Supercritical CO_2 extraction of waxes from date palm (*Phoenix dactylifera*) leaves: optimisation, characterisation, and applications.

Karima Abdullah Al Bulushi

Doctor of Philosophy

University of York

Chemistry

May2018

Abstract

The low cost, abundant, underexploited and underutilised renewable agricultural waste residue, date palm leaves (*Phoenix dactylifera*), were extracted using supercritical carbon dioxide (scCO_2) to obtain valuable waxes. The extraction process was optimised using second order factorial design to obtain high yield of waxes. Date palm leaves exhibited relatively high yield of wax of 3.49%, as compared to other agricultural residues extracted with scCO_2. Diverse range of lipophilic compounds were characterised and quantified including *n*-alkanes, free fatty acids, free fatty alcohols, long chain aldehydes, sterols and wax esters. Waxes extracted at different extraction pressure and temperature exhibited significant difference in melting profile (ranging from 35 °C for extractions at 40°C and 80 Bar to melting points of 78 °C for extractions at 100 °C and 400 Bar). Thus, suggesting the opportunity to tailor extraction to meet a target application. ScCO_2 extraction has several advantages over organic solvent extraction which were demonstrated in this work.

Date palm leaves wax was tested as structuring agent for sunflower oil along with other commercial natural waxes. Date palm wax based oleogel exhibited low critical gelling concentrations compared to other waxes. Chemical composition and crystal morphology for the waxes and their gels were further explored to gain better understanding of their gelling behaviour. Date palm wax exhibited good gelling ability and high thermal stability compared to other commercial waxes. The rheological profile for date palm wax based oleogel was comparable with other natural waxes making it a promising structuring agent in food industry.

The scale up of scCO_2 extraction was studied at semi-pilot scale and resulted in comparable yields, chemical composition and melting profile of wax to the lab scale. Attempts to further reduce the complexity of the wax by fractional extraction, yielding three different wax fractions with varying in texture, composition and physical properties. Economic aspects of the extraction process were explored to further assess the viability of the process. Cost of Manufacture of date palm wax was initially €14.01 kg^{-1} wax, which could be further reduced to €8.80 kg^{-1} wax by biomass pelletising. If the extracted biomass was utilised to generate electricity the costs are further reduced to 3.88 kg^{-1} wax.

Table of Contents

List of tables

List of Figures

Acknowledgment

Thanks for almighty Allah first and foremost for the great success and strength he provided ever in my life. I would like express my sincere gratitude to my supervisor Dr. Andrew J. Hunt for the great support and guidance throughout my PhD. I am truly grateful to have such supportive understanding supervisor who was there for advice, encouragement and help. I would like to thank Professor Mike North my second supervisor for his help and understanding towards the end of my PhD. I would like also to thank Professor James Clark for giving me the opportunity to do my PhD. I would like to thanks Ministry of Higher Education in Oman for the scholarship and thanks goes to my employer Ministry of Man Power for giving me the study leave.

A special thanks goes to Dr. Thomas Attard for his invaluable help and advice throughout the years. A big thank you to Dr. Con Robert McElroy and Dr. Thomas Attard for help with supercritical rig and fixing it all the times I blocked it. Thanks to Karl Heaton from Mass spectrometry Centre of Excellence in York for carrying out the APCI mass spectrometry and to Dr. Meg Stark for assistance with SEM imaging. Thanks to Philip from Professor Davis Smith group for the help with rheometer and rheological data. Special Thanks to Dr. Ashok Petal from Gent University for the valuable advice on the rheological sample preparation and loading. I would like to Thanks Mr. Paul Elliot for equipment training and general help in laboratory. I am grateful to Dr. Mario de Bruyn for conducting further analysis on my wax samples. My thanks also dedicated to Mrs Alison Edmonds for her great support, help in making my life possible at York and introducing me to Cathy De Bruyn. A special thank for Andrew Maneffa for chatting with me when nobody else did, Andrea and Fergal for being so kind and helpful and Imm for being there when I needed to complain. A big thanks for all who proof read my thesis including Dr. Andrew Hunt, Dr. Thomas Attard, Dr. Con Robert McElroy, Dr. James Sherwood, Andrew Maneffa, Buthaina and Fergal.

A special thanks for my lovely super supportive husband Yousef Al Bulushi and my two kids Sara (6 years) and Amin (4 years) for being there to make me smile, for making things more challenging and being awesome. A massive thanks to my Mum, Dad, my brothers and sisters for all their support love and encouragement in my entire life.

Declaration

I declare that this thesis is a presentation of original work and I am the sole author. Some of the results are obtained by, or in collaboration with other workers, who are fully acknowledged in the text. This work has not previously been presented for an award at this, or any other, University. All sources are acknowledged as References.

Karima Abdullah Al Bulushi

May 2018

Chapter 1

Introduction

1 Introduction

1.1 The aim and scope of the project

The preliminary aim of this project is to valorise date palm leaves (Phoenix *dactylifera*) and add value using a benign and green extraction technology. Previous studies identified waxes as a potential high-value product from date palm leaflets. The aims of the project can be summarised as follows:

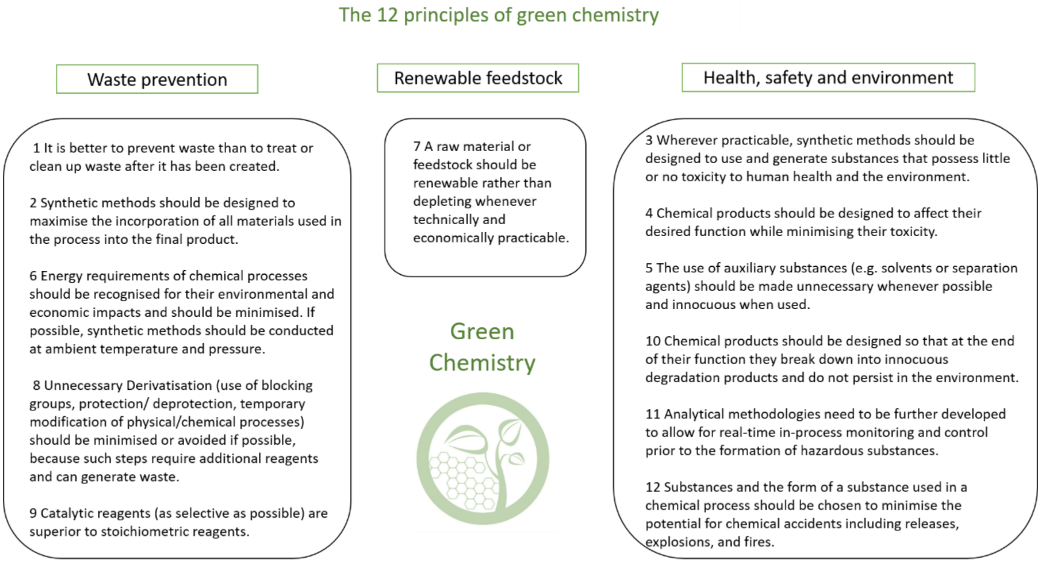
- Extraction of surface lipids from date palm leaves using a $scCO_2$ extraction process: The $scCO_2$ extraction is optimised using a second-order factorial design where temperature and pressure are varied to investigate their effects on percentage yield. The extracted waxes are characterised and quantified. Conventional Soxhlet extraction with heptane is compared with $scCO_2$ extraction.

- The potential application of the extracted wax in food industry: Waxes are known for their ability to gel vegetable oil, which make them a potential replacement for some unhealthy fats that are used currently in the food industry as shortening agents in baking and confectionary. Date palm leaf wax is investigated in oleogel preparation and subsequently tested to compare the quality of the novel oleogels with those produced from other natural waxes.

- Scale up, fractionation and economic aspects of the extraction process: The scale up of $scCO_2$ extraction of the waxes to semi-pilot scale is conducted to assess the viability of upscaling process. Fractional extraction for the waxes is attempted to reduce the complexity of extracts and obtain a range of compounds. The cost of the extraction of the waxes is estimated on industrial scale to further gain insight into the viability of the process.

1.2 Green and sustainable chemistry

Sustainable development was a term first coined in the Bruntland Commission (United Nations Commission on Environment and Development) in 1987 as "*meeting the need for the present without compromise the ability of future generations to meet their own needs*" and has been widely adapted as a culture in all aspects of modern life.[1] It is the most comprehensive term in industrial and social development that takes into consideration the society, environmental, and economical elements. Therefore, in order to achieve sustainable development, all of the aforementioned elements must be taken into consideration. The vast majority of the world industrial economies heavily depend on fossil fuel as a source of energy and chemicals.[2] Fossil fuels are depleting at an alarming rate and this, coupled with the increasing world demand for energy and chemicals (especially in developing countries), makes it all the more important for sustainable development to be considered by governments, societies, economies and individuals.[2, 3] Green chemistry is considered to be one of the most attractive tools and technologies for achieving sustainable development on a molecular level.[4] Indeed it exploits a set of principles that efficiently use raw materials and minimise or eliminate the generation of hazardous substances in the design, manufacture and application of chemicals.[1, 4] The 12 principles of green chemistry were first introduced by Paul Anastas and John Warner in 1998.[1] These can be listed under three basic elements: waste prevention through efficient utilisation of the raw materials, consideration of health, safety and environmental issues and the use of renewable feedstock as depicted in Figure 1-1.The work in this study falls under the principle 7 in that agricultural waste is utilised as a renewable feedstock for extracting high value chemical and because CO_2 is employed as an environmentally friendly, abundant and renewable solvent for the extraction of natural products. Moreover, CO_2 leaves no waste solvent and thus, also fulfils principle number 1 due to the preventing waste. This fact is particularly noteworthy as many extraction technologies involve the use and subsequent generation of significant quantities of hazardous solvents and their resulting waste.[5]



1 It is better to prevent waste than to treat or clean up waste after it has been created.

2 Synthetic methods should be designed to maximise the incorporation of all materials used in the process into the final product.

6 Energy requirements of chemical processes should be recognised for their environmental and economic impacts and should be minimised. If possible, synthetic methods should be conducted at ambient temperature and pressure.

8 Unnecessary Derivatisation (use of blocking groups, protection/ deprotection, temporary modification of physical/chemical processes) should be minimised or avoided if possible, because such steps require additional reagents and can generate waste.

9 Catalytic reagents (as selective as possible) are superior to stoichiometric reagents.

7 A raw material or feedstock should be renewable rather than depleting whenever technically and economically practicable.

3 Wherever practicable, synthetic methods should be designed to use and generate substances that possess little or no toxicity to human health and the environment.

4 Chemical products should be designed to affect their desired function while minimising their toxicity.

5 The use of auxiliary substances (e.g. solvents or separation agents) should be made unnecessary whenever possible and innocuous when used.

10 Chemical products should be designed so that at the end of their function they break down into innocuous degradation products and do not persist in the environment.

11 Analytical methodologies need to be further developed to allow for real-time in-process monitoring and control prior to the formation of hazardous substances.

12 Substances and the form of a substance used in a chemical process should be chosen to minimise the potential for chemical accidents including releases, explosions, and fires.

Figure 1-1 The 12 principles of green chemistry[3, 4]

1.3 Extraction Techniques

Solvents play an essential role in many chemical processes in facilitating a chemical reaction or the separation of a product.[6, 7] In extraction, solvents are crucial in dissolving and separating the target solute from the solid matrix.[6, 7] in most cases, the extraction efficiency is affected by the solvent as it determines two important factors: (1) the solubility of the solute and or solutes and (2) solvent ability to penetrate into the matrix.[6] Usually, a solvent is chosen based on the nature of the target solutes and matrix.[6] Polar hydrophilic solvents are used to extract polar solutes and nonpolar solvents are selected for hydrophobic compounds.[6]

Some of the current solvents used in industry for extraction of spice, oleoresins and hop including hexane, have health, safety, and environmental issues associated with them.[8] For instance, hexane is flammable, reported as neurotoxic and is a hazardous air pollutant according to US environmental protection agency (EPA).[9] Hexane is widely used in the extraction of soybean oil and the use of it is now restricted by maximum residue level in the food and beverage industry.[8] As a consequence, stringent legislation and many new initiatives established by various chemical industries such as the Responsible Care Global Charter have been introduced in order to continuously improve the environmental, health and safety credentials for chemical processes.[10] In this context, the Food and Drug Administration (FDA) has classified solvents such as benzene and carbon tetrachloride as class 1 solvent which should be avoided in pharmaceutical industries unless their use is unavoidable.[11] Other solvents including hexane and toluene (under class 2) are also restricted.[11] Recently Jerome and Gu proposed 12 criteria that a green solvent should possess as illustrated in Figure 1-2.[10]

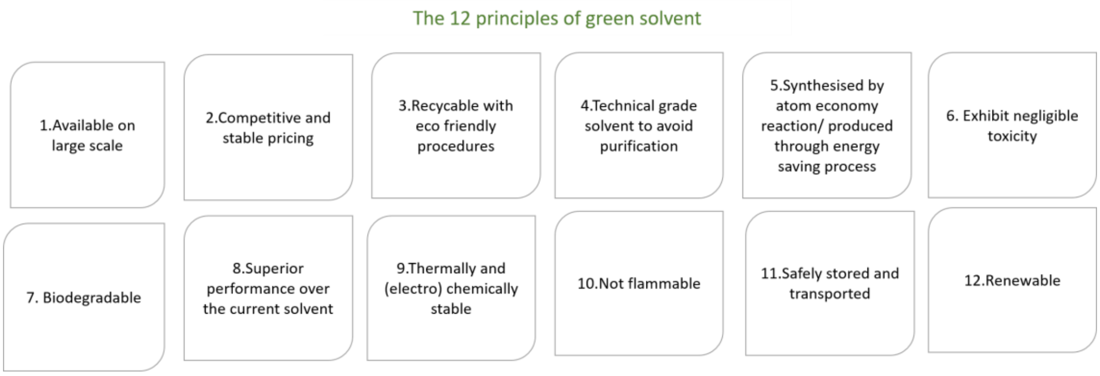


Figure 1-2 The 12 principles of green solvents.[10]

Unfortunately, an organic solvent that satisfies all these criteria does not exist. However, in recent years, there has been increased interest in discovering and utilising greener solvents, and there is currently extensive research dedicated to finding suitable green alternatives to volatile organic compounds (VOCs).[8] For instance, researching bio-based solvents derived from biomass is gaining enormous attention as a result of the rise in petroleum prices, the reduction in carbon foot print for renewably-derived substances and their possibility to provide safer, biodegradable and less toxic chemicals.[8, 10] More specifically, in the extraction of the natural products, new extraction techniques have emerged which use safer solvents like supercritical carbon dioxide ($scCO_2$) or require a minimum amount of solvent such as microwave assisted extraction (MAE) and ultrasonic assisted extraction (UAE).[12] Most often, new extraction techniques are compared to conventional extraction technology such as Soxhlet as a bench mark.

1.3.1 Soxhlet extraction

Extraction is considered as one of the most important steps in many analytical protocols in order to prepare and concentrate the desired analytes and particularly if they are solid samples.[13] During the extraction process, the desired compound or group of compounds are preferentially transferred from the matrix into a different surrounding phase. Soxhlet extraction is a well-recognised classical technique for solvent extraction, designed by Franz von Soxhlet in 1879 and it remains a useful technique to this day.[6, 13] It has been widely used for the extraction of natural compounds, and there are many standard procedures such as US EPA official methods, AOAC and British standards that are based on Soxhlet extraction.[6] In fact, Soxhlet extraction is used as a reference extraction method for assessing the performance of alternative solid-liquid extraction techniques including supercritical fluid extraction (SFE), ultrasound assisted extraction (UAE) and microwave assisted extraction (MAE).[6] In a typical Soxhlet extraction, the sample is transferred into a thimble (usually made from paper or glass) which is then placed in a glass extraction chamber that is connected to a flask which contains the extraction solvent. A condenser is fitted above the flask as shown in Figure 1-3. When the solvent boils, the extraction chamber fills gradually with condensed solvent from the distillation flask until it reaches a maximum level where it rinses back into distillation flask by siphon, carrying with it the extracted solute. This is referred to as a cycle and is repeated every 10-15 minutes.[6, 13]

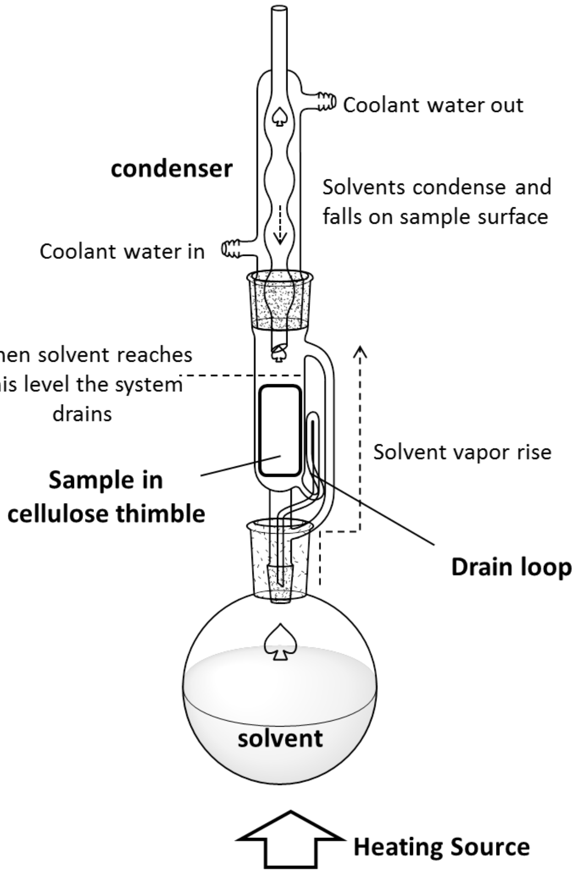


Figure 1-3 A typical Soxhlet extraction set up.[13]

Soxhlet extraction is an attractive, simple and cheap extraction technique which requires little training and relatively low financial expenditure.[13] It is an effective extraction technique as the sample is repeatedly brought into contact with fresh solvent, aiding the displacement of the transfer equilibrium.[13] In fact, it offers a higher yield when compared to any other extraction technique including supercritical CO_2 extraction and microwave assisted extraction (MAE) .[13]

However, long extraction times are required and the use of large amounts of solvent (often non-renewable) leads to the generation of considerable quantities of waste which creates issues in terms of disposal costs, as well as environmental problems. Furthermore, extractions are often performed at elevated temperature, thus leading to the potential decomposition of thermally labile compounds. Moreover, an evaporation condensation work up is typically needed at the end of the extraction to recover the desired sample.[6, 13] Finally, Soxhlet extraction is difficult to automate and is restricted to solvent selectivity.[13]

Having considered all of these drawbacks, conventional Soxhlet extraction was used as a starting point to develop new extraction approaches based on this system with the aim of reducing extraction time using microwave heating or ultrasound and automating the technique.[6, 13]

1.3.2 Supercritical carbon dioxide extraction

A supercritical fluid is a substance that is above its critical temperature and pressure (T_c, P_c).[14, 15] Varying the temperature and pressure of a supercritical substance can change their physical properties.[14-16] The phase diagram in Figure 1-4 is a simplified plot of vapour pressure and temperature, which aids in understanding this phenomena. Stable chemical compounds exhibit a triple point (TP) and critical point (CP).[15] At the triple point, all three forms of matter, solid, liquid and gas coexist in equilibrium. The curve of gas-liquid in the phase diagram starts at the triple point, which represents the equilibrium coexistence of the vapour and liquid phases. Moving from the TP towards the CP along the vapour-liquid curve results in a decrease in the density of the liquid phase due to thermal expansion and an increase in the density of the gas phase due to increase in pressure.[15] Upon reaching the critical point, the density of liquid phase and gas phase become identical and the meniscus separating the liquid and gas phase, diminishes, resulting in a single phase.[16] The phase diagram in Figure 1-4 corresponds to that of carbon dioxide, which has a critical point of 73.8 bar and 31.1 °C.

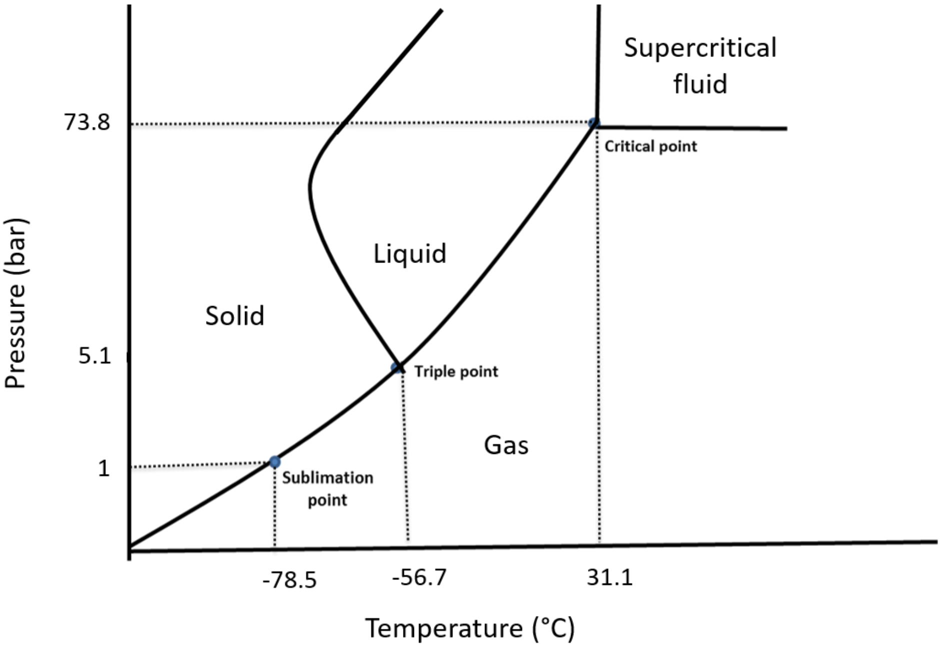


Figure 1-4 Phase diagram for carbon dioxide (CO_2).[17]

Supercritical critical fluid extraction (SFE) and more specifically supercritical carbon dioxide ($scCO_2$) is one of the most widely used extraction techniques for natural products from biomass.[18] Although there is a broad range of compounds that can be used as solvent for SFE (listed in Table 1-1), carbon dioxide is by far the most commonly used solvent in SFE due to its readily accessible low critical point at T_c (31.1 °C) and P_c (73.8 bar) Table 1-1. Moreover, carbon dioxide is benign, renewable and widely available. It is relatively inexpensive, non-flammable and has minimal toxicity. $ScCO_2$ is considered as a generally recognized as safe (GSAR) solvent and therefore it is regulated only by general manufacturing practise (GMP).[16] The relatively high critical pressure for CO_2 is a slight disadvantage as it makes the risk associated with the extraction process relatively high. However, this pressure has become routine in industrial extraction process such as decaffeination of coffee.[19] In addition, the $scCO_2$ extraction is associated with high capital cost, which can be offset if the extraction process is incorporated as a first step in a holistic biorefinery where $scCO_2$ is considered as a first step and the post extracted biomass are passed in downstream processing.[20]

Table 1-1 Critical Temperature (Tc) and pressure (Pc) values for various solvents.[21]

Solvent	Critical Temperature T_c (°C)	Critical pressure P_c (bar)
Ethylene	9.3	42.5
Ethane	32.2	48.8
Ethanol	240.8	61.4
Carbon dioxide	31.1	73.8
Propane	96.7	42.5
Ammonia	132.5	112.8
Toluene	318.6	41.1
Benzene	289.0	48.9
Water	374.2	220.5

Carbon dioxide in its critical state has the properties of both a gas and liquid, with its density being similar to a liquid and viscosity similar to a gas, as listed in Table 1-2.[16] Indeed, the high density of the $scCO_2$ enhances the solubility of the solutes while the low viscosity enables the rapid penetration of the solvent into the solid matrix and therefore facilitates a lower extraction time.[16, 22] The high diffusivity combined with high tuneable solvent strength makes $scCO_2$ a very attractive solvent for extraction.[17] For instance, $scCO_2$ can be used in the extraction of lipid molecules as it has a polarity similar to hexane and toluene.[23-25] Therefore, lower molecular compounds which are soluble in hexane, should also be soluble in $scCO_2$. One of the main advantages of using $scCO_2$ in the extraction of natural matrices is the selective extraction of non-polar compounds by fine-tuning the solvent strength, which is achieved by varying temperature and pressure.[14, 22] Varying temperature and pressure alter the density of the fluid (Figure 1-5) and hence change in density dependent properties such as solubility parameter, partition coefficient and dielectric constants which are all crucial in the extraction process.[14, 22] Indeed, carbon dioxide is gaseous at room temperature and atmospheric pressure making product recovery easy resulting in a solvent free extract. This makes it an advantageous extraction technique when it comes to food, pharmaceutical and cosmetic industries, where solvent residues must be at an absolute minimum.[12, 14].

Table 1-2 The physical properties of gas, liquid and supercritical carbon dioxide.[17]

Physical Properties	Gas	Supercritical	Liquid
Density ($g.cm^{-3}$)	10^{-3}	0.1-0.9	1
Viscosity (Pa. s)	10^{-5}	10^{-4}	10^{-3}
Diffusion coefficient ($cm^2. s^{-1}$)	0.1	10^{-3}	10^{-5}-10^{-6}

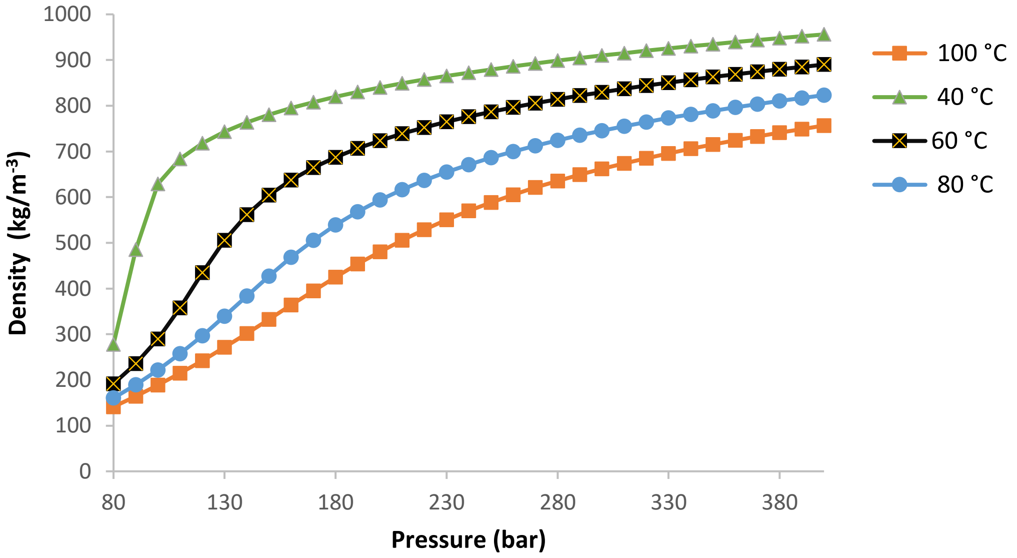


Figure 1-5 The effect of increasing pressure on the density of CO_2 at various temperatures.[26]

Most extractions have been conducted in a temperature range of 40-70 °C and a pressure of 100 - 400 bars.[18] The optimum extraction condition is achieved by tuning the temperature and the pressure in order to increase the solvent power of $scCO_2$.[17] $ScCO_2$ has low polarity which make the applications of $scCO_2$ very limited to nonpolar compounds.[16, 17] Studies on ternary system (two solutes and supercritical fluid) showed that the solubility of the less soluble solute is enhanced more significantly by the higher entraining effect of other solute compared to a binary system (only one solute and supercritical fluid).[27-29] This phenomena is known as entrainer or co-solvent effect where the solubility is enhanced in the presence of other solute which act as co-solvent/entrainer.[27]The addition of small amount of volatile compound such as methanol or ethanol enhances the solubility of polar compounds as well as non-polar compounds.[27] Similarly, addition of other solutes in binary system enhance the solubility.[16, 17, 29] Figure 1-6 shows the modifier solvent used with $scCO_2$. Ethanol is the most commonly used modifier with $scCO_2$ followed by methanol and water.[18] Ethanol is an innocuous solvent, which is safe for both humans and the environment compared to other solvents such as hexane and methanol especially when the extraction is in food, pharmaceutical and cosmetics industries.[16]

$ScCO_2$ extraction has been industrially applied in the decaffeination of coffee and tea as well as extraction of the following: bitter acids from hops for brewing industry,[19, 30, 31] flavours and aromas from spices and herbs,[30, 32, 33] nicotine from tobacco,[30] and edible oils.[34, 35] In the last two decades, it has been extensively exploited in the extraction of different compounds from oil, fatty acids, carotenoids, triglycerides, lipids and phenolic compounds.[18] Detailed mapping of $scCO_2$ extraction form various vegetable matrices are highlighted by several reviews.[36, 37]

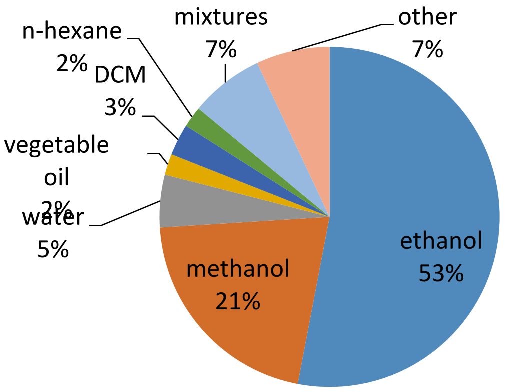


Figure 1-6 Most employed modifier solvents for SFE of vegetable matrices in publications from 2000-2013.[18]

1.4 Waxes

Waxes in their strictest chemical definition are long chain alkyl esters resulting from an esterification of long chain fatty acids with long chain aliphatic alcohols. Nevertheless, the term wax or more specifically plant wax is used to describe collective lipid components covering aerial surfaces of the plant tissue.[38, 39]

The outermost layer of the plant tissue is the cuticle, which covers the aerial surface of the plant as a continuous extracellular membrane.[40, 41] The cuticle is composed of the cuticle proper, which is sandwiched between two layers of interior intracuticular and exterior epicuticular waxes as illustrated in Figure 1-7.[38-41] The cuticle proper is a layer of cutin, which is composed of crossed linked fatty acids and usually bonded by a layer of wax.[38-41] Intracuticular wax consists of amorphous mixtures of mostly cyclic lipids such as triterpenoids and some long-chain primary alcohols embedded in the cutin matrix as shown in Figure 1-7.[39-42] The epicuticular wax is the surface lipids, which consist mainly of long-chain alkanes and fatty acids forming the crystalloids or smooth film at the outermost of the cuticle.[39-42] This hydrophobic nature of the plant cuticle has many roles including prevention of loss of water, protection of the plant from bacterial and fungal pathogens and the shielding of plants from UV-B radiation.[43]

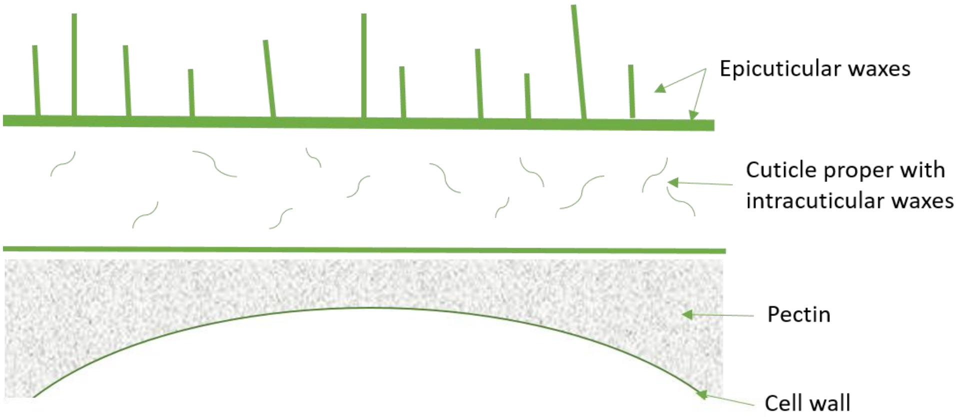
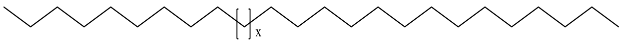
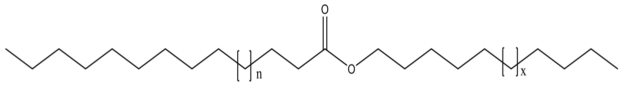
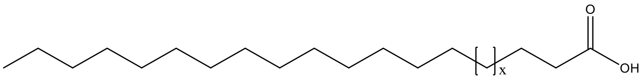
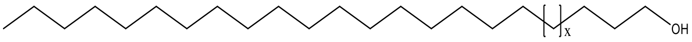
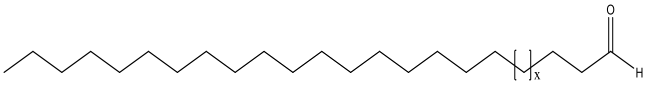
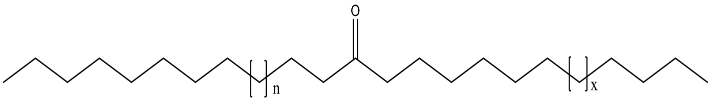
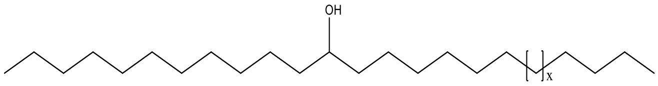
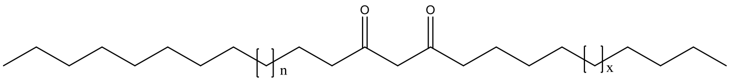


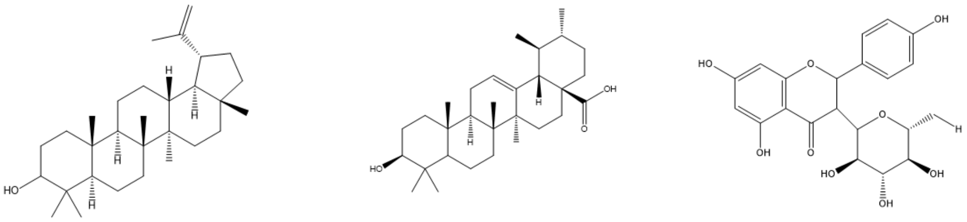
Figure 1-7 Graphic structure of epidermal cell and plant cuticle.[41]

The vast majority of plant waxes consists of long chain aliphatic compounds (Table 1-3) including hydrocarbons, wax esters, alcohols, free fatty acids, aldehydes and less commonly secondary alcohols, ketones, β-diketones and diesters. Most cyclic compounds found within plant waxes are sterols, flavonoids and triterpenes as in Figure 1-8.[40, 41, 44]

Plant wax hydrocarbons usually are a mixture of *n*-alkanes with chain lengths varying from C_{21} to C_{37} and with the odd-chained compounds being predominant over even-chain molecules.[40, 41, 44] The most abundant chain lengths are C $_{31}$ and C_{29}, which often account for 90% or more of the hydrocarbon fraction in waxes.[40, 41, 44] Nevertheless, there are many exceptions to the generalisation in regard to chain length, structure and abundance. For instance, shorter chain lengths were reported for algae with C_{17} being the major component while longer chain lengths were found in cane grass wax C_{62}.[40] Even chain length hydrocarbons are usually found as minor constituents with their chain length differing by one carbon atom from those of the major odd hydrocarbons.[44, 45]

Table 1-3 Major components of plant waxes.[38, 40]

Compounds	Chemical structure	Chain length and dominance
***N*-alkanes**		C_{21}-C_{33} odd numbered
Wax esters		C_{34}-C_{64} even numbered
Fatty acids		C_{16}-C_{32} even numbered
Primary alcohols		C_{22}-C_{32} even numbered
Aldehydes		C_{22}-C_{32} even numbered
Ketones		C_{23}-C_{33} odd numbered
Secondary alcohols		C_{23}-C_{33} odd numbered
B-Diketones		C_{23}-C_{33} odd numbered



Triterpenols Triterpenoid acids Flavonoids

Figure 1-8 Major cyclic compounds in plant cuticular wax

Wax esters are an abundant class of compounds in plant waxes and can account up to 80% of the total wax composition. Unlike the hydrocarbons, they predominantly have an even number chain length. The most common are monoesters, which typically arise from the esterification of long even chain fatty acids (C_{12}- C_{30}), and fatty alcohols (C_{20} – C_{32}). The resulting long alkyl chain esters have a chain length ranging from C_{32} to C_{64}.[46] Free fatty acids are also common in plant waxes and usually have even chain lengths ranging from C_{16} to C_{34}.[44] These fatty acids are mostly saturated and unbranched but, branched and unsaturated fatty acids have been reported.[44]

Primary alcohols found within plant wax typically straight, long-chain, even number compounds with a chain length range of C_{22} – C_{34}. There is usually one alcohol chain length which dominates the alcohol fraction such as hexacosanol (C_{26}) or octacosanol (C_{28}) and this depends on the plant.[40, 44] Aldehydes are closely related to alcohols in terms of chain length and usually have same chain lengths as alcohols.[47] Generally, their occurrences are minor as they are intermediate for further oxidation of fatty acids to alcohols.[47] However, octacosanal (C_{28}) found as major component in sugar cane wax.[48, 49] Ketones found in plant waxes are closely related to hydrocarbons with comparable chain length.[44, 50] The carbonyl group in ketones is usually found in the middle of the chain. For instance, nonacosan-15-on (C_{29}) and hentriacontan-16-one (C_{31}) are found in waxes where nonacosane (C_{20}) and hentriacontane (C_{31}) respectively are major components in the hydrocarbon fraction.[51-53] While the chain length reported for β-diketones in plants were typically in range C_{27}-C_{33}.[54] Secondary alcohols and ketones often occur together although the amount may vary considerably.[50, 55]

1.4.1 Biosynthesis of the long chain aliphatic plant wax constituents

The synthesis of aliphatic compounds in plant waxes occurs in the epidermal cells by a very complicated process which involves joining C_2 building blocks of acetyl-coenzyme A (Acetyl Co-A) and forming a long straight-chain with 24-32 carbon atoms. Figure 1-9 simplifies the wax synthesis process. Aliphatic compounds are synthesised in the epidermal cells in three stages. First, the *de novo* synthesis of the C_{16} and C_{18} fatty acids which occurs in the plastids.[40, 56-58] Both C_{16} and C_{18} serve as a central intermediate precursor for the synthesis of the other plant wax components. The second stage involves the elongation of C_{16} and C_{18} fatty acids into very long-chain fatty acids (VLCFAs).[40, 56-58] The elongation takes place in the endoplasmic reticulum in repeated reactions via fatty acid elongase (FAE) to produce VLCFAs with chain lengths of 24-32 carbon atoms.[39, 56] In the third stage, the primary wax components are assembled in the endoplasmic reticulum via two pathways: acyl-reduction and decarbonylation pathways.[39, 43, 56] The acyl-reduction pathway produces primary alcohols and wax esters while the decarbonylation pathway produces compounds including alkanes, secondary alcohols, aldehydes and ketones.[43, 56]

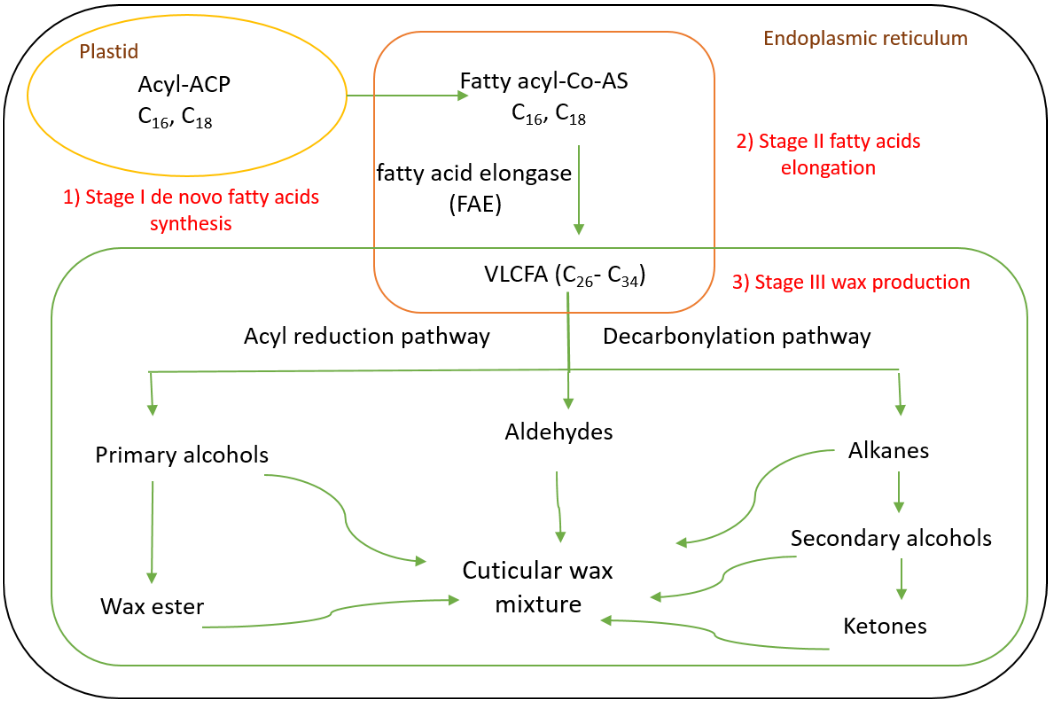


Figure 1-9 The biosynthesis of plant cuticular wax.

1.4.2 Conventional vegetable waxes

1.4.2.1 Carnauba wax

Carnauba wax is obtained from the leaves of the Brazilian palm tree (*Copernicia cerifera*). It is the hardest natural wax known so far with its high melting point above 80 °C.[59] It has a high content of wax esters (about 85%) but fewer fatty acids.[59, 60] Because of its unique properties carnauba wax is found in many applications ranging from pharmaceuticals, cosmetics to polishing products and electronics.[59] However, carnauba wax production is exclusive to Brazil due to restricted growth of the carnauba palm tree in Brazil.[59]

1.4.2.2 Candelilla wax

Candelilla wax is produced from *Euphorbia antisyphilitica*, a shrubby spurge grown in the northwest of Mexico and southwest of USA.[61, 62] Candelilla wax has potentially many applications ranging from cosmetics and pharmaceuticals to varnishing products.[61, 62] However, around 90% of candelilla wax is used in cosmetics especially in mascara, lipstick and cosmetic pencils due to its unique properties including texture (make it easily bendable with other component without causing the stiffens of the product) ,absorbed in skin easily and imparting high gloss factor.[63] Cosmetic products which have candelilla wax show temperature stability and a long shelf life.[61] Candelilla wax consists of odd numbered *n*-alkanes (C_{29}- C_{33}) and even numbered monoesters (C_{28}- C_{34}) along with free fatty acids, alcohol and sterols.[61]

1.5 Demand for natural waxes.

Waxes are important constituents of many cosmetics, personal care products, pharmaceutical coatings as well as varnishing materials.[64] Indeed, there is a huge demand for the waxes in the world driven by the economic growth of developing countries as well as increasing applications of the waxes in rheology, surface and health industries. Figure 1-10 shows the estimated demand of waxes by applications in 2010 with the total estimated demand of 9,590 lb.[65, 66]

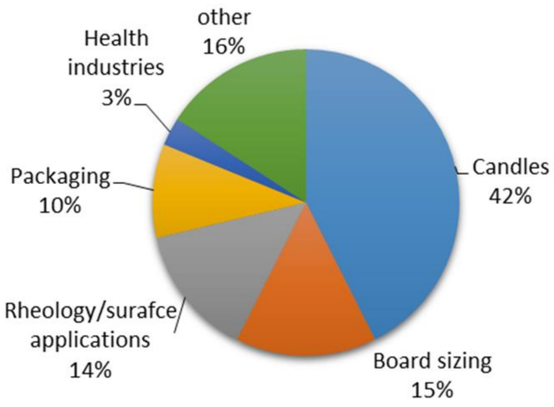


Figure 1-10 The estimated market of waxes by application in 2010.[67]

The most consumed waxes in the world are petroleum waxes followed by synthetic waxes as depicted in Figure 1-11.[65-67] However there is currently weak end-user demand for paraffin waxes as they are unsustainable and their prices are driven by volatile oil prices.[65-67] Furthermore, the shutdown of group I base oil refineries, which was the main feedstock for the petroleum waxes is limiting the supply of the petroleum waxes.[68] Moreover, the new technologies adopted progressively by the refineries such as catalytic dewaxing do not give wax as by product.[64, 68] Therefore, the need for the synthetic waxes (produced from Fischer–Tropsch process) and vegetable waxes are increasing.[65-67] Overall, it is anticipated that there will be a strong demand growth for waxes and relatively weak supply growth which will result in an increased shortage.[65-67] Although the markets share of the vegetable waxes is only 4% which is marginal compared to other waxes, this number is expected to increase as many cosmetics and health care manufacturers are seeking natural waxes so that their products are accredited with natural and organic labels.[69]

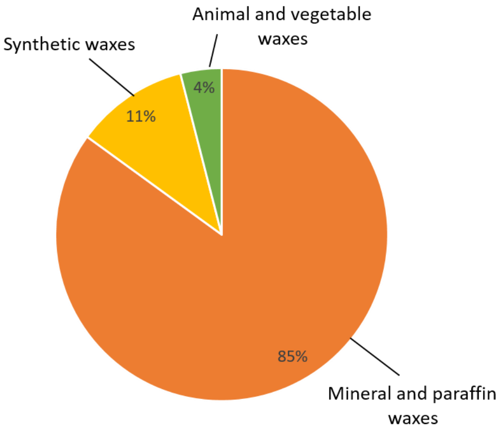


Figure 1-11 Global demand for waxes by type.[67]

1.6 Date palm tree (*Phoenix dactylifera L.*)

1.6.1 History, distribution and taxonomy of date palm tree

Date palm tree is considered to be one of the most important fruit crops in arid and semiarid climates. Early records show that date cultivation probably started as early as 3000 BC and originated most likely in the ancient Mesopotamia area (currently south Iraq) or western India.[70-72] Primarily, date palm has been cultivated in North Africa and the Arabic peninsula as well as the Middle East. However, in the last three centuries, it was introduced in new production areas including Australia, India, Pakistan, Mexico, southern Africa, South America, and the United States.[71]

Dates are considered an important income and staple food for people in many countries.[73] Indeed, it plays a role in politics and economies of many countries.[73] For instance, Sagal in a recent paper studied the significance of the date palm in the pre-oil stage in Kuwait and how the Sabah family built their economic and political power indirectly by planting the date palm and therefore establishing trade of dates.[73]

Date palm (*Phoenix dactylifera L.*) belongs to family *Palmaceae* which has about 200 genera.[71, 74] The date palm is under the Phoenix (*Coryphoideae Phoeniceae*) genera which contains a dozen species native to the tropical and subtropical area.[74] The date palm tree can grow up to 1500 m latitude and can live for 100 years.[71, 73] It can tolerate the harsh weather conditions such as drought and high temperature.[71, 73] A mature date palm tree can produce an average of 80-120 kg of fruit per year.[71, 73] Figure 1-12 shows a schematic drawing of the date palm tree. The date palm tree consists of four basic parts: the root, the trunk, the leaves and the fruit stalk and reproductive organs (spathe and inflorescence).

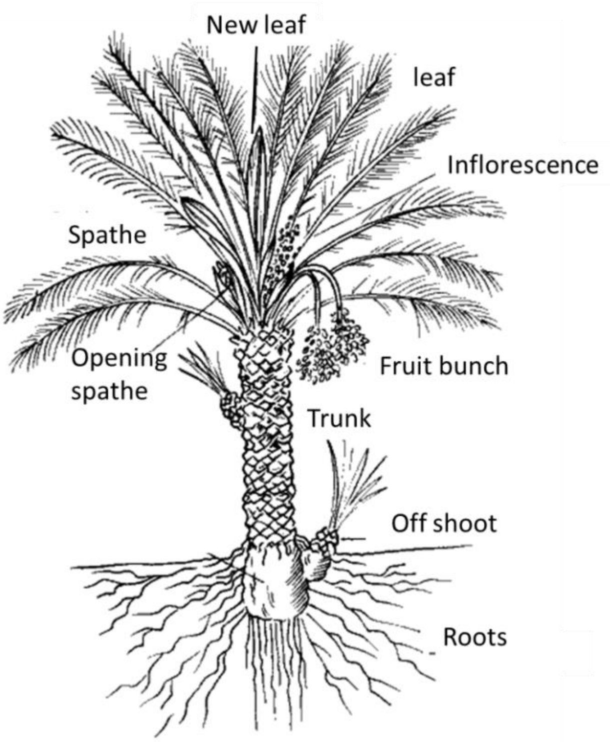


Figure 1-12 Schematic drawing of the date palm tree.(copyright permission granted from FAO under Creative Commons)

1.6.2 World production of dates

According to Food and Agriculture Organization of the United Nations (FAO) statistics, the total harvested area for date palm tree around the world in 2014 was 1.1 million hectare (ha) with a total number of the date palm trees around 110 million assuming an average density of 100-125 tree/ha.[75] Indeed, the date producing countries listed in FAO database are 38 countries based in Asia, Africa, South America and south Europe which shows the diverse countries and climate the tree can withstand as shown in Figure 1-13. Figure 1-14 shows the top 10 countries according to date palm harvesting area.

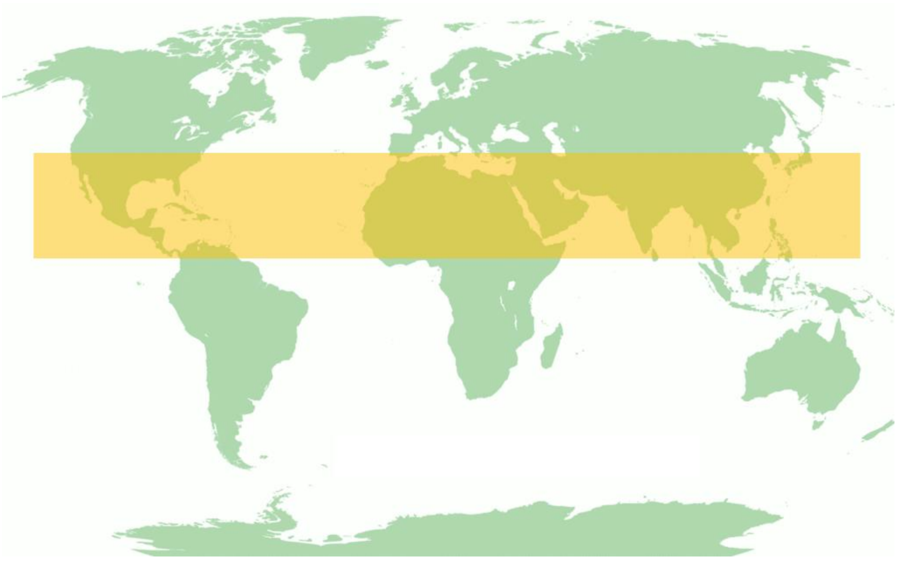


Figure 1-13 Approximate date palm distribution around the world.

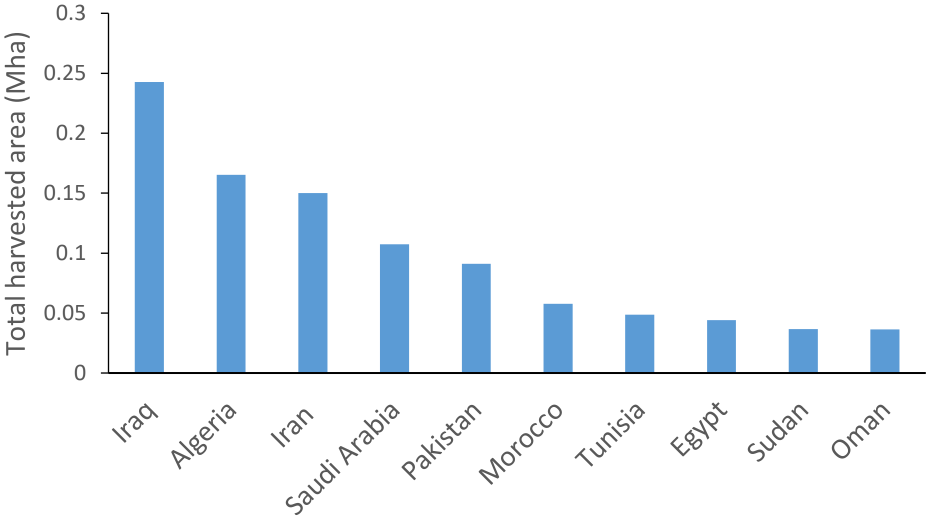


Figure 1-14 Top 10 countries in total harvested area of date palm trees.[75]

1.6.3 Date as a valuable fruit

Date fruit is a valuable, low-cost food consumed by inhabitants residing in arid and semi-arid climates. It has been a major staple food in the diet of people for thousands of years illustrating its importance.[70, 73] Moreover, it is the only fruit which can be consumed in three ripening stages; *kalal*, *rutab* and *tamer*.[76] The *tamer* or dehydrated fruit is a common favourite form available commercially. Although date fruit is considered as a dry fruit, it is not comparable to other dry fruits in that it more closely resembles a fresh fruit.[76] There are over 600 varieties estimated according to their shape and organoleptic properties, with some of the popular varieties including Dejlt noor, Ajwa, Bahri, Lulu, Farad, Fatimi, Khlas, Khinzi and many others.[77] Dates have four maturation stages, which are usually described by the Arabic words: *kimiri*, *khalal*, *rutab* and *tamer*. In the *kimiri* stage, the date is in its youngest from, green in colour and has hard a texture while in *the khalal* stage it turns yellow or purplish pink or red depending on its cultivar and still retains a firm texture. In the *rutab* stage, the fruit is less stringent with a soft texture and with the colour becoming darker. Finally, at the *tamer* stage, the fruit is fully mature, has a soft texture and is dark in colour. It is noteworthy that the chemical and phytochemical composition of the date varies in each maturation stage.[76] Dates are rich in sugar, minerals, vitamins and phytochemicals. Many studies have been conducted to identify and characterise the chemical and phytochemical composition of the date fruit and there are many recent reviews in this field.[76-78]

1.7 Date palm leaf

The leaf of the date palm is complex consisting of base, spins, midrib and leaflets. Figure 1-15 shows a schematic diagram of the leaf. The leaf is typically wide at the base or midrib and narrows on moving towards the leaf tip.[74] The leaves are 3-6 meters in length and their average life is 3-7 years. An adult date palm tree forms approximately 10-26 new leaves annually and an equivalent amount needs to be pruned in order to maintain the tree.[74] Typically, each tree produces around 13.5-50 Kg of dry fronds (leaves) annually. According to FAO statics, approximately 1.1 million ha of date palm trees were planted around the world, estimating an average density of 100-125 trees/ha. This means that potentially, 1.5-6.9 million tons of dry fronds are available annually to be utilised as biomass.[75, 79]

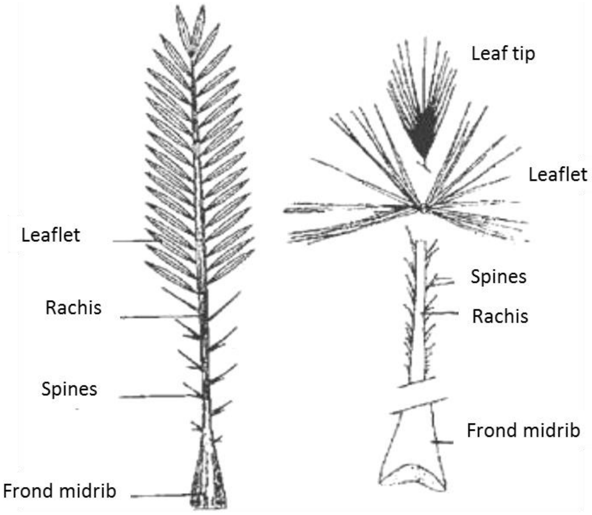


Figure 1-15 Schematic diagram of date palm leaf (copyright permission granted from FAO under creative commons).[74]

Dry fronds have many traditional applications detailed in section 1.7.1.[80-83] However, considerable amounts of these dry fronds are regarded as an agro-waste and the general practice in many date producing countries is to burn them which leads to numerous environmental problems.[81-83] Recently, many studies showed that the date palm leaf could be potentially utilised in various applications (as discussed in section 1.7.2)).[81, 83-89] With the current global attitude towards sustainability, there is a tremendous amount of ongoing research focused on exploiting available agricultural wastes as resources for chemical and fuel feedstocks; to replace petroleum-based energy and chemicals. [81, 83-89]

1.7.1 Traditional applications of date palm leaf

Date palm leaves have many traditional applications ranging from basketry and fencing to feed for animals and tooth picks. Some of the traditional applications of date palm are discussed below.

