**Supercritical extraction of waxes and lipids from biomass: a valuable first step towards an integrated biorefinery.**

Thomas M. Attarda, Natalia Bukhankob, Daniel Erikssona, Mehrdad Arshadib, Paul Geladib, Urban Bergstenb, Vitaliy L. Budarina, James H. Clarka and Andrew J. Hunta,c\*

a Green Chemistry Centre of Excellence, Department of Chemistry, University of York, YO10 5DD, UK

b Department of Forest Biomaterials and Technology, Swedish University of Agricultural Sciences, Umeå, Sweden

c Materials Chemistry Research Center, Department of Chemistry, Faculty of Science, Khon Kaen University, Khon Kaen, 40002, Thailand

\*Corresponding Author: andrew@KKU.ac.th

Waxes; Lipids; supercritical extraction; valorisation; biorefinery; separation

**Abstract**

The decline in petrochemical wax supply coupled with the ever-growing demand for bio-products means that the development of a sustainable process to renewably sourced waxes is paramount. This review focuses on recent advances in supercritical extraction as a clean efficient process for extracting waxes from waste biomass as a part of a holistic biorefinery. The use of supercritical carbon dioxide leads to reductions in solvent waste and leaves no solvent residues meaning that the biomass can further processed without the need for energy-intensive solvent removal steps. This technology crucially improves the downstream conversion of residual cellulosic biomass for the sustainable production of sugars, consumer products and biofuels (up to a 40% increase in ethanol production) leading to higher energy efficiencies and higher economic returns. Supercritical carbon dioxide extraction is not only an important technology for the cleaner production of waxes, it is a sustainable pre-treatment of biomass as part of an integrated holistic biorefinery and significantly, it can improve the safety of products, e.g. less off gassing of wood pellets.

1. **Introduction**

Waxes and lipids are utilised in a wide range of important industrial applications (Sin et al., 2014). Frequently their value and opportunity for exploitation as part of an integrated biorefinery has been overlooked. Supply issues associated with petroleum waxes are becoming more prolific as major refiners shift the production of feedstocks away from those needed for wax manufacture and the introduction of catalytic dewaxing technologies (The Freedonia Group, 2015). This reduction in petroleum waxes production combined with a predicted growth in demand may lead to supply restrictions by 2020 (Kline, 2015). These factors, combined with the growing move towards greener consumer products have resulted in new opportunities for natural sustainable waxes. The current methods for extracting natural waxes includes the use of large quantities of conventional toxic organic solvents and strong acids, leading to copious amounts of solvent waste (Sin et al., 2014). However, there is growing interest in using supercritical carbon dioxide (scCO2) as an alternative solvent for the extraction of waxes and lipids.

The isolation of natural products using supercritical fluids has been conducted since the end of the 1970’s (Herrero et al., 2010). ScCO2 is used worldwide in a number of industries including such sectors as food and flavour, beverage and cosmetics/personal care. This technique may add value to some products, through labelled as natural and/or organic. Recent work has demonstrated the importance of supercritical extraction of waxes from biomass (Attard et al., 2015c) as part of a holistic biorefinery (Budarin et al., 2011).

The current state of the art and perception concerning scCO2 extraction is that it is standalone technology, which is only utilised in a number of higher value applications for example the decaffeination of coffee or extraction of hops for beer production. Whilst in recent years the academic community have been heavily focussed on the extraction of essential oils and bio-active molecules from plants and animals (Herrero et al., 2010), however, a limited number of reviews have looked into the extraction of waxes and similar lipophilic molecules from biomass using this clean technology (Sahena et al. 2009).

The overall perception of scCO2 extraction is that it is expensive, energy intensive, requires specialist equipment and skilled operators. As such, there is a reluctance to adopt this technology by the wider chemical industry. However, if used as an integrated part of a holistic biorefinery such a process can provide a number of significant benefits for cleaner production. This includes the production of sustainable waxes and natural products, replacing and eliminating the need for traditional solvents used in wax extraction, that are deemed to be problematic, toxic or damaging to the environment. Whilst recent research has highlighted that ScCO2 extraction can improve the production efficiency of cellulosic derived biofuels such as ethanol, with improved yields of up to 40% from the residual biomass post extraction. Viewing scCO2 extraction as an important tool to be used as part of an integrated holistic biorefinery may improve the economic viability and increase the use of such a technique.

This review examines the current research into the use of scCO2 extraction as a method for the clean production (extraction) of valuable waxes and lipid components from biomass, in addition to examining the markets and applications for these sustainable products. It demonstrates some of the important benefits over traditional methods. For the first time, this work highlights the potential significance of scCO2 extraction, in terms of sustainability and its utilisation as clean production method of waxes and importantly in improving the downstream production of sugars, personal care products and biofuels from post extraction residues, as part of an economically viable holistic integrated biorefinery.

1. **Scope and Method**

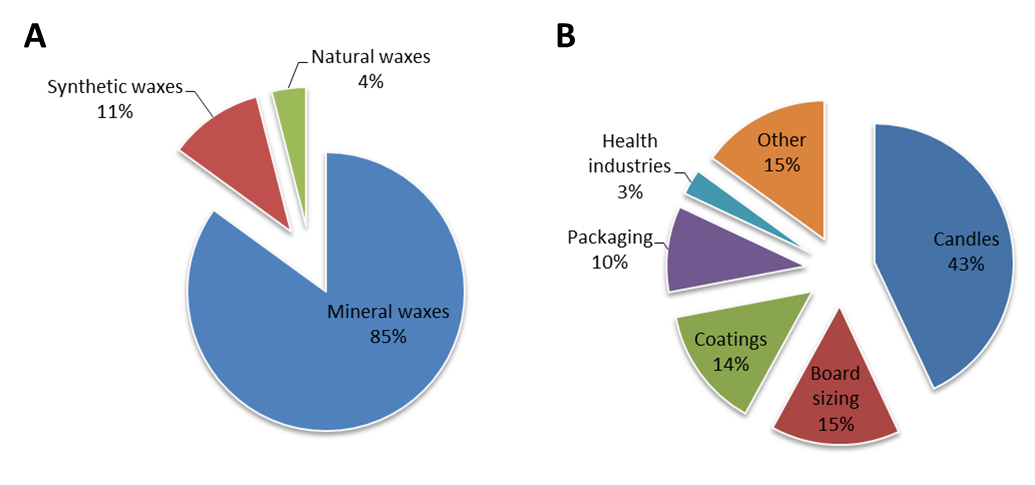
This review focuses on the latest research about supercritical extraction of waxes and lipids from waste agricultural and forestry residues. Supercritical extraction was selected, as its implementation would lead to a significant reduction in the volumes of solvent used and waste generated. Secondly, supercritical extraction demonstrated to have a positive effect on biomass downstream processing, resulting in higher yields of products and improved efficiency. Thirdly, natural waxes will play a more important role in sustainably meeting the markets demand for this product in light of the decline in petroleum wax production.

It should be noted that the review deals with valorisation of biomass including waste residues from agricultural and forestry sectors. This review focuses on waxes as extensive reviews are already available on the supercritical extraction of bioactive molecules from various plants and animals. Unlike other review articles on scCO2 extraction, this work does not focus on its use as a standalone technology, but rather as a step within a holistic biorefinery.

**3. Waxes**

**3.1 Current status**

Currently, petroleum wax is still the most important source of wax, contributing to approximately 85% of the total wax production followed by synthetic waxes (11%) and natural waxes (animal and plant waxes – 4%), see Figure 1A (Kline, 2011 and 2015). The strict chemical definition for ‘waxes’ is the esterification products from the reaction between long-chain fatty acids with long-chain primary alcohols (Barthlott et al., 1998). However, ‘plant wax’ is often used as a broad term referring to the complex mixture of hydrophobic compounds that cover the aerial tissues of herbaceous plants (Kunst and Samuels, 2003). The main application of waxes is candles, which accounted for 43% of the total application of waxes in 2010. Board sizing (15%) also contributed significantly to the wax market in 2010 (Kline, 2011). Another application where waxes are extensively used is in coatings (14% in 2010) due to their very hydrophobic nature; which includes coatings for food packages, paper, wires, wrapping and tablets (Kline, 2011). A number of different polishes contain waxes as a laminator and protective coating. They are also present in a variety of cosmetic formulations for products such as skin cream, lipsticks and depilatory wax (Garets, 1992). Lanolin is an example of wax utilised in cosmetics due to its emollient properties. Waxes are utilised as coatings in fresh fruits and vegetables (such as apples and peppers) to improve shelf life (Fei and Wang, 2017). Waxes are often added in formulations to improve function or performance e.g. small amounts of carnauba wax are normally incorporated into candles as a hardener, paraffin is blended with rubber in order to increase product stiffness and beeswax is added to improve flexibility (Hamilton, 1995). Since different waxes have varying advantages and disadvantages, a combination of waxes are utilised in some applications e.g. lipstick formulations utilise three types of waxes: lanolin (as an emollient), candelila wax (for hardness and rigidity) and carnauba wax (to increase the toughness of the lipstick) (Edmundson and Jacks, 1989; El-Nokaly et al., 2001). Figure 1B summarises the global wax applications in 2010.

****

**Figure 1 A) Types of waxes utilised in 2010 (Petroleum waxes accounts for 98% of mineral waxes) B) Wax applications (global) in 2010**

The global wax market was dominated by Asia in 2013, where the major refiners and largest production of petroleum wax are located. There has been an increase in the global demand of waxes from 4.35 million tonnes in 2010 to 4.54 million tonnes in 2013 (Kline, 2011, 2015). In the US alone, wax demand is expected to increase by 1.8% annually until 2019, with a total demand of around $3.2 billion (The Freedonia Group, 2015). Globally, it is predicted that there will be an increase in the value of the wax market from $8.2 billion (2013) to $9.0 billion in 2020 (with a compound annual growth rate (CAGR) of 2.6% from 2014 to 2020) (Kline, 2015). The demand for wax is forecasted to grow to 5.46 million tonnes in 2020 (Kline, 2011). The major industries currently “driving demand for wax” are the cosmetics and packaging industries due to the beneficial properties of waxes. In order to meet the increasing demand in Europe and North America, substantial quantities of wax are exported from Asia (Kline, 2015).

**Figure 2 Predicted wax market in 2020.**

It is expected that in the near future, there will be a number of supply issues associated with petroleum waxes due to the shift by major refiners away from the production of lower quality base oil (Group 1 Base oil refineries), the main feedstock for petroleum waxes (The Freedonia Group, 2015; Kline, 2015). Refiners are progressively switching to catalytic dewaxing technologies, which do not produce wax by-products (The Freedonia Group, 2015). This reduction in petroleum waxes, combined with the predicted growth rate in demand means that supply issues maybe experienced by 2020 (Figure 2).

These factors combined with a transition to greener products by consumers have resulted in new opportunities for natural waxes in particular plant waxes. Synthetic waxes are generally synthesised via the Fischer-Tropsch process or ethylene-based polymerisation process and are currently derived from a non-renewable feedstock (Totten et al., 2003). The utilisation of plant waxes from agricultural residues would be the preferred option, avoiding competition with food production.

Natural waxes consist of animal and plant (vegetable waxes) and are obtained from renewable biomass feedstocks, potentially “provide valuable net savings” in greenhouse gas emissions (Deswarte et al., 2007). However, the life cycle analysis of the most common commercially available animal and plant waxes indicates that the process for obtaining these waxes (including isolation and transportation) is not environmentally friendly. Two of the most commonly used plant waxes, candelila wax and carnauba wax, are derived from tropical areas and have significant contribution to the carbon footprint (Deswarte et al., 2007). The overreliance on these waxes has led to overexploitation of the plants and their production is only possible through heavy government subsidiaries (Deswarte et al., 2007). The process of obtaining these waxes has a number of health hazards associated with it, including hazardous chemical and solvent use (Hamilton, 1995). Hundreds of tons of candelila wax every month are exported by refineries and distributors from Mexico, while approximately 22,400 tonnes of carnauba wax was produced by Brazil in 2006 (Arato et al., 2014; Blake et al., 2014). There are various methods by which waxes are industrially extracted, ranging from boiling the leaves in concentrated hazardous sulfuric acid (as is the case with candelila wax) to solvent extraction using solvents such as commercial hexane (Kent, 2013). The exploitation of other sources of plant wax, such as local agricultural residues, coupled with the use of cleaner, greener technologies would allow for a more sustainable, environmentally-friendly process for supplying waxes.

