Optimisation of foam-mat freeze-drying conditions for blueberry powder and evaluation of powder properties

Sandi Darniadi

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The candidate confirms that the work submitted is his own, except where work which has formed part of jointly-authored publications has been included. The contribution of the candidate and the other authors to his work has been explicitly indicated below. The candidate confirms that appropriate credit has been given within the thesis where references has been made to the work of others. Details of jointly-authored publications are outlined on the next page.

Further details of the work from jointly-authored publications and the contributions of the candidate and the other authors to the work are included below:

This thesis contributed to the following publication based on Chapter 6:

- Comparison of blueberry powder produced via foam-mat freeze-drying versus spray drying: evaluation of foam and powder properties (*Journal of the Science of Food and Agriculture: submitted March 29, 2017*)

Manuscripts in progress for submission:

- Total monomeric anthocyanins, total phenolic content, and individual anthocyanin of foam-mat freeze-dried and spray-dried blueberry powder
- Foam-mat freeze-drying of blueberry juice by using trehalose-β-lactoglobulin and trehalose-bovine serum albumin as matrices

Details of authorship contribution:
Sandi Darniadi: conducted the experimental design, data analysis, data interpretation, laboratory work and wrote a draft of publication.
B.S. Murray: guidance, supervision, contributed to answer the reviewer's comments and manuscript editor.
P. Ho: guidance, supervision.

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Abstract

The aim of this study was to develop blueberry powder via foam-mat freeze-drying (FMFD) and to investigate the foam and powder properties. The spray-drying (SD) method was also carried out as a comparison to foam-mat freeze-drying. Foam-mat freeze-drying of blueberry juice was conducted using constant temperature (-55 ^{0}C) and vacuum pressure (0.04 mbar) for 24 h, while spray-drying was tested using two feed flow rates, 180 (SD 180) and 360 (SD 360) mL h^{-1}, and other conditions of spray-drying were kept constant. Foam properties were compared amongst foam made with ratio 2.8 of maltodextrin/whey protein isolate (M3W1), trehalose/β-lactoglobulin (T3BL1), and trehalose/bovine serum albumin (T3A1). The M3W1 foam was found less stable than those with T3BL1 and T3A1 since the M3W1 gave high foam density. FMFD powder made with M3W1 had higher moisture content (3.5%), longer rehydration time (90 s), and lower bulk density (0.32 g cm^{-3}) over the T3BL1 and T3A1. The FMFD powders made with trehalose/pure proteins generated pores and ordered structures, while the M3W1 powder had broken glass-like structures. The M3W1 reconstituted powder gave high a*/redness which was probably attributed to high total monomeric anthocyanins (TMA) content (8.5 mg Cyn3Gl g^{-1} solids). The MD/WPI as matrices had reduced losses in the total phenolic content (TPC) and individual anthocyanin than T3A1 or T3BL1. The TPC and TMA retention of M3W1 reconstituted powder were recorded as 73 and 95%, respectively. FMFD and SD powders made with MD/WPI 0.4-3.2 were also compared. The FMFD had a higher yield, lower moisture content, longer rehydration time and lower bulk density compared to those of spray-dried (SD) powders. The FMFD samples were purple powders and flake-like shaped of particles, while SD samples were bright pink powders. The SD powder

particles were smooth, spherical and smaller sized than the FMFD powder. The TMA of FMFD powders showed higher levels than those of SD samples. Delphinidin-3-glucosiede (Del3Gl), Cyanidin-3-glucoside (Cyn3Gl), and Malvidin-3-glucoside (Mal3Gl) retentions were greater, in the order: FMFD > SD.

List of accepted abstracts

- Keeping the good stuff: drying techniques to increase antioxidant, in the 1st Student. Conference on Sustainable Futures, University of Leeds. February 2, 2017
- Developing of blueberry powder via foam-mat freeze-drying and its comparison with spray-drying, in the 3rd Annual Food Science & Nutrition PhD Conference – University of Leeds. November 16, 2016
- Foam-mat freeze drying versus spray drying of blueberry juice, in the 18th IUFoST World Congress of Food Science and Technology, Dublin-Ireland. August 21-26, 2016
- Transforming liquid blueberry to solid powder for healthy food, in the PGR Conference – University of Leeds. December 4, 2015
- Effect of freezing temperature and weight of juice on the freeze-dried blueberry powder, in the 2nd Annual Food Science & Nutrition PhD Conference – University of Leeds. November 16, 2015
- The effect of the addition of maltodextrin and whey protein isolate on the foaming properties of blueberry juice and powder properties, in the Future Food Horizon, The Institute of Food Science and Innovation – University of Chester. October 21-22, 2015
- Freeze-drying of foam juice: How we could make a better quality of blueberry powder, in the PGR Conference – University of Leeds. December 4, 2014
- Optimisation of foam-mat freeze-drying conditions for blueberry powder and evaluation of powder properties, in the 1st Annual Food Science & Nutrition PhD Conference – University of Leeds. September 24, 2014

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Abbreviations

Cyn3Ara	Cyanidin-3-arabinose
Cyn3Gal	Cyanidin-3-galactoside
Cyn3Gl	Cyanidin-3-glucoside
Del3Gal	Delphinidin-3-galactoside
Del3Gl	Delphinidin-3-glucoside
FMFD	Foam-mat freeze-drying, foam-mat freeze-dried
GAE	Gallic acid equivalent
HPLC	High performance liquid chromatography
M3W1	Maltodextrin/whey protein isolate
Mal3Ara	Malvidin-3-arabinose
Mal3Gal	Malvidin-3-galctoside
Mal3Gl	Malvidin-3-glucoside
MD	Maltodextrin
Peo3Gal	Peonidin-3-galactoside
Pet3Ara	Petunidin-3-arabinose
Pet3Gal	Petunidin-3-galactoside
Pet3Gl	Petunidin-3-glucoside
SD	Spray-drying, spray-dried
SD	Standard deviation
T3A1	Trehalose/bovine serum albumin
T3BL1	Trehalose/β-lactoglobulin
TMA	Total monomeric anthocyanins
TPC	Total phenolic content
WPI	Whey protein isolate

Chapter 1

Chapter 1 General Introduction

1.1 Aim of the research

This study is inspired by the idea of using polysaccharides and proteins to generate foam in fruit juice system and to be freeze-dried for reducing the drying time and producing fruit juice powder. Also, the spray-drying was conducted as this drying process is a common technique for producing fruit powders from juices. There is little information about determining types and concentrations of polysaccharides and proteins in freeze-drying of fruit juice. The aims of this thesis are:

- To develop foam by the addition of polysaccharides and proteins into the blueberry juice
- To study the foam capacity and stability of blueberry juice and understand the relationship to drying process
- Foam-mat freeze-drying experiment using maltodextrin + whey protein isolate, trehalose + β-lactoglobulin (T3BL1), and trehalose + bovine serum albumin (T3A1) as matrices
- Spray-drying experiment using maltodextrin + whey protein isolate as carrying matrices
- To investigate the influence of foam-mat freeze- and spray-drying processes on the physical properties of blueberry powder
- To evaluate phenolic compounds and anthocyanins from the reconstituted blueberry powder, either made via foam-mat freeze-drying or spray-drying
- Comparing the physicochemical properties of foam-mat freeze-dried and spray-dried powders

1.2 Plan of the thesis

This thesis is composed of the following chapters:

- Chapter 1: General Introduction
- Chapter 2: General Materials and Methodology
- Chapter 3: Effect of maltodextrin to whey protein isolate ratios on the physicochemical properties of foam-mat freeze-dried blueberry powder and reconstituted product
- Chapter 4: Effect of trehalose and pure protein types on the physicochemical properties of foam-mat freeze-dried blueberry powder and reconstituted product
- Chapter 5: Effect of feed flow rates and wall material ratios on the physicochemical properties of spray-dried blueberry powder and reconstituted product
- Chapter 6: General Discussion
- Chapter 7: Overall Conclusions

1.3 Foam-mat drying

In 1917, Campbell patented the method for drying of foamed evaporated milk, followed by patents for the drying of foamed egg albumin.[1] Due to several advantages including fast drying process, low drying temperature, favourable rehydration, and retaining the volatiles during drying, foam-mat drying has received renewed attention over the past decade.[2] Foam is defined as a mass of small gas cells separated by thin films of liquid and formed by bubbles, giving a gas dispersed in a liquid.[3] A typical foam structure is shown in Figure 1-1. The wall of the bubble is called a lamella, and the dispersed phase is surrounded by a plateau border.[4] The size of gas bubbles ranges from about 10 μm to several mm, whereas density extends from nearly zero to about 200 kg m^{-3}. The density of foams in the foam-mat drying is confined to the 300-600 kg m^{-3}.[1]

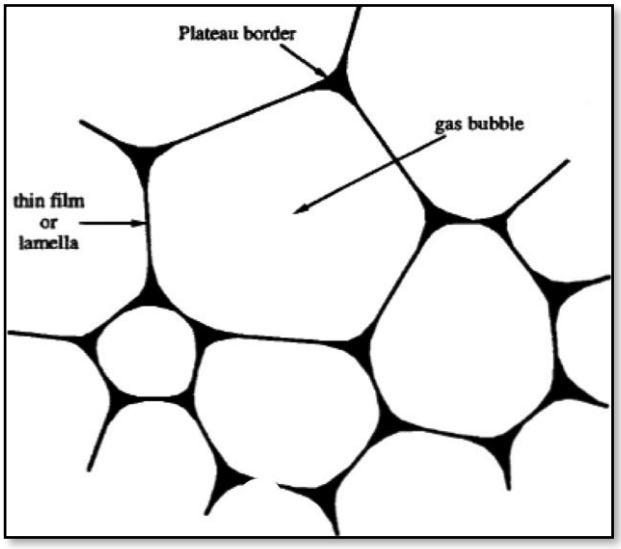


Figure 1-1 Structure of foam (adapted from Muthukumaran et al.[4])

Foams are highly fragile and delicate. The high level of surface energy at the air-water interface make them thermodynamically unstable.[4,5] In foam-mat drying, a liquid material is converted into a stabilised foam by whipping after the addition of edible

foaming agents.[3,6] Therefore, it is necessary to add foaming agents and foam stabilisers to induce foaming and enhance the stability of the foams once formed, respectively.[7,8]

Figure 1-2 shows two pictures of the foam prepared by adding 0.5% w/w of egg white powder to apple juice and whipping for 5 min; Figure 1-2A was taken immediately after whipping, whereas Figure 1-2B was taken after leaving the foam for 20 mins. The foam is then spread out in a sheet or mat and dried by means in the foaming mass expose to the larger surface area for moisture evaporation.[3] The stable foam can then be dried by several methods such as hot air (the most common method), vacuum, microwave, and freeze-drying techniques until the moisture content of the product is reduced to a certain level.[9–12] Rajkumar et al.[3] had developed a continuous foam-mat dryer consists of foaming unit, chute, Teflon belt, heating coils and blower with supporting frame (Figure 1-3)

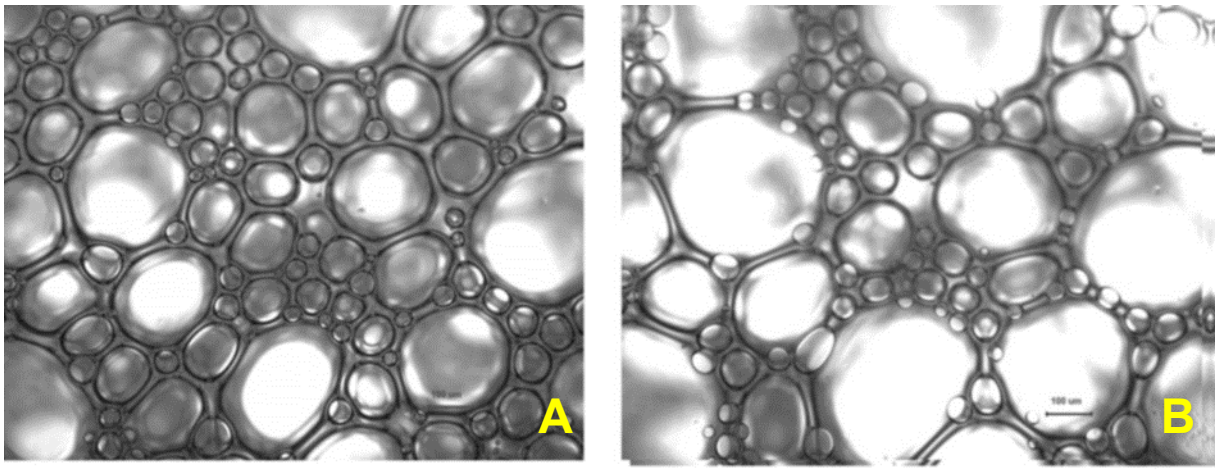
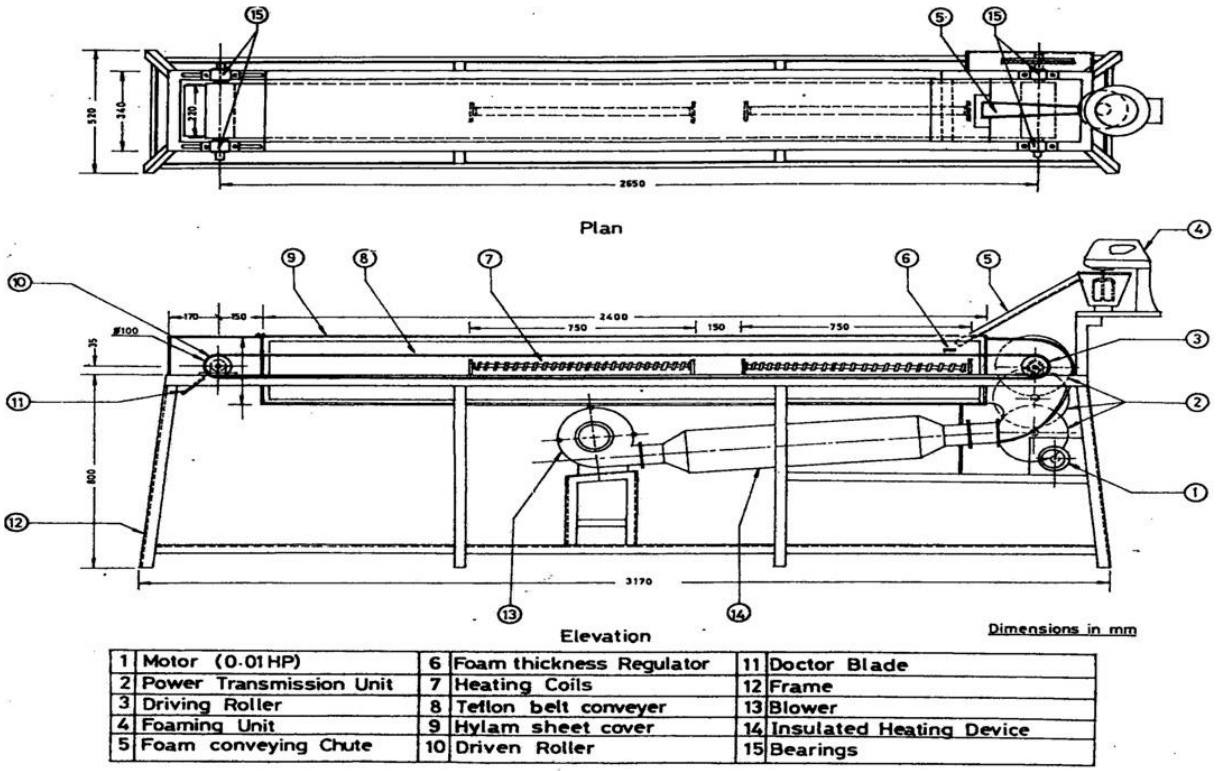


Figure 1-2 Foam of apple juice with 0.5% w/w egg white powder immediately after whipping (A) and after 20 min of the rest time (B) (adapted from Kudra & Ratti[1])



1	Motor (0-01 HP)	6	Foam thickness Regulator	11	Doctor Blade
2	Power Transmission Unit	7	Heating Coils	12	Frame
3	Driving Roller	8	Teflon belt conveyer	13	Blower
4	Foaming Unit	9	Hylam sheet cover	14	Insulated Heating Device
5	Foam conveying Chute	10	Driven Roller	15	Bearings

Figure 1-3 Continuous type foam-mat dryer (adapted from Rajkumar et al.[3])

The advantages of this process are as follows: being relatively simple and inexpensive process, rapid drying rates at lower temperatures, the produced powder is capable of instant rehydration in cold water and enhanced product quality, etc.[1,2,13,14] One difficulty that has previously been experienced with this process, however, is the lack of stability of the foam during the heating cycle.[1] If the foam does not remain stable, cellular breakdown occurs causing serious impairment of the drying operation.[1] The drying mechanism of low-density foams probably different from that as of dense foams, even though both are the gas-liquid system. The heat transfer rates are also different because the thermal conductivity of the gas is much lower than that of liquid. Also, the foam structure should play a major role in water movement during drying of low-density foams, if mass transfer controls the process.[1] Foam-mat drying technique can be used

for heat-sensitive, sticky, viscous, and high sugar content food products.[3] Foam-mat drying has been applied for the drying of banana[8,10], sour cherry[6], tamarind[15], apple juice[16], alphonso mango pulp[3], pinneaple[17], mandarin[13], tomato[18], red guava juice[19], Aloe vera[20] and starfruit[21].

1.4 Freeze-drying

Freeze-drying or lyophilisation is a well-known dehydration method resulting in a high-quality product and is the most expensive. The ability of freeze-drying to maintain high quality is due to the low temperatures in developing dehydrated products.[22] This drying technique is usually used to maintain the physical characteristics such as colour, structure and shape. Freeze-drying can maintain chemical and bioactive compounds, e.g. anthocyanidins, cathecins, flavones, tannins, and isoflavones.[23,24] Moreover, freeze-dried products have longer shelf life and retain the original flavour and aroma.[23]

Freeze-drying is based on sublimation of a frozen product. The best starting point is the phase diagram of water to understand the process properly (Figure 1-4 A), where the sublimation line is placed at a temperature lower than the water triple point (0.01 ^{o}C = 273.16 ^{o}K) and at vapour pressure below 612 Pa (or 4580 mTorr). The basic process of freeze-drying is freezing, vacuum, primary drying (sublimation) and final drying (desorption).[22] The product is firstly placed in a freezer to decrease the ambient temperature for some time until completely frozen (segment A in Figure 1-4 B). Freezing takes place in the commercial freeze-dryer chamber directly, but sometime it can be completed in other freezers. Freezing rate is an important variable during freezing since it directly affects the pore size produced after sublimation of the ice and has better mass flow during dehydration and reconstitution.[25] For example, fast

freezing develops nucleation and formation of intracellular small ice crystals, while slow freezing produces large extracellular ice crystals which damage fruits tissue.[25]

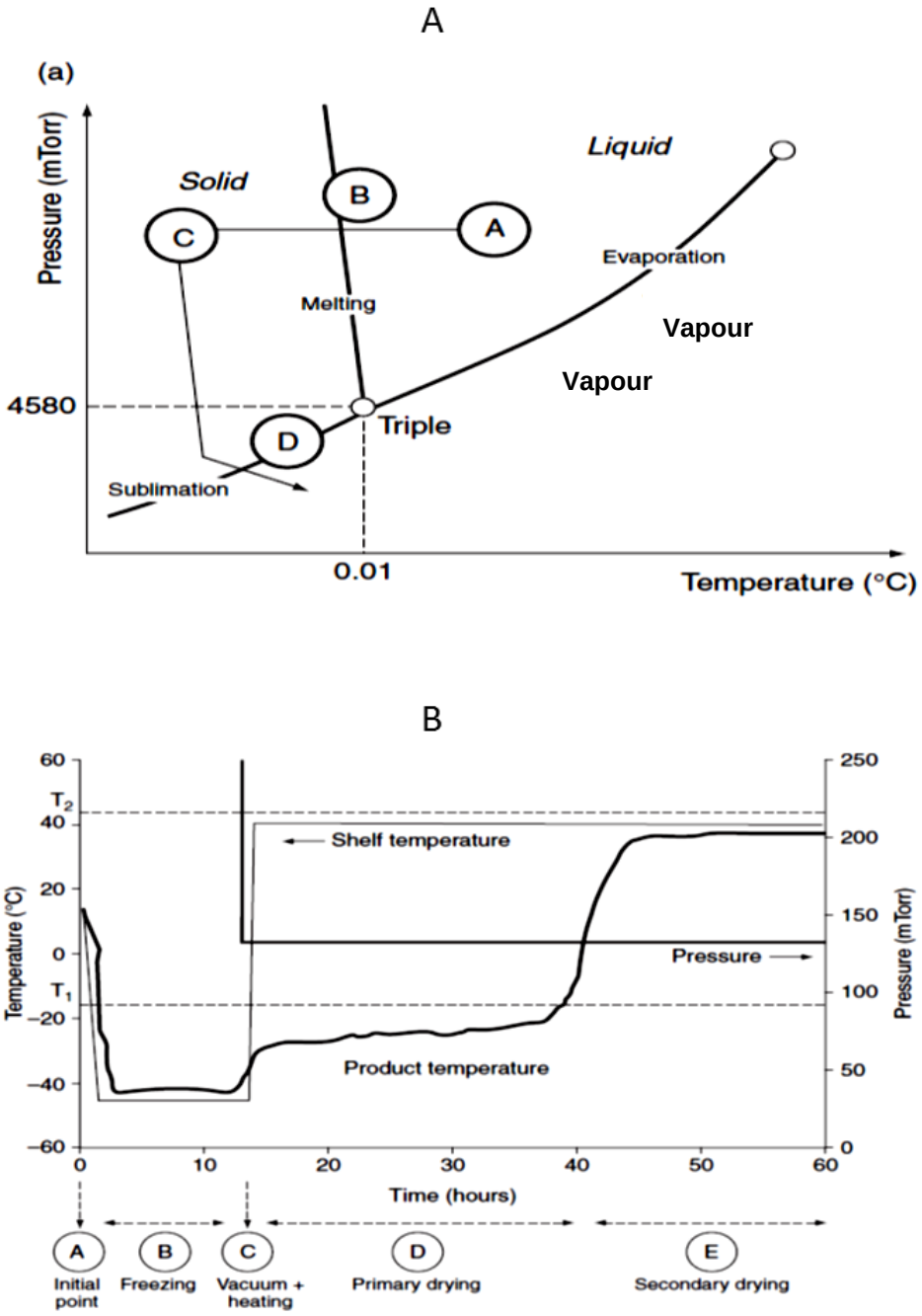


Figure 1-4 Phase diagram of water (a) product temperature profile during freeze-drying (b) (adapted from Ratti[22])

Once the product is frozen, a vacuum is applied in the drying chamber to lower the total pressure and increase the temperature of the shelves. Heat transfer by conduction applies to the products in the drying chamber and also by radiation from upper shelves and surroundings. Sublimation of ice crystal from the frozen products occurs during the primary drying and obtains a porous dry cake (segment D Figure 1-4 B). It takes place for some time the vacuum condition. The moisture content of products falls due to sublimation in the primary drying and desorption during secondary drying (segment E Figure 1-4 B).[22]

Figure 1-5 presents a schematic diagram of a typical batch freeze-dryer, where the main components including: a vacuum system (level 4-27 Pa or 13.5 Pa pressure in commercial freeze-dryer), a heat transfer system (cooling to 223.15 K or heating to 343.15 K), and a condenser (213.15 K or lower).[22] Heat transfer is usually performed through hollow and fluid filled shelves, for which freezing or heating temperatures can be controlled. Condensers are critical pumps maintaining freeze-drying conditions, while the vacuum pump removes the non-condensable gases of the environment.

Freeze-drying is the choice of drying process to guarantee paramount quality in final powdered products. This drying technique, however, is more expensive than other dehydration methods. For instance, Table 1-1 shows fixed and operation costs of freeze-drying compared to other dehydration methods for lactic acid bacteria drying.[26] As we can see from this particular information, freeze-drying costs double vacuum-drying, 6 to 8 times more than spray-drying.

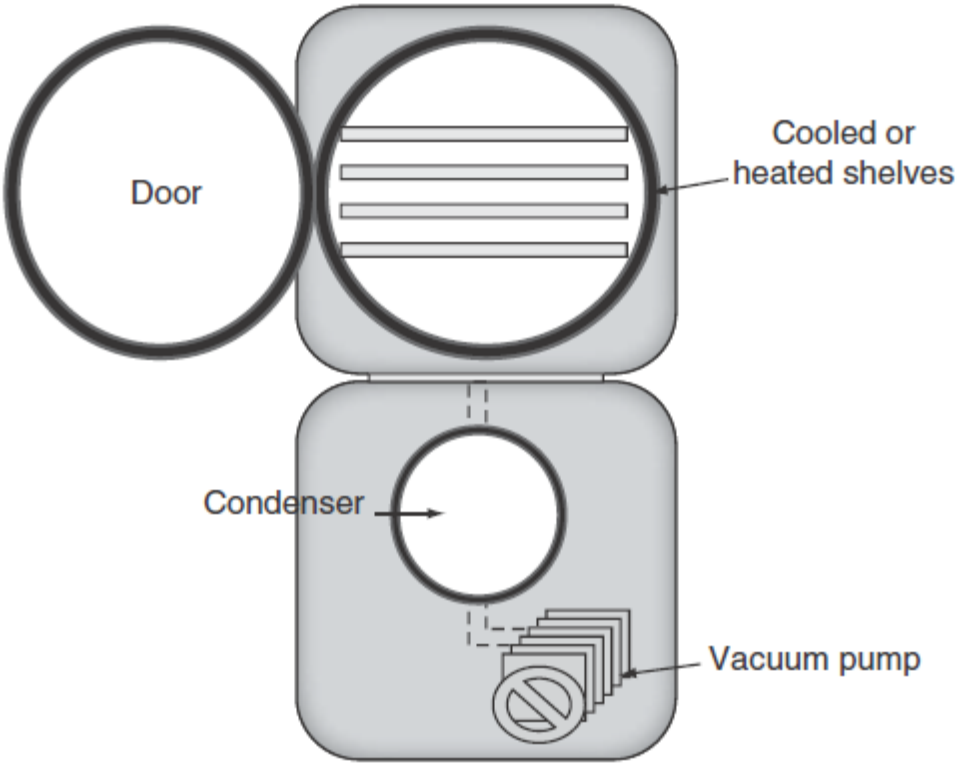


Figure 1-5 Schematic diagram of a freeze dryer (adapted from Ratti[22])

Table 1-1 Operational and fixed costs of different drying methods for lactic acid bacteria dehydration

Drying process	Fixed cost (%)	Operation cost (%)
Freeze-drying	100.0	100.0
Vacuum-drying	52.2	51.6
Spray-drying	12.0	20.0
Drum-drying	9.3	24.1
Fluidized bed-drying	8.8	17.9
Convective hot-air drying	5.3	17.9

Source: Santivarangkna et al.[26]

1.5 Foam-mat freeze-drying

Any technological improvement to classical vacuum-freeze drying to reduce energy cost was addressed to the following goals: (a) to improve heat transfer to help sublimation; (b) to cut drying times, to reduce the vacuum; (c) to avoid the need for condensers.[22] Researchers in the food area such as Sharma & Arora (1995), found that the decrease in sample thickness helps to reduce the sublimation time and the exposure time of the dry layer to high temperatures. On the other hand, some researchers successfully used foaming to decrease processing time during conventional hot-air drying of liquids.[6,10,17,21]

Foam-mat drying has been recognised over decades to drying heat-sensitive food materials, including fruit juice. In foam-mat drying, the product to be dried is converted into foam before drying. Karim & Wai[5] defined that foam-mat drying is a process to produce stable foam using foam agent in fruit juice before drying. The foam-mat drying process has three steps. A stable foam is needed at the first step of the foam mat drying process. It can be done through whipping the mixture of fruit juice to form a stiff foam. The foam is then poured into a tray to obtain a thin mat and air dried. Dried cakes are ground to obtain a fine powder at the final stage. Compared to other drying technologies such as spray drying, foam-mat drying can reduce drying time and use a lower temperature. The foam can promote a larger surface area exposed to the drying air which accelerates the moisture removal process.[21]. This foam gives an advantage of increasing the total surface area available for drying thereby improving the drying rate as well.[12,16]

Foam-mat freeze-drying method is the incorporation of foam-mat drying and freeze-drying to develop dehydrated food from the liquid. Foam-mat freeze-drying aims to dehydrate food from aqueous form, reduce time during vacuum condition in freeze drying process and maintain the nutrition and phytochemical in the final product.[12,27] Foam-mat freeze-drying process includes four primary steps: (a) adding a foaming agent and a foam stabiliser into fruit juice and whipping the mixture to form a stiff foam by using kitchen mixer (whisker) or blender.[16,17] Whipping process is done at room temperature with velocity 3000-4000 rpm for 3-7 min,[4,16,17] (b) freezing the thin layer of foam juice, (c) freeze-drying the foam to obtain a dried cakes or a dried layer, and (d) grinding the dried cake to obtain free flowing powder.[4] The dried product was scrapped and pulverised using a domestic grinder or a kitchen miller, and then was sieved (40 µm screen mesh).[6,17] The foam-mat freeze-drying process is shown in Figure 1-6.

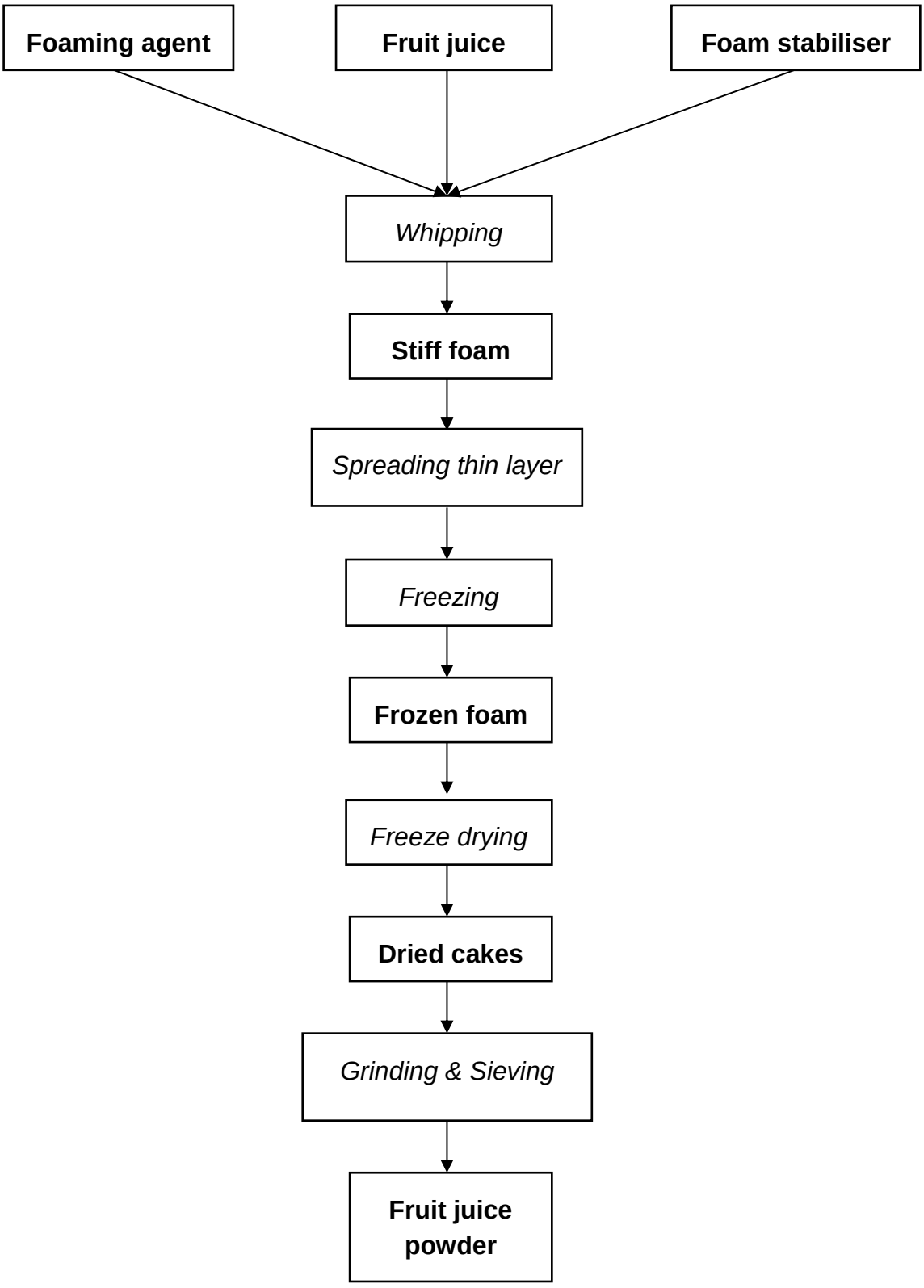


Figure 1-6 Foam-mat freeze-drying process for fruit juice powder

Table 1-2 Size range of particles by various drying process

	Average size (μm)
Bench spray-dryer	5-20
Pilot spray-dryer	20-40
Commercial spray-dryer (two stage)	200-400
Agglomerated powders	200-200

Source: Woo et al.[28]

1.6 Spray-drying

Spray-drying is a drying technique to convert the liquid materials directly into solid or semi solid particles.[29] Spray-drying involves dehydrating finely atomised (sprayed) droplet in a hot convective medium, converting the droplets into fine solid particles (Figure 1-7). The main advantage of this drying technique is the ability to dehydrate the liquid feed material and to produce the material in a micronized particle form simultaneously.[28] The spray-drying process can give high quality powders with low water activity and considerably lower moisture content, resulting in easy storage and transportation.[29] Therefore, the production of instant food powders[30,31] and heat sensitive materials, such as flavours, enzymes, and probiotics[32] employ the spray-drying process. Several parameters in spray drying process influence the physicochemical properties of the end product. The final product mainly depends on the inlet and outlet air temperatures, air flow rate, feed flow rate, atomiser speed, types of the carrier or wall materials and their concentration. Spray-drying is often selected as it can process materials very rapidly while providing relative control of the particle size distriburtion.[33] Particles size produce in spray-drying mainly depend on the spray drying process used (Table 1-2). These different particle size can be used for various

applications. The different morphologies and structures of food particles can also be found in spray-dried powder produced using various spray-drying processes. The typical spray-dried particles are as follows: ballooned, shrivelled, hollow, fragmented, smooth surface, and crystalline or agglomerated micro particles.[28]

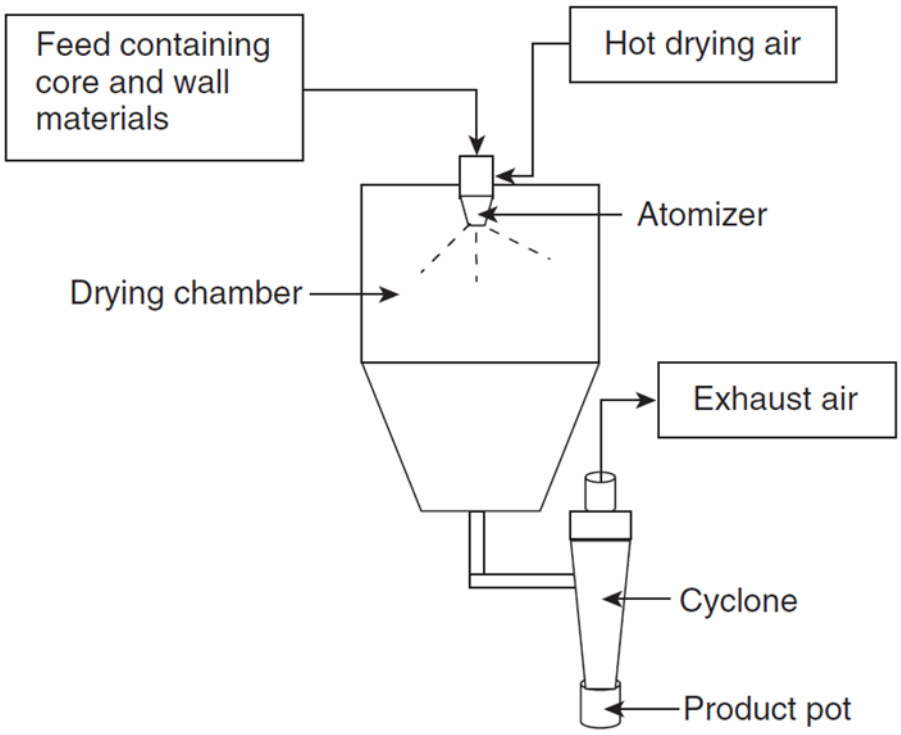


Figure 1-7 Typical schematic diagram of spray-drying for food powder (source: Fang et al.[32])

The first stage of spray-drying is the formation of feed solution or dispersion. The feed solution is commonly prepared by concentrating the liquid. This preparation is to increase the solid content by reducing the amount of moisture. The feedstock in large scale spray-dryers normally concentrates to 50-60%, while in the laboratory-scale spray-dryer has < 50% due to clogging issue in the atomiser.[29,30] Wall material or

carrier agent is added to help the concentration process. Obviously, the selection of an appropriate wall material is an essential step in the spray-drying process. Specific properties of the wall material should be considered, such as high solubility in water, good film-forming, low hygroscopicity, good emulsifying properties, protection core material, low cost, and bland in taste.[32] Some commonly used wall materials for spray drying including carbohydrates (maltodextrin, modified starch, cyclodextrin, and gums), proteins (whey protein, caseinates, skimed milk powder, egg proteins, soy protein, and gelatine), and other biopolymers (modified celluloses, Maillard reaction products, and soluble soy polysaccharides).[32,34] For specific spray-drying such as fruit juice, which contains low molecular sugar (fructose, sucrose), the wall material used, for instance: maltodextrin and gum Arabic, is able to increase the glass transition temperature and avoid the stickiness problem.[35,36]

The concentration of wall material also influenced the spray-dried powder properties. For example, maltodextrin used in spray-drying of sugar-rich fruit juice was > 35%.[37] Addition of such significant amount of wall material increases the cost and may alter the original flavour. However, Quek et al.[38] found the use of low concentration of wall material in spray-drying of watermelon. According to the authors, 5% w/w of maltodextrin recovered higher powder than those of 3%-treated samples. The concentration of maltodextrin > 10% w/w caused loss of their attractive red-orange colour. The wall material concentration also affected the moisture content of powder. Goula & Adamopoulos[39] reported that moisture content of the tomato powder had increased with the increase of maltodextrin concentration. This study confirmed the production of orange juice powder.[31] However, a different result was reported in spray-drying of pineapple.[35] Goula & Adamopoulos[31,39] also indicated that a decrease in bulk

density in tomato juice powder and orange juice powder is due to an increased in maltodextrin concentration. Particle sized were found to be larger in the high concentration of maltodextrin. These large particles may be related to the feed viscosity, which considerably increased with maltodextrin.[29] Maltodextrin have low hygroscopicity level which obtained different spray-dried powder hygroscopicity. As a result, a higher concentration of maltodextrin reduces the hygroscopicity of powder.[29,40] Maltodextrin addition in the feed solution did not reduce the powder solubility. This is related to the fact that maltodextrin is very soluble in water.[41]

Several powder properties have been influenced by feed flow rate in the spray-drying process. Bulk density, particle size, and moisture content of the orange spray-dried powder increased with increased feed flow rate.[30] Conversely, the wettability time and insoluble solids of the orange spray-dried powder reported being decreased by increase in the feed flow rate. Tonon et al.[40] suggested a negative effect on the moisture content in the acai juice powder by increased feed flow rate. It can be explained by shorter contact time between particles and heat. Therefore, heat transfer is less efficient in high flow rate and thus caused lower evaporation. The feed flow rate showed a negative effect on process yield.[40] The increase in feed rate resulted in lower process yield probably due to the slower heat and mass transfer in spray-drying.[40] Inlet temperature and feed flow rate also influence particle hygroscopicity. According to Tonon et al.[40], decreased inlet temperature and increased feed flow rate resulted in lower hygroscopicity value in the acai juice powder. The lower hygroscopicity of the powder was opposite to the moisture content, i.e., the lower their capacity to adsorb ambient moisture, which is related the lower water concentration gradient between the product and the surrounding air. The feed flow rate did not influence anthocyanin.

However, anthocyanin was affected by inlet air temperature due to its heat-sensitive compounds. Moreover, according to Bhandari et al.[42] anthocyanin in spray-dried bayberry powder reduced at high temperature and the anthocyanin was more degraded relatively to other phenolic compounds.

1.7 Fruit juice powder and powder properties

Powders are particulate solid state materials containing discreet particles of size ranging from nanometers to millimeters. One gram of powder of the average particle size of 20 microns will contain 10^8 particles.[43] The bulk powder properties are the combined effect of particle properties. Various terms are used to indicate the particulate solids in bulk, such as powder, granules, flour and dust, though all these materials can be treated under the powder category. These common terminologies are based on the size or the source of the materials. For instance, granular have an average size less of than 100 µm.[43]

Fruit juice powders are processed products with functional ingredients, and the most economical advantages compare to the fresh fruit or juice.[44] Fruit juice powders are more stable and have longer shelf life.[44] Moreover, these powders are widely used in many food products such as ready-to-drink beverages, flavouring additives, food colourant, in yogurt, cake, and milk. Most fruit juice powders have an amorphous structure. These powders are more porous, open and disordered. Therefore, molecules of the fruit juice powder are simply influenced by surrounding media such as water vapor and volatile.[43] The amorphous form is a non-equilibrium meta-stable state of materials.[45] The production of fruit juice powder is not easy due to the presence of low molecular weight sugars such as sucrose, glucose, fructose and some organic acids.[37,45] They have high molecular mobility at relatively low temperatures which

confers a sticky nature to the dried juice.[46] Sticky point (T_s) is defined as the value at which a powdery material will start caking.[46] Sticky point temperature can be increased by increasing glass transition temperature (T_g).[46] T_g is the temperature above which hard amorphous solids will transform into soft, rubbery materials due to increase in mobility/decrease of viscosity.[46,47] Stickiness does not occur when higher molecular weight carbohydrates such as maltodextrin and/or gum Arabic are dried.[48] These carbohydrates can increase the T_g of fruit juice. Thus, the hygroscopicity of fruit powders reduces. They are widely used as drying aids.[39,49,50]

Fruit juice powder, like other food powders, have several characteristics based on fundamental, functional, and defective properties. Bhandari[43] suggested that fundamental properties of the powder namely moisture, shape, bulk density, microstructure, size and distribution, glass transition and melting, surface composition and morphology. Flow-ability, compressibility, and reconstitution are examples of functional qualities of the powder. Dustiness, stickiness, caking, and specks are defective properties of the powder. The ingredients and application of powders incorporated with fundamental characteristics are functional.

1.8 Blueberry and anthocyanins

Blueberries are a perennial crop and are categorised under family Ericaceae, genus Vacinuum, and subgenus Cyanococcus. The famous berries which belong to this genus include huckleberries, cranberries, bilberries, and loganberries. In general, blueberries have two primary categories, i.e., wild-growing lowbush blueberries (*Vaccinium augustifolium*) and cultivated highbush blueberries. Three different blueberries include Northern (*V*. *corymbosum*), southern (a hybrid of *V*. *corymbosum*, *V*. *ashei*, *V*. *darrowi* Camp.), and rabbiteye (*V*. *ashei* Reade) fall under highbush type.[51]

The physical properties of blueberries are dark blue skin with creamy white to green flesh and high liquid levels. Moreover, the lowbush blueberries are much smaller (<1 g) than highbush type and are utilised in processed products such as pie, and pastry filling, pancakes, muffin, jams, and sauces.[51]

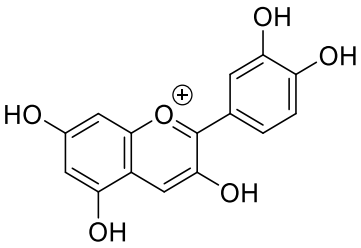
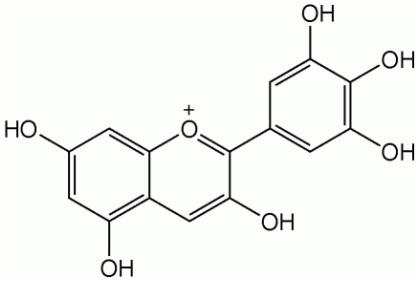
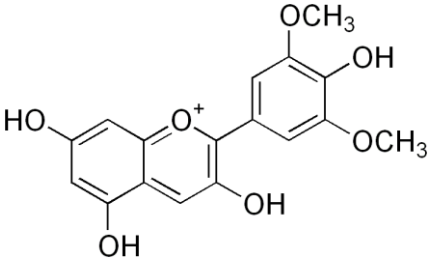
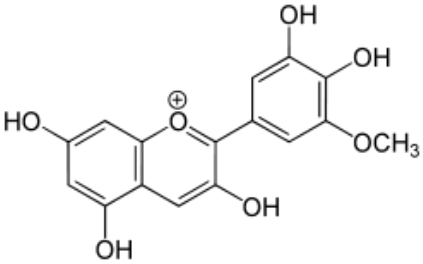
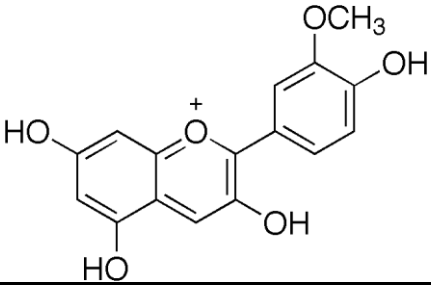
Blueberries have abundant anthocyanins with significant antioxidant activities compared to other fruits and vegetables (Table 1-3). The anthocyanins are glycosidic and acyloglycosidic forms of anthocyanins that are polyhydroxy derivatives of 2-phenylbenzopyrilium (flavilium salts). In general, anthocyanins are connected by three linkages (C_6-C_3-C_6), which are common for flavonoids.[52] Anthocyanins can be further sub-divided into five forms based on their structural composition as shown in Table 1-4 with the colours associated with them.[53] They are cyanidin, delphinidin, malvidin, petunidin, and peonidin.