- The whole leaves are used as a fencing material, whereby they are stuck into the ground and held together with layers of rope made from the leaflet. These fences are used to partition houses providing privacy and allowing proper ventilation. Moreover, the leaves are used to provide shade for newly planted offshoots. The whole leaves have also been used for religious purposes such as Palm Sunday celebration.[80]
- The midrib is that part of the leaf which acts as a base for the leaf, which encircles the palm and remains as part of the trunk. The base of the midrib is broad and

flattened becoming narrow towards the leaflet. The midrib is widely used to make crates (after removing the leaflets) which are used as fruit crates, birdcages and chicken coops. Furthermore, the midribs are also used as a fibre source for rope making, fishing rods and as a fuel source since it can be burned to obtain charcoal.[80]

- Leaflets vary in length from 15 to 100 cm and width from 1 to 6 cm, with the number of leaflets on the midrib varying from 120 to 240. Leaflets are widely used in woven material and basketry. Moreover, they can be used to make cords and act as stiffening materials for cushions and mattresses.[80]
- Spines are leaflets converted to tough, pointed pins, which are situated at the lower end of the midrib. They vary in length from very short up to 20 cm and a width of very thin up to 1 cm. Spines are used as toothpicks and in fish traps.[80]
- Date palm leaves can be used for livestock and as soil amendments.[80]

1.7.2 Potential applications for date palm leaf from literature

1.7.2.1 Activated and dehydrated carbon from the leaflet for wastewater treatment

In recent studies, activated carbon and dehydrated carbon from date palm leaflets showed the ability to remove some heavy metals as well as toxic medical waste.[87, 90] El-Shafey *et al.* prepared carbonised sorbent from the leaflet of date palm and tested it in the removal of Cu^{2+} and Ag^{+}.[87] The sorption of the Cu^{2+} was high with equilibrium being achieved in 2 h, while the sorption of the Ag^{+} was slower in comparison and required 60h to reach equilibrium.[87] The activation energy for the Cu^{2+} sorption was 16 KJ/mol which suggested a diffusion controlled ion exchange mechanism while for Ag^{+} the activation energy of 44.3 KJ/mol indicated a chemically controlled process.[87] In a more recent paper, El-Shafey investigated the absorption of dehydrated carbon and activated carbon prepared from date palm leaflets on the drugs fexofenadine and diphenhydramine.[90] Both activated and dehydrated carbon showed the ability for drug adsorption with the latter giving superior performance when compared with the former.[90] The leaf ash was shown to be a low-cost absorbent for the removal of Pb(II) from aqueous solution with a very high absorption capacity of 35 mg/g at 40 °C.[91]

1.7.2.2 Date palm leaf as a source of energy

Date palm leaves and seeds can be characterised as being of high calorific value as well as having a high volatile content and can thus be used as a good source of energy, chemicals and bio-char.[83] Malki *et al.* designed a biomass power plant for burning date palm waste to cogenerate electricity and distilled water.[81] Moreover, the high heating value was 17600 kJ/ kg for the date palm waste biomass.[81] The calculation of a typical plant in Iran showed that nearly 62 GW (with desalination) and 75 GW (without desalination) of electricity are obtained from burning around 140,000 ton of waste/year.[81]

1.7.2.3 Particle boards and composites from date palm leaf fibre

Date palm leaf also been utilised as a source of natural fibre to reinforce synthetic polymers such as polyvinyl alcohol with an optimum fibre content of 28%.[86] Usually, date palm leaf fibres are treated chemically to free the fibre from volatile organic substances and lignin in order to convert them into a form compatible with the composite.[86] The chemical treatments of the fibres enhanced the tensile strength and improved the dispersion of the fibres in polyvinyl alcohol matrix.[86] Moreover, date palm leaf fibre was utilised in making a wood plastic composite with polycarbonate, polystyrene and polyvinyl chloride for outdoor applications.[92] The reinforcement of date palm leaves as a source of cheap fibre enhances the overall mechanical strength and physical properties of the composite, giving characteristics close to commercial wood.[92] Furthermore, date palm leaflet is a promising fibrous material for making binderless particle boards as it shows high mechanical strength due to high lignin and soluble component content.[93]

1.7.2.4 Paper and pulps from date palm leaf

Date palm frond is a fibrous material and contains a high cellulose content in the range 33-44% (w/w) as highlighted in Table 1-4. Therefore, it could have potential applications in the pulp and paper industries as studies showed that it could be mechanically or chemically treated to produce good quality paper.[94, 95]

Table 1-4 Chemical composition of date palm frond

Cellulose (%)	Hemi-cellulose (%)	Lignin (%)	Reference
35	28	27	96
33.5	26	27	97
44	26.2	29.8	98

It was found that the rachis gives a better result than the leaflets in terms of yield 44% versus 33% respectively due to the chemical composition of the leaflets.[89] The pulp properties such as viscosity, water retention volume and fibre length made from leaflets were promising, suggesting that it can be used as a blend with rachis or other pulps.[89] Gosh *et al.* produced handmade pulp and paper mechanically from date palm leaf without any chemical treatment.[94] The leaves were soaked in water , subjected to mechanical beating and finally the sheet was formed from the diluted pulp slurry.[94] Indeed they showed that mixing the leaves with the cotton enhances their mechanical strength as well as the yield.[94]

1.7.2.5 Bioethanol from date palm leaves

Recent studies demonstrated that date palm leaflets have a great potential for bioethanol production.[79, 84, 85, 99] Fang *et al.* employed hydrothermal pre-treatment on leaflets and rachis and have achieved high glucan (>90%) and xylan (>75% leaflets, >79% rachis) recovery from both.[85] The optimal condition for digestion was found to be 210 °C/10 min with 100% glucan conversion for the leaf and a fermentable ethanol yield of 96%, while the rachis showed a conversion of 78% and ethanol yield of 80%.[85]. Interestingly, Fang *et al.* proposed a method using seawater as the reaction media for the hydrothermal pre-treatment of the leaflets and showed comparable results with fresh water as a reaction medium.[84] Moreover, Asraf *et al.* estimated the potential bioethanol production from leaflets to be 150-300 Kg ethanol/tonne of dry matter making the date palm residue a good lignocellulosic biomass for ethanol production.[79]

1.8 Chemicals from date palm

Kurtz has reported that the yield of cuticle wax from the date palm leaflets depended on the thickness of the cuticle which the authors claimed that is decreased with plant ageing (Table 1-5).[100] The wax has been extracted with petroleum ether[100] The obtained wax exhibited high melting points ranging from 78.5 to 80 °C.[100] The wax acid content increased as the plant matured which might relate to the synthesis of the wax esters which also exhibited an increase as the plant matured.[100]

Table 1-5 Variations with age of *Phoenix dactylifera* of the characteristics of the wax.[100]

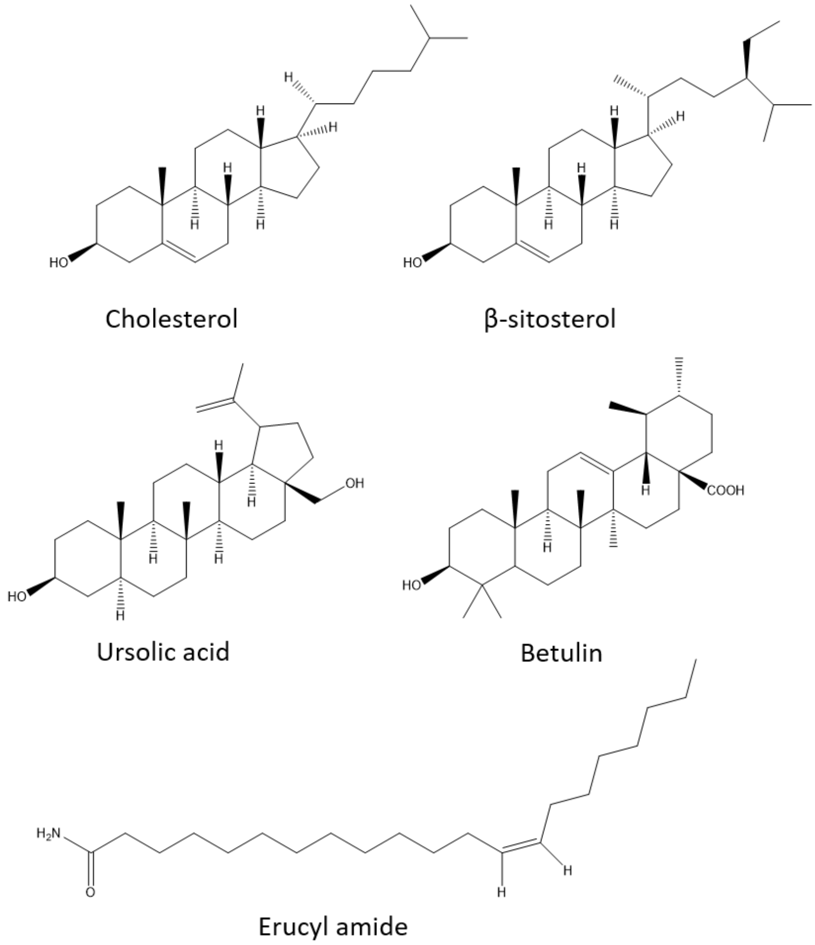
Age (months)	Yield %	Cuticle thickness (µm)	Melting point (°C)	Iodine No	Acid No	Ester No
0	0.17	1.75	80.0	2.19	20.17	30
3	0.15	2.60	79.5	1.67	10.73	49
12	0.10	3.50	77.5	0.97	13.96	43
18	0.19	3.50	80.0	1.44	13.28	41
24	0.15	3.00	78.5	2.44	12.41	43
36	0.15	3.50	79.5	2.37	13.25	40

Fatty acid ethyl esters have been identified in leaflet extracts obtained from soaking the leaflets in hexane for 15 days. Ifzal *et al.* reported fatty acids ethyl esters with fatty acid chain length ranging from C_{14} to C_{26} in the date palm leaflets. The major ethyl esters were hexadecanoic, 9-octadecanoic and octadecanoic ethyl esters.[101]Moreover Terrak *et al.* reported the presence of fatty acid methyl esters with the most abundant being palmitic acid methyl ester and α-linolic acid methyl ester.[102]

Recently, a study exploring the structure and composition of date palm leaf wax showed that the crystalloid of the date palm leaf wax is rodlets.[103] Moreover, these rodlets are distributed irregularly on the entire leaf but well developed around the stomatal pores in younger leaves.[103] However, severe erosion observed in older leaves led to rodlet structures completely disappearing or only being partially present in the fold of leaflets or around the stomatal pores.[103] The leaf wax was extracted by dipping in methanol followed by dewaxing in dichloromethane.[103] The primary compounds found within the wax were *n*-alkanes in the dichloromethane extract while the methanol extract contained fatty acids, fatty alcohols, ketones, sterols, triterpenoids and amides.[103] Table 1-6 and Figure 1-16 show some of the compounds identified by Khelil *et al*.[103] Moreover, two terpenoids were identified in the wax; ursolic acid and betulin (Figure 1-16) and only two sterols including cholesterol and β-sitosterol.[103]

Table 1-6 Compounds identified in date palm leaf.[103]

Compounds	Chain length	Most abundant	Notes
***n*-alkane**	$C_{18} – C_{35}$	C_{31}	even and odd
Fatty acids	$C_{10} – C_{29}$	C_{16}	even and odd saturated and unsaturated
Fatty alcohols	$C_{12} – C_{30}$	C_{22}, C_{21}, C_{20}, C_{19}	even and odd
Ketones	$C_{21} – C_{29}$	C_{23}, C_{22}, C_{21}	even and odd



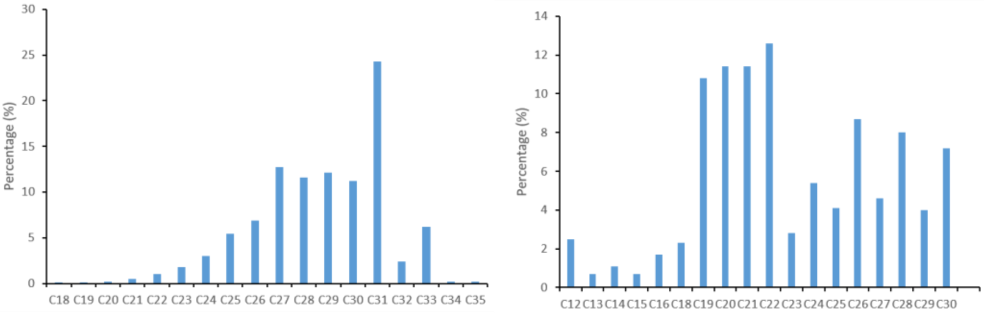
Cholesterol β-sitosterol

Ursolic acid Betulin

Erucyl amide

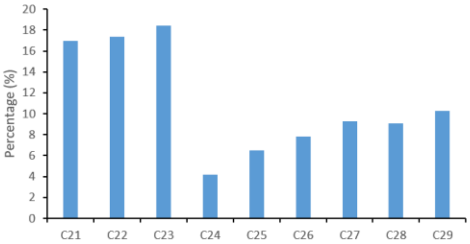
Figure 1-16 Chemicals identified in date palm leaf wax.[103]

n-Alkanes within the leaf wax ranged between C_{18}-C_{35} with C_{31} (24.3%) predominating (Figure 1-17 (A)).[103] Interestingly, odd and even numbered fatty acids were found within the extract with chain length ranging from C_{10} to C_{29}.[103] The most abundant fatty acid was palmitic acid C_{16}.[103] Alcohols (C_{12}- C_{30}) were also identified with the most abundant being C_{22} (12.6%) followed closely by C_{21}, C_{20} (both 11.4%) and C_{19} (10.8%) as shown in Figure 1-17 (B).[103] Ketones were also present (C_{21}- C_{29}) with the most abundant being C_{23} (18.4%), C_{22} (17.4%) and C_{21} (17.0%) as highlighted in Figure 1-17 (C).[103]



A (*n*-alkanes)

B (*alcohols*)



C (*ketones*)

Figure 1-17 Distribution of A (*n*-alkane), B (alcohols) and C (ketones).

1.9 Introduction to the work in this thesis

This literature review has shown that there is a considerable quantity of neglected, under-utilised and abundant agriculture waste generated from date palm trees. Date palm leaves have been reported to have a crystalline wax layer, which provides an excellent opportunity to extract high-value chemicals for industrial application.[100, 103] Very few works have been conducted to extract and analyse the waxes from the date palm leaves.[100, 103] Furthermore, conventional organic solvents were used to extract this wax layer. No work has been reported regarding the use of $scCO_2$ extraction as a sustainable and renewable solvent and extraction technology. Moreover, no optimisation or scale-up extractions have been previously consideredand fractionation of the wax from date palm leaves to obtain extracts of higher quality/purity has not been explored yet. The economics of the wax extraction has also not been studied. Finally, no studies have investigated the potential applications of the extracted wax.

With the above research gap highlights, the work in this thesis can be split into three main parts:-

Chapter 2 describes the $scCO_2$ extraction of wax from date palm leaves as a green and environmentally friendly extraction technology. An experimental design was carried out to optimise the extraction conditions to maximise the yields. Detailed chemical characterisation and quantification of wax extracts obtained using different extraction conditions was conducted. The effect of temperature and pressure on the $scCO_2$ extraction yield and chemical composition was investigated. The $scCO_2$ extraction was compared and contrasted with conventional organic solvent extraction. The effect of the chemical composition on the physical properties of the wax was explored. Finally, potential applications for the waxes were highlighted.

Chapter 3 explored the application of the extracted date palm leaf wax in the preparation of the oleogels. Rheological analysis was performed in order to evaluate the strength of the oleogels. Thermal and microscopic analysis was conducted to elucidate the structure of the wax network. The oleogels prepared from date palm wax were compared and contrasted with oleogels prepared from other commercial waxes.

Chapter 4 examines the scale up of the $scCO_2$ extraction of the wax from date palm leaves. The fractionation of the wax was carried out to tailor the extraction process and the different fractions were analysed with regard to their chemical composition and physical properties. The economic aspect of the extraction was explored to assess the feasibility of the extraction process.

Chapter 2

Supercritical CO_2 extraction of date palm leaves wax: optimisation, characterisation and quantification

2. Chapter 2 Supercritical CO_2 extraction of date palm leaves wax: optimisation, characterisation and quantification

2.1 Introduction

Current society is heavily reliant on petroleum oil for both energy and chemicals. In term of waxes, more than 70% of global wax supply is from paraffin waxes.[104, 105] Plant waxes and lipids are potentially suited to provide a renewable source of high value chemicals as a replacement to current petroleum feedstock. Natural waxes current contribute only 4% to the global wax market.[104, 105] However, the demand for natural waxes is expected to rise, powered by several factors including: the diminishing supply of petroleum oil due to the shift by oil refineries to catalytic dewaxing technologies and the volatility of oil price combined, with consumer preference towards greener, sustainable and natural products.[106, 107]

Agricultural pruning waste from date palm is one of the potential sources of natural wax.[100, 101, 103] The date palm (Phoenix dactylifera) is one of the most cultivated palm trees around the globe.[71, 73] It is commonly found in the arid band stretching from North Africa to the Middle East.[71, 73] According to Food and Agriculture Organization of the United Nations (FAO) the total harvested area of date palm trees worldwide in 2014 was 1.1 million ha, with 60% located in Arabian Gulf countries.[75] To maintain the growth of the tree, certain parts need to be pruned such as dry fronds, stem fibres and trunk fibres.[72, 82] Thus, enormous volume of waste are generated annually, which is typically burnt or thrown away. Typically, 13-20 Kg of dry frond (leaves) waste are generated by each tree annually, with an estimated volume of 1.5-6.9 million tonnes globally.[82, 108]

Traditionally lipids and cuticle wax are extracted using harmful organic solvents, which possess many health, safety and environment concerns apart from being nonselective. For instance; hexane a common solvents for the extraction of the lipids is recognized as a hazardous air pollutant by the US environmental protection agency (EPA).[9] Hexane also is a neurotoxin which affects the central nervous system.[109, 110] Supercritical CO_2 has been demonstrated as an alternative to the extraction of the cuticle waxes from plants.[5, 111, 112] $ScCO_2$ has low surface tension, high diffusivity and low viscosity which makes it an ideal solvent for use in extraction methodologies.[21] Tuning the temperature and pressure of the extraction alters the solvents properties, which provides an extra advantage in the extraction of the natural products.[16, 21, 111] $ScCO_2$ leaves no solvent residues within the making it an ideal extraction technique for industries including pharmaceutical, food and cosmetics sectors where removal of trace solvent contamination is crucial.[14, 17]

Herein, cuticle waxes from date palm leaves were extracted with $scCO_2$. The extraction conditions were optimised using second order factorial experimental design. The detailed chemical composition of the waxes was carried out and the major hydrophobic components were identified. Potential applications for extracted waxes were highlighted. $ScCO_2$ extracted waxes were compared with those obtained in heptane Soxhlet extraction of date palm leaves.

2.2 $ScCO_2$ extraction of the wax from date palm leaves

Many factors affect $scCO_2$ extraction processes including solvent properties, sample matrix and the interaction of the solvent with the solute in the matrix.[24, 113] For instance, solvent properties are determined by temperature, pressure, density, viscosity, etc.[113] A screening design is employed to determine the most important factors and their interactions.[113] Usually, two level factorial design is applied in the screening experiment as it allows determination of which factors have most influence over extraction and how they interact, while carrying out the minimum numbers of runs.[113] This is in contrast to the single variable at time approach which requires a large number of runs, especially when there are a number of factors.[113, 114] The number of factors and the level of the factors are used to classify the factorial designs.[113, 114] For instances, 2-2 or 2×2 factorial design means it has two factors and two level of the factors while 2-3 or 2×3 factorial design has 3 factors and each factor at two levels.[113, 114]

The milled date palm leaf was extracted with $scCO_2$ using a range of temperature and pressure in an initial experimental design. The 2×2 factorial design was used as screening approach with temperature and pressure being the two chosen factors. Studies showed that the temperature and pressure are the most influential factors defining the density and hence the solvent properties of $scCO_2$.[115, 116] The two factors levels were high and low (Table 2-1). An extra experiment was set to give a midpoint of the system and overall five experiments were carried out, as shown in Table 2-1.

Typically in a factorial design, the actual variable is converted into coded variables to provide a better uniformity as the model coefficients are dimensionless and hence directly comparable.[113, 114] The significance of temperature and pressure has been studied using a dimensionless factor coordinate system where the convention of (-1) for low level and (+1) for high level for each factor has been adopted. The coordinate at the centre point (E) was set at 0 as it coincided with the origin of the system. The coded variable and experimental variable are listed in Table 2-1.

Table 2-1 Experimental design matrix for optimisation of $scCO_2$ extraction for wax from date palm leaf.

Experiment point	Coded coordinate values (X1)	(X2)	Experimental conditions T (°C)	P (bar)	Yield (%)	CO_2 density ($g.cm^{-3}$)
A	Low (-1)	Low (-1)	40	80	0.10 ±0.05	0.278
B	High (+1)	Low (-1)	100	80	0.05 ±0.00	0.141
C	Low (-1)	High (+1)	40	400	0.97 ±0.020	0.956
D	High (+1)	High (+1)	100	400	3.49 ±0.23	0.757
E	Medium (0)	Medium(0)	70	240	1.23 ±0.43	0.724

The plot was designed such that it gives an effective range of temperature (40 °C to 100 °C) and pressure (80 bar to 400 bar). As illustrated in Figure 2-1 the experimental domain is defined by the four points (A, B, C and D) at the maximum and minimum operating temperature and pressure forming the corner of the square. An extra point (E) was implemented as the mid- point of the selected working range to examine any curvature in the response and thus minimise the risk of the non-linear relationship.[117, 118] The extraction time for all experiments was set at 4 hours as Sin et al demonstrated that this extraction time was sufficient to extract 90% of the wax from wheat straw.[5] The CO_2 flow rate in all extractions was set at 40 g min^{-1} giving a total of 9.6 Kg of CO_2 per extraction.

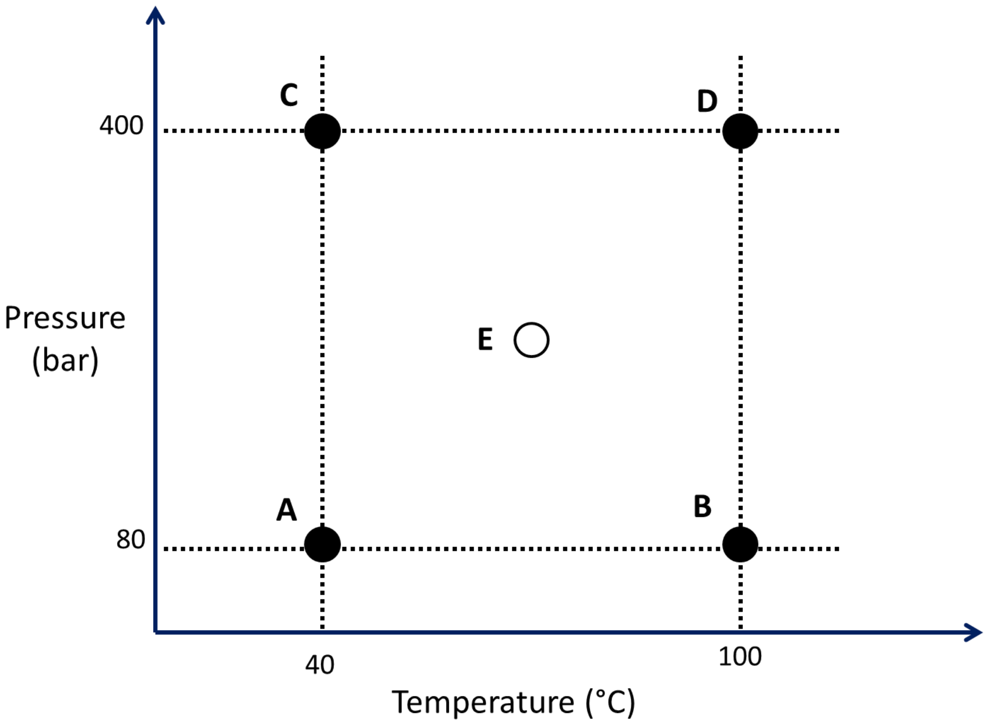


Figure 2-1 The 2x2 factorial design experimental domain for the extraction conditions optimisation.

A multiple linear regressions model was used to analyse the design. Equation 2-1 shows the first order polynomial function employed in the regression analysis:

$$\boldsymbol{Y = b_0 + b_1X_1 + b_2X_2 + b_{12}X_1X_2} \textbf{ Equation 2-1}$$

Equation 2-1: First order polynomial function

Where Y is the percentage crude yield, b1 and b2 describes the main effect of the coded coordinate values of the X1 and X2, b12 accounts for the interaction between X1 and X2 and b_0 is the response at the centre point E. The coefficients values of the first order polynomial function above were calculated using Equations 2-2 to 2-5.

$$\boldsymbol{b_0 = \frac{1}{4}(+y_1 + y_2 + y_3 + y_4)} \textbf{ Equation 2-2}$$

$$\boldsymbol{b_1 = \frac{1}{4}(-y_1 + y_2 - y_3 + y_4)} \textbf{ Equation 2-3}$$

$$\boldsymbol{b_2 = \frac{1}{4}(-y_1 - y_2 + y_3 + y_4)} \textbf{ Equation 2-4}$$

$$\boldsymbol{b_{12} = \frac{1}{4}(+y_1 - y_2 - y_3 + y_4)} \textbf{ Equation 2-5}$$

Equations 2-2, 2-3, 2-4 and 2-5 (top to bottom): Calculation of the coefficients for the first order polynomial function.

$$\boldsymbol{Y = 1.15 + 0.61X_1 + 1.07X_2 + 0.65X_1X_2} \quad \textbf{Equation 2-6}$$

Equation 2-6: First order function modelling the $scCO_2$ extraction of date palm leaf wax.

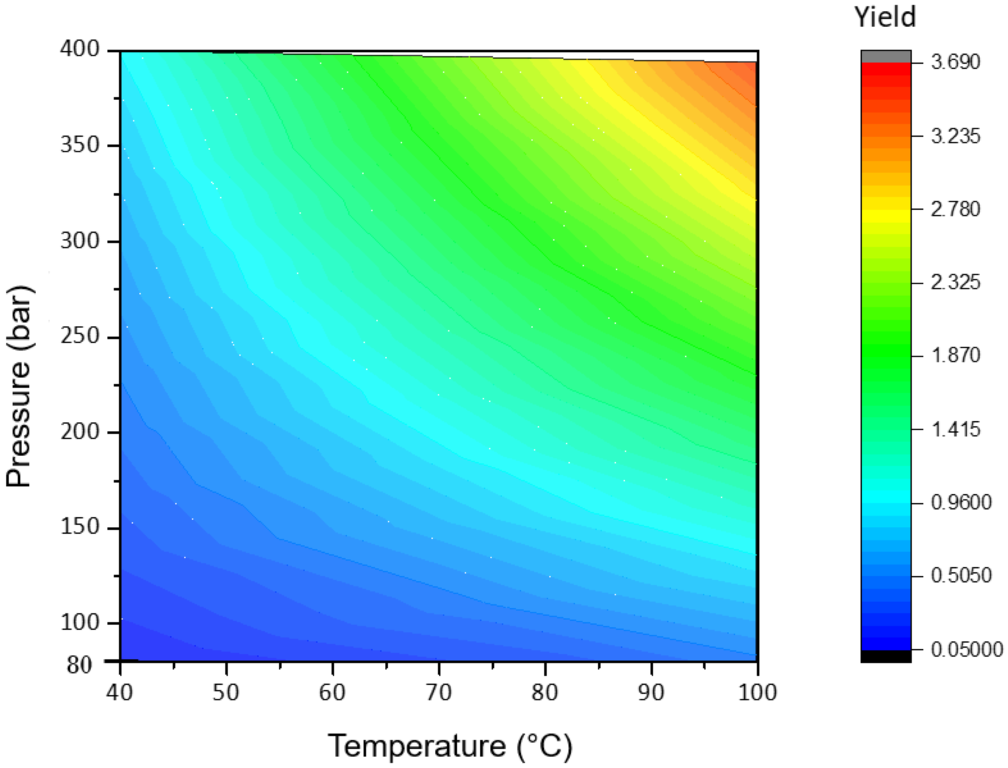


Figure 2-2 The effect of temperature and pressure on the extraction yield of date palm leaves wax.

The percentage crude yields at the different conditions changed from 0.05 to 3.49% (Figure 2-2) indicating a compositional variation during the extraction. Therefore, a change in the solvation properties of $scCO_2$ with different temperatures and pressures leads to a change in the solubility of wax components in the solvent.[30, 119, 120]Temperature and pressure dictate the dielectric constant and density of CO_2.[30, 119, 120]

The counterplot in Figure 2-2 and Equation 2-6 provides detailed insight of the effect of the temperature and pressure on the extraction yield. A linear relationship observed from Figure 2-2 indicates that both increases in temperature and pressure result in higher yields. Interestingly, all the coefficients of Equation 2-6 are positive, which further confirms that pressure, temperature and their combination have an effect on the extraction yield. At fixed temperature, the yield improved significantly from 0.10% (80 bar, 40 °C) compared to 0.97% (400 bar, 40 °C). A similar, yet more significant improvement in the percentage yield was observed at higher temperature, moving from 0.05% (80 bar, 100 °C) to 3.49% (400 bar, 100 °C), which implies that the density of CO_2 is a crucial factor in the extraction process. Increasing the pressure at constant temperature increase the CO_2 density and therefore the solubility of the wax in CO_2.[116, 119, 121] However, the highest yield of 3.49% was achieved under high pressure and temperature (400 bar, 100 °C) which corresponds to a density of 0.757 g cm^{-3} CO_2. When the highest density of 0.956 g cm^{-3} was applied (400 bar, 40 °C), moreover, only a nominal yield of 0.97% was achieved. Therefore, density is not the only factor that governs the extraction yield. Temperature also plays an important role as higher yields are obtained at higher temperatures 100 °C. The positive coefficient of the temperature (X1) in Equation 2-6 further confirms the effect of temperature in the extraction. Although increasing temperature has a synergistic effect on the density of the CO_2 and hence lower yield expected.[116, 119, 121] Elevated temperatures play a role in overcoming the lattice energy and enhances wax extractability.[119, 120] The literature proposes that there is a crossover pressure value, where below this value increasing the temperature result in decreased solubility.[122, 123] The effect of crossover pressure is clearly observed at low pressures. The extraction at 80 bar and 40 °C yielded 0.10% wax, while extraction at 80 bar and 100 °C, only exhibited a minimal yield of 0.05%, hence the temperature suppressed the solubility at this low pressure. In contrast, above the crossover pressure, increase in temperature enhanced the solubility and the yield of the wax increased from 0.97% (400 bar, 40 °C) to 3.49% (400 bar, 100 °C).[122, 123] Also, the coefficient b_{12} is 0.56 which is considerable, indicating the significant combined effect of both temperature and pressure on the solubility of wax components in CO_2.

The yield for the experimental point E (70 °C and 240 bar) has been calculated using equation 2-6 and compared with the observed yield. The predicted value from equation 6 was 1.16% while the experimental yield was 1.23% with a difference of a 0.07 and a 5.69% error. The model overall showed a good prediction for the percentage yield of date palm wax. Sin *et al*. have adopted a similar optimisation model for extraction of the wax from wheat straw, which also demonstrated a relatively a good prediction for the extraction yield.[5]

Typically, reported yields of waxes from other waste agricultural biomass extracted with $scCO_2$ are less than 2%. For instance, the yield of waxes from sugarcane leaves, corn stover and wheat straw extracted with $scCO_2$ under optimised conditions were 1.6, 0.9 and

1.8% respectively.[5, 20, 124] In contrast, the maximum yield obtained from date palm leaves at 400 bar, 100 °C was 3.49% which is significantly higher than the yields reported from other agricultural residues.[5, 20, 124] The high yield combined with the huge volume of this agricultural residue worldwide makes date palm waste a promising commercial source of natural wax.

The dynamic study was implemented to verify the optimum extraction time for a maximum wax yield. Three linear regions can be identified in the overall extraction curve profile in Figure 2-3 which are: constant extraction rate period (CER), falling extraction rate period (FER) and diffusion control extraction rate period (DCR). In CER period, the mass transfer of the solute is determined by the convection of the solvent film with the matrix and hence easily accessible solute is extracted.[121, 125, 126] Literature has reported that the in CER period about 50-90% of the extract is obtained and therefore the extraction ends shortly after CER.[121, 125, 126] In the extraction of date palm leaves 70% of the total yield is recovered at the end of CER period. Typically, reports in the literature have highlighted that after CER a falling period starts where no significant increase in yield is observed. [121, 125, 126] However, this is not the case for the extraction of date palm waxes, where a significant increase in the yield is observed from 2.5 % to 3.4 % (Figure 2-3). Most of the accessible solute has been removed from the matrix after the FER and both the convection and diffusion in the matrix must be considered in the increase of the yield.[121, 125, 126] DCR then dominates where the diffusion controls the mass transfer and therefore the curve plateaus.[121, 125, 126] The study determined that 90% of the total extractable wax could be recovered after ca. 75 min, while 97% could be isolated after 120 min as shown in Figure 2-3.

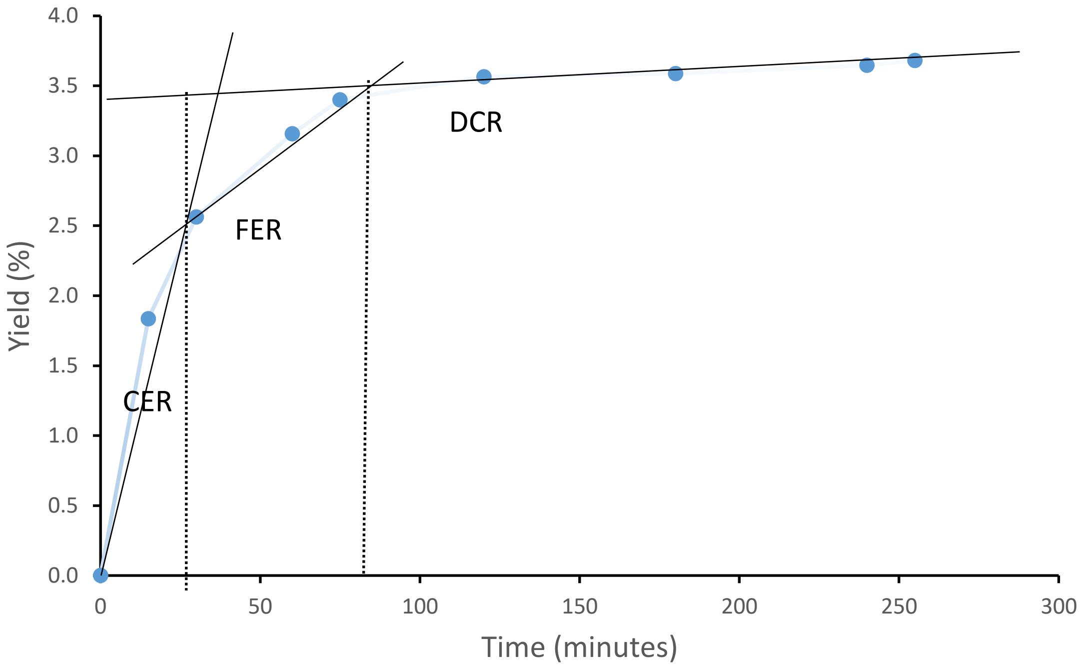


Figure 2-3 The overall extraction curve of date palm leaf wax extraction obtained at 400 bar and 100 °C.

2.3 Chemical Composition of Date palm leaf wax

Available literature describing the chemical composition of the date palm leaves lipids is scarce and limited.[100, 101, 103] Each extract of date palm leaf wax was analysed by GC and GC/MS by the method described in experimental section 5.8 to 5-10. The compounds were identified using a combination of standards, mass spectra fragmentation pattern, NIST (version 2008) mass spectra library, Kovats indices, and published spectra. Since electron ionisation (EI) fragmentation pattern does not show the molecular ion, field ionisation (FI) was used to find the molecular weight of some compounds. Figure 2-4 demonstrates the chromatogram of date palm leaf wax extracted at 400 bar and 100 °C.

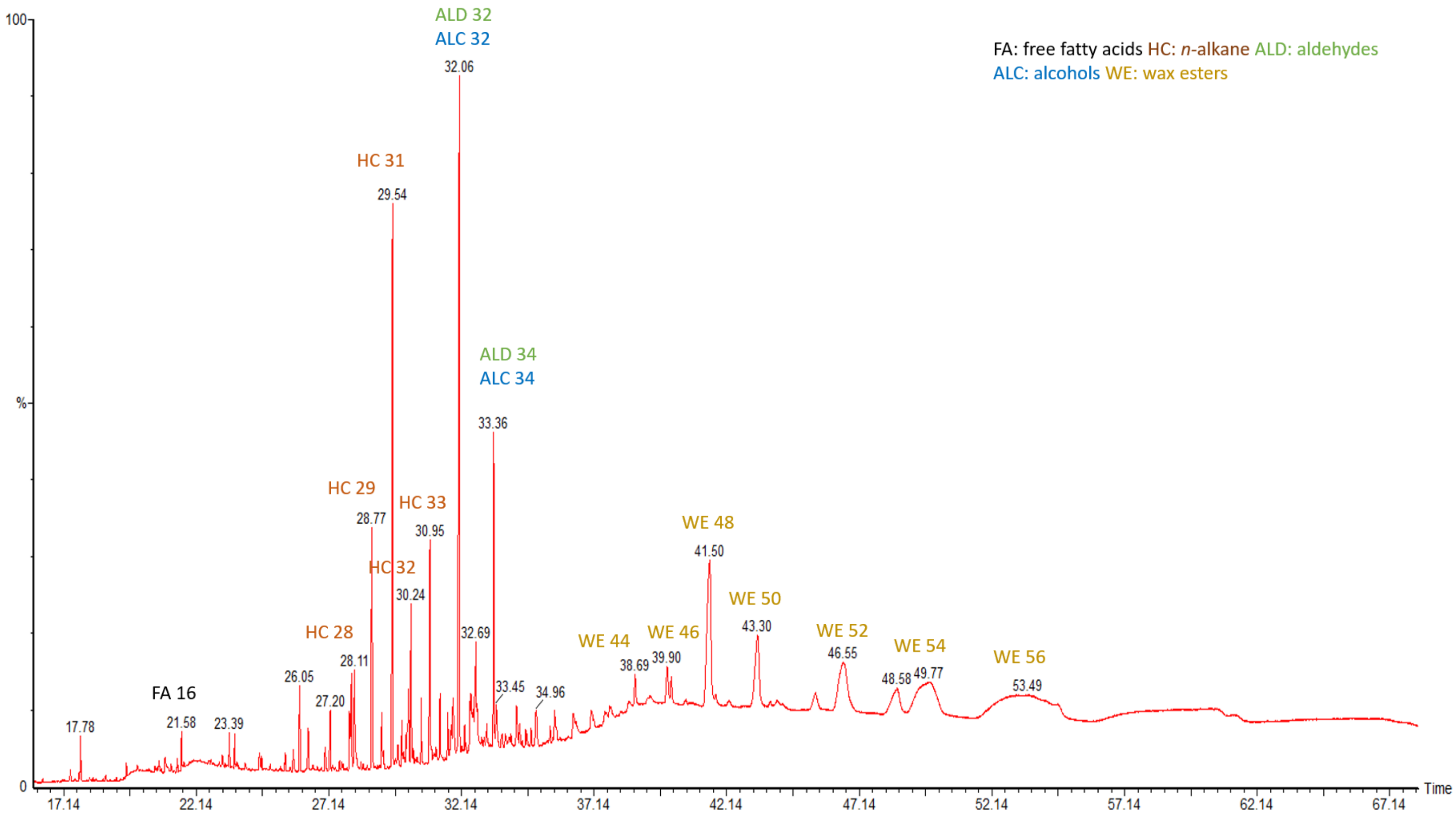


Figure 2-4 The Chromatogram of date palm leaf wax extracted at 400 bar 100 °C.

2.2.1 Hydrocarbons

Typically, plants produce a range of n-alkanes (C_{21}-C_{37}) with strong odd over even predominance with only one or two dominant chain length.[127] The distribution of the chain length varies among different species or even different parts of the same plant.[127] For instance, C_{27}-C_{29} are most abundant in woody plants while C_{31} is more common in Gymnosperms.[127] Moreover, in sugarcane the most abundant chain length in leaves was tritriacontane (C_{33}) compared to rind and bagasse, which was hentriacontane (C_{31}).[124]

The *n*-alkanes in date palm wax were identified by GC/MS and by calculating the Kovats indices (KI). Kovats retention indices developed by Kovats in 1958 are considered the most practical way to characterise the retention of compounds in gas chromatography systems.[128, 129] Kovats uses the logarithm of the isothermal retention time of the interest compounds and the n-paraffin eluting before and after them to determine the retention indices as shown in Equation 2-7 below.[128, 129]

$$\boldsymbol{KI = 100 \times \left[n + (N-n)\frac{\log(t'_r\, unknown) - \log(t'_r\, n)}{\log(t'_r\, N) - \log(t'_r\, n)}\right]} \qquad \textbf{Equation 2-7}$$

Equation 2-٧ Kovats index (KI) calculation as proposed by Kovats for isotherm gas chromatography system.

The isothermal Kovats indices equation has been modified by Van Den Dool and Dec Kratz to include the linear temperature programmed GC (Equation 2-٨).[128]

$$\boldsymbol{KI = 100 \times \left[n + (N-n)\frac{(t'_r\, unknown) - (t'_r\, n)}{(t'_r\, N) - (t'_r\, n)}\right]} \qquad \textbf{Equation 2-8}$$

Equation 2-8 Modified Kovats index (KI) calculation for linear programmed gas chromatography system.

Where: KI is Kovats index, t'_r is retention time, n and N are the numbers of carbon atom for the smaller and larger reference alkane respectively.

Even and odd chain hydrocarbons have been identified in date palm wax with the odd hydrocarbons predominating. Figure 2-5 shows EI mass spectrum of C_{31} alkane *n*-hentriacontane. The *n*-alkanes exhibit a unique fragmentation pattern with the lower m/z peaks separated by 14 units indicating a loss of a CH_2 group (57, 71, 85, etc).[130] The peaks appear in a cluster of fragment ions as C_nH_{2n}, C_nH_{2n-1} and C_nH_{2n+1} which is the largest peak in the cluster.[130] The molecular ion M^+ can rarely be seen in EI spectrum of alkane due to highly energetic nature of EI.[130] Therefore; the EI spectrum would not be enough to assign the chain length and comparison with standards and Kovats indices (KI) can give valuable information. Table 2-2 shows the identified *n*-alkanes in date palm leaf wax with the calculated KI.

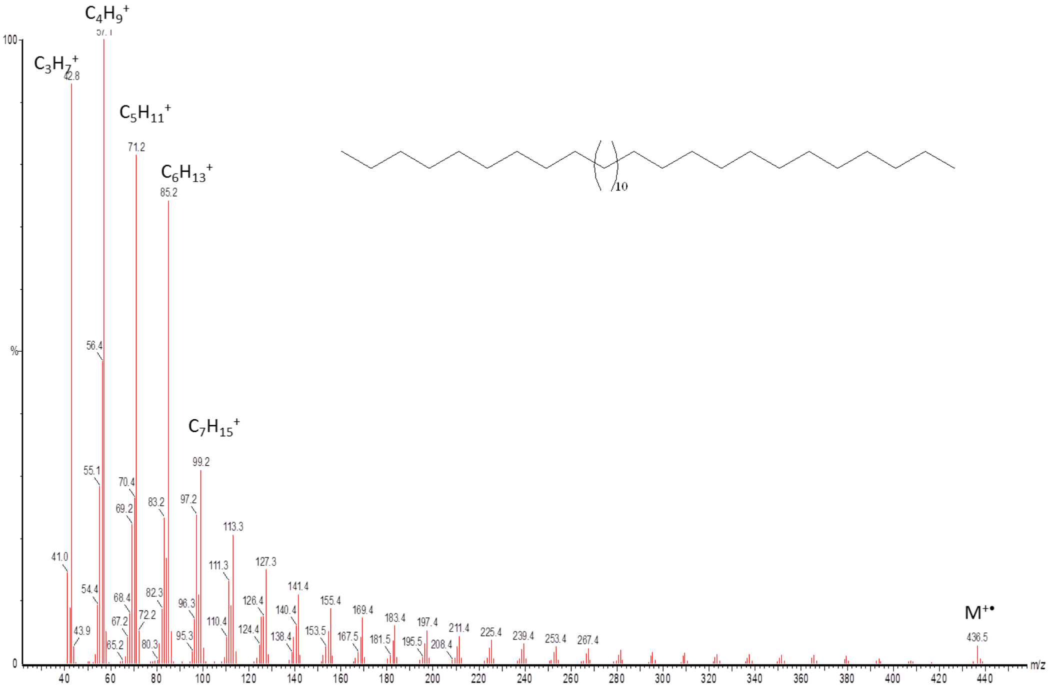


Figure 2-5 EI mass fragmentation of n-hentriaconatne.

Table 2-2 A list of identified n-alkanes in date palm leaf wax.

***n*-alkane**	**Carbon number**	**Calculated KI**	**Literature KI[128, 129]**
***n*-octacosane**	C_{28}	2804	2800
***n*-nonacosane**	C_{29}	2890	2900
***n*-triacontane**	C_{30}	2986	3000
***n*-hentriacontane**	C_{31}	3091	3100
***n*-dotriacontane**	C_{32}	3185	3200
***n*-tritriacontane**	C_{33}	3290	3300

The chain length of identified *n*-alkanes within date palm leaf extracts ranged between C_{28} to C_{33}. Both odd and even chain hydrocarbons have been identified and quantified. Kelil *et al*. reported the presence of n-alkanes of the chain length (C_{18}-C_{35}) with hentriacontane (C_{31}) being the most dominant in date palm leaves wax.[103] Figure 2-6 show the distribution of *n*-alkanes within each extract.

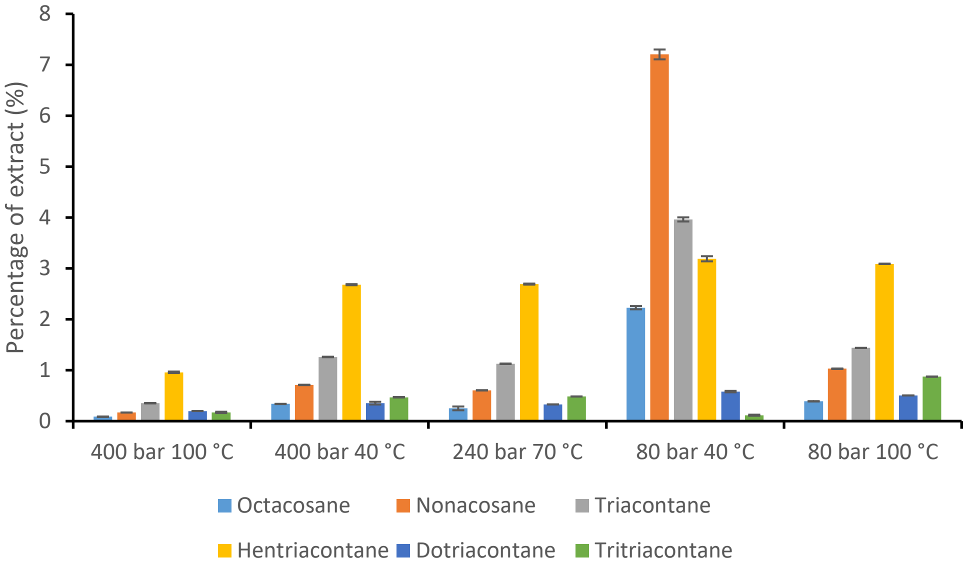


Figure 2-6 Percentage of individual n-alkanes in $scCO_2$ extracts.

The profiles of *n*-alkanes were similar with most abundant *n*-alkane being hentriacontane (C_{31}) (Figure 2-6). An exception was the extract obtained at (80 bar, 40 °C) where nonacosane (C_{29}) (Figure 2-6) was the most abundant. Interestingly, the same extract also exhibited the highest percentage of hydrocarbons. It is observed that hydrocarbons required a relatively low pressure and temperature to obtain their maximum recovery. This can be further explained by studying the effect of the solubility of n-alkanes in CO_2 reported in the literature and listed in Table 2-3. Schmitt and Reid showed that the lowest molecular weight *n*-alkanes have a higher solubility compared to a longer *n*-alkanes at a given pressure.[131] Therefore, this indicates that there are other factors that govern the extraction of *n*-alkanes such as diffusivity in higher density CO_2.[131, 132] For instance, Wako *et al*. showed that the diffusivity of the fatty acids methyl esters decreased as the chain length increased.[133] Therefore, a hydrocarbon with a higher chain length might diffuse less readily and as a consequence lower volumes will be extracted compared to shorter chain hydrocarbons.[134]

Table 2-3 Solubility of some long chain n-alkane in $scCO_2$.[131, 132]

***N*-alkanes**	**Temperature (°C)**	**Pressure (bar)**	**Density (g cm^{-3})**	**Solubility (kg kg^{-1} CO_2)**
Tetracosane (C_{24})	69.85	187-264	0.626-0.755	1.50-5.00
Hexacosane (C_{26})	69.85	158-260	0.541-0.747	0.38-3.09
Octacosane (C_{28})	69.85	162-240	0.557-0.725	0.25-1.42
Dotriacontane (C_{32})	69.85	185-310	0.626-0.797	0.23-1.38

Overall, the highest amount of hydrocarbons found in extracts obtained at 400 bar, 100 °) (897.4 ±14.7 µg g^{-1} of dry biomass) followed by extract obtained at 240 bar, 70 °C (856.1 ±5.7 µg g^{-1} of dry biomass) as listed in Table 2-4. This is consistent with the model discussed earlier where both high pressure and temperature enhance the recovery of different wax groups.

Table 2-4 Distribution and quantity of long chain n-alkanes in scCO$_2$ extracts, in µg g^{-1} of the dry plant.

Compounds	400 bar, 100 °C	400 bar, 40 °C	240 bar,70 °C	80 bar, 40 °C	80 bar, 100 °C
Octacosane (C_{28})	40.1 ±0.4	37.5 ±0.3	39.1 ±5.7	40.1 ±0.6	1.8 ±0.0
Nonacosane (C_{29})	78.4 ±0.6	79.0 ±0.5	94.5 ±0.2	129.7 ±1.7	4.8 ±0.0
Triacontane (C_{30})	163.2 ±0.8	139.9 ±0.5	175.8 ±0.5	71.3 ±0.7	6.8 ±0.0
Hentriacontane (C_{31})	444.3 ±6.8	297.5 ±1.2	419.9 ±1.6	57.4 ±0.9	14.5 ±0.0
Dotriacontane (C_{32})	91.9 ±0.2	39.2 ±3.0	51.2 ±0.2	10.5 ±0.3	2.4 ±0.0
Tritriacontane (C_{33})	79.5 ±5.9	52.1 ±0.3	75.5 ±0.3	2.1 ±0.2	4.1 ±0.0
Total n-alkanes	897.4 ±14.7	645.2 ±5.7	856.1 ±8.4	311.1 ±4.4	34.4 ±0.0

Despite their simple structure, *n*-alkanes play an essential role in biochemistry and physiology of insects and many other organisms. For instance, alkanes are well known for their semiochemicals functions in insects.[135] Semiochemicals derived from the Greek semion (signal) are naturally occurring biochemical that are used by insects and other organisms in communication and interaction.[135, 136] Therefore, semiochemicals that attract or repel insects, or that enhance or inhibit the action of some chemical, offer potential applications in pest control and crops protection which can replace the use of harmful insecticides and pesticides.[135, 136]

2.3.2 Long chain free fatty acids

Free fatty acids that have been identified in date palm were predominantly even chain length ranging from C_{10} to C_{34}. The majority of the fatty acids identified within date palm leaves waxes were saturated, with only two unsaturated fatty acids identified including oleic acid ($C_{18:1}$) with one double bond and linoleic acid ($C_{18:2}$) with two double bonds. The EI spectra for hexadecanoic acid ($C_{16:0}$) and 9-octadecenoic acid ($C_{18:1}$) are displayed in Figure 2-7 and 2-8 respectively. The identification of fatty acids was confirmed by fatty acids trimethylsilyl (TMS) ester derivatives. This derivatisation enhances the volatility and reduces the polarity of these compounds and hence improves the peak shapes in the GC chromatogram.[137] The EI spectrum of silylated fatty acid showed a weak molecular ion. However, the ion which corresponds to loss of methyl group from the TMS group $(M-15)^+$ was abundant and was used to assign the molecular ion.[138]

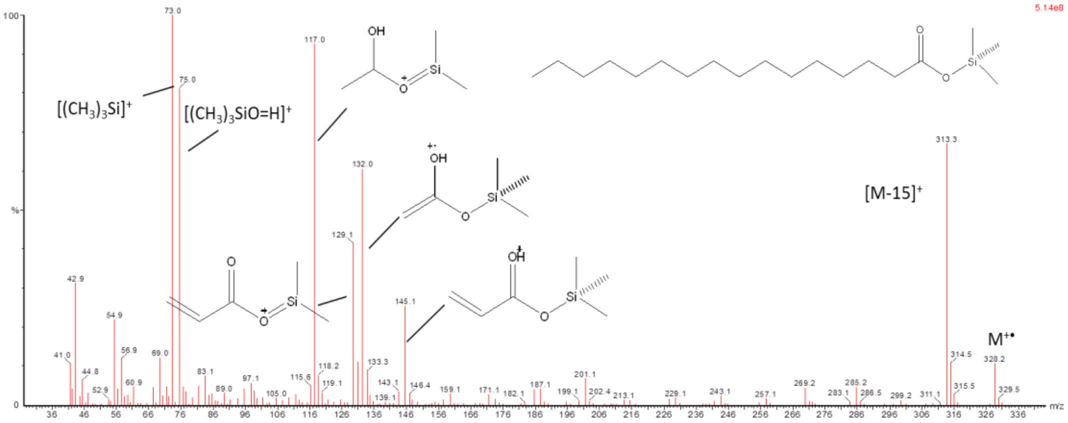


Figure 2-7 Mass spectrum of hexadecanoic acid TMS ester.

