea L. var. italica) by liquid chromatography-uV diode-array detection-electrospray ionisation mass spectrometry. *J Chromatogr A,* 1054**,** 181-93.

VALLS-PEDRET, C., LAMUELA-RAVENTOS, R. M., MEDINA-REMON, A., QUINTANA, M., CORELLA, D., PINTO, X., MARTINEZ-GONZALEZ, M. A., ESTRUCH, R. & ROS, E. 2012. Polyphenol-rich foods in the Mediterranean diet are associated with better cognitive function in elderly subjects at high cardiovascular risk. *J Alzheimers Dis,* 29**,** 773-82.

VAN GELDER, B. M., BUIJSSE, B., TIJHUIS, M., KALMIJN, S., GIAMPAOLI, S., NISSINEN, A. & KROMHOUT, D. 2007. Coffee consumption is inversely associated with cognitive decline in elderly European men: the FINE Study. *Eur J Clin Nutr,* 61**,** 226-32.

VAN PRAAG, H., LUCERO, M. J., YEO, G. W., STECKER, K., HEIVAND, N., ZHAO, C., YIP, E., AFANADOR, M., SCHROETER, H., HAMMERSTONE, J. & GAGE, F. H. 2007. Plant-derived flavanol (-)epicatechin enhances angiogenesis and retention of spatial memory in mice. *J Neurosci,* 27**,** 5869-78.

WANG, A. Y., ZHOU, M. Y. & LIN, W. C. 2011. Antioxidative and anti-inflammatory properties of *Citrus sulcata* extracts. *Food Chemistry,* 124**,** 958–963.

WARDLE, J., HAASE, A. M. & STEPTOE, A. 2006. Body image and weight control in young adults: international comparisons in university students from 22 countries. *Int J Obes (Lond),* 30**,** 644-51.

WECHSLER, D. 1981. WAIS-R manual: Wechsler adult intelligence scale-revised. *The Psychological Corporation, New York.*

WECHSLER, D. 1999. *Wechsler abbreviated scale of intelligence*, The Psychological Corporation, New York.

WEDICK, N. M., PAN, A., CASSIDY, A., RIMM, E. B., SAMPSON, L., ROSNER, B., WILLETT, W., HU, F. B., SUN, Q. & VAN DAM, R. M. 2012. Dietary flavonoid intakes and risk of type 2 diabetes in US men and women. *American Journal of Clinical Nutrition,* 95**,** 925-933.

WESTENHOEFER, J., BELLISLE, F., BLUNDELL, J. E., DE VRIES, J., EDWARDS, D., KALLUS, W., MILON, H., PANNEMANS, D., TUIJTELAARS, S. & TUORILA, H. 2004. PASSCLAIM--mental state and performance. *Eur J Nutr,* 43**,** II/85-II/117.

WESTERTERP-PLANTENGA, M. S. 2010. Green tea catechins, caffeine and body-weight regulation. *Physiol Behav,* 100**,** 42-6.

WIGHTMAN, E. L., HASKELL, C. F., FORSTER, J. S., VEASEY, R. C. & KENNEDY, D. O. 2012. Epigallocatechin gallate, cerebral blood flow parameters, cognitive performance and mood in healthy humans: a double-blind, placebo-controlled, crossover investigation. *Human Psychopharmacology-Clinical and Experimental,* 27**,** 177-186.

WILLIAMS, C. J., FARGNOLI, J. L., HWANG, J. J., VAN DAM, R. M., BLACKBURN, G. L., HU, F. B. & MANTZOROS, C. S. 2008a. Coffee consumption is associated with higher plasma adiponectin concentrations in women with or without type 2 diabetes: a prospective cohort study. *Diabetes Care,* 31**,** 504-7.

WILLIAMS, C. M., EL MOHSEN, M. A., VAUZOUR, D., RENDEIRO, C., BUTLER, L. T., ELLIS, J. A., WHITEMAN, M. & SPENCER, J. P. 2008b. Blueberry-induced changes in spatial working memory correlate with changes in hippocampal CREB phosphorylation and brain-derived neurotrophic factor (BDNF) levels. *Free Radic Biol Med,* 45**,** 295-305.

WILLSON, K. C. 1999. *Coffee, Cocoa and Tea: Origin and distribution of coffee, cocoa and tea,* United Kingdom, CABI Publishing.

WOODWARD, M. & TUNSTALL-PEDOE, H. 1999. Coffee and tea consumption in the Scottish Heart Health Study follow up: conflicting relations with coronary risk factors, coronary disease, and all cause mortality. *J Epidemiol Community Health,* 53**,** 481-487.

WOOTTON-BEARD, P. C., MORAN, A. & RYAN, L. 2011. Stability of the total antioxidant capacity and total polyphenol content of 23 commercially available vegetable juices before and after in vitro digestion measured by FRAP, DPPH, ABTS and Folin-Ciocalteu methods. *Food Research International,* 44**,** 217-224.

WU, C. H., LU, F. H., CHANG, C. S., CHANG, T. C., WANG, R. H. & CHANG, C. J. 2003. Relationship among habitual tea consumption, percent body fat, and body fat distribution. *Obesity Research,* 11**,** 1088-1095.