Table 1-3 Anthocyanins from fruits and vegetables

Food	Anthocyanins (mg 100 g^{-1})
Fruit	
• Apple, Red Delicious, raw	12.3 ± 1.9
• Blackberry, raw	245 ± 68.0
• Blackcurrant, raw	476 ± 115.4
• Blueberry, cultivated, raw	365 ± 56.5
• Blueberry, wild, raw	487
• Cherry, sweet, raw	122 ± 21.3
• Cranberry, raw	144
• Grape, raw	36.7
• Raspberry, raw	390
• Strawberry, raw	21.1
Vegetables	
• Eggplant, raw	85.7
• Cabbage, red, raw	322
• Lettuce, red, leaf, raw	2.2
• Red radish, raw	100

Modified from Wu et al.[54]

Table 1-4 Anthocyanins presents in blueberries, structures, sugar moiety, and colour

Anthocyanin based on	Basic structure	R (sugar moiety)	Colour
Cyanidin		Galactose, Glucose, Arabinose	Orange-red
Delphinidin		Galactose, Glucose, Arabinose	Blue-red
Malvidin		Galactose, Glucose, Arabinose	Blue-red
Petunidin		Galactose, Glucose, Arabinose	Blue-red
Peonidin		Galactose, Glucose, Arabinose	Orange-red

Source: Barnes et al.[53]

Most of the anthocyanins have monosaccharides attached to them such as glucose, galactose, and arabinose.[55] Anthocyanins are also responsible for many different colours. Three factors which effect the anthocyanin colours are the structure, pH and the presence of co-pigments.[56] The primary structure (lower than pH 2.5) obtain red colours, while in the second structure (pH 4-6) exhibits combination between anhydrobases (violet) and pseudobases (colourless). A yellow (chalcone) colour is formed above a pH 8 (Figure 1-8). Anthocyanins are water soluble flavonoids and contribute significantly to the antioxidant. Researchers have reported that anthocyanins have functional effects on human health due to their antioxidant activities may prevent some chronic diseases i.e. cardiovascular diseases, neurogenerative diseases, diabetes, rheumatoid arthritis, cancer, and cataracts.[57,58] Owing to the anthocyanins' positive charge and hydroxyl groups, these compounds can easily donate protons to the free radicals. Possibly this protects cells from oxidative damage that leads to various diseases.[59]

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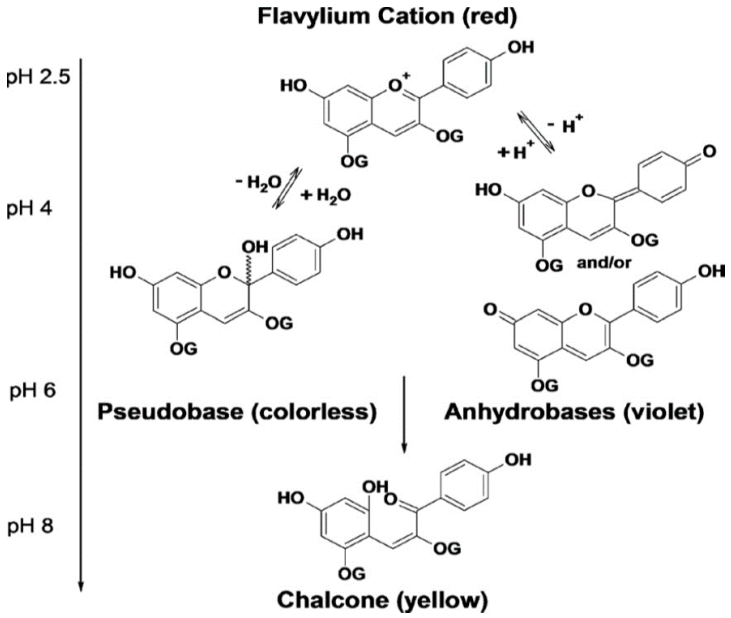

Figure 1-8 Colour variation of anthocyanins in different pH solution (adapted from Barnes et al[53])

1.8.1 Effect of processing on the blueberry anthocyanins content

Processing of blueberries can influence the bioavailability of nutrients, mainly through the changes in anthocyanins. Researchers reported that processing conditions changed anthocyanins in blueberries. Firstly, anthocyanins decrease during processing due to their antioxidant properties and enzyme polyphenol oxidase (PPO) activity. According to Routray et al.[51], the oxidation of phenolic compounds by PPO that form the O-quinones stimulated the degradation of anthocyanins.

Secondly, the anthocyanins can be degraded during the thermal process, temperature above 70^{o}C in particular.[60] However, some studies reported that dehydration process of blueberries in the range 40-60^{o}C has less influence on the total anthocyanins.[61] Furthermore, the thermal degradation generates oxidation reactions and breaks the covalent bond of anthocyanins. Varian processed blueberry products such as cereal blueberry-rich products, juices, jams, and purees are examples of thermally processed products in which anthocyanin loss occurs during dehydration processes. In deterioration during thermal processing, several studies suggest that the most significant parameters were water activity (a_w), moisture content, temperature, O_2, humidity, and light.[58,62]

Anthocyanins can also be affected by freezing conditions and cold storage. Referring to the research of Fracassetti et al.[63], anthocyanin content in blueberries were stable or at least a slight increase in cold storage or high-oxygen atmosphere due to absence/presence of oxygen. They also revealed that freeze-dried blueberry powder could maintain the stability of anthocyanins up to 130 days at 25 ^{o}C. Lastly, acidity also influences anthocyanins during processing of blueberries.

1.9 Protein as foaming and drying agent

1.9.1 Whey protein isolate

Whey is the liquid remaining after milk has been curdled and strained for cheese production, and is composed primarily of β-lactoglobulin (>50% of the total whey protein) and α-lactalbumin, bovine serum albumin (BSA), and immunoglobulins.[64] There are three forms of whey based on the protein content i.e. whey isolate, whey concentrate, and whey hydrolysate. The highest of pure protein content is whey protein isolate. Whey protein is one of the emulsifiers frequently used in the food industry including processed meats, bakery products, pasta, ice cream, and infant food.[64] The main reason for its wide application in foods is its ability to provide the formation and stabilization of oil-in-water emulsions.[65] The ability of whey protein to form stable emulsions depends on emulsion composition (including pH, and mineral content, salt, sugar, surfactant, and polysaccharide contents) and environmental condition (temperature and pressure).[66,67]

According to Akhtar & Dickinson,[67] a significant characteristic of protein in food products is foaming. The interfacial behavior of protein reflects physical interactions and are influenced by the composition and conformation of the protein at the air-water interface. An understanding of protein interaction during foam formation is necessary to optimise their preparation and use in processing. They also suggested that the foaming properties of whey protein are significantly correlated with β-lactoglobulin content and with the extent of whey protein denaturation. Studies on whey protein isolate (WPI) as a foaming agent and drying additives have been reported.[68–71] WPI affected the powder recovery more than 50% on high sugar spray dried powder.[36,42] The remarkable increase in recovery from the addition of WPI was attributed to the

combination of surface-active properties of proteins because they preferentially migrate to the surface of the droplets/particles, and form a glassy state film upon drying, which can resist heat stress during the drying process. Fang et al.[72] reported that dairy protein such as WPI was more effective than plant protein in spray drying sucrose, as they can lower the surface tension of solution before drying, resulting in higher powder recoveries with more coverage on the particle surface.

1.9.2 β-lactoglobulin

β-lactoglobulin is a major protein in bovine milk, representing about 50% of total whey protein and 12% of the total protein milk. It was among the first proteins to be crystallised, and is a typical globular protein, studied extensively and very well characterized. The molecular mass of β-lactoglobulin is 18.3 kDa, stabilized by two internal disulfide cross-links, and gives good foaming properties.[73] β-lactoglobulin contains 162 amino acids with cysteine, methionine, histidine, lysine, threonine, arginine, proline, tryptophan, tyrosine, and phenylalanine being the most potential amino acids considered to be oxidized.[74] Tryptophan, threonine, methionine, and histidine were proved to have oxidative tendency due to their hydrogen–donating ability. Cysteine in the position of 121 has mild antioxidant potency due to the only free sulfhydryl group in the protein, which can act as hydrogen donor.[74]

β-lactoglobulin, as a natural member of lipocalin family, exhibits a strong binding affinity for various hydrophobic and amphiphilic ligands, including fatty acids, retinol, β-carotene, phospholipids, vitamin D, folic acid, and phenolic compounds.[75] The lipocalin family consists of small secreted proteins that typically bind and transfer small hydrophobic ligands and interact with sell-surface receptors.[75] Around neutral pH, β-

lactoglobulin is mainly dimeric, whereas between pH 2 and 3, it is associated into monomers due to electrostatic repulsion between subunits.[76] The crystal structure shows that the 162-amino acid-long single peptide chain of β-lactoglobulin forms a calyx composed of an eight-stranded antiparallel β-sheet. Although the interaction between whey proteins and anthocyanin not been widely studied, several studies have reported that milk proteins (including β-lactoglobulin) could protect anthocyanins from degradation.[36,77–79]

1.9.3 Bovine serum albumin (BSA)

Serum albumin has a potential as drug carrier protein in blood plasma and an important role in the transportation and distribution of endogenous and exogenous substances. Bovine serum albumin (BSA) is similar to human serum albumin (HSA) in tertiary structure with high sequence homology of 76.52%.[80] BSA protein samples are highly pure, cheap and easily obtained. Normal bovine milk contains a low level of blood serum albumin (0.1-0.4 g/L; 0.3-1.0% of total N), presumably as a result of leakage from blood. BSA is quite a large molecule (molecular mass c. 66 kDa; 582 amino acids); its amino acid sequence is known.[81]

The maximum foam stability of BSA (Isoelectric point pI = 4.9) has been reported in the pH range 5-6, illustrating the effect of electrostatic repulsive forces on the extent of molecular adsorption and packing in the film and influencing film elasticity. The mechanical strength of BSA films in the pI range results in more stable foams. BSA utilised for foam preparation showed maximum surface viscosity, film elasticity, and surface yield stress in the pH range 5-6. BSA gave maximum foam stability in the pH

range 5-6 demonstrating the relationship between film properties and foaming properties.[82]

1.9.4 Foam capacity

The property of proteins to form stable foams is important in the production of a variety foods.[82] Food foams are usually very complex systems, including a mixture of gases, liquids, solids, and surfactants. Proteins in foams contribute to the uniform distribution of fine air cells in the structure of foods. Body and smoothness of food foams are related to the formation of air bubbles that allow volatilisation of flavours with enhanced palatability of the foods.[82] The foaming properties of proteins are influenced by the source of protein, methods, and thermal parameters of processing, including protein isolation, temperature, pH, protein concentration, mixing time, the method of foaming. Among many the factors influencing foaming capacity of proteins, the type of foaming equipment and method of agitation are important. Speed of whipping is important to foam properties and consumer acceptance.[82]

1.9.5 Foam stability

Foam stability requires the specific properties of protein films as formation of cohesive, viscous, elastic, continuous, air-impermeable film around each gas bubble.[82] Foams with high stability are prepared with high-molecular-weight globular proteins that produced thick adsorbed films. Foam stability is influenced by the self-healing ability of proteins, i.e., their ability to move from an area of low interfacial tension to a region of high interfacial tension (Marangoni effect). Drainage of water from foam can be reduced if polar side chains of protein polypeptides interact with molecules of water within the lamella. Foam stability is affected by the film thickness, mechanical strength,

protein-protein interaction and environmental factors such as pH and temperature. Higher stability was found for thicker films because of mechanical strength and better textural properties. The resistance of protein foam to coalescence and collapse of air bubble is determined by ability of the protein to foam a multimolecular matrix.[82]

1.10 Sugars as foam stabiliser and drying agent

1.10.1 Maltodextrin

Maltodextrins are hydrolysis products of starches with Dextrose Equivalent (DE) lower than 20 (for DE > 20 the term syrup solids or dextrins is used).[64] They represent a mixture of saccharides with a broad molecular weight distribution between polysaccharides and oligosaccharides and are available as white powders mostly or concentrated solutions.[64] In contrast to native starches, the maltodextrins are soluble in water. As a digestion product of starch, the maltodextrins contain linear and branched amylose and amylopectin degradation products the size of which extends from oligomers to macromolecules. In the sol state, these molecules are hydrated and expanded, and the extended helical regions are interrupted by short, disordered regions. Therefore, maltodextrins have a significant portion of average chain length long enough to form thermally reversible gels.[83]

Variations in DE values results in maltodextrins with varying physicochemical properties. Hygroscopicity, solubility, osmolality, and their effectiveness to reduce the freezing point increase with increasing DE, while viscosity, cohesiveness, and coarse crystal prevention increase as DE decreases.[64] Maltodextrins have the ability to form gels and retain water, and therefore are used in the food industry as a texture modifier, either for gelation, retention of water, and to a certain extent substitution of fat. They

perform multifaceted functions in food systems, including (1) bulking, (2) providing resistance to caking, (3) adding texture and body, (4) forming films, (5) binding flavour and fat, (6) serving as oxygen barriers, (7) giving surface sheen, (8) aiding dispersibility and solubility, (9) freezing control and preventing crystallization, and (10) as product extenders.[83] Maltodextrin (MD) is a low-cost, bland component ideal for the formulation of the carrier matrix. Also, the high glass transition temperature (T_g) of low DE MD provides good physical stability to the dried powders.

1.10.2 Trehalose

Trehalose, as well as sucrose, is a non-reducing disaccharide with the same chemical formula ($C_{12}O_{11}H_{22}$) but slightly different structure.[84] The sucrose molecule is composed of one fructose and one glucose ring, while trehalose is composed of two glucose rings, and it is one of the most stable known sugars, whose glycosidic bond has a low free energy of activation (K_0 of 119 sec^{-1} as opposed to K_0 of 4.44 x 10^4 sec^{-1} for sucrose), making the trehalose structure very stable regarding hydrolysis. Both disaccharides have been studied extensively and one of the most promising saccharide cryoprotectants, trehalose, has only recently received more attention.[85–87] Some mechanisms have been suggested for an explanation of trehalose and sucrose effectiveness. These include their ability to form glasses, to mimic the hydrogen bonding character of water, to increase the surface tension of the bulk solvent, to prevent thermotropic phase separations in lipid bilayers and to prevent the fusion of membranes.[84]

It is well known that the glass transition temperature (T_g) of trehalose is much higher than that of sucrose[88,89] in the anhydrous state the T_g of trehalose and sucrose may

be considered to be about 105–115 ^{0}C and 60–62 ^{0}C respectively. Trehalose is a non-reducing sugar that has a high chemical stability under low pH conditions. Therefore, it does not react with amino acids or proteins by Maillard browning reaction.[84,86,90] Trehalose also has a low cariogenic potential when compared to sucrose and moderate glycemic index with low insulinemic response.[88]

Trehalose is an alternative to sucrose in the dehydration of fruit juices as it shows a relatively higher T_g. Komes et al.[84,90] observed an improvement in aroma retention in dehydrated strawberry and apricot purees in the presence of trehalose compared to sucrose. Galmarini et al.[88] observed that strawberry puree presented better sensory properties when dried with the addition of trehalose in comparison with sucrose and maltodextrin. Stability is greatly influenced by the water sorption behavior, the glass transition, and the molecular mobility characteristics of the powder. Such data can be used for selecting appropriate storage conditions and packaging systems that optimise retention of flavour, colour, nutrients, bioactive components, and physical stability.

Chapter 2

Chapter 2 General Materials and Methodology

2.1 Blueberry juice and drying additives

Concentrated organic blueberry juice was purchased from a local supermarket in Leeds, England (Figure 2-1). The blueberry juice was labelled as not containing additional water, sugar, additives and preservatives. The juice was stored at 4 ^{0}C after opening. Maltodextrin (MD) (Sigma-Aldrich, USA) 16.5-19.5 dextrose equivalent (DE) (PubChem CID: 107526), trehalose, whey protein isolate (WPI) (Fonterra, NZ), bovine serum albumin (BSA) and β-lactoglobulin (Sigma-Aldrich, USA) were employed as a foam stabiliser and foaming agent. Physicochemical properties of organic blueberry juice are shown in Table 2-1.

Figure 2-1 Organic pure blueberry juice

Table 2-1 Physicochemical properties of organic blueberry juice

Properties	Amount
Moisture content (%)	89.78 ± 0.08
Total soluble solids (g kg^{-1})	10.23 ± 0.8
pH	2.36 ± 0.01
Density (g cm^{-3})	1.031 ± 0.0
Total phenolic content (mg GAE g^{-1} solids)	47.45 ± 0.02
Total monomeric anthocyanins (mg Cyn3Gl g^{-1} solids)	8.92 ± 0.03
Anthocyanidins (mg g^{-1} solids):	
Delphinidin-3-glucoside	1.38 ± 0.06
Cyanidin-3-glucoside	1.33 ± 0.07
Malvidin-3-glucoside	1.0 ± 0.08
Colour values:	
L*/brightness	0.08
a*/redness	0.45
b*/yellowness	0.06

2.2 Chemical and solvents

The chemicals Gallic acid, sodium carbonate and Folin-Ciocalteu's phenol reagent were purchased from Sigma-Aldrich (USA). Delphinidin-3-glucoside, cyanidin-3-glucoside, and malvidin-3-glucoside were purchased from Extrasynthese (Genay Cedex, France). Acetonitrile and methanol (HPLC grade) were purchased from VWR Intl. Chemical (France). Polyphenolic standards were dissolved in methanol (Sigma-Aldrich, UK) and working solutions were prepared daily. All other solution preparations were made using ultra-pure water produced by a Milli-Q Plus system (Millipore Corporate, MA, USA).

2.3 Foam capacity: density and overrun

Foam capacity was measured according to the method of Sadahira et al.[91], with slight modifications. A 250 mL measuring cylinder was carefully filled up with the foam. The weight of foam was recorded and then the foam density and overrun were calculated as follows:

ρ *(g cm^{-3}) = mf / volume of foam* Eq. 1

Overrun (%) = 100 (mi-mf) / mf Eq. 2

Where *mi* is the mass of the initial solution (unwhipped sample), and *mf* is the mass of the whipped sample with the same volume of *mi*.

2.4 Foam stability: drainage

Foam drainage was conducted according to the methods of Raharitsifa et al.[16] with slight modifications. A Buchner funnel was filled to the top with 50 mL of each foam. Liquid drained by gravity from the foam was collected in a 50 mL graduated cylinder. The volume V (mL) of liquid drained was measured directly from the graduated cylinder as a function of time over 120 min.

2.5 Product yield determination

The product yield was determined according to Shi et al.[70] with slight modification. The yield was defined as the ratio of the mass of solids powder obtained at the end of the foam-mat freeze-drying and spray-drying period, to the mass of initial substances, including MD and WPI, based on dry mass content, i.e.

$$\textbf{Powder yield} = 100 \text{ x} \frac{\textbf{Solids in powder}}{\textbf{total solids in foam or feed solution}} \quad \text{Eq. 3}$$

2.6 Moisture content and water activity (a_w) determination

The moisture content of foam-mat freeze-dried (FMFD) and spray-dried (SD) powders were measured via an HB 42-S halogen moisture analyzer (Mettler Toledo, UK) at 105 ^{0}C. The empty sample panhandler and the heating module were closed. Approximately 1 g of powder was placed in the sample pan. The drying time ranged from 2-5 minutes for each sample.

The water activity of the powders was determined by a Hygrolab C1 water activity meter (Rotronic, UK). Approximately 0.5 g of powder was placed into the sample holder. All measurements were performed in duplicate on samples immediately after they had been obtained via the drying process.

2.7 Solubility

Solubility is often considered to be the key determinant of reconstitution quality in the powder dissolution process. Most physical functionalities of food powders are based on whether they can completely dissolve to form homogeneous solution since any undissolved components could lead to solid losses and present a problem in the downstream processing.[92]

Solubility was determined as described by Ceballos et al.[25] A sample (1 g) of dried powder was gently dispersed into 100 ml of distilled water (at 30 ^{0}C) in a beaker via a Stuart CB-162 magnetic stirrer (Bibby Scientific Ltd, UK) at 400 rpm. After stirring for 5 min, the dispersions were transferred into a 50 mL Falcon tube and centrifuged at 3,000 rpm using a Universal 320 bench top centrifuge (Sartorius, UK), for 10 min. An

aliquot of 25 ml of the supernatant was removed to a pre-weighed Petri dish and immediately oven dried at 105 ^{0}C for 5 h. The supernatant was decanted and determined for water solubility using the following equation:

$$\textbf{Water solubility} = \textbf{100 x} \frac{\textbf{weight of dissolved solids in supernatant}}{\textbf{weight of sample}} \quad \text{Eq. 4}$$

2.8 Rehydration time

Rehydration of blueberry powder was determined according to the method described by Islam et al.[93] with slight modifications. For measurement, 0.5 g of powder was added to 50 mL distilled water at 26 ^{0}C in a 100 mL glass beaker. The mixture was agitated using a Stuart CB-162 magnetic stirrer (Bibby Scientific Ltd, UK) at 900 rpm until particles invisible. The time required in second for the powder to be completely rehydrated was recorded.

2.9 Bulk density

The bulk density of the powder is the mass of the powder divided by its bulk volume, and this can vary depending on how compact the powder is. A tapping machine is commonly used to measure bulk density. This consists of pouring a specified mass of powder into a graduated cylinder. The poured bulk density equals the powder mass divided by the measured poured volume. The cylinder then tapped for a specified number of taps, which moves the cylinder up and down and causes the powder to form a more compact structure, thus reducing the volume occupied by the powder. At the end of tapping, the volume is measured, and the tapped bulk density is evaluated.[94]

The bulk density of the powders was determined by gently adding 1 g of blueberry powder into an empty 10 mL graduated cylinder and holding the cylinder on an FB 15012 top mix vibrator (Fischer Scientific, UK) at 2,000 rpm for 1 min. The DB was calculated by dividing the mass of the powder by the volume occupied in the cylinder.[70]

2.10 Scanning electron microscopy (SEM)

To date, scanning electron microscopy achieves resolutions of 1 nm using high-resolution imaging instrument and about 3 nm using conventional instruments. The resolution which can be attained strongly depends on the sample. Foodstuffs need to be covered with a layer of electrically conductive substances (e.g. metal or carbon) to prevent charging effects during imaging. Furthermore, to avoid damaging the specimen, the electron beam has to be set accordingly.[95]

Figure 2-2 Cressington 108 putter coater (A) and FEI scanning electron microscope (SEM) (B)

The microstructural characteristics of powders were analysed by a Quanta 200 F (FEI, Oregon, USA) scanning microscope electron (SEM) (Figure 2-2 B).The samples were placed on an aluminium support using a double-sided adhesive tape containing conductive carbon and then coated with platinum using a Cressington 108 sputter coater (Cressington Scientific Instruments, UK) (Figure 2-2 A)The images were taken at an acceleration voltage of 3.00 kV.[96]

2.11 Colour analysis

The colour of foods has been measured in the CIELAB colour space. CIELAB colour space is an international standard for colour measurements, adopted by the Commission Internationale d'Eclairage (CIE) in 1976 (Figure 2-3).[25] The CIE system is achromatic space in rectangular coordinates (L*, a*, b*) together with another space that is defined in cylindrical coordinates (L*, H*, C*). The coordinate L*, represents brightness, and it is the difference between white (L* = 100) and black (L* = 0). The coordinate a* represents the difference between green (-a*) and red (+a*), while the coordinate b* represents the difference between blue (-b*) and yellow (+b*). In many cases, it is important to compare the colour of different samples to each other, or in relation to a standard colour. Thus, the colour difference between samples is defined as ΔE or total colour difference (TCD).[97]

To locate the colour of a sample in the CIELAB colour space, cylindrical coordinates can also be used. The coordinates are brightness (L*), chroma (saturation) (C*), and hue (H*). L* is defined as rectangular coordinate, while chroma represents the perpendicular distance from the axis of brightness to the considered point, and hue is the angle expressed in degrees determined by the straight line that bounds the origin

coordinate with the chromatic point and the axis +a. Hue is 0^0 for the axis +a*, 90^0 for the axis +b*, 180^0 for the axis –a*, 270^0 for the axis –b*, and $360^0 = 0$, when returning to the axis +a*.[97]

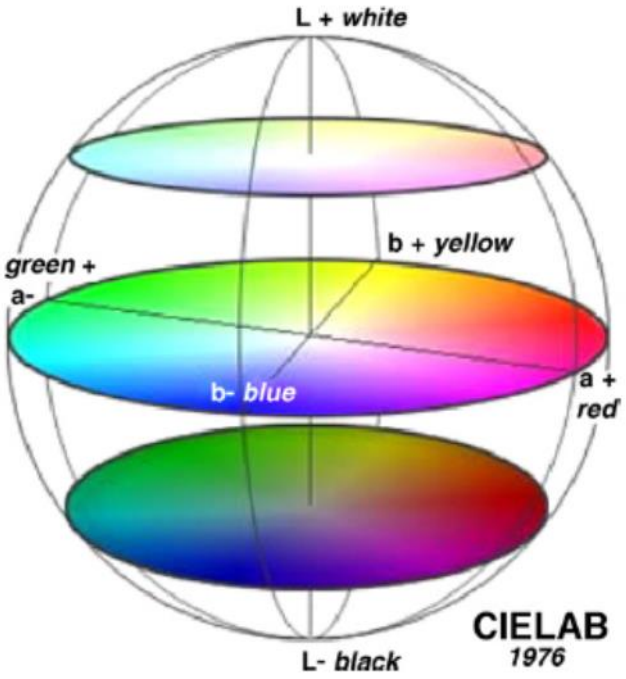

Figure 2-3 Framework of CIELAB colour model (adapted from Quek et al.[38])

The colour of samples was determined by the method described by Franceschinis et al.[98] and Mahdavee Khazaei et al.[99] The colour of blueberry FMFD and SD reconstituted solutions were examined via a Colour-Eye 7000A instrument (Macbeth, Cheshire, UK) (Figure 2-4) with illuminant D65 and 10^0 observer angle. 0.5 of powder was dissolved completely in 50 mL distilled water. The results were expressed as CIE colour values L*, a*, b* where L* was used to denote brightness, a* redness and greenness and b* yellowness and blueness. The indices of:

Hue angle:	$H^0 = tan^{-1}(b^*/a^*)$	Eq. 5
Chroma:	$C = a^*+b^*$	Eq. 6
Total colour differences:	$TCD = [(L_0 - L)^2 + (a_0 + a)^2 + (b_0 - b)^2)]^{1/2}$	Eq. 7

Where L_0, a_0 and b_0 were the L*, a* and b* of the standard and L, a, and b are the corresponding values the dissolved powder, were calculated and the mean of three replicates was reported.

Figure 2-4 Macbeth Color-eye 7000A colourimeter

2.12 Total phenolic content (TPC) analysed by Folin-Ciocalteu's method

The Folin's assay measures the total reducing capacity of the sample, and the principle behind the assay is that alkaline conditions, phenolate anions reduce the yellow molybdenum in the Folin-Ciocalteau reagent to blue colour.[100] Analysis of total phenolic content was done for both originally concentrated blueberry juice and reconstituted blueberry powders. Polyphenols in plant extracts react with the Folin-Ciocalteau's reagent to form the blue complex that can be quantified by visible-light spectrophotometry.[101]

The total phenolic content of blueberry juice and reconstituted solution was determined using Folin-Ciocalteau's method.[102] The assay contained 1 mL of concentrated blueberry juice or reconstituted blueberry powder diluted with 80% methanol solution (1:10), 5 mL of diluted Folin-Ciocalteau's phenol reagent (1:10), and 4 mL of 75 g L^{-1} sodium carbonate solution. The mixture was then kept in a water bath at 25 ^{0}C, and the absorbance reading measured at 765 nm with a spectrophotometer after 2 h. The estimation of phenolic content was performed using Gallic acid as standard.

2.13 Analysis of anthocyanins content

2.13.1 pH differential methods of total monomeric anthocyanin

Pure blueberry juice (i.e., the first juice to be freeze dried and spray dried), foam-mat freeze-dried (FMFD) and spray-dried (SD) powders were examined for monomeric anthocyanin according to a pH differential method.[103,104]. 1 mL blueberry juice was placed into a 50 mL Falcon tube and diluted using pure water to 20 mL. The blueberry juice was then filtered using a Whatman No. 4 filter paper. 4 ml of supernatant was

removed and split between two 15 mL Falcon tubes (i.e., 2 mL in each tube). 8 mL of a pH 1.0 solution containing 0.025 M KCl was added to one tube and 8 mL of a pH 4.5 solution containing 0.4 M sodium acetate to the other. The colour of the pH 1.0 solution was typically a deeper red than that of the pH 4.5 solution, due to usual pH-dependence of anthocyanins.

The blueberry powders were reconstituted by weighing 0.5 g of FMFD and SD blueberry powders into 50 mL of distilled water in a 100 mL beaker, stirred using a Stuart CB-162 magnetic stirrer (Bibby Scientific Ltd, UK) at 400 rpm, for 3 min. The solution was then filtered using Whatman No. 4 filter paper. The pH was measured before adding 9 mL of the above pH 1.0 and pH 4.5 solutions to 1 mL of the reconstituted powder solutions.

The absorbance (*A*) of the pH 1.0 and 4.5 solutions was determined at both 520 nm and 700 nm using a 6715 UV /VIS spectrophotometer (Jenway, UK). A 10 mm path length glass cuvette was used and the diluted test portions were read versus a blank cell filled with distilled water. The anthocyanin pigment concentration was then calculated as cyanidin-3-glucoside equivalents, as follows:

$$\textit{Total monomeric anthocyanins (mg } L^{-1}) = (A \times MW \times DF \times 1000) / (\varepsilon \times l) \qquad \text{Eq. 8}$$

Where *A* = (*A* at 520 nm – *A* at 700 nm) at pH 1.0 – (*A* at 520 nm – *A* at 700 nm) at pH 4.5; *MW* (molecular weight) = 449.2 g mol^{-1} for cyanidin-3-glucoside; *DF* = dilution factor; *l* = path length in cm; ε = 26 900 molar extinction coefficient (mol^{-1} cm^{-1}) for cyanidin-3-glucoside; and the factor 1000 = is required for conversion from g to mg.

2.13.2 High-performance liquid chromatography analysis (HPLC)

Preparation of anthocyanin was performed both for concentrated blueberry juice and reconstituted blueberry powder. For blueberry juice, 1 mL of concentrated blueberry juice was dissolved with pure water to 10 mL in a 15 ml Falcon tube. For blueberry powder, 0.5 g of blueberry powder was dissolved with pure water to 25 mL, using a magnetic stirrer for 3 min, and transferred to a 50 mL Falcon tube. Both Falcon tubes, 15 ml and 50 mL, were then centrifuged (3000 G; 10 min) filtered through a Whatman no.1 filter paper and used for the analysis. The preparation was repeated in duplicate.

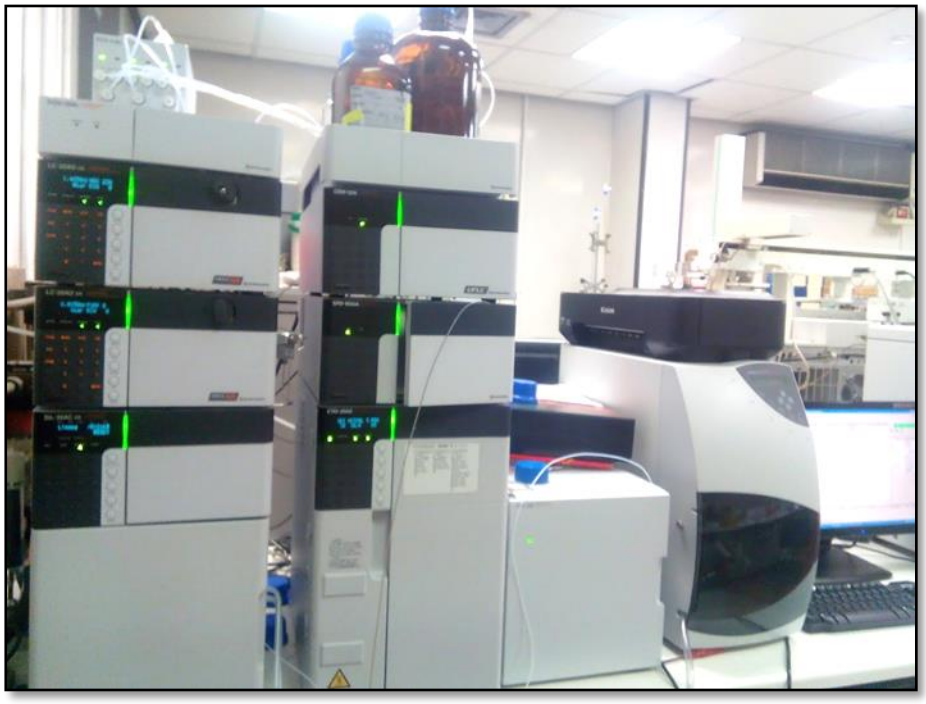

Figure 2-5 Shimadzu high-performance liquid chromatography (HPLC)

HPLC analysis was conducted according to Ifie et al.[102] HPLC identification and quantification of phenolic in blueberry juice and reconstituted powders were carried out using a UFLCXR system (Shimadzu) (Figure 2-5). It consists of a binary pump, a

photodiode array with multiple wavelengths (SPD-20A), a Solvent Delivery Module (LC-20AD) coupled with an online unit degasser (DGU-20A3/A5) and a thermostat auto sampler/injector unit (SIL-20A). The photodiode array detector was set to measure at a wavelength of 520 nm.

A two-phase gradient system consisting of 0.1 % (v/v) trifluoroacetic acid mobile phase (A) and trifluoroacetic acid/acetonitrile/water (50:49.9:0.1) mobile phase (B) was employed for the analysis. The gradient conditions were as follows: the initial state started with 92 % A and was increased to 18 % solvent B at 3.50 min, 32 % B at 18 min, 60 % B at 28 min, reaching 100 % B at 32 min, held at 100 % B for 4 min, and returning to the initial conditions for 3.5 min for the next analysis. The chromatographic separation was performed on a Phenomenex Gemini C18 column (5 µm, 250 mm x 4.6 mm) at a flow rate of 1 mL/min. The temperature of the column was maintained at 35 °C, and the injection volume was 10 µL. Identification of anthocyanins in blueberry juice and reconstituted powders was made based on comparison with standard phenolic compounds run under similar conditions regarding the retention time, UV-visible spectrum, spiking of the sample with the corresponding standard phenolic compound. The identification of anthocyanins in blueberry juice and reconstituted solution was done based on comparison with anthocyanins standards, including delphinidin-3-glucoside, cyanidin-3-glucoside, and malvidin-3-glucoside, run under similar conditions regarding the retention time, UV-visible spectrum, spiking of the sample with the corresponding standards. With the use of the system software, the similarity index (SI) was used to match each anthocyanin.

2.13.3 Linearity of HPLC method for quantification of anthocyanin in blueberry juice and reconstituted powder

Firstly, a stock solution of anthocyanins standards, e.g. Delphinidin-3-Glucoside (Del3Gl), Cyanidin-3-Glucoside (Cyn3Gl), and Malvidin-3-Glucoside (Mal3Gl) were used to prepare different concentrations of anthocyanins ranging from 50 to 500 mg L^{-1}. The correlation coefficient of the calibration curve used for estimating the quantity of the anthocyanin ranged from 0.990 to 0.996 (Figure 2-6, Figure 2-7, and Figure 2-8). In order to quantify the concentration of anthocyanins resulting from the blueberry juice and reconstituted powder, the peak area (similar retention time to the specific standard) was substituted to the linear equation as y value. After that, the x value was calculated as the concentration of anthocyanin in mg L^{-1}.

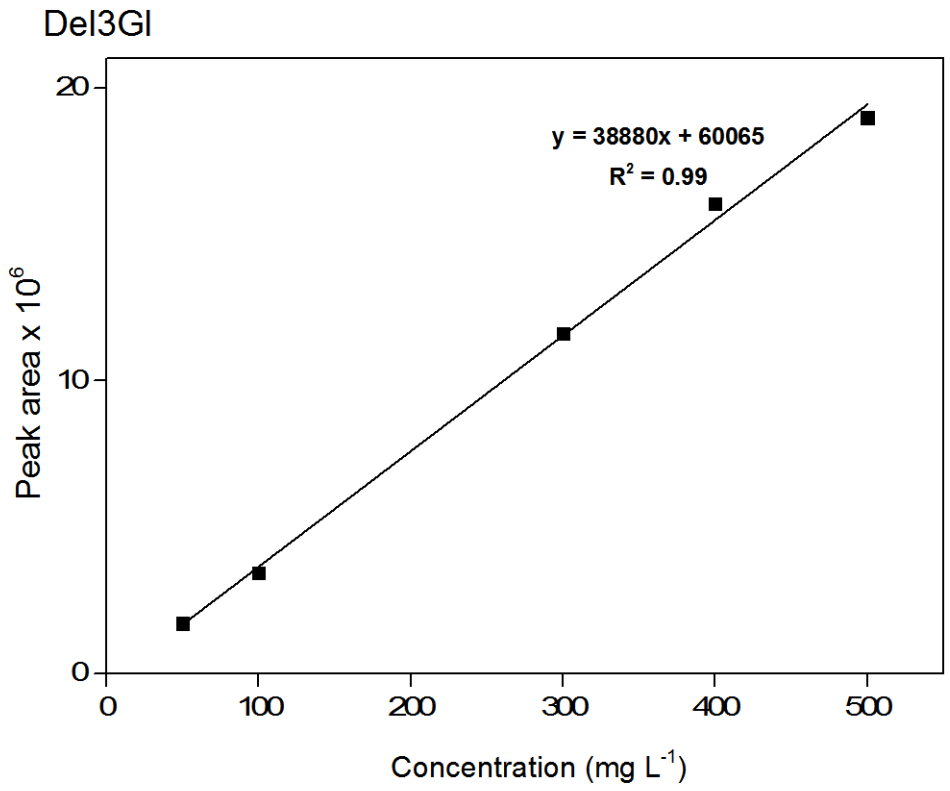

Figure 2-6 Calibration curve of Delphinidin-3-Glucoside

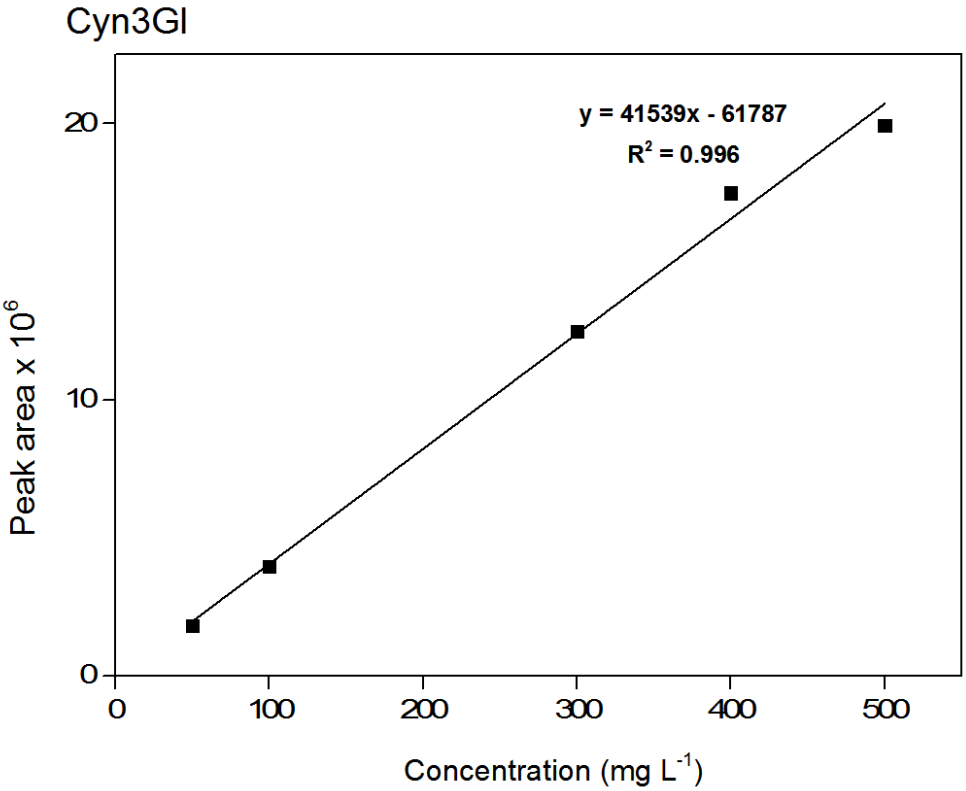

Figure 2-7 Calibration curve of Cyanidin-3-Glucoside

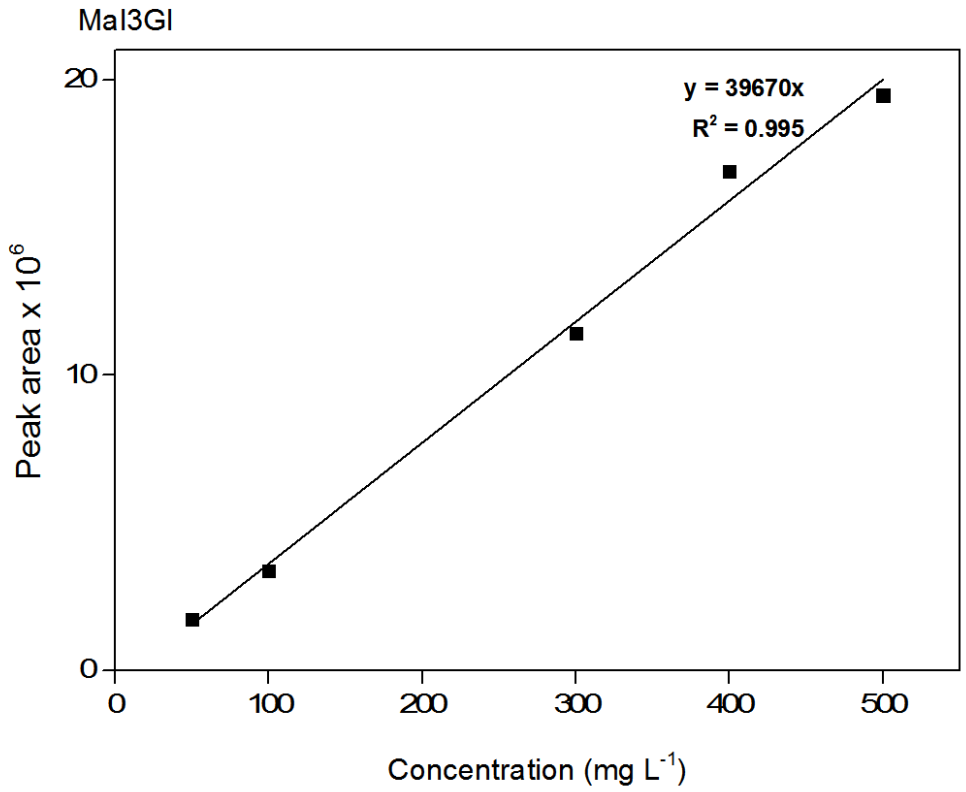

Figure 2-8 Calibration curve of Malvidin-3-Glucoside

2.13.4 Determination of anthocyanins and total phenolic content (TPC) retention

TPC and ACN retention after drying was calculated according to Fang & Bhandari[42] using the following formulas (expressed as dry matter):

TPC retention (%) = 100 x (TPC in blueberry powder / TPC in blueberry juice) Eq. 9

Anthocyanins retention (%) = 100 x (ACN in blueberry powder / ACN in blueberry juice) Eq. 10

2.14 Experimental design and statistical analysis

Randomised completely block (RCB) design with two replications was utilised in foam-mat freeze-drying and spray-drying experiment (see section 3.3 and 5.3). Analysis of variance (ANOVA) and was done to establish the presence or absence of significant differences between means. Multiple comparisons were performed using the Tukey test, and significance level was set at $p < 0.05$. All statistical analyses were carried out using Minitab 17.0.

Chapter 3

Chapter 3 Effect of maltodextrin to whey protein isolate ratios on the physicochemical properties of foam-mat freeze-dried blueberry powder and reconstituted products

3.1 Introduction

The focus of the present study was the foam-mat freeze-drying of blueberry juice, which was examined by using different ratios of maltodextrin (MD) to whey protein isolate (WPI). In this study, MD and WPI were used as a foam stabiliser and foaming agent, respectively. Maltodextrins are commonly used as bulk agents, carriers, texture modifiers, fat replacer, in the formulation of fruit leathers, and for the encapsulation of a wide range of products.[105] Whey protein has been employed as a foaming agent in foam-mat dried banana pulp.[8] Whey protein is also commonly used as wall material in drying processes particularly in sugar-rich fruit juices due to its film-forming property upon drying for overcoming the surface stickiness of sugar/protein solution.[36,70] The utilisation of MD and WPI in the foam-mat freeze-drying process is essential due to the foam obtained can increase the surface area available for drying, hence reducing the drying times and temperatures.[1,2] The foam produced also helps to lessen the loss of flavour and volatile components.[4,9]

MD and WPI addition in blueberry juice initiate a complexation of polysaccharide-protein with anthocyanin, which can retard or prevent anthocyanin degradation during drying.[77] Furthermore, complex formation between anthocyanin and other compounds, often leads to an improvement in colour stability.[56] Franceschinis et al.[96] showed that the freeze-dried blackberry juice with MD exhibited higher retention of bioactive compounds in comparison to the spray-dried product. Chung at al.[77] documented high

retention of anthocyanins and colour stability in beverage systems by application WPI and heat-denaturated WPI. As explained in section 1.8.1, anthocyanin stability is affected by factors such as pH, temperature, light, oxygen, and the presence of proteins and minerals. Foam-mat freeze-drying with MD and WPI is a promising approach for encapsulating blueberry extracts as it uses low temperatures. By using freeze-drying process, anthocyanins are dispersed and embedded throughout the polymeric matrix.[105] However, the biggest problem of foam-mat freeze-drying is the instability of the foam, which can occur both during the foaming and drying steps,[1] i.e., a collapse of porous structure occurs[8]. Hence, investigating a foam stabiliser and foaming agent concentration is critical.

To the best of our knowledge, little is known about the production of blueberry powder via foam-mat freeze-drying and optimisation of MD to WPI ratio in the blueberry juice before freeze-drying. Thus, the objectives of this present study were: (1) to develop blueberry foam-mat freeze-dried powders with different ratios of MD and WPI; and (2) investigate the effect of MD/WPI ratios on the physicochemical properties of the blueberry powders and reconstituted products.