The primary EI fragments at m/z 73 and 75 are a result of the derivatisation and correspond to $[(CH_3)_3Si]^+$ and $[(CH_3)_2SiOH]^+$ respectively.[138] The other fragments that contain the TMS group are at m/z 117,132 and 145.[138] The fragment at m/z 132 is a result of the migration of (γ-H) in McLafferty rearrangement followed by β cleavage of C-C bond where two C atoms have been removed as illustrated in Figure 2-9.[138] A further loss of a methyl group forms the ion at m/z 117 as shown in Figure 2-9.

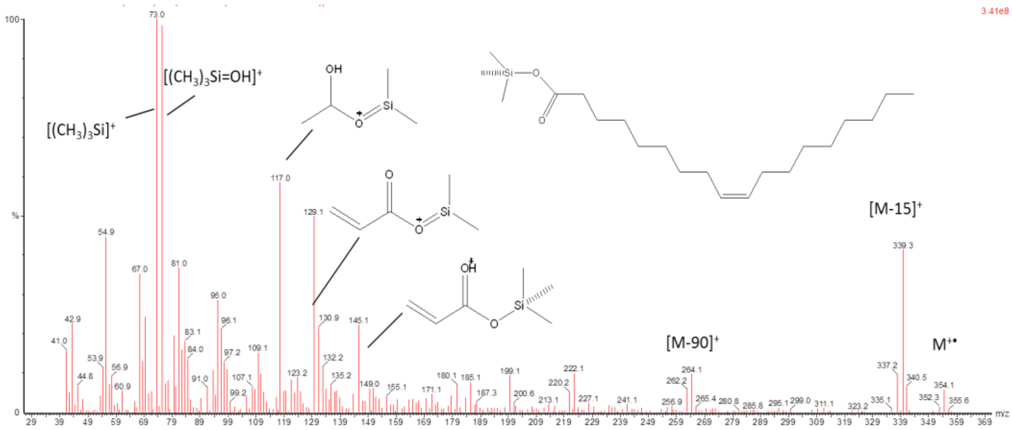


Figure 2-8 EI spectrum of 9-octadecaenoic acid TMS ester.

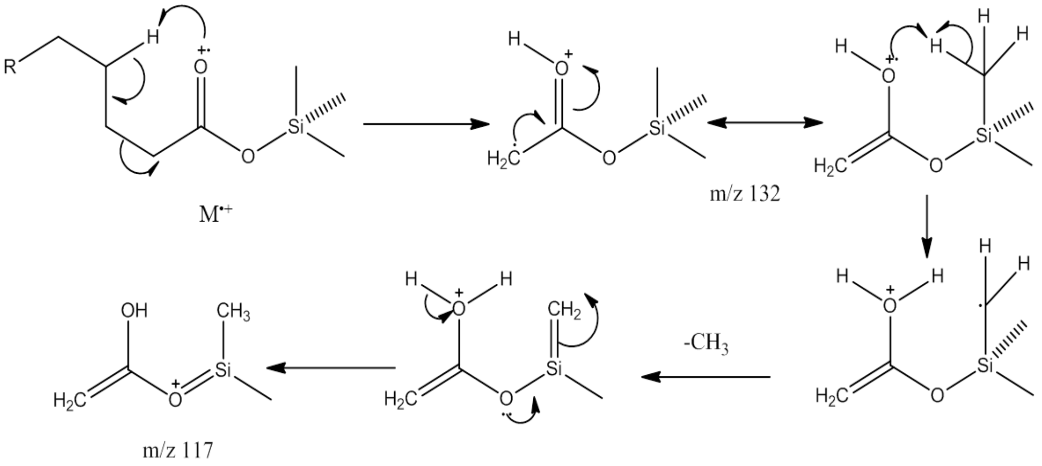


Figure 2-9 Mechanism of the formation of some fragmentation pattern at m/z 132 and 117.

Furthermore, the ion at m/z 145 is a result of the decomposition of the fatty acids derivatives as shown in Figure 2-10 which further decompose by a proton transfer mechanism to form a very stable ion at m/z 129.[138]

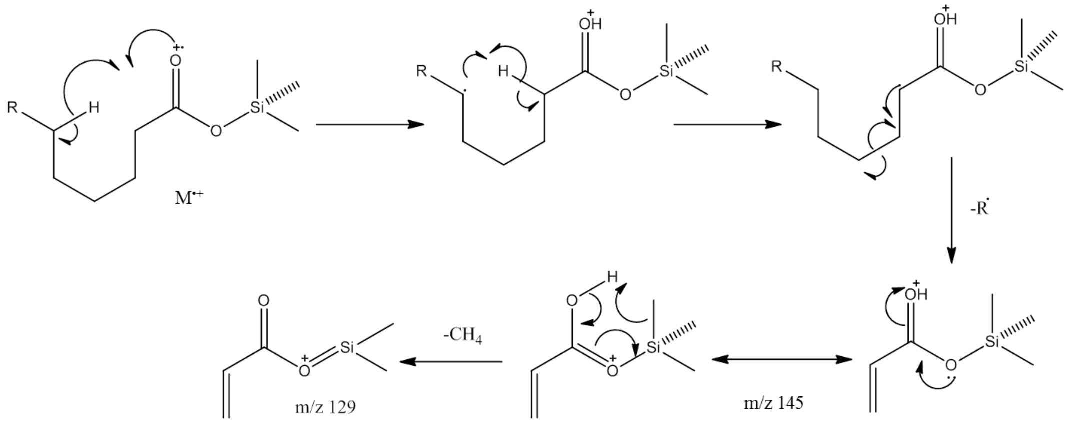


Figure 2-10 Mechanism for the formation of fragment ion at m/z 129.[22]

The distribution of the free fatty acids of different CO_2 extracts of date palm leaves is displayed in Figure 2-11. The majority of the fatty acids in date palm leaves wax were found to be even number carbon chain length, with the only odd chain acid found being heptadecanoic acid (C_{17}) in rather low loading (Table 2-5). Moreover, the most abundant fatty acids were found to be hexadecanoic acid ($C_{16:0}$), octadecenoic acid ($C_{18:1}$) and octadecadienoic acid ($C_{18:2}$) in most of the extracts. Studies showed that the most common fatty acids in plants are exclusively C_{16} and C_{18} fatty acids with up to three double bonds in some species.[139] A recent study reported a similar chain length rang of free fatty acids in date palm leaf extract.[103] Surprisingly some long chain fatty acid showed relatively high abundance in the extract obtained at higher temperature and pressure. For instance, fatty acids with chain length C_{32} were the most abundant in extracts obtained at 400 bar, 100 °C as shown in Figure 2-11.

The extracts obtained at relatively high temperature and pressure (400 bar, 100 °C) showed a generous abundance of the fatty acids (4150.8 ±280.1 $\mu g\ g^{-1}$ of dry biomass) compared to the extract obtained at the lower temperature (400 bar, 40 °C) with (90.1 ±0.2 $\mu g\ g^{-1}$ of dry biomass). This is consistent with previous observation for the enhanced yield at elevated temperature and pressure. Moreover, the increase in the extractability at high temperature might also be explained by entrainer effect. This effect is observed in a solid mixed system where the solubility of one component is increased in the presence of other components.[28] Since wax is a mixture of different compounds; some of the extractives will already be dissolved and act as co-solvent for these fatty acids.[140]

Linoleic acid (LA) is a polyunsaturated fatty acid (PUFA) that is classified as an essential fatty acid in human diet.[141] PUFA showed many health benefits including anti-cholesterolaemia, anti-inflammatory, anticancer and antibacterial effects.[141] As such, PUFAs are incorporated in many food supplements and nutraceuticals by pharmaceutical and food industries.[141, 142] Interestingly, many PUFAs can also be bio-transformed into a variety of products.[141] For instance, linoleic acid and α-linolenic acids can be converted into products such as free acids, glycerides, aldehydes, alcohols, phospholipids, etc. with potential applications in fragrance and flavours as well as agrochemicals.[141] Oleic acid is monounsaturated fatty acids, which is not considered an essential fatty acid. However, studies revealed that diets rich in oleic acid showed reduction in cholesterol levels and blood pressure as well as an induced benefit on autoimmune diseases.[143] Overall, Fatty acids have many industrial applications including as base chemicals for surfactant synthesis for use in the home and personal care sector, as polishing products and in cosmetics.[144, 145]

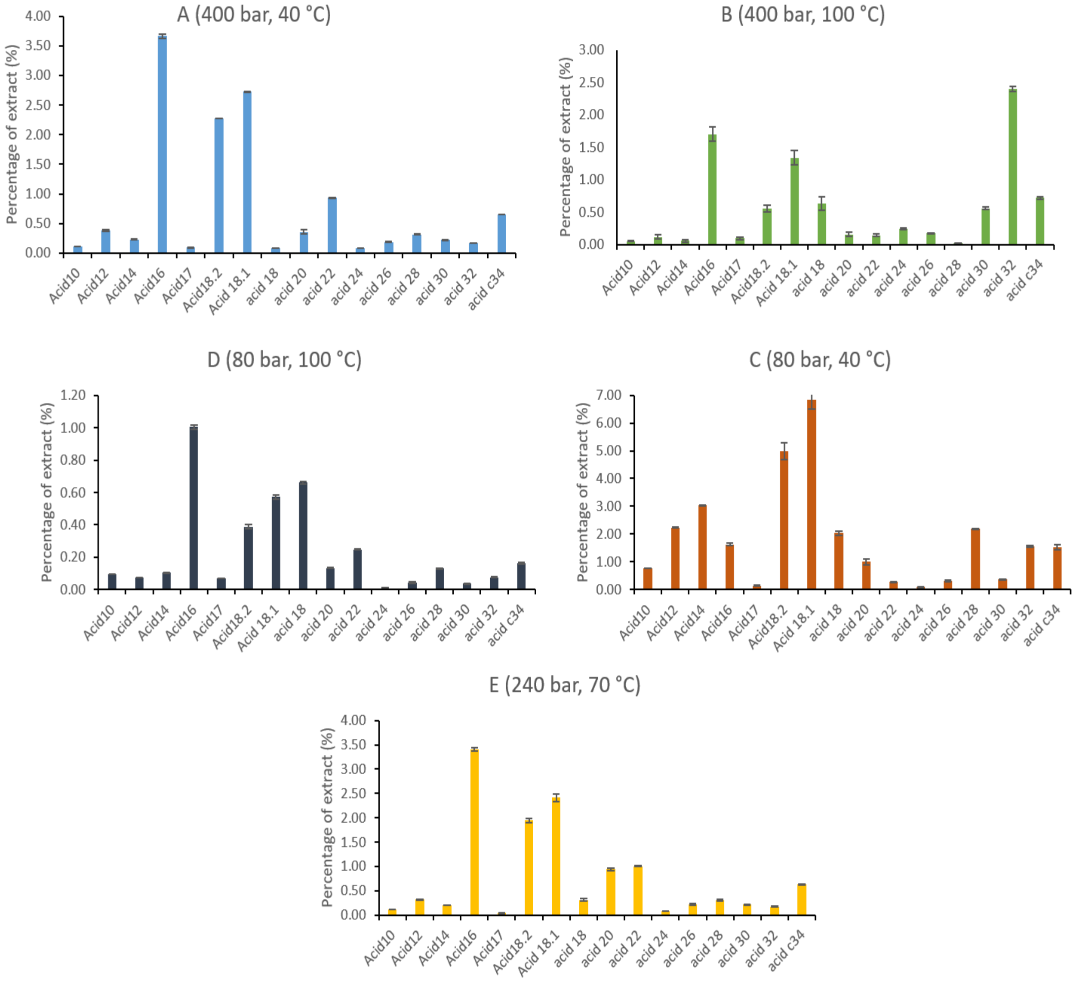


Figure 2-11 Individual free fatty acids composition in scCO$_2$ extracts of date palm leaves.

Table 2-5 Distribution and quantity of free fatty acids in $scCO_2$ extracts, in $\mu g g^{-1}$ of dry biomass.

Compounds	400 bar, 100 °C	400 bar, 40 °C	240 bar, 70 °C	80 bar, 40 °C	80 bar, 100 °C
Decanoic acid (C_{10})	24.4 ±4.0	2.1 ±0.0	16.6 ±0.1	3.7 ±0.0	0.4 ±0.0
Dodecanoic acid (C_{12})	54.9 ±13.2	6.8 ±0.3	48.4 ±0.5	10.5 ±0.1	0.4 ±0.0
Tetradecanoic acid (C_{14})	26.9 ±7.9	4.2 ±0.1	30.5 ±0.2	14.2 ±0.1	0.5 ±0.0
Hexadecanoic acid (C_{16})	787.7 ±49.7	65.9 ±0.5	524.1 ±5.4	7.7 ±0.2	4.7 ±0.1
Heptadecanoic acid (C_{17})	44.3 ±9.6	1.7 ±0.2	6.2 ±1.7	0.7 ±0.1	0.3 ±0.0
Octadecanoic acid (C_{18})	293.0 ±46.6	11.7 ±0.0	97.0 ±1.3	7.2 ±0.5	0.8 ±0.0
Eicosanoic acid (C_{20})	72.5 ±17.1	3.1 ±0.0	26.5 ±1.4	7.3 ±0.1	0.4 ±0.0
Docosanoic acid (C_{22})	66.4 ±8.9	4.0 ±0.0	32.4 ±0.6	1.7 ±0.1	0.2 ±0.0
Tetracosanoic acid (C_{24})	111.7 ±8.1	5.7 ±0.1	46.7 ±1.7	10.3 ±0.0	0.6 ±0.0
Hexacosanoic acid (C_{26})	80.8 ±2.0	3.3 ±0.1	33.4 ±1.7	1.5 ±0.1	0.2 ±0.0
Octacosanoic acid (C_{28})	9.7 ±0.9	1.6 ±0.0	13.2 ±0.1	0.4 ±0.1	trace
Triacontanoic acid(C_{30})	259.7 ±9.6	16.8 ±0.2	154.3 ±1.9	1.3 ±0.1	1.2 ±0.0
Dotriacontanoic acid(C_{32})	1111.2 ±16.3	6.4 ±0.7	144.9 ±3.5	4.7 ±0.5	0.6 ±0.0
Tetratriacontanoic acid (C_{34})	333.6 ±9.2	1.6 ±0.0	48.0 ±3.8	9.6 ±0.3	3.1 ±0.0
Total saturated acid	3276.8 ±203	134.9 ±2.3	1222.1 ±24	80.84 ±2.3	13.4±0.2
9-octadecenoic acid ($C_{18:1}$)	619.3 ±52.4	49.1 ±0.2	371.4 ±11.3	32.2 ±1.6	2.7 ±0.1
9,12-octadecdienoic acid ($C_{18:2}$)	254.6 ±24.7	41.0 ±0.0	299.4 ±6.2	23.4 ±1.4	1.8 ±0.1
Total unsaturated fatty acids	873.9 ±77.1	90.1 ±0.2	670.8 ±17.5	55.6 ±3.0	4.5 ±0.2

2.3.3 Fatty alcohols

Free fatty alcohols in date palm leaf wax were exclusively *n*-alkanol with even carbon chain length. The identification of the free fatty alcohols was confirmed by fatty alcohol (TMS) ether derivatives. Figure 2-12 shows the spectrum of TMS ether of 1-dotriacontanol. Usually, the molecular ion peak $M^{+\bullet}$ of TMS ether of fatty alcohols is very weak. However, the fragment ion corresponding to the loss of methyl group from TMS group (M-15)+ was intense and used in assigning the molecular weight for the fatty alcohols derivatives. Other peaks which contain the TMS group are present at m/z 75 and 103 for $[(CH_3)_2Si{=}OH)]^+$ and $[(CH_3)_3SiO{=}CH_2]^+$ respectively. Moreover the fragment ions at 43, 57, 71 etc. are characteristic of an alkyl chain.

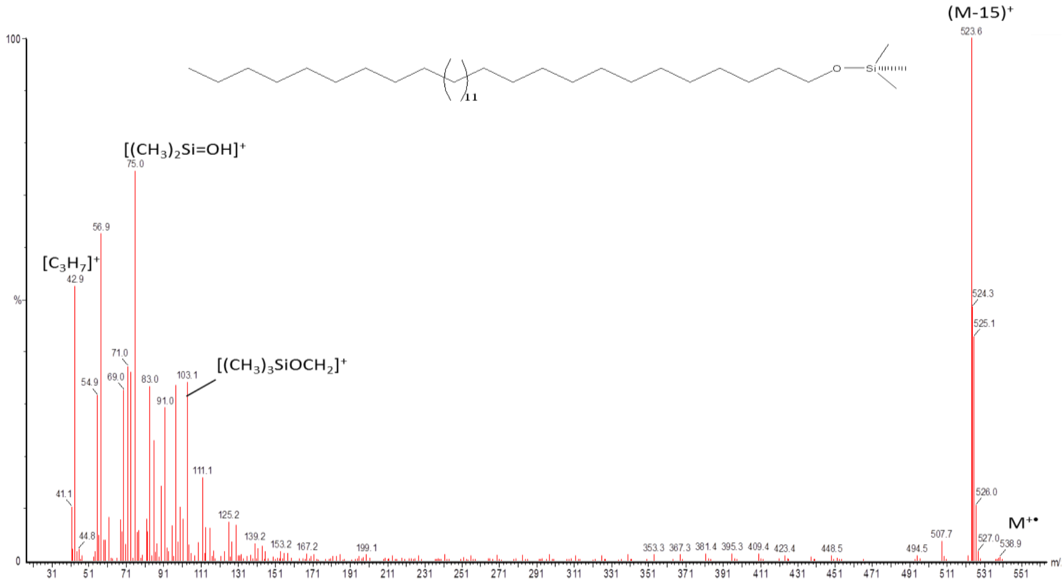


Figure 2-12 EI mass spectrum of 1-dotriacontanol TMS derivative.

Alcohols reported in date palm leaves are in the range C_{12} –C_{30} with major alcohols being between C_{19} to C_{22}.[103] However, in this study only five fatty alcohols were identified including hexacosanol (C_{26}), octacosanol (C_{28}), triacontanol (C_{30}), dotriacontanol (C_{32}) and tetratriacontanol (C_{34}) which are in agreement with the range of alcohols extracted from different biomass. For example the distribution of alcohols from sugarcane waste ranged between chain lengths C_{24} to C_{34}.[48, 124] Moreover, alcohols reported in miscanthus stems and leaves were in range (C_{22}- C_{32}).[146] While octacosanol (C_{28}) was the only alcohol identified in wheat straw wax.[5, 124] Interestingly, the most abundant alcohol in date palm leaves wax was dotriacontanol (C_{32}) followed by tetratriacontanol (C_{34}). While octacosanol (C_{28}) was the most abundant in sugarcane and wheat straw waxes.[5, 48, 112, 124] Date palm trees grows in arid and harsh climate condition while wheat straw and sugarcane come from low and moderate climate and this might explain the difference in the most abundant alcohols.

The distribution of individual fatty alcohols in all five extracts are shown in Figure 2-13. Dotriacontanol (C_{32}) and tetratriacontanol (C_{34}) were the most abundant alcohols in the composition of most extracts. The highest fatty alcohols content were found within the extract obtained at 400 bar, 100 °C and 240 bar , 70 °C with a total amount of 3115.8 ±27.0 μg g^{-1} and 1292.1 ±13.8 μg g^{-1} of dry biomass respectively (Table 2-6).

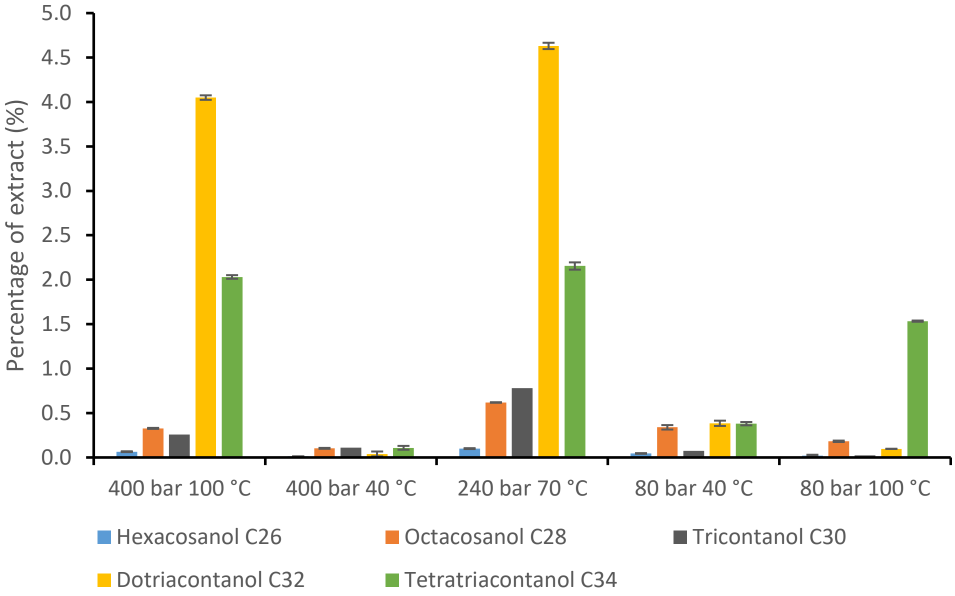


Figure 2-13 Percentage of individual fatty alcohols in $scCO_2$ extracts.

Table 2-6 Distribution and quantity of long chain fatty alcohols in $scCO_2$ extracts, in μgg^{-1} of dry biomass.

Compounds	**400 bar,100°C**	**400 bar,40°C**	**240 bar,70°C**	**80 bar,40°C**	**80 bar,100°C**
Hexacosanol (C_{26})	30.2 ±2.1	1.3 ±0.0	15.6 ±0.8	0.8 ±0.0	0.1 ±0.0
Octacosanol (C_{28})	151.6 ±2.5	11.4 ±0.1	96.5 ±0.5	6.1 ±0.1	0.9 ±0.0
Triacontanol (C_{30})	118.9 ±1.1	12.3 ±0.1	121.5 ±0.8	1.4 ±0.2	0.1 ±0.0
Dotriacontanol (C_{32})	1874.8 ±11.8	4.4 ±0.5	722.4 ±5.5	6.9 ±0.1	0.5 ±0.0
Tetratriacontanol (C_{34})	940.4 ±9.5	12.1 ±0.4	336.1 ±6.3	6.8 ±0.1	7.2 ±0.0
Total Alcohols	3115.8 ±27.0	41.5 ±1.1	1292.1 ±13.8	22.0 ±0.5	8.7 ±0.1

Mixtures of primary aliphatic fatty alcohols ranging from C_{20} to C_{32} are proven to exhibit significant cholesterol lowering effects.[147, 148] Policosanols are a mixture of fatty alcohols composed of octacosanol C_{28} (62.9%), triacontanol C_{30} (12.6%), and hexacosanol C_{26} (6.2%) which possess cholesterol-lowering properties when taken in 10-20 mg day^{-1} dose.[147, 148]

2.3.4 Fatty aldehydes

The main aldehydes found within the date palm leaves wax are dotriacontanal (C_{32}) and tetratriacontanal (C_{34}), although previous studies did not report their presence.[103] Aldehydes identified in sugarcane rind and bagasse were in range C_{26}–C_{36} with the most abundant being octacosanal (C_{28}).[124] Similarly, in miscanthus, the aldehydes were in range C_{24}- C_{30},[146] while octacosanal was the only aldehyde identified in wheat straw wax.[5] It is not surprising that the chain length of aldehyde and free alcohols are identical which suggests that the aldehyde oxidise to the corresponding fatty alcohols.[39, 149] The aldehyde structure was confirmed by studying the fragmentation pattern of the EI spectrum. Figure 2-14 shows the spectrum of dotriacontanal (C_{32}). The peak at m/z 446 represents the loss of water molecule from the molecular ion.[150, 151] Figure 2-15 shows the proposed scheme for the water loss and other fragments. Among the characteristic peak for aldehydes is that at m/z 418 which represents an expulsion of H_2O followed by CH_2=CH_2 from molecular ion.[150, 151] Hydrocarbon peaks at m/z (43, 57, and 71) are prominent in saturated aldehyde spectrum.[150, 151] Along with hydrocarbon fragments, another even numbered series of peaks appear at m/z 82, 96,110 which are believed to be characteristic of aldehyde functionality.[150, 151]

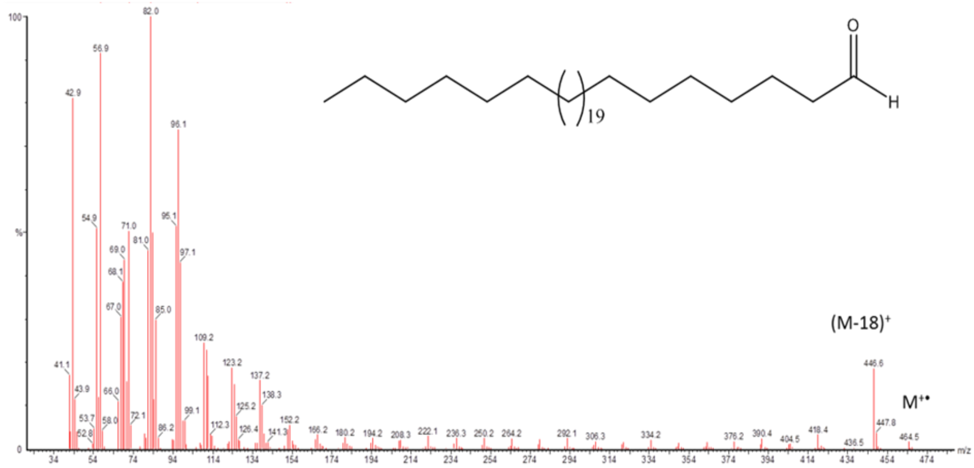


Figure 2-14 EI mass spectrum of n-dotriacontanol.

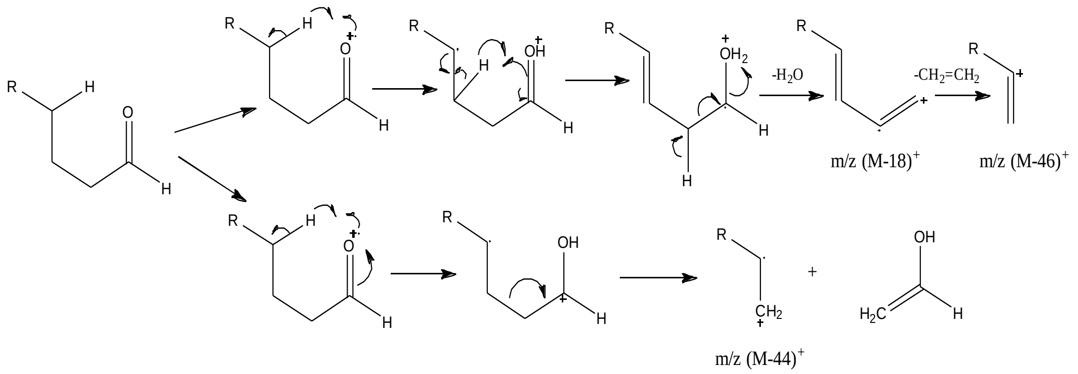


Figure 2-15 Common fragmentation in aldehyde spectrum.

Figure 2-16 show the composition of the fatty aldehydes in all five extracts, with a similar trend to that seen with fatty alcohols. The highest fatty aldehyde content found was 1897.8 ±41.4 μg g^{-1} of dry biomass at 400 bar, 100 °C and 840.6 ±4.4 μg g^{-1} of dry biomass obtained at 240 bar, 70 °C (Table 2-7) .

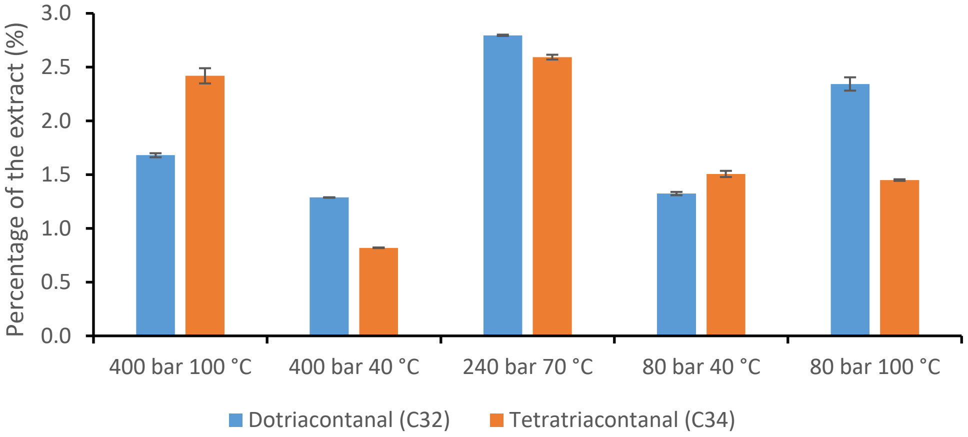


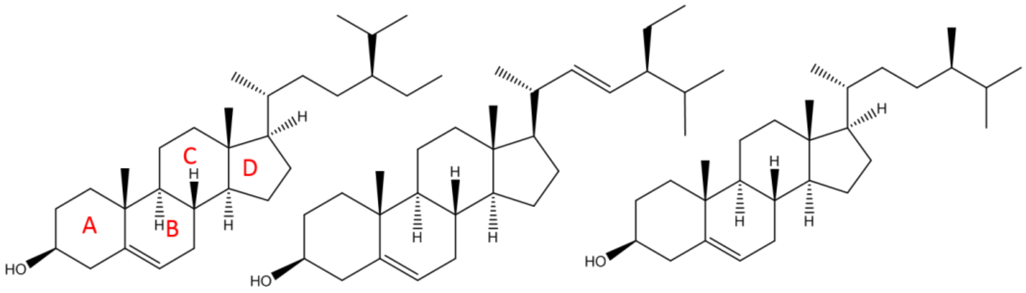
Figure 2-16 Percentage of individual aldehydes in $scCO_2$ extracts.

Table 2-7 Distribution and quantity of long chain aldehydes in scCO$_2$ extracts, in μg g^{-1} of dry biomass.

Compounds	400 bar,100 °C	400 bar,40 °C	240 bar,70 °C	80 bar, 40 °C	80 bar, 100 °C
Dotriacontanal (C32)	777.9 ±8.7	142.9 ±0.2	436.1 ±1.0	23.8 ±0.3	11.0 ±0.3
Tetratriacontanal (C34)	1119.9 ±32.7	91.0 ±0.3	404.5 ±3.4	27.1 ±0.5	6.8 ±0.0
Total Aldehydes	1897.8 ±41.4	233.8 ±0.6	840.6 ±4.4	50.9 ±0.8	17.8 ±0.3

2.3.5 Sterols

The phytosterols determined in date palm leave wax were mainly campesterol, stigmasterol and β-sitosterol, structurally represented in Figure 2-17. Most common Sterols reported for plant waxes in the literature are campesterol, stigmasterol and β-sitosterol.[5, 20, 124, 146, 152, 153] Surprisingly, a recent study indicated that date palm leave extract contained two sterols including cholesterol and β-sitosterol. In the date palm samples investigated in this thesis, β-sitosterol was also identified as one of the sterols present however; cholesterol was not detected as a major component of this plant. Cholesterol is a common sterol in mammals where it has an essential role in controlling rigidity of the cell wall, securing important proteins in the cell and regulating signal mechanism.[152-154] Other sterols that were identified in this study include the common sterols reported in plants including stigmasterol and campesterol. (Figure 2-17).



β-sitosterol Stigmasterol Campesterol

Figure 2-17 Chemical structures of campesterol, stigmasterol and β-sitosterol.

The EI mass spectrum of β-sitosterol TMS derivative are displayed in Figure 2-18 while the molecular ion and some fragmentation ions observed with steryl TMS ethers can be seen in Table 2-8.

The ion M-90 resulted from the loss of $[(CH_3)_3Si{=}OH]^+$ group which is among the common fragment in Δ5 (double bond in position 5) sterols mass spectra.[155-157] Another intrinsic and valuable fragment is the loss of the $(CH_3)_3SiO$ group along with carbon atoms C1, C2 and C3 from ring A in Figure 2-19 which forms the peak at m/z 129.[155-157] This peak results from the β-cleavage in ring A followed by hydrogen migration as illustrated schematically in Figure 2-19.

The compositions of sterols in all five extracts are shown in Figure 2-20. All extracts showed a broadly similar distribution of the sterols, with the highest percentage recovered in the extract obtained at 240 bar and 70 °C. Moreover, the highest sterol contents were found within the extracts obtained at 400 bar, 100 °C and 240 bar, 70 °C with total sterol content being 350.8 ±35.3 μg g^{-1} of dry biomass and 306.4 ±2.2 μg g^{-1} of dry biomass respectively (Table 2-9).

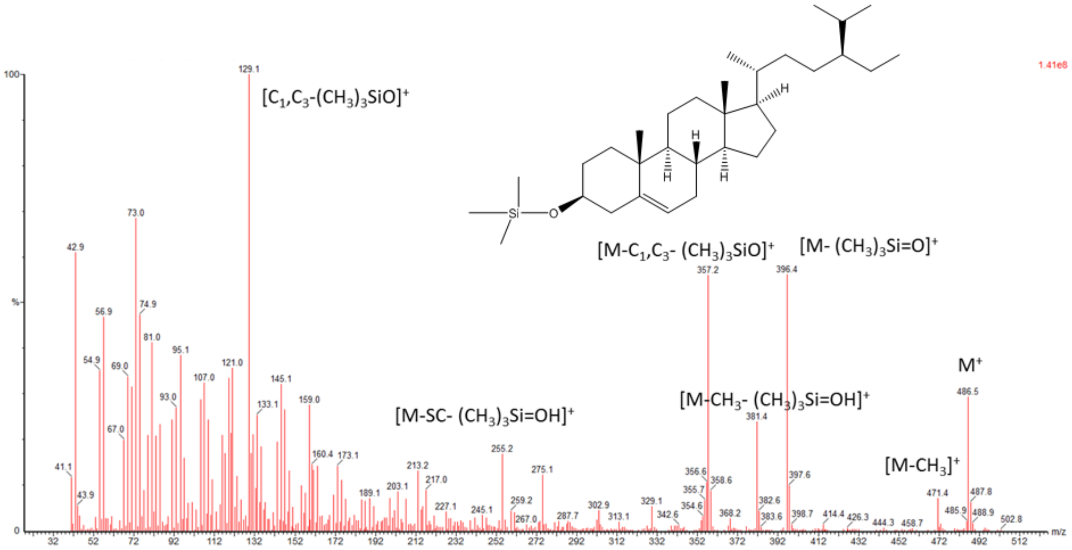


Figure 2-18 EI mass spectrum of β- sitosterol TMS derivative.

Table 2-8 Fragmentation ions associated with sterols TMS derivatives.

Fragment	Sitosterol	Stigmasterol	Campesterol
Molecular weight	414.4	412.4	400.7
M+•(TMS derivative)	486.5	484.5	472.8
[M-CH$_3$]+	471.4	469.4	457.8
[M-(CH3)$_3$Si=OH]+	396.4	394.3	382.6
[M-CH3(CH3)$_3$Si=OH]+	381.4	379.2	367.5
[M-C1,C3-(CH3)$_3$SiO]+	357.2	355.5	343.8
[M-SC-(CH$_3$)$_3$Si=OH]+	255.2	255.0	255.0
[C1,C3-(CH$_3$)$_3$SiO]+	129.1	129.1	129.1

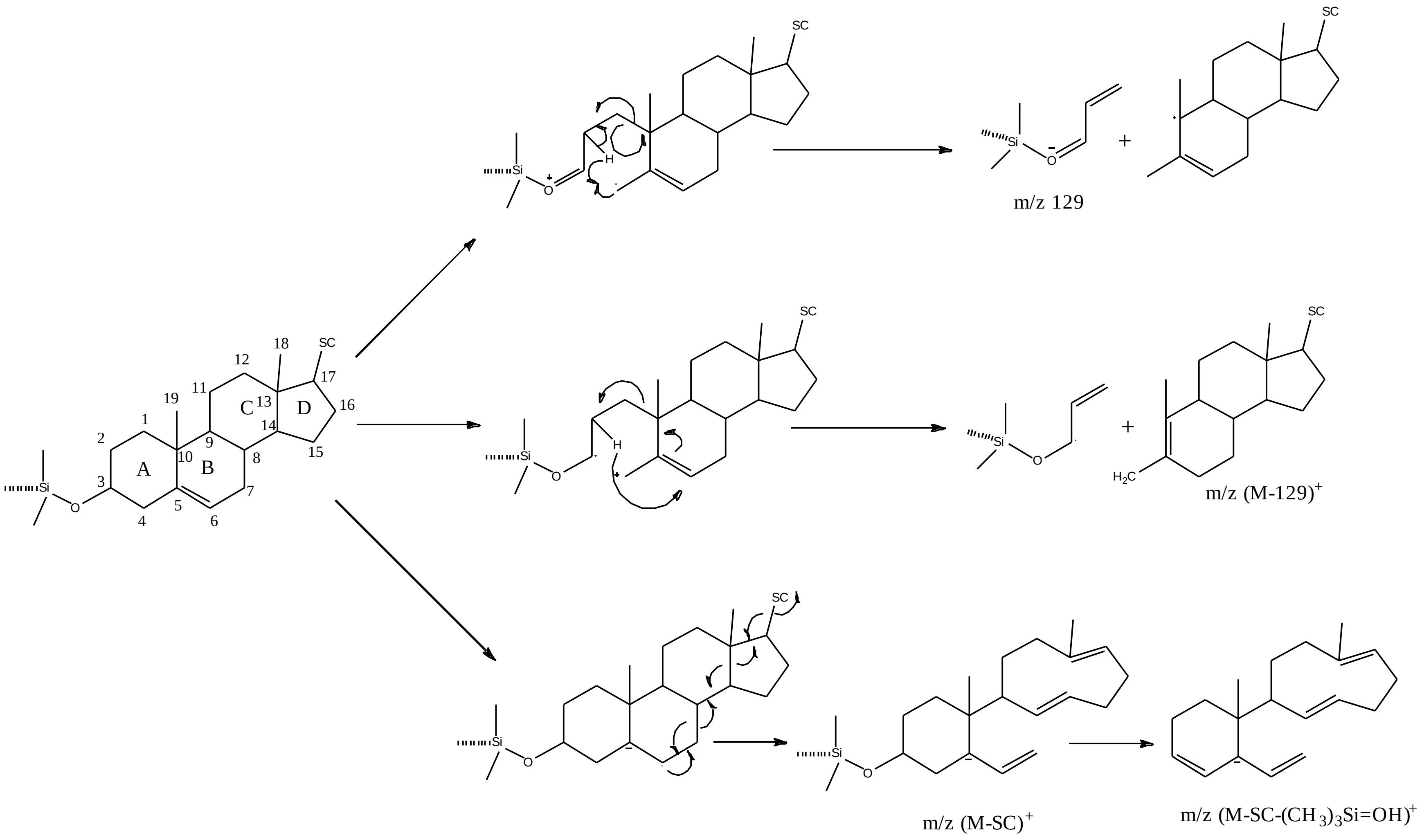


Figure 2-19 Mechanism of the formation of some fragmentation pattern with steryl TMS derivative. Note (SC: side chain, M: molecular ion)

Among the sterols, identified β-sitosterol was the most abundant one in all extracts followed by stigmasterol. In plants, campesterol and stigmasterol are both derived from β-sitosterol which explains the high abundance of β-sitosterol.[158] The high recovery of these sterols in the above two extracts are due to the enhanced high solubility at elevated pressures, as well as the increase in vapour pressure associated with higher extraction temperatures.[119, 120, 159]

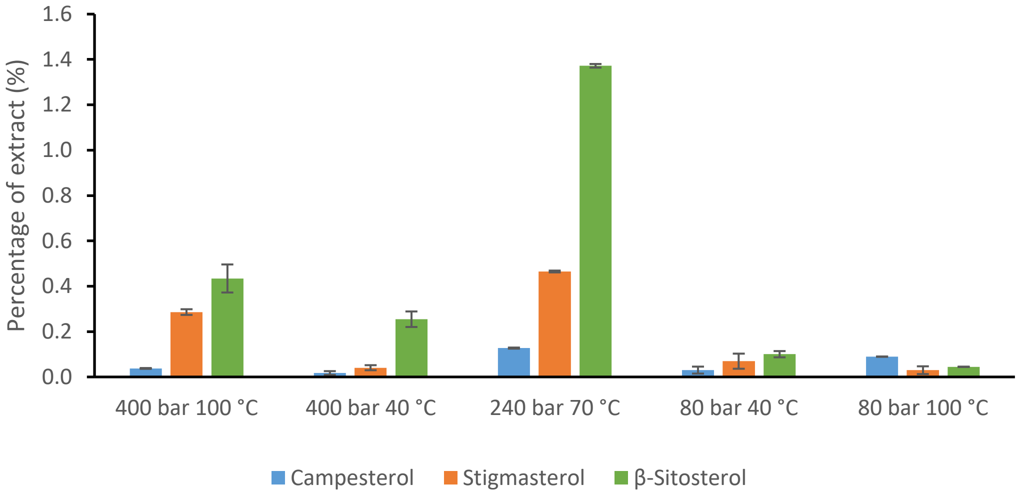


Figure 2-20 Percentage of individual phytosterols in scCO$_2$ extracts.

Table 2-9 Distribution and quantity of phytosterols in scCO$_2$ extracts, in µg g^{-1} of dry biomass.

Compounds	400 bar, 100 °C	400 bar, 40 °C	240 bar, 70 °C	80 bar, 40 °C	80 bar, 100 °C
Campesterol	17.3 ±0.8	1.9 ±0.1	19.8 ±0.4	0.5 ±0.1	0.4 ±0.0
Stigmasterol	132.4 ±5.8	4.5 ±0.2	72.5 ±0.6	1.2 ±0.2	0.1 ±0.0
β-sitosterol	201.0 ±28.6	28.3 ±0.6	214.0 ±1.2	1.8 ±0.1	0.2 ±0.0
Total Sterols	350.8 ±35.3	34.7 ±1.0	306.4 ±2.2	3.6 ±0.3	0.8 ±0.0

Plant sterols or stanols are proven to have many potential health benefits such as antioxidant, anti-inflammatory and anti-cancer activities.[158, 160, 161] Plant sterols are structurally similar to cholesterol; however their absorption in humans is less than that of cholesterol.[158, 160, 161] Indeed, a diet rich in these sterols has been proven to reduce low-density lipoprotein-cholesterol (LDL-C) levels in plasma and hence lower the risk of cardiovascular disease.[158, 160, 161] The most common phytosterols in human diet are β-Sitosterol, stigmasterol and campesterol and they are usually incorporated in food supplements to enrich the diet.[160, 161]

2.3.6 Wax esters

Wax esters identified in the date palm leaf wax range from C_{40} to C_{56}. Figure 2-21 shows the EI spectrum for the wax ester composed of C_{16} fatty acids and C_{32} alcohol. The esters are represented as follows $RCO_2R`$ with R being the fatty acid moiety and R` the alcohol moiety. Among the characteristic fragments in wax esters mass spectra are the intense fragment ions arising from fatty acid moiety $[RCO_2H_2]^+$ and $[RCO_2H]^+$ as well as some weak to medium ions resulting from fatty alcohol moiety $[CO_2R']^+$ and $[R'-1]^+$.[130]The base peak in EI spectrum for the wax ester composed of C_{16} fatty acids and C_{32} alcohol is at m/z 257 and is believed to be a result of McLafferty rearrangement where two protons are transferred to the fatty acid, as shown in Figure 2-22. A series of peaks separated by 14 mass units appear at low m/z region which indicates the presence of alkyl group.[130]

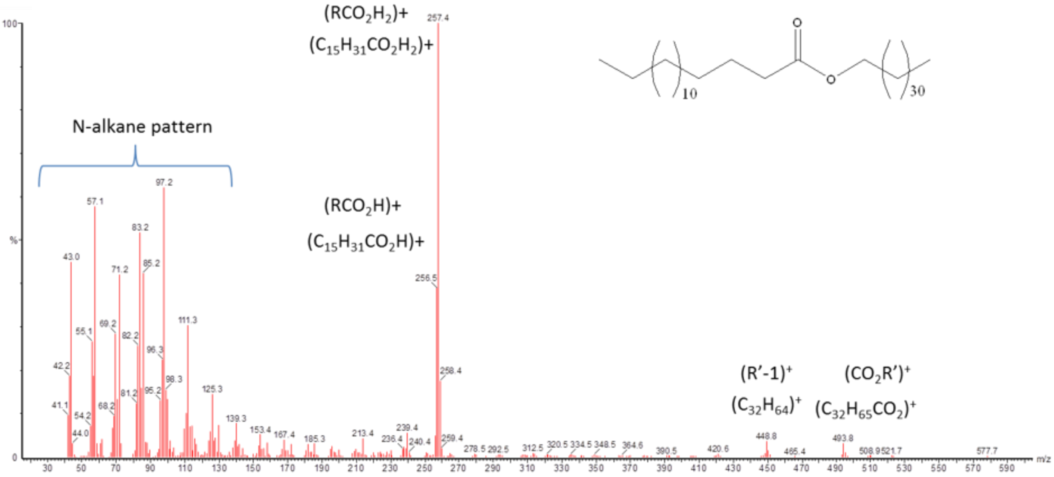


Figure 2-21 EI mass spectrum of an ester of C_{16} fatty acid and C_{32} fatty alcohol.

Table 2-10 shows the identified wax esters with some characteristic fragments. It can be concluded from the table that the esterified fatty acids ranged from C_{16} to C_{22} and esterified fatty alcohols ranged from C_{28} to C_{34}. Interestingly the most common esterified alcohols (C_{28}, C_{30}, C_{32} and C_{34}) and fatty acids (C_{16} and C_{18}) are also the most abundant in the free form which is in an agreement with the biosynthesis of wax esters.

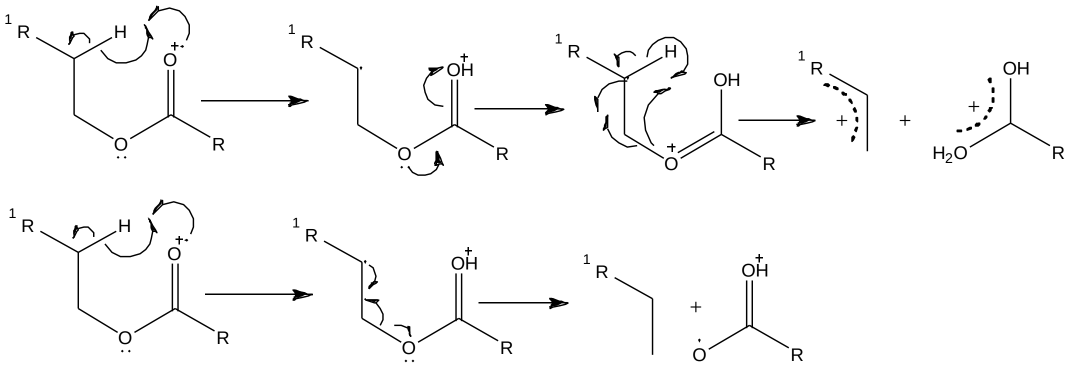


Figure 2-22 Mechanism of formation of base peaks ions $[RCO_2H_2]^+$ and $[RCO_2H]^+$ in EI spectra of wax ester.

Table 2-10 Identified wax esters in date palm leaf wax with some characteristic fragment ions.

Wax ester	Molecular weight	C acid: C alcohol	$[RCO_2H_2]^+$	$[RCO_2H]^+$	$[CO_2R']^+$	$[R'-1]^+$
C_{44}	648.7	C16:C28	257.4	256.4	437.5	392.6
C_{46}	676.7	C16:C30	257.4	256.4	465.5	420.6
C_{48}	704.7	C16:C32	257.4	256.4	493.8	448.8
C_{50}	732.8	C16:C34	257.4	256.4	521.8	476.7
C_{52}	760.8	C18:C34	285.3	284.3	521.5	476.5
		C20:C32	313.4	312.4	493.6	448.8
		C22:C30	341.2	340.4	465.4	420.5

The percentage composition of the wax esters varied in different extracts as indicated in Figure 2-23, accounting for almost 10% of the extract obtained at 240 bar, 70 °C, 8% of the extract obtained at 80 bar, 100 °C and about 5% of the extracts obtained at 400 bar, 100 °C and 400 bar, 40 °C. However it is clear from Table 2-11 that the highest recovery per g of dry plant is achieved by extracting at 400 bar, 100 °C followed by the extract obtained at 240 bar, 70 °C (2421.1 ±26.1 and 1507.7 ±24.3 µg g^{-1} biomass respectively). Even chain length wax esters were the dominant over odd chain length with C_{48} being the most abundant in most of the extracts. The highest recovery is achieved at both high temperature and pressure which is consistent with earlier observation. Stahl *et al.* stated that the solubility of the wax esters rises significantly with temperature increase.[119] For instance, a temperature rise from 20 °C to 80 °C increased the solubility of the wax esters and triglycerides by factor of 8, which further confirm the effect of the temperature in melting these high molecular compounds and hence aiding the solubilisation.[119] Therefore, a massive improvement in the yield of wax esters observed from 38.4 ±0.1 µg g^{-1} biomass at (80 bar, 40 °C) to 2421.1 ±26.1 µg g^{-1} biomass at (400 bar, 100 °C) indicating further the effect of the temperature in overcoming the wax melting point.

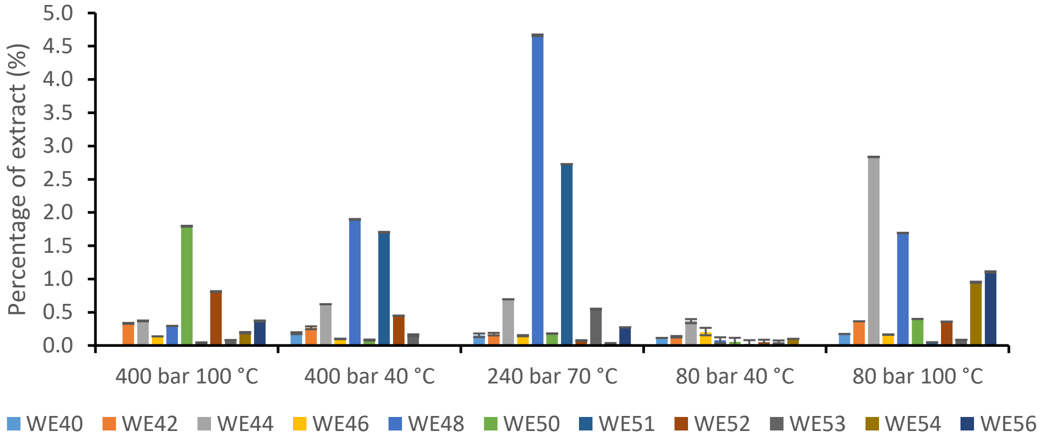


Figure 2-23 Percentage composition of individual wax esters in scCO$_2$ extracts.

Table 2-11 Distribution and quantity of wax esters in scCO$_2$ extracts, in µg g^{-1} of the dry plant.

Compounds	400 bar,100 °C	400 bar,40 °C	240 bar,70 °C	80 bar,40 °C	80 bar, 100 °C
WE40	153.9 ±3.5	20.6 ±1.6	23.8 ±4.3	2.0 ±0.2	0.8 ±0.0
WE42	171.0 ±2.9	29.6 ±2.4	26.6 ±3.0	2.4 ±0.5	1.7 ±0.0
WE44	63.4 ±0.6	68.9 ±0.2	108.4 ±0.2	6.6 ±1.2	13.3 ±0.0
WE46	136.4 ±0.5	11.2 ±0.3	23.5 ±0.8	3.6 ±0.8	0.8 ±0.0
WE48	830.4 ±3.1	210.5 ±0.7	727.6 ±1.9	1.4 ±1.1	8.0 ±0.0
WE50	375.3 ±2.9	9.1 ±0.9	27.9 ±0.3	1.0 ±0.4	1.9 ±0.0
We51	19.0 ±0.8	188.8 ±1.1	425.3 ±10.9	0.4 ±0.7	0.2 ±0.0
WE52	375.3 ±2.9	49.7 ±0.3	11.6 ±0.9	0.9 ±0.4	1.7 ±0.0
We53	37.0 ±0.6	17.0 ±1.6	85.2 ±1.2	0.9 ±0.0	0.4 ±0.0
WE54	89.0 ±4.8	-	5.2 ±0.5	1.8 ±0.0	4.5 ±0.0
WE56	170.4 ±3.6	-	42.5 ±0.3	-	5.2 ±0.0
Total wax esters	2421.1 ±26.1	605.1 ±9.0	1507.7 ±24.3	21.0 ±5.1	38.4 ±0.0

Wax esters have many industrial applications including cosmetics, personal care products, lubricants and plasticisers.[162, 163] They exhibit unusual emollient behaviour and impart stability to products due to the high melting point making them a key ingredient in personal care products and cosmetics.[162]

2.4 Melting profile for date palm leaves waxes

Differential scanning calorimetry (DSC) was used to study the melting profile for the $scCO_2$ extracted waxes at different extraction. The DSC thermograms are displayed for date palm waxes in Figure 2-24. Interestingly, different extracts showed an appreciable difference in the melting point profile. As most of the natural waxes are mixture of hydrophobic compounds and therefore they lack a defined thermal transition.[164] This is the case with date palm waxes and hence broad melting profile observed for all extracts. The melting point ranged between 30 °C to 79 °C. The lowest melting point exhibited by the extract obtained at (80 bar 40 °C) with board peaks at 30 °C and 43 °C. The board DCS profile might be due to the diverse range of compounds identified within the leave wax. Therefore, these endothermic minima correspond to thermal transition of different groups of compounds. Moreover, the low melting point is as a result of the chemical composition of the extract (80 bar, 40 °C) with more than 17% of the extract is composed from hydrocarbons, which are among the lowest molecular weight compounds within the wax and less than 2% of wax esters which are among the higher molecular compounds. . A similar profile is seen for the extract obtained at 400 bar and 40 °C with much sharper peaks at 53 °C and 62 °C due to increase in the proportion of higher molecular weight compounds such as wax esters with 6% recovery. In contrast, the extract obtained at (240 bar, 70 °C) exhibited a well-defined melting point at 71 °C. This can be attributed to richness of the extract of long chain alcohols (9%), long chain aldehydes (6%) and high wax esters content (10%) within this extracts. Unsurprisingly the highest melting point of 79 °C is associated with the extract obtained at the highest extraction conditions applied in this study (400 bar, 100 °C). Indeed, this high melting point profile is a result of high recovery of higher molecular compounds including long chain fatty acids, alcohols, aldehydes and wax esters.