**3.2 Plant waxes: Hydrophobic molecules from agricultural and forestry residues**

Epicuticular wax is normally the component investigated in extraction processes and this review will focus on these components. The main types of long-chain aliphatic compounds include ­*n-*alkanes, saturated and unsaturated fatty acids, primary alcohols, aldehydes and wax esters (Barthlott et al., 1998). Less common aliphatic compounds include secondary alcohols, ketones and β-diketones. The cyclic compounds consist of flavonoids, sterols and triterpenoids, with the latter two being the most common (Kunst and Samuels, 2009).

The chemical composition of woody feedstock, in particular pine species, contains numerous fatty and resin acids. Around 0.2–4% of extractives can be obtained from pine stemwood, which corresponds to a considerable per-hectare output for a biorefinery (Arshadi et al., 2013). The highest content of extractives (almost 20%) in wood is found in heartwood of pine stumps (Eriksson et al., 2012). Other tree parts as needles, branches and bark, which are considered as low-cost assortments, also contain high amounts of extractives and a diverse range of chemical compounds (Backlund et al., 2014). These differences in chemical content will be central for selection of different processes for separation of chemical fractions (Clark et al., 2006), characterisation (Clark et al., 2013), and isolation/extraction of speciality chemicals in a biorefinery context (Shuttleworth et al., 2014). These by-products or forestry residues play a vital role in forest management due to their low widespread usage as well as the considerable volumes generated, leading to high costs associated with harvesting and transport (Cambero and Sowlati, 2014). Although there are huge volumes of forest waste, little attention has been given to valorising this renewable feedstock (Puy et al., 2011; Puy et al., 2008).

**3.3 Applications of Hydrophobic molecules**

The complex nature of plant waxes means that they cannot only be exploited for their bulk properties but the individual constituent compounds or groups of compounds can also find use in higher value applications. Studies have shown that waxes containing *n-*alkanes having chain lengths from C21 to C29 display semiochemical properties, involved in plant-insect interactions, which could be of significant use as natural insecticides or pollination of flowers (Schiestl et al., 1999).

Saturated fatty acids are widely used in a host of applications ranging from soaps and detergents to cleaning polishes and lubricating oils (Hill, 2000; Ruston, 1952). Unsaturated fatty acids such as linoleic acid and linolenic acid have a hypocholesterolemic effect, resulting in a reduction in blood cholesterol (Horrobin and Huang, 1987). Resin acids have significant potential in pharmacological applications such as anti-thrombotic and antifungal agents (Fernández et al., 2001). Additionally studies have shown that resin acids have been used in drug-delivery applications (Ramani et al., 1996). They also can find a wide array of applications such as cosmetics, paints, varnishes, printing inks and chewing gum (Fulzele et al., 2003).

Long-chain fatty aldehydes, such as octacosanal, have shown to help prevent and treat osteoporosis (Chinen, 2006). Long-chain alcohols (*n-*policosanols) have well-documented nutraceutical applications, especially for the treatment and prevention of numerous cardio-vascular related conditions (Marinangeli et al., 2010). Policosanols are also utilised in cosmetic applications as anti-acne agents, an emollient and for the control of sebum secretion (Majeed et al., 2007).

High-molecular weight wax esters are utilised in various valuable applications such as coatings, lubricants, hard wax polishes, plasticisers, food applications (organogels) and cosmetics (Gunawan et al., 2005; Dassanayake et al., 2009)

Plant sterols (phytosterols) can significantly reduce plasma LDL-cholesterol levels (incorporated in numerous food products such as margarine etc.) (Moghadasian and Frohlich, 1999) and also display pharmaceutical properties, acting as anti-cancer compounds (Bradford and Awad, 2007). Triterpenoids also exhibit medicinal and pharmaceutical properties; friedelin and its derivatives are vascularising agents and display anti-cancer (Moiteiro et al., 2004), anti-analgesic (Pires et al., 2009), anti-bacterial activity (Frame, 2003) and anti-inflammatory properties (Nakamura et al., 1997; Zhang et al., 2006). The applications are summarised in Table 1.

**Table 1. Hydrophobic molecules found in waxes and their applications.**

|  |  |  |
| --- | --- | --- |
| Aliphatic Compounds | Structure | Applications |
| n-alkanes |  | Insecticides |
| Primary alcohols |  | Lowers blood cholesterol, Nutraceuticals, Cosmetics |
| Aldehydes |  | Osteoporosis treatment |
| Fatty acids |  | Soaps, detergents, polishes, lubricating oils |
| Wax esters |  | Coatings, lubricants, polishes, organogels, plasticisers, cosmetics |
| Secondary alcohols |  | Coatings |
| Resin acids |  | Anti-thrombotic and antifungal agents, cosmetics, paints, varnishes, printing inks, chewing gum |
| Sterols |  | Cholesterol-lowering, Anti-cancer agents, Nutraceuticals |
| Terpenoids |  | Anti-bacterial, anti-analgesic, anti-inflammatory, anti-cancer agents. |

1. **Conventional Organic extraction of waxes.**

Most organic solvent extraction of waxes reported in literature were carried out simply for the characterisation of the compounds constituting the plant cuticle, whereby the wax was extracted by immersing or dipping the biomass in solvent for a short time (Szafranek and Synak, 2006). % extraction yield of waxes was not taken into consideration.

**Table 2. Soxhlet extraction of waxes in literature**

|  |  |  |  |
| --- | --- | --- | --- |
| Literature | Biomass | Solvent | Technique |
| (Sun and Sun, 2001) | Barley straw | Various solvents toluene–ethanol  (2 : 1, v/v), chloroform, MTBE, dichloromethane and  Hexane/acetone | Soxhlet extraction |
| (Xiao et al., 2001) | Rice straw | Various solvents toluene–ethanol  (2 : 1, v/v), chloroform, petroleum ether, dichloromethane and  hexane | Soxhlet extraction |
| (Nuissier et al., 2002) | Sugarcane waxes from rum factory wastes | Cyclohexane | Soxhlet extraction |
| (Sun and Tompkinson, 2003) | Wheat straw | Various solvents toluene–ethanol  (2 : 1, v/v), toluene-ethanol-methanol (1:1:1 v/v), MTBE, chloroform-methanol (2:1, v/v) | Soxhlet extraction |
| (Gutiérrez and del Río, 2005) | Pitch deposits from hemp fibre | Acetone | Soxhlet extraction |
| (Vali et al., 2005) | Rice bran | Hexane | Refluxed in solvent |
| (Wang et al., 2005) | Grain Sorgum DDG | Hexane | Soxhlet extraction |
| (András et al., 2005) | Okra seeds | Hexane/ethanol | Soxhlet extraction |
| (Morrison Iii et al., 2006) | Flax/linseed | Hexane | Soxhlet extraction |
| (Zhao et al., 2007) | Rice, wheat straw and corn stalk | Various solvents petroleum ether, chloroform, toluene, alcohol, acetone, heptane and toluene/alcohol | Soxhlet extraction |
| (Athukorala et al., 2009) | Flax straw | Hexane | Soxhlet extraction |
| (Villaverde et al., 2009) | Miscanthus giganteus stalk | Dichloromethane | Soxhlet extraction |
| (Villaverde et al., 2010) | Miscanthus giganteus bark | Dichloromethane | Soxhlet extraction |
| (Athukorala and Mazza, 2010) | Triticale straw | Hexane | Soxhlet extraction |
| (Marques et al., 2010) | Flax, hemp, sisal and abaca fibres | Acetone | Soxhlet extraction |
| (Naik et al., 2010) | Wheat straw, barley straw, pinewood, flax straw and Timothy grass | Hexane | Soxhlet extraction |
| (Asikin et al., 2012) | Sugarcane rinds and stalks | Hexane and methanol (20:1) | Soxhlet extraction |
| (Sin et al., 2014) | Wheat straw | Hexane | Soxhlet extraction |
| (Backlund et al., 2014) | *Pinus contorta* stumpwood, branches, bark, cones and needles | Hexane | Soxhlet extraction |
| (Attard et al., 2015b) | Sugarcane rind, leaves and bagasse | Hexane | Soxhlet extraction |

Soxhlet extraction is one of the most common, conventional techniques for extracting waxes from biomass and has numerous advantages. Unlike other conventional leaching techniques, the biomass continuously comes into contact with fresh solvent which displaces the extraction equilibrium (Luque de Castro and Garcı́a-Ayuso, 1998). Complete extraction of waxes can be achieved. The heat supplied to the solvent reservoir reaches the Soxhlet extractor and considerably high extraction temperatures are maintained throughout the entire system. Simultaneous extractions can be carried out by having a number of Soxhlet extractors in parallel, which increase biomass throughput (Luque de Castro and Garcı́a-Ayuso, 1998).

However, there are a number of disadvantages associated with Soxhlet extractions. Firstly, it is a time-consuming process, requiring significant volumes of solvent (leading to problems in waste disposal) and elevated temperatures maybe required to heat the solvent to its boiling point increasing the risk of thermal decomposition of thermally labile molecules (Luque de Castro and Garcı́a-Ayuso, 1998; Wang and Weller, 2006). After the extraction, the solvent has to be evaporated to concentrate the products, which is also an energy consuming process and leads to solvent waste. Finally, the extraction of specific metabolites is dependent on the selectivity of the solvent. A large number of solvents have relatively poor selectivity, making the extraction of specific metabolites challenging (Luque de Castro and Garcı́a-Ayuso, 1998; Wang and Weller, 2006).

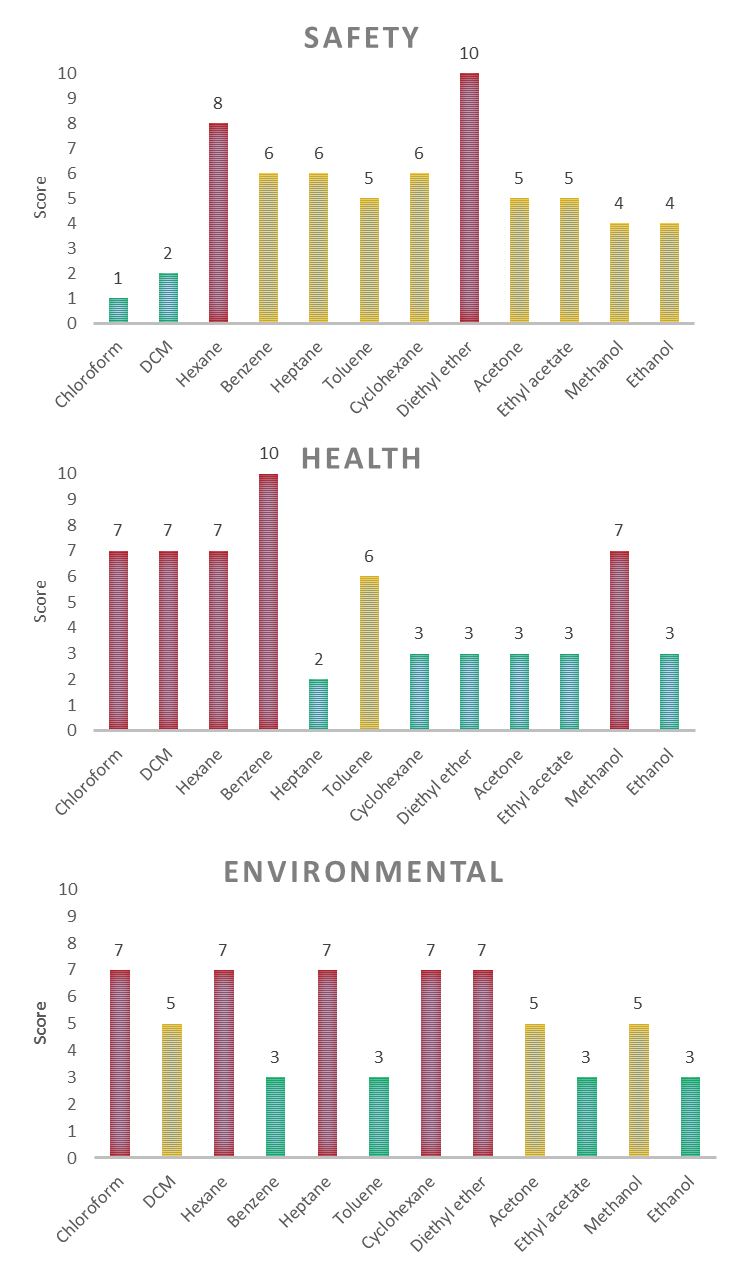
Preliminary results have shown that there is real commercial potential for utilisation of some of the waxy compounds as green replacements for some non-renewable waxes in applications including food supplements, cosmetics, flavourings, coatings amongst others. However, many of these applications have strict legislation associated with them such as restricting extraction solvent usage as well as solvent residues in flavour and fragrance cosmetics etc. Currently, one of the most commonly used solvents in commercial lipid extraction processes is commercial hexane, made up of a hydrocarbon mixture (linear and branched) having a boiling point range of 65-69 oC (Hamm et al., 2013). *n-*hexane comprises approximately 65% of the solvent (Hamm et al., 2013). It is the preferred solvent of choice in plant lipid extraction due to its wide availability at relatively low cost and favourable functional characteristics. These include moderate boiling point range, low latent heat of vaporisation, low specific heat, low solubility with water, high solubility with oils and high diffusivity through the oil seed cell walls (Kerton and Marriott, 2013).