3.2 Foam-mat freeze-drying conditions

Foam-mat freeze-drying of blueberry juice is presented in Figure 3-1. Foamed blueberry juice was prepared by whipping blueberry juice + matrices (weight ratio juice to matrices = 95:5) using a Kenwood KM 330 series mixer (Kenwood, UK) with 8 L stainless beaker, at maximum speed for 5 min and ambient temperature. The total solids fraction for all prepared foams for freeze-drying was fixed at 50 g kg^{-1}. Matrices with MD/WPI ratios are presented in Table 3-1. 85 g of the foam produced was spread

onto a round Teflon-coated pan (diameter = 180 mm, height = 30 mm) for each formulation. The foams were blast frozen using a Valera BF051ET blast freezer (Valera, Italy) at -30 ^{0}C with the average air circulation velocity at 8 m s^{-1} for 6 h, and freeze-dried using an Alpha 1-4LD Plus freeze dryer (Christ Martin, Germany) at -55 ^{0}C and vacuum pressure 0.04 mbar (Figure 3-2 and Figure 3-3), for 24 h. The dried layer obtained was then ground for 1 min using a Kenwood CH 180A mini chopper food processor (Kenwood, UK). The blueberry powders produced were stored in pre-weighed airtight dark aluminium foil and stored in the fridge at 5 ^{0}C for further analysis. The foam mat freeze-drying was conducted in duplicate.

Table 3-1 Maltodextrin (MD) and whey protein isolate (WPI) ratios used in foam-mat freeze-drying of blueberry juice

MD/WPI	Maltodextrin (% w/w)	Whey protein isolate (% w/w)	*TSS of juice + matrices (% w/w)
0.4	1.3	3.7	15
1.0	2.5	2.5	15
1.6	3.1	1.9	15
2.3	3.5	1.5	15
2.8	3.7	1.3	15
3.2	3.8	1.2	15
5.3	4.2	0.8	15
7.3	4.4	0.6	15
9	4.5	0.5	15

*Total soluble solids

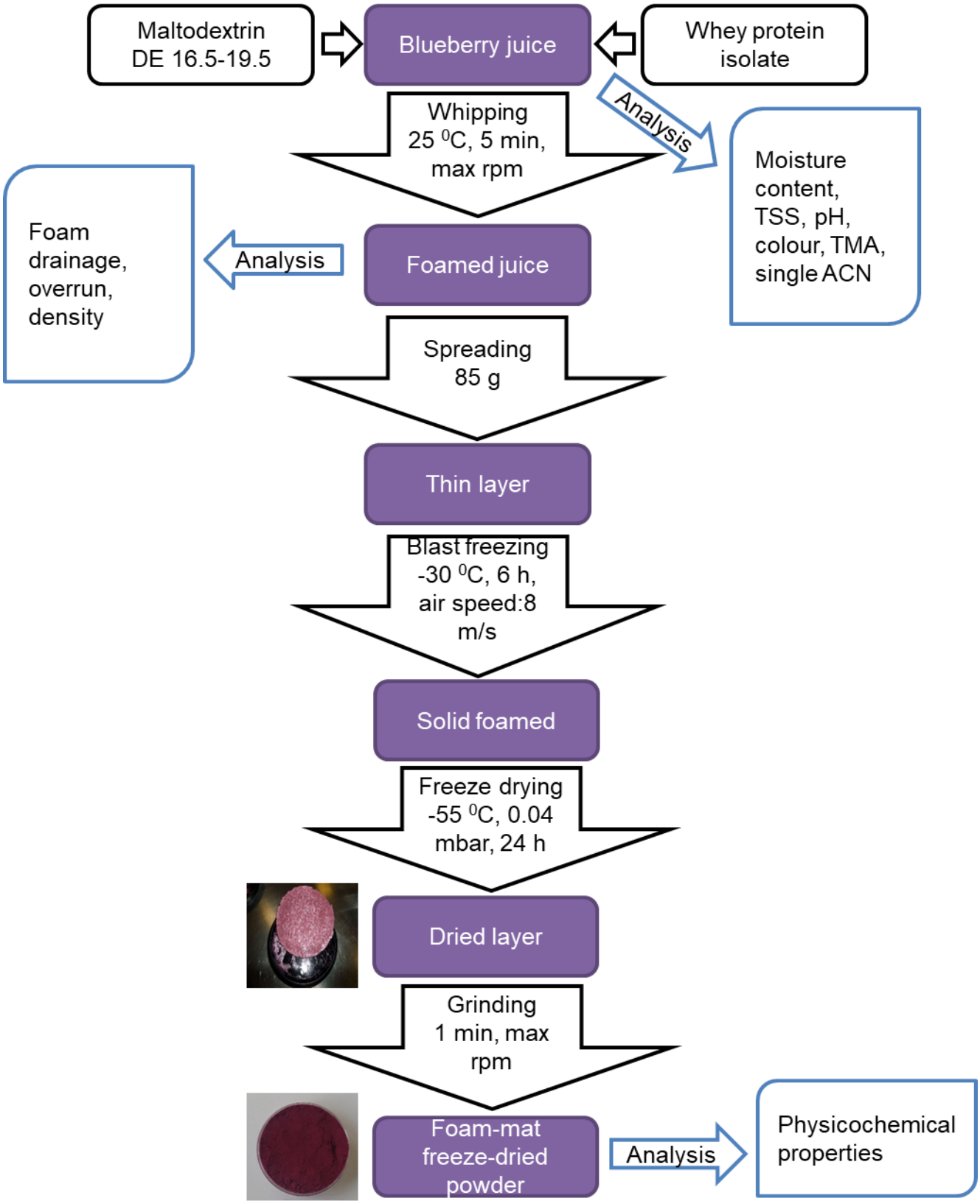

Figure 3-1 Foam-mat freeze-drying of blueberry juice with addition of maltodextrin (MD) and whey protein isolate (WPI)

Figure 3-2 Martin Christ freeze dryer Alpha 1-4LD Plus (Germany)

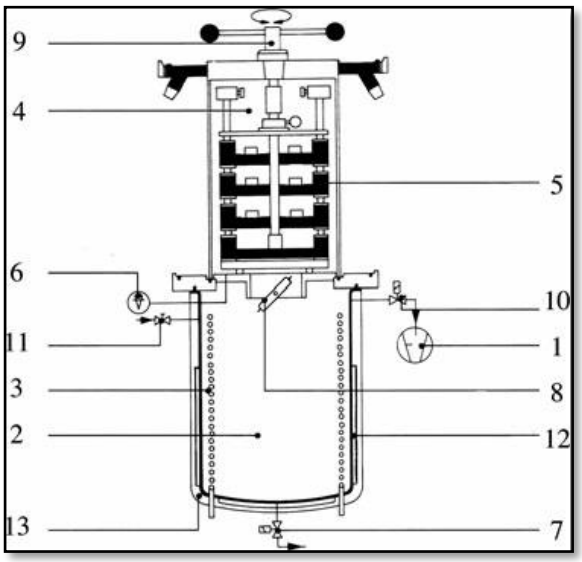

Figure 3-3 Freeze-drying equipment scheme (Acevedo et al.[106])

1) vacuum pump, 2) ice condenser chamber, 3) ice condenser,
4) drying chamber, 5) electrically heated shelves, 6) vacuum metre,
7) drain valve for defrosted water, 8) motorised intermediate valve,
9) sealing device, 10) pressure control valve, 11) micro aeration valve,
12) defrosting device, 13) insulation

3.3 Statistical analysis

The processing treatments were duplicated, and the means of the results ± range were reported. One-way analysis of variance (ANOVA) was done to establish the presence or absence of significant differences between means. Multiple comparisons were performed using the Tukey test, and significance level was set at $p < 0.05$. All statistical analyses were carried out using Minitab 17.0.

3.4 Results and Discussion

3.4.1 Physicochemical properties of blueberry juice

Physicochemical properties of blueberry juice were examined before dehydration. The blueberry juice had 89.78 ± 0.08% moisture content, 10.23 ± 0.8 g kg^{-1} total soluble solids, 2.36 ± 0.01 pH and 1.031 ± 0.0 g mL^{-1} density. The parameter colour of blueberry juice was L* (brightness) = 0.08, a* (redness) = 0.45 and b* (yellowness) = 0.06. Total monomeric anthocyanin (TMA) and total phenolic content (TPC) of blueberry juice were 8.92 ± 0.03 mg Cyn3Gl g^{-1} (solids) and 47.45 ± 0.02 mg GAE g^{-1} (solids), respectively. Individual anthocyanins contained in the blueberry juice were 1.38 ± 0.06 mg g^{-1} (solids) delphinidin-3-glucoside (Del3Gl), 1.33 ± 0.07 cyanidin-3-glucoside mg g^{-1} (solids) (Cyn3Gl), and 1.0 ± 0.08 mg g^{-1} (solids) malvidin-3-glucoside (Mal3Gl).

3.4.2 Foam capacity: density and overrun

The foamed blueberry was evaluated for foam density and overrun. Figure 3-4 shows the effect of carrier agents on the density (ρ) and overrun of foamed blueberry. As the MD/WPI was increased the overrun increased considerably, i.e., the volume fraction

of air was higher, and the density was lower.[5,91] The overrun measured ranged from 585 to 825%, whereas the foam density ranged from 0.14 to 0.16 g cm^{-3}. The overrun of foamed blueberry reached a maximum value at the MD/WPI = 1.6, i.e., the overrun was 825% and foam density 0.14 g cm^{-3}. Higher additions of MD seemed to produce the opposite effect: beyond MD/WPI = 6 there was a substantial decrease in overrun, i.e., the reduction in foam stability. This result agrees with Karim & Wai[5] who found that the overrun of foamed star fruit reduced with an increase of methylcellulose greater than 40 g kg^{-1}. Such reductions are undoubtedly due to increased viscosity of the mixture, which at a certain level makes it harder to incorporate air.

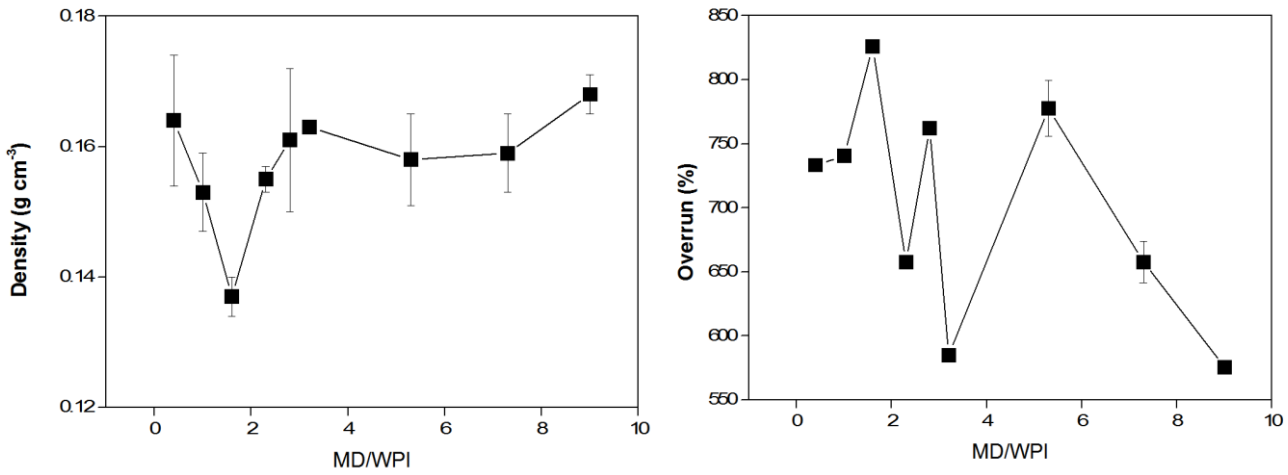

Figure 3-4 Density and overrun of foamed blueberry as a function of the mass ratio of maltodextrin to whey protein isolate (MD/WPI). Results are expressed as means ± range of duplicate determinations.

Abbasi and Azizpour[6] demonstrated the reduction of foamed sour cherry density with the increase in the concentration of egg white. This phenomenon was caused by the movement of the foaming agents from the aqueous phase to the air-liquid interface,

i.e., reduces surface tension. Thus, this mechanism leads to an increase in foaming ability and a reduction in the density.[5]

3.4.3 Foam stability: drainage

One way to determine the stability of foam is to measure the rate at which the liquid drains from it. All liquid foams are subject to drainage of the liquid from between the bubbles caused by the action of gravity.[16] Drainage is accompanied by a progressive thinning of the lamellae and may, therefore, enhance the probability of film collapse [5] The stable structure of the foam is necessary for rapid drying and ease of the removal of the dry material from trays. If the structure of foam is significantly destroyed, the drying time increases and the quality of the end product decreases.[6] Figure 3-5 shows the volume of liquid drained from the foams over 120 min for the same range of MD/WPI ratios as in Figure 3-4. The inset illustrates the result of a cut through these smooth curves at 60 min: volume drained after 60 min is plotted as function of MD concentration [MD]. From [MD] = 1.3 to 3.1 wt.% (MD/WPI ratio 0.4 to 1.6) the drainage volume decreases slightly, indicating greater foam stability, i.e., water holding capacity MD/WPI ratio, but beyond these concentrations the drainage starts to increase again. This is in agreement with the overrun results: at the higher MD/WPI ratios, foam stability decreases again. Although the aqueous phase may be more viscous at higher MD/WPI ratios, which would be expected to decrease drainage rates, the lower overrun means that the average film thickness between the bubbles will be thicker and the overall film surface area smaller, so drainage is faster. For these reasons, subsequent foam-mat freeze-drying experiments were therefore not conducted for MD/WPI ratios > 3.2.

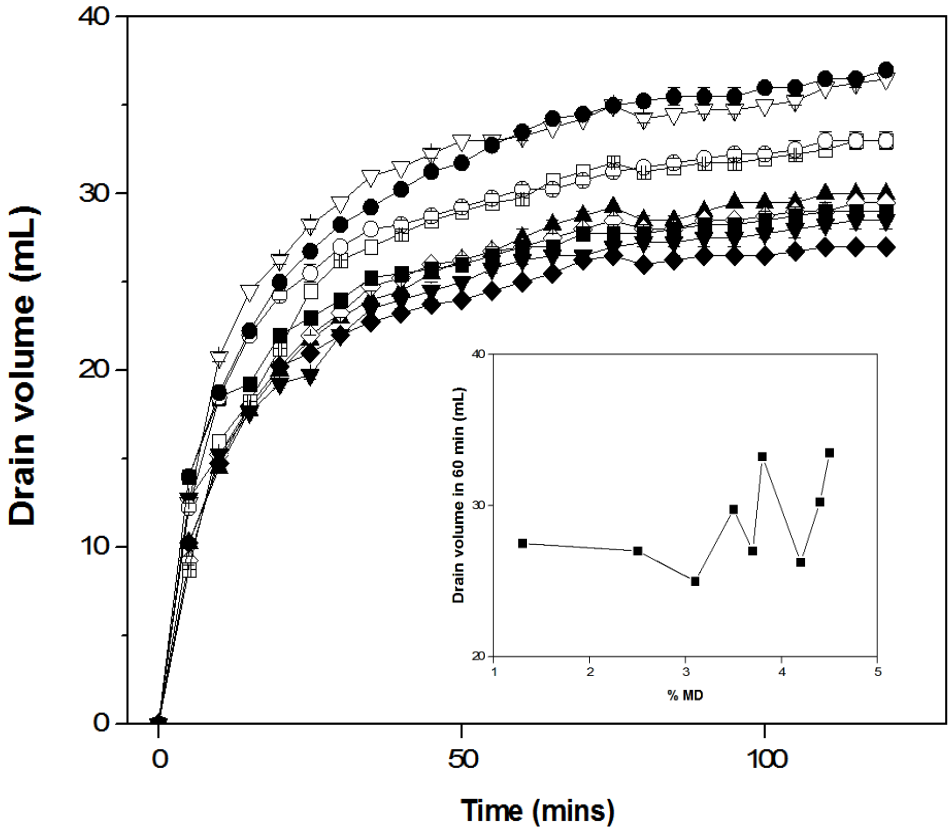

Figure 3-5 Drained liquid volume versus time of foamed blueberry juice at different mass ratios of maltodextrin to whey protein isolate: 9.0 (●), 7.3 (○), 5.3 (▼), 3.2 (▽), 2.8 (■), 2.3 (□), 1.6 (◆), 1.0 (◇), 0.4 (▲). The inset shows a cut of the drained volume after 60 min as a function of % maltodextrin (MD). Results are expressed as means ± range of duplicate determinations.

This phenomenon is similar to the observation by Muthukumaran et al.[4], who found that egg foam stability was determined by the addition of foam stabiliser (e.g. propylene glycol alginate 0.5, 0.75, and 1%). At 1% propylene glycol alginate, the drainage was lower compared to 0.75 and 0.5%. Besides, the egg foam without stabiliser obtained the highest drained liquid (foam was not stable).

3.4.4 Powder yield

The yield was calculated as the ratio mass of solids collected to the solids mass in the foam blueberry juice on a dry weight basis. Figure 3-6 presents powder yield of foam-mat freeze drying of blueberry juice containing MD and WPI. ANOVA showed that MD/WPI ratios had a significant effect (P-value = 0.000) on the powder yield. It is observed that the foam-mat freeze-drying process yielded a maximum of 79.3% at MD/WPI = 2.8, while at MD/WPI = 3.2 the powder yield decreased to a minimum, i.e., 72.4%. From the MD/WPI ratio = 0.4 to 2.3, the powder yield slightly fell by 75.3 to 74.3%. Afterward, the yield was highly increased at the MD/WPI ratio = 2.8, then dropped < 74% at the MD/WPI = 3.2. The yield was then increased until > 78% at the MD/WPI ratio 7.3 and 9.0. The losses measured for all the foam-mat freeze-dried samples was less than 30%.

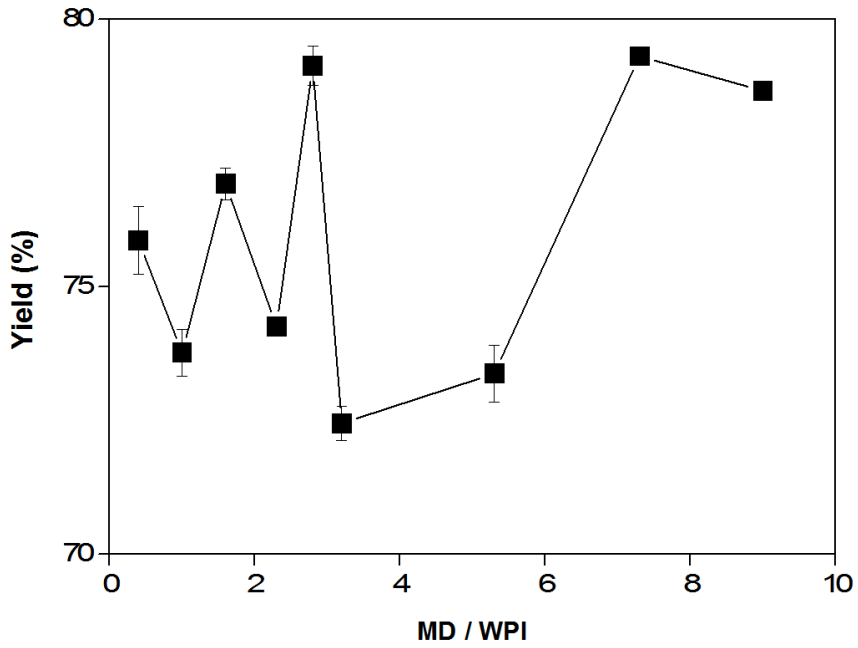

Figure 3-6 Yield of foam-mat freeze-dried powder as a function of the mass ratio of maltodextrin and whey protein isolate (MD/WPI) Results are expressed as means ± range of duplicate determinations.

This result indicates that the addition of MD and WPI to blueberry juice gave good powder recovery. Wilkowska et al.[107] recovered 78% of blueberry juice powder by using freeze-dried microencapsulation with the aid of β-cyclodextrins. Also, the authors recorded higher powder yield for the freeze-drying sample compared to the one using the spray-drying method with maltodextrin as a carrier agent, i.e., 44%. This low yield in spray-dried samples is related to the fact that only partial powder recovery achieved, while the rest remained on the wall of dryer and cyclone. With WPI, several studies suggested that addition of small amount of protein, such as WPI, in spray-drying of sugar-rich fruit juice helps to retard the stickiness problem by migrating the protein to the surface and forming glass film during drying.[36,70,108] Thus, the powder yield gained > 50%. Another study revealed that MD + WPI gave excellent HCA (hydroxy citric acid) recovery of microencapsulated *Garcinia* fruit powder, typically 90%.[109]

3.4.5 Moisture content and water activity

The important physical properties of food powder are moisture content and water activity (a_w). The type of food powder has low moisture content with the small value of a_w. These properties related to the microbial, chemical, and kinetic reaction of compounds in the food matrix. The current experimental work of foam-mat freeze-drying obtained blueberry powder in various level of moisture content and a_w. The foam-mat freeze-dried blueberry powder had moisture content range from 2.6 to 4.3% (Figure 3-7). This finding is consistent with that of Wilkowska et al.[107] who reported the moisture content of blueberry powder prepared by freeze-drying was 3.9%. However, Franceschinis et al.[96] found higher moisture content, i.e., 6%, in the freeze-dried blackberry powder. There was no significant effect (P-value = 0.309) of the MD/WPI ratios on the moisture content of foam-mat freeze-dried powders. The addition of MD

and WPI slightly changed the moisture content. This may be attributed to the fixed total soluble solids (TSS) in the original blueberry slurry before foam-mat freeze-drying. As the MD concentration increased, the moisture content of foam-mat freeze-dried powder decreased. The lowest moisture content was at the MD/WPI ratio = 3.2, i.e. 2.6%, while at the MD/WPI ratio = 0.4, the highest moisture content, i.e., 4.3%, was recorded.

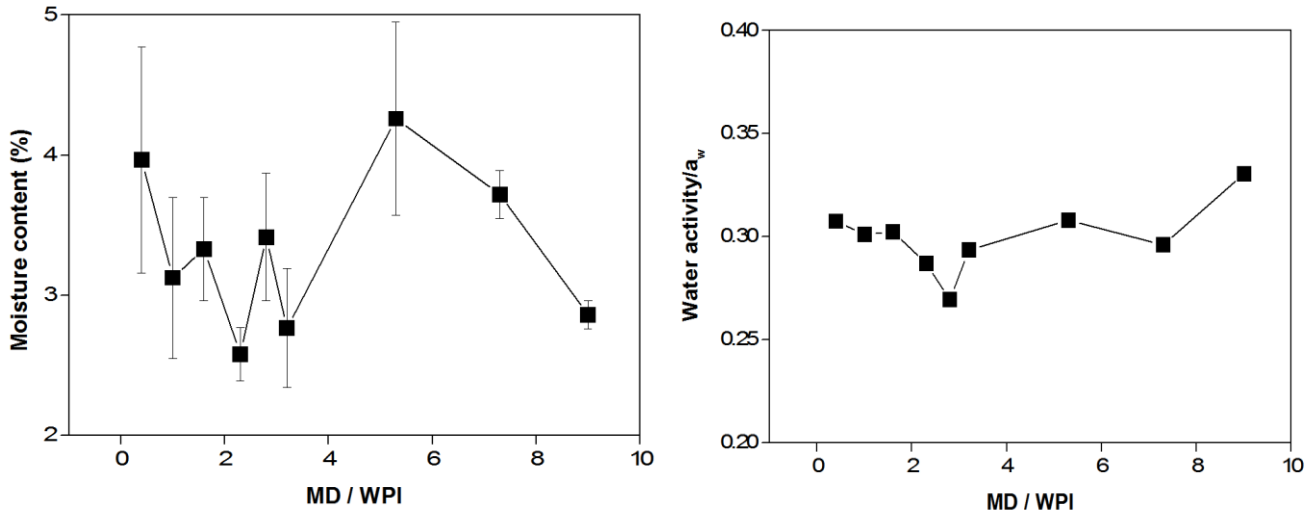

Figure 3-7 Moisture content and water activity of foam-mat freeze-dried powder as a function of the mass ratio of maltodextrin and whey protein isolate (MD/WPI). Results are expressed as means ± range of duplicate determinations.

The addition of drying additives, such as MD and WPI, into the juice with high acid and low molecular sugars (fructose and sucrose) content aimed to increase the glass transition temperature during drying. Increasing the glass transition temperature avoids collapse and stickiness problems of powders.[37,70] Furthermore, WPI aids to generate the foam while mixing and whipping processes before freeze-drying. The small bubbles

help to trap air during whipping and increase the surface area of drying. Thus, from our study, the moisture contents of powder were low, below 4.0%, giving food powders an excellent predicted stability.

As observed in Figure 3-7, there was a significant effect (P-value = 0.000) of the MD/WPI ratios on the water activity (a_w) of foam-mat freeze-dried samples. The water activity of the foam-mat freeze dried has a minimum of 0.28 and a maximum of 0.33, at the MD/WPI ratio = 3.2 and 9.0, respectively. Interestingly, from our study, all samples of foam-mat freeze-dried powder had water activity ≤ 0.45, which was acceptable for such fruit powder regarding inhibition of microbial growth and biochemical degradation.[70]

3.4.6 Solubility, rehydration time, and bulk density

Solubility is defined as the percentage of powder that completely dissolves in water. The solubility had a minimum of 95%, and a maximum of 99%, for foam-mat freeze-dried powder at the MD/WPI = 0.4 and 3.2, respectively (Figure 3-8). These results reflect those of Cano-Chauca et al.[41] who also found solubility of spray-dried mango powder using maltodextrin was above 90%. In the present study, the MD/WPI ratio had no significant effect (P-value = 0.831) on the powder solubility. This may be because MD and WPI are so soluble in water.[25,41] Therefore, the incorporation of MD and WPI with the blueberry juice made the foam-mat freeze-dried powder easier to rehydrate or dissolve in water. Ceballos et al.[25] reported that the integration of MD influenced the solubility of freeze-dried soursop fruit powder due to superior solubility in water. In our study, the solubility was similar for all the samples, attributable to the fixed total soluble solids (TSS) of foam after adding MD and WPI i.e. 50 g kg^{-1}.

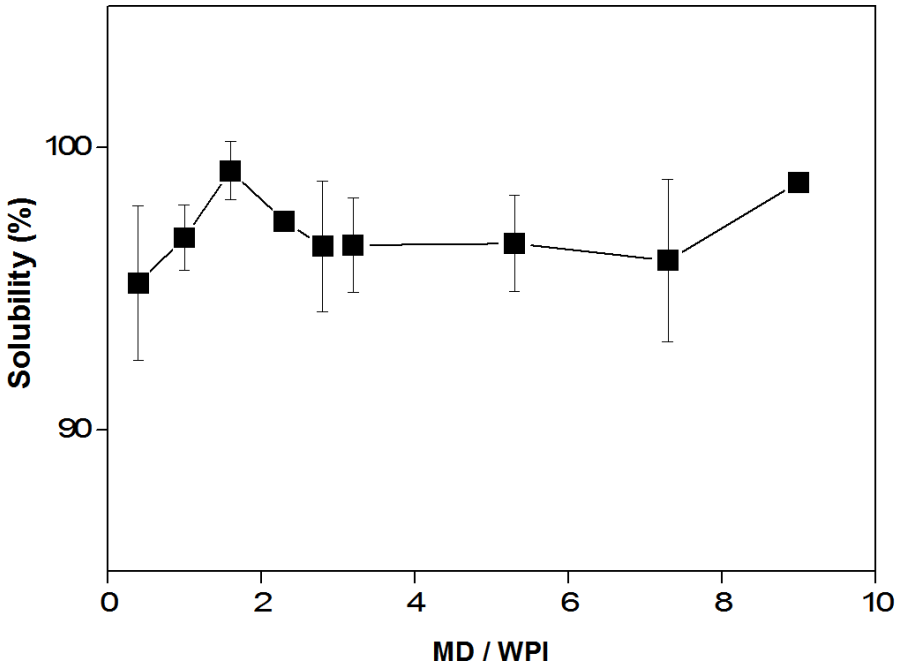

Figure 3-8 Solubility of foam-mat freeze-dried powder as a function of the mass ratio of maltodextrin and whey protein isolate (MD/WPI). Results are expressed as means ± range of duplicate determinations.

According to Figure 3-9, it is indicated that the MD/WPI ratio had a significant (P-value = 0.000) effect on the rehydration time. The quickest rehydration time was observed at the MD/WPI ratio = 3.2 and 9, i.e., 74 s. This may be due to the low moisture content of the powder as it reported in section 3.4.5. Conversely, the powder with the MD/WPI ratio = 2.8 and 5.3 had slow rehydration time, i.e., 90 s. The present results showed faster rehydration time compared to vacuum spray-dried orange powder using different maltodextrin concentrations which were in a range of 122-234 s.[93] The effect of maltodextrin on powder rehydration depends on its effect on powder moisture content. Goula & Adamopoulos[39] suggested that lower moisture content of the orange powder seems to be associated with faster rehydration due to reduced stickiness. On the other hand, the reduction in powder rehydration time was not found with increased

maltodextrin concentration, although it increases its moisture content. Concerning WPI, it affected the rehydration time of the powder. As explained in section 3.4.2 (foam capacity), WPI generating more foam at higher concentration. The more foam in the blueberry juice led to more small bubbles and more air trapped. This phenomenon agreed with that of Muthukumaran et al.[9] and Raharitsifa et al.[16] Therefore; the foam-mat freeze-drying produced a powder with low moisture content.

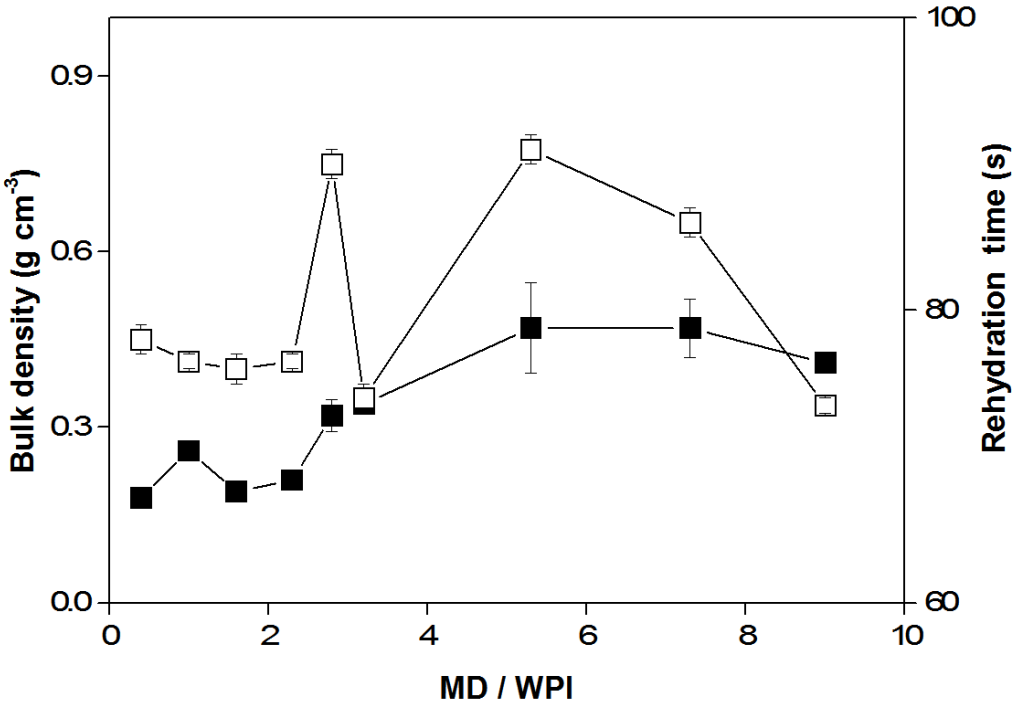

Figure 3-9 Bulk density (■**)** and rehydration time (□) of foam-mat freeze-dried powder as a function of the mass ratio of maltodextrin and whey protein isolate (MD/WPI). Results are expressed as means ± range of duplicate determinations.

According to Figure 3-9, the average bulk density of the foam-mat freeze-dried powder produced with different MD/WPI ratios varied from 0.18 to 0.47 g cm^{-3}. The MD/WPI ratio was significantly (P-value = 0.000) affected the bulk density of the powder. Bulk

density values of the foam-mat freeze-dried powders were lower than the observed values for freeze-dried blackberry powders.[96] There was no difference ($P > 0.05$) in bulk densities of the powder with the MD/WPI range of 0.4-3.2 (0.18-0.34 g cm^{-3}). Beyond these ratios, the bulk densities increased significantly (0.42-0.47 g cm^{-3}). This may be attributed to the proportion of the MD lower compared to WPI, although the total soluble solids were constant, i.e., 50 g kg^{-1}. According to Fazaeli et al.[110], bulk density decreased with an increase of maltodextrin concentration. They ascribed this behaviour to the fact that maltodextrin reduces the sticking of particles, and also an increase in the volume of air trapped in the particles due to maltodextrin is a skin-forming material. Several authors observed an inverse relation between bulk density and solubility.[31,110] In our study, it is noted that almost 100% solubility was obtained in all cases. However, some differences were observed in bulk density.

3.4.7 Particle morphology

In the SEM analysis, the foam-mat-freeze-dried samples with the MD/WPI ratio = 0.4 and 3.2 were chosen as examples. The SEM was conducted to identify the particles using 5000 x magnification. Particles of foam-mat freeze-dried powders are shown in

Figure 3-10. In general, the foam-mat freeze-dried powder particles were irregular in shape and had large size. The foam-mat freeze-dried particles at the MD/WPI ratio = 0.4 had similar appearance with those at 3.2. It is observed that most of the particles resembled broken glass or flake-like structures, probably due to their origin as bubble surface films, broken up via the food processor. A similar observation was shown in the particles of freeze-dried blackberry powder.[96] Besides, the moisture content of the

powders (see section 3.4.5) supports this result as no significant (P > 0.05) difference moisture content between samples at the MD/WPI ratio = 0.4 and 3.2

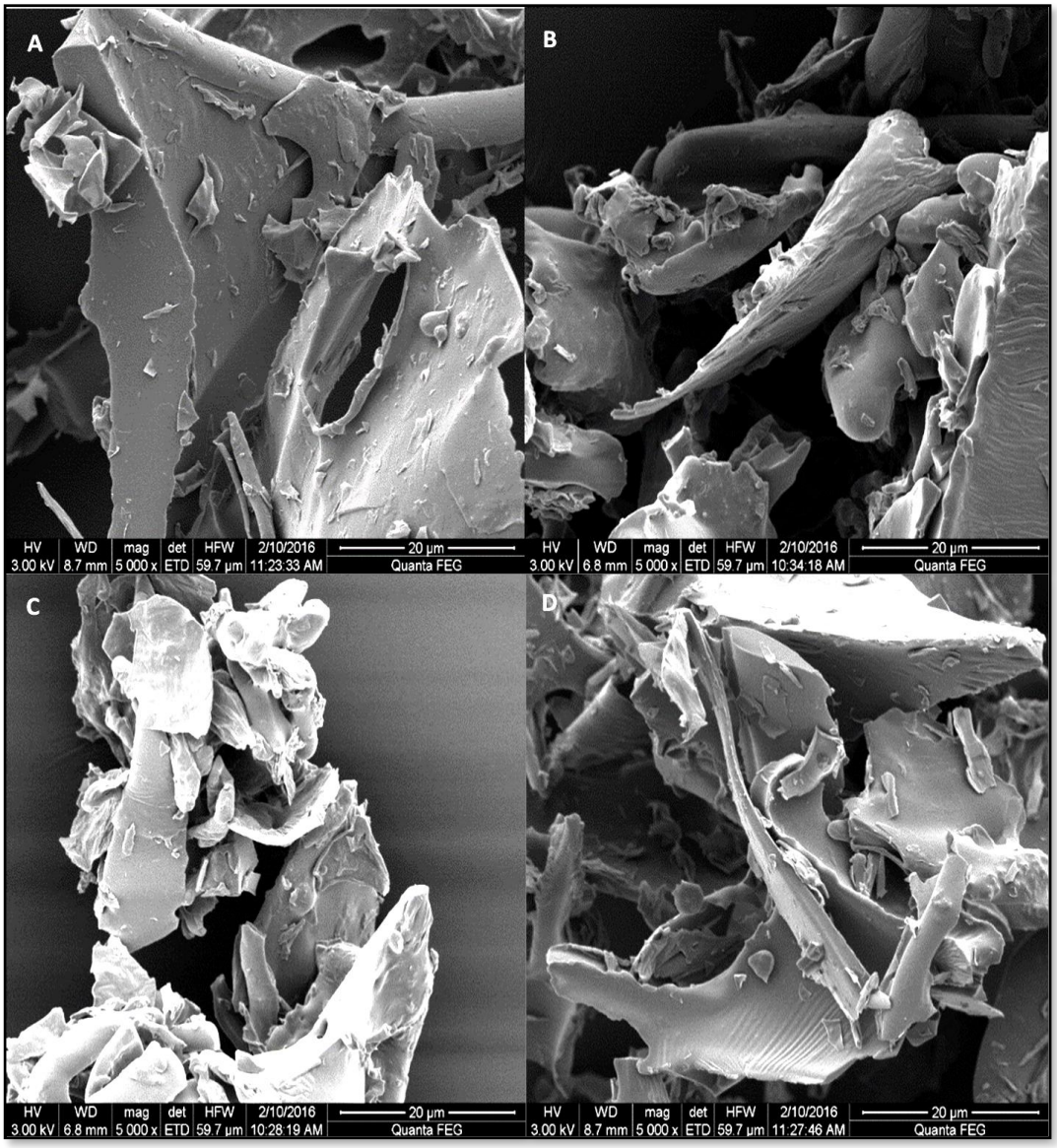

Figure 3-10 SEM micrograph (5000 x magnification) of foam-mat freeze-dried blueberry powder produced with the MD/WPI = 0.4 (A-B) and 3.2 (C-D)

The utilisation of matrices (MD and WPI) in the foam-mat freeze-drying process aimed to generate and stabilise the foam in the blueberry juice.[1,4] The air trapped by whipping in the presence of matrices can increase the surface area of drying and obtain the porous structure.[3] The porous structure presented low moisture content of powder. These physical characteristics are markedly important for food powder products.

3.4.8 Colour properties

Colour changes of the blueberry juice and foam-mat freeze-dried powder was determined using a colourimeter. Colour values include L* (brightness), a* (redness) and b* (yellowness). The original blueberry juice had 0.08, 0.45, and 0.06, for L*, a*, and b* values, respectively. The blueberry juice indicated dark purple with a small concentration of red and yellow. Photographs of the foam-mat freeze-dried powder are shown in Figure 3-11. The samples powder were then dissolved in water, filtered and centrifuged to obtain a clear solution for colour measurement.

The L* (brightness) value of reconstituted solution presented in Figure 3-12. The MD/WPI ratios had a significant effect (P-value = 0.000) on the L* values of the reconstituted solutions. The increasing of the MD/WPI ratios resulted in the decreased L* value of the reconstituted solution. The L* was a minimum of 39.8 obtained at the MD/WPI ratio = 9.0 and a maximum of 44.6 at the MD/WPI ratio = 0.4. This value may be related to the colour of MD, which is a white flour in the dry state.[64] The fixed total soluble solids used in the foamed juice, i.e., 50 g kg^{-1}, had small influence on the L* values. The L* values of foam-mat freeze-dried reconstituted solutions were greater than the blueberry juice. This phenomenon was probably attributed to the dark purple and concentrated solution observed from the blueberry juice. The L* of reconstituted

solutions were low values over the dehydrated sweet cherry products, i.e., L* = 53.2,[98] and were similar with the freeze-dried blueberry microsphere, i.e., L* = 40.6.[111]

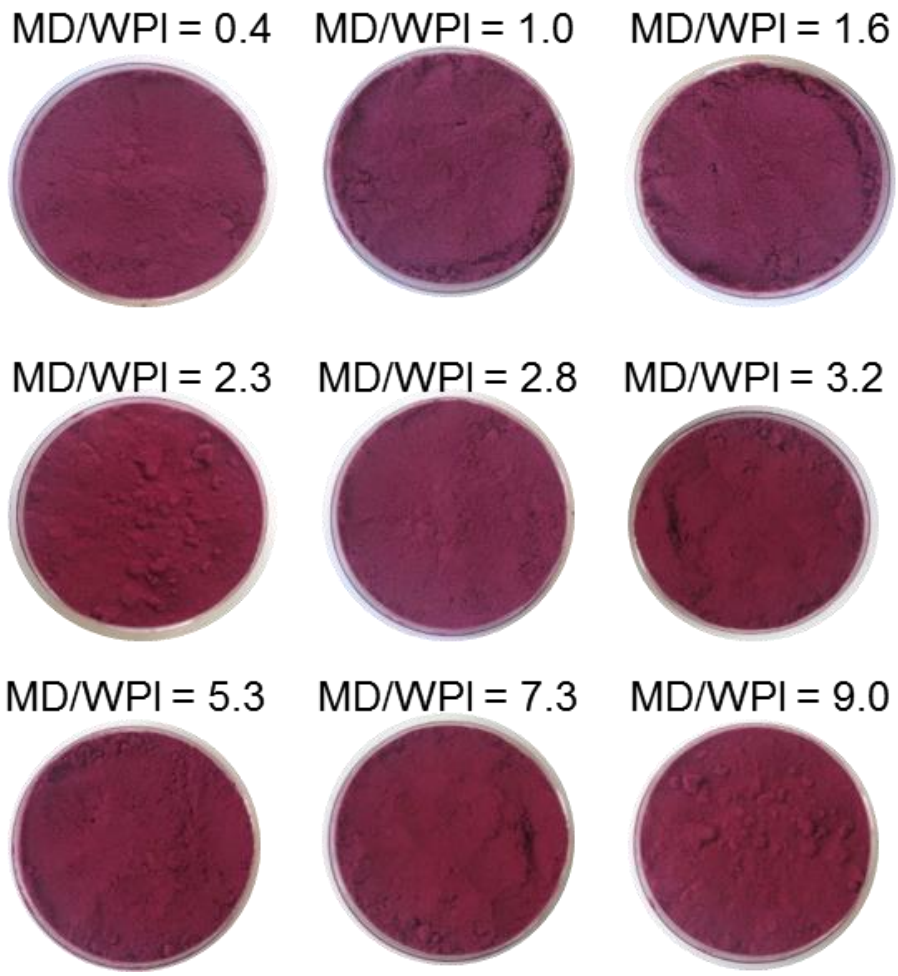

Figure 3-11 Photographs of foam-mat freeze-dried blueberry powder produced with different MD/WPI ratio

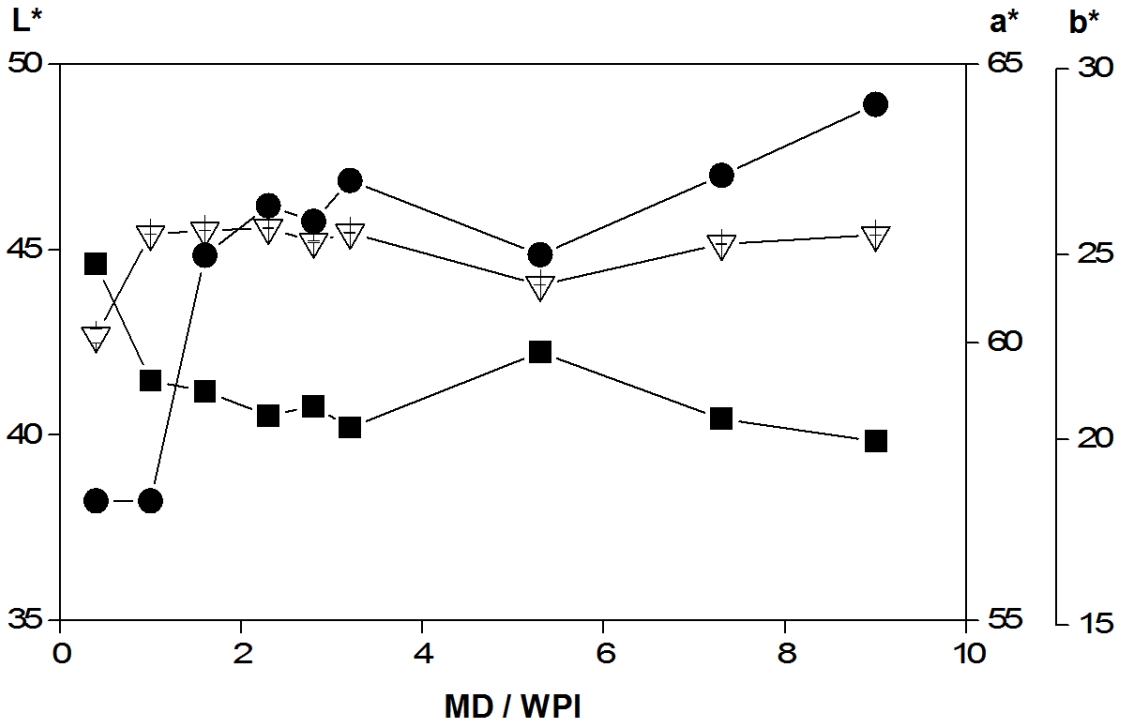

Figure 3-12 L* (■), a* (▽), and b* (●) values of reconstituted solution as a function of the mass ratio of maltodextrin and whey protein isolate (MD/WPI). Results are expressed as means ± range of duplicate determinations.

The a* (redness) value of the reconstituted solution is also presented in Figure 3-12. The ratio MD/WPI had a significant (P-value = 0.000) effect on the average a* of reconstituted solution. The a* value was > 60, which means that it was a high concentration of red.[40] The a* of the reconstituted solution at the MD/WPI ratio = 0.4 was 60, whereas at the MD/WPI = 9.0, the a* value was 62. Our result showed higher a* value compared to the freeze-dried blackberry powders containing maltodextrin[96] and freeze-dried blueberry extracted powder without mesquite gum.[112] The a* value was remarkably importance characteristics in fruit powder properties, as explained in several studies since the a* value shows the stability of colour during drying with correlation to bioactive compound such as anthocyanins.[6]

The b* (yellowness) value of the reconstituted solution is also shown in Figure 3-12. The MD/WPI ratio had a significant (P-value = 0.000) effect on the b* values. The b* value was in the range from 18 to 29. The MD/WPI 0.4 and 1.0 obtained the lower b* values, while the b* value 29 was recorded at the MD/WPI 9.0. From the results, increasing the MD/WPI ratio generally produces an increase in the b* values due to the non-enzymatic browning. Jimenez-Aguilar et al.[112] suggested lower b*, both in freeze-dried and spray-dried blueberry powder containing mesquite gum, i.e., 8.3 and 6, respectively. Freeze-drying of blackberry containing maltodextrin also performed lower b* value than foam-mat freeze-dried blueberry powder.[96] Our study shows the increasing MD/WPI ratios correspond to the increasing b* value due to may be the MD and WPI (polysaccharide and protein) reacted and changed the colour.