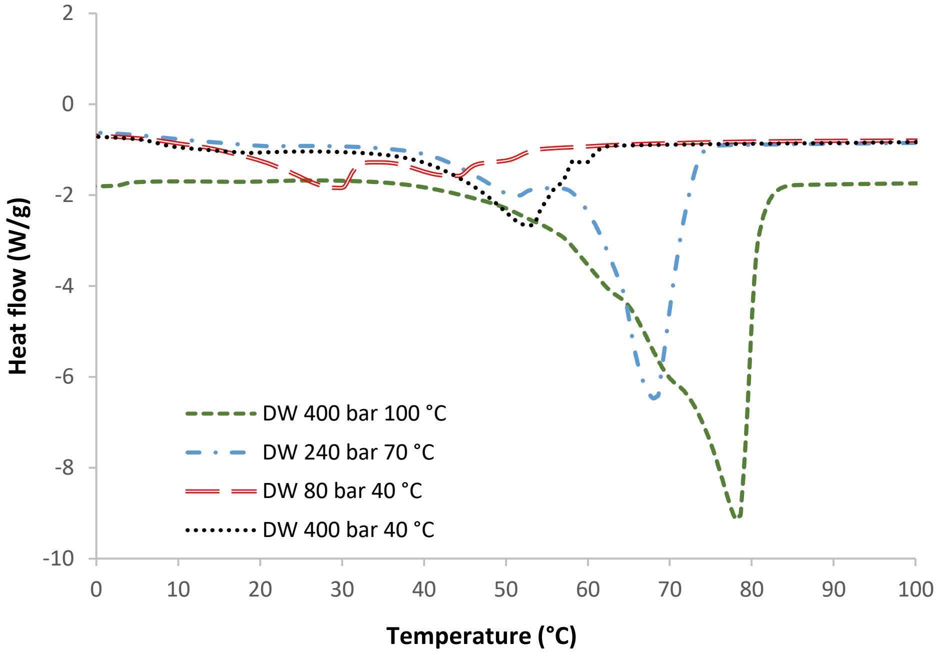


Figure 2-24 DSC thermogram showing the melting point profile for $scCO_2$ extracts.

The variation in the melting point profile for different extracts along with the compositional data discussed earlier suggest that date palm leaf wax can have a broad range of potential applications. For instance; in cosmetics manufacturing a high melting wax such as carnauba wax is used as a hardening agent to raise the melting point of the lipsticks to improve their thermal stability.[165] Moreover, high melting wax used for surface coating to prevent scratches and impart water repelling properties.[63] [165]Date palm wax extract obtained at (400 bar, 100 °C) showed a high melting point as well as high content of wax esters making it potentially suitable for such application as hardening agent, coating, as polishes, lubricants and gelling agent for liquid oil. On the other hand moderate to low melting waxes including beeswax and candillila wax are responsible for creamy texture in many cosmetics and personal care products.[63, 166] Beeswax is also used in candle making.[63, 167] Date palm extracts obtained at lower extraction conditions at (400, bar, 40 °C) exhibited the lowest melting profile and high hydrocarbon content making it suitable to impart texture in emulsion as well as natural insectside. Most of the waxes have excellent oil binding capacity and therefore they form gel which can have further application in food industry as emulsion and shortening agent.[168-171] The melting profile of date palm waxes is compared to some commercial waxes in Figure 2-25. It should be noted how versatile date palm waxes extracts are and therefore can potentially replace many commercial waxes including candelilla, carnauba and beeswax. Candelilla and carnauba waxes are both considered unsustainable as the trees from which they are harvested are restricted to limited geographical regions.[59, 62] The wax extract obtained at 70 °C, 240 bar has a melting point range very close to that of the candelilla wax, while the extract obtained at 100 °C, 400 bar showed thermal profile comparable to carnauba wax. Therefore, date palm waxes can potentially replace carnauba and candelilla waxes. Moreover, the wax extract obtained at 40 °C, 400 bar showed a thermal profile close to that of beeswax which is one of the most used wax in cosmetics as it melts at body temperature and thereof incorporated into many products.[63] The growing demand for vegan products make date palm leaves wax a promising alternative in these application.[172]

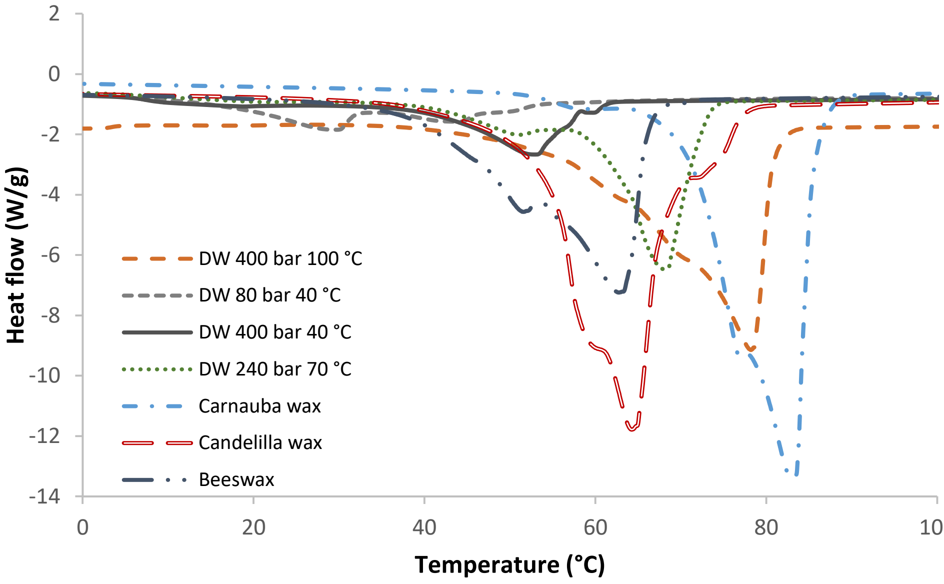


Figure 2-25 DSC thermogram showing the melting point profile commercial waxes compared to date palm wax.

2.5 Comparison between Soxhlet extraction and $scCO_2$

$ScCO_2$ extraction is commonly compared with conventional solvent extraction. Conventional solvent extraction such as Soxhlet extraction has been one of the standard technique in the extraction for more than one century and now. Moreover, it is considered as reference to which performance of other extraction technique is compared.[173, 174] $ScCO_2$ extraction is very often compared with hexane or heptane Soxhlet extraction,[175, 176] as previous studies have shown their polarities are broadly similar.[25] Figure 2-26 demonstrates the yield of Soxhlet heptane extraction compared to the $scCO_2$

extraction at various conditions. The percentage yield of Soxhlet heptane is slightly higher (4.21% ±0.21) than $scCO_2$ extract obtained at 400 bar and 100 °C (3.49% ±0.23)

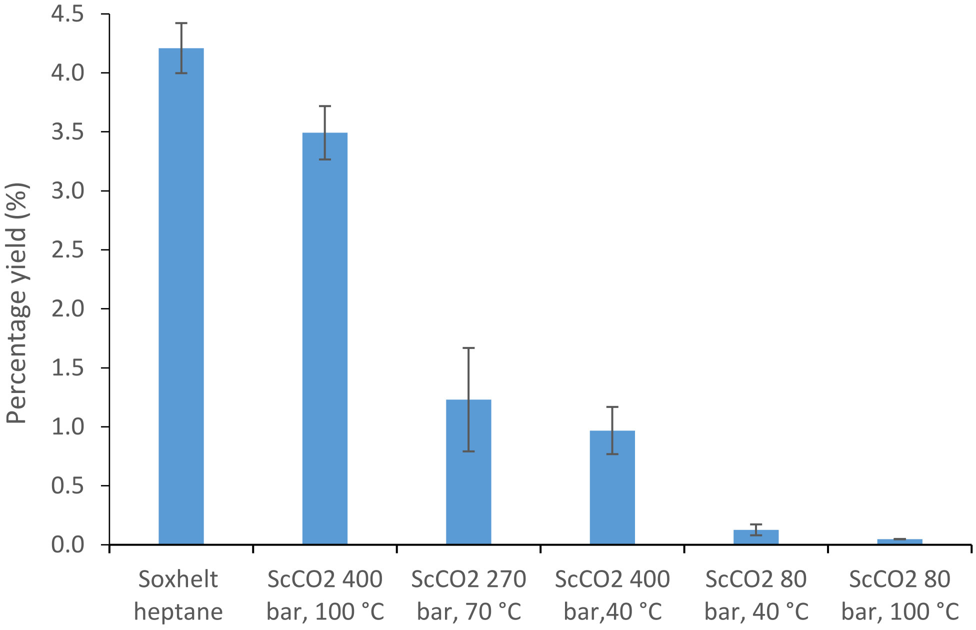


Figure 2-26 Experimental percentage yield of Soxhlet heptane extraction and $scCO_2$ extraction at different extraction temperature and pressure.

The composition of the wax obtained by heptane and $scCO_2$ is illustrated in Figure 2-27. It was found that both extracts contained the same types of compounds, with a similar distribution. However, there is slight variation in the content of individual components as noted from Table 2-12 and Figure 2-26. More hydrophobic molecules such as wax esters and *n*-alkane were preferentially extracted with heptane over CO_2. The total recovered of *n*-alkane with heptane is 964.6 ±14.7 µg g^{-1} of dry biomass versus 897.4 ±14.7 µg g^{-1} of dry biomass recovered from CO_2. This is even more profound for the recovered wax esters with heptane 3178.61 ±60.9 µg g^{-1} of dry biomass compared to CO_2 recovered wax esters 2421.1 ±26.1 µg g^{-1} of dry biomass. This is likely due to the elevated temperatures of the system during the course of extraction. In addition Soxhlet extraction is matrix independent which results in high yield more than any other leaching extraction technique[174] However, aldehydes and fatty alcohols were better recovered with CO_2. For instance, the total aldehydes extracted with $scCO_2$ at 400 bar, 100 °C was 1897.8 ±41.4 µg g^{-1} of dry biomass is much higher compared to the heptane extracts with total recovery of 368.6 ±26.1 µg g^{-1} of dry biomass. A similar trend found within the content of the fatty alcohols with 3115.8 ±27.0 µg $^{g-1}$ of dry biomass for the CO_2 extract compared to 1900.2 ±36.0 µg g^{-1} of dry biomass for heptane extract. The recovery of fatty aldehydes and fatty alcohols with $scCO_2$ over Soxhlet is owed to the nature of Soxhlet extraction as samples usually extracted at solvent boiling temperature for long time and possibility for thermal decomposition or oxidation is likely to occur.[174] Therefore, $scCO_2$ extract has advantages over heptane in the extraction of the aldehydes and polycosanols.

Figure 2-28 show the melting point profile for heptane extract compared to CO_2 extracts. The DSC exhibited two very sharp peaks at 85 and 86 °C. It is not surprising that heptane extract has the highest melting point, as the Soxhlet extraction is non-selective and exhaustive extraction technique, which usually extracts more compounds.[6]

There are numerous benefits using $scCO_2$ over Soxhlet extraction. Typically in Soxhlet extraction solvent is heated to boiling point in order to reflux it, therefore extraction is carried at one temperature and hence only one type of wax is produced. While $scCO_2$ offer different waxes product be simply varying the extraction temperature and pressure. As such, production of tailored waxes for specific applications can be achieved by $scCO_2$.

Figure 2-27 Percentage composition of different wax groups in heptane extract and $scCO_2$ extract at 400 bar, 100 °C.

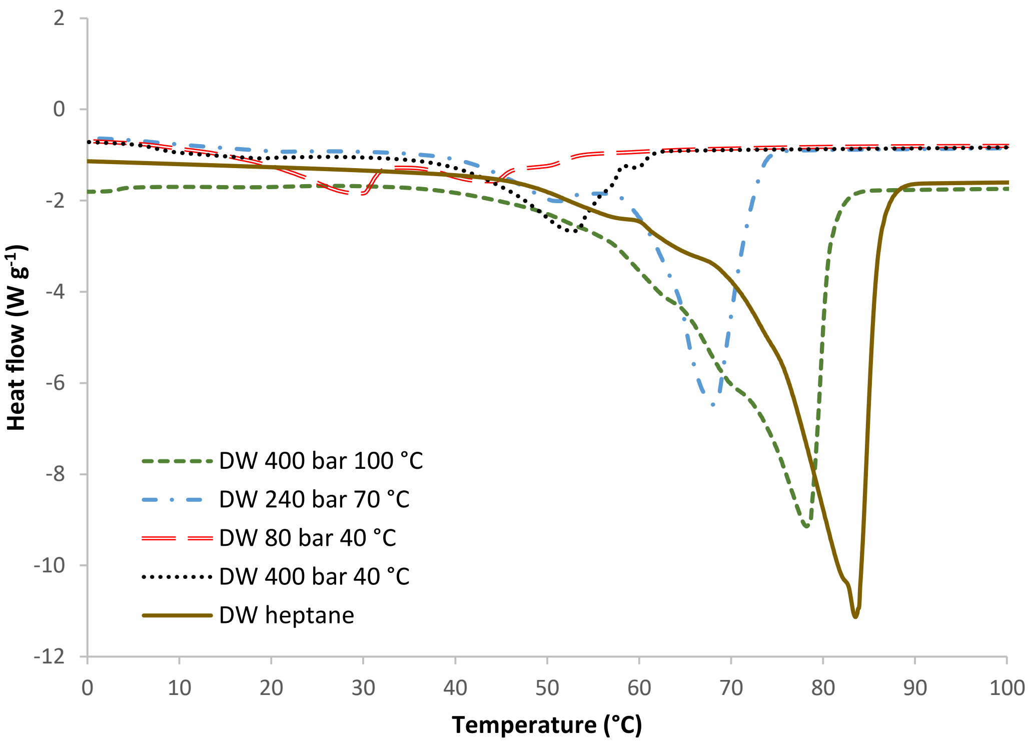


Figure 2-28 DSC thermogram showing the melting point profile for heptane extract and some $scCO_2$ extracts.

Table 2-12 Distribution and quantity of different wax compounds in $scCO_2$ extracts, in µg g^{-1} of dry biomass.

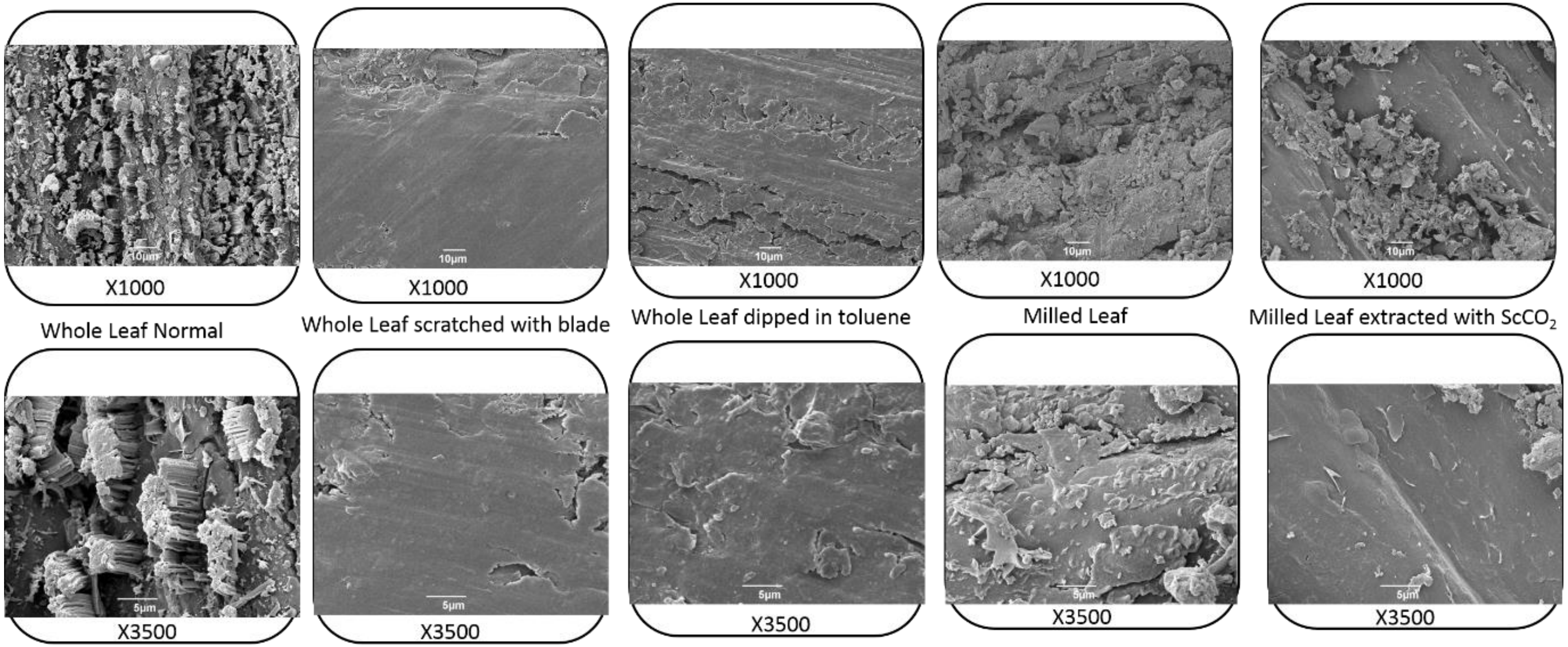
Compounds	400 bar, 100 °C	Heptane Soxhlet
Decanoic acid	24.4 ±4.0	27.6 ±0.2
Dodecanoic acid	54.9 ±13.2	65.3 ±0.8
Tetradecanoic acid	26.9 ±7.9	69.6 ±0.9
Hexadecanoic acid	787.7 ±49.7	585.3 ±6.7
Heptadecanoic acid	44.3 ±9.6	31.0 ±0.2
Octadecanoic acid	293.0 ±46.6	80.8 ±1.3
Eicosanoic acid	72.5 ±17.1	19.0 ±0.3
Docosanoic acid	66.4 ±8.9	12.8 ±0.2
Tetracosanoic acid	111.7 ±8.1	46.2 ±1.1
Hexacosanoic acid	80.8 ±2.0	182.2 ±2.8

Octacosanoic acid	9.7 ±0.9	52.1 ±1.9
Triacontanoic acid	259.7 ±9.6	149.9 ±24.9
Dotriacontanoic acid	1111.2 ±16.3	43.8 ±1.8
Tetratriacontanoic acid	333.6 ±9.2	802.8 ±21.3
Total saturated acids	**3276.8 ±203.0**	**2168.4 ±64.4**
Octadecenoic acid	619.3 ±52.4	267.2 ±2.2
Octadecdienoic acid	254.6 ±24.7	185.8 ±2.0
Total unsaturated fatty acids	**873.9 ±77.1**	**452.7 ±4.2**
Hexacosanol	30.2 ±2.1	8.0 ±0.4
Octacosanol	151.6 ±2.5	17.4 ±1.0
Triacontanol	118.9 ±1.1	90.0 ±2.2
Dotriacontanol	1874.8 ±11.8	58.7 ±1.5
Tetratriacontanol	940.4 ±9.5	1726.2 ±30.9
Total Alcohols	**3115.8 ±27.0**	**1900.2 ±36.0**
Campesterol	17.3 ±0.8	16.2 ±0.48
Stigmasterol	132.4 ±5.8	67.0 ±10.3
B-sitosterol	201.0 ±28.6	147.1 ±3.0
Total Sterols	**350.8 ±35.3**	**230.4 ±13.8**
Octacosane	40.1 ±0.4	43.5 ±2.4
Nonacosane	78.4 ±0.6	86.9 ±0.2
Triacontane	163.2 ±0.8	178.1 ±1.9
Hentriacontane	444.3 ±6.8	436.6 ±6.2
Dotriacontane	91.9 ±0.2	77.5 ±1.7
Tritriacontane	79.5 ±5.9	142.0 ±2.3
Total Hydrocarbons	**897.4 ±14.7**	**964.6 ±14.7**
Dotriacontanal	777.9 ±8.7	124.3 ±1.9
Tetratriacontanal	1119.9 ±32.7	244.3 ±24.2

Total Aldehydes	**1897.8 ±41.4**	**368.6 ±26.1**
WE40	153.9 ±3.5	53.6 ±7.3
WE42	171.0 ±2.9	96.5 ±8.0
WE44	63.4 ±0.6	845.0 ±11.8
WE46	136.4 ±0.5	603.7 ±8.5
WE48	830.4 ±3.1	180.4 ±1.5
WE50	375.3 ±2.9	29.6 ±1.3
We51	19.0 ±0.8	267.6 ±9.2
WE52	375.3 ±2.9	62.0 ±0.8
We53	37.0 ±0.6	865.4 ±7.4
WE54	89.0 ±4.8	106.2 ±2.8
WE56	170.4 ±3.6	68.7 ±2.4
Total wax esters	**2421.1 ±26.1**	**3178.61 ±60.9**
Tocopherol	12.0 ±2.1	78.6 ±1.8
2-Pentadecanone-6,10,14-trimethyl	43.1 ±1.0	37.7 ±0.2
Phytol	52.3 ±8.2	41.1 ±0.7

2.6 Scanning electron microscopy imaging for the date palm cuticle wax

Epicuticular waxes exhibit a diverse micromorphology, 3D structures and projections including platelets, rods, and tubules.[177, 178] It is believed that this variety of morphology is caused by self-assembly processes that are mainly, but not exclusively, determined by the chemical composition of the wax.[177-179] For instance, wax tubule is associated with two different chemical and morphological types.[177-179] The first type is predominantly composed of the secondary alcohol nanocosan-10-ol and its homologues as in the lotus leave.[177-179] The second type is associated with β-diketone tubule with hentriacontan-14,16-dione being the major component.[38, 179] Scanning electron microscopy images for the date palm leaf wax are displayed in Figure 2-29. The chemical composition of the date palm leaf wax showed a diverse range of compounds. Therefore, it is hard to assign this structure to a certain group, as such further studies in crystallisation and self-assembly will be needed to form a hypothesis. Figure 2-29 shows the surface of date palm leaf after subjecting to different treatments. It is observed that upon treating the leaf with solvent, the surface wax is removed. Moreover, some physical process such as scrapping the leaf surface with a blade seem to destroy and fully remove these wax structures. This suggests that the wax can potentially be removed without any necessity of using chemical solvents. Carnauba wax which is a product of a palm wax is removed mechanically by beating the dry leaf, before being refined.[59] Milling the leaf also cause the destruction of the wax structure and hence there is a potentially loss of wax yield in this physical process. The milled leaf extracted with $scCO_2$ showed the absence of these waxes structure suggesting that extraction removed these surface waxes.



Whole Leaf Normal

Whole Leaf scratched with blade

Whole Leaf dipped in toluene

Milled Leaf

Milled Leaf extracted with $ScCO_2$

Figure 2-29 SEM imaging showing the cuticle wax of the date palm leaf.

Table 2-13 Summary of the identified and quantified compounds in the date palm waxes in µg g^{-1} biomass.

Compounds	400 bar, 100 °C	400 bar, 40 °C	240 bar, 70 °C	80 bar, 40 °C	80 bar, 40 °C
Decanoic acid	24.4±4.0	2.1±0.0	16.6±0.1	3.7±0.0	0.4±0.0
Dodecanoic acid	54.9±13.2	6.8±0.3	48.4±0.5	10.5±0.1	0.4±0.0
Tetradecanoic acid	26.9±7.9	4.2±0.1	30.5±0.2	14.2±0.1	0.5±0.0
Hexadecanoic acid	787.7±49.7	65.9±0.5	524.1±5.4	7.7±0.2	4.7±0.1
Heptadecanoic acid	44.3±9.6	1.7±0.2	6.2±1.7	0.7±0.1	0.3±0.0
Octadecanoic acid	293.0±46.6	11.7±0.0	97.0±1.3	7.2±0.5	0.8±0.0
Eicosanoic acid	72.5±17.1	3.1±0.0	26.5±1.4	7.3±0.1	0.4±0.0
Docosanoic acid	66.4±8.9	4.0±0.0	32.4±0.6	1.7±0.1	0.2±0.0
Tetracosanoic acid	111.7±8.1	5.7±0.1	46.7±1.7	10.3±0.0	0.6±0.0
Hexacosanoic acid	80.8±2.0	3.3±0.1	33.4±1.7	1.5±0.1	0.2±0.0
Octacosanoic acid	9.7±0.9	1.6±0.0	13.2±0.1	0.4±0.1	-
Triacontanoic acid	259.7±9.6	16.8±0.2	154.3±1.9	1.3±0.1	1.2±0.0
Dotriacontanoic acid	1111.2±16.3	6.4±0.7	144.9±3.5	4.7±0.5	0.6±0.0
Tetratriacontanoic acid	333.6±9.2	1.6±0.0	48.0±3.8	9.6±0.3	3.1±0.0
Total saturated acids	**3276.8±203.0**	**134.9±2.3**	**1222.1±23.9**	**80.84±2.3**	**13.4±0.2**
Octadecenoic acid	619.3±52.4	49.1±0.2	371.4±11.3	32.2±1.6	2.7±0.1
Octadecdienoic acid	254.6±24.7	41.0±0.0	299.4±6.2	23.4±1.4	1.8±0.1
Total unsaturated fatty acids	**873.9±77.1**	**90.1±0.2**	**670.8±17.5**	**55.6±3.0**	**4.5±0.2**

Hexacosanol	30.2±2.1	1.3±0.0	15.6±0.8	0.8±0.0	0.1±0.0
Octacosanol	151.6±2.5	11.4±0.1	96.5±0.5	6.1±0.1	0.9±0.0
Triacontanol	118.9±1.1	12.3±0.1	121.5±0.8	1.4±0.2	0.1±0.0
Dotriacontanol	1874.8±11.8	4.4±0.5	722.4±5.5	6.9±0.1	0.5±0.0
Tetratriacontanol	940.4±9.5	12.1±0.4	336.1±6.3	6.8±0.1	7.2±0.0
Total Alcohols	**3115.8±27.0**	**41.5±1.1**	**1292.1±13.8**	**22.0±0.5**	**8.7±0.1**
Campesterol	17.3±0.8	1.9±0.1	19.8±0.4	0.5±0.1	0.4±0.0
Stigmasterol	132.4±5.8	4.5±0.2	72.5±0.6	1.2±0.2	0.1±0.0
B-sitosterol	201.0±28.6	28.3±0.6	214.0±1.2	1.8±0.1	0.2±0.0
Total Sterols	**350.8±35.3**	**34.7±1.0**	**306.4±2.2**	**3.6±0.3**	**0.8±0.0**
Octacosane	40.1±0.4	37.5±0.3	39.1±5.7	40.1±0.6	1.8±0.0
Nonacosane	78.4±0.6	79.0±0.5	94.5±0.2	129.7±1.7	4.8±0.0
Triacontane	163.2±0.8	139.9±0.5	175.8±0.5	71.3±0.7	6.8±0.0
Hentriacontane	444.3±6.8	297.5±1.2	419.9±1.6	57.4±0.9	14.5±0.0
Dotriacontane	91.9±0.2	39.2±3.0	51.2±0.2	10.5±0.3	2.4±0.0
Tritriacontane	79.5±5.9	52.1±0.3	75.5±0.3	2.1±0.2	4.1±0.0
Total Hydrocarbons	**897.4±14.7**	**645.2±5.7**	**856.1±8.4**	**311.1±4.4**	**34.4±0.0**
Dotriacontanal	777.9±8.7	142.9±0.2	436.1±1.0	23.8±0.3	11.0±0.3
Tetratriacontanal	1119.9±32.7	91.0±0.3	404.5±3.4	27.1±0.5	6.8±0.0
Total Aldehydes	**1897.8±41.4**	**233.8±0.6**	**840.6±4.4**	**50.9±0.8**	**17.8±0.3**
WE40	-	20.6±1.6	23.8±4.3	2.0±0.2	0.8±0.0
WE42	153.9±3.5	29.6±2.4	26.6±3.0	2.4±0.5	1.7±0.0

WE44	171.0±2.9	68.9±0.2	108.4±0.2	6.6±1.2	13.3±0.0
WE46	63.4±0.6	11.2±0.3	23.5±0.8	3.6±0.8	0.8±0.0
WE48	136.4±0.5	210.5±0.7	727.6±1.9	1.4±1.1	8.0±0.0
WE50	830.4±3.1	9.1±0.9	27.9±0.3	1.0±0.4	1.9±0.0
We51	19.0±0.8	188.8±1.1	425.3±10.9	0.4±0.7	0.2±0.0
WE52	375.3±2.9	49.7±0.3	11.6±0.9	0.9±0.4	1.7±0.0
We53	37.0±0.6	17.0±1.6	85.2±1.2	0.9±0.0	0.4±0.0
WE54	89.0±4.8	-	5.2±0.5	1.8±0.0	4.5±0.0
WE56	170.4±3.6	-	42.5±0.3	-	5.2±0.0
Total wax esters	**2421.1±26.1**	**605.1±9.0**	**1507.7±24.3**	**38.4±0.1**	**`21.0±5.1**
Tocopherol	12.0±2.1	0.8±0.2	6.7±0.1	0.3±0.1	-
stigmata 3,5 diene	12.5±0.6	0.9±0.0	15.1±0.2	2.6±0.1	0.1±0.0
2-Pentadecanone-6,10,14-trimethyl	43.1±1.0	37.4±0.1	40.7±0.2	55.8±1.4	0.7±0.0
Phytol	52.3±8.2	4.0±0.1	31.3±2.4	2.8±0.2	0.7±0.0

2.7 Conclusion

$ScCO_2$ extraction of waxes from date palm leaf has been successfully carried out to obtain potentially high value compounds from this waste biomass. The extractions were carried out at different extraction temperatures and pressures to find out the best extraction conditions where maximum yield is recovered at 400 bar and 100 °C. A full characterisation and quantification of the volatile compounds within the wax extracts were carried out. Results showed similar functional groups in all the extracts, however the abundance of these compounds differed significantly depending on the extraction conditions. Different extracts exhibited different melting point profiles, ranging from 30 °C to 79 °C, which suggests along with the quantification data that the wax could have a potentially wide range of applications. There was a significant difference in the chemical composition of the wax extracted by $scCO_2$ extracted and Soxhlet heptane extracted wax. The $scCO_2$ extract showed greater selectivity over heptane extract with regard to compounds such as fatty alcohols and aldehydes besides providing different wax types both in texture and melting profile.

The SEM images showed that the wax structure on the leaf surface was randomly distributed tubules. Treating the leaf mechanically destroy these structure while chemical treatments remove the wax from the surface. Future work could examine the mechanical extraction of waxes from date palm leaf to avoid the use of any solvent.

Chapter 3

Comparison of date palm wax based oleogels and other commercial waxes oleogels

3. Chapter 3 Comparison of date palm wax based oleogels and other commercial waxes oleogels

3.1 Introduction

Over the past decades, demand for healthier food products by consumers and policy makers has increased.[180-182] *Trans* fatty acids and partially hydrogenated oils (PHO) are widely used as a hard stock fat or structuring agent in food industries to impart desirable functional and structural properties.[180, 181, 183-185] However, many studies have associated the excessive consumption of *trans* fatty acids and PHO to increased risk of cardiovascular diseases.[181, 185] The US FDA announced that PHO will be removed from the generally regarded as safe (GRAS) list of substances by June 2018.[184, 186] This has forced the food industry to seek alternatives to PHO.[184, 186]

One of the approaches used currently in food industries is the use of crystalline triglyceride (TAG)molecules to structure the edible oil.[187, 188] Usually the high melting TAG is dispersed in hot liquid oil, which upon cooling, forms crystals that result in gelling of the liquid oil caused by the formation of a 3D network structure.[187] However, TAG molecules are rich in saturated fat, which are proven to be unhealthy for cardiovascular system.[184, 185, 187] Apart from that, a high concentration of TAG is needed in order to gel the liquid oil (≥20 % *w/w*).[187] Therefore, it is crucial to find alternative approaches in structuring oils, which reduce the amount of saturated fatty acids, or even completely eliminate them and replace them with unsaturated fatty acids to improve the nutritional value.[184, 185]

Organogelation or oleogelation has attracted attention as a promising approach for structuring liquid oil/solvent into solid-like materials.[183-186, 189, 190] Organogels are semi-solid systems which consist of liquid oil/solvent (>90%) and solid building blocks (gelators), which form three-dimensional network and trap the liquid oil.[183, 184, 186, 189-191] Certain physiochemical properties are vital for the gelator molecule (building block) to be able to gel liquid oil or solvent, such as: affinity to the oil, self-assembly properties and thermo-reversibility.[189] These building blocks form by the self-assembly of either crystalline materials (fatty acids, fatty alcohols, wax esters, natural waxes, mono glycerides, di glyceride or TAG) or low molecular weight compounds (fibres, tubules or strands) or by polymeric materials, as in Figure 3-1.[189]

Polymeric gelators immobilize the liquid oil by a network of entangled chains (physical interaction) or cross-linked chains (chemical interaction) Figure 3-2.[189, 192] While the self-assembly in crystalline and low molecular weight gelators is initiated by physical interaction established by gelator-gelator molecules and may be further stabilized by some forces including hydrogen bonding, van der Waal forces or π-stacking.[189, 192]

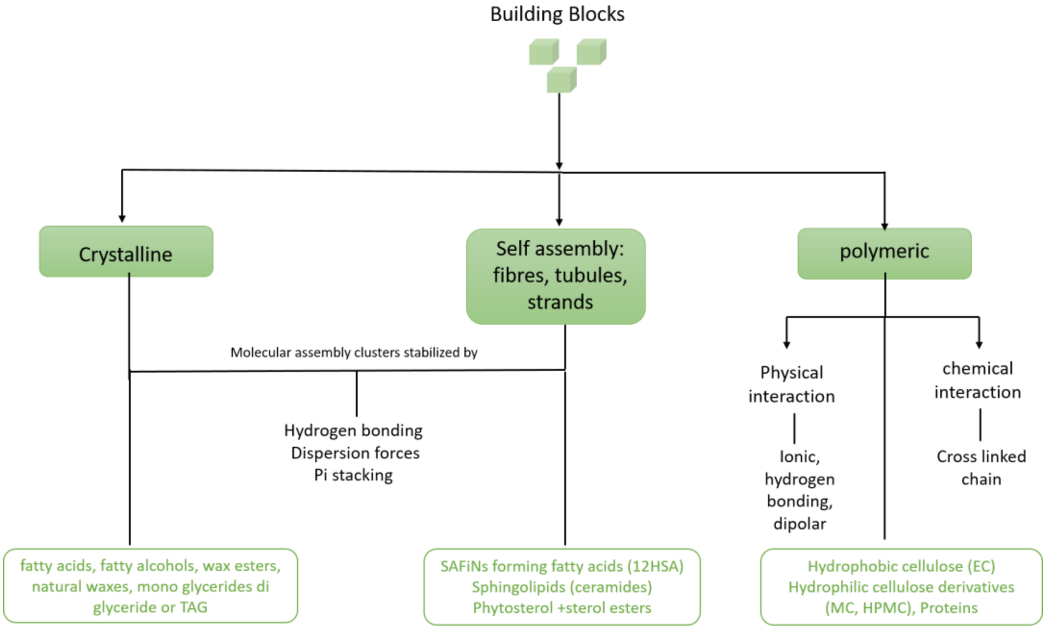


Figure 3-1 Types of building blocks (gelator) for structuring organic oil and the interaction involved in imparting the gelation properties.

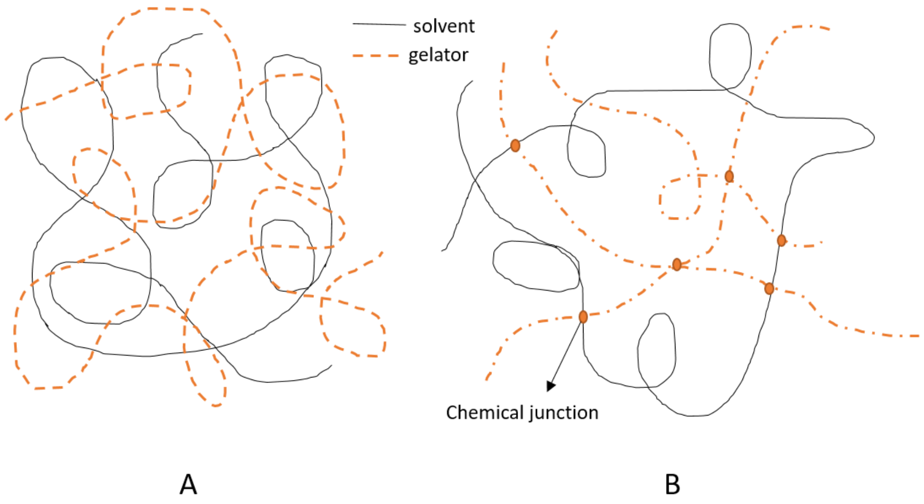


Figure 3-2 (A) Physical entanglement and (B) chemical cross linking in polymer gels.

Plant waxes have gained a considerable attention regarding structuring vegetable oils forming oleogels.[169, 188-191, 193] Natural waxes are a complex mixture of hydrophobic compounds including varied long chain length *n*-alkanes, free fatty acids, fatty alcohols and wax esters.[169, 193, 194] Upon cooling a dispersion of melted wax in organic non-polar solvent below the wax melting point, a crystalline phase starts aggregating and further interlinking to form a 3D structure that traps the liquid oil (no-polar solvent), thus forming a gel.[183, 189] There is an extensive research effort regarding the use if natural waxes as gelators for liquid oils.[184, 187, 190, 191, 193] This is owed to the fact they are abundant and that some natural waxes are approved as GRAS for food applications such as carnauba, candelilla and rice bran wax.[190, 193] Moreover, only small amount of these waxes can gel the oil, typically 1-4%, which makes it even more appealing for gelling vegetable oil.[190, 193] Wax based oleogels showed excellent oil binding capacities besides some interesting properties including thermo-reversibility between the liquid and gel state which further justify the increasing ongoing research to characterize them.[190, 193]

Among the first investigated waxes for gelling vegetable oil was candelilla wax. Candelilla wax is a FDA food approved additive.[195, 196] Toro-Vazquez *et al.* prepared organogels from candelilla wax and sunflower oil which showed thermo-reversible behaviour and was independent on the cooling rate or gelling temperature.[196] Dassanayake *et al.* compared rice bran wax with candelilla and carnauba waxes oleogels and noted high rates of gel formation and better thermal stability for rice bran wax oleogel.[197] Patel *et al.* characterised the rheological behaviour of organogels prepared from natural waxes and high oleic sunflower oil and showed that the chemical composition and crystal morphology have effect on the rheological behaviours.[193]

One of the key characterization techniques for wax-based oleogels is the rheological measurements. Rheology is defined as the science of deformation and flow of the matter when subjected to force.[198-200] Rheological data are very important in many stages of product development including process engineering to formulation to the stability and the performance of the product in industries such as food, biopharmaceuticals and polymers.[198, 200-203] An ideal Newtonian fluid will flow and its viscosity (the resistance to flow) is independent upon shear rate.[198, 200] Therefore, Newtonian fluids are directly related to shear stress (σ) and shear rate $(\dot{\gamma})$ as shown in Figure 3-3. On the other hand, an ideal Hooke elastic will not flow but will deform with a direct relationship between stress(σ) and strain (γ) as shown in figure 3-3.[198, 200]

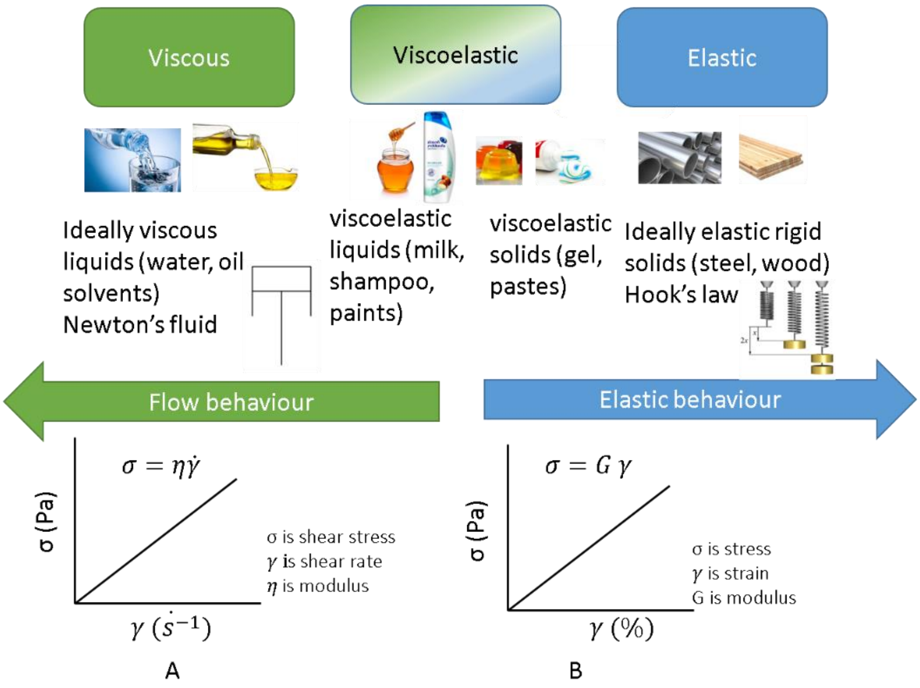


Figure 3-3 Classification of the materials based on their flow and deformation behaviour.

In between these two extremes come the viscoelastic materials that exhibit both solid-like behaviour and will flow (liquid-like behaviour) when subjected to shear force.[200, 202] Many mathematical models developed to explain the behaviour of the viscoelastic materials. Herschel–Bulkley model is one of the most commonly used model for materials that show non-Newtonian behaviour after yielding Equation 3-1:

$$\tau = \tau_y + K\dot{\gamma}^n \qquad \textbf{Equation 3-1}$$

Where τ is the stress, τ_y is the apparent stress yield, $\dot{\gamma}$ is the shear rate, K is consistency factor and n is flow index. The value of n is usually used to define shear thinning ($n < 1$) or shear thickening ($n > 1$) behaviour.

Rheological properties can be quantified and measured using an instrument called a rheometer.[200, 202] The rheometer will measure the flow of a sample upon applying deformation forces, which can either be as strain or shear rate or shear stress. The deformation manifests as either viscous flow, elastic deformation or combination of the two. Depending on the target of the rheological test, one can decide the appropriate rheometer/rheological tests for the analysis.[202, 204] For instance, the flow behaviour of the materials in a process can be predicted or the materials can be characterised without damaging the structure.[202, 204] The former is important in product delivery such as the flow of a liquid through a pipe while the later might be more relevant in early formulation of the product. [202, 204]

Dynamic or small amplitude oscillatory measurements are very useful for semi-solid and food samples.[202, 204] Indeed, it provides structural, time dependency as well as temperature stability information.[202, 204] It is a non-destructive technique as a small amount of strain is applied to the sample to generate information about the viscoelastic properties for the sample.[193, 202, 204] Usually the sample is subjected to sinusoidal small amplitude deformation in a shear field. Measuring the viscoelastic samples flow and deformation properties aid in their classification into gels (weak or strong) or viscous solids.[193, 202, 204]

Frequency-dependant functions G' and G" offer a measure of solid-like and liquid-like properties respectively.[193, 202, 204] Storage modulus (G') is the elastic component, which indicates the stored energy, while loss modulus (G") is the viscous component, which indicate the energy dissipated through generated heat. [193, 198, 200-202, 204] For instance, for gels the elastic components (G') dominate over the viscous (G") at low shear and reaches a plateau G'_{LVER} in the linear response region known as viscoelastic region (LVER) (Figure 3-4).[193, 198, 200-202, 204] The end of LVER is corresponds to the point where G' values vary by 10% from the G'_{LVER} values.[193, 198, 200, 204] Moreover, the stress at this point is referred as critical stress (τ*) and any further increase in applied shear results in sample deformation and phase change at the crossover point. The crossover point is the point at which G'=G", and corresponding stress value defined as dynamic yield stress (τ_{dy}) (Figure 3-4).[193, 198, 200, 204] AT the critical stress (τ*) the structure breakdown occurs while dynamic yield stress (τ_{dy}) represent the transition from solid to liquid. [193, 198, 200, 204]

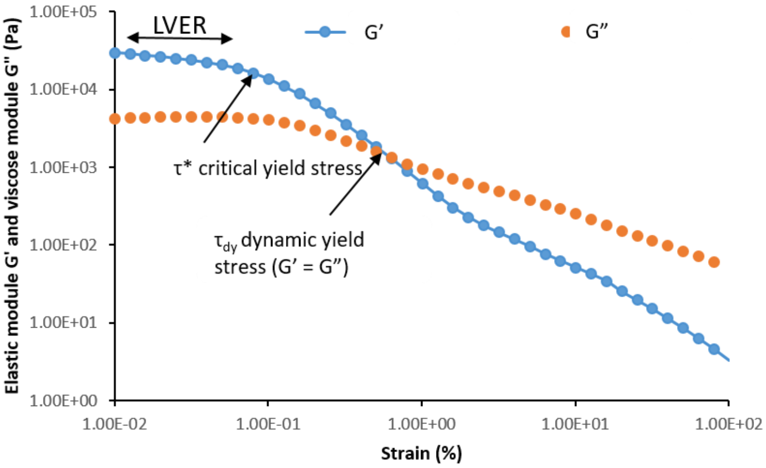


Figure 3-4 An Example of an amplitude sweep test for a gel.

A wide range of current and potential applications of organogels have been reviewed extensively in the literature.[192, 205] For instance, current applications include the selective phase recovery for the crude oil spill, gelation of flammable solvents and safe disposal of the cooking oil.[192, 205, 206] Moreover, they are used in cosmetics and personal care products to impart structure to non-polar components.[192, 205, 206] In the pharmaceutical industry, organogels can be a mean of drug delivery.[207]

As the application of organogels based on vegetable oils (oleogels) is explored in this chapter, it is crucial to point to some of the current attempts to use these organogels and more specifically oleogels in replacing the trans-fat and PHO in confectionary and food industry.[186, 187, 190] For instances; candelilla wax based oleogels in canola oil, which has been claimed to be rich in unsaturated fatty acids, were used as shortening agents for cookies,.[195] However, maintaining the desired physical properties in the final baked product is challenging.[195] Cookies prepared from sunflower wax and beeswax showed comparable texture and compositional properties compared to regular cookies (made with commercial shortening agent).[208] Moreover, the authors claimed the positive attribute to the wax based cookies were equal or better than the regular cookies, based on the consumer hedonic score.[208] Rice bran, sunflower and candelilla waxes based oleogels were tested in making margarine where the sunflower wax showed promising results in terms of the firmness and stability compared to rice bran and candelilla waxes.[171]

In this chapter, date palm wax extracted with $scCO_2$ at 400 bar and 100 °C has been tested as a structuring agent for liquid vegetable oil to form oleogels. It is crucial to compare date palm wax along with other commercial waxes to gain a better insight of its performance compared to other natural waxes. Therefore, the date palm wax was compared with other waxes at their critical gelling concentration and all subsequent measurements and discussion refer to the waxes at their critical gelling concentration.

3.2 Critical gelling concentration

Critical gelling concentration (C_g) is defined as the minimum concentration required to gel a solvent[183, 193, 194] In order to find the C_g, a series of oily dispersions were prepared with different wax concentrations in sunflower oil. The oily dispersions were heated to 90 °C to dissolve the waxes, and subsequently cooled to room temperature and then further to 5 °C in the fridge for 24 hours. The gelation was simply observed by inverting the vials to identify the ranges where the gel will not flow under gravitational forces. Table 3-1 lists the critical gelling concentration for date palm wax along with commercial waxes including rice bran, sunflower, candelilla and carnauba waxes in sunflower oil.

Table 3-1 Table Critical gelling concentration for date palm leaf wax (extracted with $scCO_2$ at 400 bar and 100 °C) and commercial waxes.

Type of waxes	Abbreviation of the wax	Abbreviation of the wax based oleogel	Critical gelling concentration (C_g)	Literature reported gelling concentration	References
Date palm	DW	SFDW	2%	-	Current study
Candelilla	CL	SFCL	1%	1-2%	169, 193, 194, 197
Carnauba	CR	SFCR	5%	4-5%	169, 193, 194, 197
Sunflower	SF	SFSF	4%	0.5-1%	169, 193, 194, 197
Rice bran	RB	SFRB	2%	1-2%	169, 193, 194, 197

The minimum gelling concentration of the date palm wax was 2% (*w/w)* in sunflower oil (SFDW2% used thereafter to refer to date palm wax-based oleogel at critical gelling concentration). Although lower concentration 1% formed a gel, it showed a phase separation upon storage for 1 week. The gelling concentration for the commercial waxes were 1% for candelilla wax (SFCL1% used thereafter in the discussion to indicate the candelilla wax based oleogel), 2% for the rice bran wax (SFRB2% used thereafter to refer to rice bran wax based oleogel), 4% for sunflower wax (SFSF4% used thereafter to refer to sun flower wax based oleogel) and 5% for carnauba wax (SFCR5% used thereafter to refer to carnauba wax based oleogel). In this study the critical gelling concentration for sunflower wax was relatively high (compared to literature sources) which can be owed to the presence of impurities as discussed later. In general, the reported values in the literature varies depending on the type of vegetable oil used, as well as the grade and purity of the waxes.[169, 193, 194, 197] For instance, the critical gelling concentration of sunflower wax in soybean oil and high oleic sunflower oil was only 0.5% while that of candelilla wax was from 1-2%.[169, 193, 194, 197] Moreover, carnauba wax was deemed with critical gelling concentration from 4-5% in soybean oil and sunflower oil.[169, 193, 194] Rice bran wax is also reported among the waxes which gel vegetable oil at low concentration in the range 0.5-1%.[169, 197] It can be noted that date palm forms a stable gel at 2% which is midway with respect to other commercial waxes. This further shows that date palm leaves wax have an advantage in requiring less amount of wax to form, the gel compared to carnauba and sunflower waxes. Figure 3-5 shows the physical appearance of the oleogels prepared from date palm wax and other commercial waxes at critical gelling concentration. In terms of physical appearance, candelilla and date palm waxes formed opaque gel with no suspensions, whereas the other waxes formed a suspension gel. Since crude date palm wax was used with no further purification, it resulted in a yellowish gel. It would be interesting to explore different methods to remove the colour and impurities from date palm leaves wax in future studies, as colour is a very important factor in many foods. Most commercial waxes undergo bleaching and purification steps to improve the colour, with some common practices using hazardous chemical including sulphuric acid and hydrogen peroxide.[63]

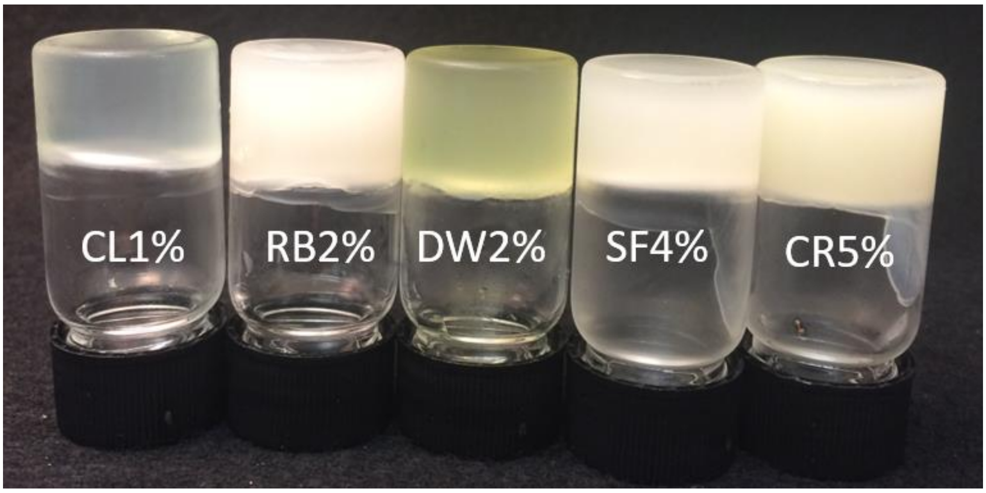


Figure 3-5 Oleogels prepared from natural waxes in sunflower oil at their corresponding critical gelling concentration.

In order to form a gel, it is believed that the gelator must not be too soluble nor too insoluble in a solvent to obtain the balance of gelator-gelator interactions and gelator-solvent interactions.[188, 189, 191, 197, 209] Insoluble gelators will precipitate out while a highly soluble gelator will interact strongly with solvent, preventing gel formation, as the crystals will lose the ability to reorganise in a network.[188, 189, 191, 197, 209] It can be noted from the critical wax concentration in sunflower oil that the wax solubility is in the following order in sunflower oil CL < RB, DW < SF < CR. Assuming all other factors are identical, such as crystal size and shape, one can judge the solubility of the waxes in sunflower oil. As noted, higher concentration of carnauba wax indicated its high solubility in sunflower oil while the lower concentrations were required from candelilla wax. Therefore it has lower solubility in sunflower oil. However, there are many other factors contribute to the critical gelling concentration including type of the vegetable oil, wax morphology, which is governed by chemical composition.[188, 189, 191, 197, 209] It is therefore crucial to characterise different waxes to truly understand the effect of their composition on the gelation properties.

3.3 Chemical composition of all waxes

The ability of the natural waxes to gel vegetable liquid oil is owed primarily to their chemical composition, which also affects the crystal morphology.[188, 194, 210] Considering that most of these natural waxes originate from different species, no two waxes are identical in their chemical composition. Other factors such as harvesting season, purification and processing steps are also important.[188, 194, 210] Therefore, it is vital to identify the chemical composition to understand wax behaviour in gelling the vegetable oil. Figure 3-6 summaries the chemical composition of the waxes explored in this study, including date palm (DW used thereafter in the text and similarly to other waxes abbreviation used), candelilla (CL) , carnauba (CR), sunflower (SF) and rice bran (RB) waxes.

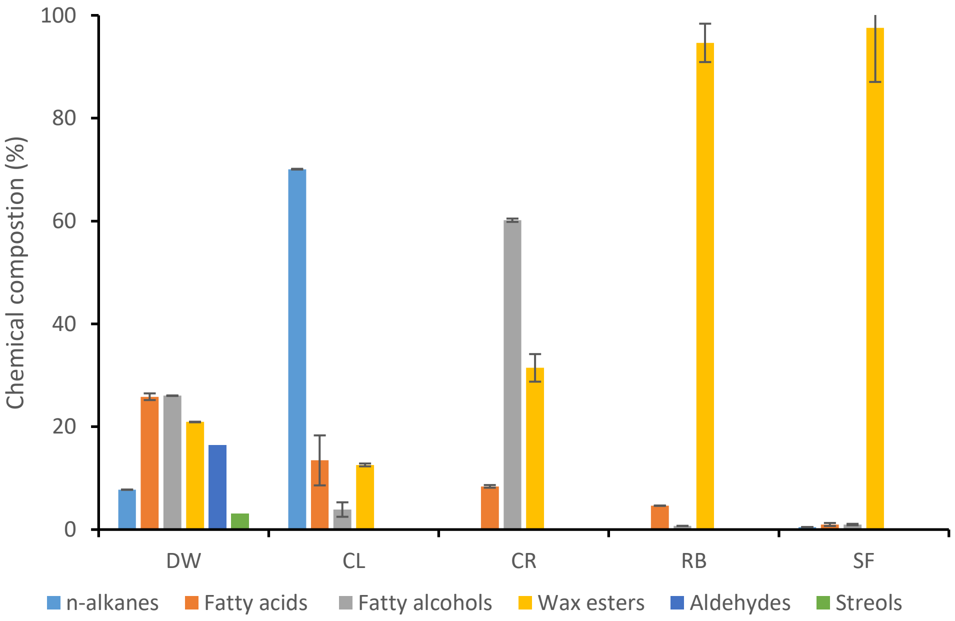


Figure 3-6 Percentage chemical composition of the natural waxes identified by GC-FID and GC/MS (DW: date wax, CL: candelilla wax, CR: carnauba wax, RB rice bran wax, SF sunflower wax).