There have been growing concerns regarding the use of hexane in extraction processes. Besides the fact that it is petroleum-derived, it has been listed by the Environmental Protection Agency (EPA) as a hazardous air pollutant in the Clean Air Act (1990) (DeSimone, 2002). Hexane has been found to be a neurotoxin, having severe adverse effects on the nervous system (Schaumburg and Spencer, 1976). The EPA Toxic Release Inventory has reported that over 20 million kg of hexane per year are released into the atmosphere (DeSimone, 2002). There have already been a number of restrictive measures implemented when using hexane. In 1999, it was added to the list of chemicals in the U.S. Toxic Release Inventory. Regulations were issued by the U.S. Environmental Protection Agency (EPA) in 2001 to control hexane emissions (Kerton and Marriott, 2013). Hexane was included in the ‘List of Toxic Substances in Schedule I of the Canadian Environmental Protection Act’ in 1999. This looks into regulating the use and release of toxic substances (Canadian Environmental Protection Act, 2003). Legislation has been implemented by the EU since 1999 relating to solvents allowed for the preparation of food ingredients (EU, 2009a, 2010). The latest EU legislation, which describes both the permitted extraction solvents as well as their maximum residual levels (MRL), lists hexane as a restricted solvent by MRL (EU, 2009a, 2010). In Japan solvents can only be used for extracting ingredients if they are approved under the specifications and standards for Food Additives. Hexane is a restricted solvent with an MRL of 25 mg/kg (Ministry of Health and Welfare, 2000). In the US, hexane is found in the Code of Federal Regulations with an MRL specified as 25 mg/kg (Kerton and Marriott, 2013).

In pharmaceutical and nutraceutical products, hexane and cyclohexane are classified as class 2 solvents. Class 2 solvents refer to solvents that should have very limited use and presence in the material due to possible causative agents of irreversible toxicity. The limited presence of class 2 solvents in materials is in absolute parts per million or permissible daily exposure (PDE in mg). In the case of hexane and cyclohexane, the PDE (mg/day) and concentration limits (ppm) are 2.9 mg/day and 290 ppm for hexane and 38.8 mg/day and 3880 ppm for cyclohexane (Kerton and Marriott, 2013). Regulation (EC) no 1223/2009 of the European Parliament and of the council of 30 November 2009 on cosmetic products listed hexane as a prohibited substance in cosmetic products (EU, 2009b).

A recent selection guide has been developed by the Innovative Medicines Initiative (IMI)-CHEM 21 public-private partnership, whereby industrial solvents have been analysed and ranked based on safety, health and environmental criteria (EHS criteria) (Prat et al., 2015). Four ranks have been assigned based on these criteria: Recommended, Problematic (can be utilised on a lab scale but certain measures have to be applied on a pilot scale), Hazardous (high constraints on a pilot scale, solvent substitution highly recommended) and highly hazardous (avoid using such solvents).

Figure 3 illustrates EHS data from the study relating to commonly used extraction solvents. A score of 1-10 (with 10 being the most hazardous) is given for each solvent, with a combination of the three criteria leading to a rank. The guide indicated chloroform and diethyl ether to be highly hazardous solvents; Dichloromethane (DCM), hexane and benzene to be hazardous; heptane, toluene and cyclohexane to be problematic while acetone, ethyl acetate, ethanol and methanol are recommended.

****

**Figure 3 Diagram illustrating safety, health and environmental criteria of commonly used extraction solvents.**

However, these solvents would still be problematic and undesirable for the extraction of waxes and lipids due to their polarity. All these solvents are more polar (higher values as shown in Table 3) than solvents such as hexane and toluene, leading to the co-extraction of a number of unwanted additional products such as sugars and further energy-intensive separation or purification processes. An ideal solvent for wax and lipid extraction would be one that has similar solvent properties to non-polar solvents such as hexane, chloroform and toluene without the negative safety, health and environmental issues associated with them. One such solvent is scCO2.

1. **Supercritical carbon dioxide extraction of waxes**

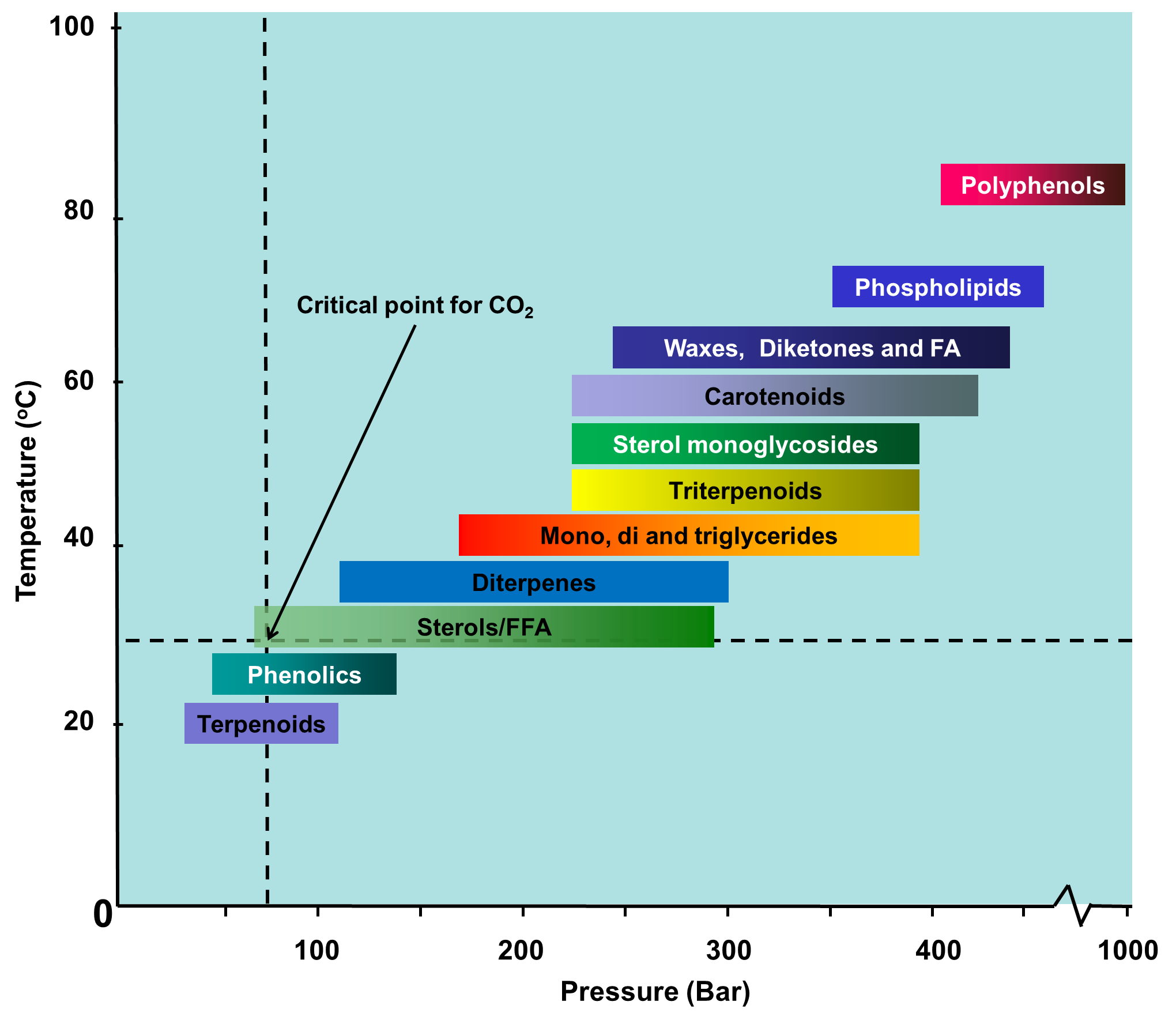
ScCO2 is the most commonly used supercritical fluid due to the relatively low critical temperature (31.1 oC) and accessible pressure, which enables the benefits of near-critical operation at temperatures lower than 35 oC. Carbon dioxide is widely available from several industrial processes, cheap, non-flammable and has minimal toxicity. scCO2 is commonly used in industry such as in the extraction of hops and decaffeination of coffee (McHugh and Krukonis, 1994; Subramaniam et al., 1997).

**Table 3 values for a numerous solvents**

|  |  |
| --- | --- |
| Solvent | values |
| Tetramethylsilane (TMS) | 0 |
| Hexane | 0.009 |
| ScCO2 | 0.012 – 0.034 |
| Toluene | 0.099 |
| DCM | 0.309 |
| Methanol | 0.762 |
| Water | 1 |

Table 3 indicates the values for numerous solvents and there is a high similarity in the polarity of scCO2 with hexane and toluene. A general rule of thumb is that low molar mass compounds having appreciable vapour pressures that dissolve in hexane should also be soluble in scCO2 (McHugh and Krukonis, 1994). It has been shown that scCO2 is an excellent solvent for extracting plant waxes and lipids as a result of its solvent tuneability (Kwan et al., 2016). Slight changes in temperature and pressure applied lead to significant variations in solvent density, which in turn result in a change in density-dependent solvent properties such as the dielectric constant, solubility parameter and partition coefficient (Leitner, 2000). By fine-tuning the solvent power, non-polar compounds can be selectively extracted, something which cannot be done with conventional organic solvent extraction (Lang and Wai, 2001; Zougagh et al., 2004). Solvent removal from the lipid products is easy and efficient, through a simple depressurisation step, avoiding any energy-intensive solvent removal processes. Recycling of CO2 is possible after extraction.

However, capital installation costs are still considerably high as well as the running costs (due to the high pressures often required) (Hunt et al., 2010). The extraction units require technically skilled operators and the process is not suitable for materials that have high water content. Nevertheless, a host of literature has demonstrated the effective implementation of scCO2 extraction for obtaining numerous waxes and lipids from a variety of biomass (Hunt et al., 2010).