WU, X., BEECHER, G. R., HOLDEN, J. M., HAYTOWITZ, D. B., GEBHARDT, S. E. & PRIOR, R. L. 2004. Lipophilic and hydrophilic antioxidant capacities of common foods in the United States. *J Agric Food Chem,* 52**,** 4026-37.

YOUDIM, K. A., SHUKITT-HALE, B. & JOSEPH, J. A. 2004. Flavonoids and the brain: interactions at the blood-brain barrier and their physiological effects on the central nervous system. *Free Radic Biol Med,* 37**,** 1683-93.

YUAN, J.-M., SUN, C. & BUTLER, L. M. 2011. Tea and cancer prevention: Epidemiological studies. *Pharmacological Research,* 64**,** 123-135.

ZAHODNE, L. B., GLYMOUR, M. M., SPARKS, C., BONTEMPO, D., DIXON, R. A., MACDONALD, S. W. S. & MANLY, J. J. 2011. Education does not slow cognitive decline with aging: 12-year evidence from the Victoria Longitudinal Study. *Journal of the International Neuropsychological Society,* 17**,** 1-8.

ZAMORA-ROS, R., ANDRES-LACUEVA, C., LAMUELA-RAVENTOS, R. M., BERENGUER, T., JAKSZYN, P., BARRICARTE, A., ARDANAZ, E., AMIANO, P., DORRONSORO, M., LARRANAGA, N., MARTINEZ, C., SANCHEZ, M. J., NAVARRO, C., CHIRLAQUE, M. D., TORMO, M. J., QUIROS, J. R. & GONZALEZ, C. A. 2010. Estimation of Dietary Sources and Flavonoid Intake in a Spanish Adult Population (EPIC-Spain). *Journal of the American Dietetic Association,* 110**,** 390-398.

ZAMORA-ROS, R., FERRARI, P., GONZÁLEZ, C., TJØNNELAND, A., OLSEN, A., BREDSDORFF, L., OVERVAD, K., TOUILLAUD, M., PERQUIER, F., FAGHERAZZI, G., LUKANOVA, A., TIKK, K., ALEKSANDROVA, K., BOEING, H., TRICHOPOULOU, A., TRICHOPOULOS, D., DILIS, V., MASALA, G., SIERI, S., MATTIELLO, A., TUMINO, R., RICCERI, F., BUENO-DE-MESQUITA, H. B., PEETERS, P. M., WEIDERPASS, E., SKEIE, G., ENGESET, D., MENÉNDEZ, V., TRAVIER, N., MOLINA-MONTES, E., AMIANO, P., CHIRLAQUE, M.-D., BARRICARTE, A., WALLSTRÖM, P., SONESTEDT, E., SUND, M., LANDBERG, R., KHAW, K.-T., WAREHAM, N., TRAVIS, R., SCALBERT, A., WARD, H., RIBOLI, E. & ROMIEU, I. 2013a. Dietary flavonoid and lignan intake and breast cancer risk according to menopause and hormone receptor status in the European Prospective Investigation into Cancer and Nutrition (EPIC) Study. *Breast Cancer Research and Treatment,* 139**,** 163-176.

ZAMORA-ROS, R., KNAZE, V., LUJAN-BARROSO, L., KUHNLE, G. G. C., MULLIGAN, A. A., TOUILLAUD, M., SLIMANI, N., ROMIEU, I., POWELL, N., TUMINO, R., PEETERS, P. H. M., DE MAGISTRIS, M. S., RICCERI, F., SONESTEDT, E., DRAKE, I., HJARTAKER, A., SKIE, G., MOUW, T., WARK, P. A., ROMAGUERA, D., BUENO-DE-MESQUITA, H. B., ROS, M., MOLINA, E., SIERI, S., QUIROS, J. R., HUERTA, J. M., TJONNELAND, A., HALKJAER, J., MASALA, G., TEUCHER, B., KAAS, R., TRAVIS, R. C., DILIS, V., BENETOU, V., TRICHOPOULOU, A., AMIANO, P., ARDANAZ, E., BOEING, H., FORSTER, J., CLAVEL-CHAPELON, F., FAGHERAZZI, G., PERQUIER, F., JOHANSSON, G., JOHANSSON, I., CASSIDY, A., OVERVAD, K. & GONZALEZ, C. A. 2012. Dietary intakes and food sources of phytoestrogens in the European Prospective Investigation into Cancer and Nutrition (EPIC) 24-hour dietary recall cohort. *European Journal of Clinical Nutrition,* 66**,** 932-941.