As noted from Figure 3-6 that some waxes including rice bran and sunflower were predominantly wax ester with more than 90% of the wax composition. This is in agreement with the reported composition of SF and RB waxes. Doan *et al*. reported the chain length of the wax esters in SF (C_{44}-C_{50} range) and in RB (C_{40}-C_{48}).[194] It is believed that wax esters are the key compounds responsible for the gelation properties of natural waxes. Hwang *et al.* showed that saturated long chain wax esters, as major component in wax is preferred in gelling rice bran oil over unsaturated wax esters and short alkyl chain.[169] Indeed, a smaller quantity of longer chain wax esters showed better gelling ability than a greater quantity of short chain wax esters.[169]

Wax esters are not the only component responsible for the gelation, and sometimes the presence of other components aids gelation. For instance, candelilla wax is composed mainly of hydrocarbons (70%) with C_{31} is the most prominent chain length. Candelilla wax also contains wax esters and fatty acids. Nevertheless, candelilla wax is among the first investigated waxes in gelling vegetable oil and it showed an ability to gel the vegetable oil at relatively low concentrations (1-2%), with interesting properties.[169, 196] The balance between the major to minor components as well as the effect of saturated alkyl chain length may be responsible.[169, 196]

The other waxes studied showed a relatively more heterogeneous mixture, with two or more type of compounds co-existing in considerable amount. Surprisingly, carnauba wax was found to be composed of 60% free fatty alcohols and 30% of wax esters, while the reported values in the literature suggest that a composition of 60% wax esters and 30% of alcohols.[194] This difference might be owed to the grade and purity of the wax with most of the corresponding literature not reporting the grade of the wax. Also, the waxes were analysed in this study by high temperature GC and GC-MS which is limited by the volatility of the compounds and therefore higher molecular weight compounds such as wax esters or disasters are not detected.[169, 210] Moreover, GC-FID detected only wax esters up to chain length 60-62, which suggests that further analytical techniques need to be considered to allow the analysis of higher molecular compounds such as high performance liquid chromatography (HPLC) coupled with advanced mass spectrometry technique like Matrix Assisted Laser Desorption/Ionisation (MALDI) .

Carnauba wax also contains cinnamic diesters which are derived from cinnammic acid and long chain alcohols.[169] Hwang *et al.* concluded that *p*-hydroxycinnamic aliphatic diesters are not as good at gelling the liquid oil as the straight chain saturated wax esters.[169] Also the presence of alcohols, which have the ability to form hydrogen bonds, could therefore make the wax more soluble in the vegetable oil.[169, 211]Dodrick and Zhu concluded that in order to achieve good gelation there should be minimal interaction between the solvent and the gelator molecule.[211] The -OH group in the wax will hydrogen-bond with the OH group in the solvent and solubilise it. Hence, higher concentrations are required with carnauba wax to form the gel.[211] This also effects the rheological profile, as will be discussed in section 3.7.

Date palm wax showed the most diverse chemical composition with equal composition of wax esters (C_{40}-C_{56}) alcohols (C_{32}-C_{34}), fatty acids (C_{10}-C_{34}) and aldehydes (C_{32}-C_{34}). Date palm wax also contained sterols and n-alkanes. The diverse chemical composition of date palm wax is owed to fact that most of the commercial waxes undergo many purification or bleaching processes, while the date palm used in this study used as crude wax with no further purification.[211]The interesting gelling ability for date palm wax may be attributed to balanced gelator-gelator interactions as well as gelator-solvent interaction. Moreover, date palm leaves wax have very long alkyl chains that might contribute to imparting the gelling properties. The true mechanism involved in the formation of the gel with natural waxes is complex due to the complexity of the mixture and its interaction with the vegetable oil as well as the crystal morphology and mechanism of self-assembly.[189, 191, 210]

Natural waxes are an extremely complex mixture making the determination of the exact composition challenging apart from other reasons including the hydrophobic nature, low polarity and low solubility in most organic solvents.[210] In this study, GC and GC-MS were used to analyse the waxes. However, many higher molecular compounds such as long chain wax esters have low volatility and therefore limit the effectiveness of the GC method. Recently, Doan *et al.* reported an updated technique in analysing natural waxes where preparative high performance liquid chromatography (HPLC) was used as the first step to separate the waxes into main fractions.[194] These fractions were subjected to GC-MS to identify the specific composition.[194] The authors reported a good resolution for compounds including free fatty acids, hydrocarbons and fatty alcohols.[194] However, the wax esters content was reported as separate fatty acids moiety and fatty alcohols moiety, as saponification was required in order to identify this fraction.[194] Therefore, a true composition of wax ester is limited as it is difficult to predict which acid moieties are bonded to which alcohol. Harron *et al.* developed a method of analysing waxes using reversed phase HPLC and they successfully analysed sorghum, carnauba and jojoba waxes without any derivatisation or using a special column.[212]

3.4 DSC analysis of waxes and their corresponding oleogels

Differential scanning calorimetry (DSC) analysis was performed for the neat waxes and their oleogels to characterise the phase transition behaviour. Many oleogels have potential applications in confectionary as discussed above in section 3.1, so knowledge of their phase transitions, including crystallisation and melting behaviour, is important in order to control the processing and storage conditions.[196, 209] The thermal analysis results combined with other key analysis like X-ray diffraction can provide insight into the textural and mouthfeel sensation.[196, 209]

Figure 3-7 displays the crystallisation and melting profile of neat waxes and their corresponding oleogels at critical concentration. All the waxes and their gels melted and crystallised over broad temperature ranges. The melting profile is similar to the crystallisation which indicated that the waxes underwent polymorphic phase change and/or the different components retained their packing behaviour upon cooling.[209] Rice bran wax showed a single crystallisation and melting peak at 69.6 (±0.1 °C) and 77.1 (±0.4 °C) respectively, indicating the homogenous nature of the wax with the peaks corresponds to the melting and crystallisation of a single component or type of compounds. The enthalpy of crystallisation of the neat wax (184.9±0.9 kJ/mol) is almost similar to the melting enthalpy (179.2±1.2 KJ/mol).

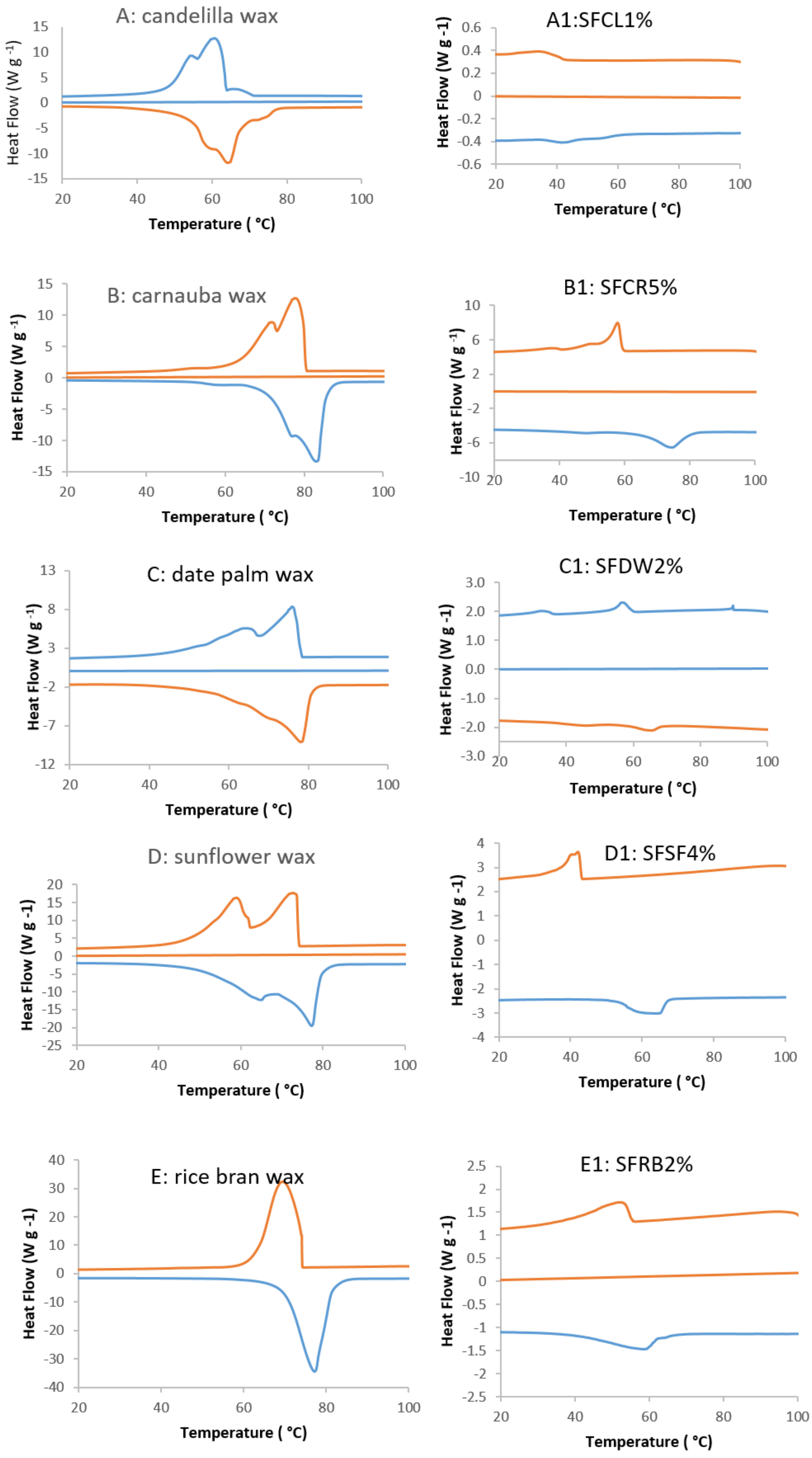


Figure 3-7 Thermograms of crystallization (exothermic) and melting (endothermic) for natural waxes and their gels at critical gelling concentration.

In section 3.3 the chemical analysis for the rice bran wax revealed more than 97% of the wax composed of wax ester. Therefore, this high purity can be attributed to the crystallisation and melting of the wax esters. The dilution effect resulted in the temperature shifting to a lower value, depressing the thermal event, and broadening the corresponding peaks of crystallisation at 52.7 (±0.7 °C), and melting at 59.7 (±1.3 °C). The crystallisation and melting of the SFRB2% required less energy than the corresponding neat waxes which can be observed in enthalpy values in Table 3-2. Similar trends were observed for rice bran wax in canola oil and rice bran oil.[188, 194]

Although sunflower wax is considered a homogenous wax with a chemical composition dominated by wax esters (<93%), it can be seen from Figure 3.3 that double endotherm (~64 °C and 77 °C) and exotherms (~59 °C, 73 °C) were observed, which provides further evidence to suggest the presence of impurities. The literature reported value for sunflower wax melting point is in the range 76-79 °C.[63, 193, 209] The difference might be owed to the presence of additives as many commercial waxes undergo refining and processing, which includes adding additives such as plasticizer.[63] This also shows the effectiveness of DSC as a tool for the characterization of adulteration in natural waxes. The dilution effect in the sunflower wax-based oleogel (SFSF4%) was similar to SFRB2% with endotherms and exotherms moving to a lower temperature. The thermogram of the SFSF4% showed a major thermal event at 63.9 (±0.3 °C) in the melting profile, and at 41.7 (±0.6 °C) in the crystallization profile. It is believed that because of the dilution the peak corresponds to the most dominant component in the wax composition.[169, 209] The enthalpy values for the SFSF4% is much lower than the neat wax due to the effect of the dilution and therefore less energy is required for the gel compared to the neat wax.

Candelilla and carnauba waxes are more heterogeneous and hence have multiple broad thermal events in their crystallization and melting profile. The dilution effect in SFCL1% is very obvious with melting and crystalizing points shifting drastically to lower values with the shape of the curve almost liners. This might be owed to the fact the only 1% of the wax is present in the SFCL1% compared to other oleogels who showed more distinct thermal profiles. Carnauba wax also showed very broad thermal events and double peaks endotherms and exotherms corresponding to crystallisation and melting of various chemical components. Because of high critical concentration in the SFCR5%, one can see very distinct thermal events shifting to lower temperature due to dilution. Candelilla and carnauba waxes' oleogels showed similarly trends in high oleic sunflower oil, canola oil and rice bran oil.[193, 194, 209]

Table 3-2 Characteristic melting and crystallization properties for natural waxes and their oleogels.

Waxes	**T_{cry}**	**T_m**	**ΔH°_{cry} (kJ/mol)**	**ΔH°_{m} (kJ/mol)**	**Oleogels**	**T_{cry}**	**T_m**	**ΔH°_{cry} (kJ/mol)**	**ΔH°_{m} (kJ/mol)**
Candelilla	60.7±0.7	64.3±0.5	138.2±1.6	135.3±2.3	SFCL 1%	33.3±0.6	42.5±0.17	0.18±0.0	0.1±0.0
Rice bran	69.6±0.1	77.1±0.4	184.9±0.9	179.2±1.2	SFRB2%	52.7±0.7	59.7±1.3	2.8±0.2	2.0±0.2
Date palm	79.1±0.9	81.92±0.7	125.5±4.6	129.2±3.8	SFDW2%	58.7±0.4	65.7±0.2	0.8±0.0	0.9±0.0
Sunflower	72.6±0.2	77.3±0.6	154.2±1.1	157.3±1.9	SFSF4%	41.7±0.6	63.9±0.3	3.7±0.1	1.9±0.2
Carnauba	77.7±0.8	83.0±0.8	138±2.2	152.9±1.4	SFCR5%	60.1±4.6	74.7±1.6	5.5±0.2	5.6±0.1

Finally, the date wax used here was a crude wax and therefore multiple thermal peaks are observed in the crystallisation and melting profile in Figure 3-7. Date palm leaf wax was the most diverse in term of chemical composition and the double peaks in the crystallisation and melting profile therefore correspond to different components including fatty alcohols and wax esters. In SFDW2% thermal events corresponding to the crystallisation and melting shifted to lower temperature and broadened significantly because of dilution effect. The enthalpies of crystallisation and melting also shifted to much lower values compared to neat wax.

Overall, all waxes and their corresponding oleogels showed similar crystallisation and melting profiles. The melting point for the wax-based oleogels from highest to lowest are as follows: SFCR5%, SFDW2%, SFSF4%, SFRB2% and SFCL1%. Generally higher concentration of crude wax will increase the melting point for the corresponding gel. Doan *et al.* showed that increasing the concentration of the berry and sunflower waxes in their corresponding oleogel resulted in an increase of the melting and crystallisation points.[209] It is crucial to remember that waxes here have been studied at the critical gelling concentration, therefore it is difficult to conclude the effect of the concentration. However, it is interesting how date palm at only 2% showed higher melting point compared to other waxes which required higher concentration including carnauba and sunflower waxes.

3.5 Oil binding capacity

Oil binding capacity is defined as the ability of a system to retain its bonded liquid oil.[188, 213] It is believed that there are two mechanisms involved in bonding the liquid oil within a crystalline network illustrated in Figure 3-8.[188, 213] The first involves entrapment of the liquid oil due to capillary forces within the surrounding physical network, while the second involves adsorption of the liquid oil onto the solid surface of the crystal.[188, 213] The oil binding capacity is usually measured by gravimetric techniques to induce oil release from the gel.[188, 213] These techniques includes gravitational, centrifugal and capillary forces, which release the oil by dripping, phase separation and wicking respectively.[188, 213]

Oil binding capacity can be determined by two approaches, the first approach is to evaluate total oil exuded over specific time which is usually referred as 'long term oil binding capacity'; the second approach measures how quickly the oil exudes and is referred as short term oil binding capacity.[188, 213] Both short and long term oil binding capacities provide valuable information regarding the application of the oleogel. For example, long term oil binding capacity is useful in a situation involving storage and long term stability, where low exuded oil is desirable.[188, 213] Short term oil capacity is more appropriate if immediate processing is required where it would be desirable to have slow exudation of the oil.[188, 213]

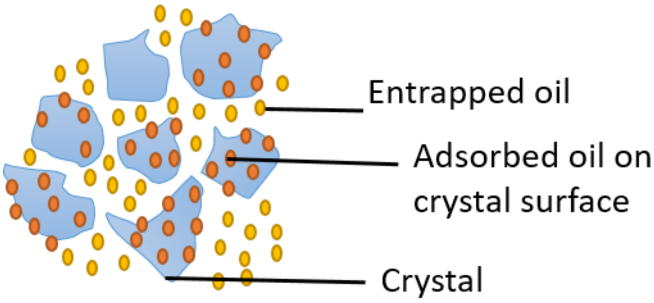


Figure 3-8 Oil binding mechanism within a crystalline network.

Figure 3-9 shows the oil lost for all wax oleogels measured after the first 15 minutes and after 60 minutes. Both of these are considered as short term and provide information on how strong the oil is bonded to the wax crystals with fast oil loss indicating poor oil binding capacity. Examining Figure 3-9 the order of exuding oil from the gel is in the following order of increasing mass: SFCL1%, SFSF4%, SFRB2%, SFDW2% and SFCR5%. Therefore, SFCR5% has the weakest oil binding capacity in the short term. Moreover, it releases the oil very fast indicating that most of the oil is entrapped in the pore of the crystals. SFDW2% showed slightly weak oil binding capacity but better than carnauba wax, with 10% of oil exuded after 15 minutes, compared to carnauba with 25% exuded after 15 minutes. Chemically, both waxes showed a heterogeneous composition, so the oil binding ability must be owed to other factors such as the crystal size and shape, as discussed in section 3.6.

SFRB2% showed a moderate oil loss with improved oil binding capacity over both carnauba and date palm waxes. On the other hand, SFCL1% and SFSF4% showed the strongest oil binding capacity on short term and hence the minimum oil loss observed in these two oleogels. This indicates that the dominant mechanism is adsorption to the crystal surface. Blake and Mongmery studied oil binding capacity of the natural waxes at their critical gelling concentration in in canola oil.[188, 213] They noted the following order, in increasing oil binding capacity: CL2% > CL1% >CR4% > SF1% >RB1%, with candelilla showing the highest ability to retain the oil and rice bran wax showing the weakest oil binding.[188, 213] The differences in results might be owed to the grade of waxes used as well the type of vegetable oil used. Oil binding behaviour results from the affinity of the oil to the wax crystal due to the molecular interaction between them, hence it would be interesting to explore this aspect in future study.[214] Crystal morphology shown to have a great effect in the oil binding capacity, therefore next section 3.6 investigate the crystal shape on the oil binding capacity and gelling properties.[188, 213] Due to time constrains long-term oil binding capacity was not studied.

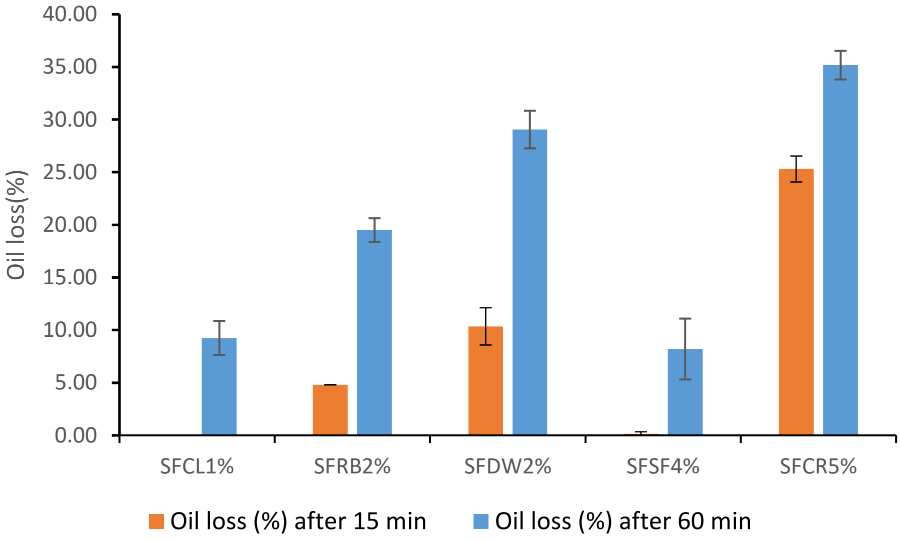


Figure 3-9 Percentage of oil loss from wax oleogels after 15 min and 60 minutes.

3.6 Crystal morphology

It is believed that the morphology of the wax crystal network plays a significant role in determining the physiochemical properties of the wax oleogels.[188, 210, 213] Therefore, it is important to acquire a basic understanding of the crystal shape that can explain their corresponding gel functionality.[188, 210, 213] The most common technique used to study wax morphology is optical light microscopy, taking advantage of its ability to differentiate between solid and liquid phases.[188, 210, 213] Furthermore, the wax crystal appears bright in contrast with the dark liquid oil phase.[188, 210, 213] Figure 3-10 shows the optical light microscopy for date palm and other commercial wax oleogels at the critical gelling concentration, with the exception to candelilla wax, as the crystals were not visible in critical gelling concentration. It was observed that different wax displayed different crystal morphology. For instances; RB2% displayed a needle-like crystal structure with large dimensions. Similar morphology is reported for the rice bran wax oleogels, and it is believed that needle like structure is desirable in the formation of the gel.[197] It is thought that needle-like crystal morphology forms a mesh at the inter-crystalline phase forming a good gel and therefore enabling entrapment of the oil in the scaffolding of the crystal.[195, 197] A greater gelation efficiency is usually observed for waxes with a highly homogenous composition due to greater compatibility between crystals.[195, 197] Studies showed that the co-existence of two or more types of crystals might have synergetic effect on the crystal packing and hence the gel properties.[209, 215] Rice bran wax contained more than 90% of wax esters and hence rice bran wax showed good oil binding capacity. Date palm oleogel was observed to have a needle-like or platelet-like structure with a relatively irregular arrangement and a larger crystal size than that of the rice bran wax. This can be further explained by the heterogeneous molecular constituents of date palm wax, as detailed in section 3.3. The presence of different molecular components has a profound effect on the crystal packing, therefore, high compatibility between the major and minor components is desired to allow for tighter packing of the crystals.[169, 197, 209, 215-217]

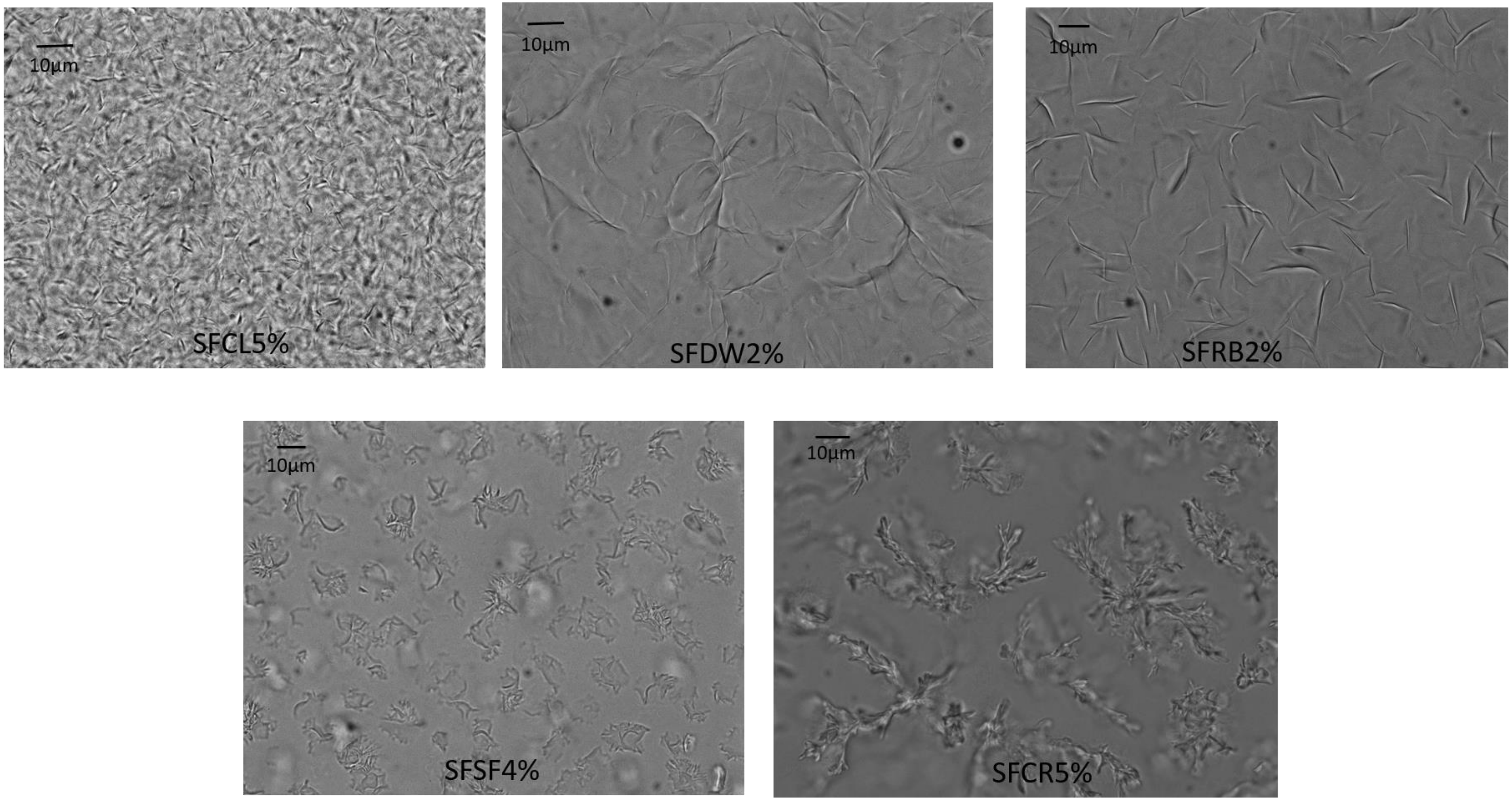


Figure 3-10 Optical light microscopy images for wax based oleogel at highest magnification x40.

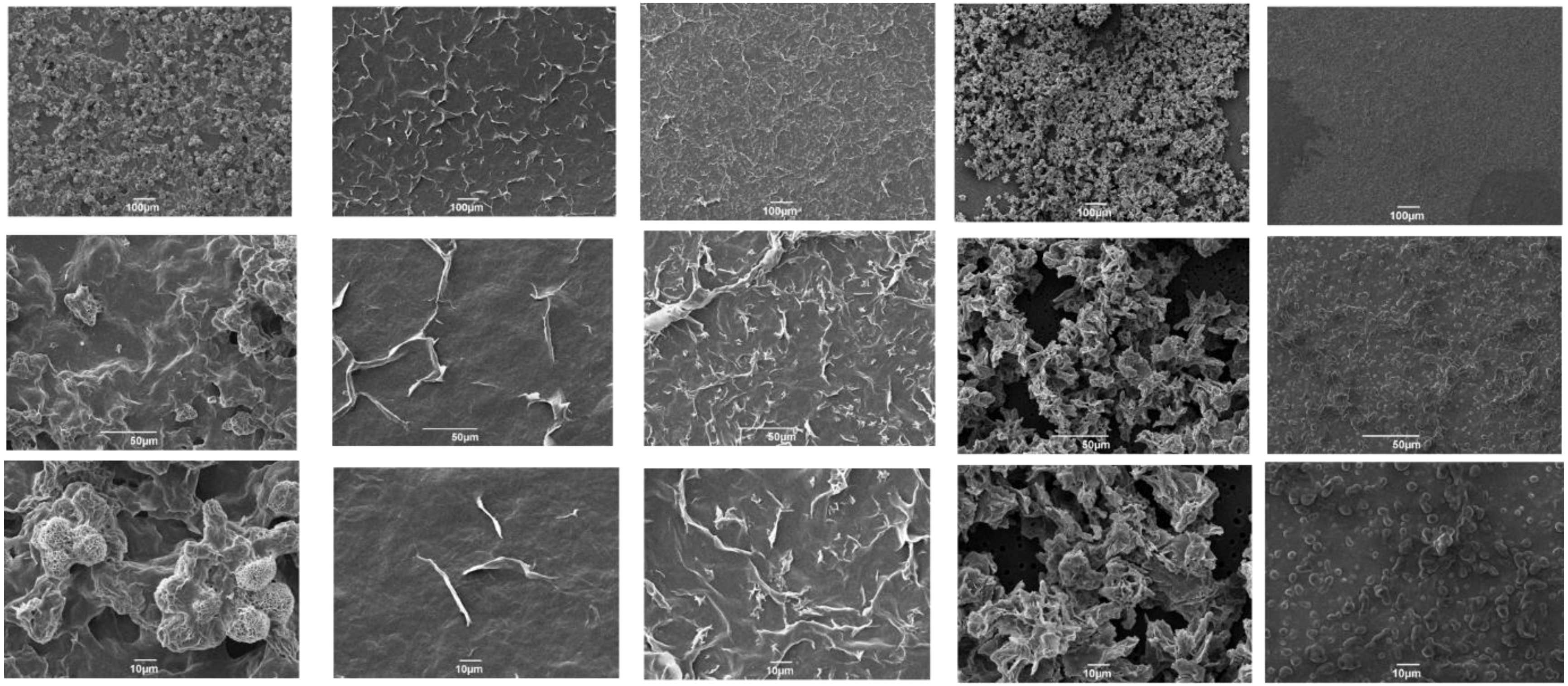
Co-crystallisation of impurities within the crystal structure is observed in candelilla and carnauba waxes (both heterogeneous in term of chemical composition) which further hindered tighter crystal packing and the formation of an ordered crystal structure.[169, 197] Interestingly, sunflower wax showed a dendritic structure with relatively small crystals compared to rice bran and date palm wax. The reported crystal morphology of sunflower wax in high oleic sunflower oil was deemed to be a rod like structure, while more recent studies concluded that the crystals are platelet structure.[193, 209, 213] This is might further confirm the impurities such as plasticizer in the sunflower wax besides the DSC profile and the high concentration needed to form gel. Blake *et al.* reported a fibrous structure for sunflower wax and it believed that because of this fibrous structure a gel forms at relatively lower concentrations of wax, with interesting rheological properties.[213]

Carnauba wax oleogel showed a dendritic crystal structure with relatively large size compared to all other waxes. The larger crystal size is owed to the high concentration required to gel the oil. This is in agreement with reported morphology of the carnauba wax in canola oil, high oleic sunflower oil and rice bran oil.[193, 194, 209] Interestingly no crystals were visible with candelilla wax oleogel at the critical gelling concentration. Candelilla wax had the lowest gelling concentration and is well dispersed in the oil as observed from the translucent appearance of the gel. Therefore, one can expect very small crystal sizes, which are not visible by optical microscopy. A higher concentration of candelilla wax oleogel was prepared and the crystal appears as a needle/platelet like structure, however it is difficult to conclude how concentration might affect the crystal shapes. Platelet-like structure was reported for candelilla wax in peanut oil, canola oil and high oleic sunflower oil.[193, 209, 218]

Optical microscopy has some limitations including limited resolution and length scale, which prevent visualizing smaller crystals such as those found in candelilla wax.[210, 218] Furthermore, the presence of liquid oil phase creates difficulties in distinguishing the crystals.[210, 218] Blake and Marangoni suggested the use of cryogenic electron microscopy to overcome these limitations, where the samples were de-oiled prior to the analysis and immobilized by freezing in liquid nitrogen at -210 °C.[218] Moreover, same authors concluded that the needle-like shapes observed were protruding edges of platelet for rice bran, sunflower and candelilla waxes with the study suggesting that the oil gelation cannot be explained only by the needle-like structure but other factors such as surface area and roughness may contribute.[218]

In this study, scanning electron microscopy images were obtained for de-oiled wax samples to gain further insight in the crystal structure. Figure 3-11 displays the scanning electron microscopy images at various magnification. Sunflower wax displayed a dendritic structure with a highly porous surface area. This might explain the excellent binding capacity exhibited by sunflower wax in this study. Moreover; the small pore size traps oil more efficiently. This, combined with the high surface area, assures better adsorption of oil. Carnauba wax also showed a dendritic structure with irregular large crystal shape. This can explain the poor oil binding capacity of the carnauba wax as entrapped oil is easily exuded from such big crystal structure due to the low surface area exhibited. Low surface area means less efficient absorption and hence poor oil binding capacity.

Rice bran wax exhibited a platelet-like structure as mentioned earlier and further confirmed by SEM. Interestingly date palm showed a very similar albeit irregular platelet crystal compared to rice bran wax. Candelilla wax showed spherulite structure with a relatively small crystal structure. Candelilla wax showed very strong oil binding capacity which can be owed to the fact that its large surface area associated with smaller crystal size, and therefore the dominant mechanism in oil binding is the adsorption.



Sunflower wax

Rice bran wax

Date wax

Carnauba wax

Candelilla wax

Figure 3-11 Scannig electron microscopy (SEM) images for wax based oleogels at various magnification.

3.7 Rheological profile for oleogels of date palm wax and commercial waxes

Small amplitude oscillatory measurements were carried for all studied wax based oleogels. The amplitude sweep test is considered foremost amongst rheological tests carried out for viscoelastic materials.[198-200] Indeed, the amplitude sweep further confirms the formation of the gel from viscous sol and enables identification of the LVER.[198-200] The G' values can give a relative strength of the gel and the length of the LVER can give further insights into the structure of the gel.[198-200] Figure 3-12 shows the amplitude sweep test for all tested oleogels carried out at 1 Hz and 5 °C. The elastic modulus G' is higher than the viscous modulus G" for all waxes, which further confirms the formation of gels. Examining closely the G' values in Table 3-3, one can see the relative strength of the gel, with SFCL1% and SFSF4% being the highest followed by SFRB2%. Interestingly, SFDW2% and SFCR5% showed relatively similar strength and were the lowest amongst other oleogels. Surprisingly, both SFCL1% and SFSF4% showed the strongest oil binding capacity as discussed in section 3.5, which further confirms the role of the crystal morphology in imparting the gelation properties. On the other hand, SFCR5% and SFDW2% showed the lowest oil binding capacities and hence the lower values for G'_{LVER}.

Stress yield is defined as the minimum stress required to initiate flow in the sample.[198-200] The stress yield values help in understanding the product performance, stability and shelf life.[198-200] There are many methods to measure stress yield including model fitting, stress ramp and oscillatory amplitude stress sweep.[199, 219] In oscillatory stress sweep test the stress yield can be measured by two approaches. The first one being the onset of non-linearity in the LVER region corresponding to the stress yield (critical stress yield, τ^*).[193, 199, 219] While the second approach considers the crossover point where G'=G" as the stress yield point since the complete breakdown of the structure occurs (dynamic stress yield, τ_{dy}).[193, 199, 219]

Table 3-3 shows the values for both τ^* and τ_{dy} and it can be noted that SFSF4% exhibited exceptional high critical stress yields both τ^* and τ_{dy}. Ashok *et al.* reported that sunflower wax forms a soft gel in high oleic sunflower oil and exhibited τ_{dy} at 9.9 Pa which is much lower than the value reported.[193] On the other hand, SFCR5% showed the lowest stress yields (2.0 ±0.2 Pa for τ^* and 3.3 ±1.2 Pa for $\tau_{d.y}$). These results are in disagreement with reported values and can be attributed to several factors including the type of vegetable oil, the grade of the wax, presence of impurities in the wax and the critical gelling concentration apart from other factors including the instrument and sample loading on the rheometer.[193] Interestingly, SFRB2% showed τ_{dy} three times than the τ^* which indicates the stability of the gel over a wide range of stress and the corresponding microstructure not breaking immediately (not all forces breaking at once).[220] Despite having the highest G'_{LVER}, CL1% showed a moderate stress yield and a narrower LVER indicating the breakage of microstructure at once. SFDW2% showed relatively low τ^* but a higher τ_{dy} and wider LVER resembling more SFRB2%. SFDW2% showed higher yield stress values compared to SFCR5% and hence better stability. This provides further evidence that date palm wax can be potentially an effective and economic replacement for the overlooked carnauba wax. Overall, the reported values for both stress yields are in agreement with literature with the exception of sunflower wax based oleogels.[193, 194, 209, 220]

Table 3-3 Rheological characteristics for wax based oleogels reported as average of three measurements.

Sample	Elastic modulus G'_{LVER} (pa)	Critical yields stress τ^* (pa)	Dynamic yield stress τ_{dy} (pa)
SFCL1%	2.98×10^4	7.8 ±0.7	11.2 ±3.1
SFRB2%	1.22×10^4	10.4 ±0.5	29.7 ±10.7
SFDW2%	8.33×10^3	5.4 ±0.2	14.2 ±4.1
SFSF4%	2.37×10^4	28.8 ±0.6	32.9 ±2.8
SFCR5%	5.35×10^3	2.0 ±0.2	3.3 ±1.2

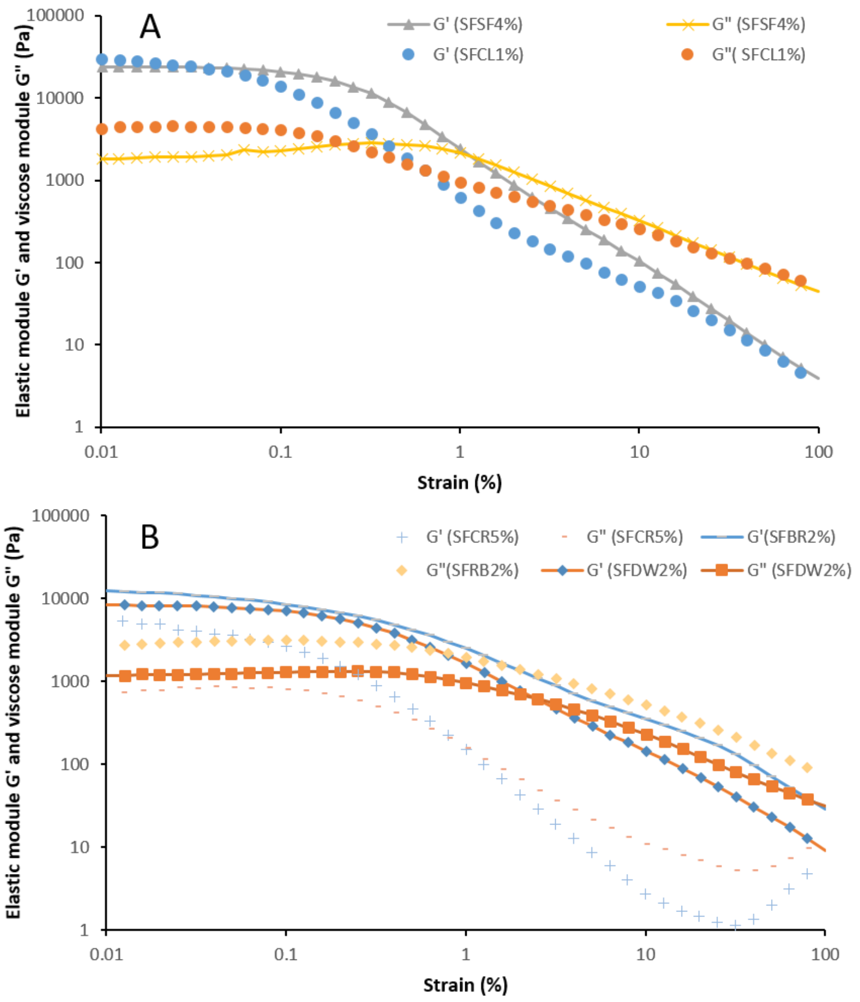


Figure 3-12 Amplitude sweep test for oleogels prepared A: candelilla and sunflower waxes B: date palm, rice bran and carnauba waxes at 1 Hz and 5 °C.

A frequency sweep was carried out for all studied waxes oleogels and the results are displayed in Figure 3-13. In a typical frequency sweep test the frequency is varied while the strain is held constant within the LVER.[198-200] The test gives valuable information on the time dependent effects on the gel with low frequency considered relatively long time and high frequency corresponds to shorter time scale.[198-200] In Figure 3-13 the G' and G" values were stable and hence almost liner curve in range 1 to 400 (rad/s). This indicates that the wax based oleogels had good tolerance to the rate of the deformation. This also an indication of the structural stability, with the bonds formed being stable within the time frame of the experiment.[220] Moving to higher frequency results in structural breakdown, and this was observed for all studied waxes, albeit more for carnauba wax, as shown in Figure 3-13, indicating higher stability of all other waxes compared to carnauba. All gels exhibited similar frequency dependence with candelilla, rice bran sunflower and date palm waxes showing better stability than carnauba wax. Doan *et al.* observed a similar trend with berry and sunflower waxes in rice bran oil with both waxes oleogel being stable in the frequency range 0.1 -500 (rad/s).[209] Petal *et al.* showed that carnauba wax oleogel in high oleic sunflower oil showed the least frequency dependence and better stability compared to other waxes including sunflower, berry, fruit and candelilla and beeswax.[193] This is in disagreement with what has been observed in this study and can be owed to several factors, including the grade of the wax, type of vegetable oil, the instrument, as well as the experimental factors associated with the measurements highlighted in section 3.8.

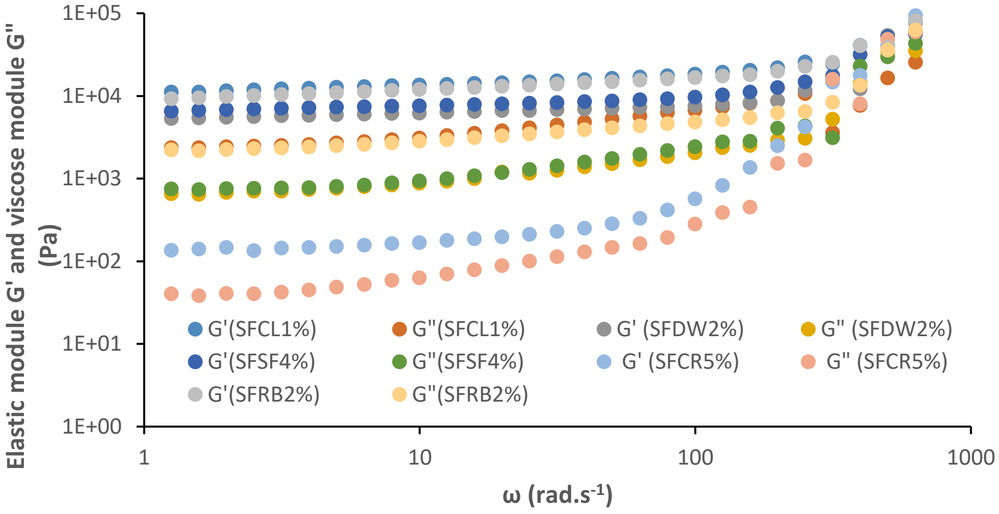


Figure 3-13 Frequency sweep for wax based oleogels at critical gelling concentration carried at 5 °C and 0.02% strain.

A temperature sweep was carried out for all oleogels at critical gelling concentration to gain a better insight to the corresponding gelling temperature. Figure 3-14 shows the temperature sweep for wax based oleogels. It can be noted an increase in G' values was observed as temperature decreased until the inflection point is reached where G'= G" where the phase changes occur. The SFCL1% exhibited the lowest gelation temperature at (34.36 ±0.001 °C) which is slighter lower than the crystallisation temperature observed in the DSC profile at 36.67 (±0.16 °C), but within the range. This was also the inflection point or crossover where G'= G" and phase change from liquid to solid occurred indicating the aggregation of the crystals in clusters. It is believed that upon cooling, a molten system that large crystal starts forming in a single dimension and their sizes and number increases with decreasing the temperature. Potential transition of the crystals from 1D to polymorphic 3D occurs during the cooling, resulting in 3D crystalline network.[216, 220] It is quite surprising that for SFCL1%, the G' values kept increasing and never reached a steady value or a linear region taking in consideration that the test was carried in LVER region. Further tests are needed in order to fully understand the behaviour of the SFCL1%. Similar trends were observed with SFSF4% in terms of the G' values with a gelation occurring at a higher temperature of 43.75± 0.2 °C which is slightly higher than the crystallisation peak observed in the DSC profile in Table 3-2. SFDW2% and RB2% showed a similar gelation behaviour with increase in G' values as temperature decreased. However, after gelation (crossover point) the increase in the G' values were very small and the curve levelled off, resulting in the LVER region. In terms of the gelation temperature, SFDW2% showed slightly higher gelation temperature at 58.8 ±0.1 °C compared to SFRB2% at 54.4 ±0.2 °C. Finally, SFCR5% exhibited a gelation temperature at 57.8 ±0.2 °C very close to that of the SFDW2%. This shows the advantages of date wax over carnauba wax in requiring less material and exhibiting higher gelation temperatures.

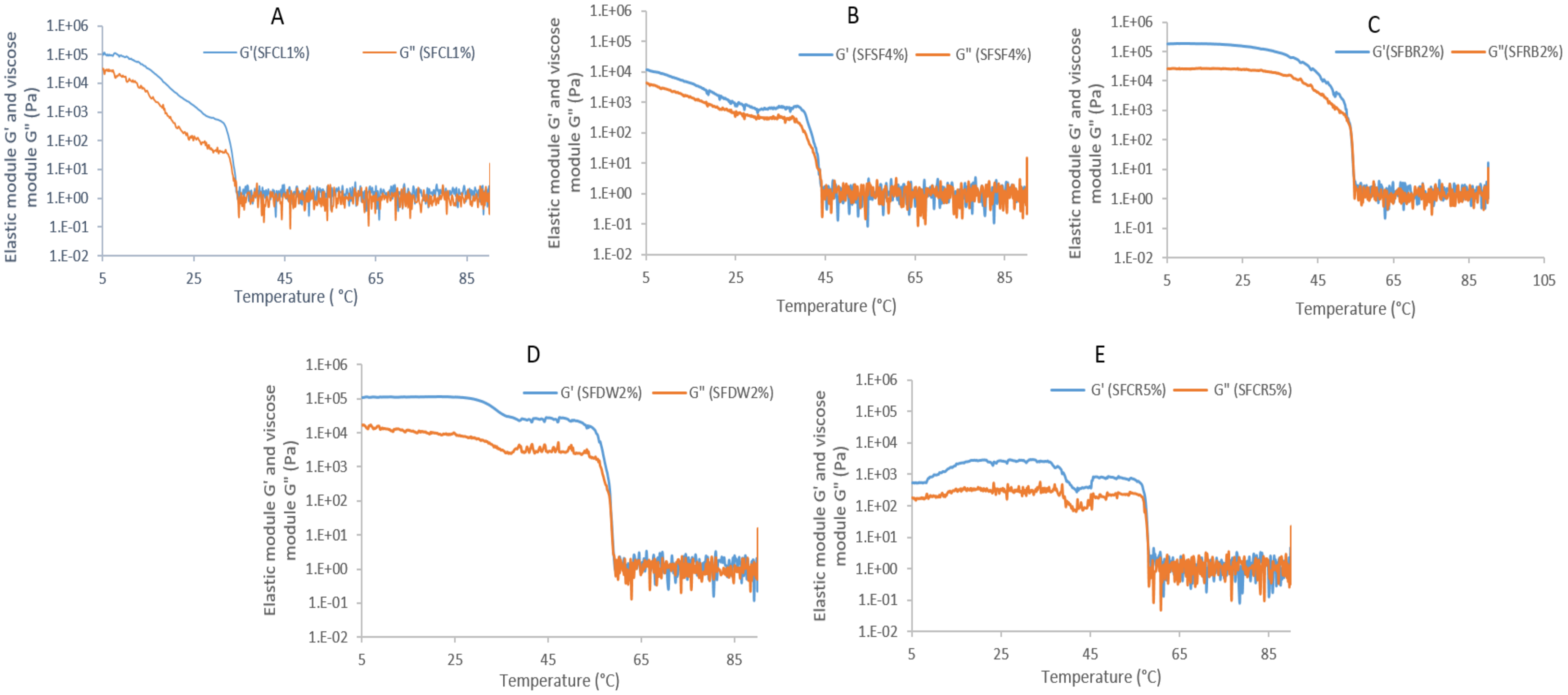


Figure 3-14 Temperature sweep for wax based oleogels at 0.02% strain and 1 Hz.

Thixotropy is defined as continuous decrease of sample viscosity with time when subjected to a flow (usually as shear rate or shear stress) and subsequent recovery of the original (rest) viscosity upon eliminating/discontinuing the flow.[199, 201, 202, 221] Thixotropy describes complex rheological behaviour but can be explained based on the microstructure of the sample.[199, 201, 202, 221] It is believed that it is a result of a weak attraction between the particles, which result in the formation of flocs, Figure 3-15.[221, 222] Upon applying a mechanical stress, the weak inter-particle bonds break during the flow resulting in breaking the network, and therefore separating the flocs and further decreasing the size of the flocs.[221, 222] When decreasing the shear rate or removing the stress, the crystals regrow and the particulate network reforms, resulting in attaining the original viscosity (or close value) with complete recovery Figure 3-15. Less desirable is the lower viscosity value, which is referred as partial recovery.[221, 222]

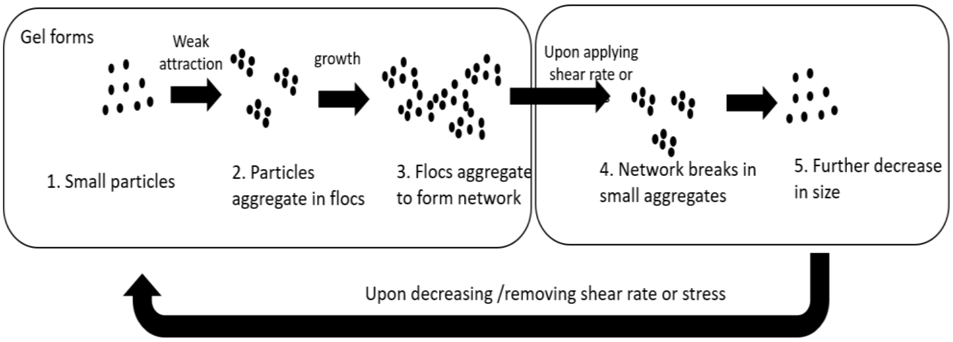


Figure 3-15 Behaviour of the particle when shear stress or rate applied and removed.

Time dependant viscosity is an important parameter in product formulation.[199, 201] Indeed, for some applications gradual viscosity change is desired where in other case accelerated recovery of viscosity is preferred after sudden change in shear stress/rate.[199, 201] It allows for an estimation and prediction of the materials behaviour upon agitation or pumping etc. and the effect of ceasing the agitation on how fast the structure recovery obtained.[199, 201] Usually thixotropic materials show shear thinning combined with time dependency where the viscosity decreases with increasing the time.[199, 201] There are many tests to measure the thixotropy including thixotropic loop, constant shear rate and creep recovery.[221] Herein, a specific rheological test is employed called three intervals thixotropic test (3-ITT) which is composed of three intervals: 1) a reference shear rate 2) high shear rate 3) the regeneration interval.[222] Moreover, in the first step the shear is assumed at the rest to be so low the shear rate is applied within the LVER region.[222] In second step, high shear is applied in order to break the internal structure of the gel or sample, mimicking the high shear processes in industry such as agitation, pumping or shaking.[222] The third interval again low shear applied in order to see how well and fats the material recovery occur.[222] Figure 3-16 shows the 3-ITT tests for the studied oleogels at their critical gelling concentration.

Candelilla wax based oleogel showed outstanding recovery of the structure and viscosity with 95% recovery, which indicates excellent structure recovery. This might be owed to several factors associated with the microstructure of the sample including the dominancy of hydrocarbon in the chemical composition, the spherical crystal structure and suggesting more homogenous bonds between the crystals and the aggregated flocs. Ashock *et al.* reported a recovery of 58% for the candelilla wax based oleogel prepared from high oleic sunflower oil.[193] Rice bran and sunflower wax both performed a recovery of around 70%. Generally, a recovery of 70% and higher is desirable in food industry.[193, 199, 201] This might be owed to the homogenous nature of these two waxes and therefore homogenous bonds between the aggregate crystals. Surprisingly the literature reported only 43% recovery for sunflower wax based oleogel, which is far less than that observed in this study.[193] However, taking into consideration that the corresponding critical gelling concentration was much lower for sunflower wax based oleogel than the one reported here, it is difficult to justify the differences. Date palm and carnauba waxes showed poor structure recovery, with only 40% being observed. This might be owed to the diversity in terms of chemical composition for these two waxes, as discussed earlier. Therefore, the bonding between crystals and aggregates are more heterogeneous and non-uniform which, upon shearing, break the aggregated clusters into smaller units. The restructuring of the network upon removal of the shear is eluded because shear force overcame the Brownian motion. Similar weak recovery is observed for the carnauba wax based oleogel in sunflower oil with only 15% recovery.[193]

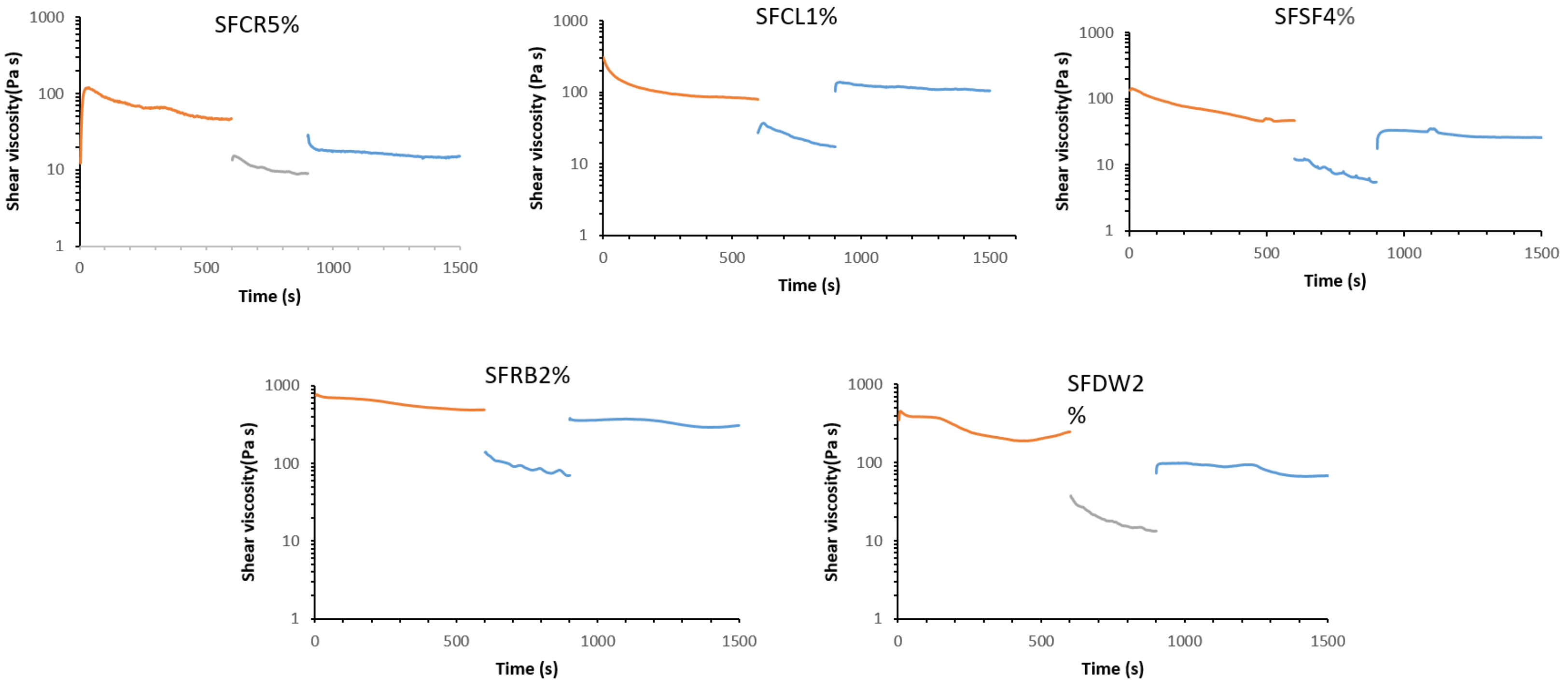


Figure 3-16 The 3-ITT tests for wax based oleogels at 5 °C.