It is important to have an understanding of the supercritical extraction conditions, their influence on solubility behaviour of lipids and the resulting wax composition, to develop efficient extraction and fractionation processes. Several studies have investigated the influence of temperature and pressure on the solubility of pure lipids, including alkanes (Shi et al., 2015), fatty acids, fatty acid esters (Güçlü-Üstündağ and Temelli, 2000), sterols (Güçlü-Üstündağ and Temelli, 2004), mono-, di-, and triglycerides and phospholipids (Hammam, 1992). These studies provide an understanding of the appropriate conditions required for optimal extraction. Figure 4 presents a generalised scheme indicating some relative pressures and temperatures for the extraction of families of compounds. However, selecting appropriate conditions for selective extractions or fractionations can be more challenging in complex multicomponent systems where entrainer effects can enhance the solubility (Anderson and Siepmann, 2008). In addition, the physical state of a molecule, its hydrogen bonding ability, molecular weight or polarity may also play key roles in the solubility of lipids. 

**Figure 4 Graphical representation of some relative pressures and temperatures for the extraction of families of compounds (FFA = free fatty acid and FA = fatty alcohol).**

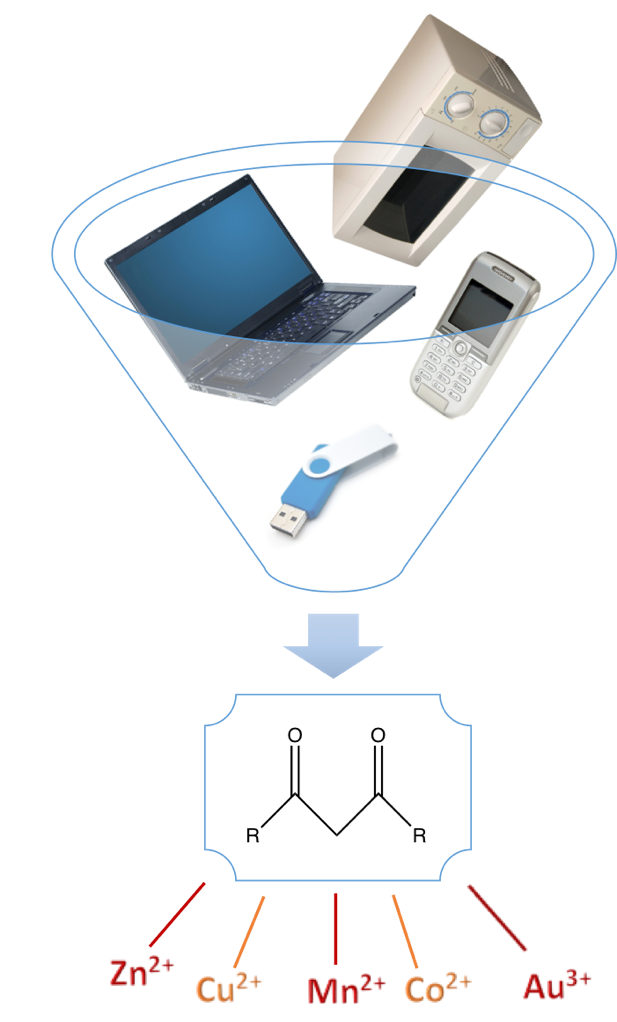
Research has utilised the Chrastil equation to determine the operating conditions such as density, pressure, and temperature on the solubility of a range of molecules (Güçlü-Üstündağ and Temelli, 2004). For many classes of molecule, a general trend in scCO2 extraction is declining solubility with increasing molecular weight or polarity (Güçlü-Üstündağ and Temelli, 2000). For example, the solubility data of analogous di-, triglycerides demonstrated that dilaurin was less soluble than trilaurin above 200 bars due to its greater polarity (Hammam, 1992). The opposite effect dominates below 200 bar, resulting from the lower molecular weight of dilaurin (Hammam, 1992). Increased pressure also leads to enhanced solubility of lipids under isothermal conditions (McElroy et al., 2018). Typically, increased solubility can occur with elevated temperature at a constant density (Güçlü-Üstündağ and Temelli, 2000). The physical state of a lipid can influence its solubility. As the temperature within an extraction system increases, a decrease in solubility is generally observed for molecules in the liquid state, while the opposite is true for solids and results in enhanced solubility or extraction rates (Güçlü-Üstündağ and Temelli, 2000). The final considerations in optimising extraction conditions must include, reactor design, pre-processing of biomass (milling, pelletizing and grinding) and other process conditions such as flow rate and extraction duration, which all play a key role in the process. Table 4 illustrates the literature concerning the scCO2 extraction of waxes from agricultural waste residues.

**Table 4. Supercritical extraction of waxes from various agricultural waste residues.**

|  |  |  |  |
| --- | --- | --- | --- |
| Literature | Biomass | Conditions | Study |
| Morrison *et al.* 2006 | Flax/linseed | scCO2 and scCO2 extraction (552 bar, 60 oC)  Ethanol co-solvent 1, 5 and 10% | Physical Properties, Characterisation/quantification |
| Deswarte *et al.* 2006 | Wheat Straw | (100 bar/300 bar, 60 oC) | Physical Properties, Characterisation |
| Deswarte *et al.* 2007 | Wheat Straw | 300 bar, 40 oC | Extraction and application of waxes |
| Athukorala *et al.* 2009 | Flax straw | (300 bar, 70 oC) | Physical Properties, Characterisation/quantification |
| Athukorala *et al.* 2010 | Triticale | (250/300/350/400 bar, 70 oC) | Physical Properties, Characterisation/quantification  Process optimisation |
| Budarin *et al.* 2011 | Wheat straw | 100 – 300 bar, 40 – 100 oC | Incorporation into a biorefinery |
| Sin *et al.* 2014 | Wheat straw | 75 – 400 bar, 32 – 100 oC | Characterisation/quantification  Process Optimisation  Solubility Studies |
| Lucas *et al.* 2007 | Sugarcane crude wax | 350 bar, 100 oC/20% KOH | Extraction of *n-*policosanols |
| Prado *et al.* 2011 | Sugarcane residue | 350 bar, 60 oC | Scale-up supercritical extraction process |
| Attard *et al.* 2015 | Sugarcane rind, leaves, bagasse | 350 bar, 50 oC | Characterisation/quantification  Process Optimisation |
| Attard *et al.* 2015 | Maize stover | 80 - 400 bar, 35 - 65 oC | Characterisation/quantification  Process Optimisation + Effect of SFE on Fermentation of biomass |
| Attard *et al.* 2016 | *Miscanthus giganteus and Miscanthus sinnsis (leaves and bark)* | 80 – 400 bar, 35 – 65 oC | Characterisation/quantification  Process Optimisation + Effect of SFE on Hydrolysis of biomass |
| Vagi *et al.* 2007 | Industrial Tomato waste | 300 /460 bar, 40/80 oC | Extraction of tocopherols, carotenoids and sitosterols |
| Lau *et al.* 2008 | Palm-pressed mesocarp fibre | 100 – 300 bar/ 40 oC | Extraction of tocopherols, carotenoids and sitosterols |
| Kawahito *et al.* 2008 | Loquat seeds | 300 and 450 bar, 40, 60 and 80 oC | Extraction of phytosterols |
| Martins *et al.* 2016 | *Eichhornia crassipes* biomass | 200 – 300 bar, 50 oC | Extraction of phytosterols |
| de Melo *et al.* 2016 | *Eichhornia crassipes* biomass | 200 bar, 40 oC | Optimisation of phytosterol extraction |
| Ortega *et al.* 2017 | Saw Palmetto | 350 – 450 bar, 40 oC and 60 oC | Extraction of free fatty acids and phytosterols |

Extensive work has been conducted on straw wax. Morrison *et al.* carried out supercritical extraction of waxes from flax processing waste. Flax is generally processed for its fibres and during the process, significant quantities of dust are generated (Morrison Iii et al., 2006). Utilisation of this dust could lead to an added-value co-product, which would significantly aid the flax industry. The extraction was carried out at high pressure and temperature (557 bar and 60 oC) and a much higher % crude yield was obtained with scCO2 (7.4%) compared to hexane (4%). The wax was composed mainly by four groups of compounds; *n-*policosanols, fatty acids, fatty aldehydes and wax esters. A wide range of *n-*policosanols were identified, ranging from C18 to C30, constituting the major constituents of the wax (350 – 380 mg/g of wax) (Morrison Iii et al., 2006). Similarly, Athukorala *et al.* studied the scCO2 extraction of waxes from flax straw and in addition to the compounds identified by Morrison lii *et al.*, phytosterols and *n-*alkanes were also present (Athukorala et al., 2009). Optimisation of the scCO2 extraction process was conducted by investigating the effect of flow rate, pressure and temperature on the % crude wax yield. The highest yields were obtained using a flow rate of 61.6 g/min-1, a pressure of 377.8 bar and temperature of 74.7 oC. It was concluded that the most important factor on the crude yield was temperature (Athukorala et al., 2009).

Deswarte *et al.* and Sin *et al.* investigated the scCO2 extraction of waxes from wheat straw (Deswarte et al., 2006; Sin et al., 2014). In 2014, the estimated global production of wheat grain was *ca.* 713 million tonnes. It has been estimated that for every tonne of wheat grain produced, 1.3 tonnes of wheat straw are generated and is considered to be of very low economic value (Sin et al., 2014). Sin *et al.* modelled the scCO2 extraction of waxes using the Chrastil model. It was found that the crude yield as well as the % of wax esters could be predicted using the Chrastil model resulting in maximum extractions. In both studies by Deswarte et al. and Sin et al., besides the typical group of compounds present in the wax (fatty acids, fatty alcohols, fatty aldehydes, *n*-alkanes, sterols, and wax esters), the wheat straw wax was found to be unique among the straw waxes in that it contained two ß-diketones; 14,16-hentriacontanedione and 16,18-triatriacontanedione as the major components of the wax (Deswarte et al. 2006, Sin et al., 2014). These diketones were also found by Athukorala and Mazza in triticale straw wax (a cross between wheat and cereal rye), (Athukorala and Mazza, 2010). These diketones are highly sought after due to their unique chelating properties (Fanou et al., 2007). It has been demonstrated that isolation of diketones from the wheat straw wax is simple and efficient, leading to diketone products of high purity (Asemave et al., 2016). Recent work has successfully demonstrated the use of these extracted diketones as natural, bio-derived chelators for metal ions extraction (Ni2+, Co2+, Cu2+ and Cr3+) (Asemave et al., 2016) (Figure 5). This is particularly relevant especially due to the rapidly growing need for elemental sustainability, due to the rapid depletion of a number of metals and the need for their recovery from waste (Hunt et al., 2015).

**Figure 5. Use of diketones to recover metals for elemental sustainability**

Waxes from sugarcane residues (sugarcane rind, leaves and bagasse) have been thoroughly investigated due to the large volumes generated during the harvesting and milling for sugar. One such study, investigated these residues for their wax content in order to potentially add value to this waste (Attard et al., 2015b). The sugarcane rind wax was dominated by long-chain fatty aldehydes and *n-*policosanols, constituting approximately 83% of the total wax composition. The significant proportion of these compounds exhibit nutraceutical properties makes their isolation and purification commercially viable. In contrast, wide varieties of compounds were identified in the leaves with the major group being triterpenoids followed by unsaturated and saturated fatty acids and phytosterols. This demonstrated that different sugarcane waxes could be utilised in various applications. In the majority of cases, scCO2 extracted larger quantities of lipophilic molecules as compared to hexane extraction, in particular the triterpenoid molecules (Attard et al., 2015b). *n-*policosanols from sugarcane were also extracted by Lucas *et al.*, who conducted the scCO2 extraction of *n-*policosanols from sugarcane crude wax (de Lucas et al., 2007). This is a by-product of sugarcane production, obtained from filter mud by extracting with heptane. Optimal conditions were found to be 350 bar and 100 oC using 20% KOH during the saponification stage to optimise extraction yields (de Lucas et al., 2007). Following on from this, Prado *et al.* looked into developing a scale-up supercritical fluid extraction process for Brazilian raw materials including sugarcane residue. Three main compounds that were extracted included 1-octacosanol, stigmasterol and β-sitosterol (Prado et al., 2011).