The chroma (C*) value was proportional to the strength of the colour and indicates its degree of saturation. The C* of the reconstituted solutions is displayed in Figure 3-13. There was significant (P-value = 0.000) differences in the C* values between the powder with MD/WPI 0.4 and 9.0, where were obtained 63 and 68, respectively. The increasing MD/WPI ratio resulted in the increasing of C* values. Similar results have been reported in the freeze-dried blackberry and soursop fruits.[25,96] The hue angle (H^0) or tonality vary from 0° (pure red colour), 90° (pure yellow colour), 180° (pure green colour) to 270° (pure blue colour).[113] The H^0 of the sample with MD/WPI 9.0 was the highest (25.1) and had significant differences (P-value = 0.000) compared to other samples (Figure 3-13).The lowest H^0 was recorded at the MD/WPI 0.4 (17) and 1.6 (16.5). The H^0 of reconstituted solutions was calculated < 30; this result suggests all samples can be described as red samples.[114]

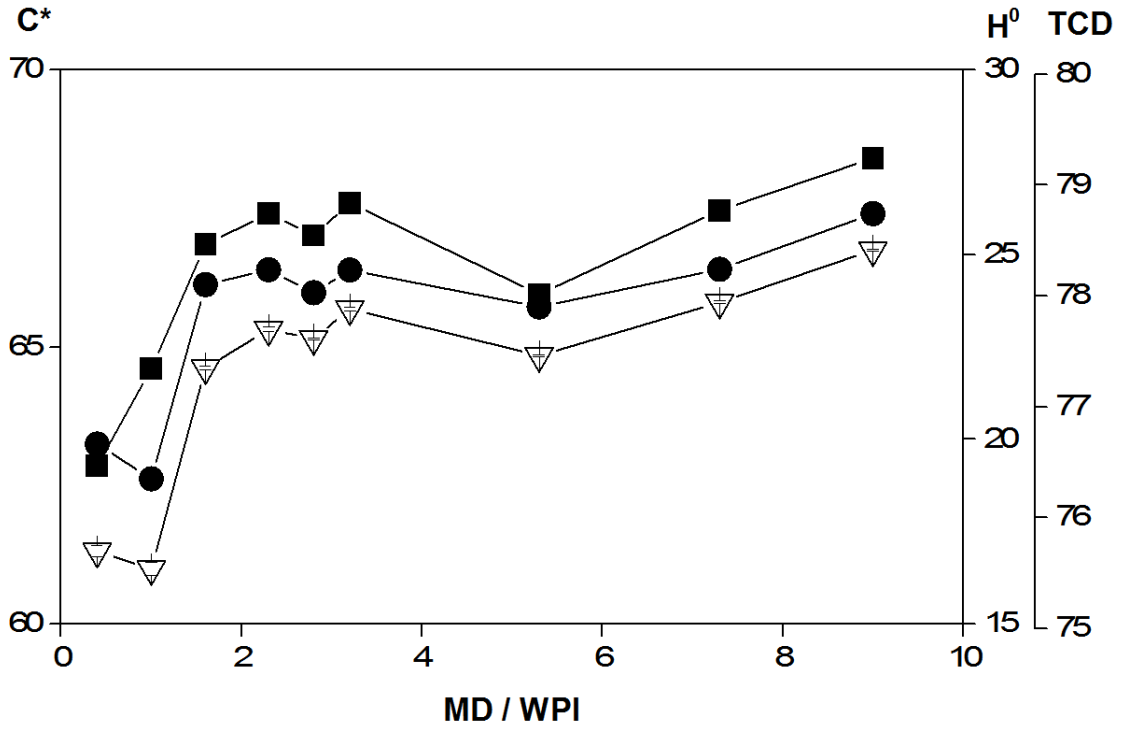

Figure 3-13 C* (■), H^0 (▽), and Total Colour Density/TCD (●) of reconstituted solution as a function of the mass ratio of maltodextrin and whey protein isolate (MD/WPI). Results are expressed as means ± range of duplicate determinations.

The total colour difference (TCD) was in the range of 76.4-78.7 (Figure 3-13).The MD/WPI ratios = 9.0 had statistically higher (P-value = 0.000) TCD than other MD/WPI ratios. The lowest TCD value was observed in the samples with MD/WPI 1.0. The increased MD/WPI ratio resulted in increased the TCD values. This may be related to the MD and WPI were white flours which increased the L*, a* and b*. Thus the TCD was slightly higher.

3.4.9 Total phenolic Content (TPC)

Phenolic compounds changes are found during the drying process, as they are affected by moisture and temperature.[115] They also determine the colour and chemical

compounds in the product. The total phenolic content (TPC) of foam-mat freeze-dried blueberry powder is presented in Figure 3-14. Increasing of the MD/WPI ratio resulted in increased total phenolic content of the reconstituted solution. The MD/WPI ratio had no significant (P-value = 0.332) effect on the TPC. The TPC is a minimum of 29 mg GAE g^{-1} solids and a maximum of 43 mg GAE g^{-1} solids, for powder produced at the MD/WPI ratio = 0.4 and 9.0, respectively. This result was much higher TPC in comparison with the freeze-dried blackberry powder, with the TPC of 6.6 and 5 mg GAE g^{-1} solids, for MD-treated and trehalose-treated, respectively.[96]

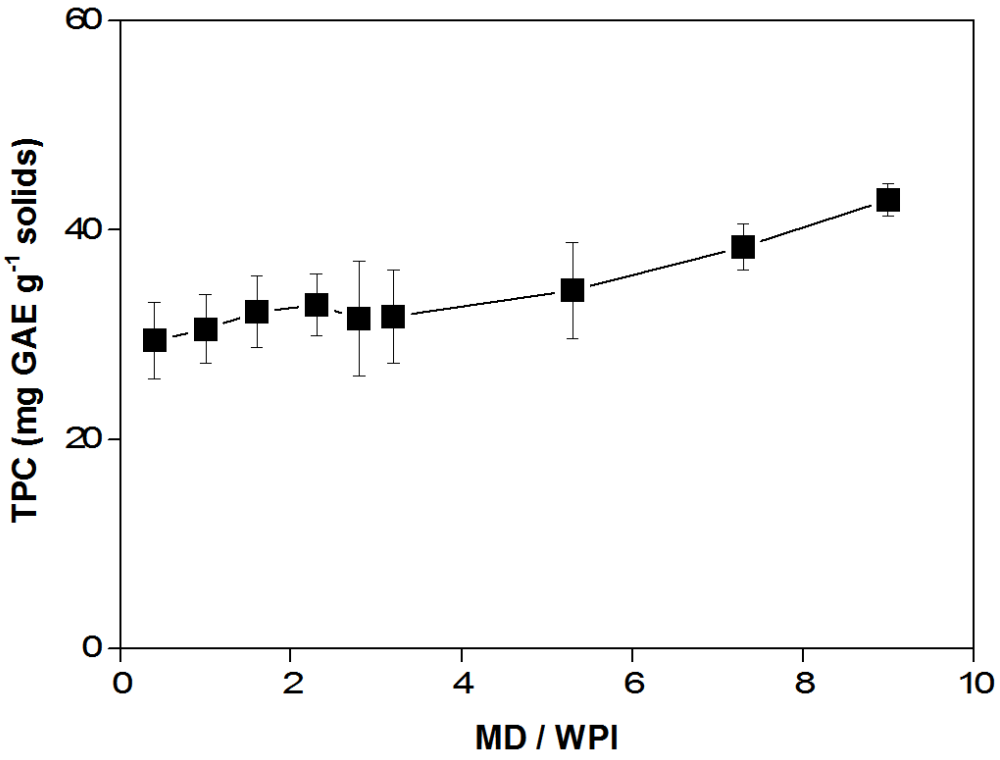

Figure 3-14 Total phenolic content (TPC) of foam-mat freeze-dried powder as a function of the mass ratio of maltodextrin and whey protein isolate (MD/WPI). Results are expressed as means ± range of duplicate determinations.

Our study also resulted in significantly higher TPC compared to the conventional freeze-drying of two different cultivars blueberry (whole fruits), which recovered about 21.5 and 25 mg GAE g^{-1} solids.[116] These results show that the application of MD and WPI in the blueberry juice before freeze-drying gave better protection of the phenolic compounds in the blueberry powder. Despite MD and WPI addition, the drying method did affect the TPC in the finished product. Yan and Ker[117] used vacuum-belt dryer and freeze-dryer to dry apple pomace. They found that the TPC of powder was 48, 44, and 52 mg GAE g^{-1}, for vacuum-belt drying at 95, 110 ^{0}C, and freeze-drying, respectively. However, at 80 ^{0}C with a vacuum-belt dryer, the TPC was similar to the freeze-dried apple pomace powder.

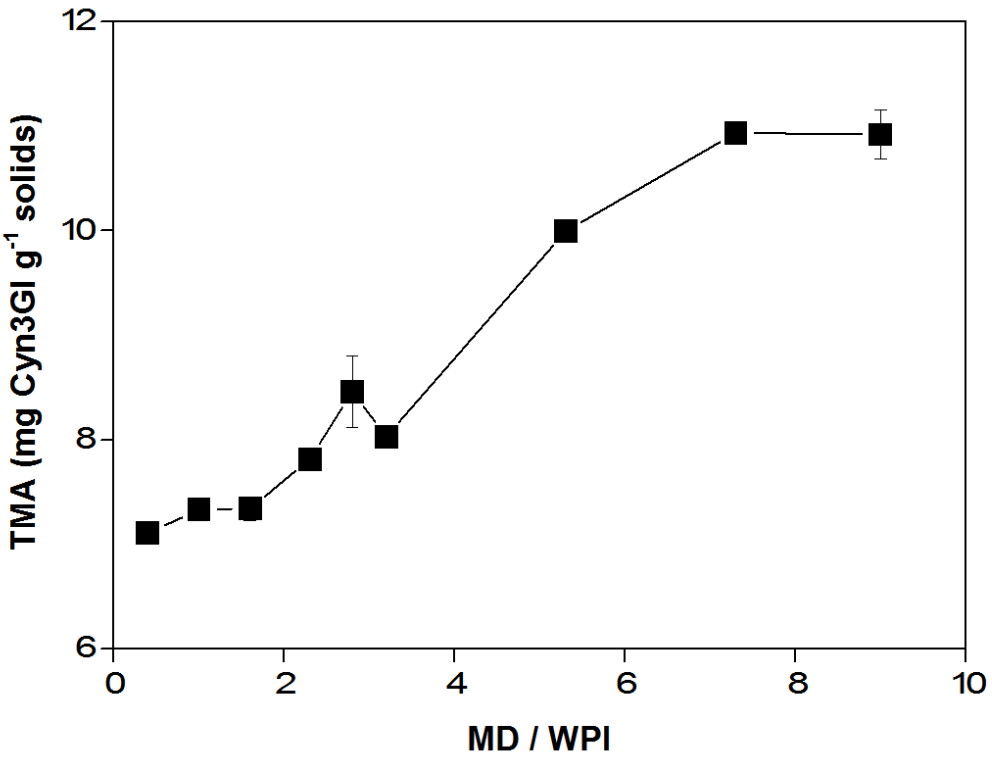

Figure 3-15 Total monomeric anthocyanin (TMA) of foam-mat freeze-dried powder as a function of the mass ratio of maltodextrin and whey protein isolate (MD/WPI). Results are expressed as means ± range of duplicate determinations.

3.4.10 Total monomeric anthocyanin (TMA)

The MD/WPI ratio had a significant (P-value = 0.000) effect on the total monomeric anthocyanin (TMA) content of foam-mat freeze-dried powders (Figure 3-15). Increased the MD/WPI ratio increases the total monomeric anthocyanins of the samples.The low content of TMA was observed at the MD/WPI 0.4 (7.1 mg Cyn3Gl g^{-1} solids), 1.0 and 1.6 (7.3 mg Cyn3Gl g^{-1} solids). Conversely, at the MD/WPI ratio = 7.3 and 9.0 resulted in significant increases of TMA content, i.e. 11 mg Cyn3Gl g^{-1} solids. The difference of TMA content amongst all the samples may be attributed to the function of MD in protecting anthocyanins during freeze-drying. Celli et al.[105] suggested that MD is an active matrix to prevent the degradation of anthocyanins in freeze-dried microencapsulated lowbush blueberries. According to the authors, the ratio MD: blueberry extract = 1:2.5 (g mL^{-1}) was the best formulation for producing the freeze-dried blueberry powder.

Duangmal et al.[118] found that maltodextrin (MD) provided superior stability than trehalose and slow anthocyanin degradation in the freeze-dried powder of Roselle anthocyanin extract. Further, they explained that MD had lower hygroscopicity than trehalose which caused less stability of anthocyanin. The addition of dextrins in anthocyanins pigments, extracted from tart cherries and stored, were more stable than those without additives.[119] This can be explained by complexing of the flavylium cation form of anthocyanins with dextrins prevented their transformation to other, less stable forms.[119] The previous studies have also demonstrated that MD, WPI, and MD + WPI can retard anthocyanin degradation.[42,77,96] In the case of WPI, Chung, et al.[77] suggested that WPI increases stability to the anthocyanin. Also, modified WPI, such as heated-denatured WPI (HD-WPI), was more effective at inhibiting colour fading than

the WPI, which suggested that unfolding of the protein may have increased the interaction with anthocyanin, possibly due to greater exposure of the hydrophobic group. In our study, the addition of WPI into the blueberry juice was without heat treatment. Therefore, we assumed that there was little interaction between anthocyanin and WPI, either before or after foam-mat freeze-drying.

3.4.11 Individual anthocyanins

Anthocyanins extracted from blueberry juice and reconstituted powders were analysed by HPLC-PAD, as shown in Figure 3-16. The retention time of these three of Del3Gl, Cyn3Gl, and Mal3Gl standards were 15.2, 17.5 and 21.5 min, respectively (see Figure 3-16A) with peak heights 12.3, 15.2 and 12.2 mAUx10^{-4}, respectively. More than 10 peaks were visible between retention times 13.5 to 34.0 min in blueberry juice (Figure 3-16 B), however, the retention times of 15.2, 17.5 and 21.5 min with corresponding peak heights of 3.7, 3.5 and 2.5 mAUx10^{4}, respectively, were assumed to be due to Del3Gl, Cyn3Gl and Mal3Gl, respectively, based on the relative distance between and their relative peak heights and previous descriptions of the main anthocyanins in blueberry juice.[120,121] However, another study found 13 peaks in blueberry juice (cv. Rubel), with the total monomeric anthocyanin of 10.8 mg g^{-1} dry weight.[122] In addition, the individual anthocyanins in blueberry juice (cv. Rubel) were as follow: Del3Gal (mass = 465.2), Del3Gl (465.2), Cyn3Gal (449.2), Del3Ara (435), Cyn3Gl (449.2), Pet3Gal 0(479.2), Cyn3Ara (419), Pet3Gl (479.2), Peo3Gal (463.2), Pet3Ara (449), Mal3Gal (493.2), Mal3Gl (493.2), and Mal3Ara (463).

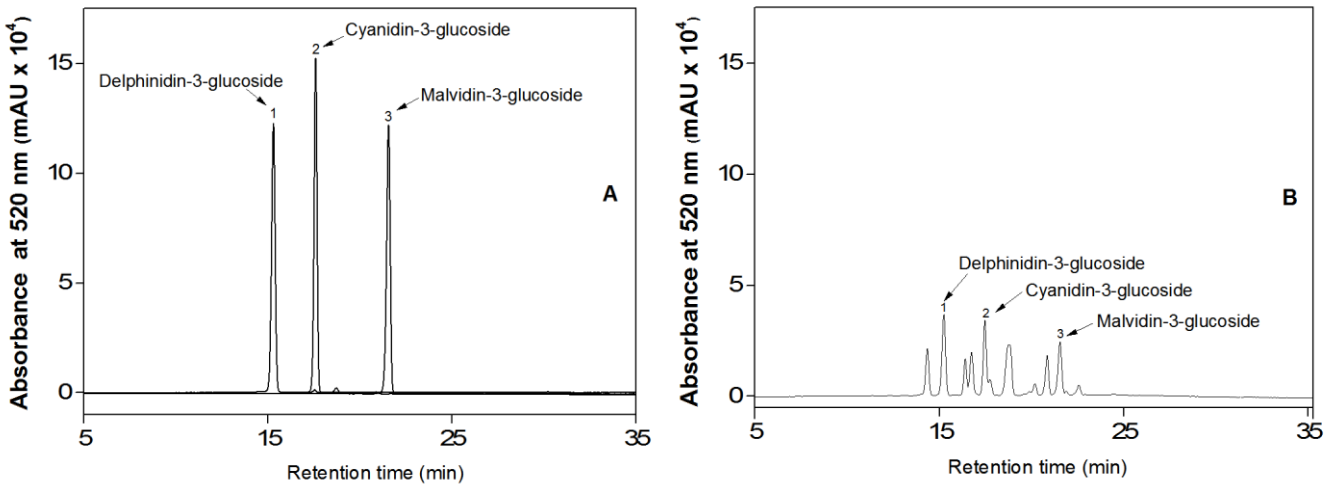

Figure 3-16 HPLC chromatogram of anthocyanin profiles from anthocyanins standards (A) and blueberry juice (B)

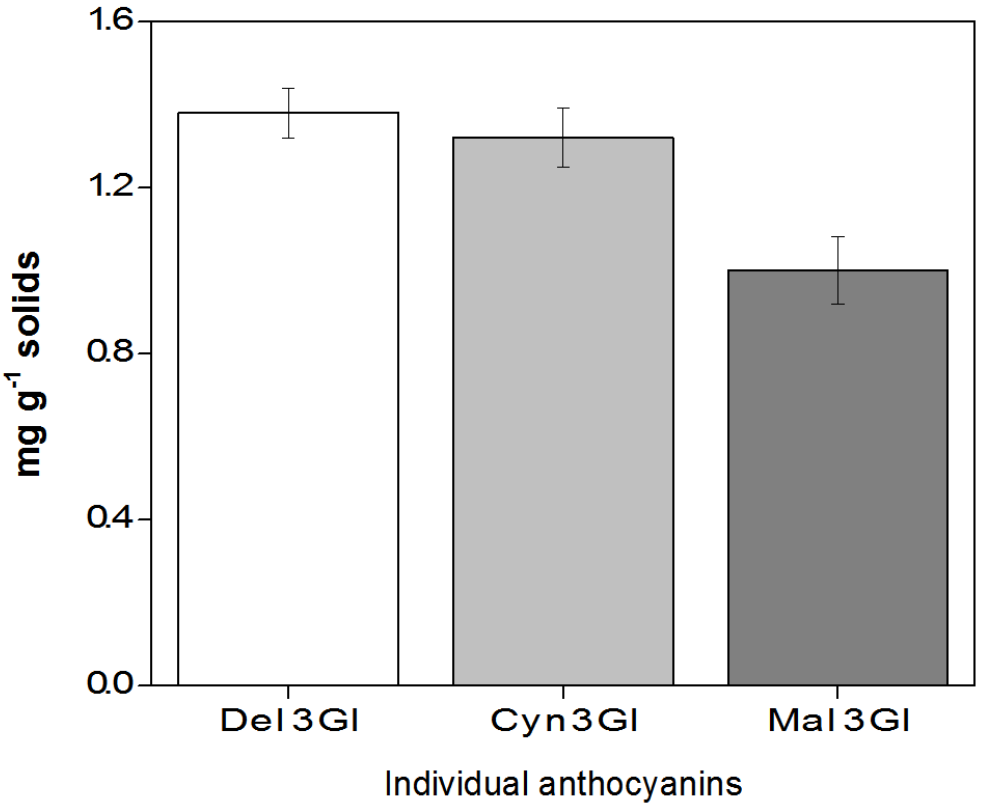

Figure 3-17 Individual anthocyanin concentration of blueberry juice. Results are expressed as means ± range of duplicate determinations.

The individual anthocyanin content of initial blueberry juice and foam-mat freeze-dried blueberry powders was then quantified based on the peak and retention time against three anthocyanin standards. As shown in Figure 3-17, it is observed that the initial blueberry juice contained 1.38, 1.32 and 1 mg g^{-1} (solids), for this Del3Gl, Cyn3Gl, and Mal3Gl, respectively. Del3Gl and Cyn3Gl had higher concentrations over Mal3Gl. This result is contrary to that of Lee et al.[122] who found Del3Gl, Cyn3Gl, and Mal3Gl of blueberry juice was 0.59, 0.05, and 1.7 mg g^{-1} solids, respectively, in blueberry juice (cv. Rubel). Mal3Gl was the highest anthocyanin. This difference may be attributed to the blueberry variety and the juice processing.

Del3Gl of foam-mat freeze-dried blueberry powder is presented in Figure 3-18. There was no significant (P-value = 0.507) difference in Del3Gl amongst all the samples. The concentration of Del3Gl ranged from 1.05 to 1.17 mg g^{-1} (solids). The decreasing trend was found in Del3Gl by increasing the MD/WPI ratio. With Cyn3Gl, there was no significant (P-value = 0.601) difference in Cyn3Gl amongst all the samples. As observed in Figure 3-19, the Cyn3Gl content of samples ranged from 1.29 to 1.37 mg g^{-1} (solids). The HPLC analysis was also identified and quantified for Mal3Gl. As observed from Figure 3-20, there was no significant (P-value = 0.562) difference in Mal3Gl amongst all the samples. The Mal3Gl is a minimum of 0.80, and a maximum of 0.88, for the powder at the MD/WPI ratio = 7.3 and 0.4, respectively.

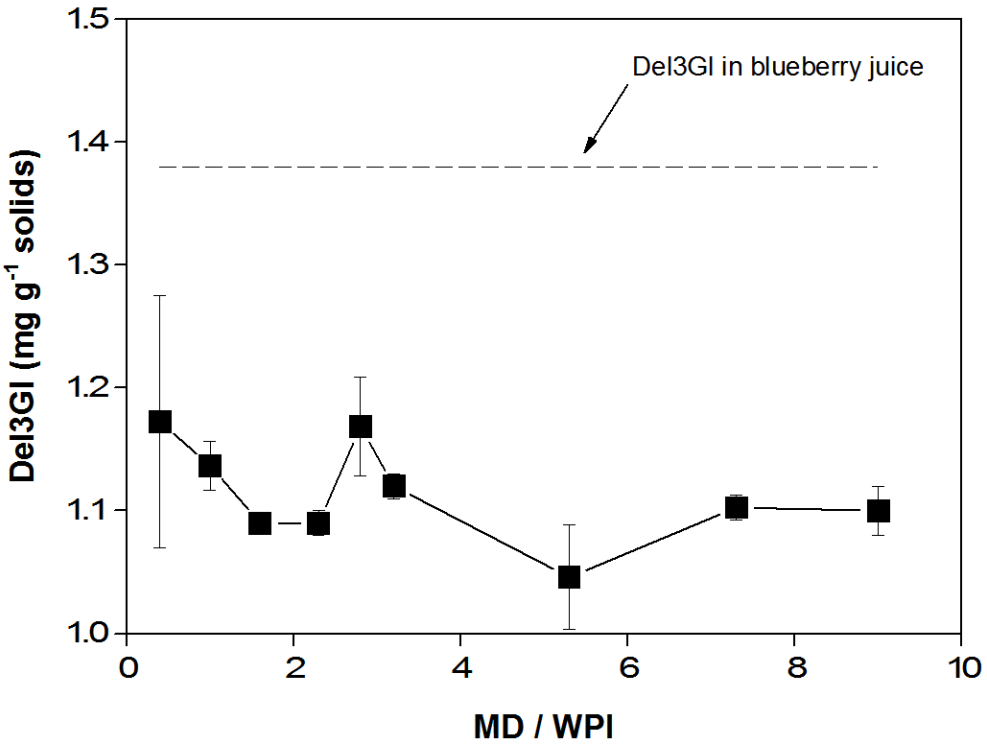

Figure 3-18 Delphinidin-3-glucoside (Del3Gl) of reconstituted foam-mat freeze-dried powder (■) and blueberry juice (---).Results are expressed as means ± range of duplicate determinations.

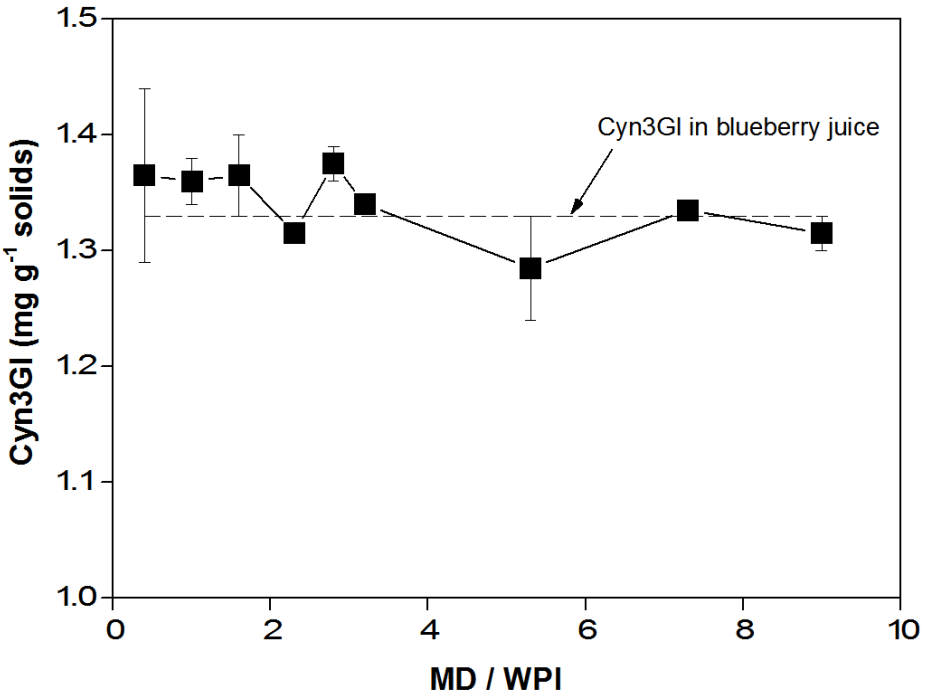

Figure 3-19 Cyanidin-3-glucoside (Cyn3Gl) of reconstituted foam-mat freeze-dried powder (■) and blueberry juice (---).Results are expressed as means ± range of duplicate determinations.

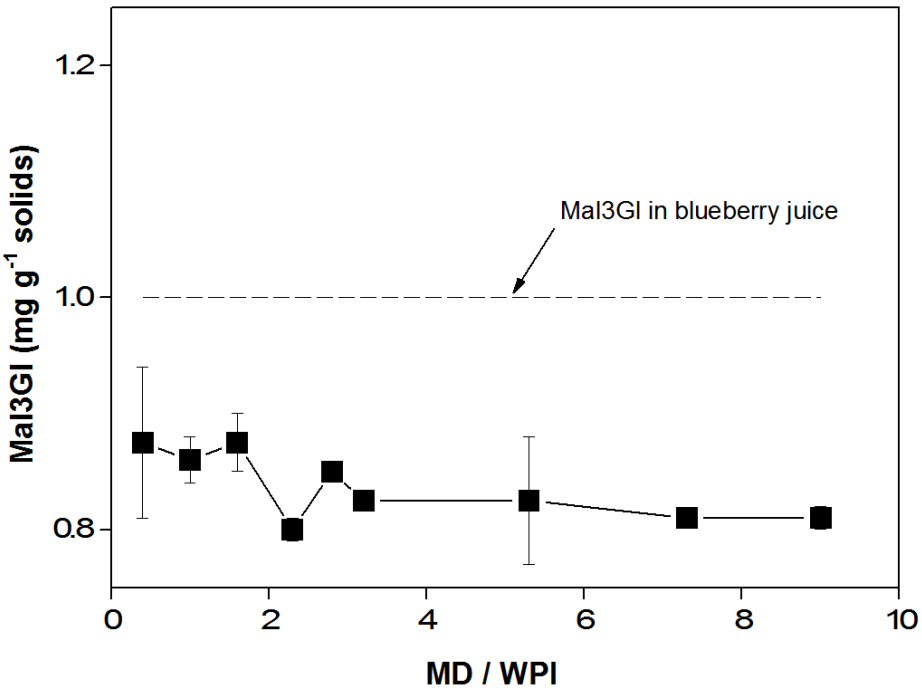

Figure 3-20 Malvidin-3-glucoside (Mal3Gl) of reconstituted foam-mat freeze-dried powder (**■**) and blueberry juice (**---**).Results are expressed as means ± range of duplicate determinations.

Overall, individual anthocyanins were affected by the different ratio of MD and WPI via foam-mat freeze-drying. The highest concentration of individual anthocyanin was Cyn3Gl, followed by Del3Gl and Mal3Gl. This outcome is contrary to that of Lee et al.[120] who concluded that Del3Gl, Cyn3Gl, and Mal3Gl of blueberry were 1.43, 0.27 and 2.0 mg g^{-1} (solids). They also revealed that the percentage of these anthocyanins was 10.9, 2.1, and 15.3 % from the total monomeric anthocyanins. Fracassetti et al.[63] reported that anthocyanin degradation in freeze-dried wild blueberry powder and found that the individual anthocyanin linked to galactose such as Cyn3Gal (cyanidin-3-galactoside), Mal3Gal (malvidin-3-galactoside), and Pet3Gal (Petunidin-3-galactoside) are more heat-stable. In another word, the individual anthocyanins bound to glucose and arabinose exhibited a faster degradation rate than those linked to galactose.

Another study conducted by Ichiyanagi et al.[123], which is also contradictory to our result, documented that the ranking order was arabinoside > galactoside > glucoside from the most to the least stable. On the other hand, our results agree with the findings of Trost et al.[124], from which individual anthocyanin stability in a blueberry-aronia nectar stored for over 207 days at 30 ^{0}C was higher for Cyn3Gl and Pe3Gl (peonidin) and lower for Pet3Gl (petunidin), Mal3Gl, and Del3Gl. The rankings were glucoside > galactoside > arabinoside from the most to the least stable. This phenomenon can be explained by the anthocyanins bound to glucose and galactose had superior stability than arabinose due to the steric hindrance, which is larger for the hexose sugars.[124]

3.4.12 Retention of TPC, TMA, and individual anthocyanin

The TPC retention of the powders obtained various values 68 to 99% (Table 3-2). There was no significant (P-value = 0.334) difference of TPC retention amongst all the samples. Increasing the MD/WPI ratio led to increasing the TPC retention. This result is consistent with that of Wilkowska et al.[107] who found the TPC retention of freeze-dried blueberry powder containing β-cyclodextrin was a maximum of 98.5%. Turan et al.[113] documented the TPC retention of freeze-dried powder from blueberry juice and extracted containing MD were 95 and 97%, respectively. The high TPC retention of the foam-mat freeze-dried blueberry powder is understandable due to low drying temperatures and vacuum conditions.[116,125]

Table 3-2 Effect of MD/WPI ratio on the retention of TPC, TMA, and individual anthocyanins

MD/WPI Ratio	*TPC (%)	TMA (%)	*Delphinidin-3-glucoside (%)	*Cyanidin-3-glucoside (%)	*Malvidin-3-glucoside (%)
0.4	68 ± 8	80 e	85 ± 7	103 ± 6	88 ± 7
1.0	71 ± 8	82 de	82 ± 1	103 ± 2	86 ± 2
1.6	74 ± 8	82 de	79	103 ± 3	88 ± 3
2.3	76 ± 7	82 ± 1de	79 ± 1	100	80 ± 1
2.8	73 ± 13	95 ±4 c	85 ± 3	104 ± 1	85
3.2	73 ± 10	90 cd	81± 1	102	83 ± 1
5.3	79 ± 11	95 ±4 c	76 ± 3	97 ± 3	83 ± 6
7.3	89 ± 5	112 b	80 ± 1	101	81
9.0	99 ± 3	122 a	80 ± 2	100 ± 1	81 ± 1

**No significant differences amongst values. Mean values ± range of duplicate determination, followed by a different single letter in each column if significantly different ($p < 0.05$, Tukey's test), whereas ab, for example, indicates values that are not significantly different from the corresponding a and b values.*

The TMA retention of foam-mat freeze-dried powder is also presented in Table 3-2.The MD/WPI ratio had a significant (P-value = 0.000) difference in the TMA retention. Increasing the MD/WPI ratio increased TMA retention. The TMA retention was a minimum of 80% and a maximum of 122%, calculated from the MD/WPI ratio = 0.4 and 9.0, respectively. The powder prepared at the MD/WPI ratio = 7.3 and 9.0 showed higher TMA retention than other samples at low MD/WPI ratio, thus demonstrating that the wall material ratios are important parameters protection to the bioactive components, especially anthocyanins.[105] Turan et al.[113] revealed that the anthocyanin retention of freeze-dried blueberry microcapsules obtained with MD or MD + gum arabic was 83-99% or 83-100%, respectively. These results were comparable to the range (80-120%) in our study.

Individual anthocyanins retention are also shown in Table 3-2. There were no significant (P-value = 0.507) differences in the Del3Gl retention from all the samples. The blueberry powder prepared using MD/WPI ranged from 79 to 85%. A similar phenomenon was observed for Cyn3Gl, and there were no significant (P-value = 0.601) differences amongst all the samples. The Cyn3Gl retention ranged from 97 to 104%. In the case of Mal3Gl, the retention value was not statistically (P-value = 0.562) different amongst all the samples. The Mal3Gl retention was a minimum of 81%; and a maximum of 88% for the sample contained the MD/WPI ratio = 9.0 and 0.4, respectively. These results were found to be higher than those of Wilkowska et al. [107] who found that the Del3Gl, Cyn3Gl, and Mal3Gl retention were of 28, 28.5, and 21.5%, respectively

3.5 Summary

Blueberry powder was successfully produced via foam-mat freeze-drying with the aid of maltodextrin (MD) DE 16.5-19.5 and whey protein isolate (WPI). Foam and physicochemical powder properties were evaluated. The density of foamed blueberry juice prepared with the MD/WPI ratio = from 0.4 to 9.0 was in a range from 0.14 to 0.16 g cm^{-3}. The overrun of foamed blueberry reached a maximum value at the MD/WPI ratio = 1.6, i.e., 825%.

The powder yield ranged 72-79%, with 99-100% solubility for all the foam-mat freeze-dried powders. The highest yield was from the blueberry powder with MD/WPI 2.8. All samples of foam-mat freeze-dried powder had a moisture content in a range 2.6-3.9% and water activity ≤ 0.45. Most of the particles resembled broken glass or flake-like structures and porous. The colour of reconstituted solution showed L* = 40-45, a* = 60-62, b* = 18-27. All the samples can be described as red samples according to H^0 values < 90. The reconstituted powder made with MD/WPI 9.0 had the highest a* and lowest L* values.

The TPC (total phenolic content) was in a range from 29 to 43 mg GAE g^{-1} solids for all the samples powder. The TMA (total monomeric anthocyanins) was in a range of 7 and 11 mg Cyn3Gl g^{-1} solids and was found higher at the high MD/WPI ratio, i.e. 9.0, than at low ratio. TPC and TMA retention were 68-99% and 80-122%, respectively. All the foam-mat freeze-dried powder samples showed Del3Gl 1-1.2 mg g^{-1} (solids), with the calculated retention of 79-85%; Cyn3Gl 1.3-1.4 mg g^{-1} (solids), with 97-104% retention; Mal3Gl 0.80-0.88 mg g^{-1} (solids), with 81-88% retention.

Chapter 4

Chapter 4 Effect of trehalose and pure protein types on the physicochemical properties of the foam-mat freeze-dried blueberry powder and reconstituted products

4.1 Introduction

Foam-mat freeze-drying was also conducted with alternative matrices of trehalose + pure β-lactoglobulin (T3BL1) and trehalose + bovine serum albumin (T3A1). Trehalose was chosen because of its supposed special properties in acting as a cryoprotective agent.[90] Trehalose is an alternative to sucrose in the dehydration of fruit juices as it shows a relatively higher glass transition temperature (T_g). Galmarini et al.[88] observed that strawberry puree presented better sensory properties when dried with the addition of trehalose in comparison with sucrose and MD. β-lactoglobulin is the main surface active ingredient of WPI.[72,73] Bovine serum albumin (BSA) has been used in other model studies of foaming.[126] Although these agents are more expensive than MD or WPI, it was of interest to see if the more pure ingredients conferred any particular advantages in the foam-mat freeze-drying of blueberry juice.

The objectives this present study were: (1) to develop blueberry foam-mat freeze-dried powders with different matrices, including trehalose + pure β-lactoglobulin (T3BL1) and trehalose + bovine serum albumin (T3A1), (2) investigate the effect of T3BL1 and T3A1 on the physicochemical properties of the blueberry powders and reconstituted products.

4.2 Foam-mat freeze-drying conditions

All of the foam-mat freeze-dried conditions were similar as described in section 3.2, except the matrices used. Trehalose + β-lactoglobulin (T3BL1) and trehalose + bovine serum albumin (T3A1) were prepared with the ratio of sugar/protein = 2.8 (w/w %) by dissolving them in the blueberry juice. This ratio was chosen due to the highest yield according to the previous experiment (see section 3.4.4)

4.3 Statistical analysis

The processing treatments were triplicated, and the means of the results ± standard deviation were reported. Two sample t-test was done to establish the presence or absence of significant differences between means and significance level was set at $p < 0.05$.

4.4 Results and Discussion

4.4.1 Foam capacity: density and overrun

As described in Figure 4-1, T3BL1 sample had statistically (P-value = 0.003) higher foam density, i.e., 0.15 g cm^{-3}, than T3A1, i.e. 0.13 g cm^{-3}. The higher foam density of T3BL1 was followed by the low overrun, i.e., 608%, while the T3A1 sample had higher overrun (P-value < 0.001), i.e., 658%. In foam-mat drying, the drying rates of foamed materials depend greatly on its stability, density, and bubble size.[1] The low density of foam results in higher drying rates due to more porous and open structure of the foam. Thus, the final powder was easy to pulverise. Also, the porous structure also influences the low moisture of the powder. Ratti and Kudra[1] suggested that excessive density of foamed materials led to prolonged drying time. Thus, this situation caused poor quality

of dried products. However, foams of lower density dry faster. In the case of the same foam density, the foams contain smallest bubbles and highly homogeneity is most suitable for drying.

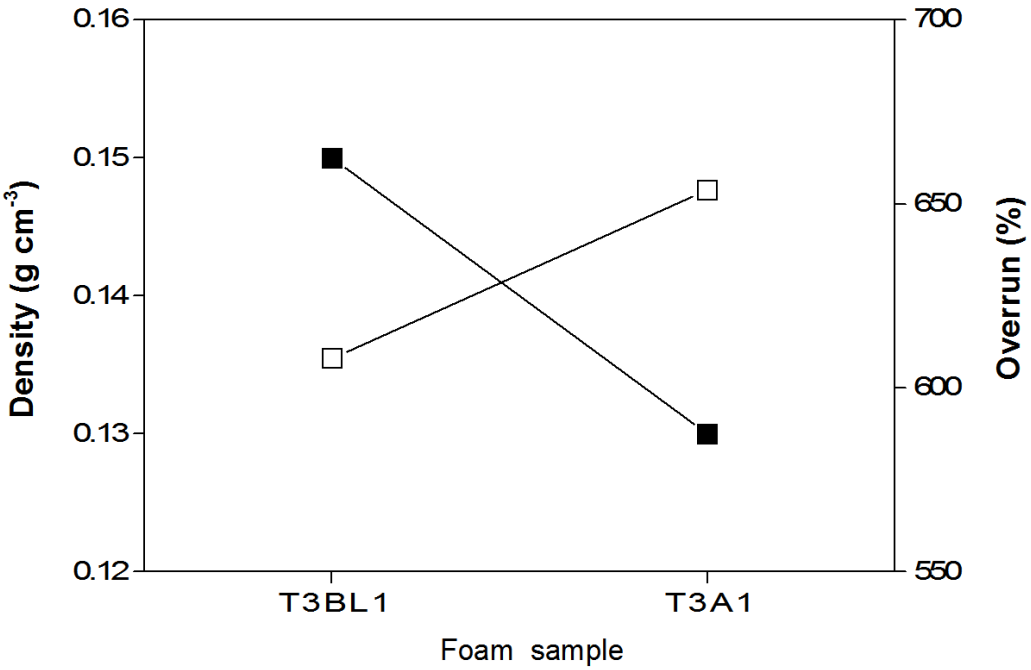

Figure 4-1 Foam density (■), overrun (□) of foamed blueberry as a function of trehalose + beta lactoglobulin and trehalose + bovine serum albumin. Results are expressed as means ± SD of triplicate determinations.

The foaming capacity of protein, namely β-lactoglobulin and bovine serum albumin (BSA), is influenced by environmental conditions such as pH, heating, and additives. Protein food foams are mostly manufactured outside the isoelectric pH range of the proteins.[82] Electrostatic attraction between proteins are at a maximum pI (isoelectric point), and more proteins adsorb at the interface reducing interfacial tension. As reported by Kim & Kinsella,[127] the maximum surface pressure was found at the near pI of BSA and decreased rapidly below pH 5 and above pH 6. A rapid adsorption of β-

lactoglobulin that increased at pH 5.3 was reported. Maximum surface viscosity was in the pH range 5-6 and decreased by 40% at pH 7.0.[128] Under this conditions (pH range) foams prepared with β-lactoglobulin exhibited maximum strength. In the present study, the pH of blueberry juice was < 3.0. This low pH possibly reduced the foaming capacity of β-lactoglobulin and BSA. For instance, overrun was 1346 % via whipped for 15 mins with pH 8 solution of β-lactoglobulin. For BSA, maximum foam formation was reached at pH 5, as this is nearest to its pI.[126]

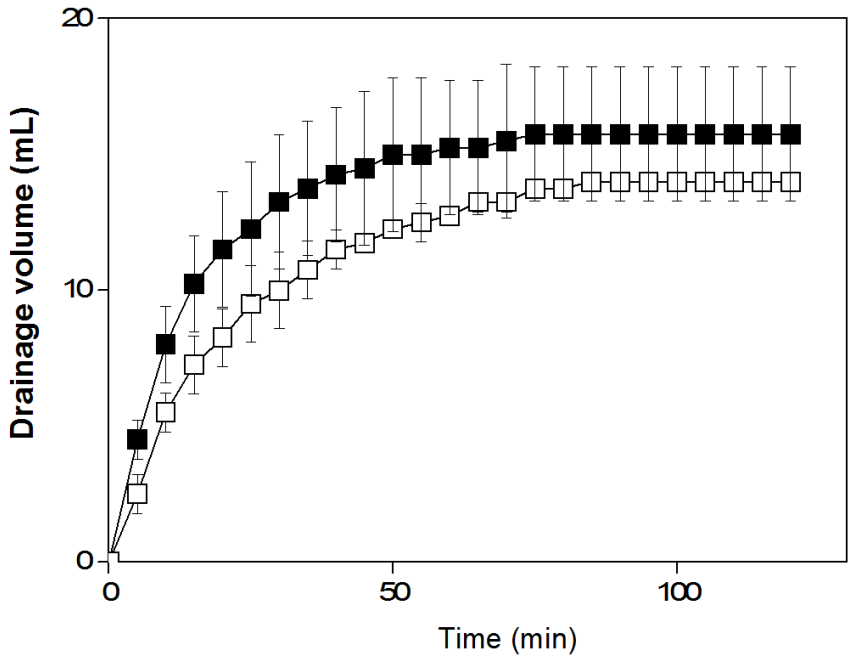

Figure 4-2 Drainage volume versus time of foamed blueberry juice with trehalose + beta lactoglobulin (T3BL1) (■) and trehalose + bovine serum albumin (T3A1) (□). Results are expressed as means ± SD of triplicate determinations.

4.4.2 Foam stability: drainage

The comparison of foam drainage during 120 min is presented in Figure 4-2.From 0-15 min, the foam produced with T3BL1 had a higher volume of liquid (less stable) in comparison with T3A1 (more stable). Afterwards, there was no significant effect on

foam stability between T3BL1 and T3A1. The maximum drain volume was 16 and 14 mL, for T3BL1 and T3A1, respectively. The rate of drainage usually falls with time and depends on the viscosity of the bulk liquid phase. Kim and Kinsella[127] reported that foam stability of bovine serum albumin (BSA), as measured by drainage rates shows maximum in the range pH 5-6. At this pH range, higher inter- and intramolecular interaction were observed resulting in a more cohesive film elasticity. In the present study, we assumed that the foam stability of blueberry juice was lower due to pH < 5.0 of the foam. This low pH caused the surface yield stress decreases rapidly.

4.4.3 Powder yield

Figure 4-3 presents the yield of foam-mat freeze-dried powder prepared with trehalose + β-lactoglobulin (T3BL1) and trehalose + bovine serum albumin (T3A1). The two matrices had a significant (P-value = 0.001) effect on the yield. The T3BL1 sample exhibited 66% of yield, while the T3A1 sample presented 67%. The low foam density of the T3A1 sample resulted in the high yield. Conversely, the T3BL1 obtained higher foam density, from which low yield was found. As explained earlier in the section 4.4.2, the foam capacity, i.e., density, affects the drying rates, where the low foam density had more porous structure and higher drying rates.[1] Thus, the T3A1 sample with low density had higher yield compared to T3BL1 powder. Both foam sample, however, had higher stability compared to the foam prepared by MD + WPI (see section 3.4.3) due to lower drained liquid. Mechanical stability is required before drying, to avoid collapse during feeding and deposition on the dryer. Practically, foams that do not collapse for at least one hour are considered mechanically stable.[1]

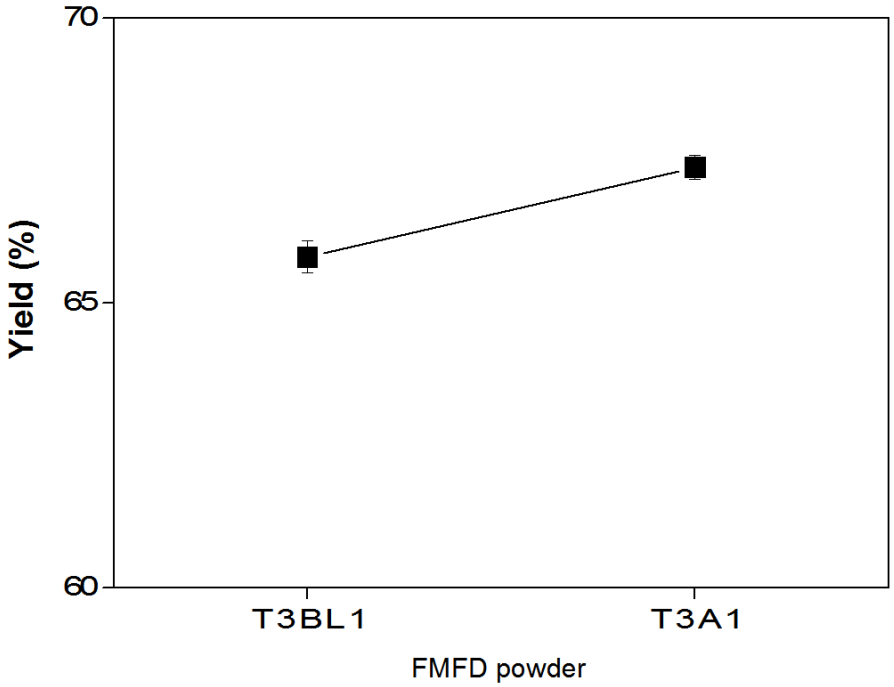

Figure 4-3 Yield of foam-mat freeze-dried powder as a function of trehalose + beta lactoglobulin (T3BL1) and trehalose + bovine serum albumin (T3A1). Results are expressed as means ± SD of triplicate determinations.