3.8 Difficulties with rheological measurements

There are many challenges and difficulties associated with rheological measurements. It is believed that there is no exact single value for viscosity or modulus but rather these two are function of many other factors including the time scale, sample loading, amplitude and environmental variables.[223] In a recent review, Ewoldt *et al.* described the challenges and errors associated with the measuring of rheological properties for soft, water based and biological materials.[223] Among the challenges highlighted by the authors were those related to the rheometers, including instrument specification, inertia and geometry.[223] While others related to the material including thermal stability, particle settling and migration, surface tension and edge fracture.[223] Some of these challenges and artefacts are also encountered in measuring the rheological profile of polymer melts.[224] Wax based oleogels are fragile and soft as the molecular interaction involved in their formation are relatively weak. The challenges encountered in measuring the rheological profile for wax based oleogels were mainly associated with the fragile nature of the gel. For instances; the temperature difference from the time the sample is removed from fridge and loading it on rheometer is important to be kept to the minimum in order to assure consistency in the measurements. Moreover, sample loading is also an important aspect of the measurements and with soft gels it is advised to scoop the sample once from the vial as multiple scooping introduces shear that might break the structure. Table 3-4 summarise the gel properties of date palm wax along with other commercial waxes.

Table 3-4 Summary of gel properties for wax based oleogels.

Wax oleogels	Critical gelling concentration %(w/w)	Melting temperature	Oil loss %(w/w) after 15 min	Oil loss %(w/w) after 60 min	Crystal structure	Elastic modulus G' $_{LVER}$ (pa)	Critical yields stress τ* (pa)	Dynamic yield stress τ_{dy} (pa)
SFCL1%	1	42.5 ±0.17	0.00 ±0.00	9.26 ±1.62	Spherulite	2.98×10^4	7.8 ±0.7	11.2 ±3.1
SFRB2%	2	59.7 ±1.3	4.80 ±0.00	19.51 ±1.11	Platelet	1.22×10^4	10.4 ±0.5	29.7 ±10.7
SFDW2%	2	65.7 ±0.2	10.35 ±1.78	29.05 ± 1.79	Platelet	8.33×10^3	5.4 ±0.2	14.2 ±4.1
SFSF4%	4	63.9 ±0.3	0.15 ±0.19	8.20 ± 2.89	Dendritic	2.37×10^4	28.8 ±0.6	32.9 ±2.8
SFCR5%	5	74.7 ±1.6	25.30 ±1.23	35.17 ±1.36	Dendritic	5.35×10^3	2.0 ±0.2	3.3 ±1.2

3.9 Conclusion

In this study the application of date palm leaf wax as a structuring agent for vegetable oil was assessed alongside other commercial waxes. A date palm wax based oleogel showed low critical gelling concentration compared to some other waxes including sunflower and carnauba, which potentially makes it more economically viable in terms of that lower concentration needed. The chemical composition of the waxes is important to understand their gelling behaviour. While homogenous composition is preferred for better gelling properties, the compatibility between the major and minor components within the wax plays a vital role. Date palm wax based oleogels showed good thermal stability with high gelling temperature making it promising for applications where a more thermally stable gel is required. The oil binding capacity was relatively weak for the date palm wax based oleogel compared to other waxes including candelilla, sunflower and rice bran waxes. However, this can be further improved if the wax was refined or/and the preparation method improved by controlling the cooling rate or applying shear during cooling. The crystal morphology revealed plate like structures for the date palm wax base oleogel, which is the favoured structure to form a gel with desirable properties. The rheological profile for the date palm wax based oleogel was within the range with other waxes' oleogels making date palm wax a promising alternative that can compete with other natural waxes. There are many variables which influence the gelling ability of the waxes in vegetable oil including chemical structure, crystal morphology, affinity of the wax to the oil and the balance in molecular interaction that forms the gel which make it difficult to explain the mechanism of the gelation and careful study required to optimise wax based oleogels.

Chapter 4

Scale up, fractionation and economic of $scCO_2$ extraction of waxes from date palm leaves

4. Chapter 4 Scale up, fractionation and economic of $scCO_2$ extraction of waxes from date palm leaves

4.1 Introduction

$ScCO_2$ extraction has been demonstrated as an alternative greener extraction technology for added value compounds from natural matrices. The numerous advantages of $scCO_2$ include high solvation power for non-polar hydrophobic compounds, high mass transfer and the ability to obtain solvent free extracts make this a promising technique when considering the use of extracts in products such as personal care, cosmetics and food ingredients. In chapter 2 $scCO_2$ utilised in the extraction of waxes from date palm leaves on a lab based scale with a promising recovered yield. It was crucial to take the process to the next step and consider the scale up at semi-pilot scale to gain a better understanding of the process viability. It is believed that the information gained from an initial pilot scale demonstration in term of time, process conditions and costs can be applied for an industrial scale process.[225] There are several approaches for scale up including maintaining constant kinetics parameters, using mathematical models to predict the process behaviour at scale and some simpler approaches by changing the solvent to feed ratio.[225]

$ScCO_2$ can be used as a fractionation technique to reduce the complexity of natural matrices which add further value to the final products. Fractions with different properties can be achieved by selectively adjusting the pressure and temperature. This gives $scCO_2$ a significant advantage to tailor the desired product by simple manipulation of the pressure and temperature of the extraction.

It is important to get an estimation of the manufacturing cost of the process to assess it is economic viability and highlight the main cost associated with the pocess. The scale up and cost of manufacturing for $scCO_2$ of date palm leaves to obtain wax has previously not been investigated in the literature.

In this chapter, three aspects of wax extraction were explored. Firstly, a preliminary scale up of the $scCO_2$ extraction at pilot scale up was attempted to gain better insight and assess the viability of the process at larger industrial scale. Secondly, $scCO_2$ used as a fractionation technique to further reduce the complexity of the wax and obtain fractions with different properties. Finally, the economic aspects of the extraction process were assessed.

4.2 ScCO$_2$ scale up of date palm leaves wax

There are several approaches for scale up including maintaining constant kinetics parameters, using mathematical models to predict the process behaviour at scale up and developing empirical equations based on the geometry of the extractor bed[225-230]. All these approaches are costly in term of time and the design required for the pilot scale.[225, 231] On the other hand, adapting simpler approach by selecting single criterion could aid in developing a scale up process easily and with minimum time and cost.[231] One of the simpler method adopted by Meireles et al was retaining a constant solvent to feed ratio showed promising results.[231, 232]

The approach adopted for scaling up scCO$_2$ extraction of date palm leaves wax was to increase the extraction vessel from 0.5 L to 5 L as well as increasing the biomass feed from 0.1 Kg to 1 Kg, while the solvent to feed ratio kept constant. The scale up extraction was carried at optimal extraction condition at 400 bar and 100 °C, which was determined from a second order factorial design detailed in section 2.2. The kinetic of the extraction was studied in section 2.2 using a 0.5 L vessel as in Figure 4-1 (reproduced from Figure 2-3)

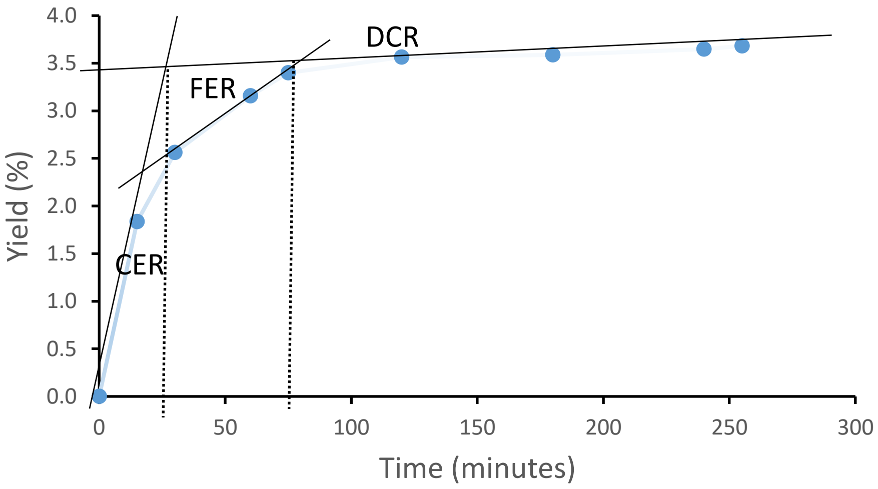


Figure 4-1 The overall extraction curve (OEC) for date palm leaves wax at 400 bar, 100 °C.

The OEC gives important information regarding the estimation of the optimum extraction time. It was found that 90% of the extract is obtained after 75 minutes from the start of the extraction. Typically, in industry the extraction is marked complete if 90% of the solute extracted.[225] Hence the ideal operating condition occur in the first stage of the extraction CER region.[225] Therefore, the optimum extraction time employed in the scale up was 75 minutes. The yield of the scale up extraction was 3.1% *w/w,* this value is similar to the yield obtained at the lab scale (3.49% *w/w*). This further illustrates that this simple yet reliable method can help in creating a benchmark procedure, as no previous research considered $scCO_2$ extraction of waxes from date palm leaves. It is noteworthy to mention that the actual yield might be higher than reported owed to the nature of the wax and the loss occurring in the tube walls. Date palm leaves wax exhibited high melting profile and hence the high temperature of the extraction 100 °C needed to overcome the crystalline point. However, typically in $scCO_2$ extraction equipment the extractor vessel is heated to the set extraction temperature, while the tubing remain at ambient temperature, which can result in solidification and precipitation of the wax in the tubing from extractor unit to the separator unit. While this was not obvious problem at the lab scale, it must be considered for the scale up and with wider pipelines, the precipitation is more profound. Further work needed to overcome this problem in terms of improving the design of the instrument (the addition of heated tubing or use of a co-solvent) to facilitate the movement of the extracted wax through the tubing. The chemical composition of the extracted wax was similar to the lab based extraction, which further confirmed the viability of the process on large scale. Figure 4-2 show the normalised chemical composition for both the lab scale and scale up runs both extracted at 400 bar and 100 °C.

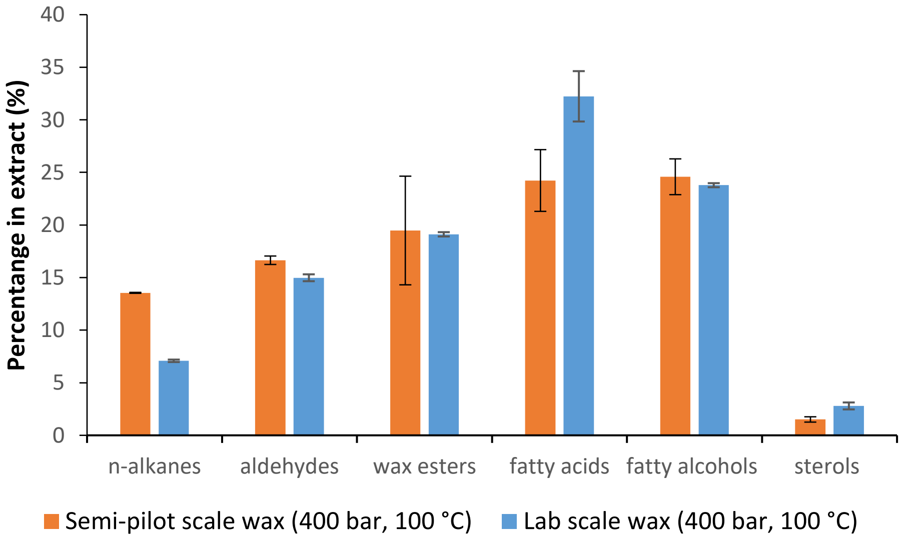


Figure 4-2 Chemical composition of date palm leaves waxes extracted on lab scale and scale up at 400 bar, 100 °C.

No single class of compounds dominated the composition but, a complex mixture of different compounds was observed. Interestingly, fatty acids (32%) were the most abundant compounds on the lab scale run wax followed closely by fatty alcohols (24%). While the wax from the scale up run similar portions of these fatty alcohols and fatty acids. Moreover, higher percentage of hydrocarbons (13.5%) extracted (almost double) on scale up compared to the lab based (7%). On the other hand, lower proportions of sterols were observed for the scale up run compared to the lab scale. The overall compositions of the both waxes are comparable and slight variation might be due to the natural variation within the biomass composition. It is crucial to point that only 27% of the wax composition is identified for the lab based wax while only 14% of the wax from scale up. This indicates that there is a big portion of wax yet to be characterised. As future work, it will be interesting to look at additional analytical technique to quantify and characterise date palm wax. Dr Debyrn from Green Chemistry Centre of Excellence at the University of York attempted further characterisation for date palm leaves wax using Matrix Assisted Laser Desorption/Ionization (MALDI) mass spectrometry technique and this data can be found in appendix B.

The extracted wax showed relatively high melting profile with a sharp endotherm associated with the melting centred around 81 °C (Figure 4-3). Several shoulders in the profile, can be attributed to the heterogeneous nature of the wax. Compounds including hydrocarbons being lower molecular weight could contribute to the shoulders around 46 °C and 60 °C. On the other hand, wax esters and higher molecular alcohols and aldehydes are on higher molecular weight scale and hence the high melting profile. This high melting profile makes date palm among the hard waxes which can have potential application as hardening agent and applications were higher thermal stability required which makes date palm waxes unique in term of properties. Carnauba wax is one of the hardest natural commercial wax available in the market exhibits melting point ~82 °C and is used in various application in cosmetics such as lipstick to increase the toughness,[233, 234] It is also incorporated in candles as hardening agent,[235] as well as coating of fresh fruits to improve the shelf life etc.[236]

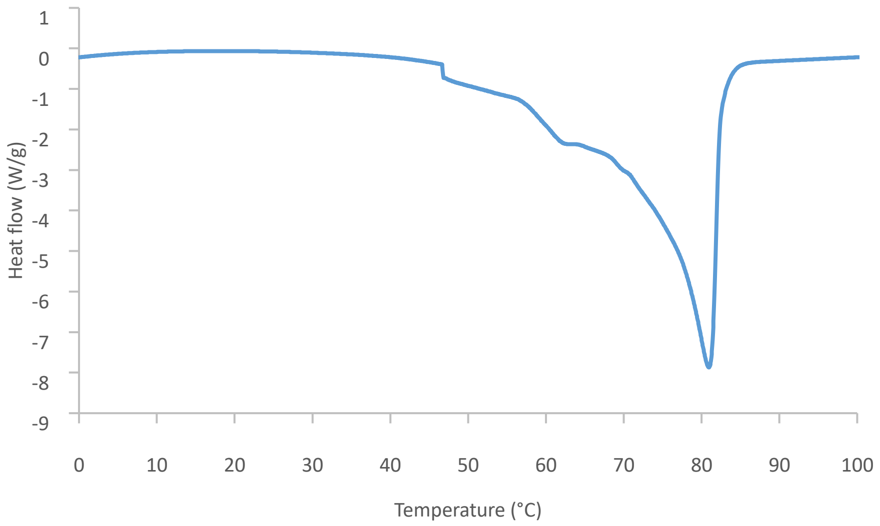


Figure 4-3 DSC thermogram for date palm leaves wax extracted at scale up run at 400 bar and 100 °C.

$ScCO_2$ extraction is advantageous in extraction of high value chemicals such as nutraceuticals or pharmaceuticals, where maximum yield is desired and less workout to isolate the extract. If the value of the product decrease then the economic dictate increasing the concentration of the extract.[225] Therefore, it would be interesting to reduce the complexity of the wax by fractionation mean to further add value to the process and making it economically more viable by obtaining higher value products.

4.3 Supercritical fractionation for date palm leaves wax

Typically, in conventional extraction methods involving solvents and the resulting crude products contains unwanted co-extracted groups of compounds. The same effect obtained with $scCO_2$ at single operating temperature and pressure. However, one of the key advantages of the $scCO_2$ is that the extract selectivity can be improved and products with higher value obtained by performing fractional separation.[237] In fractional separation, the compounds are precipitated in consecutive steps by dropping the pressure and temperature successively.[237] This results in greater homogeneity in fractions leading to a reduction in downstream separations with higher value. Fractional separation can be achieved by altering the density of CO_2 and thus decreasing the solubility of compounds.[237] Typically, $scCO_2$ extraction unit have a series of fractional separator units which are set at various temperatures and pressures, thus the density of CO_2 is different in each unit and hence the solvation properties of CO_2. When the extract (solutes molecules) in Figure 4-4 pass through fractional separation unit some of the molecules will be soluble hence they will pass to the next separator unit while other molecules are insoluble will precipitate. This results in fractionation of different types of compounds.

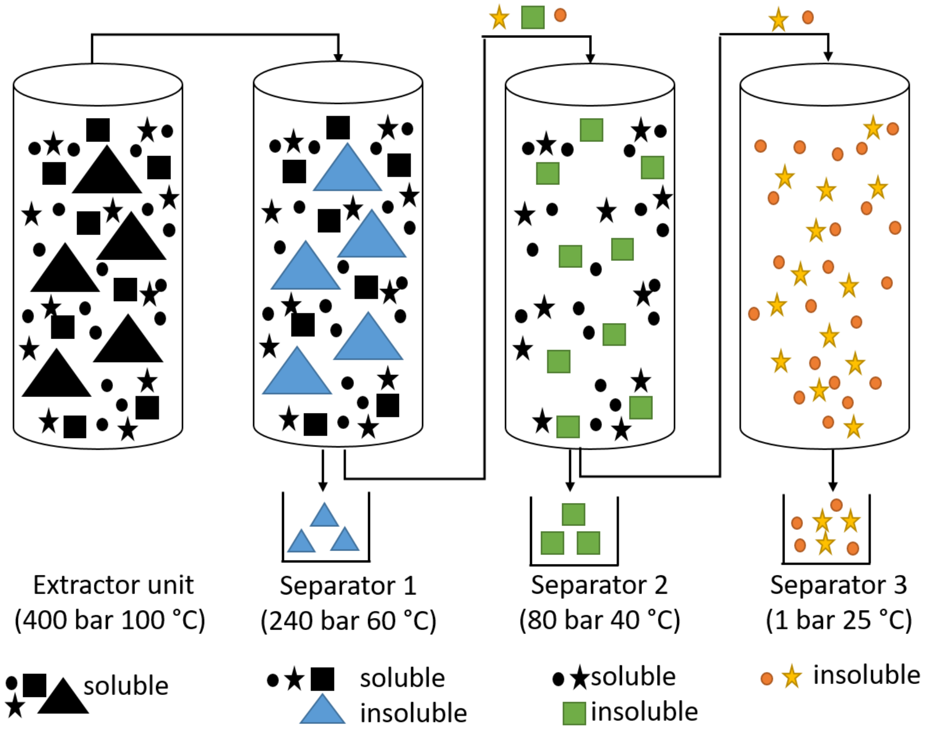


Figure 4-4 Schematic diagram exemplifying the fractionation of molecules with scCO₂.

The fractionation was carried out directly from the milled leaves and fractionation occurred instantaneously with extraction. Figure 4-5 shows the experimental set up for the fractionation of date palm leaves wax.

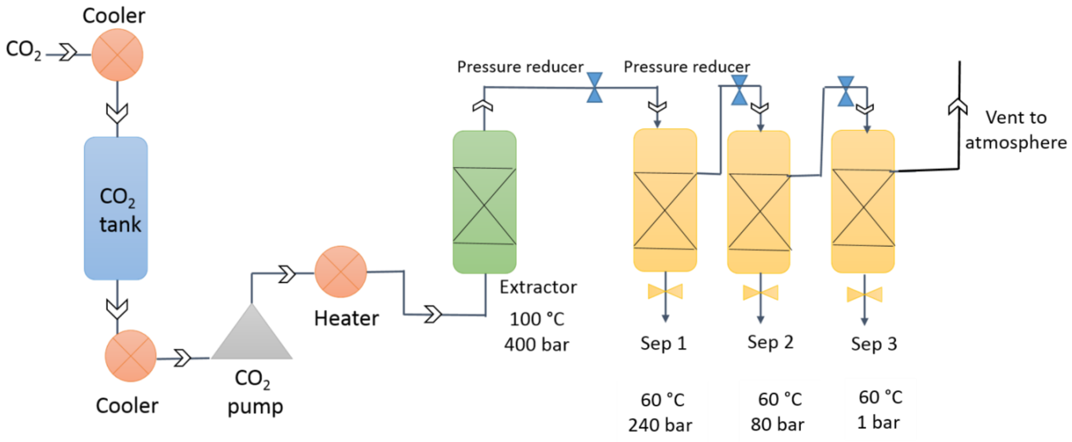


Figure 4-5 Schematic diagram for fractionation of date palm leaves wax.

Fractional separation for date palm leaves wax was achieved at three different pressure (240, 80 and 1 bar) while the temperature kept constant at 60 °C throughout all separator. The three fractions demonstrated different textural properties, colours (Figure 4-6), chemical composition (Table 4-1, Figure 4-7) and melting profiles (Figure 4-8).

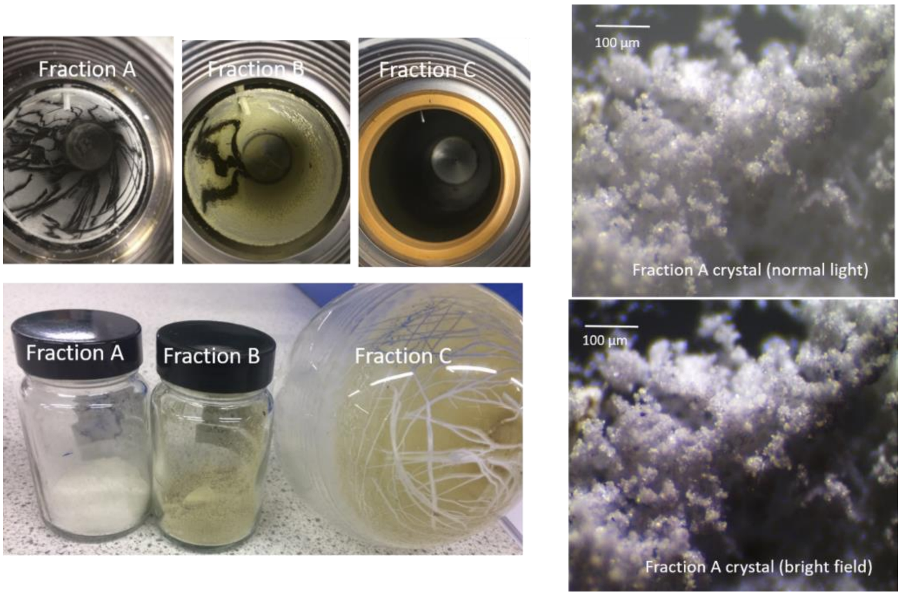


Figure 4-6 Texture and colour of fraction A (240 bar, 60 °C), fraction B (80 bar, 60 °C) and fraction C (1 bar, 60 °C) Left, fraction A visualised under optical microscopy in normal light and bright field.

Table 4-1 Chemical composition for fraction A (240 bar, 60 °C), B (80 bar, 60 °C) and C (1 bar, 60 °C) of date palm leaves wax .

Compounds	Fraction A (240 bar, °60) (µg/g biomass)	Fraction B (80 bar, °60) (µg/g biomass)	Fraction C (1 bar, °60) (µg/g biomass)
Decanoic acid	-	1.57 ±0.7	0.24 ±0.11
Dodecanoic acid	-	1.78 ±0.70	0.51 ±0.3
Tetradecanoic acid	-	1.31 ±0.25	0.45 ±0.1
Hexadecanoic acid	1.02 ±0.72	15.05 ±0.98	4.74 ±0.11
Heptadecanoic acid	-	1.84 ±0.32	0.63 ±0.02
Octadecanoic acid	0.24 ±0.28	2.91 ±2.38	0.45 ±0.1

Eicosanoic acid	-	0.80 ±0.03	0.21 ±0.0
Docosanoic acid	-	1.94 ±0.03	0.32 ±0.1
Tetracosanoic acid	-	3.90 ±0.49	0.70 ±0.00
Hexacosanoic acid	-	4.73 ±0.46	0.50 ±0.04
Octacosanoic acid	1.16 ±0.42	3.44 ±0.26	0.44 ±0.02
Triacontanoic acid	4.08 ±0.12	6.37 ±0.13	0.82 ±0.4
Dotriacontanoic acid	3.18 ±0.4	11.22 ±0.8	1.49 ±0.12
Tetratriacontanoic acid	-	0.44 ±0.5	0.9 ±0.2
Total saturated fatty acids	**9.68 ±1.94**	**57.30 ±8.76**	**14.20 ±1.62**
Octadecenoic acid	-	2.43 ±0.61	0.46 ±0.1
Octadecdienoic acid	-	1.83 ±0.16	0.63 ±0.02
Total unsaturated fatty acids		**4.36 ±0.77**	**1.09 ±0.12**
Octacosanol	1.21 ±0.66	1.11 ±0.10	0.63 ±0.1
Triacontanol	1.84 ±0.99	7.31 ±0.24	0.25 ±0.1
Dotriacontanol	0.76 ±0.23	43.18 ±2.63	4.47 ±0.19
Tetratriacontanol	1.86 ±0.23	16.95 ±1.28	1.74 ±0.6
Total Alcohols	**5.67 ± 2.11**	**68.55 ±4.25**	**7.09 ±0.99**
Campesterol	-	1.35 ±0.10	0.14 ±0.2
Stigmasterol	-	5.46 ±0.03	0.49 ±0.2
B-sitosterol	-	11.28 ±0.46	1.06 ±0.04
Total Sterols		**18.24 ±0.59**	**1.69 ±0.44**
Hexacosane	-	0.22 ±0.1	0.989 ±0.02
Heptacosane	-	0.53 ±0.53	1.74 ±0.1
Octacosane	-	0.76 ±0.4	2.49 ±0.1
Nonacosane		1.70 ±0.15	2.82 ±0.00
Triacontane	-	2.86 ±0.1	3.74 ±0.1
Hentriacontane	-	2.28 ±0.00	8.80 ±0.4
Dotriacontane	-	1.21 ±0.21	0.84 ±0.1
Tritriacontane	-	3.10 ±0.19	2.30 ±0.2
Total Hydrocarbons	**-**	**37.94 ±1.68**	**23.72 ±1.20**
Dotriacontanal	2.98 ±0.12	19.16 ±0.51	4.34 ±0.1
Tetratriacontanal	0.99 ±0.7	7.84 ±0.22	1.01 ±0.4
Total Aldehydes	**3.98 ±0.82**	**27.00 ±0.73**	**5.35 ±0.50**
WE40	3.63 ±0.00	-	0.11 ±0.00

WE44	-	-	0.35 ±0.02
WE46	-	-	0.38 ±0.1
WE50	-	7.15 ±1.20	1.81 ±0.14
WE52	-	-	0.37 ±0.16
Total wax esters	**3.63 ±0.00**	**7.15 ±1.20**	**3.02 ±0.42**

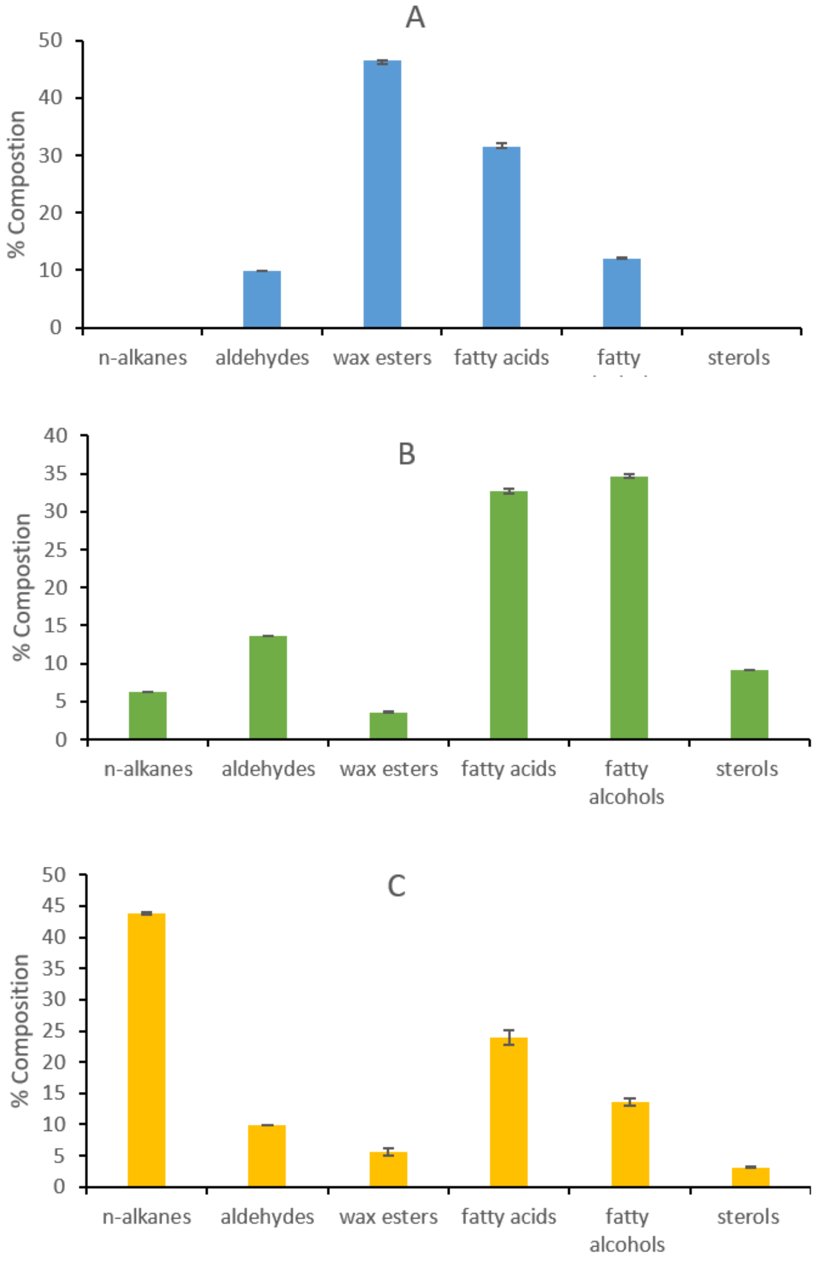


Figure 4-7 Percentage composition of groups of compounds for date palm leaves wax fraction A (240 bar, 60 °C), fraction B (80 bar, 60 °C) and fraction C (1 bar, 60 °C).

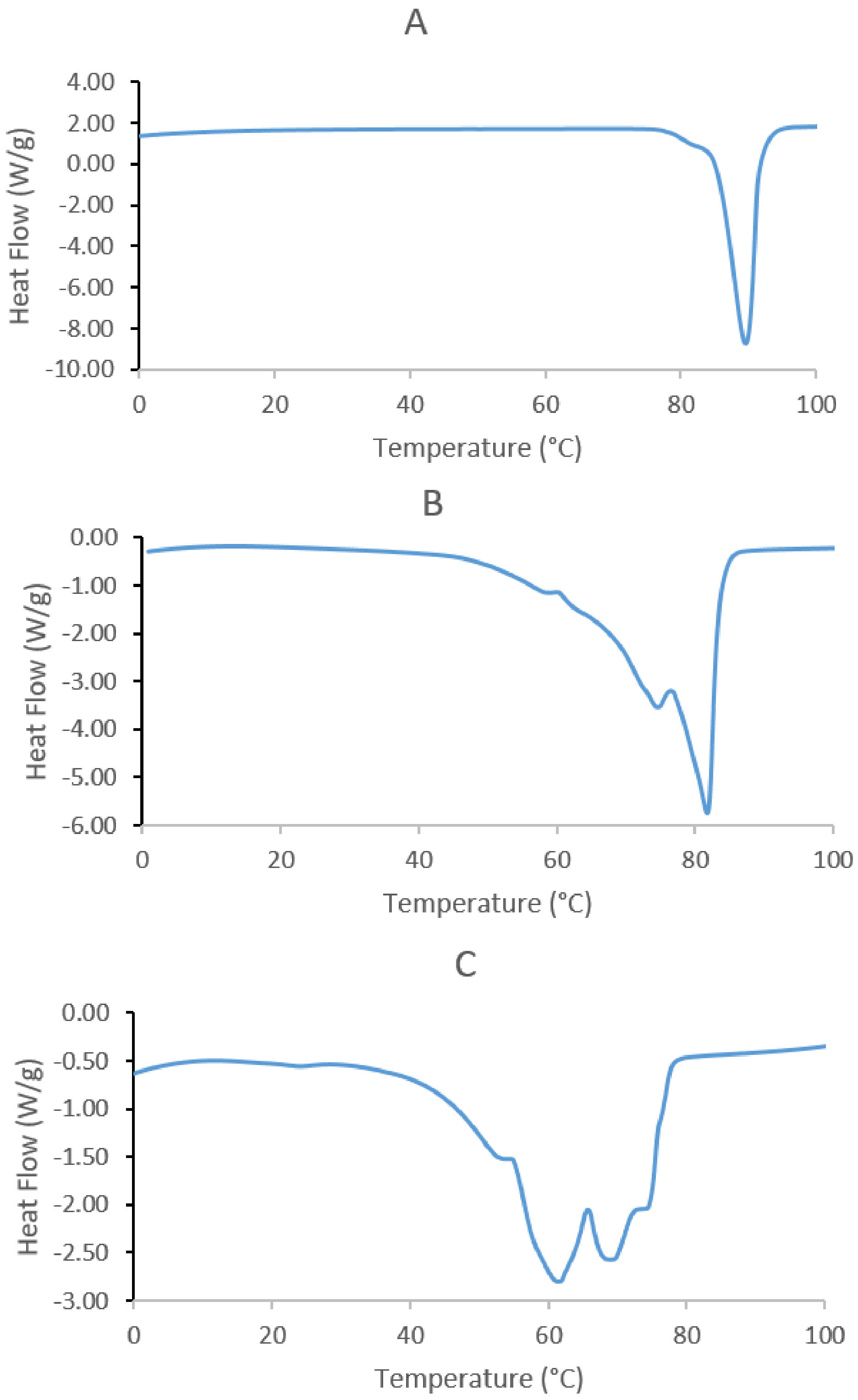


Figure 4-8 DSC thermograms for date palm leaves wax for fraction A (240 bar, 60 °C), fraction B (80 bar, 60 °C) and fraction C (1 bar, 60 °C).

Fraction A obtained at (240 bar and 60 °C) was a white yellowish very fine and light weight powder Figure 4-7. Fraction A showed an interesting crystalline structure when visualised under optical microscopy suggesting the purity of this fraction. Fraction A also showed the highest melting point with a very sharp crystalline endothermic event centred at 90 °C. This suggested that this fraction might contain higher molecular weight compounds. Unfortunately, the fraction exhibited limited solubility in most of the organic solvent and would dissolve in hot toluene. However, upon cooling it formed a gel, which further limited its analysis (Figure 4-9). The properties of this fraction may find use in the preparation of oranogels as previously discussed in chapter 3, further work is needed to confirm this hypothesis.

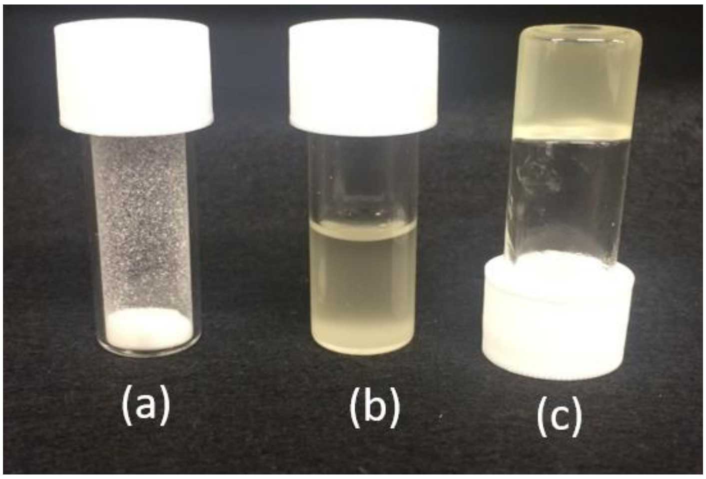


Figure 4-9 Date palm leaves wax Fraction A (240 bar, 60 °C) (a) powder sample (b) suspension in toluene (c) gel in toluene formed after heating the suspension and cooling it down to room temperature.

The normalised chemical composition is displayed in Figure 4-7. Interestingly, fraction A exhibited higher chain fatty acids (C_{28} –C_{32}), Fatty alcohols, aldehydes and wax esters. However, that total identified in fraction A was only 4% and it is believed that higher molecular weight compounds are present, which cannot be analysed by GC due to their limited volatility. To further confirm the presence of higher molecular compounds in fraction A, (Atomic Pressure Chemical Ionisation) APCI mass spectrometry was performed for the fraction A and the mass spectrum displayed in Figure 4-10 and Table 4-2.

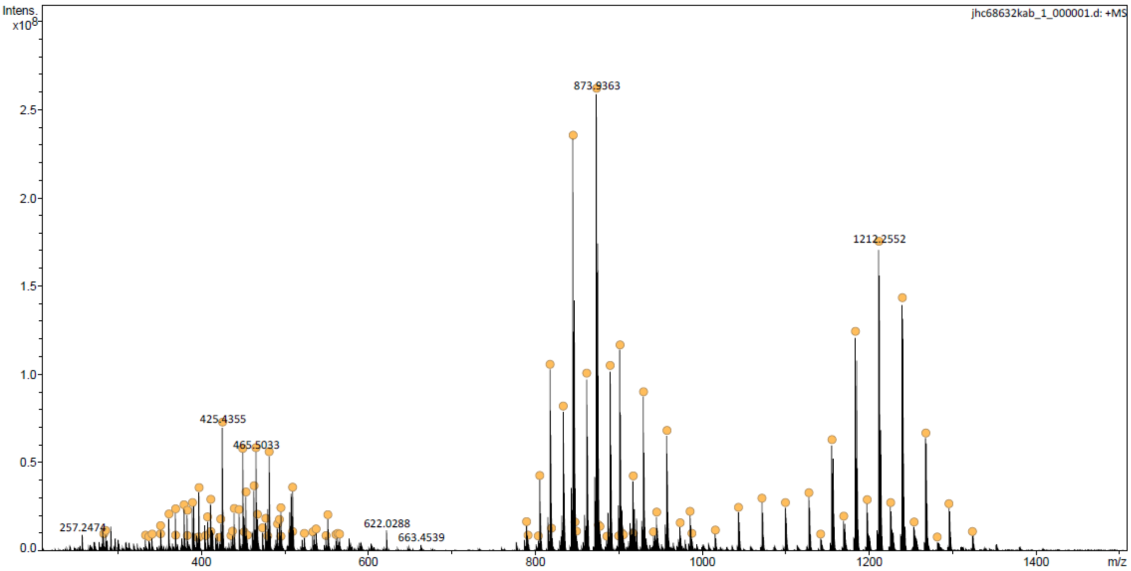


Figure 4-10 APCI mass spectrum for date palm leaves wax fraction A (240 bar, 60 °C).

Interestingly, molecular masses as high as 1300 m/z were observed, which further confirm the presence of higher molecular weight compounds. A smart formula software used to predict the molecular formula that matches with molecular weight in Table 4-2. Some parameters were used to limit the prediction of the formula detailed in experimental section 5.11. The m sigma value is a measure of how well the isotopic pattern matches with that of the predicted formula, with lower sigma value indicating a better fit. Surprisingly, some of the higher molecular weights matched are predicted to contain 3 and 4 oxygen atoms which can be potentially compounds including dicarboxylic acids, hydroxyl acids, di hydroxy acids, epoxy acids di esters, and hydroxy esters as well as aromatic and or cyclic compounds (Figure 4-11). Some of the compounds including hydroxyl carboxylic acid, di hydroxyl carboxylic acids, epoxy hydroxyl acids and hydroxyl acids esters have been reported as building monomers for plant cutin.[238, 239] Future work would need to focus of additional analysis of fractions, where analytical techniques such as high performance liquid chromatography, size exclusion chromatography, supercritical chromatography etc. coupled with advance mass spectrometry techniques will gain a better insight into the wax composition. Fraction A exhibited several interesting properties including the colour (light colour and hence avoiding bleaching process), the texture (fine powder making it easier to incorporate into products) and the exceptional high melting point (very high melting point make it among hard wax and hence loads of application as coating, instrument, and hardening agent). Date palm leave wax has the ability to gel the sunflower oil as showcased in chapter 3, it will interesting to test fraction A as gelator molecule and how might fractionation enhances the gelling ability in future study. This further shows an advantage using $scCO_2$ extraction and fractionation over conventional extraction technique in obtaining waxes with different properties such as melting point, texture and colour Having the solvent free extracts which enable their use the extract for high value application including pharmaceutical, personal care and food industries is an advantage.[64]

Table 4-2 Smart formula software results for the higher molecular compounds in fraction A (240 bar, 60 °C).

Measured m/z	Ion Formula	Formula m/z	err [ppm]	m Sigma
791.7854	$C_{52}H_{103}O_4$	791.7851	-0.4	25.6
803.8582	$C_{55}H_{111}O_2$	803.8579	-0.4	24.8
805.8374	$C_{54}H_{109}O_3$	805.8371	-0.3	38.3
817.8738	$C_{56}H_{113}O_2$	817.8735	-0.4	12.1
819.8533	$C_{55}H_{111}O_3$	819.8528	-0.6	28.9
833.8687	$C_{56}H_{113}O_3$	833.8684	-0.4	37.1
845.905	$C_{58}H_{117}O_2$	845.9048	-0.2	16.8
847.8845	$C_{57}H_{115}O_3$	847.8841	-0.5	40.8
849.8637	$C_{56}H_{113}O_4$	849.8633	-0.4	3.5
861.9001	$C_{58}H_{117}O_3$	861.8997	-0.5	18.3
873.9363	$C_{60}H_{121}O_2$	873.9361	-0.2	10.5
875.9159	$C_{59}H_{119}O_3$	875.9154	-0.5	30.6
877.8952	$C_{58}H_{117}O_4$	877.8946	-0.6	9.8
885.9728	$C_{62}H_{125}O$	885.9725	-0.3	34.9
889.9313	$C_{60}H_{121}O_3$	889.931	-0.3	45
899.9884	$C_{63}H_{127}O$	899.9881	-0.3	33.4
901.9677	$C_{62}H_{125}O_2$	901.9674	-0.3	17.8
903.9473	$C_{61}H_{123}O_3$	903.9467	-0.6	23.8
905.9263	$C_{60}H_{121}O_4$	905.9259	-0.4	14.4
917.9263	$C_{61}H_{121}O_4$	917.9259	-0.4	11.8
917.9627	$C_{62}H_{125}O_3$	917.9623	-0.4	49.5
929.999	$C_{64}H_{129}O_2$	929.9987	-0.3	39.6
942.0355	$C_{66}H_{133}O$	942.0351	-0.4	41.1
945.9943	$C_{64}H_{129}O_3$	945.9936	-0.7	30.6
958.0305	$C_{66}H_{133}O_2$	958.03	-0.5	21.6
974.0254	$C_{66}H_{133}O_3$	974.0249	-0.5	34.2

986.062	$C_{68}H_{137}O_2$	986.0613	-0.7	18.9
988.0046	$C_{66}H_{131}O_4$	988.0042	-0.4	35.5
1016.0363	$C_{68}H_{135}O_4$	1016.0355	-0.8	21.1
1044.0674	$C_{70}H_{139}O_4$	1044.0668	-0.6	30.1
1072.0989	$C_{72}H_{143}O_4$	1072.0981	-0.7	17.2
1100.13	$C_{74}H_{147}O_4$	1100.1294	-0.5	28.5
1128.1615	$C_{76}H_{151}O_4$	1128.1607	-0.7	23
1142.1773	$C_{77}H_{153}O_4$	1142.1763	-0.8	34.7
1156.1928	$C_{78}H_{155}O_4$	1156.192	-0.7	11
1170.2085	$C_{79}H_{157}O_4$	1170.2076	-0.7	14.7
1184.2241	$C_{80}H_{159}O_4$	1184.2233	-0.7	9.5
1198.2396	$C_{81}H_{161}O4$	1198.2389	-0.6	40.5
1212.2552	$C_{82}H_{163}O_4$	1212.2546	-0.5	12.2
1226.2708	$C_{83}H_{165}O_4$	1226.2702	-0.4	31.9
1240.2866	$C_{84}H_{167}O_4$	1240.2859	-0.5	25.1
1254.3019	$C_{85}H_{169}O_4$	1254.3015	-0.3	44.6
1268.3174	$C_{86}H_{171}O_4$	1268.3172	-0.2	19.3
1282.3338	$C_{87}H_{173}O_4$	1282.3328	-0.8	44.2
1296.3497	$C_{88}H_{175}O_4$	1296.3485	-0.9	18.2
1324.3804	$C_{90}H_{179}O_4$	1324.3798	-0.5	28.9

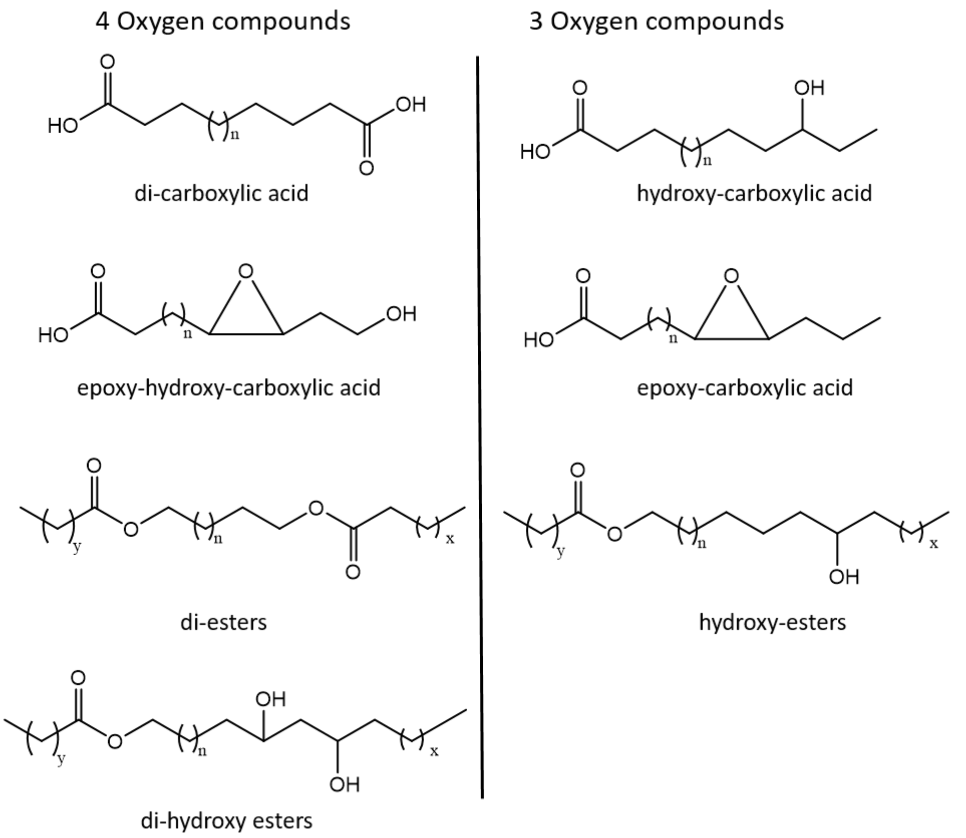


Figure 4-11 Some of potential 3 and 4 Oxygen atoms compounds in fraction A (240 bar, 60 °C).

Fraction B was dark yellow powder (Figure 4-6) and the DSC thermogram exhibited a sharp endothermic event centred at 81 °C and two shoulders at 75 °C and 59 °C (Figure 4-8). In term of melting profile, colour and texture, this fraction resembles the whole wax with no fractionation. The chemical composition in figure 4-7 shows that fatty alcohols (78.54 ±4.14 µg/g biomass), fatty acids (57.30 ±7.95 µg/g biomass) and aldehydes (27.44 ±0.75 µg/g biomass) are among the most abundant compounds in the extract. Interestingly, considerable amount of sterols (18.09 ±0.53 µg/g biomass) found in this extract which is the highest compared to the other extracts. Solubility studies of sterols in pure CO_2 showed an increase in solubility moving from CO_2 critical point to higher temperatures and pressures which can further explain the richness of extract B (80 bar, 60 °C) with sterols.[159] The abundance of sterols further added value to the fraction, as many of sterols are incorporated in nutraceutical products.[160, 161] Further test application for this fraction would be exciting to find the best application specially that the fraction exhibited high melting profile.

Fraction C was sticky in texture with greenish colour (Figure 4-6). The DSC profile showed broad multiple shoulders and endothermic minima centred at 61 °C and 69 °C (Figure 4-8). This can be explained by studying the chemical composition presented in Figure 4-7 and Table 4-1. Interestingly, more than 40% of fraction C was hydrocarbons (23.45 ±1.2 µg/g biomass), which are amongst the lower molecular compounds within the wax composition. Fatty acids (13.50 ±2.35 µg/g biomass) were the second most abundant followed by fatty alcohols (7.09 ±0.99 µg/g biomass). The high hydrocarbon content in this fraction might allow for relatively easy isolation and purification for tailored applications such as cosmetics and personal care products. Hydrocarbons are used by insect as mean of interaction and communication (insect semiochemicals) and hence they can be incorporated in neutral strategies to control the pest.[135]

It is noteworthy to indicate that the fractionation experiment was done on large scale, however, technical problem resulted from solidification or precipitation in the piping the experiment was run for only 1 hour hence lower quantities of individual compounds. Nerveless, the results reported here demonstrate promise with all the three fractions showing different melting ranges, colour and texture further emphasising the possibility of obtaining tailored wax fraction for a single feedstock. As well as the advantage of using $scCO_2$ in avoiding further purification and fractionation steps, which typically requires use of significant quantities of organic solvent and resources. Further work would focus on reducing the precipitation of wax during the extraction. With these promising results obtained for date palm leave wax, it is important to look at the economic aspects associated with the $scCO_2$ date palm leaves wax extraction in order to assess the viability of the process on the industrial scale.

4.4 Economics of $scCO_2$ extraction of date palm leaves wax

This section estimates the manufacturing cost of date palm leaves wax using $scCO_2$ extraction using a methodology proposed by Turton *et al*. Several studies employed this methodology to estimate cost of manufacturing associated with supercritical fluid extraction processes and reported its effectiveness and reliability.[240-242] It is assumed that the extraction facility will be based in the Middle East due to high availability of the date palm trees in the region and hence reducing the cost of transportation. Therefore, all the costs associated with the process were based on figures from this region such as transportation, electricity, raw materials and labour. Often, $scCO_2$ extraction is associated with high manufacturing cost, which prevents this technology from being used for the recovery of low value products and is only utilised for high value products. However, the advantages associated with $scCO_2$

extraction as green tool should outweigh the higher cost. Moreover, if the $scCO_2$ extraction is considered as a part of biorefinery system where extraction of biomass is the first step, will further reduce the cost.

4.4.1 Cost of manufacturing (COM)

Typically, three types of expenses involved in calculating the manufacturing cost for a chemical product Table 4-3 including:[243]

1) Direct costs (DC): these costs deal with the operating expenses that vary with rate of production. It includes cost associated with raw materials, operational labour, utility, waste treatment and others listed in table 4.3.[243]
2) Fixed costs (FC): these costs are not affected by the production level and they are charged at fixed rate even if the plant is not operational. It includes costs such as depreciation, insurance and taxes.[243]
3) General expenses (GE): these costs are not directly related to the manufacturing process and are associated with maintain the business including: administration cost, research and development and sale and distribution costs.[243]

Table 4-3 Typical individual expenses associated with the main three cost when calculating COM.

Direct costs (DC)	Fixed Costs (FC)	General Expenses (GE)
raw materials , waste treatment , operating labour, maintenance and repair, utility, direct supervisory and clerical labour, operating supplies, laboratory charge, patent and royalties	Depreciation, local taxes and insurance , plant related costs	administration costs , distribution and selling costs , research and development

Therefore the cost of manufacturing (COM) is sum of all above costs:

$$COM = DC + FC + GE \qquad \textbf{Equation 4-1}$$

The three costs to the COM can be determined in term of five main costs including:

1. Fixed capital investment (FCI)
2. Cost of operating labour (C_{OL})
3. Cost of utility (C_U)
4. Cost of waste treatment (C_{WT})
5. Cost of raw materials (C_{RM})

Typically, a range of multiplication factors are required to evaluate individual cost, with mid-point value is used for each range when no information is available (as proposed by Turton *et al.*) and reproduced in Table 4-4 .[243]

Table 4-4 Multiplication factors to estimate individual cost item associated with manufacturing cost.[243]

Cost item	Range of multiplication factors	Value used in text
1. Direct costs (DC)		
A. Raw materials	C_{RM}	C_{RM}
B. Waste treatment	C_{WT}	C_{WT}
C. Utilities	C_{UT}	C_{UT}
D. Operating labour	C_{OL}	C_{OL}
E. Direct supervisory and clerical labour	(0.1 – 0.25) C_{OL}	$0.18C_{OL}$
F. Maintenance and repair	(0.02 – 0.1)FCI	0.6FCI
G. Operating supplies	(0.1 – 0.2) Line 1.F	0.009FCI
H. Laboratory charge	(0.1 – 0.2) C_{OL}	0.15 C_{OL}
I. Patent and royalties	(0 – 0.6) COM	0.3COM
Total Direct costs (DC)	C_{RM}+ C_{WT}+ C_{UT}+ $1.33C_{OL}$ +0.03COM +0.069FCI	
2. Fixed costs (FC)		
A. Depreciation	0.1FCI	0.1FCI
B. Local taxes and insurance	(0.14 – 0.5)FCI	0.032FCI

C. Plant related costs	(0.5 0.7) (Line 1.D + Line 1.E + Line 1.F)	0.708 C_{OL} +0.36 FCI
Total fixed Costs (FC)	0.708 C_{OL}+ 0.68FCI + depreciation	
3. General Expenses		
A. Administration costs	(0.15)(Line 1. D + Line 1. E +Line 1. F)	0.177C_{OL}+0.009FCI
B. Distribution and selling costs	(0.2 – 0.2)COM	0.11COM
C. Research and development	0.05COM	0.50COM
Total General Expenses (GE)	0.177 C_{OL} +0.009FCI + 0.16COM	
Total costs (COM): C_{RM} + C_{WT} + C_{UT} + 2.215 C_{OL}+ 0.190COM + 0.146FCI + deprecation		

The three following equation can be generated upon applying the mid-point value for each cost category:

$$DC = C_{RM} + C_{WT} + C_{UT} + 1.33\ C_{OL} + 0.069\ FCI + 0.03COM \qquad \textbf{Equation 4-2}$$

$$FC = 0.708\ C_{OL} + 0.68FCI + depreciation \qquad \textbf{Equation 4-3}$$

$$GE = 0.177\ C_{OL} + 0.009\ FCI + 0.16COM \qquad \textbf{Equation 4-4}$$

Adding the three cost category and solving the equation for COM give the following equation

$$COM = 0.180FCI + 2.73C_{OL} + 1.23\ (C_{RM} + C_{WT} + C_{UT}) \qquad \textbf{Equation 4-5}$$

Adding the depreciation of 0.10FCI to the above equation gives the final equation as follow:

$$COM = 0.280FCI + 2.73C_{OL} + 1.23\ (C_{RM} + C_{WT} + C_{UT}) \qquad \textbf{Equation 4-6}$$

4.4.2 Fixed Capital cost (FCI)

The fixed capital cost (FCI) was calculated based on an industrial supercritical extraction unit which consists of two 0.4 m^3 extractor unit, a series of separator units for fractionation, CO_2 reservoir, a pump and a heater for CO_2 illustrated in simplified process diagram at Figure 4-12.