The scCO2 extraction of fatty acids, phytosterols, tocopherols and carotenoids (including lycopene and ß-carotene) from industrial tomato waste was conducted by Vagi *et al.* (Vági et al., 2007). The effect of temperature and pressure on lipid yield found that above 300 bar and 40 oC, the extracts contained high quantities of tocopherols and phytosterols, while the highest amounts of carotenoids were obtained at 460 bar and 80 oC (Vági et al., 2007). Similar results were observed in a continuous scCO2-extraction technique developed by Lau *et al.* to extract sterols, tocopherols and carotenoids from palm-pressed mesocarp fibre; whereby a pressure range of 100 – 300 bar and a temperature of 40 oC was implemented (Lik Nang Lau et al., 2008). At 100 bar, vitamin E and squalene were selectively extracted while bulky triglycerides were removed when the pressure was raised to 200 bar. As was the case with Vagi et al., at a higher pressure (increased to 300 bar), a carotene enriched fraction was obtained. Phytosterols were found in all extracts but were obtained in higher quantities at higher pressure (Lik Nang Lau et al., 2008).

Kawahito *et al.* investigated the scCO2 extraction of phytosterols from loquat seeds, a waste product from loquat fruit (Kawahito, 2008). Extractions were carried out on roasted powder and unroasted ground seeds using various temperatures and pressures (40, 60 and 80 oC/300 and 450 bar). It was found that there was a higher total extract and ß-sitosterol recovery when using the roasted powder loquat seeds (Kawahito, 2008). This was attributed to the smaller particle size (0.3 mm) of the roasted powder, which leads to a higher surface area of the seed (allowing greater contact with scCO2) as well as an increase in broken cell availability resulting in higher quantities of phytosterol extracted. Ortega et al. conducted the supercritical extraction of free fatty acids (FFAs) and ß-sitosterol from Saw Palmetto using conditions of 300 – 450 bar, in the absence and presence of a co-solvent (Ortega et al. 2017). The FFA content was affected by temperature. It was postulated that at higher temperatures (at constant pressure), the compounds dissolved more in the solvent due to an increase in volatility. There was an increase in the ß-sitosterol content with increasing pressure. For both FFA and ß-sitosterol, there was an increase extraction yields due to the higher solubility of these compounds in ethanol (Ortega et al., 2017).

Recent studies have focused on valorising water hyacinth (*Eichornia crassipes*), a fast-growing invasive aquatic plant species. This vegetal species has raised environmental concerns in areas such as Europe and Africa. A number of valorisation pathways are being investigated in order to provide additional value to those economies that have been affected its invasion. One pathway was to look at extracting the unusually high quantities of stigmasterol (a precursor in the synthesis of synthetic progesterone) found in the leaves and stalk. Martins et al. conducted supercritical extractions, using pure and modified CO2 (ethanol co-solvent). The highest yield of sterols obtained was 38.26 wt % (26.35 wt % comprising stigmasterol) using conditions of 300 bar and 2.5% ethanol co-solvent (Martins et al. 2016). Following on from this, de Melo et al. developed a supercritical process selective towards stigmasterol. They found that conditions of 200 and 40 oC led to the selective extraction of stigmasterol - 32% wt of the total extracts, which is three times higher when compared to Soxhlet extraction with dichloromethane (de Melo et al. 2016).

ScCO2 extraction has also been utilised as a versatile technique for the extraction of lipophilic extractives from different parts of forestry (wood) biomass (Fojtová et al., 2010). Utilisation of scCO2 with and without co-solvents were demonstrated to be efficient in isolation of fatty and resin acids from Scots pine sawdust (a by-product from the timber industry) and wood pellets (produced from Scots Pine sawdust) (Arshadi et al., 2012). 84% of the lipids and resin acids were removed from using conditions of 350 bar and 55 oC for 6 hours. The fatty acids recovered had chain lengths of C8 – C18 while the resin acids included abietic acid, dehydroabietic acid, oxodehydroabietic acid, pimaric acid and isopimaric acid (Attard et al., 2016a). In 2013, there was around 12 million tonnes of wood pellets produced mostly from saw dust in Europe alone, which would lead to around 312,000 tonnes of extract per year for liquid fuels and chemicals if the conditions in this study are implemented (Attard et al., 2016a).

Fojtová *et al.* reported about suitability and high efficiency of scCO2 extraction using chloroform as a modifier for terpenes isolation from coniferous wood needles and tree-leaves wood (Fojtová et al., 2010). It was found that scCO2 (300 bar, 130 oC) extracted the highest amount of terpenes when compared to other extraction techniques including accelerated solvent extraction, steam distillation, soxhlet extraction, sonication and solvent agitation. By optimising the supercritical extraction process (pressure, temperature, modifier etc.), they were able to significantly reduce the extraction time from the needles when compared to conventional extraction methods (Fojtová et al., 2010). Zhao et al. studied the correlation between the scCO2 extraction of fatty acids with their heat yield value (HYV), and compared it to Soxhlet extraction (Zhao et al., 2016a and2016b). The authors reported that scCO2 extraction could be done with milder conditions (45 °C, 40-50 MPa) and shorter extraction times (80 mins) to isolate fatty-extracts and that the scCO2 extracts had a higher HYV (Zhao et al., 2016b) .

One of the huge advantages of supercritical extraction is the possibility of incorporating fractional separation (during the extraction process) to improve extraction selectivity and obtains products of higher value (Reverchon and De Marco, 2006). Unfortunately, there is no means of preventing these unwanted families of compounds from being extracted with conventional solvents. The same applies to scCO2 extractions carried out using a single pressure and temperature. However, with fractional separators, it is possible to fractionate or separate the crude products, in which different families of compounds precipitate out in successive steps. There is a substantial advantage for using scCO2 extraction over conventional organic extraction.

This fractionation can be achieved through changing the CO2 density, lowering the solubility of the desired molecules in scCO2. An extraction unit normally possesses several fractional separators, arranged in series and set at different pressures and temperatures. Each separator has a different CO2 density and the solvation properties of scCO2 for each separator are different. When molecules are passing through a fractional separator, some will be soluble at the specific CO2 density range of the separator, while others will not be soluble (as they are only soluble at a particular CO2 density range). The latter will precipitate out while the former will pass into the next fractional separator. Figure 6 summarises this concept as shown in a recent study on the fractional separation of wax molecules from maize stover (Attard et al., 2015c).

 **Figure 6 Graphical illustration of the fractionation of molecules with scCO2.**

Supercritical fractionation has been extensively applied in essential oil isolation and purification (Reverchon et al., 1995); for e.g. in the supercritical fractionation of rosemary leaves to separate the oleoresin into two fractions, each one having a different antioxidant activity (Ibáñez et al., 1999).Considerable work has focused on the separation of essential oils from epicuticular waxes as a whole (Reverchon et al., 1995). This is particularly useful as often, both families of compounds are co-extracted and fractionation prevents the need for any post-extraction purification techniques while obtaining two pure added-value products. The separation is based on the fact that at very low temperatures (*ca.* -5 oC to 5 oC), plant waxes are completely insoluble in CO2 while essential oils are fully soluble which leads to the separation of the two (Reverchon and De Marco, 2006). This has been applied to the fractionation of lavender essential oils and waxes, majoram leaves, peppermint leaves and oregano leaves (Reverchon and De Marco, 2006; Simándi et al., 1998). 118 Da porto *et al.* recently carried out a fractionation on hemp inflorescences (Da Porto et al., 2014). At a pressure of 100 bar and 40 oC and the use of two fractional separators, they managed to collect the waxes on the surface of the flowers in the first fractional separator while all the volatile aroma compounds were collected in the second separator (Da Porto et al., 2014). In a recent study, Baldino *et al.* conducted a supercritical fractionation of active compounds from *Ruta* graveolens, known to have furanocoumarinic compounds that contain active properties (Baldino et al. 2017). Using optimised conditions of 200 bar and 40 oC and a fractional separator, the cuticular waxes (comprising 78% of the total compounds) were separated from the furanocoumarinic compounds resulting in the purification of two products (Baldino et al. 2018). Highlighting another advantage of scCO2 extraction over traditional solvent extraction, whereby no fractionation can occur, energy-intensive and time-consuming processes must be implemented post-extraction for separation.

Besides separating waxes as a whole from other unwanted compounds, it is also possible to fractionate the crude wax product into various fractions allowing for the concentration of certain waxy compounds. Work by Attard *et al*. has demonstrated that it is possible to obtain different wax fractions directly from maize stover biomass using fractional separators (Attard et al., 2015c). Three different wax fractions were obtained each having a distinct melting point range. The first fraction was found to have a relatively high-melting point range, enabling its use in instrument and automobile polishes. The second wax fraction was found to be dominated by phytosterols, which have significant nutraceutical properties. The large abundance of these phytosterols should make their isolation and purification relatively straightforward. The final fraction had a very low melting point range and was found to be very promising as an anti-foaming agent in detergent formulations (Attard et al., 2015c).

1. **Supercritical Extraction of waxes/lipids and the biorefinery: a holistic approach**

Currently, commercial supercritical extraction processes are frequently stand-alone technologies. However, from an economical perspective, the supercritical extraction of waxes is not feasible as a standalone technology. Studies have demonstrated numerous benefits of incorporating the supercritical extraction as a first step in a holistic biorefinery (Attard et al., 2015c). One of the major aims of a biorefinery is the concept of holistic utilisation, whereby all of the biomass is utilised to maximise value, whilst ideally generating no waste. Currently, supercritical extraction is not widely utilised in a biorefinery leading to the loss of the lipophilic molecules. scCO2 extraction could be a first stage, prior to aggressive physical pre-treatment (Attard et al., 2015c)). Since, scCO2 extraction leaves no solvent residues, is a non-destructive technology and only removes the outer lipid layer covering plant surfaces, following extraction the biomass undergo further downstream processing (Morais et al., 2014). This would not be practical for conventional solvent extraction, as the biomass will contain considerable quantities of solvent residue and an additional solvent removal step would be required, which would be both time-consuming and energy-intensive.

Budarin *et al.*  proposed a model in which scCO2 extraction of waxes is integrated as a first stage in a wheat straw biorefinery, to extract added-value products prior to microwaves pyrolysis of wheat straw to generate high quality char and oils (Budarin et al., 2011).

Studies have looked into introducing scCO2 extraction of waxes in an integrated maize stover biorefinery (Attard et al., 2015c). ScCO2 extraction and fractionation of waxes from maize stover was conducted prior to hydrolysis and fermentation for ethanol and surfactant production. Importantly, this work demonstrated that there is a positive effect on the downstream processing of the biomass. ScCO2 extraction of the waxes prior to physical pre-treatment resulted in a 40% increase in ethanol production (when compared to non-scCO2 extracted maize stover) as well as greater potential growth for the production of surfactants (Attard et al., 2015c).

Another study investigated the effect of supercritical extraction on the downstream processing of *miscanthus giganteus* (Attard et al., 2016b). ScCO2 extraction of waxes from leaves of *miscanthus giganteus* was carried out followed by subsequent enzyme saccharification of the leaves and the results were compared to non-extracted *miscanthus giganteus* leaves. It was found that a 20% increase in the total sugars released was obtained with the scCO2 extracted leaves when compared to the non-extracted leaves (Attard et al., 2016b). The results from this study correlate to studies conducted on the effect of cuticular waxes of C4 biomass. It has been stated that waxes are critical inhibitors of fermentation. It was found that sweet sorghum cuticular waxes prevent acidogenesis and solvatogenesis phase of acetone-butanol-ethanol fermentation by *Clostridium acetobutylicum* ABE 1201 (Cai et al., 2013). The main constituents found in the wax are fatty acids, fatty alcohols and fatty aldehydes. By removing the layer of waxy lipids covering the plant surface, the hydrothermal pre-treatment and enzyme access to polysaccharides are greatly improved resulting in better hydrolysis and subsequent fermentation. CO2 has been used in lignocelullosic (LCM) pretreatment (not as an extraction solvent). Small pores of recalcitrant lignocellulosic materials are easily penetrated by CO2 at high pressures, which cause a structural change leading to easier access for the hydrolysing enzymes to xylan and glucan (Morais et al. 2014).