Concerning the effect of trehalose on the yield, our results were lower to those study of Wilkowska et al.[107] who found 78% yield of freeze-drying of blueberry using β-cyclodextrin as a drying agent. The possible explanation of this result is because glass transition temperature of the β-cyclodextrin was 292 ^{0}C,[129] higher than the glass temperature of trehalose, i.e., 115 ^{0}C.[84] Also, the glass transition temperature of trehalose is lower than maltodextrin, which has glass transition temperatures in the range 140-180 ^{0}C.[130] These three polysaccharides were commonly used to reduce caking, stickiness, and improve flowability.[84,129,130] The higher the glass transition temperatures, the less sticky the powder. Thus, the yield of the freeze-dried powder significantly increased.

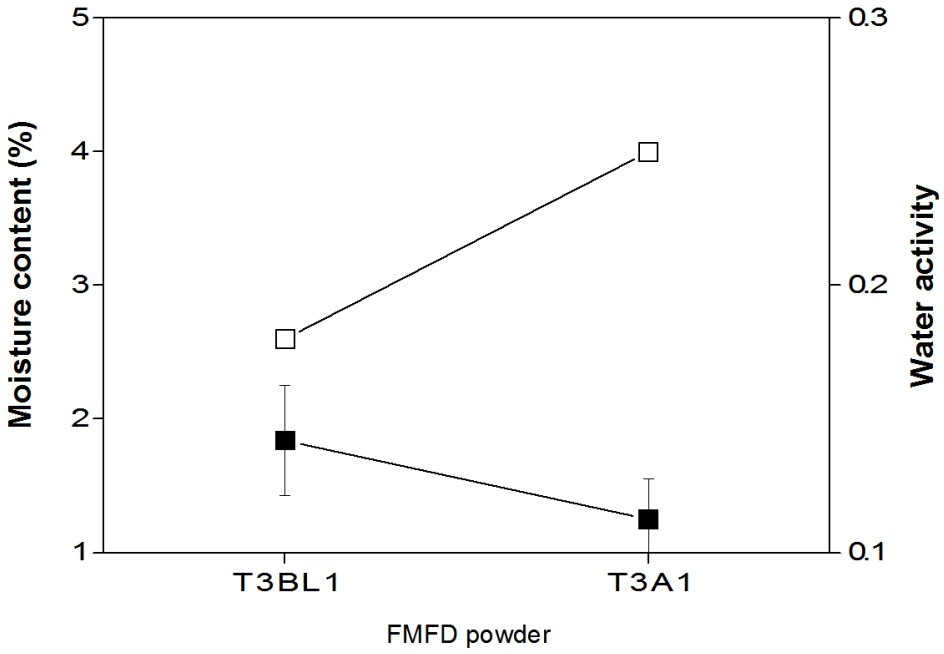

Figure 4-4 Moisture content (■) and water activity/a_w (□) of foam-mat freeze-dried powder as a function of trehalose + beta lactoglobulin (T3BL1) and trehalose + bovine serum albumin (T3A1). Results are expressed as means ± SD of triplicate determinations.

4.4.4 Moisture content and water activity (a_w)

Figure 4-4 shows the effect of trehalose and pure proteins on the moisture content of foam-mat freeze-dried blueberry powder. It is observed from the figure; there was no significantly (P-value = 0.113) measured difference in moisture content between T3BL1 (1.8 ± 0.4%) and T3A1 (1.3 ± 0.3%) samples. This result was lower compared to freeze-dried blackberry powder using maltodextrin + trehalose, i.e. 6.1%,[96] and freeze-dried blueberry powder using maltodextrin alone, i.e., 3.3%.[107] Also, both powders, T3BL1 and T3A1, had smaller moisture content than our findings for foam-mat freeze-drying using maltodextrin + whey protein isolate, which had a moisture

content in a range between 2.6% and 3.9% (see section 3.4.5). The low moisture content could be related to the difference matrices used.

The a_w of foam-mat freeze-dried blueberry powder was 0.18 and 0.25 for T3BL1 and T3A, respectively (Figure 4-4). Between two samples T3BL1 and T3A1, there was statistically (P-value = 0.000) measured differences in the a_w. Our result showed a_w below 0.3, which extends powder stability because the low a_w values represent less free water available for microbial growth and biochemical reaction and therefore longer shelf life.[131]

4.4.5 Solubility, rehydration time, and bulk density

Solubility of T3BL1, i.e., 98.3% ± 0.06 was significantly (P-value = 0.001) different from the T3A1 sample, i.e., 96.7% ± 0.04 (Figure 4-5). This result may be explained by the fact that the β-lactoglobulin is more water soluble than bovine serum albumin at the same temperature.[82] However, both products showed higher solubility > 95%, which was related to the fact that trehalose has high solubility in water.[87,89] Our results seemed to be consistent with those of Franceschinis et al.[96] who reported high solubility of freeze-dried blackberry powders. The solubility of maltodextrin-treated blackberry powder was 99.8%, while the trehalose/maltodextrin- treated blackberry powder was 99.5%. Conversely, Ceballos et al.[25] suggested that the water solubility of freeze-dried soursop powder obtained with the addition of 18% maltodextrin was in a range from 82-86%.

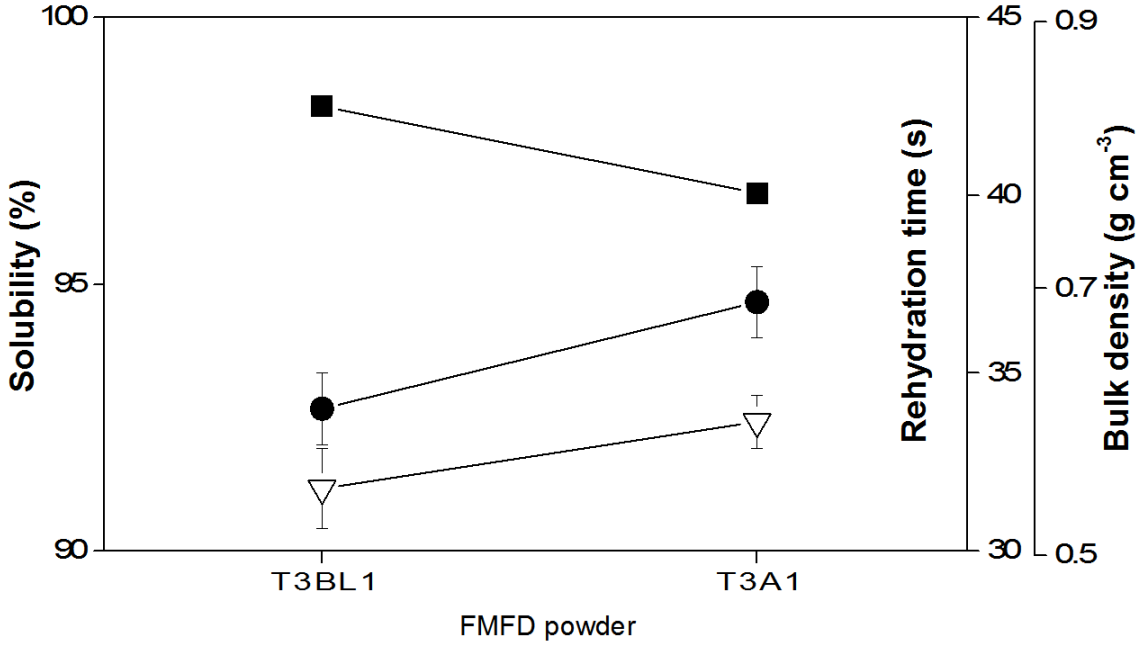

Figure 4-5 Solubility (■), rehydration time (●), and bulk density (▽) of foam-mat freeze-dried powder as a function of trehalose + beta lactoglobulin (T3BL1) and trehalose + bovine serum albumin (T3A1). Results are expressed as means ± SD of triplicate determinations.

The rehydration time of foam-mat freeze-dried blueberry powder obtained with β-lactoglobulin (T3BL1) and bovine serum albumin (T3A1) was 34 s and 37 s respectively (Figure 4-5). A t-test of observed results showed that T3BL1 was statistically (P-value = 0.021) faster rehydration time than the T3A1 sample. The present study exhibited faster rehydration time in comparison with that of Islam et al.[93] who suggested that the rehydration time of orange powder was in a range 122-253 s. Freeze-drying consists of the production of ice crystals and their sublimation at minimal pressures.[22] This procedure results in food particles with an open pore structure, which absorbs water easily when they are reconstituted.[22]

Functional and economic reasons make bulk density an important property of the powdered product.[68] The bulk density of foam-mat freeze-dried powders was affected by the matrices (Figure 4-5). Bulk density values were 0.55 ± 0.03 and 0.6 ± 0.02 g cm^{-3}, for T3BL1 and T3A1 samples, respectively. There were no significant (P-value = 0.052) differences between both products. These results were understandable because the two foam-mat freeze-dried powders contained equal amount of trehalose and pure proteins. Results of bulk densities were slightly higher to those of Franceschinis et al.[96] who freeze-dried blackberry with the addition of maltodextrin and trehalose. Also, the bulk densities of T3BL1 and T3A1 blueberry powders were higher to the foam-mat freeze-dried blueberry powder obtained with maltodextrin and whey protein isolate (see section 3.4.6.). They were in a range of 0.18 – 0.47 g cm^{-3}. It is also important to note that the powder particles (Figure 4-6) can affect the bulk density values.

4.4.6 Particle morphology

Figure 4-6 (A and B) correspond to trehalose + β-lactoglobulin (T3BL1) at 1000 x and 2500 x magnification. Figure 4-6 (C and D) correspond to trehalose + bovine serum albumin (T3A1) using the same magnification. The foam-mat freeze-dried blueberry products indicated irregular particles and the structural resemblance between both samples. Similar particles were observed in the shrimp powder,[7] soursop powder,[25] blackberry powder,[96] apple juice powder,[12] and blueberry powder[107,113], which were obtained by using freeze-drying and foam-mat drying. During freeze-drying, the freezing conditions determine the size of the generated crystals and thus the pore size distribution of the final product.[132]

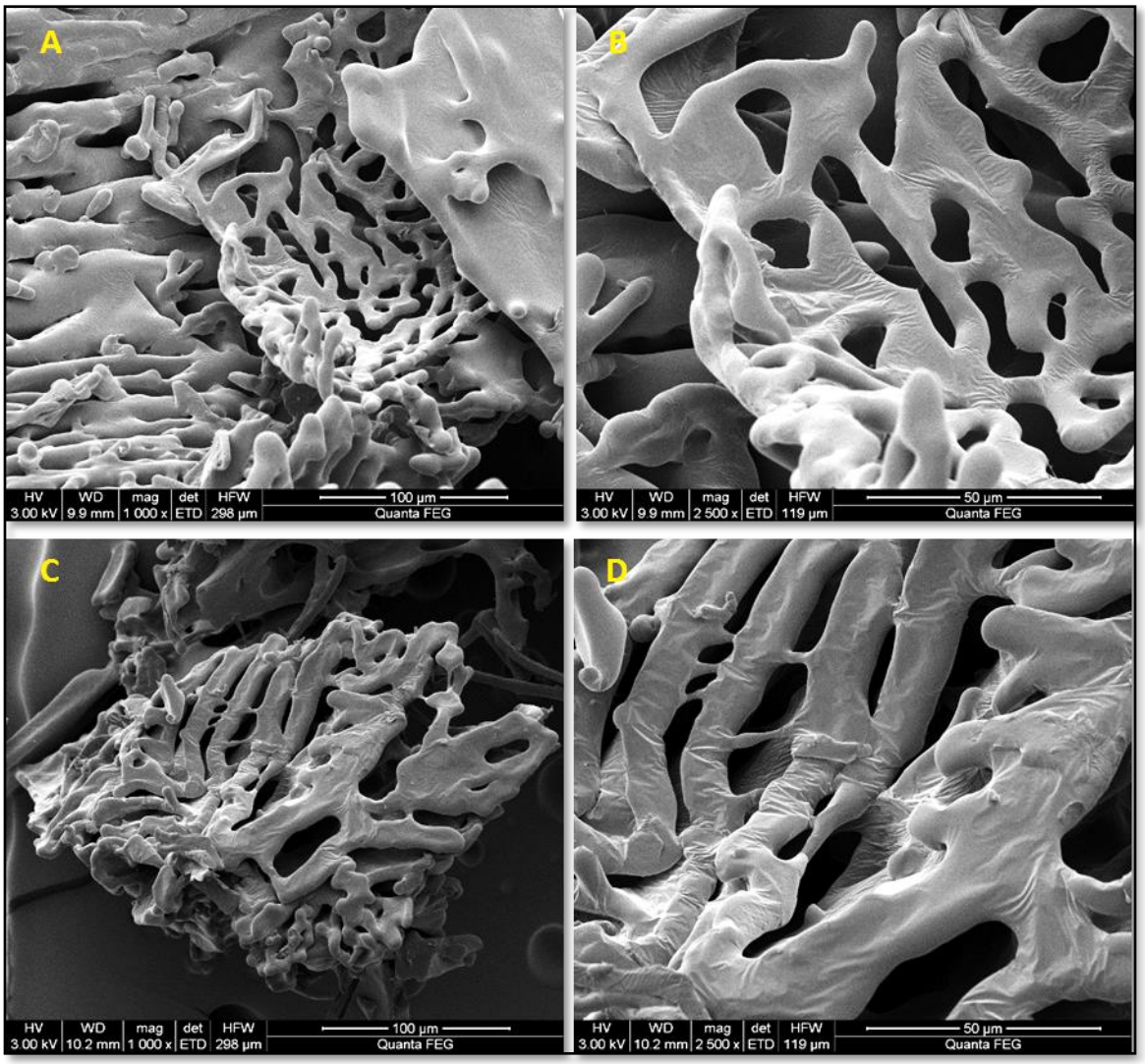

Figure 4-6 SEM Micrograph of foam-mat freeze-dried powder produced with trehalose + β-lactoglobulin (A and B) and trehalose + bovine serum albumin (C and D), at magnification 1000 x and 2500x respectively

The lower the freezing temperature, the smaller the ice crystal size due to the increase viscosity and decrease in the ice crystal growth. Therefore, the morphology of freeze-dried powder depends on the pore size and distribution and the condition of pulverisation. Our study showed that the pore size of two foam-mat freeze-dried products were the same due to the higher freezing rate and similar pulverisation

process. This result, however, did not confirm the solubility and rehydration time discussion presented above (section 4.4.5). Regarding the foam, the whipping process before freeze-drying generated fine bubbles in the blueberry juice. Palzer et al.[132] suggested that highly porous solid foams are obtained and depends on the amount of gas incorporated. Furthermore, the mean bubble size and the span of the bubble size distribution influenced the stability of the foam after rehydration of the foamed powders.

Figure 4-7 Photographs of foam-mat freeze-dried blueberry powder produced with trehalose + β-lactoglobulin (T3BL1) and trehalose + bovine serum albumin (T3A1)

4.4.7 Colour properties

The photographs of the foam-mat freeze-dried powder obtained with trehalose + β-lactoglobulin (T3BL1) and trehalose + bovine serum albumin (T3A1) are shown in Figure 4-7. The colour was evaluated in liquid samples, and because of this, the foam-mat freeze-dried blueberry powders were re-diluted with water, filtered and centrifuged to obtain a clear solution for colour measurement. The original blueberry juice indicated

dark purple with a small concentration of red and yellow (L = 0.08, a = 0.45, and b = 0.06). Regarding the t-test of colour (L* a* b*) properties, there were significant (P < 0.05) differences for T3BL1 and T3A1 samples, which implied that final colour of samples could be ascribed to the matrices and the foam-mat freeze-drying process.

A t-test of observed result shows that T3BL1 reconstituted solution was significantly (P-value = 0.000) lower L* value (brightness), i.e., 23.5 ± 0.04, or darker in comparison with the T3A1 sample, i.e., 39.6 ± 0.04 (Figure 4-8). As both matrices (β-lactoglobulin and bovine serum albumin) are white in the dry state, they showed brighter colour than the original blueberry juice. In the case of a* value of foam-mat freeze-dried reconstituted solutions, it is observed that pure proteins influenced the a* values. The T3BL1 showed statistically (P-value = 0.000) lower redness level (a* = 37.9) than the T3A1 product (a* = 44.9). The β-lactoglobulin had a direct impact on the b* (yellowness) of foam-mat freeze-dried blueberry reconstituted solutions. The b* value (P-value = 0.000) of T3BL1 sample was recorded as 19.2, while lower b* value was recorded from the T3A1 reconstituted solution, i.e. 15.4. Jimenez et al.,[112] reported the effect of the freeze-drying process of blueberry concentrated extract (without matrices) on the colour changes. According to the authors, the freeze-dried blueberry samples had L* = 30.2, a* = 41.1, and b* = 8.3. These results are in line with those of the present studies, except the b* was higher over the foam-mat freeze-dried blueberry samples.

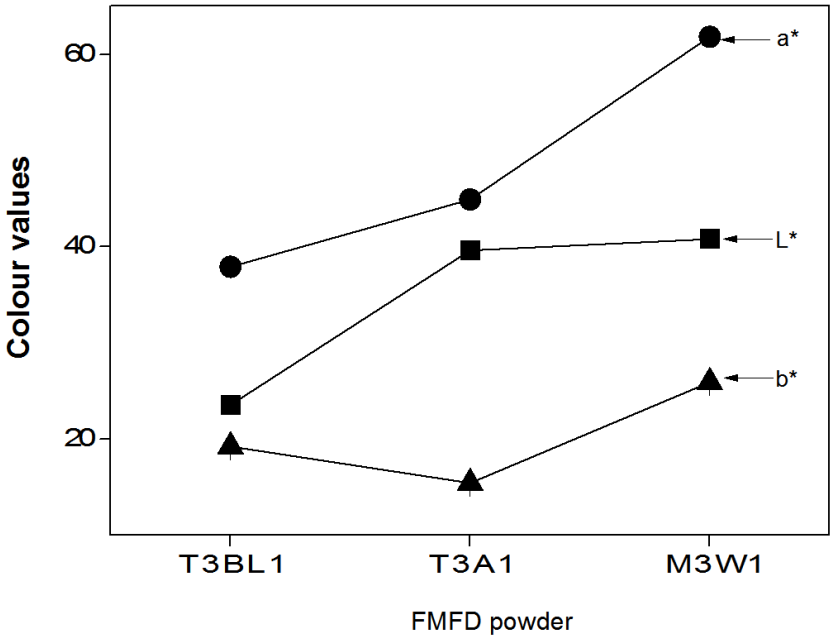

Figure 4-8 L* (■), a* (▽), and b* (●) values of reconstituted solution as a function ratio of trehalose + β-lactoglobulin (T3BL1) and trehalose + bovine serum albumin (T3A1). Results are expressed as means ± SD of triplicate determinations.

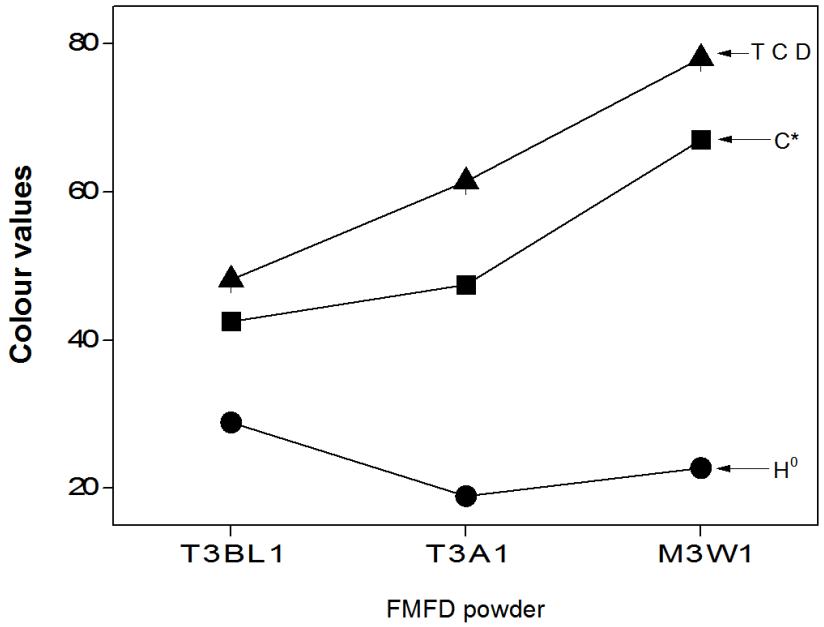

Figure 4-9 C* (■), H^0 (▽), and Total Colour Density/TCD (●) of reconstituted solution as a function of trehalose + β-lactoglobulin (T3BL1) and trehalose + bovine serum albumin (T3A1). Results are expressed as means ± SD of triplicate determinations.

Chroma/C*, hue angle/H^0, and total colour differences/TCD values of the foam-mat freeze-dried reconstituted solutions are presented in Figure 4-9. The C* and TCD values of T3BL1 were significantly (P-value = 0.000) lower than T3A1, while the H^0 of T3BL1 was statistically (P-value = 0.000) higher than T3A1 sample. In the case of the T3A1 solution, C* and TCD values of solution were increased. The C* and TCD of T3A1 solution were recorded as 47.5 and 61.4, respectively. Conversely, the T3BL1 exhibited low C* = 42.5 and TCD = 48.1. The H^0 value of the T3BL1 solution was found to be higher (28.9) according to T3A1 sample (18.9), and differences were found statistically important. However, both samples had a hue of $<45^0$, which can be described as red or red-orange.

4.4.8 Total phenolic content (TPC)

The total phenolic content of foam-mat freeze-dried blueberry powders, T3BL1 and T3A1, was recorded as 17.7 ± 0.08 and 14.5 ± 0.3 mg GAE g^{-1} solids, respectively. There was a significant (P-value = 0.000) difference in total phenolic content between both foam-mat freeze-dried samples. As shown in Figure 4-10, the total phenolic content of T3BL1 sample was higher in comparison with T3A1 sample. These results were consistent to those of Turan et al.[113] who reported that the total phenolic content of freeze-dried blueberry powder produced from blueberry juice and extract with the use of maltodextrin/gum arabic was 13.81 and 16.63 mg GAE g^{-1} solids, respectively. In the case of studies carried out by Wilkowska et al.,[107] phenolic content of *Vaccinium mrytillus* blueberry freeze-dried with the use of β-cyclodextrin was 1.4 mg GAE g^{-1} solids. Conversely, Franceschinis et al.[96] suggested that freeze-dried blackberry powder prepared by using maltodextrin/trehalose exhibited lower total phenolic content, i.e. 4.9 mg GAE g^{-1} solids, than our results. The total phenolic content of

freeze-dried highbush blueberries produced from fresh fruits, cultivars Duke and Reka, was 25.2 and 18.3 mg GAE g^{-1} solids, respectively.[116]

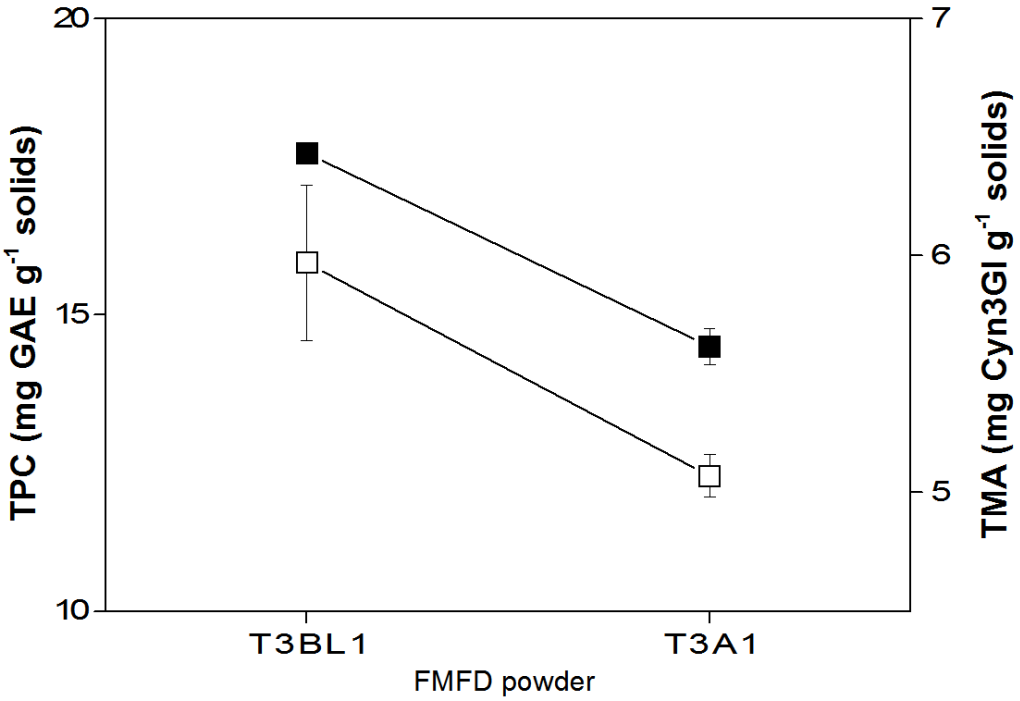

Figure 4-10 Total phenolic content (■) and total monomeric anthocyanins (□) of the reconstituted solution as a function of trehalose + β-lactoglobulin (T3BL1) and trehalose + bovine serum albumin (T3A1). Results are expressed as means ± SD of triplicate determinations.

4.4.9 Total monomeric anthocyanin (TMA)

The total monomeric anthocyanins of foam-mat freeze-dried blueberry powders, T3BL1 and T3A1, was recorded as 5.9 ± 0.33 and 5.1 ± 0.09 mg Cyn3Gl g^{-1} solids, respectively (Figure 4-10). As shown in the figure, the total monomeric anthocyanins of T3BL1 sample was significantly higher (P-value = 0.000) in comparison with T3A1 sample. Duangmal et al.[118] suggested that the addition of trehalose retarded anthocyanin degradation in freeze-dried Roselle powder. Turan et al.[113] showed that

anthocyanin content of freeze-dried blueberry juice and the extract was 22.7 and 60.7 mg Cyn3Gl g^{-1} solids, respectively. Thus, the retention of anthocyanin reached 89% and 98%, respectively. This differs from the finding presented here, probably due to the different matrices and *Vaccinium* species. In addition, the anthocyanin content of freeze-dried blueberry powder prepared by β-cyclodextrin was found higher, i.e., 11 mg Cyn3Gl g^{-1} solids, than the present results. The total monomeric anthocyanins content of the present study was much lower than other studies can be explained by the fact that complexation between anthocyanin and β-lactoglobulin; or anthocyanins and bovine serum albumin were less effective in preventing anthocyanins degradation.

4.4.10 Individual anthocyanins

Individual anthocyanin content of the foam-mat freeze-dried blueberry powder obtained with trehalose + β-lactoglobulin (T3BL1) and trehalose + bovine serum albumin (T3A1) is presented in Figure 4-11.Three individual anthocyanins, namely delphinidin-3-glucoside (Del3Gl), cyanidin-3-glucoside (Cyn3Gl), and malvidin-3-glucoside (Mal3Gl), were identified and quantified as their concentrations are high in the blueberry. There were no significant differences in Del3Gl (P-value = 0.87), Cyn3Gl (P-value = 0.92), and Mal3Gl (P-value = 0.45) between T3BL1 and T3A1 samples.

Del3Gl of T3BL1 sample was calculated as 0.63 mg g^{-1} solids, while T3A1 sample exhibited 0.67 mg g^{-1} solids. Cyn3Gl of T3BL1 sample was recorded as 0.85 mg g^{-1} solids, whereas T3A1 sample obtained 0.89 mg g^{-1} solids. In the case of Mal3Gl, the T3BL1 and T3A1 reconstituted solution were observed of 0.52 and 0.53 mg g^{-1} solids, respectively. Wilkowska et al.[107] revealed that individual anthocyanin of blueberry powder was influenced by freeze-drying and matrices. They suggested that freeze-

dried blueberry powder had Del3Gl = 1.82, Cyn3Gl = 1.04, and Mal3Gl = 1.0 mg g^{-1} solids. According to these results, the present study showed the lower concentration of all three individual anthocyanins measured. It was noticed that the type of matrix used in the freeze-drying process is an important factor for preventing degradation of anthocyanins.

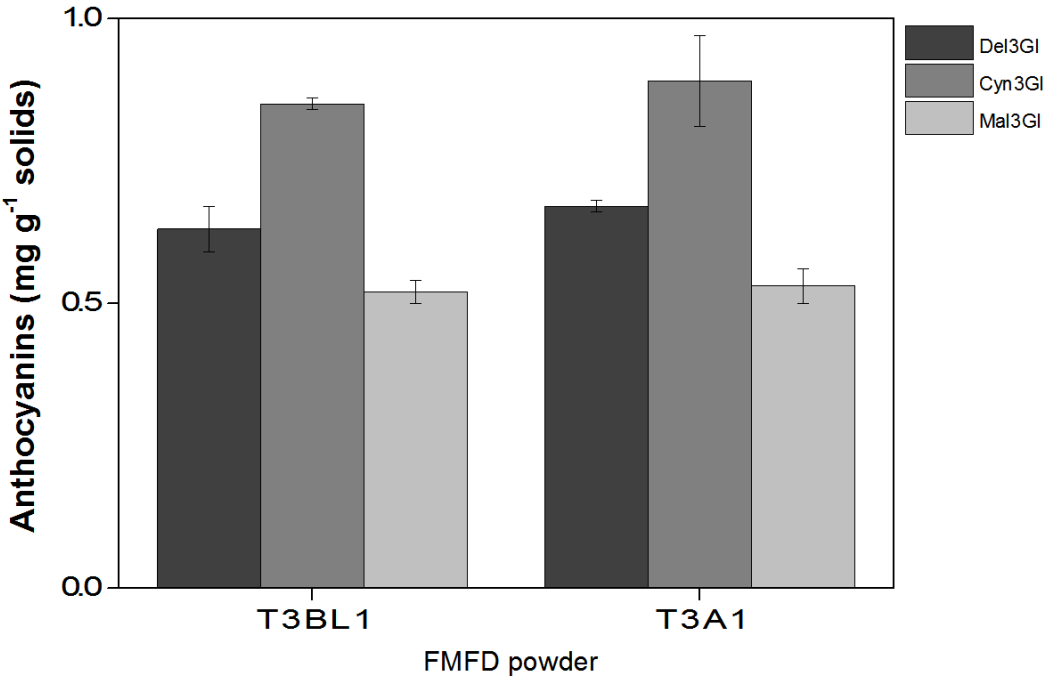

Figure 4-11 Del3Gl, Cyn3Gl, and Mal3Gl of the reconstituted solution as a function of trehalose + β-lactoglobulin (T3BL1) and trehalose + bovine serum albumin (T3A1). Results are expressed as means ± SD of triplicate determinations.

He at al.[133] suggested that added whey protein of into grape skin anthocyanin extract (GSAE) solution reduced the loss of anthocyanins and prevented colour fading, and increased the thermal, oxidation and photo stability of GSAE. This was attributed to the complexation of β-lactoglobulin and Mal3Gl via hydrophobic interaction with Ks of 0.67 x 10^3 M^{-1} at 297 K and pH 6.3. In the case of bovine serum albumin (BSA), Shi et

al.[134] reported that Cyn3Gl could be bound within the hydrophobic cavity in site II of BSA. This binding process of Cyn3Gl with BSA is spontaneous, and the main interaction forces of Cyn3Gl with BSA are Van der Waals and hydrogen bonding. Regarding BSA interaction with Del3Gl, Zuo et al.[80] suggested that Del3Gl mainly bound to BSA in the site I, which was in the large hydrophobic cavity of subdomain IIA. Furthermore, the pH and other metal ions had a significant impact on the binding. The process of binding Del3Gl with BSA was a natural molecular interaction, and hydrogen bonds and Van der Waals forces played a major role in the interaction.

4.4.11 Retention of TPC, TMA, and individual anthocyanin

Figure 4-12 shows the retention values of total phenolic content and anthocyanins of foam-mat freeze-dried blueberry powder produced with trehalose and pure proteins. There were significant (P-value = 0.000) differences in total phenolic compounds (TPC) and total monomeric anthocyanins (TMA) retention for both freeze-dried blueberry powders produced with T3BL1 and T3A1. In the case of individual anthocyanin, however, There were no significant differences in the retention of Del3Gl (P-value = 0.355), Cyn3Gl (P-value = 0.308), and Mal3Gl (P-value = 0.803). The TPC retention of foam-mat freeze-dried powdered samples was 41 and 34% for T3BL1 and T3A1, respectively. With the TMA retention, T3BL1 sample reached 57%, while T3A1 powder recovered 46%. These results were much lower to those of Jimenez et al.[112] who calculated the total phenolic content and total monomeric anthocyanins concentrations of freeze-dried blueberry powder without matrix as 97 and 100%, respectively.

After foam-mat freeze-drying, the individual anthocyanin retention of blueberry powders was found to be higher than those of Wilkowska et al.[107] They reported that

the retention of Del3Gl, Cyn3Gl, and Mal3Gl was 28, 28.5, and 21.5%, respectively. In the present study, the T3BL1 sample had Del3Gl, Cyn3Gl, and Mal3Gl retention of 46, 64, and 53%, respectively. On the other hand, the T3A1 sample had Del3Gl, Cyn3Gl, and Mal3Gl retention of 48, 67, and 54%.

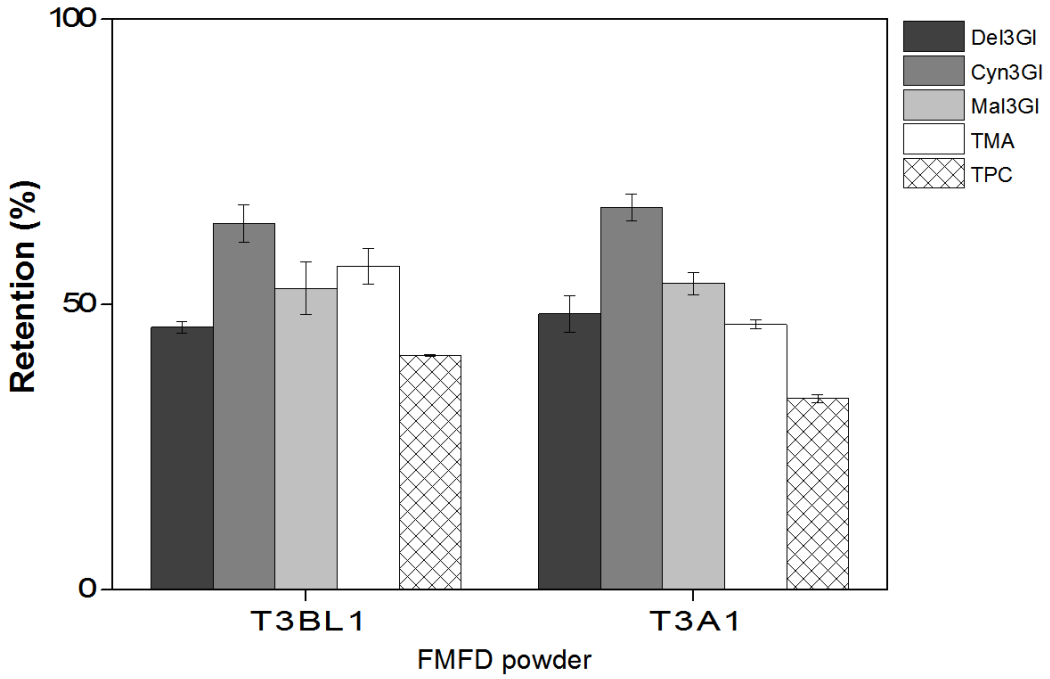

Figure 4-12 Retention of individual anthocyanins, TMA and TPC of foam-mat freeze-dried blueberry powder as a function of trehalose + β-lactoglobulin (T3BL1) and trehalose + bovine serum albumin (T3A1). Results are expressed as means ± SD of triplicate determinations.

4.5 Summary

Foam-mat freeze-dried blueberry powders had been successfully made with different matrices of trehalose and pure proteins. The sugar/protein ratio = 2.8 was used in this experiment. The higher density of foam was recorded from the T3BL1 sample compared to the T3A1-treated. However, both foam samples were not statistically different in stability with each other. The product yield of T3BL1 was 66%, whereas T3A1 presented yield of 67%. Both foam-mat freeze-dried blueberry powders, T3BL1 and T3A1, had a low value of moisture content (1.8 and 1.3%) and water activity (0.18 and 0.25), which were considered as microbiologically table products. In term of powder rehydration properties, both powders T3BL1 and T3A1 had different rehydration time, i.e. T3BL1 < T3A. As a result, the T3BL1-treated powder had slightly higher solubility than those of the T3A1-treated powder. The solubility of both foam-mat freeze-dried blueberry powders was > 95%. Bulk densities of the T3BL1 and T3A1 were observed equal in value. The foam-mat freeze-dried blueberry products indicated irregular particles and with structural resemblance between both samples. The T3BL1 reconstituted solution was darker and less red than the T3A1 solution. However, both samples had a hue of < 45^0, which can be described as red or red orange. In the case of chemical properties, the T3A1-treated sample highly reduced retention of the total phenolic content and total monomeric anthocyanins. On the other hand, the blueberry powder made with T3BL1 was better in prevention of anthocyanin and phenolic compound degradation than in the T3A1 sample. The individual anthocyanins of foam-mat freeze-dried blueberry powders had equal degradation either made with T3BL1 or T3A1 treatment.

Chapter 5

Chapter 5 Effect of feed flow rates and wall material ratios on the physicochemical of spray-dried blueberry powder and reconstituted product

5.1 Introduction

In this chapter, the spray-drying method was tested as an alternative drying method for producing food powders from liquid form, and the physicochemical of spray-dried blueberry powder was investigated and discussed. A comparison between powder properties of spray-dried and foam-mat freeze-dried blueberry powders will be discussed in Chapter 6.

Spray-drying of fruit juice with its high content of low molecular sugars and organic acids is relatively difficult. These conditions result in low values of glass transition temperature and consequently is related to several problems, such stickiness and caking.[32,37,135] To ease the drying process, it is common to add some carriers to the juice or concentrate. There are many studies on production of honey powder, watermelon, orange, mango and various concentrated fruit juice powders by spray-drying with whey protein, maltodextrin and gum Arabic as a carrier.[30,70,135,136] They utilised polysaccharides alone and/or polysaccharides + polysaccharides in spray drying process.

Shi et al.,[70] and Fang et al.[72] have successfully used proteins, either from plant or animal, as wall material in spray-drying of high-sugars content liquid. Previous studies have successfully spray-dried berry juice, including bayberry juice[36,42], black mulberry juice[110], blackberry juice[96,137], and blueberry juice.[111–113,138].

Another issue regarding spray-drying of blueberry juice is that utilisation of high drying temperatures (> 100 ^{0}C) could increase degradation of anthocyanins.[63,111] Jimenez-Aguilar et al.[112] reported that the anthocyanin losses of spray-dried blueberry powders obtained at 140 ^{0}C, i.e., 4%, less than those samples at 160 ^{0}C, i.e., 20%. However, higher temperatures in spray-drying of berry juices have several advantages in term of physical properties of the powder, including low moisture content and a_w, form a structure to fine powders; and low bulk density and particle density.[29] The feed flow rate has a negative impact on the yield and moisture content.[40] This is related to the slower heat and mass transfer occurring when the process was carried out with higher feed flow rates. Higher flow rates imply a shorter contact time between the feed and drying air. Also, the higher feed rates caused dripping inside the drying chamber of feed that is not atomised, resulting in a lower process yield.[40] Furthermore, Chegini & Ghobadian[139] reported that an increase in the inlet temperature and feed flow rate led to the high wall deposit and low yield.

To our knowledge, to date, only type and concentration of wall materials have been tested in spray drying of blueberry.[107,111,113,138] Therefore, the objective of the current study was to investigate the influence of feed flow rates and wall material ratios on the physical properties of the spray-dried blueberry powder and total phenolic content, total monomeric anthocyanins, and individual anthocyanin of reconstituted powder.

5.2 Spray drying conditions

The spray drying process is presented in Figure 5-1. Feed solutions were prepared with a weight ratio of juice to matrices = 9:1. The total solids fraction of all spray-dried solutions was fixed at 100 g kg^{-1}. Matrix weight ratios of MD/WPI were prepared at 0.4, 1.0, 1.6, 2.3 and 3.2, i.e., as for FMFD (Table 5-1). The spray-drying was performed in a Buchi B-290 mini spray dryer (Buchi Laborthecnik AG, Switzerland) (

Figure 5-2). The drying conditions were kept constant for each run with an inlet temperature of 150 ^{0}C, outlet air temperature of 101 ^{0}C, aspirator rate 100% (35 m^3 h^{-1}), air pressure 0.41 bar and nozzle tip diameter 1.5 mm. The feed rates used were 180 and 360 mL h^{-1} and spray drying results at these two feed rates referred to as SD 180 and SD 360. After each drying process, the blueberry powders were collected from the cyclone and stored in the dark in pre-weighed, air-tight containers in a refrigerator at 5 ^{0}C for further analysis. The spray-drying processes were all performed in duplicate for each set of conditions.

5.3 Statistical analysis

The processing treatments were duplicated, and the means of the results ± range were reported. Two-way analysis of variance (ANOVA) was done to establish the presence or absence of significant differences between means. Multiple comparisons were performed using the Tukey test, and significance level was set at $p < 0.05$. All statistical analyses were carried out using Minitab 17.0.