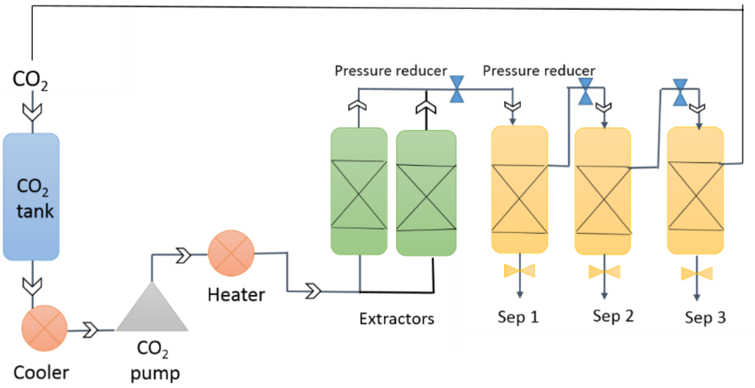


Figure 4-12 Simplified process diagram for the extraction process

The cost of such industrial unit is €1,400,000 for 2000 tonne per annum processing scale.[18, 240] The industrial unit price includes the cost of construction and set up of the planet as well as initial training based on an email communication with Apeks® Supercritical. The annual fraction of FCI calculated by taking 10% depreciation rate in account and hence the FCI multiplied by 10% as proposed by Turton *et al*.[243] The initial quantity of CO_2 required to fill the reservoir is considered among the FCI however, the cost is negligible compared to the cost of the extraction unit.[240]

4.4.3 Cost of labour (C_L)

The operating labour cost (C_{oL}) estimated in term of man-hour per operation hour. The total operation time was assumed 330 days per year and continuous 24 hours per day shift with total of 7920 h of extraction. Therefore two operator allocated per shift with the hourly wage of €5.00, which attributed to the work carried out solely with extraction of the waxes.[244] Typically, the overall wages will be higher considering that the plant is part of a biorefinery and the worker will have additional duties within the biorefinery. It is important to note that the hourly wage is based on the geographical location of the plant which is Middle East where the average wage for a skilled labour is in range (€5-8 per hour).[245]

4.4.4 Cost of raw materials (C_{RM})

The raw materials costs (C_{RM}) associated with all materials that are used in the production including the solid biomass, which contains the waxes and the CO_2 lost in the extraction process. The cost of biomass typically includes the price of the date palm leaves as well as all the cost associated in processing the date palm leaves making them appropriate for extraction including cleaning, drying, storage, milling and palletising. Date palm leaves can be considered as a by-product of current date production industries and therefore costs such as agricultural machinery, land renting and pesticides are minimised. Therefore, only the costs attributed to the biomass preparation (harvesting, storage, cleaning, pelletising and transportation) will be used. Typically, the date palm leaves are pruned from the tree as dry and can be used directly without any further drying. However, considering that most aired environment are dusty the leaves accumulate dust and debris. Therefore, it is necessary to wash the leaves with water to remove the dust and then air dry them and store them. It is assumed that the processed biomass will be transported at least 100 km. The total cost of milled date palm leaves was estimated to be €23.79 tonne^{-1} and €69.74 tonne^{-1} of pelletised biomass.[246, 247]

4.4.5 Waste treatment cost (C_{WT})

The cost of waste treatment (C_{WT}) is assumed to be negligible as the CO_2 is recycled and the only waste to be considered is the exhausted biomass and loss of CO_2 from the system on depressurisation.[240] The exhausted biomass can be utilised in a biorefinery system and can be used to produce further materials, chemicals or energy products, while the leaked CO_2 from system is negligible. Therefore, it can be assumed that the extraction process generates little to no waste and hence C_{WT} value is €0.

4.4.6 Utility cost (C_{UT})

The cost of the utility (C_{UT}) is associated mainly with the electric power needed for CO_2 pump, heater and refrigerator. The cost of electricity calculated utilising the enthalpy values of CO_2 at the extraction condition 400 bar, 100 °C. The bed density of milled date palm leaves was found to be 0.39 g cm^{-3}and it is estimated that for industrial scale 151 kg of milled date palm leaves can be loaded in the extractor unit. The flow rate for CO_2 at the laboratory based extraction unit was 2.4 kg hr^{-1} and the calculated flow rate for industrial scale process would be 3042.03 kg CO_2hr^{-1} or 20.1 kg CO_2 kg^{-1} milled biomass hr^{-1}. The cost of electricity was assumed to be €0.70/kwh. Therefore the cost associated with the CO_2 pump can be calculated as follow:

$$\boldsymbol{CO_2 enthalpy\ at\ 400\ bar, 100^{\circ}C\ = 378.88\frac{kJ}{Kg}CO_2}$$

$$\boldsymbol{1\ kg\ of CO_2\ = 378.88\ kJ\ CO_2}$$

$$\boldsymbol{3042.03\ kg\ of\ CO_2 = 1,152.564.34\ kJ\ CO_2}$$

$$\boldsymbol{P(kw) = \frac{1,152.564.33}{3600} =\ 320.17 kwh}$$

$$\boldsymbol{cost\ of\ electrcity = 320.17\ Kwh\ \times 0.07€\mathrm{K}wh^{-1} = €22.41/h}$$

Therefore the cost associated with CO_2 pump is €22.41/h.

Typically, CO_2 has to be heated from the 4 °C to the desired extraction temperature, which is 100 °C in the extraction of waxes from date palm leaves. Therefore, it is crucial to calculate energy cost associated with the heating process in CO_2 heater. This was done assuming 50% efficiency using the equation:

$$\boldsymbol{Q = MC_p \Delta T} \qquad \textbf{Equation 4-7}$$

Where Q is the energy, M is the mass of CO_2, C_p specific heat capacity of CO_2 and ΔT change in temperature of CO_2. The mass of CO_2 used in the extraction is 3042.03 Kg and the C_p at 100 °C is 0.871 kJ kg^{-1} k^{-1} and ΔT is 96 °C.

$$\boldsymbol{Q = 3042.03\ \times 0.871\ \times 96 = 2545362\ kJ}$$

Assuming 50 % efficiency then

$$\boldsymbol{Q = 508,725\ KJ\ per\ hour\ (508\ MJ)}$$

Therefore the energy required for the CO_2 heater is 508 MJ per hour. If the biomass after extraction is utilised to obtain the energy then it could cover the cost associated with the heater. The energy released when burning date palm leaves is 17.9 MJ Kg^{-1} therefore, burning 28.42 kg per hour or 18% of the post extracted biomass and thus the costs associated with the heaters are negligible.[248, 249]

The cost associated with refrigerators was calculated using the Equation 4-7. Again the M is the mass of CO_2 3042.03 Kg and the C_p at room temperature for CO_2 0.846 kJ kg^{-1} k^{-1} and ΔT is the temperature difference of the water (the fluid circulated in the loop) from room temperature 20 °C to 4 °C is 16.

$$\boldsymbol{Q = 3042.03\ \times 0.846\ \times 16 =\ 41176.9kJ}$$

Since refrigeration is more expensive than heating as it involves the use of electric power, it was estimated by calculating the coefficient of performance COP (COP at 20 °C and 4 °C obtained from Turton *et al.*)[243] as follow

$$\boldsymbol{COP\ 20^{\circ}C = 0.08}$$

$$\boldsymbol{COP\ 4^{\circ}C = 0.15}$$

$$\boldsymbol{Q =\ 41176.9kJ \times \frac{0.15}{0.08} = 77206.7}$$

$$\boldsymbol{P(kw) = \frac{77206.7}{3600} = 21.44\ kwh}$$

$$cost\ of\ electrcity = \ 21.44\ kwh\ \times 0.07€\text{Kwh}^{-1} = €1.50/h$$

Therefore the costs associated with the CO_2 refrigeration are €1.50 per hour extraction. The total cost of the utility C_{ut} is the cost associated with CO_2 pump and refrigeration are €23.91 per hour.

4.4.7 Total cost of manufacturing (COM)

The total cost of manufacturing (COM) for supercritical CO_2 extraction of waxes from date palm leaves can be calculated from Equation 4-6

$$COM = 0.280FCI + 2.73C_{OL} + 1.23\ (C_{RM} + C_{WT} + C_{UT})$$

$$COM = 0.280(1400000) + 2.73(79200) + 1.23\ (32750.79 + 0.0 + 18999384.95)$$

$$COM = 392000 + 216216 + 23409526.9$$

$$COM = \frac{€881495.57/year}{1378.46\ tonne/year} = €640\ per\ tonne\ date\ palm\ leaves$$

$$COM = €14.01\ per\ kg\ of\ wax$$

The total COM associated with extraction of wax from milled date palm milled leaves is estimated to be €14.01 Kg^{-1} of date palm wax Figure 4-13 shows simple mass balance for the extraction process.

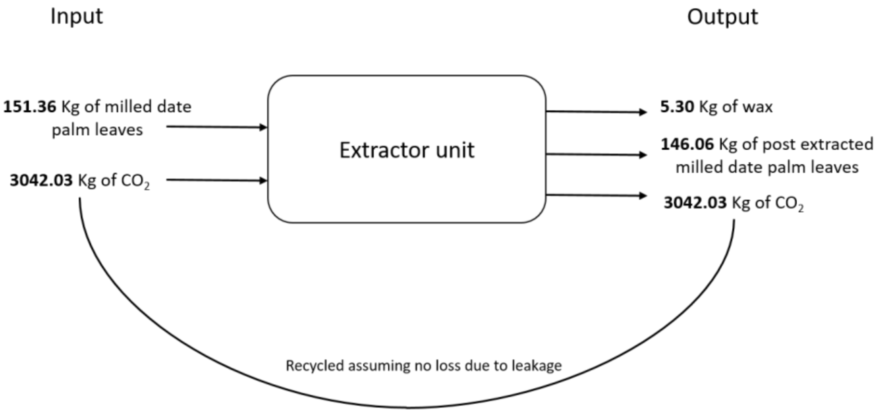


Figure 4-13 Simple mass balance for the extraction process.

The COM calculated above was based on milled date however; typical industrial scale plants load biomass as pellets. The process of pelletisition increase the C_{RM} and C_{UT} three fold as the palletisation enhances the bulk density of the biomass from 40-400 kg M^{-3} to 600-1,200 kg M^{-3} (almost three fold) and hence increase the loading capacity for the extractor unit.[20, 146, 250, 251]. The calculation for pelletised biomass shown below

$$COM = 0.280(1400000) + 2.73(79200) + 1.23\ (288401.42 + 0.0 + 568154.84)$$

$$\boldsymbol{COM = \frac{€1661780.20/year}{4135.38\ tonne/year} = €402\ per\ tonne\ date\ palm\ leaves}$$

$$\boldsymbol{COM = €8.80\ per\ kg\ of\ wax}$$

Although using pelletised biomass increase the cost of C_{RM} and C_{UT} however, the overall COM reduced to €8.80 kg^{-1} wax. It is noteworthy that the calculation of the raw materials C_{RM} was assumed to be for the supercritical extraction as standalone process. If scSCO$_2$ extraction was assumed as part of biorefinery where the post extracted biomass will undergo downstream processing then that will further reduce the raw material cost and hence the overall COM. Moreover, if the post extracted biomass were combusted to generate electricity as least added value step then this can result in further reducing the COM as calculated below. It was assumed that best available technologies used for the energy conversion with 43% efficiency.[252]

$$\boldsymbol{Mass\ of\ milled\ date\ palm\ leaves\ per\ extraction = 151.36\ kg}$$

$$\boldsymbol{Mass\ of\ pelletised\ date\ palm\ leaves = 15136\ \times 3 = 454\ kg}$$

$$\boldsymbol{Mass\ of\ the\ \ wax\ extracted\ (3.5\%\ yiled) = 454 \times 0.035 = 15.89\ kg}$$

$$\boldsymbol{Mass\ of\ post\ extracted\ leaves = 454 - 15.89 = 438.11\ kg}$$

For each extraction run 85.26 kg of date palm leaves is required to heat the extractors to cover the cost of heating pump and hence the available date palm leaves is given by following equation:

$$\boldsymbol{438.11 - 85.26 = 352.85\ kg\ avialble\ of\ pelletised\ date\ palm\ leaves}$$

Energy generated from pelletised date palm leaves combustion can be calculated as follow:

$$I\ kg\ of\ date\ palm\ leaves = 17.9\ MJ$$

$$17\ MJ\ \times 352.85 = 6230.92\ MJ/extraction$$

$$Electrcity\ generated\ assuming\ 43\%\ effcicency = 6230.92 \times 0.43 = 2679.29\ MJ$$

$$Electrcity\ generated\ assuming\ 43\%\ effcicency = 2679294.7\ KJ\ per\ extracion$$

$$Cost\ of\ electrcity\ Middle\ East = €0.07\ per\ Kwh$$

$$Vlaue\ of\ electrcity\ genertaed\ per\ extraction = P(Kw)$$
$$= \frac{2679294.7\ KJ}{3600} \times 0.67(time\ of\ extracion) \times €0.07\ Kwh^{-1} = €78.15$$

$$Value\ of\ electrcity\ per\ tonne\ of\ date\ palm\ leaves = €78.15\ \times \frac{1000}{454} = €172.14$$

$$Value\ of\ electrcity\ per\ kg\ of\ wax = €4.92$$

Therefore the COM after burning the post extracted biomass to generate electricity is determined by subtracting the value of electricity from the cost of wax production as follow

$$COM = €8.80 - €4.92 = €3.88\ per\ kg\ of\ wax$$

Therefore, the overall COM further decreased from €8.80 Kg^{-1} wax for pelletised date palm leaves to €3.88 kg^{-1} wax when burning the post extracted biomass to generate electricity. Other green technologies can be coupled in the biorefinery to add more value to the date palm leaves including microwave technology for pyrolysis to obtain chemical, fuel and energy. Studies showed that when scCO$_2$ extraction of the waxes performed as a first step in a holistic biorefinery resulted in enhancing the post extracted biomass for downstream process including fermentation and pyrolysis.[20, 146, 251] Therefore it is recommended that scCO$_2$ extraction of waxes is not carried as standalone technology but incorporated in a biorefinery system. Table 4-5 summarise the cost associated with manufacturing of wax from date palm leaves.

Table 4-5 Table cost associated with manufacturing date palm leaves wax from milled biomass and pelletised biomass

Parameters	Milled biomass	Pelletised biomass
Extraction pressure (bar)	400	400
Extraction temp (°C)	100	100
Enthalpy (kJ kg^{-1})	378.880	378.880
Loading (kg run^{-1})	151.360	454.080
Flowrate (kg kg^{-1}h^{-1})	20.098	20.098
cost electricity (€kwh^{-1})	0.070	0.070
Product (kg run^{-1})	115.881	347.642
FCI (€)	1400000	1400000
C_{OL} (hour)	5	5
C_{RM} (tonne)	23.79	69.74
C_{WT} (€)	0	0
C_{UT} (hours)	23.91	71.74
C_{UT} CO_2 Pump (hours)	22.41	67.23
C_{UT} CO_2 Heater (tonne hr^{-1} sawdust)	0.04	0.13
C_{UT} CO_2 refrigerator (hours)	1.50	4.50

C_{UT} biomass heater (tonne hr^{-1} sawdust)	0.0024	0.0072
wax year (tonne year^{-1})	64.73	194.20
COM per tonne date palm leaves (€ tonne^{-1})	640.28	402.26
COM per tonne wax (€ tonne^{-1})	13616.46	8554.63
COM per kg wax (€ kg^{-1})	**14.01**	**8.80**
Combusting post extracted date palm leaves		
Residual date palm leaf after extraction (kg run^{-1})		347.642
Residual date palm leaf after extraction (kg year^{-1})		0.348
Residual date palm leaf after extraction (tonne year^{-1})		4129.99
Energy generated burning dewaxed date palm leaf residue (MJ run^{-1})		6222.79
Electricity generated assuming 43% efficiency (MJ run^{-1})		2675.80
Electricity generated assuming 43% efficiency (kJ run^{-1})		2675799.74
Value of electricity generated (€ run^{-1})		78.04
Value of electricity generated (€ tonne^{-1} dewaxed date palm leaves)		224.50
Value of electricity generated (€ kg^{-1} dewaxed date palm leaves)		4.77
COM per kg wax with electricity generation (€ kg^{-1} of wax)		**3.88**

One-at-time sensitivity analysis was carried in order to get insight of the most influential parameters on COM Figure 4-14 . It was found that FCI have the greatest effect on the COM followed by C_{UT}, while the C_{RM} has the lowest effect on the COM. This is in agreement with reported on sensitivity analysis of $scCO_2$ extraction of waxes from maze stover.[241]

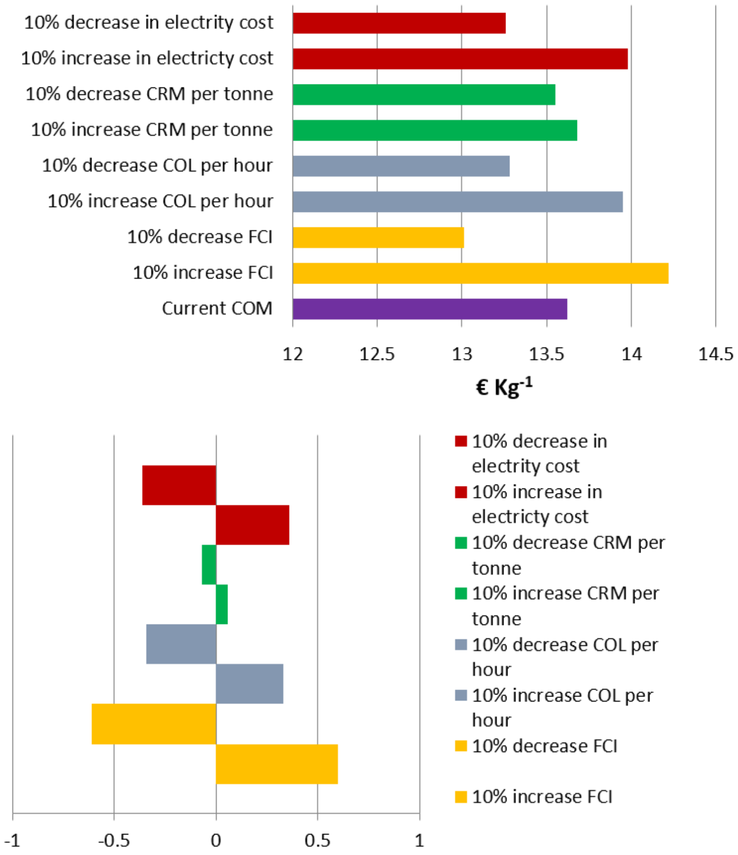


Figure 4-14 One-at-time sensitivity analysis (above) the total COM per Kg of extracted wax when increasing and decreasing the main associated costs by 10% (Below) the difference in COM in respect to the original COM by varying the individual costs parameters by 10%.

The cost of $scCO_2$ extraction of waxes from miscanthus and maiz stover along with date palm wax are listed in Table 4-6. It can be noted that the COM of date palm wax is much lower compared to COM of waxes obtained from miscanthus and maize stover whether the biomass used in milled form or pelletised form. This can be explained by taking in consideration several factors including the yield, the raw material cost as well as the utility cost. For instance, date palm leaves wax yield (3.49%) is massively higher than miscanthus stem (0.50%) and maize stover (0.86%), which indicate that the high yield of date palm wax played a crucial role in lowering the COM. Moreover, the COM of miscanthus and maize stover waxes were both based on Europe region while date palm wax COM is based in gulf region where utility cost and labour costs are significantly lower. Nerveless, the result obtained for COM of date palm wax show are promising and highlighting the feasibility of commercial production better than other biomass using $scCO_2$ extraction technology.

Table 4-6 The Cost of manufacturing waxes from miscanthus , maiz stover and date palm leaves using scCO2 extraction technology.

Product	% yield	Cost in € Kg^{-1} of wax (milled biomass)	Cost in € Kg^{-1} of wax (pelletised biomass)	Cost in € Kg^{-1} of wax (combustion of post extracted biomass)	Reference
Miscanthus wax	0.50	148.00	93.52	4.77	146
Maize stover wax	0.84	88.98	57.10	4.56	20, 241
Date palm leaves	3.49	14.01	8.80	3.88	This study

The US wax market import prices in 2015 for non-petroleum waxes including beeswax, candelilla and carnauba wax were €7.8/Kg, €2.4/kg and €6.0/kg respectively.[64, 253] Therefore, production of waxes from date palm leaf could be promising with the cost of manufacturing falling within the price range of commercial non-petroleum waxes.

4.5 Conclusion

Scale up of supercritical extraction of the wax from date palm leaves was carried out successfully. The yield, chemical composition and thermal profile were very similar to the lab based experiment, which further demonstrated the promise and viability of the scale up approach with a constant solvent to feed ratio. Despite the yield was comparable with lab based, it is believed that the actual yield might be higher as solidification of the wax in the pipeline occurred. Therefore it is crucial to carry on further research in term of the design of the extraction plant in order to overcome the solidification problem. Only 14% of the extracted wax was characterised by GC and GCMS and hence further analytical technique need to be considered to fully characterise the wax.

The second part of the chapter look at fractionation for date palm wax and the point of extraction. Three different fractions were obtained with different texture, colour, chemical composition and thermal profile highlighting further the possibility to tailor the wax composition with only $scCO_2$ fractionation. Fraction A obtained at high pressure 240 bar exhibited a very sharp melting at 90 °C, with a fine crystalline powder texture and very light yellow colour makes this fraction interesting in term of applications including organogels. In term of chemical composition APCI mass spectrometry demonstrated the presence of higher molecule compounds which cannot be analysed via GC. Hence, further analytical techniques should be explored to further understand the chemical composition of fraction A. Fraction B resembled the unfractionated wax in term of colour, texture and thermal profile. The chemical composition of this extract was rich with long chain fatty acids, fatty alcohols, fatty aldehydes and sterol making it interesting in food or nutraceutical related application as fatty alcohols and sterols are incorporated in many cholesterol reducing products. Fraction C obtained at lower pressure exhibited high percentage of hydrocarbons making it of potential interest for use in applications such as pesticides due to the previously reported semiochemical properties of waxes containing high proportional of hydrocarbons towards insects.[135]

The third part of the chapter discussed a techno-economic assessment of supercritical extraction of date palm leaves wax. The COM was estimated based on Turton *et al.* methodology and was based on five main cost including fixed capital cost (FCI), labour cost (C_{OL}), raw materials cost (C_{RM}), waste treatment cost (C_{WT}) and utility cost (C_{UT}). The COM for date palm leaves wax found to be €14.01 Kg^{-1} for milled date palm leaves. This cost calculated assuming that the date palm leaves feedstock was only utilised for the extraction of waxes. If $scCO_2$ extraction is viewed as first step of a holistic biorefinery where the biomass will be utilised in further down streaming process, then this can reduce the cost of the raw materials further and hence the COM. If the pelletised date palm leaves were considered exclusively for the extraction of the waxes that will further reduce the cost to €8.80 Kg^{-1} as pelletisation increase the bulk density and hence the loading capacity. If the pelletised biomass is combusted after extraction to harness energy then this will further reduce the COM to €3.88 Kg^{-1}. One a time sensitivity analysis showed that FCI and C_{UT} have the greatest effect on the COM, while CRM has the least effect. Compared to the other commercial natural waxes in the market the COM is very promising.

Chapter 5

Experimental

5. Experimental

5.1 Materials

Samples of date palm leaves (Phoenix dactylifera L.) were sourced from Oman. The samples were rinsed with water to remove the dust and dried in sun for 3 weeks. The dry leaflets were milled by a Glen Creston Limited hummer mill to pass through a 2 mm size screen prior to extraction and stored in paper bags.

Organic solvents dichloromethane, toluene, ethanol, methanol and ethyl acetate were analytical grade ≥99% and purchased from Fisher Scientific UK Limited. Liquid CO_2 cylinder with purity of ≥99% was obtained from BOC group and used with no further purification.

Supelco ASTM® D5442 C16-C44 qualitative retention time mix (contains 8.3% *w/w* each of: n-hexadecane, n-octadecane-, n-eicosane, n-docosane, n-tetracosane, n-hexacosane,n-octacosane, n-triacontane, n-dotriacontane, n-hexatriacontane, n-tetracontane and n-tetratetracontane), oleic acid (≥97%), linoleic acid (≥99%), 1-octacosanol (≥90%), dodecanal (≥95%), hentriacontane (≥98%), strearyl palmitate (≥99), stigmasterol (95%) N,O-Bis(trimethylsilyl)trifluoroacetamide (≥99%) were purchased from Sigma-Aldrich UK Limited. Tetradecane (≥99%) was used as internal standards purchased from Sigma-Aldrich UK limited. Carnauba wax grade 2 purchased from Acros Organics. Candelilla wax was purchased from Aldrich. Sunflower seed wax (Kahlwax2811, batch No: F1626035-002), rice bran wax (Kahlwax 6607L, Batch No.: F1631019-001) were kindly provided by H Foster & Co Ltd as gift samples. Sunflower oil was bought from a local supermarket Asda. TLC K60 silica gel aluminium plates obtained from Merck. , phosphomolybedic acid hydrate obtained from Sigma-Aldrich UK limited. Filter membranes, Isopore track-etched polycarbonate with 0.2 µm pore size and 25 mm diameter was obtained from Sigma-Aldrich UK limited.

5.2 Lab scale extraction of date palm wax with supercritical CO_2

The $scCO_2$ extraction was conducted with a Thar Technology (Pittsburgh, PA, USA) SFE 500 mL system, equipped with a high pressure pump, a back pressure regulator, an electric heater, a temperature controller, sample and product holder, and a PC computer for controlling extraction temperature, pressure and solvent flow rate. Liquid CO_2 from a cylinder (99.95 % purity; BOC) was used as solvent. Milled date palm leaf was placed in extractor vessel (100 g), heated to extraction temperature as in Table 5-1 controlled by thermostat and monitored by computer. The flow of CO_2 was set at 40 g min^{-1} and the pressure to desired value as in Table 5.1. ScCO_2 was passed through the extractor vessel for 4 hours. The scCO_2 was depressurised over a period of 20 minutes to 75 minutes depending on the extraction pressure. The extracted wax collected from stainless-steel separator vessel by rinsing twice with approximately 100 mL of toluene. The solvent was removed in vacuum to dryness. The wax product was weighed and the % yield calculated. The plant materials removed from the extractor vessel and the system was cleaned using 10% of toluene in dynamic mode for 45 minutes followed by 10% of ethanol for 20 minutes. The co solvent pump was then turned off and the CO_2 was allowed to pass through the system for additional 20 minutes to dry the system. For the optimisation of the scCO_2 extraction process the extraction conditions were chosen listed in Table 5-1 below.

Table 5-1 ScCO_2 extraction conditions for the extraction of date palm leaf.

Experiments	Temperature (°C)	Pressure (bar)
A	40	80
B	100	80
C	40	400
D	100	400
E	70	240

5.3 Semi-pilot scale extraction and fractionation of date palm leaves wax

The scCO_2 extraction of date palm leaves wax was carried out on semi-pilot scale using a Thar technologies SFE-500 (Pittsburgh, PA, USA). About 1.15 Kg of milled date palm leaves were loaded into 5 L extraction vessel. The extraction temperature and pressure were set (100 °C, 400 bar). The extraction vessel was heated to 100 °C and allowed to equilibrate for 10 minutes. An internal pump was used in order to achieve the chosen pressure of 400 bar and the pressure of the system was retained by ABPR. A flow rate of 150 g min^{-1} of liquid CO_2 and the extraction was carried out for 4 hours. At the end of the extraction the system is depressurised at rate 1 bar per 20 sec and the wax collected from separator unit.

5.4 Fractional extraction of date palm leaves wax

≈1.20 Kg milled date palm leaves were loaded in 5 L extractor vessel. The extraction temperature and pressure were set at (100 °C, 400 bar). The extraction vessel was heated to the target temperature and allowed to equilibrate while the pressure is obtained by internal pump. Three separator units employed with temperature set at 60 °C. The pressure of three separator vessels were set manually via manual back pressure regulator to 240 bar, 80 bar and 1 bar. A flow rate was set at 150 g min^{-1} of liquid CO_2. Due to wax crystallisation at the tubing the extraction was conducted for 1 hour. At the end of the extraction the system is depressurised. The pressure is manually released from the separator units 1 and 2 and the extracts is collected from the three separator units.

5.5 Study of time effect on extracted wax yield

For studying time effect on extraction yield a Thar Technology (Pittsburgh, PA, USA) SFE 500 mL system used (similar instrument for the lab based extraction). $ScCO_2$ extraction of dry milled date palm leaves (115.45 g) was carried out at 400 bar and 100 °C for 4 hours at constant flow of CO_2 at 40 g/min. The extract was collected after 30, 60, 120, 180, 240, 300 minutes. This is done by stopping temporarily the CO_2 pump after the above specified time and rinsing the separator vessel with 50- 100 mL of toluene. The solvent then removed by vacuum to dryness, the sample weighed to determine the yield and construct the dynamic extraction curve Figure 2-3.

5.6 Extraction of date palm wax with hexane using Soxhlet extraction

Milled dry palm leaf (10 g) was placed in cellulose thimble and placed in Soxhlet apparatus fitted with a round bottom flask containing 200 mL heptane. The solution was heated at 120 °C and allowed to reflux for 5 hours. Soxhlet extracted with 250 mL heptane for 5 hours. The resulting solution was filtered immediately using fluted filter paper to remove any leaf residue and concentrated to dryness by rotary evaporator. Extracts were air dried at room temperature to remove trace solvent and moisture. The extracts were weighed until constant weight achieved. The extraction was carried out in duplicate and average % yield calculated.

5.7 Preparation of oleogels from date palm leaves wax and commercial waxes

1-4 % (*w/*w) of date palm ($scCO_2$ extracted at 100 °C, 400 bar), carnauba, candelilla, rice bran and sunflower waxes were dispersed in 20 mL of sunflower oil. The mixture was stirred at 200 rmp and heated to 90 °C for 30 minutes making sure all waxes dissolved. The hot solution was then poured in small vials and cooled at room temperature for 1 hours and then at fridge at 5 °C for 24 Hours. The formation of the gel is confirmed by flipping the vials. Minimum concentration required to gel sunflower oil of each wax is used thereafter in subsequent measurements from section 5.14 to 5.18.

5.8 Gas chromatography (GC) analysis for the waxes.

Approximately 20 mg of wax sample has been analysed by GC. The samples have been analysed by HEWLET PACKARD HP 6890 Series GC system. The column used for analysis was Zebron DB5HT with following dimensions: 30.0 m × 0.25 mm × 0.25 µm and 15.0 m × 0.25 mm × 0.25 µm. The inlet temperature set up at 340 °C with a total flow of 26.4 mL. The sample injection volume was 1 µL and the split ratio of 5:1. The initial oven temperature was 60 °C held for 1 min. and ramped at 8 °C min^{-1} until temperature reaches 360 °C, which held for 30 minutes. The carrier gas was Helium with flow of 2.2 mL min^{-1} and average velocity of 55 cm sec^{-1}. Flame ionization detector was used and the temperature of the detector set up at 360 °C. Nitrogen was used as make up gas with flow 32.8 mL min^{-1}.

Quantification of the key wax compounds were carried out by mean of internal standard and extremal standards calibration curves. The calibration curves were plotted with a minimum of five different concentrations, with a standard along with tetradecane as an internal standard. The standards were chosen to be as close as possible to the corresponding wax components in term of chain length and/or functional group. The area and mass ratio were calculated according to equation 1, 2.The best line fit was generated from the area and mass ratio.The R_2 was obtained.

$$\boldsymbol{Area\ ratio} = \frac{\boldsymbol{area\ of\ standard}}{\boldsymbol{area\ of\ internal\ standard}} \qquad \textbf{Equation 5-1}$$

$$\boldsymbol{Mass\ ratio} = \frac{\boldsymbol{mass\ of\ standards\ in\ 1\ mL\ (mg)}}{\boldsymbol{mass\ of\ internal\ standards\ in\ 1\ mL\ (mg)}} \qquad \textbf{Equation 5-2}$$

The standards that were used were oleic acid, linoleic acid, 1-octacosanol, dodecanal, hentriacontane, hexadecanoic acid octadecyl ester (Palmitic acid stearyl ester), stigmasterol and phytol. Silylated calibration curves were also produced for fatty acids, alcohols and phytol.

5.9 Derivatisation for GC analysis of waxes.

Derivatisation was carried for wax samples in order to increase the volatility, enhance peaks shape and help in identification. Trimethylsilylation of the wax sample was carried out using 200 µL N,O-Bis(trimethylsilyl)trifluoroacetamide with trimethylchlorosilane and 1 mL of toluene were added to about 20 mg of wax sample. The reaction was completed by heating the sample on a hot plate at temperature 70 °C for 30 minutes. The sample was allowed to stand for a few minutes prior to GC and GC/MS analysis.

5.10 Gas chromatography /mass spectrometry (GC/MS) procedure for analysis of waxes

The extracted wax samples (approximately 20 mg mL^{-1} toluene) were analysed by GC/MS. The samples have been analysed by Perkin Elmer Clarus 500 Gas chromatography coupled with by Perkin Elmer Clarus 560 Mass spectrometry equipped with split injector and an auto-sampler. The high temperature capillary column was analysis was DB5HT with the following dimensions: 30 m × 0.25 mm × 0.25 µm. Helium was used as carrier gas with flow rate of 1 mL min^{-1}. The initial oven temperature was 60 °C held for 1 min and then ramped at 8 °C min^{-1} to 360 °C where it held for 30 min. The injection volume was 0.5 µL. The Perkin Elmer Clarus 560 Mass spectrometry was in electron ionisation mode at 70 eV. Compounds were identified by comparing their spectra with the library.

Table 5-2 Mass spectra fragmentation of some of the compounds identified within date palm wax.

Compounds	Mass spectra fragmentations m/z (intensity)
Hexadecanoic acid	328 (10), 313 (16),145 (30), 132 (64), 117 (99), 73 (100), 55 (24), 43 (30)
Octadecanoic acid	356 (18), 341 (68), 145 (34), 132 (65), 117 (100), 73 (100)
Octacosanoic acid	497 (29), 482 (70), 145 (48), 132 (70), 117 (90), 73 (100), 53 (54), 43 (60)
Dotriacontanoic acid	553 (42), 538 (65), 145 (62), 132 (88), 117 (100), 73 (99), 43 (38)
Tetratriacontanoic acid	581 (40), 566 (39), 145 (60), 132 (90), 117 (100), 73 (99), 57 (54), 43 (52)
Octadecanoic acid	356 (8), 339 (48), 145 (25), 129 (55), 117 (65), 73 (100), 55 (45), 43 (25)
Octadecdienoic acid	352 (15), 337 (95), 262 (50), 178 (25), 156 (38), 135 (45), 95 (50), 81 (100), 67 (98)
Octacosanol	468 (100), 75 (52), 57 (40), 43 (38)
Triacontanol	496 (100), 75 (60), 57 (30), 43 (28)
Dotriacontanol	528 (100), 508 (8), 75 (75), 57 (65), 43 (43)
Tetratriacontanol	552 (100), 536 (5), 75 (80), 57 (72), 43 (55)
Stigmasterol	495 (10), 48 (13), 432 (10), 394 (18), 255 (18), 129 (39), 96 (40), 83 (100), 69 (72), 57 (80), 43 (80)

B-sitosterol	486 (34), 471 (10), 396 (60), 381 (25), 357 (60), 145 (32), 129 (100), 73 (60), 43 (80)
Octacosane	395 (5), 97 (30), 85 (74), 71 (90), 57 (100), 43 (60)
Nonacosane	409 (3), 97 (35), 85 (77), 71 (92), 57 (100), 43 (77)
Triacontane	420(5), 97 (78), 85 (74), 71 (80), 57 (99), 43 (100)
Hentriacontane	436 (3), 97 (30), 85 (75), 71 (82), 57 (100), 43 (95)
Dotriacontane	448 (5), 97 (90), 83 (61), 71 (63), 57 (75), 43 (100)
Tritriacontane	464 (6), 97 (29), 85 (77), 71 (92), 57 (75), 43 (100)
Dotriacontanal	464(3), 446 (17), 82 (80), 96 (75), 57 (75), 43 (100)
Tetratriacontanol	493 (2), 474 (17), 82 (100), 96 (88), 57 (68), 43 (80)
WE46	465 (2), 420 (5), 355 (3), 285 (10), 257 (95), 97 (100), 83 (95), 71 (75), 57 (90), 43 (65)
WE48	494 (8), 449 (5), 257 (100), 97 (65), 83 (55), 71 (41), 57 (60), 43 (45)
WE50	522 (5), 477 (8), 285 (10), 257 (100), 97 (78), 83 (74), 71 (55), 57 (65), 43 (45)
WE52	369 (3), 341 (4), 313 (42), 285 (20), 264 (20), 97 (99), 83 (100), 71 (90), 57 (88), 43 (42)

5.11 Atmospheric pressure chemical ionisation mass spectrometry

The atmospheric pressure chemical ionisation (APCI) mass spectrometry was carried for fraction 1 (collected at 240 bar, 60 °C) by Karl Heaton from Centre of Excellence in Mass spectrometry at York. This was done on Burker Compact^{TM} Time of flight (TOF) mass spectrometry with APCI capillary voltage of 4.5 kV. Nitrogen was used as nebuliser dry gas at flow rate of 2.5 L min^{-1}. The dry gas temperature was 250 °C and the vaporizer temperature set at 400 °C. The sample was introduced by syringe infusion at 180 µL min^{-1}. Spectra were obtained over the range of m/z 250–1350, with a scan speed of 1000 amu s^{-1}.SmartFormula® software was used to predict the formula with the following parameters: any number of carbon atom C, any number of hydrogen atom H, maximum number of Oxygen atom O is 4, no nitrogen atom N allowed in formula, no Sodium atom Na allowed in formula, maximum mass measurements error was 5 ppm and maximum msigma value of 50 allowed.

5.12 Scanning Electron Microscopy (SEM) for date palm leaflets.

Intact date palm leaflet, leaflet scratched with sharp blade, leaflets milled socked in toluene for 48 hours, milled leaflet sample and milled leaflets after $scCO_2$ extraction samples were coated with pallidum in order to make them conductive in the SEM. A fully computer-controlled scanning electron microscope (SEM - JEOL JSM-6490LV) with a tungsten-heated filament was used to scan each palladium-coated specimen. A specimen was sealed in the SEM chamber and the air was evacuated. Each specimen was scanned with electron beam 30 kV in high vacuum.

5.13 Differential scanning calorimetry (DSC) analysis

Thermal analysis has been conducted with the modulated DSC; TA Instruments MDSC Q2000 Thermal Analysis. The samples (3-6 mg) were weighed in T-Zero standard aluminium hermetic pans and placed in a test cell along with a reference pan. The samples were heated from 25 °C and ramped at 10.00 °C min^{-1} to 100 °C where they held for 1 min at this temperature. Next, the sample cooled to -80.00 °C in a rate of 5.00 °C min^{-1} and held at -80.00 °C for 1 min. Then , they were heated to 100 °C at a rate of 5.00 °C min^{-1}. DSC curve of the last DSC cycle was used in melting point determination.

5.14 Oil Binding Capacity for wax based oleogels

Oil binding capacity was measured according to method reported by Blake and Marangoni[188] where 1.0 -1.5 g of wax based oleogel is placed in 15 mL plastic centrifuge vials and centrifuged for 15 min. and 60 min. in Thermo Scientific Heraeus Megafuge 40R centrifuge at a speed of 3500 rpm at 20 °C . The vials were weighted before and after adding the oleogel. After each centrifuge cycle the realised oil decanted by inverting the vial for 1 min. and weighting the vials. Oil loss percentage was reported as mean of three replicate measurements. The percentage oil loss is calculated according to equation 5-3.

$$\boldsymbol{Oil\ loss}\ (\%) = \frac{\boldsymbol{initial\ sample\ mass - mass\ after\ decanting\ oil}}{\boldsymbol{initial\ sample\ mass}} \times \mathbf{100} \qquad \textbf{Equation 5-3}$$

5.15 Rheological characterisation

All rheological measurements were done on controlled stress Malvern Pro rheometer. Preliminary stress sweep test carried out with shear strain from 0-100% at studied temperatures 5 °C in order to determine the linear visco elastic (LVER) region. All subsequent test used the % strain value determined from this test. Frequency sweep test was carried out at 0.02% strain at 1-100 Hz at set temperature. Temperature sweep test were carried out from either 90 °C to 5 °C at 1 Hz and 0.02% shear strain in order to study the gelling temperature. Stress yield for all samples were carried out from 0-200 Pa. Three Interval Thixotropy Test (3ITT) were performed by alternating low (0.1 s^{-1}, 10 minutes), high (1 s^{-1}, 10 minutes) and low (0.1 s^{-1}, 10 minutes) shear rate at the set temperature. Flow frequency test was carried out by first subjecting the sample to a shear rate ramp from 0.01 s^{-1} to 20 s^{-1} and then frequency sweep at 0.2% strain and 0.01-10 Hz at 5°C.

5.16 Optical microscopy

The microstructure of the wax was studied with normal light microscopy Axiovert200 Zeiss equipped with AxioCam HRC camera and halogen lamp HAL 100. All microscopic images taken with highest magnification lens (x63). The oleogel sample, microscopic slide, and slipcover were heated at 90 °C. A very thin of hot liquid oleogel is smeared on microscopic slide and then covered with coverslip. The slide was cooled at room temperature for 24 hours and then the microscopic images taken.

The normal light and bright filed microscopic images for the fraction A powder were obtained using Leica D6E stereo microscope equipped with EC3 camera. The images taken at 40x magnification.

5.17 Scanning electron microscopy of oleogels samples

Oleogels samples prepared by heating the oleogels at 90 °C and a very thin layer of the mixture was deposited on a poly carbonate membrane and allowed to cool at room temperature for 24 hours. The oleogels samples were then sprayed with palladium nanoparticles. The samples were scanned with electron beam 30 kV in high vacuum sealed chamber. A fully computer-controlled scanning electron microscope (SEM - JEOL JSM-6490LV) with a tungsten-heated filament was used to scan each palladium-coated oleogel samples.

Chapter 6

Conclusions and future work

6. Chapter 6 Conclusions and future work

6.1 Conclusions

In this study, waste date palm leaves were explored for their lipid content with the aim to add value to this waste under exploited biomass. $ScCO_2$ extraction of waxes from date palm leaves were carried out successfully and an experimental design developed to optimise the extraction. The best extraction condition where the highest yield (3.49%) achieved were 400 bar and 100 °C. The extracted waxes at various temperature and pressure were characterised and quantified by GC and GC/MS. The main groups of compounds identified were free fatty acids, long chain fatty alcohols, fatty aldehydes, hydrocarbons, sterols and wax esters. All extracts exhibited similar compositions however; the abundance of these compounds differed significantly with extraction conditions with greater temperatures and pressures favouring the extraction of higher molecular compounds including wax esters, higher chain aldehydes, alcohols and fatty acids. While the lower temperature and pressure extraction conditions enhanced the extraction of hydrocarbon and sterols, which are amongst the lower molecular compounds within the wax composition. Different extracts revealed different thermal profiles, with melting points in range 30 °C to 79 °C. The possibility of tuning wax composition and properties opens up the opportunity to tailor the wax extraction to meet the needs of specific applications. This is a distinct advantage of $scCO_2$ over conventional organic solvent extraction as a simple manipulation of pressure and temperature in $scCO_2$ can produce waxes with different properties, while only one type of wax can be obtained with conventional organic solvents.

$ScCO_2$ extraction was compared with heptane Soxhlet extraction. There were significant differences in chemical composition, with $scCO_2$ showing the enhancement over heptane for the extraction of fatty alcohols and fatty aldehydes, which can be nutraceutical products. For the first time, this study reported $scCO_2$ extraction as green and clean extraction technology for date palm leaves with relatively high percentage yield compared to other agricultural residues. Moreover, the high melting point of date palm leaf wax makes it an extremely hard wax, which can potentially applications in the replacement of many less sustainable natural waxes including candelilla and carnauba waxes where their production is restricted to geographical region.

Natural waxes are known for their ability to gel; vegetable oil which have many applications in food industry as shortening fat and in confectionary. The ability of date palm leaves wax (extracted with $scCO_2$ at 400 bar and 100 °C) to gel sunflower oil was explored.

The minimum gelling concentration of date palm leaves wax to gel sunflower oil was 2%, which was in good agreement with other commercial waxes and was in fact lower than carnauba and sunflower waxes making it more efficient at lower concentrations needed. The oil binding capacity for the oleogel from date palm wax showed weaker oil retention compared to candelilla, rice bran and sunflower waxes and better oil retention than carnauba wax. It is believed that chemical composition and crystal morphology for the wax plays role in imparting gelling properties. Date palm wax showed mixture of different compounds with high alkyl chain and it is believed that they played role in imparting the gelling properties for the wax.

The crystal morphology was a platelet like structure, which is proven to be the preferred morphology for waxes to gel oils. Gel prepared from date palm wax showed high thermal stability making it promising for application, where extra thermal stability is crucial like in the confectionary industry.

The rheological measurements were carried out to further explore the gelling efficiency and the rheological properties for date palm wax based oleogel. These properties were comparable to other natural commercial waxes. Date palm wax based oleogel showed lower stress yield compared to sun flower and rice bran waxes oleogels and higher than carnauba and candelilla waxes oleogels. Frequency sweep test for date palm oleogel showed that the gel is stable at lower frequency range, however structure breaks down result at higher frequencies. The temperature sweep showed higher temperature stability compared to sunflower, rice bran and candelilla waxes. Date palm wax oleogel showed weak recovery with only 40% recovered in three interval thixotropic test. This is a first time date palm wax tested for the gelling of vegetable oil and open up doors for future studies in order to improve the gelling properties and optimise the gel formation. Moreover, due to the nature of the weak gel many factors can affect the gelling properties including, type of liquid oil, chemical composition and morphology. Further works need to better understand the gelling mechanism by studying the chemical and physical interaction between liquid oil and wax molecules to optimise the gel preparation.

The successfully extraction of wax from date palm leaves with unique properties in term of high yield, high melting profile and chemical composition was demonstrated by $scCO_2$ extraction. In order to take this work further, it was crucial to investigate aspect s including scale up, fractionation and the economic assessment for the manufacturing of date palm leaves wax. The semi-pilot scaling up for $scCO_2$ extraction of date palm leaves wax was carried successfully with a yield consistent to the lab scale study. The chemical composition was comparable to the lab scale and slight variation is owed to the natural variation within the biomass. It is crucial to improve the design of the extraction unit or further optimise the extraction process to overcome precipitation of the wax in the tubing. Only 14% of the wax identified by GC and GC/MS, therefore further analytical tools need to be investigated in order to understand the true wax composition, which may give further insight into potential applications for this product.

Date palm leaves wax is complex mixture of hydrophobic molecules and hence it was vital to reduce the complexity of the wax. This is the first instance where a fractional separation with $scCO_2$ extraction technology was reported for date palm leaves wax. Three different extracts obtained by dropping the pressure of the separators units successively. The three fractions differed in their physical characteristics such as colour, texture and melting profiles. The chemical composition of each extracts was determined with GC and GC/MS and it is observed at higher pressure higher molecular compounds extracted including long chain wax esters, long chain fatty acids, fatty alcohols and aldehydes. While the lower pressure favoured extraction of smaller molecular compounds within the wax composition and hence the fraction C was rich in hydrocarbons. The results obtained are promising and opening up opportunity to tailor the extraction process to suit a particular application. Nerveless, further work on the chemical composition are essential to get further insight in the wax composition.

It was essential to estimate the associated cost of manufacturing (COM) for the wax extraction from date palm leaves to further develop the work and prove its viability. The COM was estimated based on methodology proposed by Turton *et al* [243] whereby the COM was estimated based on five main costs including; fixed capital cost (FCI), raw materials cost (C_{RM}), labour cost (C_{OL}), utility cost (C_{UT}) and waste treatment cost (C_{WT}). The costs estimated for an industrial supercritical plant based on the Middle East region with capacity of 2000 tonne of biomass per annum. The COM estimated to be €14.01 Kg^{-1} if milled date palm leaves used in the extractor unit. This COM can be further reduced by introducing the biomass as pellet to €8.80 Kg^{-1}. Although, the pelletisation process increase the C_{RM} and C_{UT} to three times than the milled biomass, however, the overall COM reduced significantly. If $scCO_2$ extraction wax is integrated in the concept of biorefinery where the exhausted biomass undergo further down streaming process then this will further reduce the COM as many of the costs then will be shared. If combustion of post extracted biomass is considered as least favourable to harness the energy then the COM of date palm wax is further reduced to €3.88

Kg^{-1}. One-at-time sensitivity analysis revealed that the main costs influence the COM are FCI followed by C_{UT}. Compared to other commercial natural waxes, date palm wax COM is promising well suited in the current wax market prices.

6.2 Future work

6.2.1 Further characterisation and quantification for date palm wax and the fraction A

A significant portion of date palm leaves wax was unidentified and in some cases can account for 90% of the extract in case of fraction A or 80% of the whole wax due to the limitation of the analysis technique. It is believed that there are higher molecular compounds within the wax composition which are beyond the GC and GC/MS and therefore it is crucial to explore other analytical tools to have a better insight into the wax composition. Some of the analytical techniques which might assist the characterisation and quantification of the higher molecular compounds such as high performance liquid chromatography, supercritical chromatography and size exclusion chromatography coupled with advance mass spectrometry techniques including MALDI, APCI would give better insight into wax composition. Obtaining a better insight into the composition will help find appropriate applications for the date palm wax as well as optimising the fractional extraction for tailored application.

6.2.2 Toxicity tests for date palm leaves wax

In this study, date palm leaves wax showed a good gelling properties for liquid vegetable oils which are used in food industries. Therefore, it is crucial to do further toxicity test to assure the safety of the wax for food applications. In addition it would be of significant interest to analyse for pesticides or herbicides which may restrict the use of such waxes in food applications.

6.2.3 Optimisation of oleogel prepared from date palm wax and potential applications of the oleogl

Date palm wax showed a good ability to gel sunflower oil and preliminary results suggesting that it can be potentially implemented in food industry as shortening agent and confectionary. Therefore, it is crucial to work further in optimising the preparation of oleogels which can be done from several aspects. Firstly, the liquid oil plays a major role in the final oleogel properties therefore screening for variety of vegetable oils used in food industry will further enhance the gelling properties. Secondly, the homogenous chemical composition of the wax is believed to impart better gelling properties, therefore it would be interesting to reduce the complexity of the wax by aiming to have more homogenous mixture or fraction. Hence, it would be interesting to test the different fractions of the date palm wax obtained from fractional extraction specially fraction A, which showed more homogenous composition and crystalline melting profile. Thirdly, optimising the experimental condition when preparing the oleogel that might be applying sheer during preparation as some studies showed applying sheer rate when the hot liquid oil and wax mixture is cooling down improved the properties of the gel.[213]

Oleogel are considered weak gel and the true mechanism for the gelling is not well understood. Understanding the mechanism of the gelling might provide further insight into the best practices to prepare the oleogel. This involve studying the chemical and physical interaction between the wax molecules (gelator) and the liquid oil (solvent) and between gelator-gelator themselves.

In the current study the rheological profile for date palm wax oleogel was promising and well suited with other natural waxes oleogel. Therefore, future work might look in application of the oleogel in food industries more specifically as shortening agent in baking products and confectionary.

6.2.4 Other potential application of date palm wax

Date palm wax exhibited high melting point in range of 79 -81 °C making it among hard waxes and very close to carnauba wax (the hardest natural wax with melting point 82 °C). This high melting profile open up opportunities in various potential applications as hydrophobic coating agent, hardening agent in cosmetics, in polishes, car waxes and for musical instruments etc. Therefore, it would be interesting to further establish applications for this wax. Initial results from experimental design and fractional extraction indicated the possibility of having wax with different composition and physical properties. Therefore, future work can look into exploring the possibility of having tailored extracted waxes to suit to suit specific application.

6.2.5 Optimisation of the fractional extraction of date palm leaves wax

Fractionation of the date palm wax showed interesting result in term of obtaining fractions with different composition and physical properties. This opens new opportunities to further enhance the separation by increasing number of separator units to obtain higher value products, which can have potential application in pharmaceutical, nutraceutical, food, cosmetic and personal care industries. In this study temperature was fixed and pressure was dropped successively in the separators units. Thus, it would be interesting to explore fixing the pressure and varying the temperature in the separator units and observe the effect on the fractions.

6.2.6 Explore the downstream process for post extracted biomass and effect of $scCO_2$ on the downstream processing.

Downstream process for the post extracted biomass have not been investigated in this study. Therefore, future work could look at utilising the post extracted biomass with the aid of green technologies (such as microwave technology) to further add more value with the aim of zero waste. It would be interesting as well to see how $scCO_2$ extraction affect the biomass for the downstream process.

6.2.7 Design improvement for the $scCO_2$ extraction unit

The scale up on semi-pilot level was carried out successfully, however a problem of the crystallisation of the wax in the tubing need to be considered and further explored. Future work can look into improving the design of the extraction unit in order to avoid crystallisation of the wax in tubing. This can be done possibly by heating the tubing to desired temperature or/and using co solvent (such as vegetable oils or ethanol) to enhance the solubilisations of the waxes and overcome the precipitation.

Chapter 7

Appendices

7. Appendices

7.1 Appendix 1 Samples of rheological data

Table 7-1 Amplitude sweep test for date palm wax based oleogel SFDW2% carried at 20 °C and 1 Hz.