ZAMORA-ROS, R., KNAZE, V., LUJAN-BARROSO, L., ROMIEU, I., SCALBERT, A., SLIMANI, N., HJARTAKER, A., ENGESET, D., SKEIE, G., OVERVAD, K., BREDSDORFF, L., TJONNELAND, A., HALKJAER, J., KEY, T. J., KHAW, K. T., MULLIGAN, A. A., WINKVIST, A., JOHANSSON, I., BUENO-DE-MESQUITA, H. B., PEETERS, P. H. M., WALLSTROM, P., ERICSON, U., PALA, V., DE MAGISTRIS, M. S., POLIDORO, S., TUMINO, R., TRICHOPOULOU, A., DILIS, V., KATSOULIS, M., HUERTA, J. M., MARTINEZ, V., SANCHEZ, M. J., ARDANAZ, E., AMIANO, P., TEUCHER, B., GROTE, V., BENDINELLI, B., BOEING, H., FORSTER, J., TOUILLAUD, M., PERQUIER, F., FAGHERAZZI, G., GALLO, V., RIBOLI, E. & GONZALEZ, C. A. 2013b. Differences in dietary intakes, food sources and determinants of total flavonoids between Mediterranean and non-Mediterranean countries participating in the European Prospective Investigation into Cancer and Nutrition (EPIC) study. *British Journal of Nutrition,* 109**,** 1498-1507.

ZAMORA-ROS, R., KNAZE, V., LUJAN-BARROSO, L., SLIMANI, N., ROMIEU, I., FEDIRKO, V., SANTUCCI DE MAGISTRIS, M., ERICSON, U., AMIANO, P., TRICHOPOULOU, A., DILIS, V., NASKA, A., ENGESET, D., SKEIE, G., CASSIDY, A., OVERVAD, K., PEETERS, P. H., MARIA HUERTA, J., SANCHEZ, M. J., QUIROS, J. R., SACERDOTE, C., GRIONI, S., TUMINO, R., JOHANSSON, G., JOHANSSON, I., DRAKE, I., CROWE, F. L., BARRICARTE, A., KAAKS, R., TEUCHER, B., BAS BUENO-DE-MESQUITA, H., VAN ROSSUM, C. T., NORAT, T., ROMAGUERA, D., VERGNAUD, A. C., TJONNELAND, A., HALKJAER, J., CLAVEL-CHAPELON, F., BOUTRON-RUAULT, M. C., TOUILLAUD, M., SALVINI, S., KHAW, K. T., WAREHAM, N., BOEING, H., FORSTER, J., RIBOLI, E. & GONZALEZ, C. A. 2011a. Estimated dietary intakes of flavonols, flavanones and flavones in the European Prospective Investigation into Cancer and Nutrition (EPIC) 24 hour dietary recall cohort. *Br J Nutr***,** 1-11.

ZAMORA-ROS, R., KNAZE, V., LUJAN-BARROSO, L., SLIMANI, N., ROMIEU, I., FEDIRKO, V., SANTUCCI DE MAGISTRIS, M., ERICSON, U., AMIANO, P., TRICHOPOULOU, A., DILIS, V., NASKA, A., ENGESET, D., SKEIE, G., CASSIDY, A., OVERVAD, K., PEETERS, P. H., MARIA HUERTA, J., SANCHEZ, M. J., QUIROS, J. R., SACERDOTE, C., GRIONI, S., TUMINO, R., JOHANSSON, G., JOHANSSON, I., DRAKE, I., CROWE, F. L., BARRICARTE, A., KAAKS, R., TEUCHER, B., BAS BUENO-DE-MESQUITA, H., VAN ROSSUM, C. T., NORAT, T., ROMAGUERA, D., VERGNAUD, A. C., TJONNELAND, A., HALKJAER, J., CLAVEL-CHAPELON, F., BOUTRON-RUAULT, M. C., TOUILLAUD, M., SALVINI, S., KHAW, K. T., WAREHAM, N., BOEING, H., FORSTER, J., RIBOLI, E. & GONZALEZ, C. A. 2011b. Estimation of the intake of anthocyanidins and their food sources in the European Prospective Investigation into Cancer and Nutrition (EPIC) study. *Br J Nutr,* 12**,** 1-10.

ZAMORA-ROS, R., KNAZE, V., ROMIEU, I., SCALBERT, A., SLIMANI, N., CLAVEL-CHAPELON, F., TOUILLAUD, M., PERQUIER, F., SKEIE, G., ENGESET, D., WEIDERPASS, E., JOHANSSON, I., LANDBERG, R., BUENO-DE-MESQUITA, H. B., SIERI, S., MASALA, G., PEETERS, P. H. M., GROTE, V., HUERTA, J. M., BARRICARTE, A., AMIANO, P.,

CROWE, F. L., MOLINA-MONTES, E., KHAW, K. T., ARGUELLES, M. V., TJONNELAND, A., HALKJAER, J., DE MAGISTRIS, M. S., RICCERI, F., TUMINO, R., WIRFALT, E., ERICSON, U., OVERVAD, K., TRICHOPOULOU, A., DILIS, V., VIDALIS, P., BOEING, H., FORSTER, J., RIBOLI, E. & GONZALEZ, C. A. 2013c. Impact of thearubigins on the estimation of total dietary flavonoids in the European Prospective Investigation into Cancer and Nutrition (EPIC) study. *European Journal of Clinical Nutrition*, 67**,** 779-782.

ZUJKO, M. E., WITKOWSKA, A. M., WASKIEWICZ, A. & SYGNOWSKA, E. 2012. Estimation of dietary intake and patterns of polyphenol consumption in Polish adult population. *Adv Med Sci,* 57**,** 375-84.