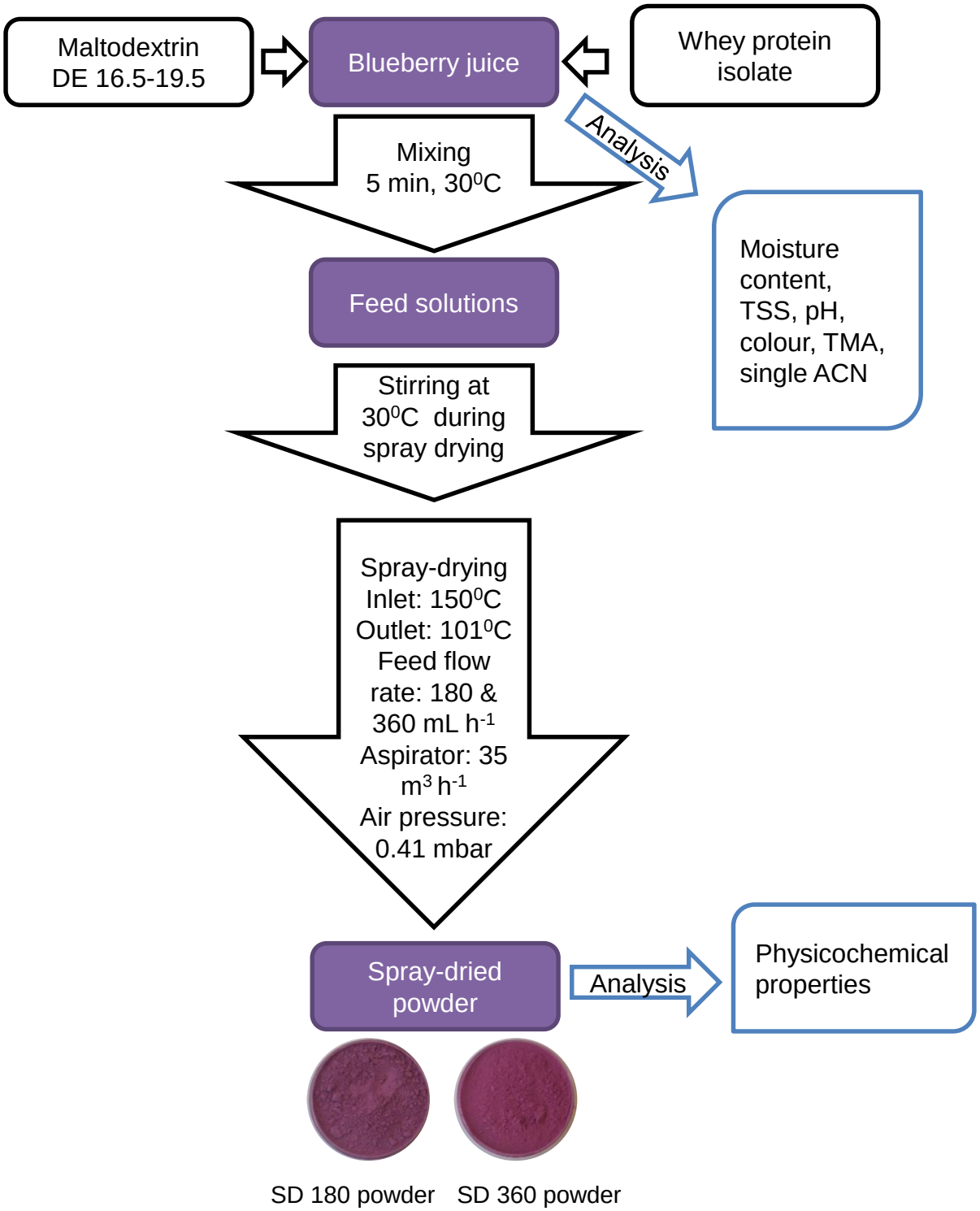

Figure 5-1 Spray-drying of blueberry juice with addition of maltodextrin (MD) and whey protein isolate (WPI)

Table 5-1 Maltodextrin (MD) and whey protein isolate (WPI) concentrations used in spray-drying of blueberry juice

MD/WPI	Maltodextrin (% w/w)	Whey protein isolate (% w/w)	*TSS juice + matrices (% w/w)
0.4	2.6	7.4	20
1.0	5.0	5.0	20
1.6	6.2	3.8	20
2.3	7.0	3.0	20
3.2	7.6	2.4	20

**Total soluble solids*

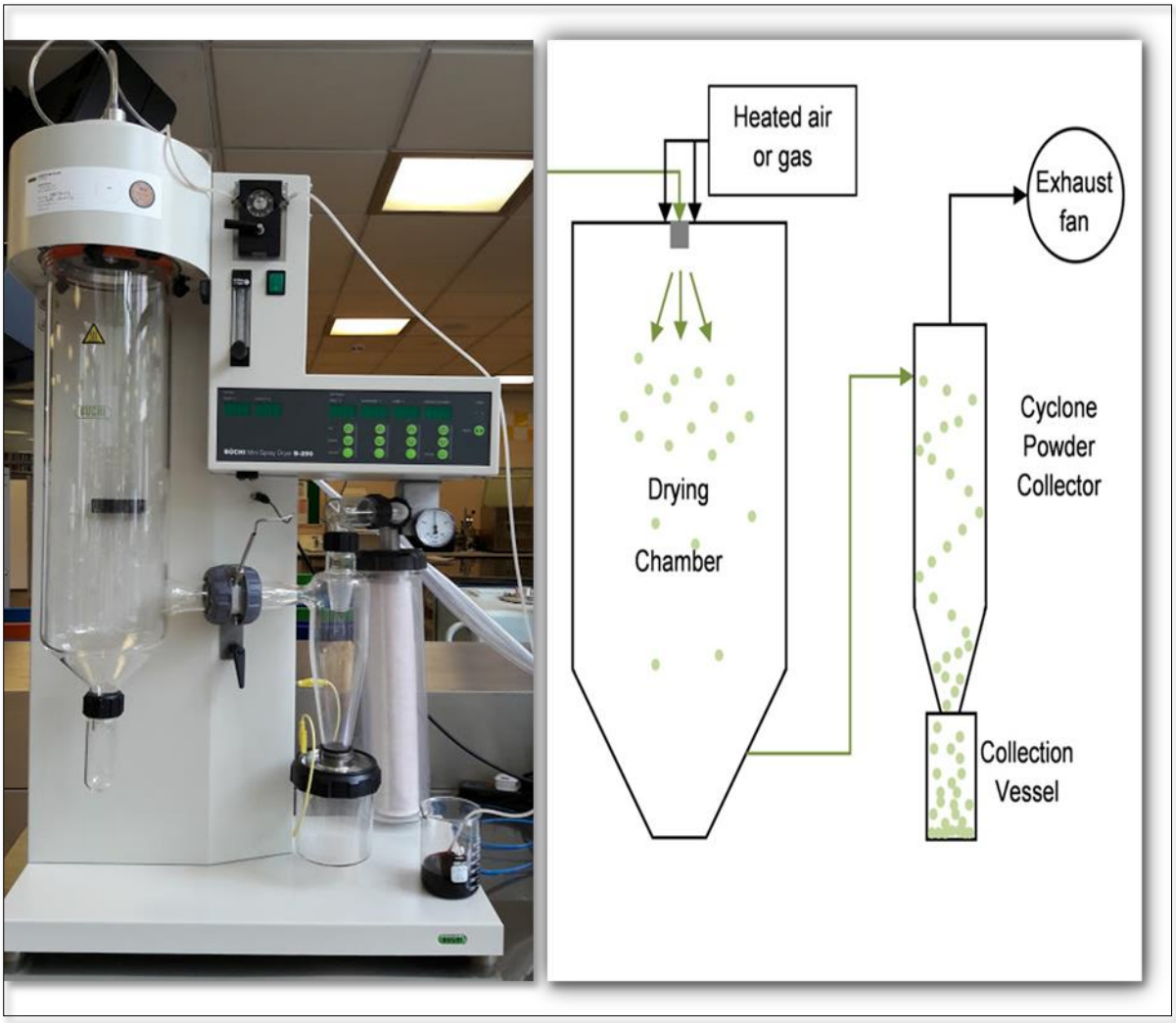

Figure 5-2 Buchi B-290 mini spray-dryer

5.4 Results and Discussion

5.4.1 Powder yield

In this study, the spray-dried (SD) powder was only collected from collection vessel and any particles deposited in drying chamber were discarded. Effect of feed flow rates and wall material ratios on the powder yield is presented in Figure 5-3. ANOVA of observed results shows that the feed flow rates and wall material ratios gave significant (P-value = 0.000) differences in the powder yield. The powder yield was a minimum of 61%, and maximum of 72%, for SD 180 sample with the MD/WPI = 0.4 and SD 360 with the MD/WPI = 2.3, respectively. These findings suggested an efficient spray drying according to the criteria of 50% powder recovery.[36] It is observed from the results that by increasing MD/WPI ratios, at two different feed rates, gave in increasing powder yield.

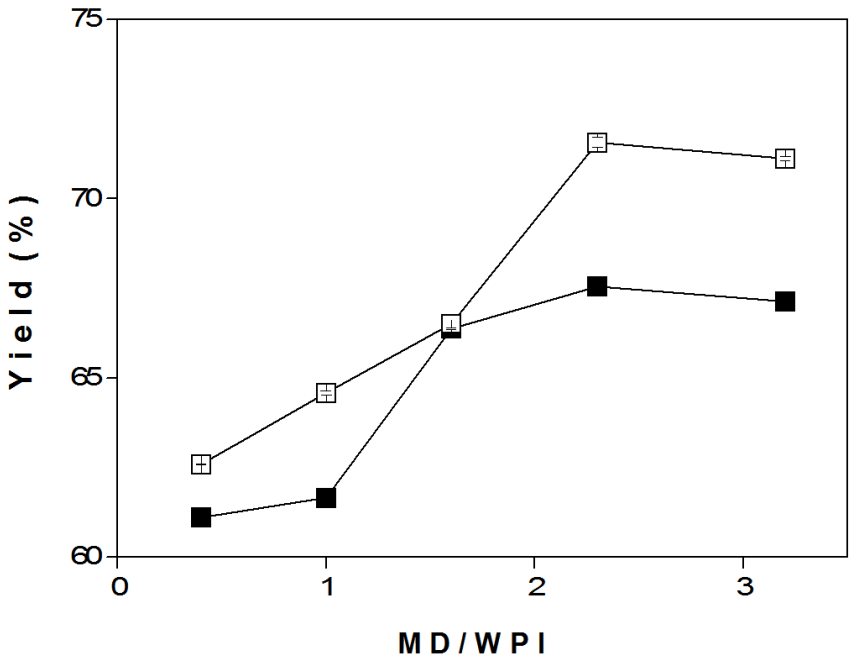

Figure 5-3 Yield of SD 180 (■) and SD 360 (□) powders as a function of the mass ratio of maltodextrin and whey protein isolate (MD/WPI). Results are expressed as means ± range of duplicate determinations.

This trend was attributed by the maltodextrin (MD) successfully in reducing the stickiness of particles in the drying chamber during spray-drying.[37] Therefore, the moisture of particles was evaporated by the high temperature and the dry particles then moved effectively to the collection vessel. Several studies found that the addition of drying aids (high molecular sugars and small amount of protein) successfully reduced stickiness of spray-dried of high-sugar food due to the maltodextrin was able to increase the T_g of feed solution and the protein can modify the surface active property (preferential migration to air/water interface) with their film forming ability upon drying.[45,72,108] Another reason is that the whey protein isolate (WPI) in the feed solution successfully increased the film coverage of the particles.[140] As a result, the particles from the nozzle (atomisation) released easily and contacted with the heat in the drying chamber.

The feed flow rates, i.e., 180 and 360 mL h^{-1}, also influenced the yield of spray-dried blueberry powder, where SD 180 had a lower (P-value = 0.000) yield than SD 360. This result is contradictory to Phisut[29] and Chegini and Ghobadian[30] who reported that the lower yield at higher feed flow rate due to heat and mass transfer from the medium (hot air) to the mixture as not as effective. Also, at a higher flow rate, a dripping of feed solution was observed in the drying chamber due to the mixture passing through to the chamber without atomisation. Thus, the yield becomes low. The possible explanation of our result is that the inlet temperature at 150 ^{0}C for spray-drying of blueberry juice was optimum at the feed flow rate 360 mL h^{-1}. Therefore, at feed flow rate 180 mL h^{-1}, an inlet temperature > 150 ^{0}C is required for increasing the heat transfer and yield.

5.4.2 Moisture content and water activity (a_w)

The moisture content of spray-dried blueberry powder is shown in Figure 5.4. It can be seen that the feed flow rates and wall material ratios have significant (P-value = 0.000) effect on the moisture content of the spray-dried blueberry powder. The lowest (1.7%) and highest (3.2%) moisture content was observed from SD 360 at MD/WPI 3.2 and 0.4, respectively. The SD 180 samples had a moisture content in a range of 2.2 to 3.0 %, while SD 360 samples were in a range of 1.7 to 3.2 %.

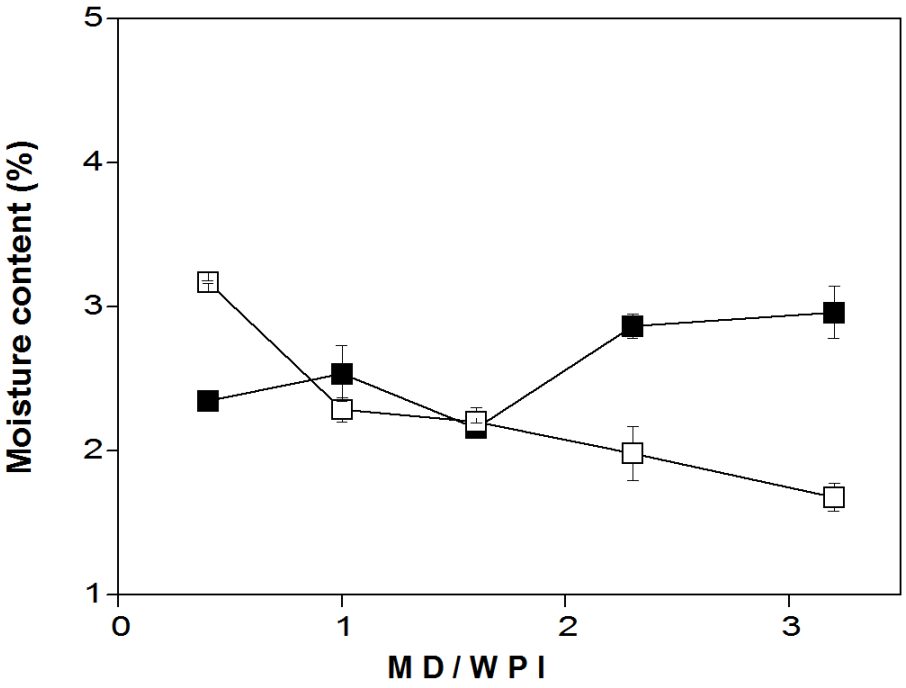

Figure 5-4 Moisture content of SD 180 (■) and SD 360 (□) powders as a function of the mass ratio of maltodextrin and whey protein isolate (MD/WPI). Results are expressed as means ± range of duplicate determinations.

These values fall within the commonly observed moisture content values in industrial spray drying.[32] The increased feed flow rate resulted in slightly higher moisture content due to the increased load in drying chamber.[29,68] The higher the feed load, the particles

have less contact time to heat in the drying chamber. Also, larger droplets containing more moisture are produced as a result of increasing the powder moisture content.[30] The presence of drying aids (MD and WPI) also affected the moisture content. The SD 360 samples show decreased trend of moisture content by the increasing of MD/WPI ratios, while those of SD 180 samples slightly increased with the increasing of MD/WPI ratios. These findings could be explained by the fact that addition of MD and WPI resulted in high feed solids and a reduction in total moisture content for evaporation.[68] However, Goula & Adamapoulos[31] showed an increase in moisture content with an increase in MD concentration. Concerning WPI, Adhikari et al.,[141] found that the additional small amount of whey protein isolate (≤ 1% w/w) in the feed solution modified the surface properties of the droplets/particles. Therefore, the particles covered by film-forming property upon drying, minimise the stickiness and moisture content.

The average water activity of the samples is presented in Figure 5-5. ANOVA of observed data shows that the feed rates and wall material concentrations had significant (P-value = 0.000) effect on the water activity. The a_w of spray-dried blueberry powder was in a range from 0.09 to 0.17 and from 0.21 to 0.23, for SD 180 and SD 360 samples, respectively. These results can be considered quite microbiologically stable as the $a_w < 0.6$.[38] According to Fazaeli et al.[110] reported that high water activity indicates more free water available for biochemical reactions and hence, shorter shelf life. The data also showed that the water activity declined with higher MD concentration. Other works have reached similar findings.[38,110]

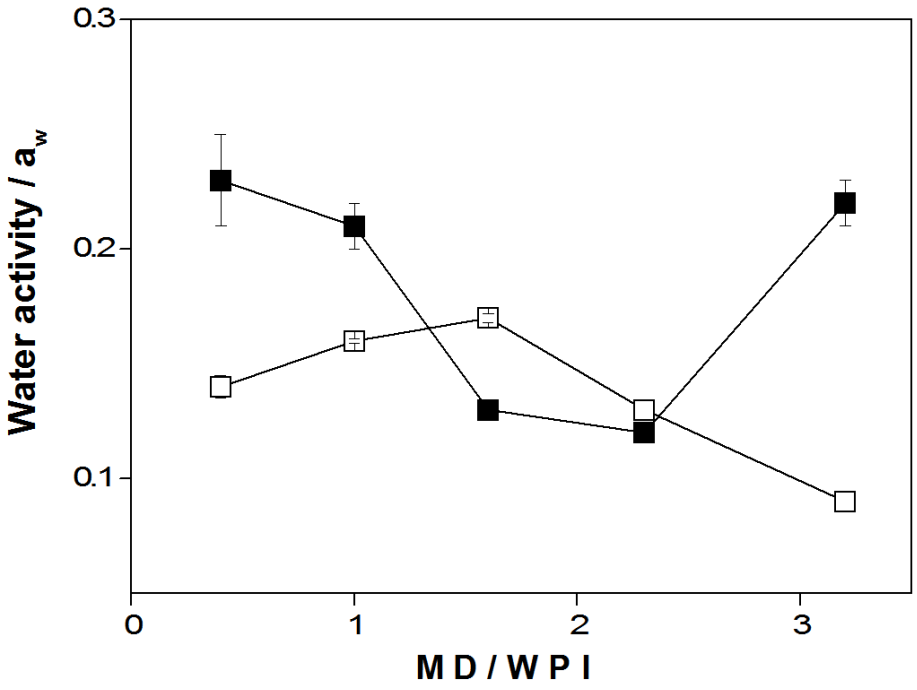

Figure 5-5 Water activity of SD 180 (■) and SD 360 (□) powders as a function of the mass ratio of maltodextrin and whey protein isolate (MD/WPI). Results are expressed as means ± range of duplicate determinations.

5.4.3 Solubility, rehydration time, and bulk density

Fruit spray-drying requires knowledge of its properties and factors affecting the process. Solubility problems occur when foods are submitted to high temperatures, and especially in products with high concentration of solids.[41] The effect of feed flow rates and wall material ratios on the solubility of spray-dried blueberry powder was analysed (Figure 5-6). ANOVA of observed results shows the feed flow rates and wall material concentrations had no significant (P-value = 0.972) effect on the solubility of the samples. The average solubility of SD 180 was in a range of 97-98.5%, while for the SD 360 samples, the solubility was in a range of 96-96.7%. The very high solubilities may be attributed to the fact that MD has high solubility in water.[31,110] Also, the inlet air temperature at 150 ^{0}C (with constant outlet air temperature at 101 ^{0}C) may

be attributed in forming a medium-soft surface layer. Consequently, water molecules diffused easier through the particles and increased the solubility of the powder. Sharifi et al.,[142] also support these findings, where they found the solubility of spray-dried barberry powders was not statistically ($P > 0.05$) different amongst all samples by the increasing MD concentrations (0-11.25 % w/w).

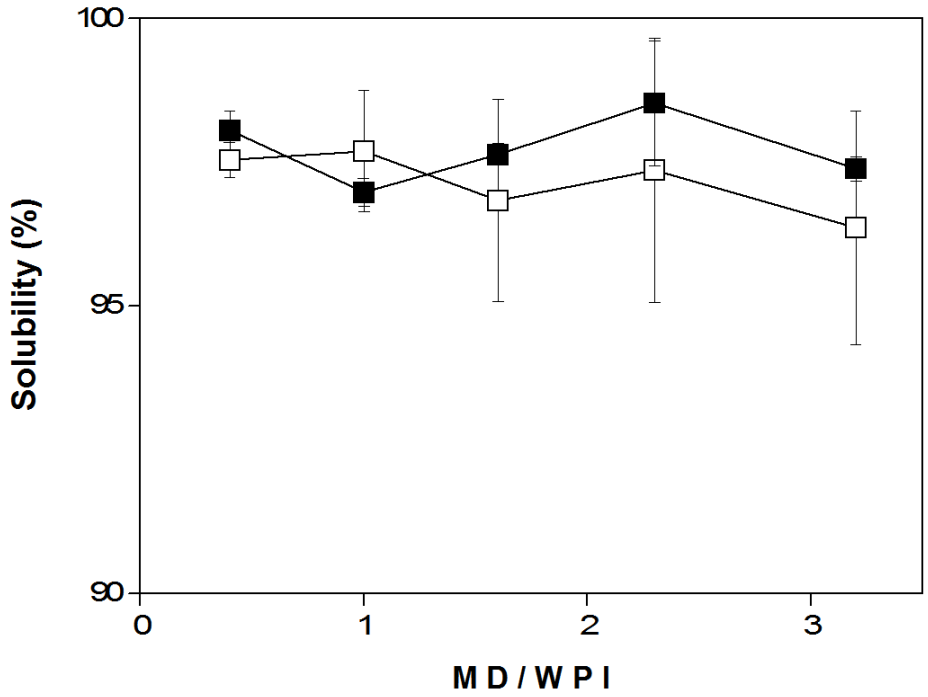

Figure 5-6 Solubility of SD 180 (■) and SD 360 (□) powders as a function of the mass ratio of maltodextrin and whey protein isolate (MD/WPI). Results are expressed as means ± range of duplicate determinations.

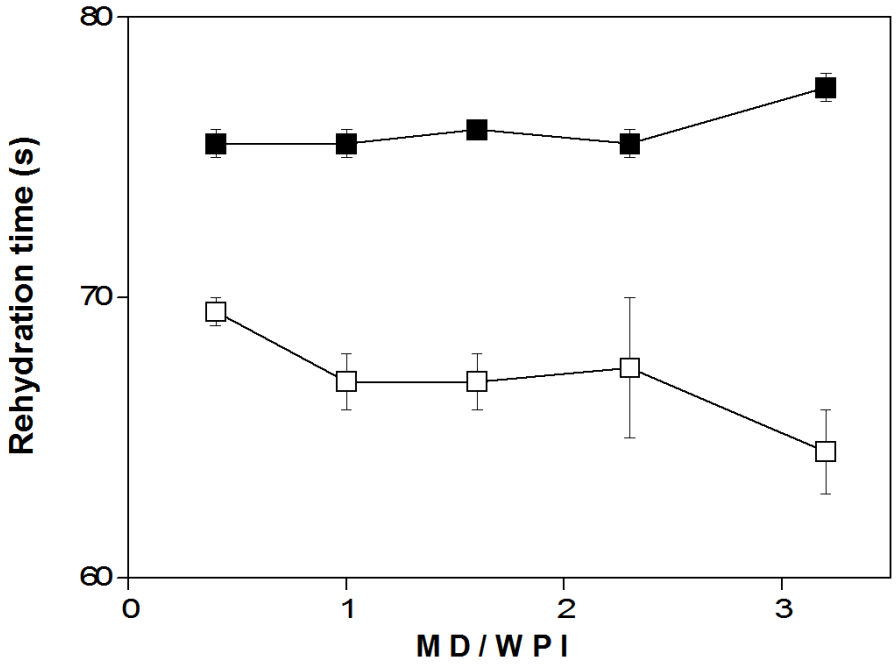

Figure 5-7 Rehydration time of SD 180 (■) and SD 360 (□) powders as a function of the mass ratio of maltodextrin and whey protein isolate (MD/WPI). Results are expressed as means ± range of duplicate determinations.

Figure 5-7 presents the rehydration time of spray-dried blueberry powder. The feed flow rates and wall materials ratio had no significant (P-value = 0.082) effect on the rehydration time. However, the feed flow rates had significant (P- value = 0.000) effect on the rehydration time. The SD 360 samples obtained faster rehydration time, i.e., 65-69 s in comparison with those from the SD 180 samples, i.e., 76-78 s. This result is contrary to previous studies which have reported that the feed rate has a negative impact on the moisture.[29,40] Higher feed rates imply in a shorter contact time between the feed and drying air and making the heat transfer less efficient and thus caused lower water evaporation.[40] Therefore, the moisture content of the powder relatively high. These types of powders had a strong tendency of agglomeration which helped to increase the reconstitution of the powders.[31,135] However, our findings reflect those

of Islam et al.[93] who also found that the rehydration of vacuum spray-dried orange juice powder was influenced by increasing MD concentration and low moisture content of powder. Again, the fast rehydration time may be due to the high MD solubility in water and the low moisture content of the powders.[41]

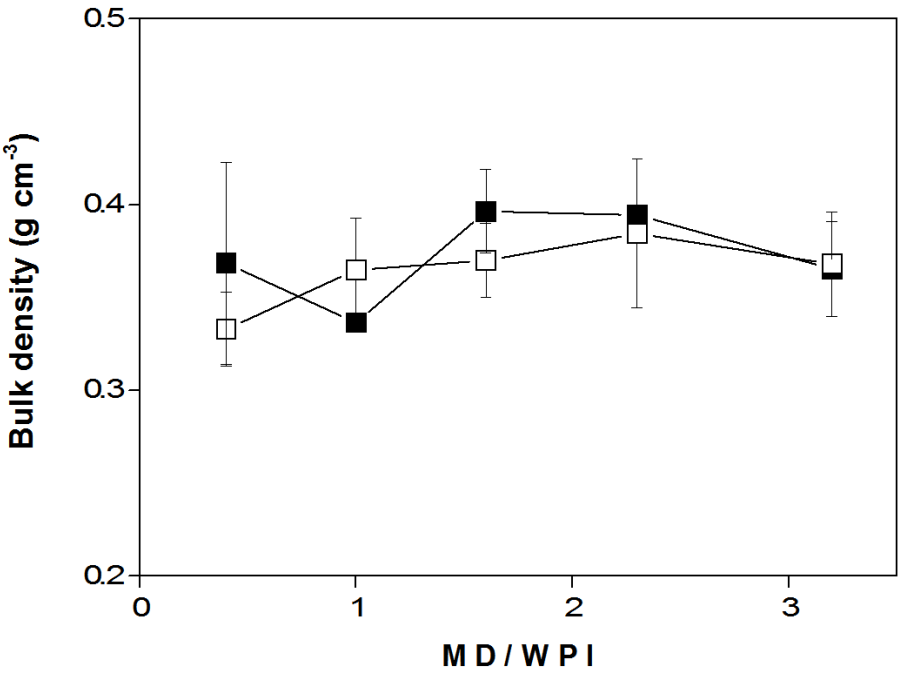

Figure 5-8 Bulk density of SD 180 (■) and SD 360 (□) powders as a function of mass ratio of maltodextrin and whey protein isolate (MD/WPI). Results are expressed as means ± range of duplicate determinations.

From Figure 5-8, it is observed that the bulk density of spray-dried blueberry powder had no significantly (P-value = 0.806) difference among all the samples produced with feed flow rate 180 and 360 mL h^{-1}. However, our findings are contrary to that of Jumah et al.[143] and Goula et al.[31] who found that the bulk density and solubility were in an inverse relation. Their studies examined the different feed rates and inlet air temperature during spray-drying. Therefore, it may be that the primary effect on the

bulk density was due to the inlet air temperature, instead of feed flow rate. At very high temperatures, since water evaporation is faster, the product dries to a more porous structure and so a lower density of the powders.[110]

5.4.4 Particle morphology

The particles produced by the spray-drying have different sizes morphologies and structures, depending on the spray drying parameters such as inlet air temperature, atomiser speed, feed flow rate, air pressures, type and concentration of drying agents.[33] In this study, the SEM was operated (5000 x magnification) to identify the particles. As shown in Figure 5-9, generally, it is observed that the spray-dried blueberry powders appeared as ballooned, shrivelled and spherical particles, typical of spray-dried powders. In the case of SD 180 samples, at low MD/WPI ratios, the particles are skin-like and hollow, have shrivelled up structures as shown in Figure 5-9A. In contrast, the shape of the particles appeared smooth spherical or shrivelled at high MD/WPI ratio as ilustrated in Figure 5-9B. This may be because the MD produces soft and porous crust. With regard to SD 360 samples, it was observed that the spray-dried powders particles were mainly with some spherical and shrivelled particles, for both wall material concentrations, as shown in Figure 5-9C and D. Tonon et al.[144] and Ersus & Yurdagel[145] observed the same behaviour for spray-dried Acai juice powder and black carrot powder, respectively. They found that the hard skin of the particles was formed at high temperatures. As a result, the hollow particles cannot deflate when vapour condenses within the vacuole as the particles move into a cooler region of the dryer.[144,145] Conversely, the skin remains moist and supple for longer at lower drying temperatures, so that the hollow particles can deflate and shrivel as it cools.[144,145]

Our findings are also consistent with Fazaeli et. al[110] who suggested that MD (DE19.5) produced mulberry powders with large amorphous and agglomerated particles. This can be attributed to the fact that the higher the maltodextrin DE, the lower glass transition temperature and the stickier the particles.

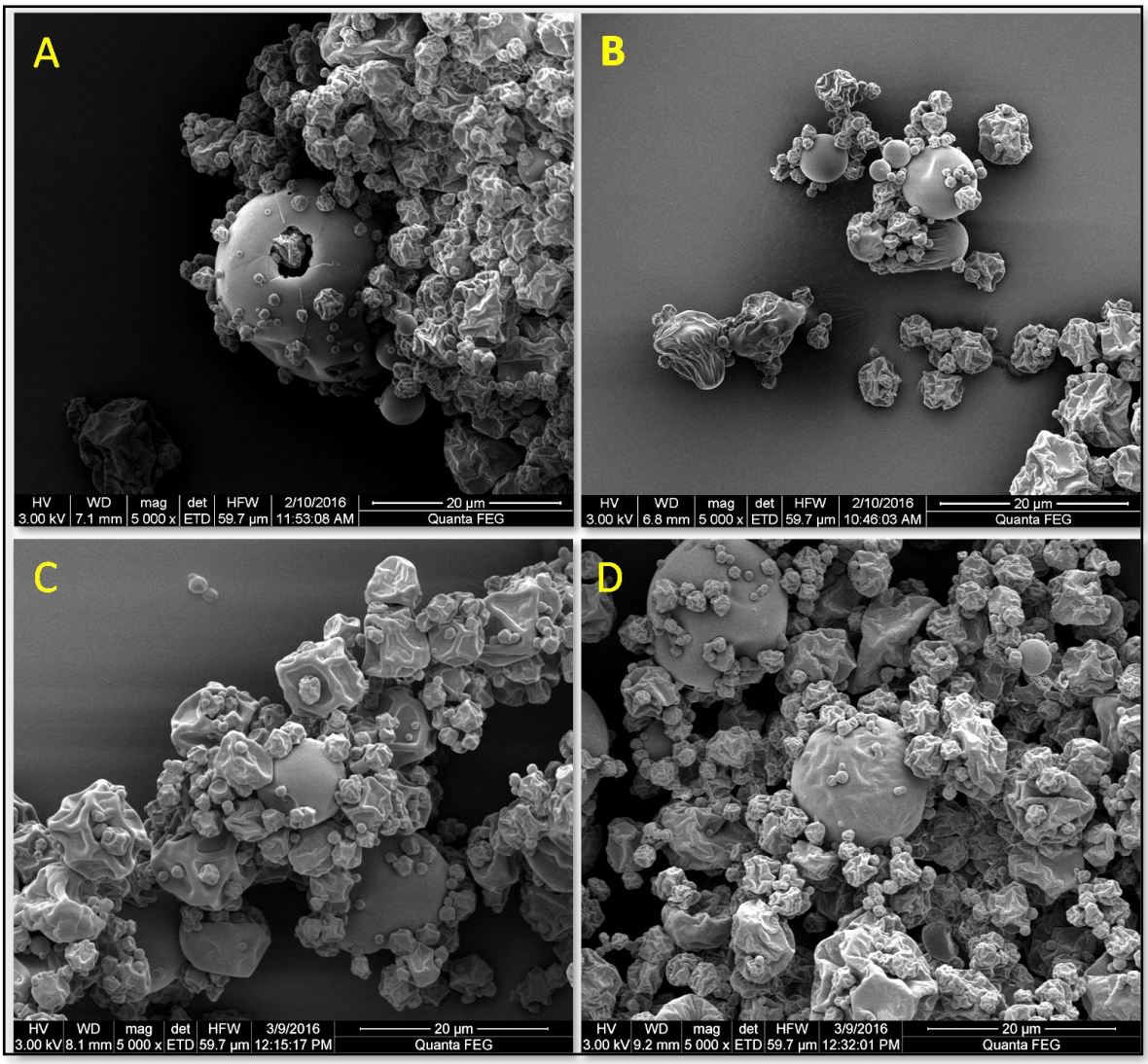

Figure 5-9 SEM micrograph of SD 180 made with MD/WPI 0.4 (A) and 3.2 (B), and SD 360 with MD/WPI 0.4 (C) and = 3.2 (D), magnification 5,000x

5.4.5 Colour properties

The feed flow rates and wall material ratios effect on the spray-dried blueberry powder were investigated by using a colourimeter with a CIELab scale (L*, a* and b*). The visual photos of spray-dried blueberry powder are shown in Figure 5-10. The colour was evaluated in liquid samples, because of that, the spray-dried blueberry powder were re-diluted with water, filtered and centrifuged to obtain a clear solution for colour measurement**.**

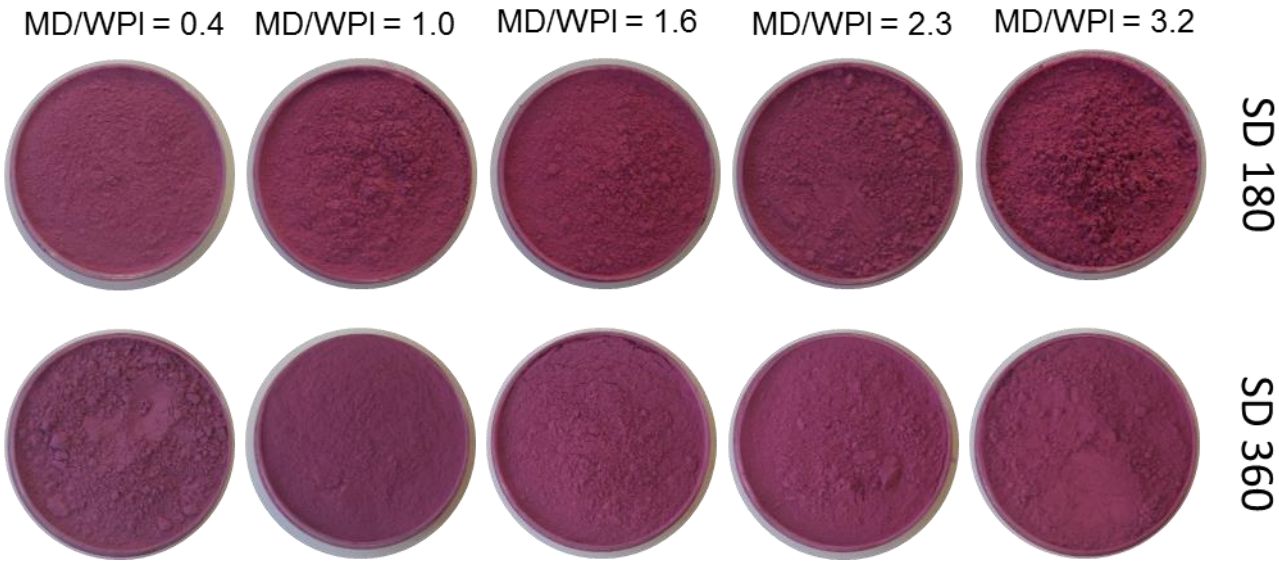

Figure 5-10 Photographs of spray-dried blueberry powder produced with feed rate 180 and 360 mL h^{-1}

According to Table 5-2, feed flow rates and wall material ratios had a significant (P-value = 0.000) effect on the L*/brightness value of the SD reconstituted solutions. The L* value was in a range from 51.9 to 53.1 and from 51.6 to 60.3, at the feed rate 180 and 360 mL h^{-1}, respectively. The L* (brightness) values were significantly ($P < 0.05$)

higher for the SD 360 samples produced at the MD/WPI ratio = 0.4 and 1.0 than those of SD 180 samples (Table 5-2). Conversely, the MD/WPI ratio = 1.6 and 3.2 of SD 180 samples gave statistically ($P < 0.05$) higher L* value than the SD 360 products. The L* values were found significant at the MD/WPI = 2.3 (for both SD 180 and SD 360 samples). This inconsistency result may be due to the dilution of powder in pure water, which is less stable in comparison with acidic water. However, these results are in line with those obtained by Franceschinis et al.[96] who found that the L* value of spray-dried blackberry powder was about 68 by using MD and trehalose as wall materials. Also, the spray-dried blackberry powder gave significantly higher L* values compared to those obtained by the freeze-drying process.[96] This result may be explained by the fact that greater proportion of wall material in spray-dried samples than freeze-dried samples.

Table 5-2 Brightness (L*), redness (a*) and yellowness (b*) value of blueberry juice and SD reconstituted solutions

Sample	MD/WPI	L*	a*	b*
SD 180 reconstituted solutions	0.4	52.2 ± 0.05 ^{e}	56.2 ± 0.04 ^{b}	12.6 ± 0.03 ^{c}
	1.0	51.9 ± 0.03 ^{f}	47.4 ± 0.04 ^{h}	10.2 h
	1.6	53.1 ± 0.03 ^{c}	53.0 ± 0.03 ^{f}	11.0 ± 0.01 ^{d}
	2.3	52.5 ± 0.03 ^{d}	54.7 ± 0.03 ^{d}	12.3 ± 0.01 ^{d}
	3.2	51.9 ± 0.02 ^{f}	56.3 ± 0.02 ^{b}	13.1 ± 0.02 ^{b}
SD 360 reconstituted solutions	0.4	60.3 ± 0.02 ^{a}	42.5 ± 0.03 ^{i}	7.0 ^{j}
	1.0	53.6 ± 0.02 ^{b}	49.4 ± 0.04 ^{g}	8.8 ± 0.01 ^{i}
	1.6	51.6 ± 0.03 ^{g}	53.3 ± 0.04 ^{e}	11.2 ± 0.01 ^{f}
	2.3	52.2 ± 0.02 ^{e}	55.0 ± 0.03 ^{c}	11.5 ± 0.02 ^{e}
	3.2	51.8 ± 0.03 ^{f}	57.0 ± 0.03 ^{a}	13.2± 0.02 ^{a}

Mean values ± range of duplicate determinations, followed by a different single letter in each column if significantly different ($p < 0.05$, Tukey's test), whereas ab, for example, indicates values that are not significantly different from the corresponding a and b values.

The a* (redness) values of spray-dried powder is also presented in Table 5-2. The feed flow rates and wall material ratios had a significant (P-value = 0.000) effect on the a*/redness value of the SD reconstituted solutions. The feed flow rate 180 mL h^{-1} (SD 180) obtained the a* in a range from 47.4 to 56.3 while the feed flow rate 360 mL h^{-1} (SD 360) had a* value in a range 42.5 to 57.0. The a* values increased by the increasing of MD/WPI ratio at both feed flow rates, except at the MD/WPI ratio = 1.0 of SD 180 sample. These results were higher than spray-dried blackberry powders which contain 7% MD alone (a* = 23) and 7% gum Arabic alone (a* = 19).[137] From our results, it can be explained that the high a* values can be related to anthocyanin content, because wall materials (MD and WPI) increase the feed solution viscosity and effective heat and mass transfer during drying. Therefore, the powders recovered high anthocyanin which has red colour in the dry state.

Feed flow rates and wall material ratios had a significant (P-value = 0.000) effect on the b*/yellowness value of the SD reconstituted solutions. The trend in b* values or yellowness was similar to the a* values, the increased MD/WPI ratio resulted in increasing the b* values for both feed rates (Table 5-2). An exception is observed at the MD/WPI ratio = 1.0 with SD 180 sample. The b* value of SD 180 sample was in a range from 10.2 to 13.1, whereas SD 360 was in a range from 7.0 to 13.2. These results were higher compared to spray-dried blackberry powders (e.g. b* = 3)[96,146]. The high b* value shows that the particles have undergone browning as a result of high temperature during spray-drying.

The values of chroma, hue and total colour difference (TCD) is displayed in Table 5-3. The feed flow rates and wall material ratios had a significant (P-value = 0.000) effect on the C*/Chroma value of the SD reconstituted solutions. The SD 180 samples

obtained chroma values with a range from 48.5 to 57.8, while 43 to 58.5 was recorded from the SD 360 samples. It is observed that the increase of MD/WPI ratios led to increasing the chroma values of all samples. This phenomenon can be explained by the wall materials (MD and WPI) cause slightly increased lightness along the increasing of redness and yellowness values.

Table 5-3 Chroma (C*), hue angle (H^0), and TCD of blueberry juice and SD reconstituted solutions

Sample	MD/WPI	C*	H^0	TCD
SD 180 reconstituted solution	0.4	57.6 ± 0.05 ^{c}	12.6 ± 0.02 ^{e}	77.4 ± 0.01 ^{b}
	1.0	48.5 ± 0.04 ^{h}	12.1 ± 0.00 ^{d}	70.6 ± 0.01 ^{j}
	1.6	54.1 ± 0.03 ^{f}	11.7 ± 0.01 g	75.5 ± 0.01 ^{f}
	2.3	56.1 ± 0.03 ^{d}	12.7 ± 0.01 ^{c}	76.4 ± 0.01 ^{d}
	3.2	57.8 ± 0.02 ^{b}	13.1 ± 0.02 ^{b}	77.3 ± 0.01 ^{c}
SD 360 reconstituted solution	0.4	43.0 ± 0.03 ^{i}	9.3 ± 0.00 ^{i}	73.6 ± 0.01 ^{h}
	1.0	50.2 ± 0.04 ^{g}	10.1 ± 0.01 ^{h}	73.0 ± 0.0 ^{i}
	1.6	54.5 ± 0.04 ^{e}	11.9 ± 0.00 ^{f}	74.6 ± 0.0 ^{g}
	2.3	56.2 ± 0.03 ^{d}	11.9 ± 0.01 ^{f}	76.3 ± 0.01 ^{e}
	3.2	58.5 ± 0.03 ^{a}	13.0 ± 0.01 ^{a}	77.7 ± 0.01 ^{a}

Mean values ± range of duplicate determinations followed by a different single letter in each column if significantly different ($p < 0.05$, Tukey's test), whereas ab, for example, indicates values that are not significantly different from the corresponding a and b values.

Feed flow rates and wall material ratios had a significant (P-value = 0.000) effect on the hue angle (H^0) values (Table 5-3). Overall H^0 values of spray-dried blueberry reconstituted powder were < 45^0. Therefore they can be described as red samples powder.[113] The increased MD/WPI ratio led to increasing the H^0 values. The H^0 of the spray-dried reconstituted powders was in a range 11.7-13.1^0 and 9.3-13^0, for SD 180 and SD 360 samples, respectively. These results are consistent with Jimenez-Aguilar

et al.[112] who reported H^0 values of the spray-dried blueberry powder obtained using mesquite gum at two different air inlet temperatures, i.e., 140 and 160 ^{0}C, range from 5 to 11^0. Lee et al.[114] also reported the H^0 values of grapefruit juice < 45^0 as pure red samples.

Table 5-3 also presents the total colour difference (TCD). The feed flow rates and wall materials ratios had a significant (P-value = 0.00) effect on the TCD. The SD 180 samples obtained the TCD 70.6 – 77.3, while the SD 360 samples ranged 73 – 77.7. As the MD/WPI ratio was increased the TCD increased significantly, i.e., the a* values were higher, which is attributed to the total anthocyanins content (see later). The SD 360 sample at the MD/WPI ratio = 3.2 showed higher the TCD (77.7) than the TCD of SD 180 sample (77.3) at the same proportion. This phenomenon can be explained by the higher feed flow rate had less contact with heat. Thus, the a* values remain high and less changed.

5.4.6 Total phenolic content (TPC)

Figure 5-11 shows the total phenolic content of spray-dried blueberry powder obtained at the feed flow rate 180 and 360 mL h^{-1} with different MD/WPI ratio. The feed flow rates and wall material ratios had no significant (P-value = 0.954) effect on the total phenolic content of spray-dried blueberry powder. TPC of SD 180 samples was in a range of 20.8 - 29.8 mg GAE g^{-1} solids, while 23.3 - 31.1 mg GAE g^{-1} solids was found in SD 360 samples. Jimenez-Aguilar et al.[112] reported similar phenomena in spray-dried blueberry powder with mesquite gum as wall material. They reported that the blueberry powder obtained at feed flow rates at 510 and 576 mL h^{-1} (inlet temperature 160 ^{0}C), had no significant (P > 0.05) different on the TPC values, i.e., 18.3 and 18.7

mg GAE g^{-1} solids, respectively. TPC value was also found to have no significant ($p > 0.05$) difference in the spray-dried blueberry powders obtained with low inlet temperatures (140 ^{0}C) and feed flow rates (510 mL h^{-1}), i.e., 19.71 mg GAE g^{-1} solids.[112]

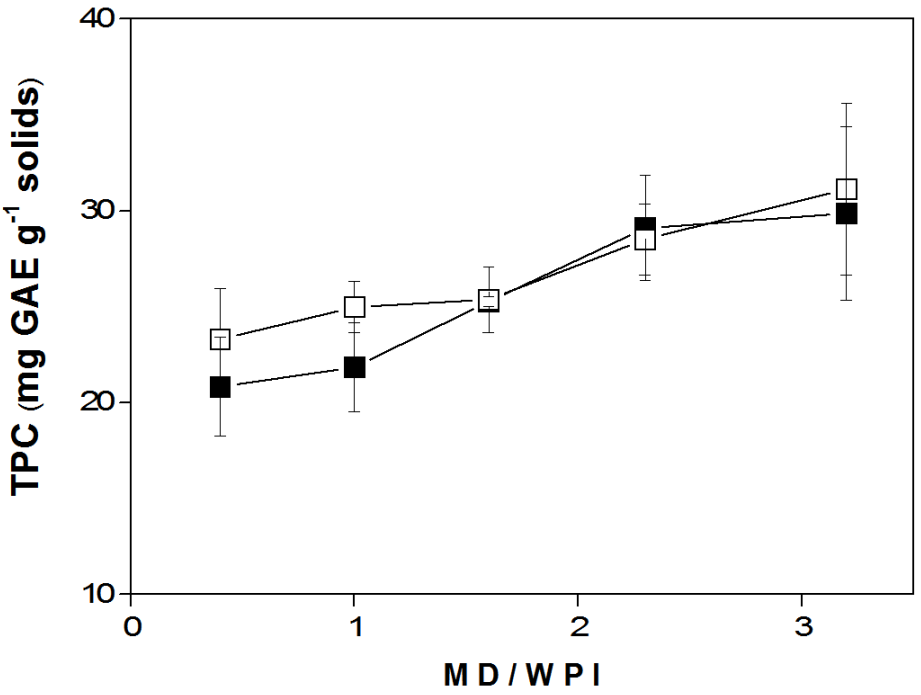

Figure 5-11 Total phenolic content (TPC) of SD 180 (■) and SD 360 (□) powders as a function of the mass ratio of maltodextrin and whey protein isolate (MD/WPI). Results are expressed as means ± range of duplicate determinations.

With the wall material ratios, TPC of spray-dried blueberry powder was found to increase by increasing MD/WPI ratios at both feed flow rates, 180 and 360 mL h^{-1}. These results suggested that incorporation of MD and WPI into blueberry juice was able protecting the phenolic compounds from heat exposure in the main drying chamber. Lim & Dolan[138] also reported that increasing MD concentrations, i.e., 70, 90 and 95% w/w solids, could raise the total phenolic content in spray-dried blueberry

powders with blueberry by-product as a raw material. The averages of three samples were 24.61, 30.15, and 33.53 mg GAE g^{-1} solids, with the percentage in total phenolic per gram of blueberry solids after spray drying 24%, 17%, and 11%, for samples that contained 95%, 90%, and 70% MD, respectively.