Recent work has focused on utilising cereal, oilseed grains and dried distillers’ grains with solubles (DDGS) as renewable feedstock sources for CO2 biorefineries. Temelli *et al.* proposed that scCO2 could remove the lipids, while conventional processes would then fractionate the remaining carbohydrates and proteins (Temelli and Cifci, 2015). Post-scCO2 extraction, the meal residues (such as canola) contain higher amount of protein and carbohydrates when compared to conventional organic extraction. The CO2 process would focus on the extraction of lipids, their fractionation and conversion to other products as well as particle formation for bioactive delivery (Temelli and Cifci, 2015).

Albarelli *et al*. proposed implementing scCO2 in a biorefinery for valorising annatto seeds. ScCO2 would be a useful first step in generation tocotrienol-abundant and bixin-rich extracts (Albarelli et al., 2016). Process modelling and simulation tools showed that supercritical fluid extraction is vital from an economic standpoint as it leads to higher bixin-rich extracts. A positive influence on the economic indicators was found due to higher tocotrienol production. Efforts to further expand the viability of a biorefinery through scCO2 extraction and algal growth, whereby the electricity, CO2, heat and ethanol produced from a sugarcane biorefinery are utilised for the extraction of lipids and carotenoids from microalgae (Albarelli et al., 2016).

Qi *et al.* investigated the effect of removal of waxes on the enzymatic hydrolysis of sugarcane bagasse (Qi et al., 2016). Hydrolysis of the original and scCO2-extracted bagasse was conducted using an enzyme cocktail consisting of 29 FPU cellulose. In the case of the scCO2-extracted bagasse, the cellulose and xylose digestibilities were found to be 18.7% and 10.3%,while for the original unextracted bagasse, the digestibilities were found to be 13.1% (cellulose) and 8.9% (xylose) (Qi et al., 2016).

A recent study illustrated the significance of supercritical extraction as part of a forestry biorefinery (Attard et al., 2016a). The study looked into the impact that supercritical extraction has on the off-gassing of wood pellets during storage. ‘Off-gassing’ refers to the spontaneous release of CO2, CO and CH4 during storage of these pellets through auto-oxidation. Such reactions are common on storage of saw dust, wood pellets or wood chips and may led to the rapid heating of biomass leading to fires. Whilst, in an enclosed environment the gasses themselves can lead to a number of accidents causing serious harm. In this study, the authors proposed that the scCO2 extraction of the sawdust prior to pelletization would remove the fatty and resin acids, reducing the off-gassing that would occur during storage of the pellets and generate value added products. The key results are summarised in Figure 7 below (Attard et al., 2016a).

**Figure 7 The emission (ppm) of CO2, CO and CH4 of non-extracted pellets and scCO2 extracted pellets (11 kg of each) during storage for 30 days.**

From Figure 7 it can be see that there was a drastic reduction in the emissions of CO2 (-85%), CO (-85%) and CH4 (-94%) from the scCO2-extracted pellets when compared to the non-extracted pellets. Another crucial point was that there was no significant difference in the density, production, calorific content and durability between the two sets of pellets showing that supercritical extraction has no negative effect on the physical properties of the pellets.

Supercritical extraction can not only lead to the extraction of added-value wax products but also improve the downstream processing of the biomass. scCO2 extraction of added value wax products from the surface of the biomass prior to the application of destructive technologies could be a viable first stage of and integrated biorefinery (Figure 8).



**Figure 8. Incorporation of scCO2 extraction in an integrated biorefinery.**

1. **Supercritical extraction: Economics**

A major drawback of scCO2 extraction is the perceived cost of the technology, due to the high level of initial investment and relatively high pressures required to maintain the supercritical state. Recent economic analysis of scCO2 looked at the fixed capital investment (FCI), labour costs (COL), cost of raw materials (CRM; land rent, rhizome costs, agricultural machinery, harvesting, milling, storage, transportation *etc.*), cost of waste treatment (CWT) and utility costs (CUT; costs associated with the heating, refrigeration and the CO2 pumps). The cost of wax production using scCO2 could be economically viable when considering certain parameters. The form in which the biomass is received is one such factor (cut, milled or pelletized). Pelletization of biomass would result in a higher biomass loading per extraction which would lead to lower overall costs (despite it being more expensive than cutting or milling) as larger amounts of wax are obtained per extraction. The part of the plant chosen for extraction is also another factor, as leaves contain the highest wax content resulting in a four-fold reduction in cost was observed (for both maize and miscanthus). Finally, utilisation of the biomass post-scCO2 extraction (e.g. combustion of biomass for electricity generation) results in a further reduction in costs. In the case of maize biomass, the wax extraction costs were found to be €4.60 per kg of wax, while the wax extraction costs for miscanthus were found to be approximately €6 per kg of wax (Attard et al., 2015a and 2016b). Global wax reports for 2015 have shown that the average price of non-petroleum waxes imported in the US was €5.75 per kg of wax; which included beeswax (€7.66 per kg of wax), carnauba wax (€7.15 per kg of wax) and candelila wax (€2.68 per kg of wax) (Argus, December 2015). The waxes obtained from maize and miscanthus fall within this range. The cost of wax would depend on different factors such as the purity of the wax product as well as its application (Argus, December 2015). The studies by Attard *et al.* suggested that combustion of the biomass post-scCO2 extraction could be used to add value through energy generation (Attard et al., 2015a and 2016b). However, based on the studies that utilise the residual biomass for the production of sugars, surfactants or liquid biofuels, the enhanced yields obtained may improve the costs further. Future work should focus on the economic assessment of a full biorefinery process to confirm the cost effective nature of this technology.

1. **Future Challenges**

In order to implement supercritical extraction as a technology within a biorefinery, it is important to address several key challenges, which may hinder its exploitation. Firstly, biomass selection for is vital, i.e. getting the right agricultural residues, which are abundant and have high wax yields. Plant breeding that not only optimises the yield of food, but also improves the characteristics of the residual biomass such as increased wax content, could be an part of developing economically viable bio-economies. This will lead to higher quantities of product and lower total COM associated with the supercritical extraction. Other future work could focus on the pre-treatment and hydrolysis of bound-lipids. Supercritical extraction of waxes currently focuses on the extracuticular waxes, i.e. those lipids found on the surface of the plant. It would be beneficial to develop clean cost-effective pre-treatment techniques to remove the lipids bound to the plant cell walls (the intracuticular waxes) in order to increase the yields of waxes.

An important challenge that should be addressed for all biorefineries, is to develop sustainable, cost effective and efficient separation technologies to retain functionality. One way to carry this out is to use scCO2 for extraction and as an in-situ separation technology. Such activities have been successfully implemented in the separation of pyrolysis oils (Mudroboyina *et al.* 2016).

1. **Conclusions**

This review demonstrates the extensive opportunities for both supercritical extraction as well as bio-derived waxes. Such waxes may find use in a wide range of higher value applications including chelators, pharmaceuticals, cosmetics, foods and nutraceuticals. This technique has been demonstrated to improve production of sugars by as much as 20%, whilst yields of biofuels such as ethanol can be increased by up to 40% over feedstocks not extracted by scCO2 . Significantly utilisation of scCO2 could lead to lower volumes of solvent waste and reduced VOC emissions (the EPA Toxic Release Inventory (TRI) indicated that more than 20,000 tonnes of hexane are released to atmosphere from extraction) (Sin et al., 2014). Higher product selectivity and fractionation at the point of extraction or collection with scCO2 extraction, importantly reduces the need for further energy or resource intensive purification steps. Finally, no residual solvent in the extracts means that scCO2 extraction is a cleaner, more efficient process when compared to traditional extraction. ScCO2 extraction is not only a suitable alternative technology for recovering waxes from waste biomass, but, it also offers the opportunity to improve the downstream processing of the residual biomass. This enhances hydrolysis and fermentation yields, as well as generating safer products (such as wood pellets) leading to more energy-efficient and economically viable biorefineries. The use of scCO2 extraction can be a cost effective method for wax extraction when utilised as part of a biorefinery. This review has demonstrated that scCO2 extraction can be an effective process for wax extraction and improving the downstream treatment of biomass as part of an integrated biorefinery.

**Acknowledgements**

The authors would like to thank the financial support of the Swedish Research Councils FORMAS (CETEX project – grant number 2014-12813-27337-64).

**References**

Act, C.P.E., 2003. Order Adding Toxic Substances to Schedule 1 to the Canadian Environmental Protection Act. Canada Gazette Part II., Canada, 137, 1864 - 1877.

Albarelli, J.Q., Santos, D.T., Cocero, M.J., Meireles, M.A.A., 2016. Economic Analysis of an Integrated AnnattoSeeds-Sugarcane Biorefinery Using Supercritical CO2 Extraction as a First Step. Materials 9, 494.

Anderson, K.E., Siepmann, I.J., 2008. Solubility in Supercritical Carbon Dioxide: Importance of the Poynting Correction and Entrainer Effects. J. Phys. Chem. B, 112 (36), 11374–11380.

András, C.D., Simándi, B., Örsi, F., Lambrou, C., Missopolinou-Tatala, D., Panayiotou, C., Domokos, J., Doleschall, F., 2005. Supercritical carbon dioxide extraction of okra (Hibiscus esculentus L) seeds. J. Sci. Food Agric. 85, 1415-1419.

Arato, M., Speelman, S., Van Huylenbroeck, G., 2014. The contribution of non-timber forest products towards sustainable rural development: The case of Candelilla wax from the Chihuahuan Desert in Mexico. Nat. Res. Forum. 38, 141-153.

Argus, December 2015. Argus Global Waxes. Incorporating Wax Data. https://www.argusmedia.com/Oil-Products/Argus-Global-Waxes/~/media/0841EC796DC64919A6FAA8AC8DABA01C.ashx., London, UK, Accessed 12th May 2017.

Arshadi, M., Backlund, I., Geladi, P., Bergsten, U., 2013. Comparison of fatty and resin acid composition in boreal lodgepole pine and Scots pine for biorefinery applications. Ind. Crops Prod. 49, 535-541.

Arshadi, M., Hunt, A.J., Clark, J.H., 2012. Supercritical fluid extraction (SFE) as an effective tool in reducing auto-oxidation of dried pine sawdust for power generation. RSC Adv. 2, 1806.

Asemave, K., Byrne, F., Farmer, T.J., Clark, J.H., Hunt, A.J., 2016. Rapid and efficient biphasic liquid extraction of metals with bio-derived lipophilic [small beta]-diketone. RSC Adv. 6, 95789-95792.

Asikin, Y., Takahashi, M., Hirose, N., Hou, D.-X., Takara, K., Wada, K., 2012. Wax, policosanol, and long-chain aldehydes of different sugarcane (Saccharum officinarum L.) cultivars. Eur. J. Lipid. Sci. Technol.114, 583-591.

Athukorala, Y., Mazza, G., 2010. Supercritical carbon dioxide and hexane extraction of wax from triticale straw: Content, composition and thermal properties. Ind. Crops Prod. 31, 550-556.

Athukorala, Y., Mazza, G., Oomah, B.D., 2009. Extraction, purification and characterization of wax from flax (Linum usitatissimum) straw. Eur. J. Lipid Sci. Technol. 111, 705-714.

Attard, T., McElroy, C., Hunt, A., 2015a. Economic Assessment of Supercritical CO2 Extraction of Waxes as Part of a Maize Stover Biorefinery. Int. J. Mol. Sci. 16, 17546-17564.

Attard, T.M., Arshadi, M., Nilsson, C., Budarin, V.L., Valencia-Reyes, E., Clark, J.H., Hunt, A.J., 2016a. Impact of supercritical extraction on solid fuel wood pellet properties and off-gassing during storage. Green Chem. 18, 2682-2690.

Attard, T.M., Hunt, A.J., Matharu, A.S., Houghton, J.A., Polikarpov, I., 2014. Biomass as a Feedstock, in: Clark, J., Deswarte, F., Introduction to Chemicals from Biomass. John Wiley & Sons, Ltd, UK, pp. 31-46.