Complex shear strain (%)	First measurement		Second measurement		Third measurement		Average values	
	G’ (Pa)	G” (Pa)	G’ (Pa)	G” (Pa)	G’ (Pa)	G” (Pa)	G’ (Pa)	G” (Pa)
9.99E-03	1.61E+04	2.27E+03	4.44E+03	605.7	4.46E+03	682.8	8.33E+03	1.19E+03
0.01268	1.61E+04	2.19E+03	4.43E+03	639	4.44E+03	711.9	8.33E+03	1.18E+03
0.015762	1.60E+04	2.32E+03	4.41E+03	643.3	4.40E+03	718.1	8.27E+03	1.23E+03
0.019708	1.60E+04	2.28E+03	4.39E+03	635.8	4.37E+03	720.1	8.25E+03	1.21E+03
0.024803	1.59E+04	2.25E+03	4.35E+03	648.7	4.27E+03	719.2	8.17E+03	1.21E+03
0.031559	1.58E+04	2.25E+03	4.29E+03	658.1	4.22E+03	737.3	8.10E+03	1.22E+03
0.039626	1.56E+04	2.31E+03	4.20E+03	670.5	4.13E+03	744.7	7.98E+03	1.24E+03
0.049824	1.54E+04	2.31E+03	4.10E+03	681.4	4.01E+03	756.3	7.84E+03	1.25E+03
0.062659	1.50E+04	2.35E+03	3.98E+03	696.9	3.84E+03	770.6	7.61E+03	1.27E+03
0.079212	1.45E+04	2.37E+03	3.83E+03	709.5	3.70E+03	772.2	7.34E+03	1.28E+03
0.099899	1.40E+04	2.40E+03	3.65E+03	717.9	3.53E+03	771.3	7.06E+03	1.30E+03
0.125851	1.32E+04	2.42E+03	3.45E+03	721	3.31E+03	772.1	6.66E+03	1.30E+03
0.158488	1.24E+04	2.45E+03	3.23E+03	720.5	3.09E+03	766	6.24E+03	1.31E+03
0.199767	1.13E+04	2.48E+03	2.99E+03	715	2.84E+03	755.5	5.71E+03	1.32E+03
0.251801	9.96E+03	2.54E+03	2.74E+03	701.6	2.58E+03	739.1	5.10E+03	1.33E+03
0.316964	8.52E+03	2.56E+03	2.48E+03	679.9	2.31E+03	715.8	4.44E+03	1.32E+03

0.399747	7.14E+03	2.53E+03	2.20E+03	647.8	2.05E+03	684.3	3.80E+03	1.29E+03
0.503429	5.81E+03	2.43E+03	1.94E+03	614.6	1.77E+03	642.1	3.17E+03	1.23E+03
0.634	4.61E+03	2.27E+03	1.69E+03	575.1	1.51E+03	591	2.60E+03	1.15E+03
0.799279	3.59E+03	2.09E+03	1.45E+03	531	1.26E+03	540.2	2.10E+03	1.05E+03
1.00697	2.74E+03	1.91E+03	1.22E+03	486.9	1.04E+03	493.8	1.67E+03	9.64E+02
1.2663	2.05E+03	1.74E+03	1.00E+03	444	843.8	448.3	1.30E+03	8.77E+02
1.59406	1.52E+03	1.55E+03	819.5	403.8	678	404.2	1.01E+03	7.86E+02
2.00855	1.12E+03	1.36E+03	660.4	367	536.1	363.7	7.72E+02	6.97E+02
2.52856	843.5	1.19E+03	525.3	332	422.3	324.9	5.97E+02	6.16E+02
3.18863	638.1	1.01E+03	415.1	298.8	331.2	287.6	4.61E+02	5.32E+02
4.01742	495.3	861.2	331.5	266.5	260.2	252.6	3.62E+02	4.60E+02
5.06576	388.6	720.7	267.5	234.4	203.9	219.4	2.87E+02	3.92E+02
6.39009	306.9	597.1	217.2	204.1	161.5	191.1	2.29E+02	3.31E+02
8.06605	242.2	485.7	176.2	177	129.5	166.2	1.83E+02	2.76E+02
10.1754	189	388.5	142.6	154	105.2	144.4	1.46E+02	2.29E+02
12.8302	145	304.9	114.7	133.7	85.7	124.7	1.15E+02	1.88E+02
16.1595	108.6	234.9	91.99	116	69.52	106.8	9.00E+01	1.53E+02
20.3357	78.94	175.4	73.66	100	55.76	90.34	6.95E+01	1.22E+02
25.4982	57.01	134.7	58.63	85.45	44.26	75.91	5.33E+01	9.87E+01
32.0172	41	104.8	46.16	72.33	34.88	63.62	4.07E+01	8.03E+01
40.2038	29.45	82.96	35.96	60.85	27.31	53.48	3.09E+01	6.58E+01
50.5103	21.03	66.46	27.66	51.09	21.21	45.21	2.33E+01	5.43E+01
63.4536	15	53.52	20.83	42.79	16.21	38.43	1.73E+01	4.49E+01
79.7795	10.77	43.77	15.29	35.79	12.13	32.7	1.27E+01	3.74E+01
100.31	7.798	36.44	10.95	29.9	8.829	27.77	9.19E+00	3.14E+01

Table 7-2 Frequency sweep test for date palm wax based oleogel SFDW2% carried at 20 °C and 0.02% strain.

Frequency (rad s^{-1})	First measurement		Second measurement		Third measurement		Average values	
	G’ (Pa)	G” (Pa)	G’ (Pa)	G” (Pa)	G’ (Pa)	G” (Pa)	G’ (Pa)	G” (Pa)
0.628318	4.45E+03	669.6	5.36E+03	673.1	4.92E+03	781.5	4.91E+03	708.0667
0.791052	4.69E+03	660.5	5.34E+03	565.8	5.20E+03	729.2	5.08E+03	651.8333
0.995884	4.69E+03	602.4	5.54E+03	611.9	5.39E+03	754.1	5.21E+03	656.1333
1.253494	4.83E+03	554.7	5.61E+03	700.5	5.52E+03	714.5	5.32E+03	656.5667
1.578335	4.93E+03	577.9	5.74E+03	579.9	5.68E+03	784.4	5.45E+03	647.4
1.986742	5.02E+03	600.8	5.84E+03	654.1	5.70E+03	794.7	5.52E+03	683.2
2.501334	5.11E+03	645.9	5.95E+03	637.8	5.88E+03	835.9	5.65E+03	706.5333
3.14913	5.24E+03	623.3	6.08E+03	650.7	6.01E+03	845.5	5.77E+03	706.5
3.964687	5.30E+03	649.9	6.16E+03	686.8	6.11E+03	883.8	5.86E+03	740.1667
4.99073	5.40E+03	675.3	6.26E+03	704.1	6.17E+03	928.1	5.94E+03	769.1667
6.28318	5.48E+03	714.2	6.38E+03	734	6.33E+03	955.5	6.06E+03	801.2333
7.910524	5.55E+03	749.4	6.46E+03	757.4	6.40E+03	996.3	6.14E+03	834.3667
9.95884	5.64E+03	789.1	6.55E+03	806	6.53E+03	1.06E+03	6.24E+03	886.3667
12.53494	5.75E+03	842	6.65E+03	839.9	6.67E+03	1.13E+03	6.36E+03	935.9667
15.78335	5.86E+03	904.4	6.76E+03	889	6.81E+03	1.20E+03	6.48E+03	997.1333
19.86742	5.96E+03	965.9	7.06E+03	1.36E+03	6.93E+03	1.29E+03	6.65E+03	1205.967
25.01334	6.08E+03	1.06E+03	6.98E+03	1.03E+03	7.02E+03	1.41E+03	6.69E+03	1167.333
31.4913	6.20E+03	1.15E+03	7.08E+03	1.12E+03	7.20E+03	1.53E+03	6.83E+03	1265
39.64687	6.34E+03	1.27E+03	7.24E+03	1.23E+03	7.34E+03	1.68E+03	6.98E+03	1390.333
49.9073	6.49E+03	1.40E+03	7.39E+03	1.35E+03	7.49E+03	1.83E+03	7.12E+03	1524.333
62.8318	6.66E+03	1.52E+03	7.59E+03	1.49E+03	7.61E+03	2.04E+03	7.29E+03	1685.667
79.10524	6.84E+03	1.71E+03	7.80E+03	1.61E+03	7.72E+03	2.21E+03	7.45E+03	1843.667
99.5884	7.08E+03	1.91E+03	8.07E+03	1.81E+03	7.83E+03	2.45E+03	7.66E+03	2057.333

125.3494	7.46E+03	2.24E+03	8.45E+03	2.09E+03	7.96E+03	2.83E+03	7.96E+03	2384.667
157.8335	7.85E+03	2.44E+03	9.14E+03	2.16E+03	8.12E+03	2.98E+03	8.37E+03	2526
198.6742	8.54E+03	2.36E+03	1.00E+04	2.91E+03	7.92E+03	3.48E+03	8.83E+03	2913.667
250.1334	1.20E+04	2.79E+03	1.29E+04	4.41E+03	1.02E+04	2.06E+03	1.17E+04	3083.333
314.913	7.10E+03	3.83E+03	2.27E+04	4.91E+03	1.85E+04	7.07E+03	1.61E+04	5268.667
396.4687	2.93E+04	2.87E+04	6.55E+03	5.39E+03	981.8	8.43E+03	1.23E+04	14170
499.073	4.37E+04	3.08E+04	6.69E+04	3.03E+04	5.13E+04	4.23E+04	5.39E+04	34456.67
628.318	2.59E+04	5.68E+04	1.14E+05	1.81E+04	9.26E+04	3.13E+04	7.74E+04	35376.67

7.2 Appendix 2 Further characterisation for date palm wax extracted with $scCO_2$ at 400 bar and 100 °C carried by Dr. Mario Deburyn.

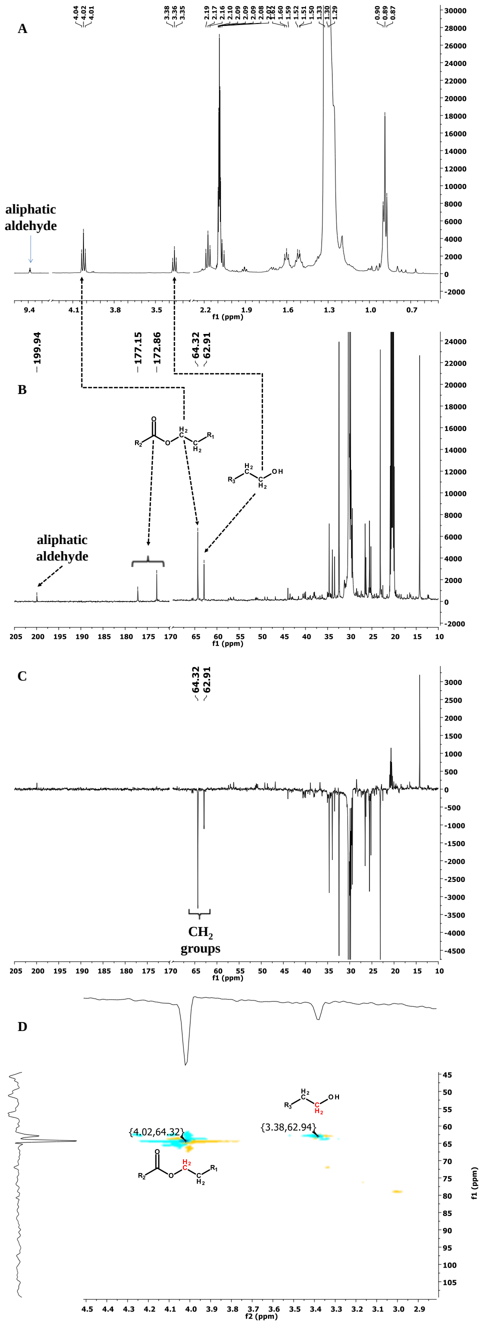


Figure 7-1 ^{1}H NMR (A), ^{13}C NMR (B), DEPT (C) and HSQC (D) spectra of palm date wax at 80 °C in toluene-d8.

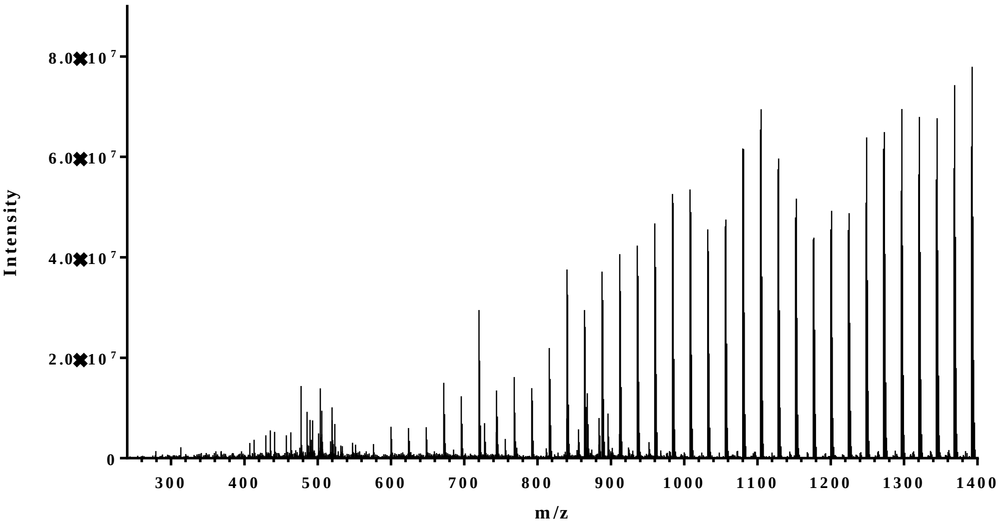


Figure 7-2 MALDI_FTICR analysis of palm date wax using S1300.

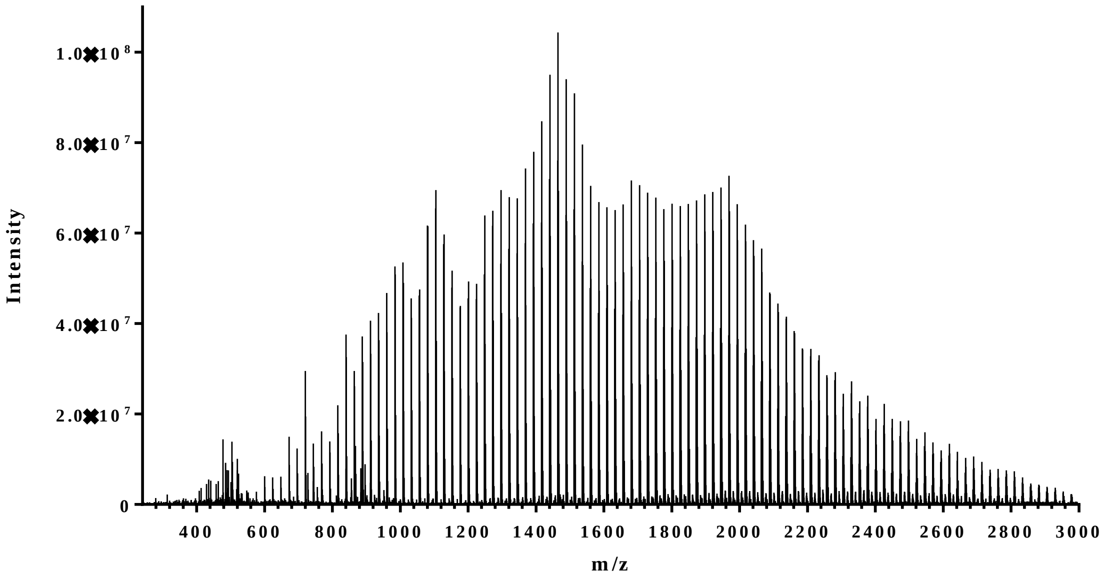


Figure 7-3 MALDI_FTICR analysis of palm date wax using S1300 (extended view).

List of Abbreviation

A	Alpha
ASTM	American Society for Testing and Materials
≈	Approximately
AOAC	Association of Analytical Communities
ATM	Atmospheric pressure
APCI	Atmospheric pressure chemical ionisation
B	Beta
C	Carbon
CO_2	Carbon dioxide
cm^3	Centimetre cubed
COP	Coefficient of performance
CER	Constant extraction rate
COM	Cost of manufacturing
C_{OL}	Cost of operational labour
C_{RM}	Cost of raw materials
C_{UT}	Cost of utility
C_{WT}	Cost of waste treatment
C_g	Critical gelling concentration
P_c	Critical pressure
τ^*	Critical stress
T_c	Critical temperature
T_{cry}	Crystallisation temperature
°C	Degrees Celsius
Δ	Delta
DCM	Dichloromethane
DSC	Differential scanning calorimetry
DCR	Diffusion Control extraction Rate period
DAG	Diglyceride
DC	Direct cost
τ_{dy}	Dynamic yield stress
G'	Elastic modules

EI	Electron ionisation
ΔH	Enthalpy change
EPA	Environmental Protection Agency
€	Euro
FER	Falling extraction rate
FAE	Fatty acids elongase complex
FAS	Fatty acids synthase complex
FCI	Fixed capital investment
FC	Fixed cost
FAO	Food and Agricultural Organisation of United Nation
FT-IR	Fourier transform-Infrared Spectroscopy
γ	Gamma
GC	Gas chromatography
GC/MS	Gas chromatography mass spectrometry
GE	General expense
GRAS	Generally Regarded As Safe
g	Gram
Ha	Hectare
Hz	Herts
HDL	High density lipoprotein
HPLC	High Performance Liquid Chromatography
h	Hour
H	Hydrogen
K	Kelvin
KJ	Kilo joules
Kg	Kilogram
Kw	Kilowatt
KI	Kovat's Index
LVER	Linear viscoelastic region
L	Litre
LDL	Low density lipoprotein
MS	Mass spectrometry
m/z	Mass-to-charge-ratio
MALDI	Matrix Assisted Laser Desorption

MJ	Mega joules
T_m	Melting temperature
m	Meters
μ	Micro
mg	Milligram
mL	Millilitre
mm	Millimetre
min	Minute
mol	Molar
M^+	Molecular ion
NIST	National Institute of Standard and Technology
NMR	Nuclear Magnetic Resonance
no.	Number
OEC	Overall extraction curve
O	Oxygen
p-	Para
PHO	Partially Hydrogenated Oils
pa	Pascal
π	Pi
P	Pressure
R_f	Response factor
rpm	Revolution per minute
SEM	Scanning electron microscopy
s	Second
SAFiN	self-assembled fibrillar network
$\dot{\gamma}$	Shear rate
σ	Sheer stress
Si	Silicon
τ	Stress
τ_y	Stress yield
scCO_2	Supercritical carbon dioxide
SFE	Supercritical extraction
SCF	Supercritical Fluid
T	Temperature

i.e.	That is
TLC	Thin layer chromatography
3-D	Three dimensional
3-ITT	Three Intervals Thixotropic Test
TOF	Time-Of-Flight
TR	Trace amount
TAG	Triglyceride
TMS	Trimethylsilane
US	United state
VLCFA	Very long chain fatty acids
G”	Viscous modulus
w/w	Weight/weight

Chapter 8

References

6. References

1. M. Lancaster, *Green chemistry: an introductory text*, Royal Society of Chemistry, Cambridge, 2nd edition 2010.

2. J. H. Clark, *Green Chemistry*, 2006, **8**, 17-21.

3. R. A. Sheldon, *Green Chemistry*, 2014, **16**, 950-963.

4. P. Anastas and N. Eghbali, *Chemical Society Reviews*, 2010, **39**, 301-312.

5. E. H. Sin, R. Marriott, A. J. Hunt and J. H. Clark, *Comptes Rendus Chimie*, 2014, **17**, 293-300.

6. M. Palma, G. F. Barbero, Z. Pineiro, A. Liazid, C. G. Barroso, M. A. Rostagno, J. M. Prado and M. A. A. Meireles, in *Natural Product Extraction: Principles and Applications*, The Royal Society of Chemistry, Cambridge, 2013, pp. 58-88.

7. L. Wang and C. L. Weller, *Trends in Food Science & Technology*, 2006, **17**, 300-312.

8. R. M. Francesca Kerton *Alternative Solvents for Green Chemistry* ,The Royal Society of Chemistry, Cambridge, 2nd edition., 2013,pp.1-30

9. J. M. DeSimone, *Science*, 2002, **297**, 799-803.

10. Y. Gu and F. Jerome, *Chemical Society Reviews*, 2013, **42**, 9550-9570.

11. in *Alternative Solvents for Green Chemistry* , The Royal Society of Chemistry, Cambridge, 2nd edition,2013, pp. 31-50.

12. F. Chemat, M. A. Vian and G. Cravotto, *International journal of molecular sciences*, 2012, **13**, 8615-8627.

13. M. D. Luque de Castro and F. Priego-Capote, *Journal of Chromatography A*, 2010, **1217**, 2383-2389.

14. R. L. Mendes, B. P. Nobre, M. T. Cardoso, A. P. Pereira and A. F. Palavra, *Inorganica Chimica Acta*, 2003, **356**, 328-334.

15. C. A. Eckert, B. L. Knutson and P. G. Debenedetti, *Nature*, 1996, **383**, 313-318.

16. M. A. Rostagno, J. M. Prado, in *Natural Product Extraction: Principles and Applications*, Royal Society of Chemistry, Cambridge,2013, pp. 196-213.

17. J. A. Mendiola, M. Herrero, M. Castro-Puyana and E. Ibanez, in *Natural Product Extraction: Principles and Applications*, The Royal Society of Chemistry, Cambridge, 2013, pp. 196-230.

18. M. M. de Melo, H. M. Barbosa, C. P. Passos and C. M. Silva, *The Journal of Supercritical Fluids*, 2014, **86**, 150-159.

19. K. Ramalakshmi and B. Raghavan, *Critical reviews in food science and nutrition*, 1999, **39**, 441-456.

20. T. M. Attard, E. Theeuwes, L. D. Gomez, E. Johansson, I. Dimitriou, P. C. Wright, J. H. Clark, S. J. McQueen-Mason and A. J. Hunt, *RSC Advances*, 2015, **5**, 43831-43838.

21. M. M. Hugh and V. Krukonis, *Supercritical Fluid Extraction*, Butterworth-Heinemann, 2nd edition, 1994.

22. F. M. Kerton and R. Marriott, in *Alternative Solvents for Green Chemistry (2)*, The Royal Society of Chemistry, 2013, pp. 115-148.

23. Y. Ikushima, N. Saito and M. Arai, *The Journal of Physical Chemistry*, 1992, **96**, 2293-2297.

24. J. A. Hyatt, *The Journal of Organic Chemistry*, 1984, **49**, 5097-5101.

25. I. Yutaka, S. Norio, A. Masahiko and A. Kunio, *Bulletin of the Chemical Society of Japan*, 1991, **64**, 2224-2229.

26. Thermophysical Properties of Fluid Systems, http://webbook.nist.gov/chemistry/fluid/, (accessed 21/10/2014).

27. E. Ruckenstein and I. Shulgin, *Fluid Phase Equilibria*, 2001, **180**, 345-359.

28. F. P. Lucien and N. R. Foster, *The Journal of Supercritical Fluids*, 2000, **17**, 111-134.

29. J. M. Dobbs and K. P. Johnston, *Industrial & Engineering Chemistry Research*, 1987, **26**, 1476-1482.

30. P. Hubert and O. G. Vitzthum, *Angewandte Chemie International Edition in English*, 1978, **17**, 710-715.

31. C. J. Chang, K.-L. Chiu, Y.-L. Chen and C.-Y. Chang, *Food Chemistry*, 2000, **68**, 109-113.

32. D. Ehlers, E. Czech, K. W. Quirin and R. Weber, *Phytochemical Analysis*, 2006, **17**, 114-120.

33. G. Leeke, F. Gaspar and R. Santos, *Industrial & engineering chemistry research*, 2002, **41**, 2033-2039.

34. J. P. Friedrich and G. R. List, *Journal of Agricultural and Food Chemistry*, 1982, **30**, 192-193.

35. N. Nik Norulaini, W. Setianto, I. Zaidul, A. Nawi, C. Azizi and A. Omar, *Food Chemistry*, 2009, **116**, 193-197.

36. M. Raventós, S. Duarte and R. Alarcón, *Food Science and Technology International*, 2002, **8**, 269-284.

37. M. De Melo, A. Silvestre and C. Silva, *The Journal of Supercritical Fluids*, 2014, **92**, 115-176.

38. W. Barthlott, C. Neinhuis, D. Cutler, F. Ditsch, I. Meusel, I. Theisen and H. Wilhelmi, *Botanical Journal of the Linnean Society*, 1998, **126**, 237-260.

39. L. Kunst and A. Samuels, *Progress in lipid research*, 2003, **42**, 51-80.

40. P. Kolattukudy, *Lipids*, 1970, **5**, 259-275.

41. G. Eglinton and R. J. Hamilton, *Science*, 1967, **156**, 1322-1335.

42. C. Buschhaus and R. Jetter, *Journal of experimental botany*, 2011, **62**, 841-853.

43. L. Samuels, L. Kunst and R. Jetter, *Annual Reviews Plant Biology*, 2008, **59**, 683-707.

44. A. P. Tulloch, in *Chemistry and biochemistry of natural waxes*, ed. P. E. Kolattukudy, Elsevier, Amsterdam, 1976, pp. 235-251.

45. G. Eglinton, A. Gonzalez, R. Hamilton and R. Raphael, *Phytochemistry*, 1962, **1**, 89-102.

46. G. Audisio, A. Rossini, G. Bianchi and P. Avato, *Journal of High Resolution Chromatography*, 1987, **10**, 594-597.

47. R. Croteau and I. Fagerson, *Phytochemistry*, 1971, **10**, 3239-3245.

48. Y. Asikin, M. Takahashi, N. Hirose, D. X. Hou, K. Takara and K. Wada, *European Journal of Lipid Science and Technology*, 2012, **114**, 583-591.

49. Z. Kranz, J. Lamberton, K. Murray and A. Redcliffe, *Australian Journal of Chemistry*, 1960, **13**, 498-505.

50. P. Kolattukudy, J. Buckner and T.Y. J. Liu, *Archives of biochemistry and biophysics*, 1973, **156**, 613-620.

51. A. C. Chibnall, S. H. Piper, H. A. El Mangouri, E. F. Williams and A. V. V. Iyengar, *Biochemical Journal*, 1937, **31**, 1981.

52. P. N. Sahai and A. C. Chibnall, *Biochemical Journal*, 1932, **26**, 403.

53. H. J. Channon and A. C. Chibnall, *Biochemical Journal*, 1929, **23**, 168.

54. M. Riederer and C. Muller, *Annual Plant Reviews, Biology of the Plant Cuticle*, John Wiley & Sons, Heidelberg, 2008.

55. Y. H. Choi, J. Kim, M. J. Noh, E. M. Park and K.-P. Yoo, *Korean Journal of Chemical Engineering*, 1996, **13**, 216-219.

56. L. Kunst and L. Samuels, *Current opinion in plant biology*, 2009, **12**, 721-727.

57. R. Jetter and L. Kunst, *The Plant Journal*, 2008, **54**, 670-683.

58. L. Samuels, L. Kunst and R. Jetter, *Plant Biology*, 2008, **59**, 683.

59. E. Taube, *Economic Botany*, 1952, **6**, 379-401.

60. U. Wolfmeier, H. Schmidt, F. L. Heinrichs, G. Michalczyk, W. Payer, W. Dietsche, K. Boehlke, G. Hohner and J. Wildgruber, *Ullmann's Encyclopedia of Industrial Chemistry*, 1996.

61. S. Ernst, Trade survey on succulent Euphorbia species protected by CITES and used as cosmetic, food and medicine with special focus on candelilla wax, http://www.cites.org/common/com/pc/18/X-PC18-Inf06.pdf, (accessed 29/10/2014).

62. W. Hodge and H. Sineath, *Economic Botany*, 1956, **10**, 134-154.

63. E. Endlein and K.-H. Peleikis, *SÖFW-Journal*, 2011, **137**.

64. T. M. Attard, N. Bukhanko, D. Eriksson, M. Arshadi, P. Geladi, U. Bergsten, V. L. Budarin, J. H. Clark and A. J. Hunt, *Journal of Cleaner Production*, 2018, **177**, 684-698.

65. Kline, Global Wax Demand Including Vegetable-waxes to Grow at a CAGR of 1.5%: Kline http://www.specialchem4bio.com/news/2013/07/17/global-wax-demand-including-vegetable-waxes-to-grow-at-a-cagr-of-15-kline,(accessed 19/06/2018)

66. R. Welbirg, *Outlook 14: Europe p-wax producers face weak end-user demand*, http://www.icis.com/resources/news/2013/12/31/9737152/outlook-14-europe-p-wax-producers-face-weak-end-user-demand/, (accessed 23/10/2014).

67. Kline Group, Global Wax Industry 2010: Market Analysis and Opportunity http://www.klinegroup.com/reports/brochures/y635a/brochure.pdf), (accessed 23/04/2018)

68. Global wax market size, share, development, growth and demand forecast to 2020 industry insights by type by application, research and market, https://www.prnewswire.com/news-releases/global-wax-market-size-share-development-growth-and-demand-forecast-to-2020--industry-insights-by-type-petroleum-and-mineral-wax-synthetic-wax-and-natural-wax-by-application-candles-packaging-rheologysurface-application-300215559.html, (accessed 14/04/2018)

69. Nature Label: requirements to be met by natural and organic cosmetics, http://www.biodynamic.org.uk/fileadmin/user_upload/Documents/Cosmetics/NATRUE-Label_Requirements_V2-6__10102012_EN.pdf), (accessed 14/04/2018)

70. A. E. Hadrami and J. M. Al-Khayri, *Emirates Journal of Food & Agriculture*, 2012, **24**,235-239.

71. C. T. Chao and R. R. Krueger, *HortScience*, 2007, **42**, 1077-1082.

72. R. W. Nixon, *Economic Botany*, 1951, **5**, 274-301.

73. E. Segal, *British Journal of Middle Eastern Studies*, 2014, 1-16.

74. Z. Abdelouahhab and E.J. Arias-Jiménez, *Date Palm Cultivation*, Food and Agriculture Organization of the United Nations, Rome, 2002.

75. Total Harvested Area of Date palm trees http://faostat3.fao.org/faostat-gateway/go/to/download/Q/QC/E, (accessed 08/06/2017)

76. P. K. Vayalil, *Critical Review Food Science Nutrition*, 2012, **52**, 249-271.

77. M. S. Baliga, B. R. V. Baliga, S. M. Kandathil, H. P. Bhat and P. K. Vayalil, *Food Research International*, 2011, **44**, 1812-1822.

78. Z. X. Tang, L. E. Shi and S. M. Aleid, *J Sci Food Agric*, 2013, **93**, 2351-2361.

79. M. T. Ashraf, C. Fang, T. Bochenski, I. Cybulska, A. Alassali, A. Sowunmi, R. Farzanah, G. P. Brudecki, T. Chaturvedi and S. Haris, *Emirates Journal of Food and Agriculture*, 2016, **28**, 99.

80. W. H. Barreveld, *Date Palm Products,* Food and Agriculture Organization of the United Nations, Rome, 1993.

81. M. Mallaki and R. Fatehi, *Renewable Energy*, 2014, **63**, 286-291.

82. M. Chandrasekaran and A. H. Bahkali, *Saudi journal of biological sciences*, 2013, **20**, 105-120.

83. H. H. Sait, A. Hussain, A. A. Salema and F. N. Ani, *Bioresource technology*, 2012, **118**, 382-389.

84. C. Fang, M. H. Thomsen, G. P. Brudecki, I. Cybulska, C. G. Frankær, J. R. Bastidas-Oyanedel and J. E. Schmidt, *ChemSusChem*, 2015, **8**, 3823-3831.

85. C. Fang, J. E. Schmidt, I. Cybulska, G. P. Brudecki, C. G. Frankær and M. H. Thomsen, *BioMed research international*, 2015, **2015**.

86. J. R. Mohanty, S. N. Das, H. C. Das and S. K. Swain, *Polymer Composite*, 2013, **34**, 959-966.

87. E. S. I. El-Shafey and S. M. Z. Al-Kindy, *Environmental Technology*, 2013, **34**, 395-406.

88. A. Dehghani, S. M. Ardekani, M. A. Al-Maadeed, A. Hassan and M. U. Wahit, *Mater Design*, 2013, **52**, 841-848.

89. P. Khristova, O. Kordsachia and T. Khider, *Bioresource technology*, 2005, **96**, 79-85.

90. E.-S. I. El-Shafey, H. A. J. Al-Lawati and A. Y. Al-Hussaini, *Chemistry and Ecology*, 2014, 1-19.

91. F. Boudrahem, F. Aissani-Benissad and A. Soualah, *Journal of Chemical & Engineering Data*, 2011, **56**, 1804-1812.

92. M. A. Binhussain and M. M. El-Tonsy, *Constr Build Mater*, 2013, **47**, 1431-1435.

93. N. Saadaoui, A. Rouilly, K. Fares and L. Rigal, *Mater Design*, 2013, **50**, 302-308.

94. S. K. Ghosh and D. Nag, *Journal of Indian Chemical Society*, 2010, **87**, 761-763.

95. M. El-Morsy, B. Riad and M. Mohamed, *Fibre Science and Technology*, 1981, **14**, 157-161.

96. A. Sbiai, H. Kaddami, H. Sautereau, A. Maazouz and E. Fleury, *Carbohydrate Polymers*, 2011, **86**, 1445-1450.

97. A. Bendahou, A. Dufresne, H. Kaddami and Y. Habibi, *Carbohydrate Polymers*, 2007, **68**, 601-608.

98. M. Shoaib and H. M. Al-Swaidan, *Biomass and Bioenergy*, 2015, **73**, 124-134.

99. J.-R. Bastidas-Oyanedel, C. Fang, S. Almardeai, U. Javid, A. Yousuf and J. E. Schmidt, *Bioresource technology*, 2016, **215**, 21-28.

100. E. Kurtz Jr, *Plant physiology*, 1950, **25**, 269.

101. A. Sumbul, I.Rehan, R. Munawwr, A. V. Uddin, *Journal of Chemical Society of Pakistan*, 2010, **32**, 672-676.

102. R. Gaceb-Terrak and F. Rahmania, *African Journal of Traditional ,Complementary and Alternative Medicine*, 2017, **14**(3): 242–256

103. R. Khelil, E. Jardé, F. Cabello-Hurtado, A. Ould-el-Hadj Khelil and M.-A. Esnault, *Scientia Horticulturae*, 2016, **201**, 238-246.

104. J. Taylor, Outlook 14: US paraffin wax flat on supply/demand https://www.icis.com/resources/news/2016/12/30/10061506/outlook-17-us-paraffin-wax-flat-on-supply-demand/, (accessed 19/06/2017).

105. Global wax demand including vegetable-waxes to grow at a CAGR of 1.5%: Kline, http://www.specialchem4bio.com/news/2013/07/17/global-wax-demand-including-vegetable-waxes-to-grow-at-a-cagr-of-15-kline, (accessed 19/06/2017).

106. S. L. Forbes, D. A. Cohen, R. Cullen, S. D. Wratten and J. Fountain, *Journal of Cleaner Production*, 2009, **17**, 1195-1199.

107. D. Brécard, B. Hlaimi, S. Lucas, Y. Perraudeau and F. Salladarré, *Ecological economics*, 2009, **69**, 115-125.

108. A. Chehma and H. Longo, *Production et Valorisation-Biomasse*, 2001, 59-64.

109. C. f. D. Control and Prevention, *MMWR. Morbidity and mortality weekly report*, 2001, **50**, 1011.

110. W. J. Krasavage, J. L. O'Donoghue, G. D. DiVincenzo and C. J. Terhaar, *Toxicology and Applied Pharmacology*, 1980, **52**, 433-441.

111. A. J. Hunt, E. H. Sin, R. Marriott and J. H. Clark, *ChemSusChem*, 2010, **3**, 306-322.

112. F. E. Deswarte, J. H. Clark, J. J. Hardy and P. M. Rose, *Green Chemistry*, 2006, **8**, 39-42.

113. K. Sharif, M. Rahman, J. Azmir, A. Mohamed, M. Jahurul, F. Sahena and I. Zaidul, *Journal of Food Engineering*, 2014, **124**, 105-116.

114. D. C. Montgomery, *Design and analysis of experiments*, John Wiley & Sons, Hoboken, NJ, 6th edition, 2005.

115. P. G. Jessop and W. Leitner, *Chemical synthesis using supercritical fluids*, John Wiley & Sons, Winheium, 1st edition, 1999.

116. A. Clifford, D. Walker, J. King and G. List, *Analytical supercritical fluid extraction for oil and lipid analysis*, AOCS, 1996.

117. A. Brachet, P. Christen, J. Y. Gauvrit, R. Longeray, P. Lantéri and J. L. Veuthey, *Journal of biochemical and biophysical methods*, 2000, **43**, 353-366.

118. I. J. Barnabas, J. R. Dean, W. R. Tomlinson and S. P. Owen, *Analytical Chemistry*, 1995, **67**, 2064-2069.

119. E. Stahl, K.-W. Quirin and D. Gerard, *Dense gases for extraction and refining*, Springer Science & Business Media, 2012.

120. Ü. Güçlü and F. Temelli, *Industrial & Engineering Chemistry Research*, 2000, **39**, 4756-4766.

121. C. Pereira and M. A. Meireles, *Food Bioprocess Technologies*, 2010, **3**, 340-372.

122. N. R. Foster, G. S. Gurdial, J. S. Yun, K. K. Liong, K. D. Tilly, S. S. Ting, H. Singh and J. H. Lee, *Industrial & Engineering Chemistry Research*, 1991, **30**, 1955-1964.

123. E. Chimowitz and K. Pennisi, *AIChE journal*, 1986, **32**, 1665-1676.

124. T. M. Attard, C. R. McElroy, C. A. Rezende, I. Polikarpov, J. H. Clark and A. J. Hunt, *Industrial Crops and Products*, 2015, **76**, 95-103.

125. H. Sovová, J. Kučera and J. Jež, *Chemical Engineering Science*, 1994, **49**, 415-420.

126. E. Reverchon, G. Donsi and L. Sesti Osseo, *Industrial & engineering chemistry research*, 1993, **32**, 2721-2726.

127. R. T. Bush and F. A. McInerney, *Geochimica et Cosmochimica Acta*, 2013, **117**, 161-179.

128. H. van Den Dool and P. Dec. Kratz, *Journal of Chromatography A*, 1963, **11**, 463-471.

129. E. Kovats, *Helv. Chim. Acta*, 1958, **41**, 1915-1932.

130. R. Ryhage and E. Stenhagen, *Journal of Lipid Research*, 1960, **1**, 361-390.

131. W. J. SCHMITT and R. C. REID, *Chemical Engineering Communications*, 1988, **64**, 155-176.

132. T. Chartier, E. Delhomme, J. F. Baumard, P. Marteau, P. Subra and R. Tufeu, *Industrial & Engineering Chemistry Research*, 1999, **38**, 1904-1910.

133. T. Funazukuri, S. Hachisu and N. Wakao, *Industrial & Engineering Chemistry Research*, 1991, **30**, 1323-1329.

134. T. Furuya and A. S. Teja, *Industrial & engineering chemistry research*, 2000, **39**, 4828-4830.

135. N. Agelopoulos, M. A. Birkett, A. J. Hick, A. M. Hooper, J. A. Pickett, E. M. Pow, L. E. Smart, D. W. Smiley, L. J. Wadhams and C. M. Woodcock, *Pesticide science*, 1999, **55**, 225-235.

136. G. Powell, J. Hardie and J. A. Pickett, *Entomologia experimentalis et applicata*, 1997, **84**, 189-193.

137. N. C. Shantha and G. E. Napolitano, *Journal of Chromatography A*, 1992, **624**, 37-51.

138. A. Kuksis, J. J. Myher, L. Marai and K. Geher, *Analytical Biochemistry*, 1976, **70**, 302-312.

139. A. A. Millar, M. A. Smith and L. Kunst, *Trends in Plant Science*, 2000, **5**, 95-101.

140. Z.-R. Yu, B. Singh, S. S. Rizvi and J. A. Zollweg, *The Journal of Supercritical Fluids*, 1994, **7**, 51-59.

141. I. Gill and R. Valivety, *Trends in biotechnology*, 1997, **15**, 401-409.

142. U. N. Das, *Biotechnology journal*, 2006, **1**, 420-439.

143. H. Sales-Campos, P. Reis de Souza, B. Crema Peghini, J. Santana da Silva and C. Ribeiro Cardoso, *Mini reviews in medicinal chemistry*, 2013, **13**, 201-210.

144. K. Hill, *Pure and applied chemistry*, 2000, **72**, 1255-1264.

145. N. Ruston, *Journal of the American Oil Chemists' Society*, 1952, **29**, 495-498.

146. T. M. Attard, C. R. McElroy, R. J. Gammons, J. M. Slattery, N. Supanchaiyamat, C. L. A. Kamei, O. Dolstra, L. M. Trindade, N. C. Bruce, S. J. McQueen-Mason, S. Shimizu and A. J. Hunt, *ACS Sustainable Chemistry & Engineering*, 2016, **4**, 5979-5988.

147. I. Gouni-Berthold and H. K. Berthold, *American Heart Journal*, 2002, **143**, 356-365.

148. R. Menendez, S. I. Fernandez, A. Del Rio, R. M. Gonzalez, V. Fraga, A. M. Amor and R. M. Mas, *Biol Res*, 1994, **27**, 199-203.

149. W. Boerjan, J. Ralph and M. Baucher, *Annual review of plant biology*, 2003, **54**, 519-546.

150. M. C. Pérez-Camino, W. Moreda, R. Mateos and A. Cert, *Journal of Chromatography A*, 2003, **983**, 283-288.

151. K. Christiansen, V. Mahadevan, C. Viswanathan and R. Holman, *Lipids*, 1969, **4**, 421-427.

152. E. J. Dufourc, *Journal of Chemical Biology*, 2008, **1**, 63-77.

153. C. Grunwald, *Annual review of plant physiology*, 1975, **26**, 209-236.

154. F. R. Maxfield and I. Tabas, *Nature*, 2005, **438**, 612-621.

155. R. P. Evershed, M. C. Prescott, N. Spooner and L. J. Goad, *Steroids*, 1989, **53**, 285-309.

156. M. Govind Rao, E. Perkins, W. E. Connor and A. K. Bhattacharyya, *Lipids*, 1975, **10**, 566-568.

157. C. Brooks, E. C. Horning and J. Young, *Lipids*, 1968, **3**, 391-402.

158. V. Piironen, D. Lindsay, T. Miettinen, J. Toivo and A. Lampi, *Journal of Science, Food and Agriculutre*, 2000, **80**, 939-966.

159. Ö. Güçlü-Üstündağ and F. Temelli, *The Journal of Supercritical Fluids*, 2004, **31**, 235-253.

160. A. de Jong, J. Plat and R. P. Mensink, *The Journal of Nutritional Biochemistry*, 2003, **14**, 362-369.

161. I. Rudkowska, *Maturitas*, 2010, **66**, 158-162.

162. P. S. Keng, M. Basri, M. R. S. Zakaria, M. B. A. Rahman, A. B. Ariff, R. N. Z. A. Rahman and A. B. Salleh, *Industrial Crops and Products*, 2009, **29**, 37-44.

163. L. Wenning, T. Yu, F. David, J. Nielsen and V. Siewers, *Biotechnology and bioengineering*, 2017, **114**, 1025-1035.

164. B. Ritter, J. Schulte, E. Schulte and H. P. Thier, *European Food Research and Technology*, 2001, **212**, 603-607.

165. W. A. Poucher, *Perfumes, Cosmetics and Soaps: Modern Cosmetics*, Springer Science Business Media B.V,8th edition, 2013.

166. M. G. Müller, J. A. Lindner, H. Briesen, K. Sommer and P. Foerst, *Advanced Powder Technology*, 2018, **29**(3), 781-788.

167. T. Fei and T. Wang, *Current Opinion in Food Science*, 2017, **16**, 7-14.

168. N. E. Hughes, A. G. Marangoni, A. J. Wright, M. A. Rogers and J. W. Rush, *Trends in Food Science & Technology*, 2009, **20**, 470-480.

169. H.-S. Hwang, S. Kim, M. Singh, J. K. Winkler-Moser and S. X. Liu, *Journal of the American Oil Chemists' Society*, 2012, **89**, 639-647.

170. B. Mert and I. Demirkesen, *Food Chemistry*, 2016, **199**, 809-816.

171. D. C. Zulim Botega, A. G. Marangoni, A. K. Smith and H. D. Goff, *Journal of food science*, 2013, **78**.

172. M. Dietrich, M. Fischer and D. Walcher, *Vegan Design*, LuluPress, Kuchi, 2016.

173. J. Azmir, I. Zaidul, M. Rahman, K. Sharif, A. Mohamed, F. Sahena, M. Jahurul, K. Ghafoor, N. Norulaini and A. Omar, *Journal of Food Engineering*, 2013, **117**, 426-436.

174. M. D. Luque de Castro and L. E. García-Ayuso, *Analytica Chimica Acta*, 1998, **369**, 1-10.

175. Y. Athukorala and G. Mazza, *Industrial Crops and Products*, 2010, **31**, 550-556.

176. L. Wang, C. L. Weller, V. L. Schlegel, T. P. Carr and S. L. Cuppett, *European Journal of Lipid Science and Technology*, 2007, **109**, 567-574.

177. K. Koch, A. Dommisse and W. Barthlott, *Crystal Growth & Design*, 2006, **6**, 2571-2578.

178. K. Koch, W. Barthlott, S. Koch, A. Hommes, K. Wandelt, W. Mamdouh, S. De-Feyter and P. Broekmann, *Planta*, 2006, **223**, 258-270.

179. K. Koch and H.-J. Ensikat, *Micron*, 2008, **39**, 759-772.

180. A. J. Martins, A. Vicente, R. L. Cunha and M. A. Cerqueira, *Food & Function*, 2018.

181. V. Remig, B. Franklin, S. Margolis, G. Kostas, T. Nece and J. C. Street, *Journal of the American Dietetic Association*, 2010, **110**, 585-592.

182. M. R. L'Abbé, S. Stender, C. Skeaff and M. Tavella, *European Journal of Clinical Nutrition*, 2009, **63**, S50.

183. A. R. Patel, *Edible Oil Structuring*, Royal Society of Chemistry, Cambridage, 2017, pp;3-17.

184. M. A. Rogers, A. J. Wright and A. G. Marangoni, *Soft Matter*, 2009, **5**, 1594-1596.

185. M. A. Rogers, *Food Research International*, 2009, **42**, 747-753.

186. F. C. Wang, A. J. Gravelle, A. I. Blake and A. G. Marangoni, *Current Opinion in Food Science*, 2016, **7**, 27-34.

187. A. R. Patel and K. Dewettinck, *European journal of lipid science and technology*, 2015, **117**, 1772-1781.

188. A. I. Blake, E. D. Co and A. G. Marangoni, *Journal of the American Oil Chemists' Society*, 2014, **91**, 885-903.

189. A. R. Patel, *Current Opinion in Food Science*, 2017, **15**, 1-7.

190. A. R. Patel and K. Dewettinck, *Food & function*, 2016, **7**, 20-29.

191. E. D. Co and A. G. Marangoni, *Journal of the American Oil Chemists' Society*, 2012, **89**, 749-780.

192. D. J. Abdallah and R. G. Weiss, *Advanced Materials*, 2000, **12**, 1237-1247.

193. A. R. Patel, M. Babaahmadi, A. Lesaffer and K. Dewettinck, *Journal of agricultural and food chemistry*, 2015, **63**, 4862-4869.

194. C. D. Doan, C. M. To, M. De Vrieze, F. Lynen, S. Danthine, A. Brown, K. Dewettinck and A. R. Patel, *Food chemistry*, 2017, **214**, 717-725.

195. A. Jang, W. Bae, H.-S. Hwang, H. G. Lee and S. Lee, *Food chemistry*, 2015, **187**, 525-529.

196. J. F. Toro-Vazquez, J. A. Morales-Rueda, E. Dibildox-Alvarado, M. Charó-Alonso, M. Alonzo-Macias and M. M. González-Chávez, *Journal of the American Oil Chemists' Society*, 2007, **84**, 989-1000.

197. L. S. K. Dassanayake, D. R. Kodali, S. Ueno and K. Sato, *Journal of the American Oil Chemists' Society*, 2009, **86**, 1163.

198. K. P. Menard, *Dynamic mechanical analysis: a practical introduction*, CRC press, 2008.

199. R. Brummer, *Rheology essentials of cosmetic and food emulsions*, Springer-Verlag Berlin Heidelberg, 2006.

200. H. A. Barnes, J. F. Hutton and K. Walters, *An introduction to rheology*, Elsevier, 1989.

201. P. Fischer and E. J. Windhab, *Current Opinion in Colloid & Interface Science*, 2011, **16**, 36-40.

202. M. H. Tunick, *Journal of Agricultural and Food Chemistry*, 2010, **59**, 1481-1486.

203. K. Nishinari, *Colloid and Polymer Science*, 1997, **275**, 1093.

204. I. T. Norton, F. Spyropoulos and P. Cox, *Practical food rheology: an interpretive approach*, Wiley & Blackwell, Oxford, 2010.

205. P. Terech and R. G. Weiss, *Chemical reviews*, 1997, **97**, 3133-3160.

206. N. M. Sangeetha and U. Maitra, *Chemical Society Reviews*, 2005, **34**, 821-836.

207. A. Vintiloiu and J.-C. Leroux, *Journal of controlled release*, 2008, **125**, 179-192.

208. E. Yılmaz and M. Öğütcü, *Food & function*, 2015, **6**, 1194-1204.

209. C. D. Doan, I. Tavernier, M. D. B. Sintang, S. Danthine, D. Van de Walle, T. Rimaux and K. Dewettinck, *Food biophysics*, 2017, **12**, 97-108.

210. K. Mattice and A. Marangoni, in *Edible Oil Structuring*, 2017, pp. 69-94.

211. G. Zhu and J. S. Dordick, *Chemistry of Materials*, 2006, **18**, 5988-5995.

212. A. F. Harron, M. J. Powell, A. Nunez and R. A. Moreau, *Industrial crops and products*, 2017, **98**, 116-129.

213. A. I. Blake and A. G. Marangoni, *Food biophysics*, 2015, **10**, 456-465.

214. G. Fayaz, S. A. H. Goli and M. Kadivar, *Journal of the American Oil Chemists' Society*, 2017, **94**, 47-55.

215. C. D. Doan, I. Tavernier, P. K. Okuro and K. Dewettinck, *Innovative Food Science & Emerging Technologies*, 2018, **45**, 42-52.

216. M. A. Rogers, *Edible Nanostructures: A Bottom-up Approach*, Royal Society of chemistry, Cambridage 2015, pp 1-14, 144.

217. S. Martini, C. Y. Tan and S. Jana, *Journal of food science*, 2015, **80**.

218. A. I. Blake and A. G. Marangoni, *Food Structure*, 2015, **3**, 30-34.

219. D. Bonn, M. M. Denn, L. Berthier, T. Divoux and S. Manneville, *Reviews of Modern Physics*, 2017, **89**, 035005.

220. I. Tavernier, C. D. Doan, D. Van de Walle, S. Danthine, T. Rimaux and K. Dewettinck, *RSC Advances*, 2017, **7**, 12113-12125.

221. J. Mewis and N. J. Wagner, *Advances in Colloid and Interface Science*, 2009, **147-148**, 214-227.

222. O. S. Toker, S. Karasu, M. T. Yilmaz and S. Karaman, *Food Research International*, 2015, **70**, 125-133.

223. R. H. Ewoldt, M. T. Johnston and L. M. Caretta, in *Complex Fluids in Biological Systems*, Springer, New York,2015, pp. 207-241.

224. F. J. Stadler, *Korea-Australia Rheology Journal*, 2014, **26**, 277-291.

225. C. Pronyk and G. Mazza, *Journal of Food Engineering*, 2009, **95**, 215-226.

226. L. S. Moura, R. N. Carvalho Jr, M. B. Stefanini, L. C. Ming and M. A. A. Meireles, *The Journal of supercritical fluids*, 2005, **35**, 212-219.

227. R. N. Carvalho Jr, L. S. Moura, P. T. Rosa and M. A. A. Meireles, *The Journal of Supercritical Fluids*, 2005, **35**, 197-204.

228. J. M. del Valle, O. Rivera, M. Mattea, L. Ruetsch, J. Daghero and A. Flores, *The Journal of supercritical fluids*, 2004, **31**, 159-174.

229. J. Martínez, P. T. Rosa and M. A. A. Meireles, *The Open Chemical Engineering Journal*, 2007, **1**,1-7.

230. N. Mezzomo, J. Martínez and S. R. Ferreira, *The Journal of Supercritical Fluids*, 2009, **51**, 10-16.

231. J. M. Prado, G. H. Prado and M. A. A. Meireles, *The Journal of Supercritical Fluids*, 2011, **56**, 231-237.

232. M. A. A. Meireles, in *Supercritical fluid extraction of nutraceuticals and bioactive compounds*, CRC Press, Boca Raton, 2008, pp. 243-274.

233. J. A. Szweda and C. A. Lutrario, *Journal*, 1997.

234. R. J. Edmundson and T. C. Jacks, *High-gloss, high-shine lipstick*, US patant US 4873078A,1989.

235. I. N. Duling and R. D. Cassar, *Wickless Candle*,US patent US360697A, 1971.

236. M. Chiumarelli and M. D. Hubinger, *Food Hydrocolloids*, 2012, **28**, 59-67.

237. E. Reverchon and I. De Marco, *The Journal of Supercritical Fluids*, 2006, **38**, 146-166.

238. J. Graça and P. Lamosa, *Journal of agricultural and food chemistry*, 2010, **58**, 9666-9674.

239. M. Riederer and J. Schönherr, *Planta*, 1988, **174**, 127-138.

240. P. T. Rosa and M. A. A. Meireles, *Journal of Food Engineering*, 2005, **67**, 235-240.

241. T. M. Attard, C. R. McElroy and A. J. Hunt, *International journal of molecular sciences*, 2015, **16**, 17546-17564.

242. Á. L. Santana, J. Q. Albarelli, D. T. Santos, R. Souza, N. T. Machado, M. E. Araújo and M. A. A. Meireles, *Food and Bioproducts Processing*, 2018, **107**, 25-35.

243. R. Turton, R. C. Bailie, W. B. Whiting and J. A. Shaeiwitz, *Analysis, synthesis and design of chemical processes*, Pearson Education,Upper Saddle River,New Jersay, 2008.

244. Q. Tong, *Wages structure in the United Arab Emirates*, Institute for Social and Economic Research (Zayed University) working paper, 2010.

245. Hourly wages for select jobs in construction in the Middle East, https://www.statista.com/statistics/528832/labor-cost-for-jobs-in-construction-middle-east-by-country/, (accessed 13/07/2018).

246. M. Altarawneh and A. Ebraheem, *Scientific Journal of King Faisal University, Basic and Applied Sciences*, 2013, **14**, 49-68.

247. S. Mani, S. Sokhansanj, X. Bi and A. Turhollow, *Applied Engineering in Agriculture*, 2006, **22**, 421-426.

248. Y. Elmay, G. Trouvé, M. Jeguirim and R. Said, *Fuel Processing Technology*, 2013, **112**, 12-18.

249. H. H. Sait, A. Hussain, A. A. Salema and F. N. Ani, *Bioresource technology*, 2012, **118**, 382-389.

250. P. Purohit and V. Chaturvedi, *Techno-economic Assessment of Biomass Pellets for Power Generation in India*. CEEW Working Paper,Thapar House, New Delhi, India, http://pure.iiasa.ac.at/id/eprint/13896/1/CEEW%20-%20Working%20Paper%20-%20Techno-economic%20assesment%20of%20bio-pellets%20-%2025Oct16.pdf, (acessed 25/04/2018)

251. T. M. Attard, M. Arshadi, C. Nilsson, V. L. Budarin, E. Valencia-Reyes, J. H. Clark and A. J. Hunt, *Green Chemistry*, 2016, **18**(9):2682-90

252. L. Gustavsson and R. Madlener, *Energy*, 2003, **28**, 1405-1425.

253. Argus Media Ltd, Argus Global Waxes Incorporating Wax Data, file://userfs/kaab501/w2k/argus-global-waxes.pdf, (accessed 04/04/2018).