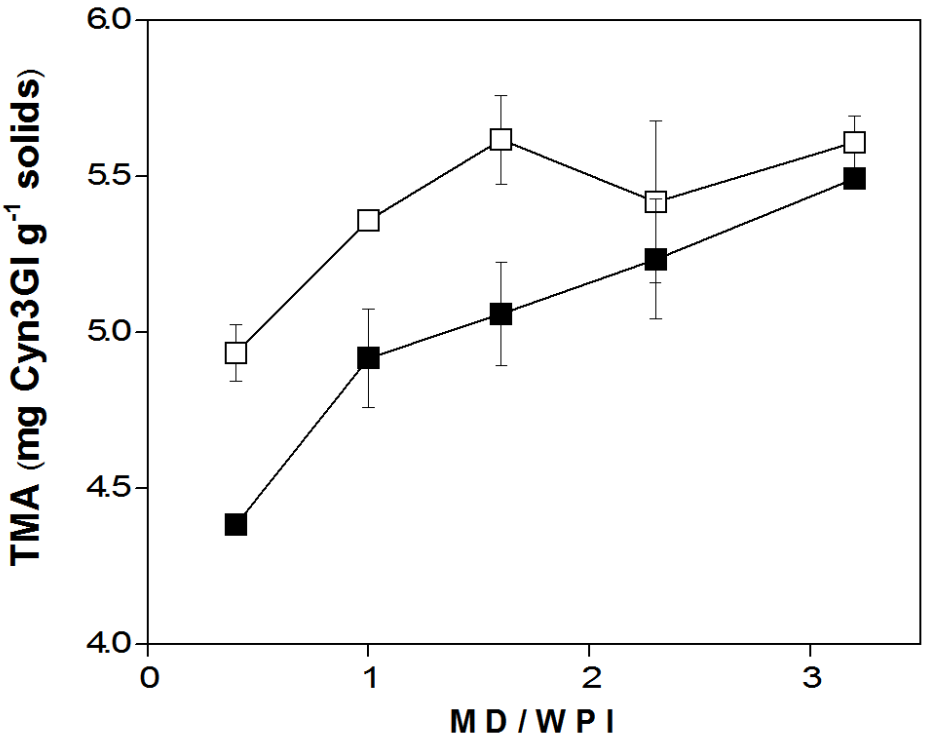

Figure 5-12 Total monomeric anthocyanins (TMA) of SD 180 (■) and SD 360 (□) powders as a function of the mass ratio of maltodextrin and whey protein isolate (MD/WPI). Results are expressed as means ± range of duplicate determinations.

5.4.7 Total monomeric anthocyanins (TMA)

Total monomeric anthocyanins of spray-dried blueberry powder containing MD and WPI obtained at feed flow rates 180 and 360 mL h^{-1} are presented in Figure 5-12. The feed flow rates and wall material ratios had no significant (P-value = 0.402) effect on the TMA of spray-dried blueberry powders. As the MD concentrations increased, the

total monomeric anthocyanins increased at both feed flow rates. There was a significant ($P < 0.05$) difference in TMA between the spray dried powder at the MD/WPI 0.4-1.6 for both feed flow rates. The TMA concentration of SD 180 samples was 4.4, 4.9, and 5.1 mg Cyn3Gl g^{-1} solids, while in the SD 360 samples, the TMA was calculated as 4.9, 5.4, and 5.6 1 mg Cyn3Gl g^{-1} solids. Beyond these MD/WPI ratios, there was no significant ($P > 0.05$) differences in the TMA of spray-dried samples; both produced at 180 and 360 mL h^{-1}. These results were higher than spray-dried blueberry extract (1.1-1.3 mg Cyn3Gl g^{-1} solids) made with maltodextrin.[138] However, the TMA levels from the current study were lower in comparison with the spray-dried blueberry powder produced using a conventional-nozzle and an ultrasonic-nozzle with the inlet temperature 125 ^{0}C, i.e., 17.71 and 19.4 mg Cyn3Gl g^{-1} dried matter, respectively.[113] From our study, the highest percentage anthocyanin retention was calculated as 62 and 63% for SD 180 and SD 360 samples, respectively. The higher value of TMA in the current study mainly caused by the higher air inlet and outlet temperatures, i.e., 150 ^{0}C and 101 ^{0}C, respectively. Another study has revealed higher TMA values compared to our results, where TMA of the spray-dried blueberry powder obtained using air inlet temperature 140 ^{0}C (outlet temperature 81 ^{0}C) was 15.61 mg Cyn3Gl g^{-1} solids, while at 160 ^{0}C (outlet temperature 95 ^{0}C) the TMA value was 11.98 mg Cyn3Gl g^{-1} solids.[112] These results revealed that the TMA values are directly related to the air inlet and outlet temperatures, instead of feed flow rates.

5.4.8 Individual anthocyanins

Individual anthocyanins content of spray-dried blueberry powders was evaluated using HPLC method with three pure anthocyanidins as standards, i.e., delphinidin, cyanidin, malvidin. Del3Gl of original blueberry juice was recorded as 1.38 mg g^{-1} solids (Figure

5-13). Del3Gl of SD 180 and SD 360 was observed lower than the original blueberry juice as also shown in Figure 5-13. Del3Gl of spray-dried powder was significantly (P-value = 0.000) affected by the feed rates and wall material ratios. The highest TMA concentration of spray-dried powder was observed from the SD 180 with MD/WPI = 3.2 (1.1 mg g^{-1} solids), while the lowest TMA concentration obtained was from the SD 360 at MD/WPI = 0.4 (0.62 mg g^{-1} solids). The SD 180 samples were slightly higher in Del3Gl concentration over the SD 360 samples, except at the MD/WPI = 1.6 (SD 180 = 6.6 mg g^{-1} solids, SD 360 = 6.75 mg g^{-1} solids). This high concentration in Del3Gl was possibly related to the higher heat and mass transfer occurring when the drying process was carried out with lower feed flow rates.[40] Thus, the powder particles have low moisture content (not agglomerate), which prevents the loss of Del3Gl by protecting the anthocyanins against oxidation.[38]

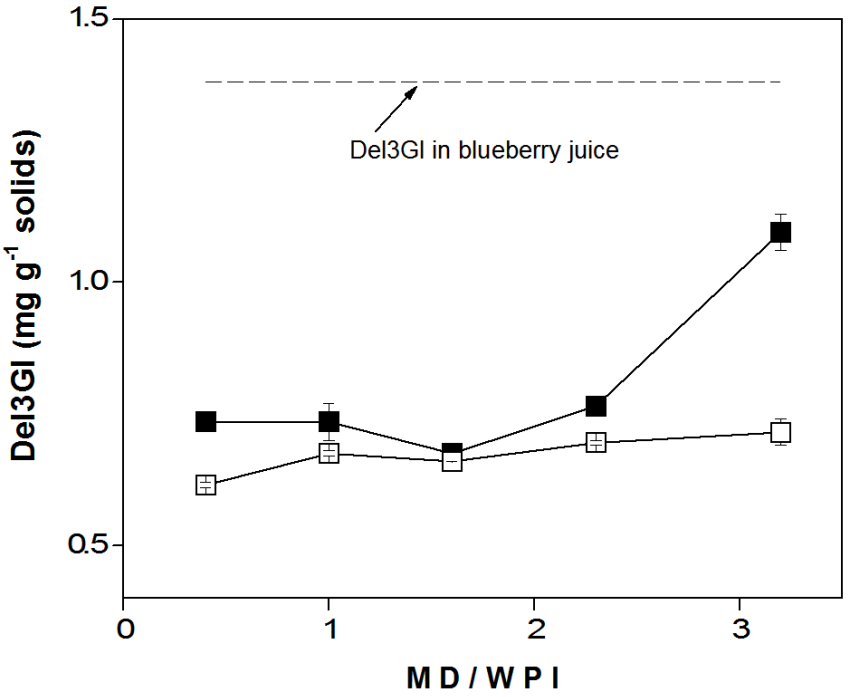

Figure 5-13 Delphinidin-3-Glucoside content of SD 180 (■) and SD 360 (□) powders as a function of the mass ratio of maltodextrin and whey protein isolate (MD/WPI). Results are expressed as means ± range of duplicate determinations.

Figure 5-14 shows Cyn3Gl of blueberry juice and spray-dried blueberry powders. The Cyn3Gl in blueberry juice was 1.32 mg g^{-1} solids. There was a significant (P-value = 0.000) effect of feed flow rates and wall material ratios on the Cyn3Gl concentration. At MD/WPI 0.4, both SD 180 and SD 360 samples had 0.85 mg g^{-1} solids. The SD 180 with MD/WPI = 3.2 obtained 1.2 mg g^{-1} solids of Cyn3Gl, which was the highest concentration. Conversely, 0.78 and 0.81 mg g^{-1} solids were the lowest concentrations of Cyn3Gl. These values were recorded from the SD 180 with MD/WPI = 1.0 and 1.6, respectively. In the case of SD 360 samples, at MD/WPI = 1.0, 1.6, and 2.3 the Cyn3Gl was higher ($P < 0.05$) concentration compared to those of SD 180 with the same ratio MD/WPI.

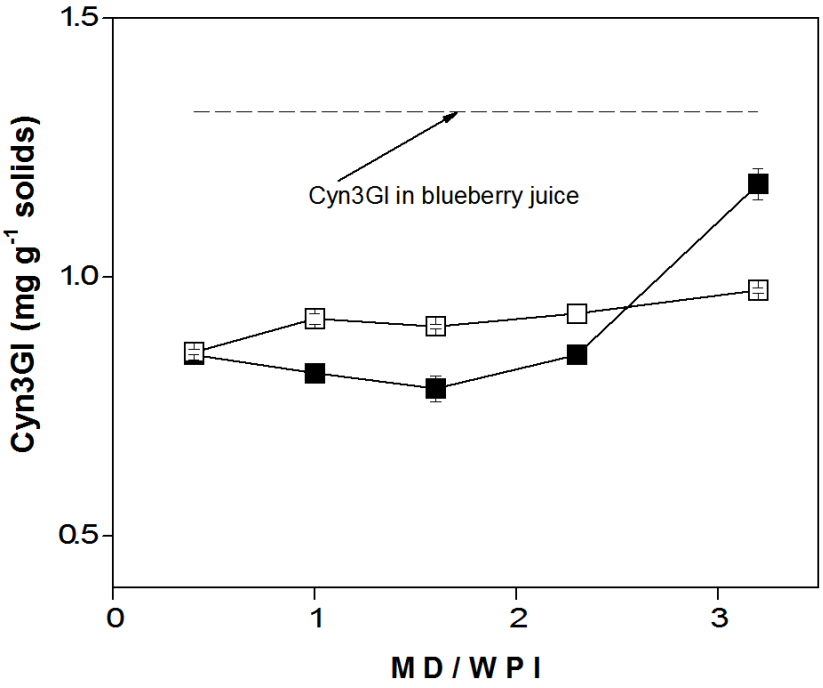

Figure 5-14 Cyanidin-3-Glucoside content of content of SD 180 (■) and SD 360 (□) powders as a function of the mass ratio of maltodextrin and whey protein isolate (MD/WPI). Results are expressed as means ± range of duplicate determinations.

Mal3Gl of blueberry juice and spray-dried blueberry powders is presented in Figure 5-15. The blueberry juice contained 1 mg g^{-1} solids of Mal3Gl. Feed flow rates and MD/WPI ratios had a significant (P-value = 0.001) effect on the Mal3Gl concentration of spray-dried powders. It was observed that SD 180 with MD/WPI =3.2 retain 0.76 mg g^{-1} solids, which was high ($P < 0.05$) concentration compared to other samples. At MD/WPI = 0.4, the SD 180 samples was greater (0.58 mg g^{-1} solids) than SD 360 (0.5 mg g^{-1} solids). There were no significant ($P > 0.05$) differences in Mal3Gl of both samples SD 180 and SD 360, with the MD/WPI = 1.0, 1.6, and 2.3. The values varied from 0.51 to 0.57 mg g^{-1} solids.

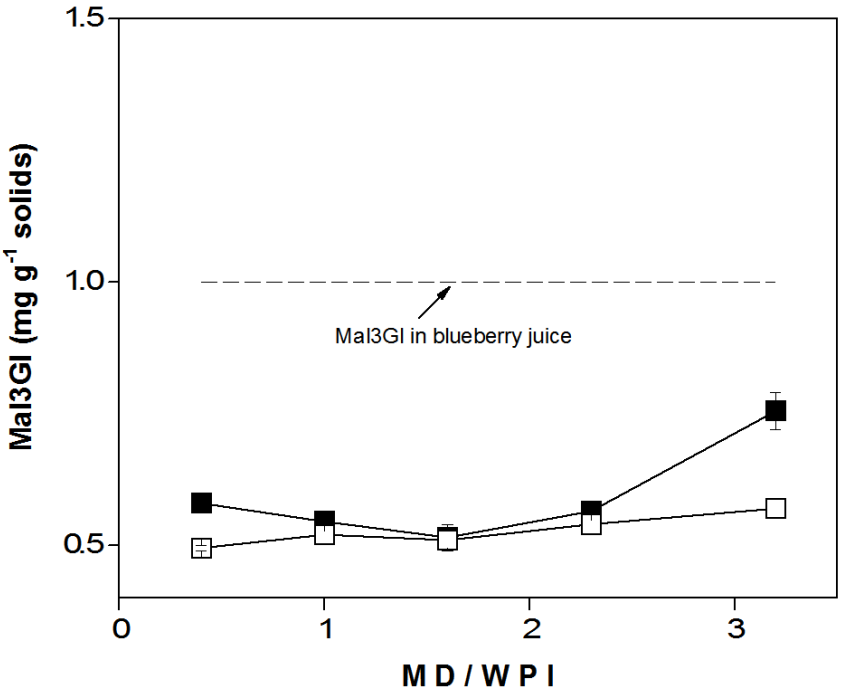

Figure 5-15 Malvidin-3-Glucoside content of SD 180 (■) and SD 360 (□) powders as a function of the mass ratio of maltodextrin and whey protein isolate (MD/WPI). Results are expressed as means ± range of duplicate determinations.

5.4.9 Retention of TPC, TMA, and individual anthocyanins

Table 5-4 shows the retention values of phenolic compounds and anthocyanins of spray-dried blueberry powder. There was no significant (P-value = 0.950) difference in total phenolic compounds (TPC) retention for all the samples. The retention of TPC in the SD 360 powder samples ranges between 54 and 72%, while in the SD 180 samples range between 48 and 69%. The spray-dried powder samples, both from SD 180 and SD 360, showed a tendency to give high retention of phenolic compounds, especially at high MD/WPI ratio; the retention of TPC increased with increasing MD/WPI ratio. According to the literature, spray-drying of blueberry using an inlet temperature 160 ^{0}C with a feed flow rate of 480 and 540 mL h^{-1} gave the highest retention of phenolic compounds, i.e., 76%.[112] In the current study, the retention of TPC is lower than this due to the lower feed flow rate used.

Table 5-4 Retention of TPC, TMA, and individual anthocyanin of spray-dried blueberry powder produced with different feed flow rates and wall material ratios

MD/WPI	*TPC (%)	*TMA (%)	Delphinidin-3-glucoside (%)	Cyanidin-3-glucoside (%)	Malvidin-3-glucoside (%)
SD 180					
0.4	48 ± 9	49	53 b	63 ± 1 b	58 ± 1 b
1.0	51 ± 4	55 ± 2	53 ± 3 bc	60 b	55 ± 1 b
1.6	58 ± 6	57 ± 2	49 ± 1 bc	58 ± 2b	52 ± 3 b
2.3	67 ± 7	59 ± 2	55 b	63 b	57 b
3.2	69 ± 14	60	79 ± 3 a	87 ± 2 a	76 ± 4 a
SD 360					
0.4	54 ± 8	55 ± 1	45 c	63 b	50 ± 1 b
1.0	58 ± 8	60 ±	49 bc	68 ± 1 b	52 b
1.6	59 ± 1	63 ± 2	48bc	67 b	51 b
2.3	66 ± 9	61 ± 3	50 bc	69 b	54 b
3.2	72 ± 15	63 ± 1	52 ± 2 bc	72 b	57 b

**No significant differences amongst values. Mean values ± range of duplicate determinations, followed by a different single letter in each column if significantly different (p < 0.05, Tukey's test), whereas ab, for example, indicates values that are not significantly different from the corresponding a and b values.*not*

The retention of total monomeric anthocyanins of spray-dried blueberry powder was in a range between 49 and 60%, for SD 180 samples; and between 56 and 62%, for SD 360 samples (Table 5-4). Increasing the feed flow rate and MD/WPI ratio led to an increase in TMA retention, this is due to less drying time and better protection of wall materials to the anthocyanins. ANOVA of observed result shows no significant (P-value = 0.372) variation in retention of TMA. The lowest retention of TMA was 49%, i.e., the SD 180 at the MD/WPI ratio = 0.4. However, SD 360 sample obtained at the MD/WPI ratio = 3.2 gave the highest retention of TMA, i.e., 62%. According to Turan et al.[113], the spray-drying of blueberry juice, using a conventional and ultrasonic nozzle, retained 70 and 77% of TMA, respectively. Our findings had low retention of TMA. It can be explained by the fact that the inlet temperature used was high, i.e., 150 ^{0}C.

Increasing the MD/WPI ratio at different feed rates led to an improve Del3Gl retention (Table 5-4). Feed flow rates and wall materials had a significant (P-value = 0.000) effect on the Del3Gl retention of spray-dried powder. The lowest retention of Del3Gl, i.e., 45% was found from SD 360 sample (at MD/WPI = 0.4), while SD 180 sample (at MD/WPI = 3.2) had the highest retention of Del3Gl, i.e., 79%.In the case of Cyn3Gl, a similar phenomenon in increasing the retention of Cyn3Gl by increased the MD/WPI ratio was found (Table 5-4) Feed flow rates and wall materials had a significant (P-value = 0.000) effect on the Cyn3Gl retention of spray-dried powder. The retention of Cyn3Gl reached about 87% for SD 180 sample (at MD/WPI = 3.2). However, the remaining samples had no significant (P > 0.05) variation measured retention of Cyn3Gl. Increasing the MD/WPI ratio led to an increase in Mal3Gl retention (Table 5-4). Feed flow rates and wall materials had a significant (P-value = 0.000) effect on the Mal3Gl retention of spray-dried powder The Mal3Gl retention of SD 180 sample was

the highest, i.e., 76%, at the MD/WPI = 3.2. However, the remaining samples had no significant ($P > 0.05$) measured retention of Mal3Gl.

5.5 Summary

Blueberry powder was successfully produced via spray-drying with the aid of maltodextrin (MD) DE 16.5-19.5 and whey protein isolate (WPI); at two different feed flow rates, 180 and 360 mL h^{-1}. Physicochemical properties of the spray-dried powder were investigated. The yield of spray-dried powder, at both the feed rates, showed an efficient spray-drying process as its values > 60%; the SD 360 showed better yield than the SD 180. These feed flow rates gave spray-dried powders with low moisture content and a_w, which were considered as non-sticky and microbiologically stable. The spray-dried powders had high water solubility, i.e., > 96% due to low bulk densities; the SD 360 samples had similar values to those SD 180 samples. However, the rehydration time of these two feed flow rates showed differences, where SD 180 samples had longer rehydration times than SD 360 samples. In term of particles, the spray-dried blueberry powders appeared as large ballooned, shrivelled or spherical particles. The L*, a*, and b* values were 51-60, 42-57, and 10-13, respectively, which were known as pink rose colour. The SD 180 and 360 samples showed similar values of the total phenolic content. Anthocyanin content of the spray-dried powder was influenced by the MD/WPI ratio and feed flow rates, where the SD 360 samples had slightly higher total monomeric anthocyanins than SD 180. The retention of total phenolic content and total monomeric anthocyanins was in the range 48-72% and 49-64%, respectively, at both feed flow rates. In the case of individual anthocyanins, the SD 180 samples showed equal concentrations with the SD 360 samples. According to the results above, optimum spray-drying conditions for producing blueberry powder

was SD 180 with MD/WPI 3.2. The physicochemical properties of SD 180 made with MD/WPI 3.2 were as follows: yield: 74%, moisture content: 2.9%, aw: 0.09, solubility: 97.4%, rehydration time: 77.5 s, bulk density: 0.37 g cm^{-3}, L* value: 51.9, a* value: 56.3, b* value: 13.1, TPC : 29.9 mg GAE g^{-1} solids, TPC retention: 69.1%, TMA content: 5.5 mg Cyn3Gl g^{-1} solids, TMA retention: 62%, Del3Gl retention: 79%, Cyn3Gl retention: 87%, and Mal3Gl retention: 76%.

Chapter 6

Chapter 6 General Discussion

6.1 Comparison of foam-mat freeze-dried blueberry powders produced with trehalose and pure proteins with those produced with MD and WPI

In this section, foam-mat freeze-dried blueberry powders, which were made with trehalose and pure proteins and with maltodextrin (MD) and whey protein isolate (WPI) were compared. They were T3BL1 (trehalose/β-lactoglobulin = 2.8), T3A1 (trehalose/bovine serum albumin = 2.8), and M3W1 (MD/WPI = 2.8). The results shown in this section were from Chapter 3 and 4. One-way ANOVA was performed to establish the presence or absence of significant differences between means. Multiple comparisons were performed using the Tukey test, and significance level was set at $p < 0.05$. All statistical analyses were carried out using Minitab 17.0.

6.1.1 Foam overrun and stability

Figure 6-1A presents the foam stability of three different carrier agents. The foam stability was measured the liquid drain from the foam. The carrier agent types had a significant (P-value = 0.000) effect on the foam stability (drain volume). It is observed that M3W1 had higher liquid (less stable foam) to those samples prepared with pure proteins (more stable foam). This was possibly attributed to the type of proteins used, where β-lactoglobulin and bovine serum albumin form a stiff foam with more air entrapped (low density). As a result, the low-density foam holds the water (less liquid drainage) than the high-density foam. This phenomenon is in agreement with Karim et al.[5,6]

Foam overrun of the blueberry foam prepared by three carrier agents are shown in Figure 6-1B. ANOVA of observed data showed there were a significant (P-value = 0.000) differences among all foams. The overrun of M3W1 was recorded as 762% and higher in comparison with T3BL1 (608%) and T3A1 (654%). Zayas[82] reported that foam overrun values ranged from 753% for WPI 5% w/w. The higher overrun in the case of M3W1 is probably more air trapped in the foam. Conversely, the lower overrun of T3BL1 is due to β-lactoglobulin requires slightly higher temperature for unfolding and formation of film.[82]

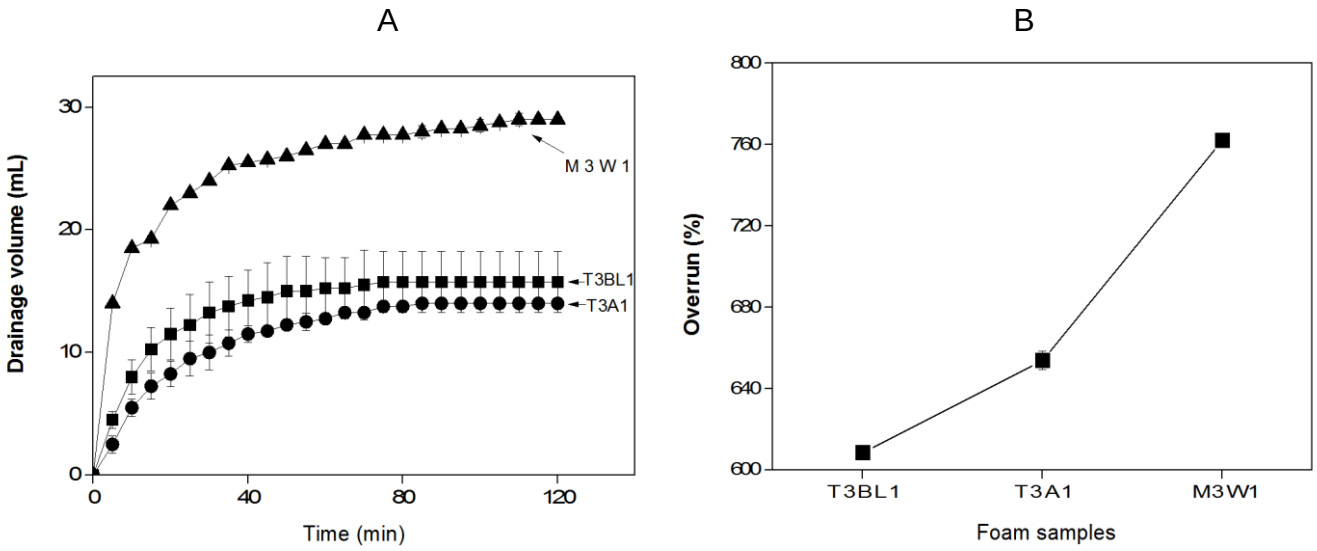

Figure 6-1 A: drain volume of blueberry juice-foam with M3W1 (▲), T3BL1 (■), and T3A1 (●). B: Overrun of blueberry juice-foam prepared by M3W1, T3BL1, and T3A1. Results are expressed as means ± range of duplicate determinations.

6.1.2 Powder yield, moisture content, and water activity/a_w

The yield of foam-mat freeze-dried powder prepared with M3W1, T3BL1, and T3A1 is presented in Figure 6-2A. ANOVA of yield showed that M3W1 had statistically (P-value

= 0.000) higher yield compared to those prepared with pure proteins. The yield of M1W3 samples reached 79%, whereas T3BL1 and T3A1 recovered 66 and 67%, respectively. The M3W1 yield was also higher according to the freeze-drying of blueberry juice prepared with β-cyclodextrin (15% w/w).[107] According to the authors, the yield of freeze-drying of blueberry juice was calculated as 78%.[107]

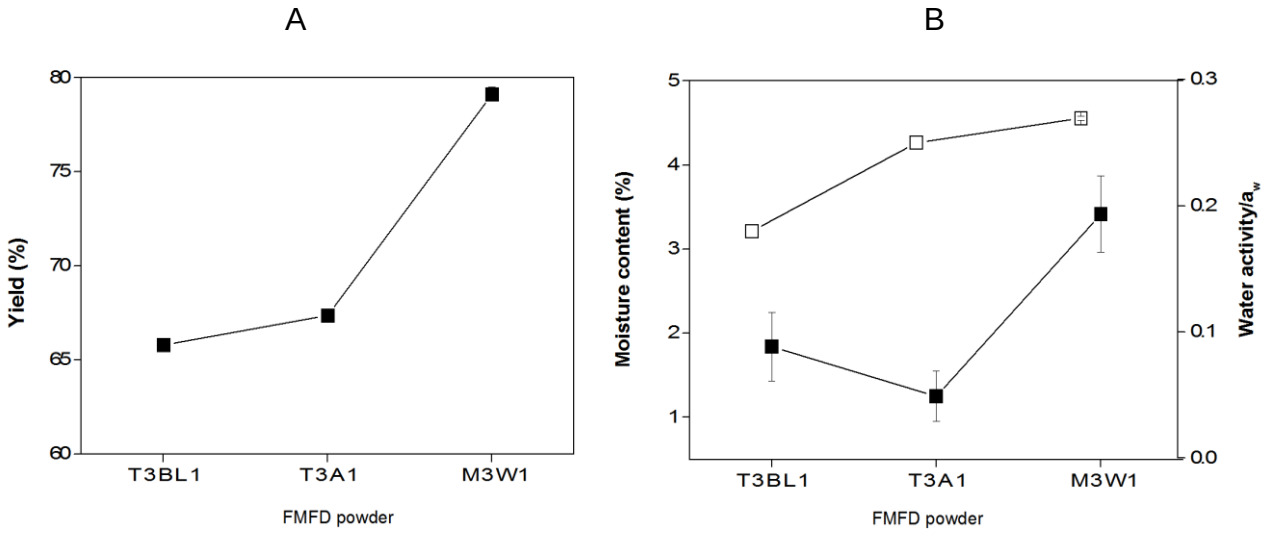

Figure 6-2 A: Yield of FMFD powders. B: Moisture content (■) and water activity/a_w (□).Results are expressed as means ± range of duplicate determinations.

Foam-mat freeze-dried powders obtained variable moisture content and a_w as shown in Figure 6-2B. There was a significant (P-value = 0.007) effect of carrier agents on the moisture content. The moisture content of M3W1 was higher (3.5%) compared to T3BL1 and T3A1. It was observed that between T3BL1 (1.8 ± 0.4%) and T3A1 (1.3 ± 0.3%) had no statistically ($P < 0.05$) difference in moisture content. The T3BL1 and T3A1 gave lower moisture content than M3W1 due to possibly the more stable foams

of T3BL1 and T3A1 (see section 6.1.1). Therefore, the stable foam led to increase the porosity of the structure, an increase in the drying rate, and a reduced the drying time.[6]

In the case of water activity, ANOVA of a_w showed that the foam-mat freeze-dried prepared with pure proteins had statistically (P-value = 0.000) lower compared to M3W1 sample. T3BL1 and T3A1 obtained a_w of 0.18 and 0.25, respectively, while a_w of M3W1 was 0.27. The low water activity values could be related the moisture loss of the samples because of the more porous structure.[7]

6.1.3 Solubility, rehydration time, and bulk density

The solubility of foam-mat freeze-dried powders prepared with T3BL1, T3A1, and M3W1 is shown in Figure 6-3A. Type of carrier agents had no significant (P-value = 0.356) on the solubility of foam-mat freeze-dried samples. The solubility was 98.3, 96.7, and 96.5%, for T3BL1, T3A1, and M3W1, respectively. These results seemed to be higher according to the foam-mat dried of sour cherry powder (solubility 43-48%) using egg white and methylcellulose as foaming agents.[6] Different type of proteins and polysaccharides in the foam-mat drying causes vary in foam structural stability. The stable foams indicate more bubbles remain during whipping and drying. Thus, these bubbles increase the porosity of the powder and its solubility.[6]

Figure 6-3B shows rehydration time of the foam-mat freeze-dried powders made with T3BL1, T3A1, and M3W1. ANOVA of rehydration time revealed that there were a significant (P-value = 0.000) differences among all the samples. The shortest rehydration time was recorded as 34 s (T3BL1), while M3W1 gave the longest rehydration time, i.e. 90 s. The bulk density of foam-mat freeze-dried powders is also presented in Figure 6-3B. There was a significant effect of carrier agent type on the

bulk density. M3W1 had a lower bulk density (0.32 g cm^{-3}) over the foam-mat freeze dried samples made with pure proteins, i.e. 0.55 and 0.6 g cm^{-3}. The high bulk density caused by the presence of water is considerably denser than the dry solids.[30,110] Thus, the particles are wetted easily and result in a reduction of rehydration time. However, the less dense powder tends to be less wettable due to entrapped air amongst particles and hence, an increase in rehydration time occurs.

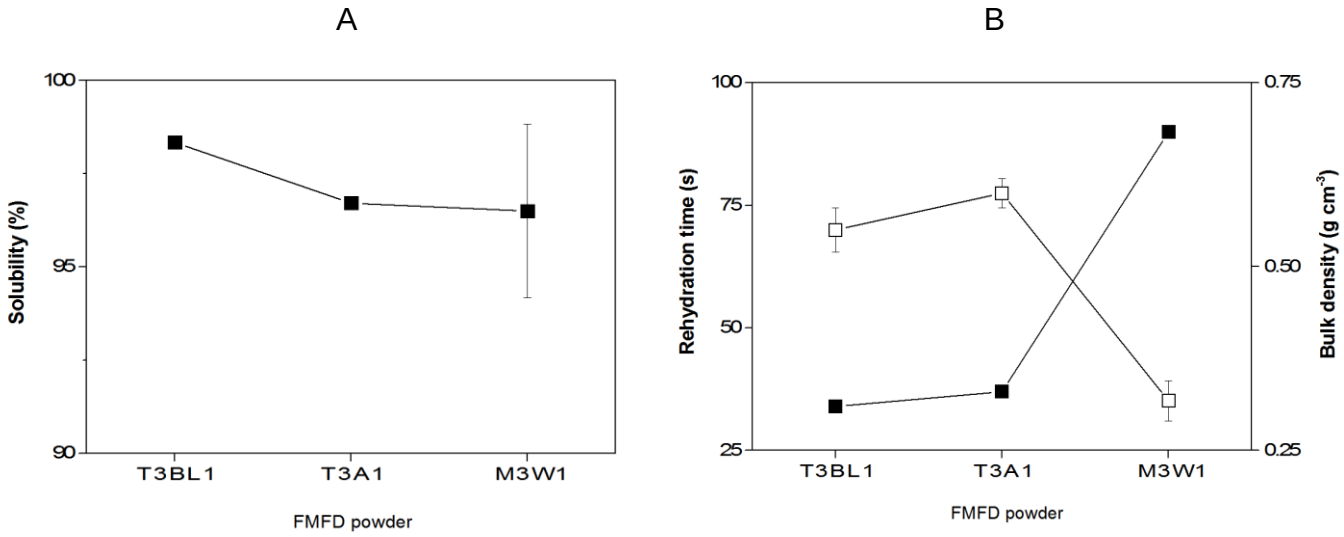

Figure 6-3 A: Solubility of FMFD powders. B: Rehydration (■) and bulk density (□) of FMFD powders. Results are expressed as means ± range of duplicate determinations.

6.1.4 Particles morphology

Figure 6-4 presents SEM micrographs of foam-mat freeze dried powders made with T3BL1, T3A1, and M3W1. The particles of three foam-mat freeze-dried powders had irregular shapes. However, the first two foam-mat freeze-dried powders made with pure proteins generated pores and ordered structures, while the M3W1 powder had broken glass-like structures. These results were attributed to the foam density and

stability (see section 6.1.1), where the T3BL1 and T3A1 had lower foam density and higher foam stability than M3W1 samples. Thus, the T3BL1 and T3A1 generated more small bubbles. Further, when sublimation was subjected to the foam, these bubbles retained similar shapes once they dried. These results are in agreement with the study carried out by Thuwapanichayanan et al.[10] In the case of M3W1, the foam drained fast and could not hold the structure due to foam instability. Thus, once the foams were dried and crushed, they showed flake-like particles. Franceschinis et al.[96] reported similar phenomena in producing blueberry powder by using the freeze-drying technique.

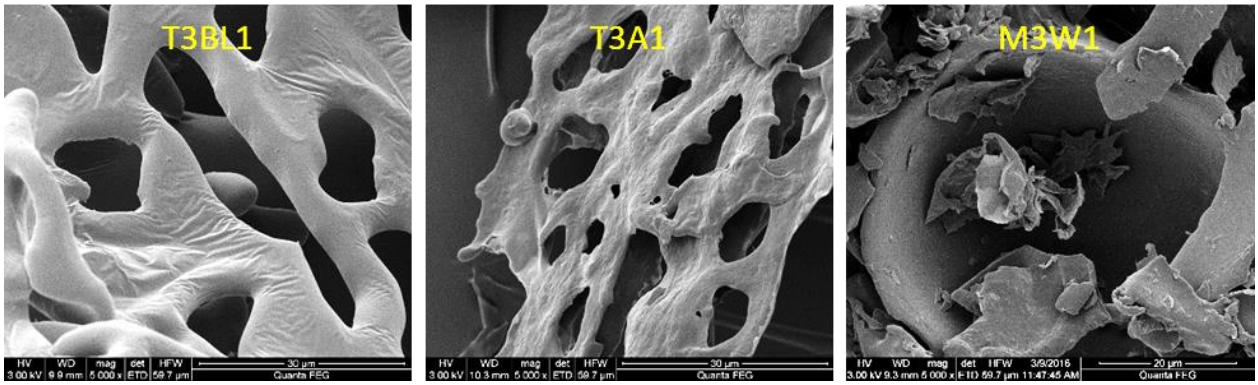

Figure 6-4 SEM micrographs of foam-mat freeze-dried powders made with T3BL1, T3A1, and M3W1, magnification 5,000x

6.1.5 Colour properties

Colour is one of the important properties of powders, which has a great influence on their desirability and final price.[7] L*, a*, and b* values of foam-mat freeze-dried reconstituted powder are shown in Figure 6-5A. The L* value of reconstituted powder

made with pure proteins was statistically (P-value = 0.000) lower than the sample made with M3W1. This phenomenon indicates that T3BL1 and T3A1 had little influence on the brightness of the reconstituted solutions. The a* value of M3W1 had higher (P-value = 0.000) a* value over the other foam-mat freeze-dried samples. The a* value is related to the anthocyanins content of foam-mat freeze-dried powders. The high a* value of M3W1 sample was attributed to high total monomeric anthocyanins (TMA) content (see section 6.1.6). In the case of b*, M3W1 also shown high-level, while T3BL1 and T3A1 gave lower (P-value = 0.000) b* values than M3W1. The study conducted by Franceschinis et al.[96] and Duangmal et al.[118] showed that there is an increase of a* and b* value by addition maltodextrin and/or trehalose on the blackberry powder and Roselle anthocyanin obtained by freeze-drying. They found lower a* (24-27) and b* (3-5) compared to the present study.

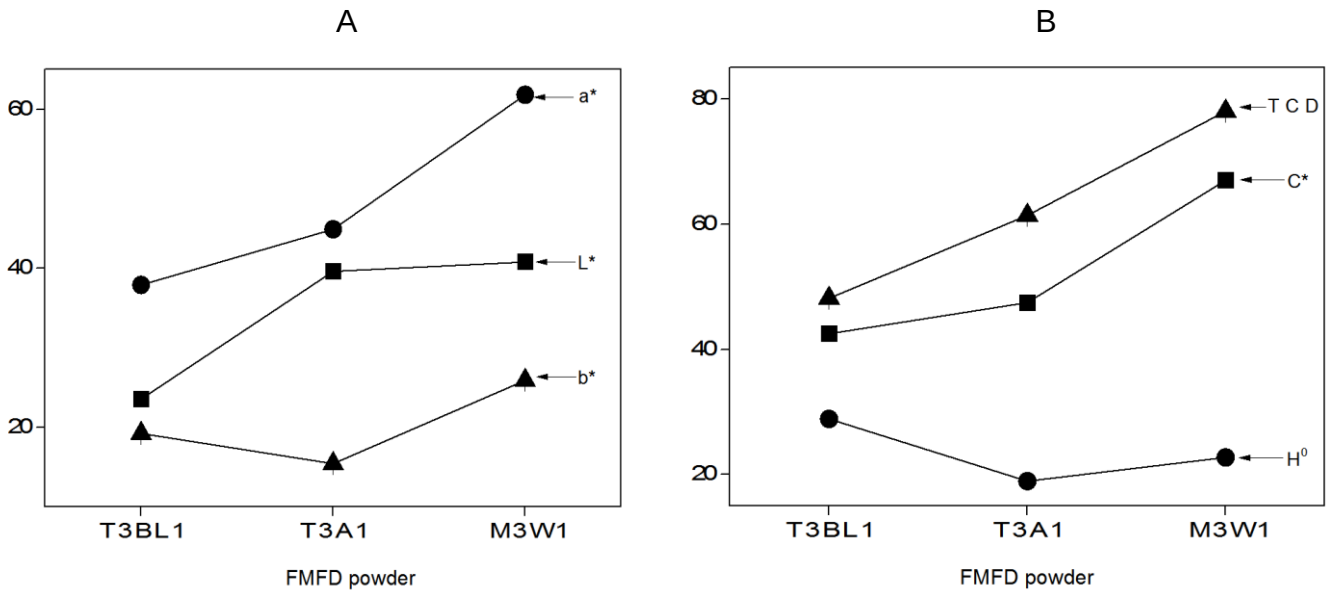

Figure 6-5 A: L*, a*, and b* values of FMFD powders. B: Hue/H^0, Chroma/C*, and total colour difference/TCD of FMFD reconstituted powders. Results are expressed as means ± range of duplicate determinations.

Total colour density (TCD), Chroma (C*), and Hue (H^0) of foam-mat freeze-dried reconstituted powders are presented in Figure 6-5B. M3W1 sample was found to be higher in total colour density (TCD) and C* values than those of T3BL1 and T3A1. TCD of M3W1 was recorded as 78, while T3BL1 and T3A1 were 48 and 6, respectively. This high TCD was attributed to high level of a* and high total monomeric anthocyanins content (see section 6.1.6). C* value of M3W1 was calculated as 67, whereas both powders made with pure proteins < 50. In the case of Hue, the T3BL1 were slightly higher (H^0 = 29) over the M3W1 sample (H^0 = 23). H^0 of the three foam-mat freeze-dried samples were found < 90, which were considered as pure red.

6.1.6 Total phenolic content (TPC), total monomeric anthocyanins (TMA), and retention of TPC and TMA

Figure 6-6A shows total phenolic content (TPC) and total monomeric anthocyanins (TMA) of foam-mat freeze-dried made with three different carrier agents. It was observed that M3W1 was superior (P-value = 0.000) in the TPC and TMA compared to both samples made with pure proteins. The M3W1 had 31.5 mg GAE g^{-1} solids and 8.5 mg Cyn3Gl g^{-1} solids for the TPC and TMA, respectively. The TPC of T3BL1 and T3A1 was calculated as 17.7 ± 0.08 and 14.5 ± 0.3 mg GAE g^{-1} solids, respectively. In the case of TMA content, T3BL1 and T3A1 had 5.9 ± 0.33 and 5.1 ± 0.09 mg Cyn3Gl g^{-1} solids, respectively. This phenomenon could be related to maltodextrin used in M3W1 sample, which was better at preserving phenolic compounds and anthocyanins compared to trehalose-treated samples.[96,118] Therefore, the M3W1 powder had a high retention of TPC and TMA (Figure 6-6B). It was recorded as 73 and 95% of TPC and TMA retention, respectively. Conversely, T3BL1 and T3A1 samples showed low retention of TPC and TMA, i.e. < 60%. Franceschinis et al.[96] reported that 73 and 75%

of TPC and TMA retention of freeze-dried blackberry powder made with maltodextrin., However, the M3W1 sample showed lower TPC retention when compared to the freeze-dried blueberry powder (TPC retention: 95%) reported in elsewhere.[113] This may be related to the blueberry cultivar was used.

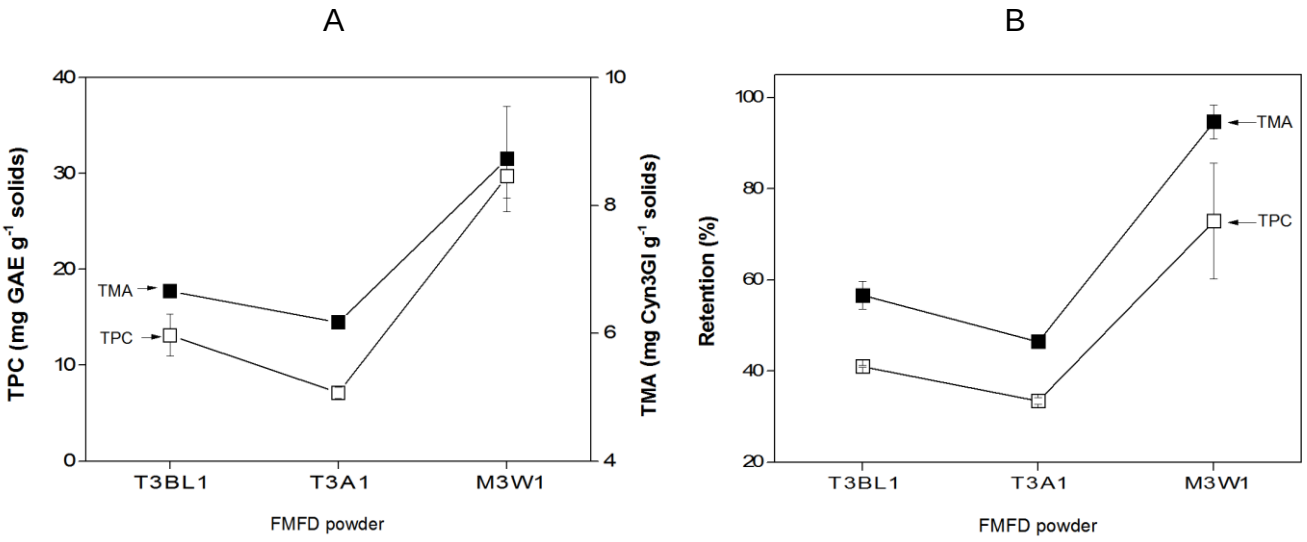

Figure 6-6 A: Total phenolic content/TPC and total monomeric anthocyanins/TMA of FMFD powders. B: Retention of TPC and TMA of FMFD powders. Results are expressed as means ± range of duplicate determinations.

6.1.7 Individual anthocyanins and retention of individual anthocyanins

Individual anthocyanins of foam-mat freeze-dried produced with T3BL1, T3A1, and M3W1 are presented in Figure 6-7A. Del3Gl concentration was found to be higher in M3W1. Also, M3W1 had high Cyn3Gl and Mal3Gl concentration. In the case of M3W1, the concentration of Del3Gl, Cyn3Gl, and Mal3Gl was recorded as 1.17., 1.38, and 0.85 mg g^{-1} solids, respectively. Retention of individual anthocyanins is shown in Figure 6-7B. M3W1 could prevent the degradation better than other foam-mat freeze-dried samples. Del3Gl retention of M3W1 was calculated as 85%, while 46 and 48% was

recorded from T3BL1 and T3A1 powders. In the case of Cyn3Gl retention, M3W1 recovered 104%, whereas T3BL1 and T3A1 recovered 64 and 69%, respectively. Again, M3W1 had a high retention of Mal3Gl, i.e. 85%. Conversely, both powder samples made with pure protein recovered 52-53%.

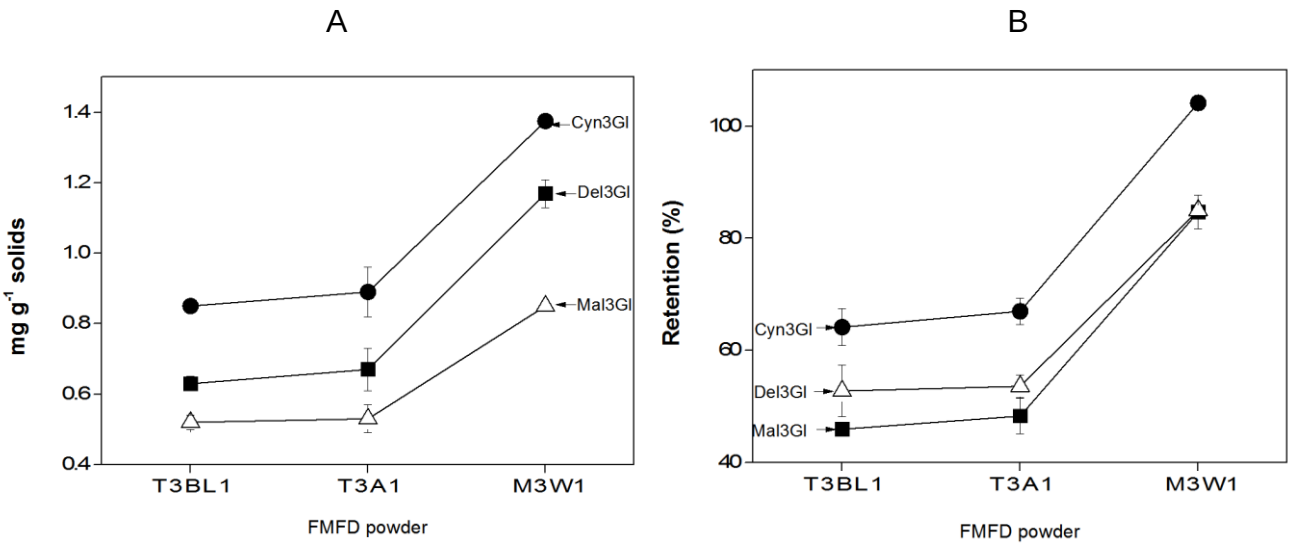

Figure 6-7 A: Concentration of Del3Gl, Cyn3Gl, and Mal3Gl of FMFD powders. B: Retention of Del3Gl, Cyn3Gl, and Mal3Gl of FMFD powders. Results are expressed as means ± range of duplicate determinations.