Attard, T.M., McElroy, C.R., Gammons, R.J., Slattery, J.M., Supanchaiyamat, N., Kamei, C.L.A., Dolstra, O., Trindade, L.M., Bruce, N.C., McQueen-Mason, S.J., Shimizu, S., Hunt, A.J., 2016b. Supercritical CO2 Extraction as an Effective Pretreatment Step for Wax Extraction in a Miscanthus Biorefinery. ACS Sustain. Chem. Eng. 4, 5979-5988.

Attard, T.M., McElroy, C.R., Rezende, C.A., Polikarpov, I., Clark, J.H., Hunt, A.J., 2015b. Sugarcane waste as a valuable source of lipophilic molecules. Ind.Crops Prod. 76, 95-103.

Attard, T.M., Theeuwes, E., Gomez, L.D., Johansson, E., Dimitriou, I., Wright, P.C., Clark, J.H., McQueen-Mason, S.J., Hunt, A.J., 2015c. Supercritical extraction as an effective first-step in a maize stover biorefinery. RSC Adv. 5, 43831-43838.

Backlund, I., Arshadi, M., Hunt, A.J., McElroy, C.R., Attard, T.M., Bergsten, U., 2014. Extractive profiles of different lodgepole pine (Pinus contorta) fractions grown under a direct seeding-based silvicultural regime. Ind. Crops Prod. 58, 220-229.

Baldino, L., Adami, R., Reverchon, E., 2018. Concentration of *Ruta graveolens* active compounds using SC-CO2 extraction coupled with fractional separation. J. Supercrit. Fluids 131, 82-86.

Barthlott, W., Neinhuis, C., Cutler, D., Ditsch, F., Meusel, I., Theisen, I., Wilhelmi, H., 1998. Classification and terminology of plant epicuticular waxes. Bot. J. Linn. Soc. 126, 237-260.

Blake, A.I., Co, E.D., Marangoni, A.G., 2014. Structure and Physical Properties of Plant Wax Crystal Networks and Their Relationship to Oil Binding Capacity. J. Amer. Oil Chem. Soc. 91, 885-903.

Bradford, P.G., Awad, A.B., 2007. Phytosterols as anticancer compounds. Mol. Nutr. Food Res 51, 161-170.

Budarin, V.L., Shuttleworth, P.S., Dodson, J.R., Hunt, A.J., Lanigan, B., Marriott, R., Milkowski, K.J., Wilson, A.J., Breeden, S.W., Fan, J., Sin, E.H.K., Clark, J.H., 2011. Use of green chemical technologies in an integrated biorefinery. Energy Environ Sci. 4, 471-479.

Cai, D., Chang, Z., Wang, C., Ren, W., Wang, Z., Qin, P., Tan, T., 2013. Impact of sweet sorghum cuticular waxes (SSCW) on acetone–butanol–ethanol fermentation using *Clostridium acetobutylicum* ABE1201. Biores Technol. 149, 470-473.

Cambero, C., Sowlati, T., 2014. Assessment and optimization of forest biomass supply chains from economic, social and environmental perspectives – A review of literature. Renew. Sustainable Energy Rev. 36, 62-73.

Chinen, I., 2006. Composition containing higher fatty acid derivative and foods and drinks. USA, US Pat. 20060127450.

Clark, J.H., Budarin, V., Deswarte, F.E.I., Hardy, J.J.E., Kerton, F.M., Hunt, A.J., Luque, R., Macquarrie, D.J., Milkowski, K., Rodriguez, A., Samuel, O., Tavener, S.J., White, R.J., Wilson, A.J., 2006. Green chemistry and the biorefinery: a partnership for a sustainable future. Green Chem. 8, 853-860.

Clark, J.H., Pfaltzgraff, L.A., Budarin, V.L., Hunt, A.J., Gronnow, M., Matharu, A.S., Macquarrie, D.J., Sherwood, J.R., 2013. From waste to wealth using green chemistry. Pure Appl. Chem. 85, 1625-1631.

Da Porto, C., Decorti, D., Natolino, A., 2014. Separation of aroma compounds from industrial hemp inflorescences (Cannabis sativa L.) by supercritical CO2 extraction and on-line fractionation. Ind. Crops Prod. 58, 99-103.

Dassanayake, L.S.K., Kodali, D.R., Ueno, S., Sato, K., 2009. Physical Properties of Rice Bran Wax in Bulk and Organogels. J. Amer. Oil Chem. Soc. 86, 1163.

de Lucas, A., García, A., Alvarez, A., Gracia, I., 2007. Supercritical extraction of long chain n-alcohols from sugar cane crude wax. J. of Supercrit. Fluids 41, 267-271.

de Melo, M.M.R., Silva, R.P., Silvestre, A.J.D., Silva, C.M., 2016. Valorization of water hyacinth through supercritical CO2 extraction of stigmasterol. Ind. Crops Prod. 80, 177-185.

DeSimone, J.M., 2002. Practical Approaches to Green Solvents. Science 297, 799-803.

Deswarte, F.E.I., Clark, J.H., Hardy, J.J.E., Rose, P.M., 2006. The fractionation of valuable wax products from wheat straw using CO2. Green Chem. 8, 39-42.

Deswarte, F.E.I., Clark, J.H., Wilson, A.J., Hardy, J.J.E., Marriott, R., Chahal, S.P., Jackson, C., Heslop, G., Birkett, M., Bruce, T.J., Whiteley, G., 2007. Toward an integrated straw-based biorefinery. Biofuels Bioprod. Biorefin. 1, 245-254.

Edmundson, R.J., Jacks, T.C., 1989. High-gloss, high-shine lipstick. USA, US Pat. 4873078.

El-Nokaly, M., Walling, D.W., Vatter, M.L., Leatherbury, N.C., 2001. Lipsticks compositions containing association structures. USA, US Pat. 6325995.

Eriksson, D., Weiland, F., Hedman, H., Stenberg, M., Öhrman, O., Lestander, T.A., Bergsten, U., Öhman, M., 2012. Characterization of Scots pine stump–root biomass as feed-stock for gasification. Biores Technol. 104, 729-736.

EU, 2009a. Directive 2009/324/EC. Official Journal of the European Communities L141, Brussels, Belgium, 3 -11.

EU, 2009b. Regulation (EC) no 1223/2009 of the European Parliament and of the council of 30 November 2009 on cosmetic products Official Journal of the European Union L342, Brussels, Belgium, 59-209.

EU, 2010. Directive 2010/59/EU. Official Journal of the European Communities L225, Brussels, Belgium, 10 - 12.

Fanou, D., Yao, B., Siaka, S., Ado, G., 2007. Heavy metals removal in aqueous solutions by two delta-diketones. J. Appl. Sci 7, 310-313.

Fei, T., Wang, T., 2017. A review of recent development of sustainable waxes derived from vegetable oils. Curr. Opin. Food Sci. 16, 7-14.

Fernández, M.A., Tornos, M.P., García, M.D., de las Heras, B., Villar, A.M., Sáenz, M.T., 2001. Anti-inflammatory activity of abietic acid, a diterpene isolated from Pimenta racemosa var. grissea. J. Pharm. Pharmacol. 53, 867-872.

Fojtová, J., Lojková, L., Kubáň, V., 2010. Supercritical fluid extraction as a tool for isolation of monoterpenes from coniferous needles and walnut-tree leaves. Cent. Eur. J. Chem. 8, 409-418.

Frame, A., 2003. Anti-bacterial plant compositions. WO2003059371

Fulzele, S.V., Satturwar, P.M., Dorle, A.K., 2003. Study of the biodegradation and in vivo biocompatibility of novel biomaterials. Eur. J. Pharm. Sci. 20, 53-61.

Garets, C., 1992. Depilatory composition. USA, US Pat. 5154919 A

Gill, I., Valivety, R., 1997. Polyunsaturated fatty acids, part 1: Occurrence, biological activities and applications. Trends Biotechnol. 15, 401-409.

Goto, S.M., M. Sasaki, M. Tanaka, M, 2008. Utilization of citrus peel by sub- and supercritical fluid technology. Sep. Purif. Technol. 61, 130–135.

The Freedonia Group, 2015. Waxes: US Industry Study with Forecasts for 2019 & 2024. https://www.freedoniagroup.com/brochure/32xx/3271smwe.pdf, USA, Accessed 12 March 2017.

Gunawan, E.R., Basri, M., Rahman, M.B.A., Salleh, A.B., Rahman, R.N.Z.A., 2005. Study on response surface methodology (RSM) of lipase-catalyzed synthesis of palm-based wax ester. Enzyme Microb. Technol. 37, 739-744.

Gutiérrez, A., del Río, J.C., 2005. Chemical characterization of pitch deposits produced in the manufacturing of high-quality paper pulps from hemp fibers. Biores Technol. 96, 1445-1450.

Güçlü-Üstündağ, O., Temelli, F., 2000. Correlating the Solubility Behavior of Fatty Acids, Mono-, Di-, and Triglycerides, and Fatty Acid Esters in Supercritical Carbon Dioxide, Ind. Eng. Chem. Res. 39, 4756-4766.

Güçlü-Üstündağ, O., Temelli, F., 2004. Correlating the solubility behavior of minor lipid components in supercritical carbon dioxide, J. Supercrit. Fluids 31, 235–253.

Hamilton, R.J., 1995. Waxes : chemistry, molecular biology and functions. Oily Press, Dundee, UK.

Hamm, W., Hamilton, R.J., Calliauw, G., 2013. Edible Oil Processing. Wiley, UK.

Hammam, H., 1992. Solubilities of Pure Lipids in Supercritical Carbon Dioxide, J. Supercrit. Fluids 5, 101-106.

Herrero, M., Mendiola, J.A., Cifuentes, A., Ibáñez, E., 2010. Supercritical fluid extraction: Recent advances and applications. J. Chromatogr. A 1217, 2495-2511.

Hill, K., 2000. Fats and oils as oleochemical raw materials. Pure Appl. Chem. 72, 1255-1264.

Horrobin, D.F., Huang, Y.S., 1987. The role of linoleic acid and its metabolites in the lowering of plasma cholesterol and the prevention of cardiovascular disease. Int. J. Cardiol. 17, 241-255.

Hunt, A.J., Matharu, A.S., King, A.H., Clark, J.H., 2015. The importance of elemental sustainability and critical element recovery. Green Chem. 17, 1949-1950.

Hunt, A.J., Sin, E.H.K., Marriott, R., Clark, J.H., 2010. Generation, Capture, and Utilization of Industrial Carbon Dioxide. ChemSusChem 3, 306-322.

Ibáñez, E., Oca, A., de Murga, G., López-Sebastián, S., Tabera, J., Reglero, G., 1999. Supercritical Fluid Extraction and Fractionation of Different Preprocessed Rosemary Plants. J. Agric. Food Chem. 47, 1400-1404.

Kent, J.A., 2013. Handbook of Industrial Chemistry and Biotechnology. Springer Science & Business Media, Switzerland.

Kerton, F.M., Marriott, R., 2013. Alternative Solvents for Green Chemistry. Royal Society of Chemistry, Cambridge, UK.

Kline, 2011. Global wax industry 2010: Market analysis and opportunities. Kline & Company Inc. http://www.klinegroup.com/reports/brochures/y635a/brochure.pdf, USA, Accessed 5th January 2017.

Kline, 2015. Wax Market Is Expected To Reach US$ 8.98 Bn In 2020. http://www.klinegroup.com/reports/brochures/Y635D/brochure.pdf, USA, Accessed 5th January 2017.

Kunst, L., Samuels, A.L., 2003. Biosynthesis and secretion of plant cuticular wax. Prog. Lipid Res. 42, 51-80.

Kunst, L., Samuels, L., 2009. Plant cuticles shine: advances in wax biosynthesis and export. Curr. Opin. Plant Biol. 12, 721-727.

Kwan, T.A., Tu, Q., Zimmerman, J.B., 2016. Simultaneous Extraction, Fractionation, and Enrichment of Microalgal Triacylglyerides by Exploiting the Tunability of Neat Supercritical Carbon Dioxide. ACS Sustain. Chem. Eng. 4, 6222-6230.