6.2 Comparison of foam-mat freeze-dried and spray-dried blueberry powders

Comparison between two drying processes, i.e., foam-mat freeze- and spray-drying, will be discussed regarding the effect of type of drying and matrices on the physicochemical properties of the blueberry powders and reconstituted solutions. The results shown in this section were from Chapter 3 and 5. Physical properties comparison of the foam-mat freeze-dried and spray-dried powders made with MD/WPI

ratio = 0.4, 1.0, 1.6, 2.3, and 3.2 will be reviewed. Two-way ANOVA was performed to establish the presence or absence of significant differences between means. Multiple comparisons were performed using the Tukey test and significance level was set at $p < 0.05$. All statistical analyses were carried out using Minitab 17.0.

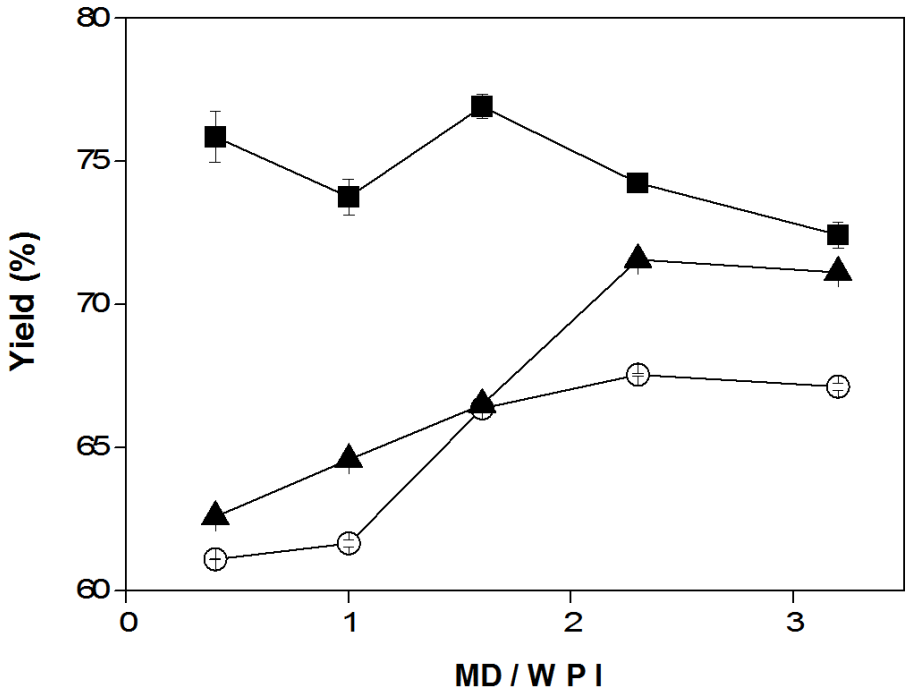

Figure 6-8 Yield of FMFD (■), SD 180 (○) and SD 360 (▲) powders as a function of the mass ratio of maltodextrin to whey protein isolate (MD/WPI). Results are expressed as means ± range of duplicate determinations.

6.2.1 Powder yield

Figure 6-8 shows the yields of blueberry powders produced by foam-mat freeze-drying (FMFD) and spray-drying (SD 180 and SD 360). In general, FMFD yields decreased with higher MD/WPI ratios (up to 3.2) while SD yields increased, although FMFD gave higher yields than SD at all MD/WPI ratios. In the case of FMFD, the MD/WPI ratio 1.6

gave the highest yield (77%). MD/WPI ratios greater than this resulted in a fall of the yield to approximately 72%. For SD powders, SD 180 gave a yield of 61% but this increased to 67% at MD/WPI = 3.2. Regarding the SD 360 process, the lowest yield (63%) was with the MD/WPI ratio 0.4, while 1.0 gave a slight increase to 64.6 % and 2.3 increased this to a maximum of 71.5%. These results agree with those of Fang and Bhandari.[42] Comparison of freeze-drying and spray-drying with MD and WPI has been reported in other food products. Shi et al.[70] obtained spray-dried honey powder recoveries > 50 % with the addition of MD and WPI, but no powder was recovered when pure honey was spray-dried. Another study revealed that MD + WPI gave excellent HCA (hydroxy citric acid) recovery of microencapsulated *Garcinia* fruit powder, typically 90%. [147]

6.2.2 Moisture content and water activity

The final moisture content and water activities of blueberry FMFD and SD powders are shown in Figure 6-9 and Figure 6-10, respectively. There was no statistically (P-value = 0.068) effect of drying methods and carrier agent on the moisture content. FMFD samples had similar moisture content compared to SD samples of similar composition, except at MD/WPI 1.6. There were two powders which had the notably highest (3.9%) and lowest (1.6%) moisture content, namely FMFD with MD/WPI = 0.4 and SD 360 with MD/WPI = 1.6, respectively. Ezihalarasi et al.[109] and Franceschinis et al.[96] suggested that spray-drying gives a lower moisture content than freeze-drying, due to the higher temperature in the spray-drying process.[96,147] Our results were in inverse linear to these study possibly due to the fixed concentration of carrier agent, i.e. 5% (w/w).

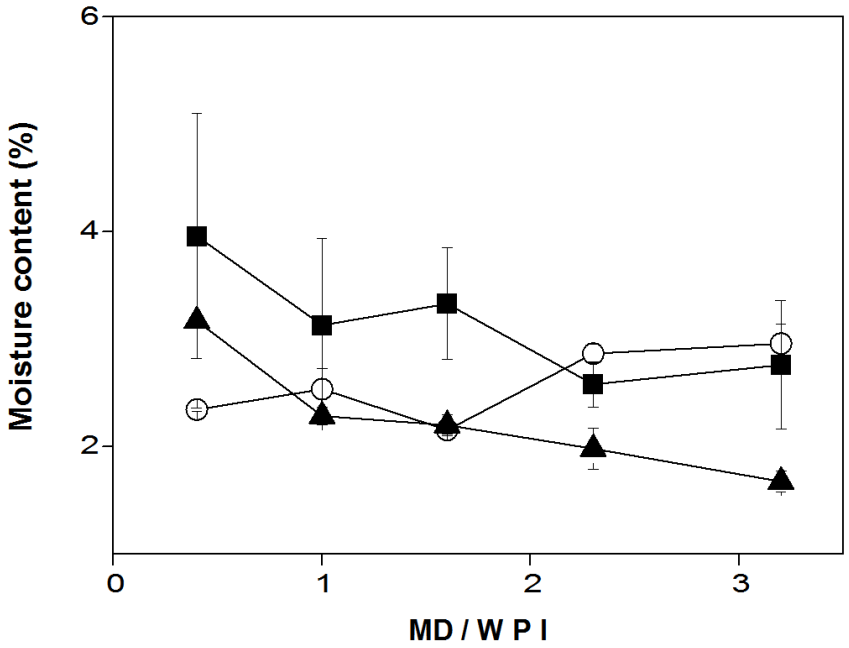

Figure 6-9 Moisture content of FMFD (■), SD 180 (○) and SD 360 (▲) powders as a function of the mass ratio of maltodextrin to whey protein isolate (MD/WPI). Results are expressed as means ± range of duplicate determinations.

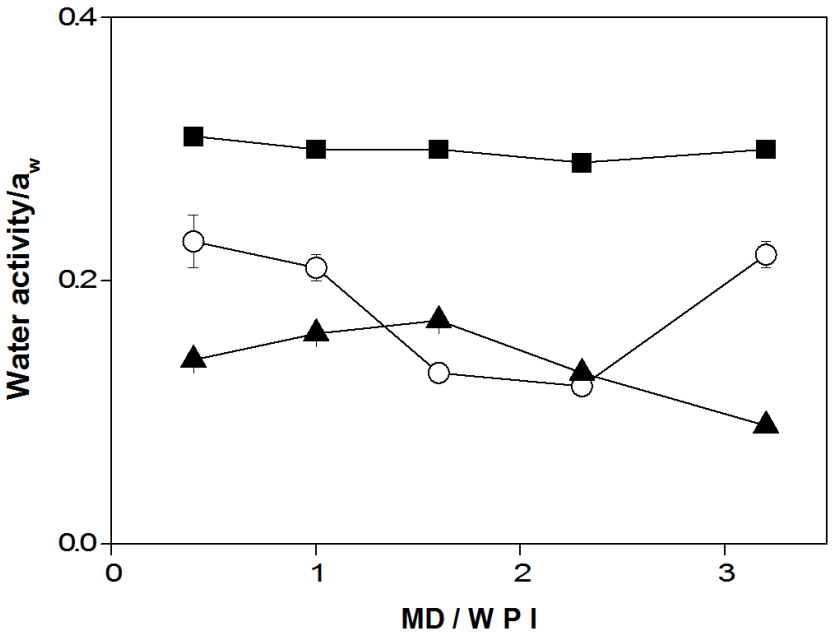

Figure 6-10 Water activity of FMFD (■), SD 180 (○) and SD 360 (▲) powders as a function of the mass ratio of maltodextrin to whey protein isolate (MD/WPI). Results are expressed as means ± range of duplicate determinations.

In this study, the water activity value of all the powders produced was less than 0.40 (Figure 6-10), which is acceptable for such powders regarding inhibition of microbial growth and biochemical degradation.[70] However, the statistical analysis confirmed that the water activity was significantly (P-value = 0.000) affected by the drying methods and carrier agents. The highest water activity was the FMFD powder produced with an MD/WPI ratio = 0.4. In contrast, the SD 180 powder with the lowest a_w was produced with the highest ratio of MD/WPI = 3.2. This powder also had the lowest moisture content.

6.2.3 Solubility

The solubility of FMFD and SD powders is compared in Figure 6-11. Drying methods and carrier agents had no significantly different effect (P-value = 0.914) on the solubility of the powders: the solubility was > 95% for all powders. For FMFD powder, MD/WPI 1.6 gave the highest solubility (99%). These results seem to be consistent to those of Francescinis et al.[96] who reported that there were no statistically differences in the solubility of freeze-dried and spray-dried blueberry powders, either made with maltodextrin alone or with trehalose and maltodextrin. The solubility of freeze-dried powder was in range 99-99.5%, while 98.5-100% was obtained in spray-dried samples.

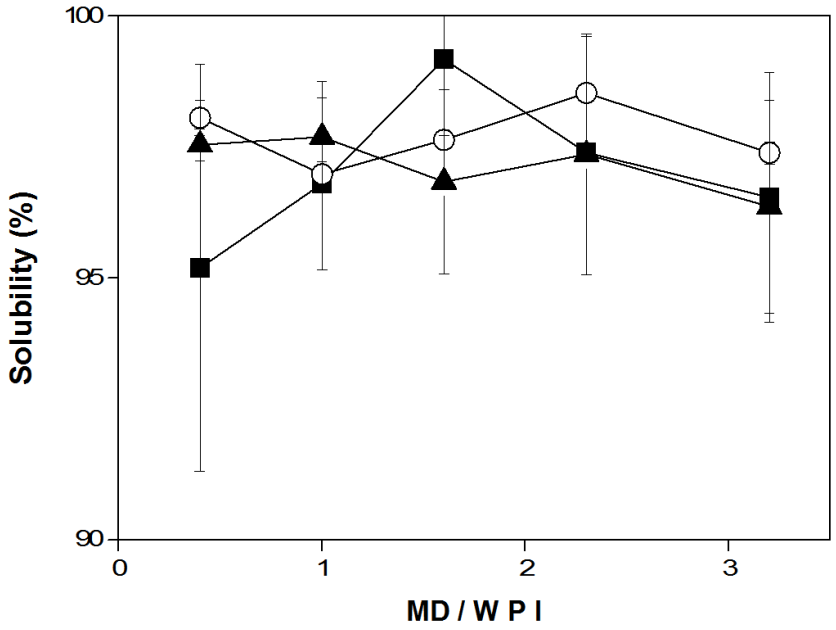

Figure 6-11 Solubility of FMFD (■), SD 180 (○) and SD 360 (▲) powders as a function of the mass ratio of maltodextrin to whey protein isolate (MD/WPI). Results are expressed as means ± range of duplicate determinations.

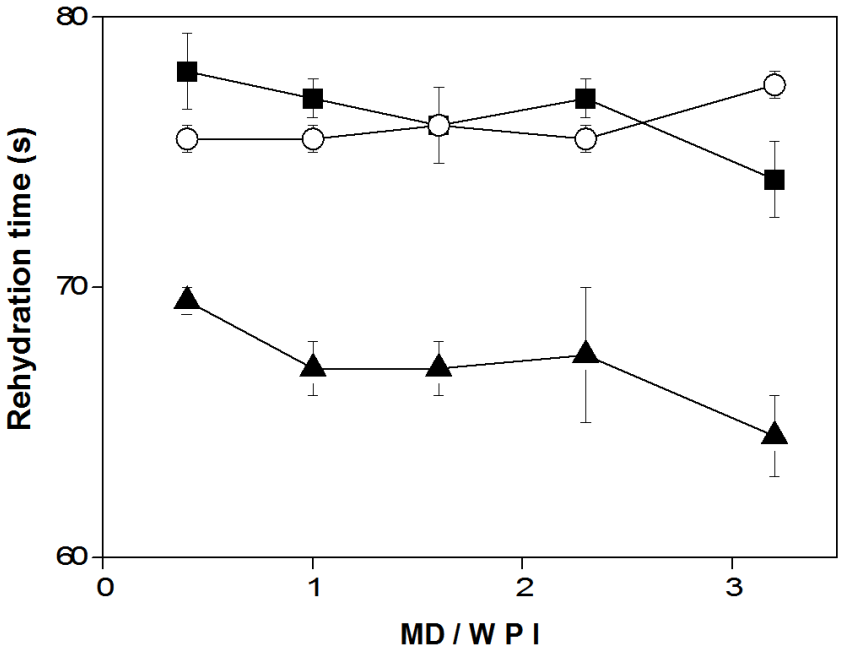

Figure 6-12 Rehydration of FMFD (■), SD 180 (○) and SD 360 (▲) powders as a function of the mass ratio of maltodextrin to whey protein isolate (MD/WPI). Results are expressed as means ± range of duplicate determinations.

6.2.4 Rehydration time

There was no significant (P-value = 0.129) effect of drying methods and carrier agents on the rehydration. However, among drying processes, SD 360 powders had significantly (P-value = 0.000) shorter rehydration times than FMFD and SD 180 powders (Figure 6-12). Rehydration times of FMFD powders were in range 74-78 s, while the SD 360 powders had rehydration times in range 65-70 s. This result was attributed due to a low moisture content of SD 360 powder. Thus, the particles absorb water quickly.

6.2.5 Bulk density

FMFD powders had significantly lower (P-value = 0.024) bulk densities than SD powders (Figure 6-13), except at MD/WPI 3.2. Since spray-dried and freeze-dried powders had very similar moisture content (see section 6.2.2), this suggests a more open and porous structure of the freeze-dried powders (Figure 6-14). However, this result contrasts with a study conducted by Turan et al.[113] for blueberry powders produced via an ultrasonic spray-dryer and freeze-dryer. Another study also found that freeze-dried blackberry powder had higher bulk density than spray-dried powder.[96]

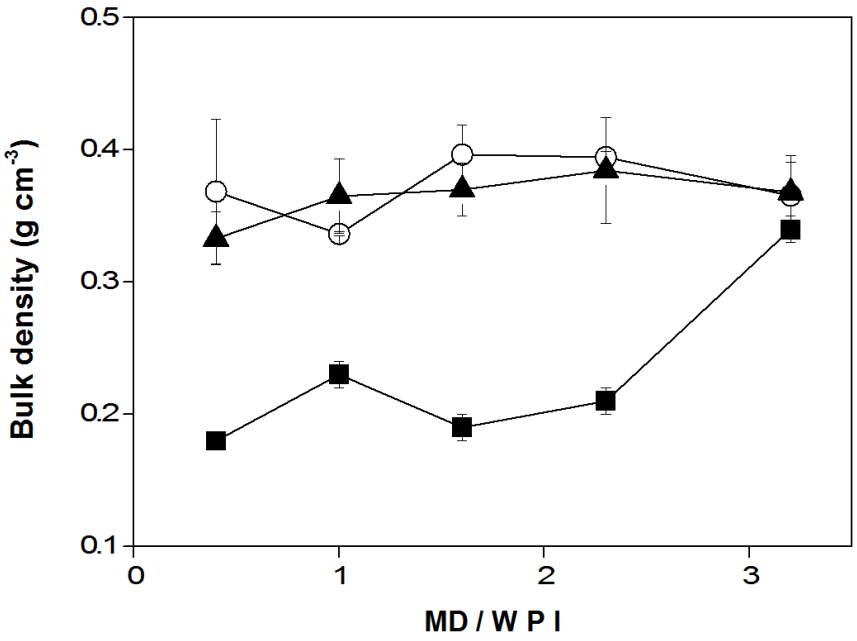

Figure 6-13 Bulk density of FMFD (■), SD 180 (○) and SD 360 (▲) powders as a function of the mass ratio of maltodextrin to whey protein isolate (MD/WPI). Results are expressed as means ± range of duplicate determinations.

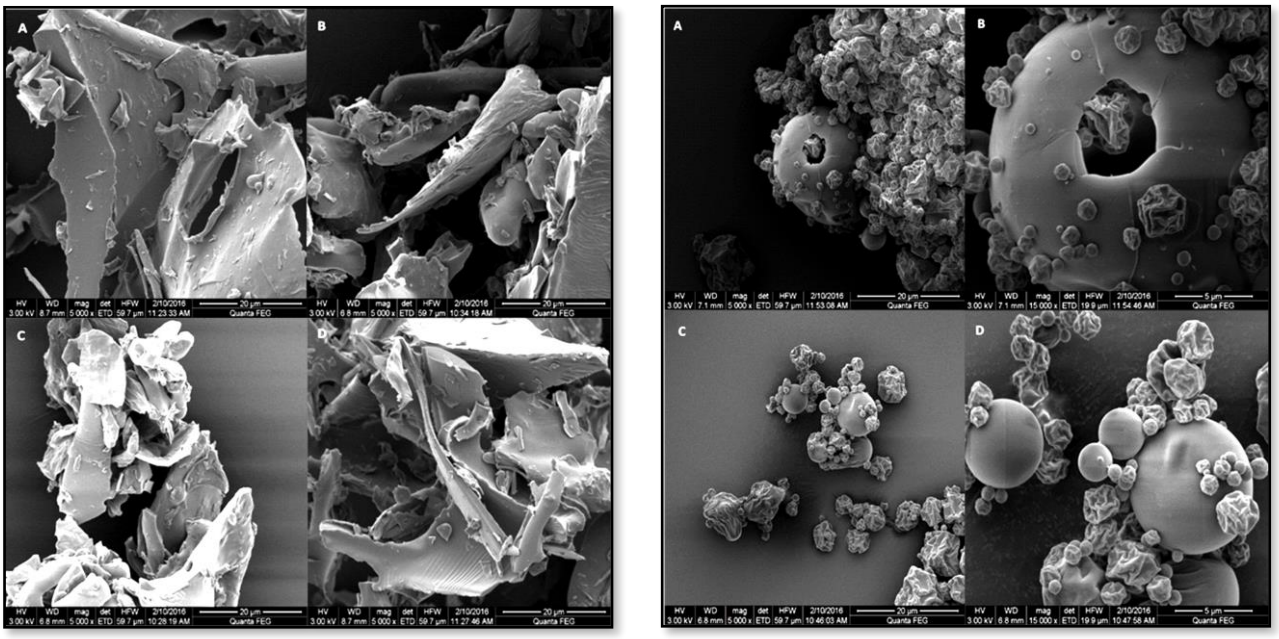

Figure 6-14 SEM Micrographs of FMFD powders (I) produced with MD/WPI = 0.4 (A, B) and 3.2 (C, D) at magnification of 5000 x, and SD 180 powders (II): with MD/WPI = 0.4 (A, B) and 3.2 (C, D) at magnifications of 5000 x and 15000 x, respectively

6.2.6 Particle morphology

The morphological characteristics of FMFD and SD powders are presented in Figure 6-14, respectively. In general, the surface morphology of FMFD powders was irregular particles of larger size than those observed for SD powders. Most of FMFD particles resembled broken glass or flake-like structures, probably due to their origin as bubble surface films, broken up via the food processor. The FMFD powders produced with MD/WPI ratios 0.4 (Figure 6-14 I A-B) had similar appearances to those produced with 3.2 ratios (Figure 6-14 I C-D). This flake-like structure is analogous to that observed in freeze-dried soursop fruit pulp[25] and blackberry.[96] In contrast, the blueberry SD powders appeared as spherical particles, typical of SD powders. Similar behaviour was observed by Tonon et al.[144] for spray-dried Acai juice and Ersus and Yurdagel[145] for black carrot juice. The shape of the particles in SD 180 powders suggested shrunken spheres at low MD (e.g., MD/WPI = 0.4) (Figure 6-14 II A-B), whereas at higher MD (e.g., MD/WPI = 3.2) spheres appeared smooth (Figure 6-14 II C-D). This may be because the higher MD produced a soft and porous crust.

6.2.7 Colour properties

The parameter colour of original blueberry juice was L* = 0.08, a* = 0.45 and b*= 0.06. The brightness, redness, and yellowness of original blueberry juice were significantly (P-value = 0.000) different from both FMFD and SD reconstituted solutions, as shown in Table 6-1.The L* of blueberry juice was very dark. In contrast, the L* of FMFD and SD reconstituted solutions were brighter colours which can attribute to the addition of MD, which is a white flour in the dry state (Table 6-1). In addition, the SD solutions

contained higher matrix proportions (e.g. 100 g kg^{-1} solids) compared to FMFD solutions (e.g. 50 g kg^{-1} solids) corresponding to higher L* value.

Table 6-1 Brightness (L*), redness (a*) and yellowness (b*) values of blueberry juice and reconstituted solutions from FMFD and SD powders containing different weight ratios of maltodextrin to whey protein isolate (MD/WPI)

Sample	MD/WPI	L*	a*	b*
Blueberry juice	0	0.08 ± 0.04 ^{j}	0.45 ± 0.03^{l}	0.06 ± 0.0 ^{m}
FMFD	0.4	44.6 ± 0.24 ^{g}	60.1 ± 0.05 ^{b}	18.3 ± 0.27 ^{d}
	1.0	41.5 ± 0.07 ^{h}	61.9 ± 0.01 ^{a}	18.3 ± 0.27 ^{d}
	1.6	41.2 ± 0.05 ^{h}	62.0 ± 0.01 ^{a}	25.0 ± 0.11 ^{c}
	2.3	40.5 ± 0.06 ^{i}	62.1 ± 0.01 ^{a}	26.3 ± 0.09 ^{b}
	3.2	40.2 ± 0.07 ^{i}	61.9 ± 0.01 ^{a}	27.0 ± 0.09 ^{a}
SD180	0.4	52.2 ± 0.05 ^{d}	56.2 ± 0.04 ^{d}	12.6 ± 0.03 ^{fg}
	1.0	51.9 ± 0.03 ^{ef}	47.4 ± 0.04 ^{j}	10.2 ^{j}
	1.6	53.1 ± 0.03 ^{c}	53.0 ± 0.03 ^{h}	11.0 ± 0.01 ^{d}
	2.3	52.5 ± 0.03 ^{d}	54.7 ± 0.03 ^{f}	12.3 ± 0.01 ^{g}
	3.2	51.9 ± 0.02 ^{ef}	56.3 ± 0.02 ^{d}	13.1 ± 0.02 ^{ef}
SD360	0.4	60.3 ± 0.02 ^{a}	42.5 ± 0.03 ^{k}	7.0 ± 0.0 ^{l}
	1.0	53.6 ± 0.02 ^{b}	49.4 ± 0.04 ^{i}	8.8 ± 0.01 ^{k}
	1.6	51.6 ± 0.03 ^{f}	53.3 ± 0.04 ^{g}	11.2 ± 0.01 ^{hi}
	2.3	52.2 ± 0.02 ^{de}	55.0 ± 0.03 ^{e}	11.5 ± 0.02 ^{h}
	3.2	51.8 ± 0.03 ^{f}	57.0 ± 0.03 ^{c}	13.2 ± 0.02 ^{e}

Mean values ± range of duplicate determinations, followed by different single letter in each column if significantly different ($p < 0.05$, Tukey's test), whereas ab, for example, indicates values that are not significantly different from the corresponding a and b values.

Regarding the a* values, FMFD reconstituted solutions had greater a* than those from SD 180 and SD 360 reconstituted solutions (Table 6-1).The FMFD reconstituted solutions had a deeper red colour compared to SD solutions. In contrast, Franceschinis et al.[96] found that the redness value was not affected by the drying method. They found

that SD blackberry powder produced with MD had the same a* value as FD powder produced with MD. However, they claimed that utilisation of other types of matrices (e.g., trehalose) had an impact on the redness. Trehalose-treated powders had lower a* than MD-treated powders, using freeze-drying and spray-drying processes. In the case of FMFD, increasing MD resulted vary a* value.

The FMFD reconstituted solutions showed higher b* than SD180 and SD360 reconstituted solutions (Table 6-1). The SD360 method resulted in the lowest yellowness value (b* = 6.94). In contrast, b* = 26.96 was obtained from FMFD powder sample. The same behaviour of b* values was founded for FMFD and SD blackberry powders.[96]

Regarding the colour intensity (Chroma), FMFD reconstituted solutions produced with MD/WPI = 2.3 and 3.2 had significantly ($P < 0.05$) higher values amongst the FMFD treatments (Table 6-2).The Chroma value of FMFD powders was also to be found higher according to SD 180 and SD 360 samples. The higher Chroma values indicate the a* (redness) and b* (yellowness) of samples were greater.[98,111] Hue angle (H^0) of the blueberry juice was 7.05, and this presented the colour in pure red. The FMFD, SD 180 and SD 360 reconstituted solutions gave H^0 values lower than 24 (Table 6-2). These values indicate pure red for all samples due to the values lower than 90.[111] ANOVA of H^0 showed that FMFD reconstituted solutions produced greater values in comparison with SD 180 and SD 360 reconstituted solutions.

Table 6-2 Chroma (C*), hue angle (H^0) and TCD of blueberry juice and reconstituted solutions from FMFD and SD powders containing different weight ratios of maltodextrin to whey protein isolate (MD/WPI)

Sample	MD/WPI	C*	H^0	TCD**
Blueberry juice	0	0.45 ± 0.03 ^{n}	7.05 ± 0.59 ^{j}	--
FMFD	0.4	62.78 ± 0.22 ^{d}	16.89 ± 0.20 ^{d}	76.66 ± 0.03 ^{f}
	1.0	64.58 ± 0.09 ^{c}	16.42 ± 0.23 ^{d}	76.35 ± 0.02 ^{h}
	1.6	66.84 ± 0.04 ^{b}	21.89 ± 0.09 ^{c}	78.10 ± 0.02 ^{b}
	2.3	67.39 ± 0.05 ^{a}	22.94 ± 0.07 ^{b}	78.23 ± 0.02 ^{a}
	3.2	67.58 ± 0.05 ^{a}	23.51 ± 0.06 ^{a}	78.24 ± 0.01 ^{a}
SD180	0.4	57.60 ± 0.05 ^{f}	12.61 ± 0.02 ^{ef}	77.35 ± 0.01 ^{d}
	1.0	48.48 ± 0.04 ^{l}	12.14 ^{fg}	70.63 ± 0.01 ^{n}
	1.6	54.14 ± 0.03 ^{j}	11.69 ± 0.01 ^{g}	75.47 ± 0.01 ^{j}
	2.3	56.08 ± 0.03 ^{h}	12.71 ± 0.01 ^{e}	76.44 ± 0.01 ^{g}
	3.2	57.83 ± 0.02 ^{f}	13.05 ± 0.02 ^{e}	77.30 ± 0.01 ^{e}
SD360	0.4	43.02 ± 0.03 ^{m}	9.29 ^{i}	73.76 ± 0.01 ^{l}
	1.0	50.17 ± 0.04 ^{k}	10.11 ± 0.01 ^{h}	73.01± 0.0 ^{m}
	1.6	54.46 ± 0.04 ^{i}	11.90 ^{g}	74.61 ± 0.0 ^{k}
	2.3	56.16 ± 0.03 ^{h}	11.86 ± 0.01 ^{g}	76.28 ± 0.01 ^{i}
	3.2	58.51 ± 0.03 ^{e}	12.99 ± 0.01 ^{e}	77.74 ± 0.01 ^{c}

*Mean values ± range of each measurement, followed by different single letter in each column if significantly different ($p < 0.05$, Tukey's test), whereas ab, for example, indicates values that are not significantly different from the corresponding a and b values. **Blueberry juice was used as a control against powder*

In order to compare the colour difference (TCD) obtained between FMFD and SD samples, the powders were reconstituted in water. Higher TCD means more colour changes correspond to changes of L*, a* and b*. Small values of total colour differences (TCD) are desirable since it is indicative of the colour stability of the powder.[113] As seen in Table 6-1, the reconstituted solutions of FMFD with MD/WPI = 1.0 presented higher TCD than SD 180 and 360. FMFD solutions produced with MD/WPI = 3.2 showed the most elevated TCD. With SD 180 and 360 powders, TCD was significantly changed with increasing MD.

6.2.8 Total phenolic content (TPC) and TPC retention

Total phenolic content of blueberry juice and reconstituted blueberry powders is presented in Table 6-3. Results showed that both drying methods and carrier agents did not significantly (P-value = 0.948) influenced the TPC of powders obtained. However, ANOVA of observed data indicated total phenolic content significantly (P-value = 0.019) varied with drying methods.

Average TPC of FMFD powders was 31.3 mg GAE g^{-1} solids, which was statistically (P-value = 0.019) higher than SD 180 samples (25.4 mg GAE g^{-1} solids) but not significantly different with SD 360 samples (26.7 mg GAE g^{-1} solids) (Data not shown). MD/WPI ratios did not appear to be a major factor for TPC values in FMFD process. However, increased MD/WPI ratio resulted in increasing TPC levels with SD 180 and SD 360. Maltodextrin is responsible for forming a dry crust around the droplets.[109] The phenolic compounds might be protected from heat exposure by this dry crust during spray-drying. Overall, it is seen that SD processing resulted in considerably lower TPC compared to the FMFD method.

As observed in Table 6-3, the TPC retention was 68-76%, 48-69%, and 54-72% for FMFD, SD 180, and SD 360, respectively. From this table, it is also observed that increased in MD/WPI ratio increase the TPC retention. There was no significant (P-value = 0.948) effect of drying methods and carrier agents on the TPC retention. However, drying methods significantly (P-value = 0.019) influenced the TPC blueberry powders, where average FMFD powders gave the highest TPC retention (73%). SD 180 and SD 360 gave low TPC retention, i.e., 60 and 62%, respectively (Data not shown). Franceschinis et al.[96] reported comparison of freeze- and spray-drying using maltodextrin as a carrier agent in producing blueberry powders. According to the

authors, total phenolic content retention of these two drying methods was 73 and 68%, for freeze-drying and spray-drying, respectively. Our result is in agreement with this study.

Table 6-3 Effect of drying methods and MD/WPI ratios on total monomeric anthocyanin (TMA), total phenolic content (TPC), TMA retention, and TPC retention

Sample	MD/WPI	*TPC	***TPC retention (%)	*TMA	***TMA retention (%)
Blueberry juice	0	43.25 ± 0.01	--	8.92 ± 0.03 ^{a}	--
FMFD reconstituted solution	0.4	29.43 ± 3.63	68 ± 8	7.11 ± 0.04 ^{c}	80 ^{b}
	1.0	30.53 ± 3.30	71 ± 8	7.33 ± 0.02 ^{c}	82 ^{b}
	1.6	32.18 ± 3.43	74 ± 8	7.34 ± 0.11 ^{c}	82 ^{b}
	2.3	32.84 ± 2.98	76 ± 7	7.82 ^{b}	82 ± 1 ^{b}
	3.2	31.73 ± 4.50	73 ± 10	8.03 ± 0.02 ^{b}	90 ^{a}
SD 180 reconstituted solution	0.4	20.84 ± 2.56	48 ± 6	4.38 ± 0.03 ^{g}	49 ^{e}
	1.0	21.86 ± 2.30	51 ± 5	4.92 ± 0.16 ^{f}	55 ± 2 ^{de}
	1.6	25.27 ± 0.27	58 ± 1	5.06 ± 0.17 ^{ef}	57 ± 2 ^{cd}
	2.3	29.10 ± 2.75	67 ± 6	5.24 ± 0.19 ^{def}	59 ± 2 ^{cd}
	3.2	29.87 ± 4.54	69 ± 11	5.49 ± 0.02 ^{de}	62 ^{cd}
SD 360 reconstituted solution	0.4	23.31 ± 2.64	54 ± 6	4.93 ± 0.09 ^{ef}	55 ± 1 ^{de}
	1.0	24.99 ± 1.33	58 ± 3	5.36 ^{def}	60 ^{cd}
	1.6	25.38 ± 1.72	59 ± 4	5.62 ± 0.14 ^{de}	63 ± 2 ^{c}
	2.3	28.52 ± 1.86	66 ± 5	5.42 ± 0.26 ^{d}	61 ± 3 ^{cd}
	3.2	31.14 ± 4.49	72 ± 10	5.61 ± 0.08 ^{d}	63 ± 1 ^{c}

Means values ± range *of each measurement followed by a different single letter in a column are significantly different ($p < 0.05$, Tukey's test). ab indicates values are not significantly different from a and b values.*
**Results expressed in* mg GAE g^{-1} blueberry solids, ***Results expressed in mg Cyn3Gl g^{-1} equivalent (blueberry solids,)* ****Blueberry juice was used as a control against powder*

6.2.9 Total monomeric anthocyanin (TMA) and TMA retention

Table 6-3 also shows the TMA content and TMA retention of foam-mat freeze-dried (FMFD) and spray-dried (SD) blueberry powders produced with different MD/WPI ratios. The monomeric anthocyanin content in the blueberry juice was 8.92 ± 0.03 (mg Cyn3Gl g^{-1} blueberry solids), while TMA of blueberry powders had 4.38-8.03 mg Cyn3Gl g^{-1} blueberry solids. Drying methods and carrier agents significantly (P-value = 0.000) influenced the TMA of blueberry powders. FMFD powders produced with different MD/WPI ratio showed significant ($P < 0.05$) differences in the TMA compared to those from SD. Regarding FMFD powders, the lowest TMA content was produced with MD/WPI ratio 0.4, while the MD/WPI ratio 3.2 gave the highest TMA. Increased the MD/WPI ratio resulted in higher monomeric anthocyanin contents of FMFD powders. The TMA of the FMFD powders was lower than that of blueberry juice powder (22.69 mg Cyn3Gl g^{-1} blueberry solids), and blueberry extract (60.72 mg Cyn3Gl g^{-1} blueberry solids) produced reported elsewhere via freeze drying.[113] SD powders showed lower TMA in comparison with FMFD powders (Table 6-3). SD 180 had the lowest TMA (e.g. 4.34 mg Cyn3Gl g^{-1} blueberry solids). However, the lower TMA values in SD powders here were in part due to the initially low value in the original blueberry juice. Furthermore, the inlet air temperature (150 ^{0}C) was higher here than in some of these other studies, which is expected to cause greater degradation of anthocyanins in the end product. Total monomeric anthocyanins retention is also presented in Table 6-3. It is observed that FMFD samples had better TMA retention (80-90%) compared to SD samples (49-63%). Turan et al.[113] also reported that freeze-drying process could retain TMA up to 90% in blueberry powder, while the spray-drying technique was found to be lower, i.e. 73%.

6.2.10 Individual anthocyanins

The concentration of individual anthocyanins from blueberry juice and reconstituted powders is presented in Table 6-4 Del3Gl of the blueberry juice sample (1.38 mg g^{-1} blueberry solids) had higher than that measured in all reconstituted powder samples. FMFD samples had 1.09-1.17 mg g^{-1} solids, while 0.62-0.77 mg g^{-1} solids was observed with SD samples. All Del3Gl concentrations measured from FMFD samples were significantly (P-value = 0.000) higher than with the SD powders, most probably due to the higher temperatures of spray-drying (Table 6-4).

As observed in Table 6-4**,** Cyn3Gl of the blueberry juice was 1.33 mg g^{-1} blueberry solids. FMFD samples had 1.32-1.37 mg g^{-1} solids, while 0.79-0.98 mg g^{-1} solids was observed with SD samples. The blueberry juice and FMFD samples had higher Cyn3Gl concentration than the SD samples. SD 180 caused a 35% reduction of Cyn3Gl except for the MD/WPI 3.2 samples, and SD 360 gave a slightly lower reduction (< 32%).

Mal3Gl concentration in the blueberry juice was measured as 1 mg g^{-1} blueberry solids (Table 6-4). FMFD samples had 0.80-0.88 mg g^{-1} solids, while 0.50-0.58 mg g^{-1} solids was observed with SD samples. FMFD samples exhibited significantly (P-value = 0.000) higher Mal3Gl concentrations compared to SD samples.

The highest individual anthocyanin of FMFD reconstituted powders was performed in Cyn3Gl, followed by Del3Gl and Mal3Gl. This outcome is contrary to that of Lee et al.[120] who found that Del3Gl, Cyn3Gl, and Mal3Gl of conventional freeze-dried blueberry powder was 1.43, 0.27 and 2.0 mg g^{-1} solids. On the other hand, our result agrees with the findings of Trost et al.[124]

In the case of individual anthocyanins retention, FMFD samples had higher Del3Gl retention (79-85%) compared to SD samples (45-55%). Cyn3Gl retention of FMFD was the highest retention 102-103%, while Cyn3Gl of SD samples was recorded as 58-72%. Mal3Gl retention was calculated as 80-88% with FMFD samples, whereas 50-58% with SD samples. These results were higher to those in the study carried out by Wilkowska et al.[107] who found retention of Del3Gl (freeze-dried sample = 28%, spray-dried sample = 31%), Cyn3Gl (FD = 28%, SD = 34%), and Mal3Gl (FD = 22%, SD = 29%).

Table 6-4 Effect of drying methods and MD/WPI ratios on individual anthocyanins

Sample	MD/WPI	*Del3Gl	Del3Gl retention (%)	*Cyn3Gl	Cyn3Gl retention (%)	*Mal3Gl	Mal3Gl retention (%)
Blueberry juice	0	1.38 ± 0.06 ^{a}	--	1.33 ± 0.07 ^{a}	--	1 ± 0.08 ^{a}	--
FMFD reconstituted solution	0.4	1.17 ± 0.10 ^{b}	85 ± 7 ^{a}	1.37 ± 0.08 ^{a}	103 ± 6 ^{a}	0.88 ± 0.06 ^{b}	88 ± 7 ^{a}
	1.0	1.14 + 0.02 ^{b}	82 ± 1 ^{a}	1.36 ± 0.02 ^{a}	103 ± 2 ^{a}	0.86 ± 0.02 ^{b}	86 ± 2 ^{a}
	1.6	1.09 ^{b}	79 ^{a}	1.37 ± 0.03 ^{a}	103 ± 3 ^{a}	0.88 ± 0.03 ^{b}	88 ± 3 ^{a}
	2.3	1.09 ± 0.01 ^{b}	79 ± 1 ^{a}	1.32 ^{ab}	100 ^{ab}	0.80 ± 0.01 ^{b}	80 ± 1 ^{a}
	3.2	1.12 ± 0.01 ^{b}	81 ± 1 ^{a}	1.34 ^{a}	102 ^{a}	0.83 ± 0.01 ^{b}	83 ± 1 ^{a}
SD 180 reconstituted solution	0.4	0.74 ± 0.01 ^{c}	53 ^{b}	0.85 ± 0.01 ^{cde}	63 ^{cde}	0.58 ± 0.01 ^{c}	58 ± 1 ^{b}
	1.0	0.74 ± 0.04 ^{c}	53 ± 3 ^{b}	0.82 ^{de}	60 ^{de}	0.55 ± 0.01 ^{c}	55 ± 1 ^{b}
	1.6	0.68 ± 0.02 ^{c}	49 ± 1 ^{b}	0.79 ± 0.03 ^{e}	58 ± 2 ^{e}	0.52 ± 0.03 ^{c}	52 ± 3 ^{b}
	2.3	0.77 ± 0.01 ^{c}	55 ^{b}	0.85 ^{cde}	63 ^{cde}	0.57 ± 0.01 ^{c}	57 ^{b}
	3.2	1.10 ± 0.03 ^{b}	79 ± 3 ^{a}	1.18 ± 0.03 ^{b}	87 ± 2 ^{b}	0.76 ± 0.04 ^{b}	76 ± 4 ^{c}
SD 360 reconstituted solution	0.4	0.62 ± 0.01 ^{c}	45 ^{b}	0.86 ± 0.01 ^{cde}	63 ^{cde}	0.50 ± 0.01 ^{c}	50 ± 1 ^{b}
	1.0	0.68 ± 0.01 ^{c}	49 ^{b}	0.92 ± 0.01 ^{cde}	68 ± 1 ^{cde}	0.52 ^{c}	52 ^{b}
	1.6	0.66 ± 0.0 ^{c}	48 ^{b}	0.91 ± 0.01 ^{cde}	67 ^{cde}	0.51 ^{c}	51 ^{b}
	2.3	0.70 ± 0.01 ^{c}	50 ^{b}	0.93 ^{cd}	69 ^{cd}	0.54 ^{c}	54 ^{b}
	3.2	0.72 ± 0.03 ^{c}	52 ± 2 ^{b}	0.98 ± 0.01 ^{c}	72 ^{c}	0.57 ^{c}	57 ^{b}

Means values ± range of each measurement followed by a different single letter in a column are significantly different ($p < 0.05$, Tukey's test). ab indicates values are not significantly different from a and b values

* Results expressed in mg g^{-1} blueberry solids

Chapter 7

Chapter 7 Overall Conclusions

7.1 Physical properties of foam-mat freeze-dried made with MD/WPI vs. with trehalose/pure proteins

Foam-mat freeze-dried (FMFD) powders were made with MD/WPI = 2.8 (M3W1), trehalose/β-lactoglobulin = 2.8 (T3BL1), and trehalose/bovine serum albumin = 2.8 (T3A1). Foam with MD/WPI was found less stable than those with trehalose/pure proteins since the MD/WPI gave high foam density. The yield of M1W3 samples reached 79%, whereas T3BL1 and T3A1 recovered 66 and 67%, respectively. The solubility of these three foam-mat freeze-dried powders was > 96% which was commonly found in other freeze-dried fruit juice powders. The M3W1 had higher moisture content (3.5%) than those samples with trehalose/pure proteins (< 2%), which was attributed to long rehydration time (90 s). The bulk density of M3W1 powders was 0.32 g cm^{-3}, while T3BL1 and T3A1 were 0.55 and 0.6 g cm^{-3}, respectively. The FMFD powders made with trehalose/pure proteins generated pores and ordered structures, while the M3W1 powder had broken glass-like structures. The FMFD powder with MD/WPI had higher a* (redness) and b* (yellowness) values than powders with trehalose/pure proteins. Conversely, L* (brightness) value of powder with MD/WPI was lower compared others. The M3W1 sample was superior in the total phenolic content (TPC) and total monomeric anthocyanins (TMA) compared to both samples made with pure proteins. The TPC and TMA retention of M3W1 were recorded as 73 and 95%, respectively.

7.2 Physical properties of foam-mat freeze-dried vs. spray dried blueberry powder

MD/WPI ratio of 0.4, 1.0, 1.6, 2.3, and 3.2 was tested via foam-mat freeze-drying (FMFD) and spray-drying (SD). FMFD method gave the highest yield (76%) at MD/WPI 1.6, while SD 180 and SD 360 gave a yield of 66.5% with similar MD/WPI composition. Beyond this ratio, the yield of SD 180 and SD 360 powders increased to maximum 67 and 72%, respectively. The moisture content of both FMFD and SD powders was comparable with a similar composition. a_w of all the powders produced was less than 0.40 which is acceptable for such powders regarding inhibition of microbial growth and biochemical degradation. In term of rehydration time, SD 360 powder was quicker (65-70 s) over the FMFD powders (74-78 s). However, bulk densities of the FMFD samples were lower than SD samples. The FMFD reconstituted solutions was less bright (L* <<) and deeper red (a* >>) compared to SD reconstituted solutions. Total phenolic content (TPC) of the FMFD was found higher than those of SD samples. The TPC retention reached 73% with FMFD, while 60 and 62% with SD 180 and SD 360, respectively. In the case of total monomeric anthocyanin, FMFD powders showed higher concentration than SD powders. TMA retention of 80-90% was recorded with FMFD samples, while 49-63% of SD samples. Del3Gl retention of FMFD was 79-85% compared to SD samples 45-55%. Cyn3Gl retention of FMFD was the highest retention 102-103%, while Cyn3Gl of SD samples was recorded as 58-72%. Mal3Gl retention was calculated as 80-88% with FMFD samples, whereas 50-58% of SD samples.

7.3 Future work

This study has demonstrated the possibility of using polysaccharides and proteins in the blueberry juice as matrices for foam-mat freeze-drying of blueberry powders. The foam-mat freeze-drying offers a novel method to keep the physical properties and bioactive compounds as high as the fresh products. Based on the findings from this work, further research could be undertaken to understand the foam-mat freeze-drying process:

- The condition of foaming processes, such as different whipping times and temperatures of liquid, could be investigated. This could obtain better foam stability for foam-mat freeze-drying.
- It would be of considerable interest to measure bubble size distribution as the surface area of foam bubbles could influence the heat and mass transfer during drying.
- It might be useful to observe different freezing rates before freeze-drying as it will effect the ice crystal size and pores of dried layer. This maybe influences the rehydration and bulk density of powders.
- There could be potential benefits in using different freeze-drying temperatures and vacuum pressures which could improve the sublimation of ice crystals and desorption process.
- There is a need to investigate the shelf life of foam-mat freeze-dried powder to have more information on the stability of phenolic compounds and anthocyanins. Furthermore, colour degradation as affected by the temperature should be evaluated.

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