Lang, Q., Wai, C.M., 2001. Supercritical fluid extraction in herbal and natural product studies — a practical review. Talanta 53, 771-782.

Leitner, W., 2000. Green chemistry: Designed to dissolve. Nature 405, 129-130.

Lik Nang Lau, H., Choo, Y.M., Ma, A.N., Chuah, C.H., 2008. Selective extraction of palm carotene and vitamin E from fresh palm-pressed mesocarp fiber (Elaeis guineensis) using supercritical CO2. J. Food Eng. 84, 289-296.

Luque de Castro, M.D., Garcı́a-Ayuso, L.E., 1998. Soxhlet extraction of solid materials: an outdated technique with a promising innovative future. Anal. Chim. Acta 369, 1-10.

Majeed, M., Gangadharan, G.K., Prakash, S., 2007. Compositions and methods containing high purity fatty alcohol C24 to C36 for cosmetic applications. USA, US Pat. 20070196507 A1.

Marinangeli, C.P.F., Jones, P.J.H., Kassis, A.N., Eskin, M.N.A., 2010. Policosanols as Nutraceuticals: Fact or Fiction. Crit. Rev. Food Sc. Nutr. 50, 259-267.

Marques, G., del Río, J.C., Gutiérrez, A., 2010. Lipophilic extractives from several non woody lignocellulosic crops (flax, hemp, sisal, abaca) and their fate during alkaline pulping and TCF/ECF bleaching. Biores Technol. 101, 260-267.

Martins, P.F., de Melo, M.M.R., Sarmento, P., Silva, C.M., 2016. Supercritical fluid extraction of sterols from *Eichhornia crassipes* biomass using pure and modified carbon dioxide. Enhancement of stigmasterol yield and extract concentration. J. Supercrit. Fluids 107, 441-449.

McElroy, C. R., Attard, T.M., Farmer, T.J., Gaczynski, A., Thornthwaite, D., Clark, J.H., Hunt,A.J., 2018. Valorization of spruce needle waste via supercritical extraction of waxes and facile isolation of nonacosan-10-ol, J. Clean. Prod. 171, 557-566.

McHugh, M.A., Krukonis, V.J., 1994. Supercritical fluid extraction: principles and practice. Butterworth-Heinemann, Oxford, UK.

Moghadasian, M.H., Frohlich, J.J., 1999. Effects of dietary phytosterols on cholesterol metabolism and atherosclerosis: clinical and experimental evidence. Am. J.Med. 107, 588-594.

Moiteiro, C., Manta, C., Justino, F., Tavares, R., Curto, M.J.M., Pedro, M., Nascimento, M.S.J., Pinto, M., 2004. Hemisynthetic Secofriedelane Triterpenes with Inhibitory Activity against the Growth of Human Tumor Cell Lines in Vitro. J. Nat. Prod. 67, 1193-1196.

Morais, A.R.C, da Costa Lopes, A.M., Bogel-Łukasik, R., 2015. Carbon Dioxide in Biomass Processing: Contributions to the Green Biorefinery Concept, Chem. Rev. 115, 3-27.

Morrison Iii, W.H., Holser, R., Akin, D.E., 2006. Cuticular wax from flax processing waste with hexane and super critical carbon dioxide extractions. Ind. Crops Prod. 24, 119-122.

Naik, S., Goud, V.V., Rout, P.K., Jacobson, K., Dalai, A.K., 2010. Characterization of Canadian biomass for alternative renewable biofuel. Renew. Energy 35, 1624-1631.

Nakamura, M., Nakasumi, T., Yoshizawa, T., Minagawa, Y., 1997. Analgesic anti-inflammatory drug. EP0776666

Nuissier, G., Bourgeois, P., Grignon-Dubois, M., Pardon, P., Lescure, M.H., 2002. Composition of sugarcane waxes in rum factory wastes. Phytochem. 61, 721-726.

Ortega, A. B., Garcia, A. C., Szekely, E., Škerget, M., Knez, Ž., 2017. Supercritical fluid extraction from Saw Palmetto berries at a pressure range between 300 bar and 450 bar. J. Supercrit. Fluids 120, 132- 139.

Pires, R.A.R., Da Silva Estima Martins, S.P.A., Das Chagas, J.A.M., Dos Reis, R.L.G., 2009. Extraction and purification of friedelin. EU, EP2070906 A1.

Prado, J.M., Prado, G.H.C., Meireles, M.A.A., 2011. Scale-up study of supercritical fluid extraction process for clove and sugarcane residue. J. Supercrit. Fluids 56, 231-237.

Prat, D., Wells, A., Hayler, J., Sneddon, H., McElroy, C.R., Abou-Shehada, S., Dunn, P.J., 2015. CHEM21 selection guide of classical- and less classical-solvents. Green Chem. 18, 288-296.

Puy, N., Murillo, R., Navarro, M.V., López, J.M., Rieradevall, J., Fowler, G., Aranguren, I., García, T., Bartrolí, J., Mastral, A.M., 2011. Valorisation of forestry waste by pyrolysis in an auger reactor. Waste Manag. 31, 1339-1349.

Puy, N., Tàbara, D., Bartrolí Molins, J., Bartrolí Almera, J., Rieradevall, J., 2008. Integrated Assessment of forest bioenergy systems in Mediterranean basin areas: The case of Catalonia and the use of participatory IA-focus groups. Renew. Sustain. Energy Rev. 12, 1451-1464.

Qi, G., Peng, F., Xiong, L., Lin, X., Huang, C., Li, H., Chen, X., Chen, X., 2016. Extraction and characterization of wax from sugarcane bagasse and the enzymatic hydrolysis of dewaxed sugarcane bagasse. Prep. Biochem. Biotechnol., 1-6.

Ramani, C.C., Puranik, P.K., Dorle, A.K., 1996. Study of diabietic acid as matrix forming material. Int. J. Pharmaceut. 137, 11-19.

Reverchon, E., De Marco, I., 2006. Supercritical fluid extraction and fractionation of natural matter. J. Supercrit. Fluids 38, 146-166.

Reverchon, E., Porta, G.D., Senatore, F., 1995. Supercritical CO2 Extraction and Fractionation of Lavender Essential Oil and Waxes. J. Agric. Food Chem. 43, 1654-1658.

Ruston, N.A., 1952. Commercial uses of fatty acids. J. Am. Oil Chem. Soc. 29, 495-498.

Sahena , F., Zaidul, I.S.M., Jinap, S., Karim, A.A., Abbas, K.A., Norulaini, N.A.N., Omar, A.K.M., 2009. Application of supercritical CO2 in lipid extraction – A review. J. Food Eng. 95, 240-253.

Schaumburg, H H., Spencer, P.S., 1976. Degeneration in central and peripheral nervous systems produced by pure n-hexane: an experimental study. Brain 99, 183-192.

Schiestl, F.P., Ayasse, M., Paulus, H.F., Löfstedt, C., Hansson, B.S., Ibarra, F., Francke, W., 1999. Orchid pollination by sexual swindle. Nature 399, 421-421.

Shi, Q., Jing, L., Qiao, W., 2015. Solubility of n-alkanes in supercritical CO2 at diverse temperature and pressure, J.CO2 Util., 9, 29–38.

Shuttleworth, P.S., De bruyn, M., Parker, H.L., Hunt, A.J., Budarin, V.L., Matharu, A.S., Clark, J.H., 2014. Applications of nanoparticles in biomass conversion to chemicals and fuels. Green Chem. 16, 573-584.

Simándi, B., Oszagyán, M., Lemberkovics, É., Kéry, Á., Kaszács, J., Thyrion, F., Mátyás, T., 1998. Supercritical carbon dioxide extraction and fractionation of oregano oleoresin. Food Res. Int. 31, 723-728.

Sin, E.H.K., Marriott, R., Hunt, A.J., Clark, J.H., 2014. Identification, quantification and Chrastil modelling of wheat straw wax extraction using supercritical carbon dioxide. C. R. Chimie 17, 293-300.

Subramaniam, B., Rajewski, R.A., Snavely, K., 1997. Pharmaceutical processing with supercritical carbon dioxide. J. Pharm. Sci. 86, 885-890.

Sun, R., Sun, X.-F., 2001. Separation and characterization of lipophilic exracts from barley straw. Sep. Sci.Technol. 36, 3027-3048.

Sun, R.C., Tompkinson, J., 2003. Comparative study of organic solvent and water-soluble lipophilic extractives from wheat straw I: yield and chemical composition. J. Wood Sci. 49, 0047-0052.

Szafranek, B.M., Synak, E.E., 2006. Cuticular waxes from potato (Solanum tuberosum) leaves. Phytochem. 67, 80-90.

Temelli, F., Ciftci, O.N., 2015, Developing an integrated supercritical fluid biorefinery for the processing of grains. J. Supercrit. Fluids 96, 77-85.

Totten, G.E., Westbrook, S.R., Shah, R.J., 2003. Fuels and Lubricants Handbook. ASTM International, Pennsylvania, USA.

Vági, E., Simándi, B., Vásárhelyiné, K.P., Daood, H., Kéry, Á., Doleschall, F., Nagy, B., 2007. Supercritical carbon dioxide extraction of carotenoids, tocopherols and sitosterols from industrial tomato by-products. Journal Supercrit. Fluids 40, 218-226.

Vali, S., Ju, Y.-H., Kaimal, T., Chern, Y.-T., 2005. A process for the preparation of food-grade rice bran wax and the determination of its composition. J. Amer. Oil Chem. Soc. 82, 57-64.

Villaverde, J.J., De Vega, A., Ligero, P., Freire, C.S.R., Neto, C.P., Silvestre, A.J.D., 2010. Miscanthus x giganteus Bark Organosolv Fractionation: Fate of Lipophilic Components and Formation of Valuable Phenolic Byproducts. J. Agri. Food Chem. 58, 8279-8285.

Villaverde, J.J., Domingues, R.M.A., Freire, C.S.R., Silvestre, A.J.D., Neto, C.P., Ligero, P., Vega, A., 2009. Miscanthus x giganteus Extractives: A Source of Valuable Phenolic Compounds and Sterols. J. Agri. Food Chem. 57, 3626-3631.

Wang, L., Weller, C.L., 2006. Recent advances in extraction of nutraceuticals from plants. Trends Food Sci. Technol. 17, 300-312.

Wang, L., Weller, C.L., Hwang, K., 2005. Extraction of lipids from grain sorghum DDG. ASAE 48, 1883-1888.

The Ministry of Health and Welfare, 2000. Japan's Specifications and Standards for Food Additives (7th Edition) section E Published by The Ministry of Health and Welfare, Japan.

Xiao, B., Sun, X.F., Sun, R., 2001. Extraction and characterization of lipophilic extractives from rice straw. I. Chemical Composition. J. Wood Chem. Technol. 21, 397-411.

Zhang, Y., Wu, X., Yu, Z., Zhu, Y.L., Chen, L., Lou, S., 2006. Composition containing total triterpenoid saponins extracted from bamboo, and the preparation method and use thereof. USA, US Pat. 20060148733.

Zhao, F., Wang, M., Shu, L., Tian, X., Liu, K., 2016a. Supercritical extracts of forest fuels in Great Xing’an Mountains. J. For. Res. 27, 1143-1151.

Zhao, F., Wang, Q., Shu, L., Yang, L., Liu, K., 2016b. Correlations between Supercritical Extracts of Coniferous Fuel and the Heat Yield Value and Ignition Point. Scientia Silvae Sinicae 52, 68-74.

Zhao, W., Zong, Z.-M., Lin, J., Song, Y.-M., Guo, X.-F., Yao, Z.-S., Zhang, L.-N., He, R.-L., Cao, J.-P., Wei, X.-Y., 2007. Dewaxing from Stalks with Petroleum Ether by Different Methods. Energy Fuels. 21, 1165-1168.

Zougagh, M., Valcárcel, M., Rı́os, A., 2004. Supercritical fluid extraction: a critical review of its analytical usefulness. TrAC 23, 399-405.