ECRONICON OPEN ACCESS

CHEMISTRY
Review Article

Food Processing by Supercritical Carbon Dioxide-Review

Omprakash H Nautiyal*

Department of Organic Chemistry, Haramaya University, Ethiopia

***Corresponding Author:** Omprakash H Nautiyal, Professor and Researcher, 102, Shubh Building, Shivalik II, Canal Road, Chhani Jakat Naka, Vadodara 390024, Gujarat, India.

Received: September 21, 2015; **Published:** January 09, 2016

Abstract

Supercritical fluid extraction technology employing CO_2 as an extract ant being the most greener technology in the area of food processing and hence many food and their ingredients important from the health point of view for combating various health issues have been attempted by many researchers to be investigated by this process. This review covers the extraction of caffeine, vitamin E, wine and beer, defatting, bio processing and response surface plot of the extraction has been found to be an important tool to monitor the progress of extraction with regards to pressure, temperature and yield of the bioactive contents of the foods. *Garcinia mangostana L*. active components and fractionation of corn bran oil are also excellent investigations being reviewed along with palm kernel oil comparatively super oil in comparison to conventional extraction methods.

Keywords: *Food processing; $SCCO_2$; Caffeine; Bio processing; Palm kernel oil*

Introduction

Carbon dioxide is gaining popularity among coffee manufacturers looking to move away from some of the classic decaffeinating solvents of the past, many of which have led to public rebellion because of real or perceived dangers related to their use in food preparation. Supercritical CO_2 is forced through the green coffee beans which are then sprayed with water at high pressure to remove the caffeine. The caffeine can then be isolated for resale (e.g. to the pharmaceutical industry or to beverage manufacturers) the water is passed through activated charcoal filters or by distillation, crystallization or reverse osmosis. Supercritical carbon dioxide is employed to remove organic chloride pesticides and metals from agricultural crops without adulterating the desired constituents from the plant matter in the herbal supplement industry (Department of Pharmaceutical Analysis, Shenyang Pharmaceutical University, Shenyang 110016, and China).

Supercritical carbon dioxide can also be used as a more environmentally friendly solvent for dry cleaning as compared to more traditional solvents such as hydrocarbons and percholroethylene. Supercritical carbon dioxide is used as the extraction solvent for creation of essential oils and other herbal distillation. Its main advantages over solvents such as hexane and acetone in this process are that it is non-toxic and non-flammable. Furthermore, separation of the reaction components from the starting material is much simpler than with traditional organic solvents, merely by allowing it to evaporate into the air or recycling it by condensation into a cold recovery vessel. Its advantage over steam distillation is that it is used at a lower temperature, which can separate the plant waxes from the oils.

In laboratories, supercritical carbon dioxide is used as an extraction solvent, e.g., in determination of total recoverable hydrocarbons from soils, sediments, fly-ash, and other media, and determination of polycyclic aromatic hydrocarbons in soil and solid wastes. Supercritical fluid extraction has also been used in determination of hydrocarbon components in water. Processes which use supercritical carbon dioxide to produce micro and nano scale particles, often for pharmaceuticals uses, are currently being developed. The gas and solvents processes, rapid expansion of supercritical solutions, and supercritical ant solvent precipitation (as well as several related methods) have been shown to process a variety of substances into particles. Recent studies have proved SC-CO_2 is an effective alternative for terminal

sterilization of biological materials and medical devices with combination of the additive PAA (per acetic acid). The SC-CO_2 only does not sterilize the media, but also kills because it does not kill the spores of microorganisms. Moreover, this process is gentle, as the morphology, ultra structure, and protein profiles of inactivated microbes are maintained [1-10].

Special applications of supercritical fluids to food processing

Carbon dioxide is the most common supercritical fluid in the food industry. Due to the non-toxicity and low critical temperature, it can be used to extract thermally labile food components and the product is not contaminated with residual solvent. Further, the extract's colour, composition, odour, texture are controllable and extraction by supercritical fluid carbon dioxide retains the aroma of the product. Supercritical fluid extraction provides a distinct advantage not only in the replacement but also extracts oils that are lower in iron and free fatty acid. Some application of SFE in food is mentioned below:

Enrichment of Vitamin E from Natural Sources

SFE have several advantages for the enrichment of tocochromanols over conventional techniques such as vacuum distillation, in particular a lower operating temperature. As starting material one can use various edible oils or their distillates. Most promising feed materials are CPO and SODD. CPO contains several tocotrienols and tocopherols at a total concentration of approximately 500 ppm. SODD may contain (after several conventional concentration steps) about 50% tocopherols. Both materials can be used for the production of enriched fractions of tocochromanols. Although it is possible to recover tocochromanols directly from CPO, it is better to produce esters of the triglycerides in order to be able to more easily separate these compounds from the tocochromanols. In this method, the triglycerides are subject to an esterification with methanol to form fatty acid methyl esters, which are easily extractable with CO_2. That means that the tocochromanols, together with other unsaponifiable matter such as squalene and sterol are enriched in the bottom phase of an extraction column. This attempt is described in more detail by. For a discussion of enriching tocochromanols, phase equilibrium data have to be considered first. FFA and tocochromanols exhibit a much higher solubility in CO_2 than the triglycerides. Hence, these components are enriched in the gaseous phase, expressed by a distribution coefficient being higher than one [11.13].

The distribution coefficient of the triglycerides is smaller than one, whereas that for the carotenes is much smaller than one, meaning that these components stay in the liquid oil phase. Thus, tocochromanols can be extracted as the top phase product in a separation column, whereas carotenes remain in the bottom phase product together with triglycerides. For recovering the carotenes together with the tococromanols the above mentioned esterification to volatile (CO_2 soluble) methyl esters makes possible to recover tocochromanols and carotenes (together with squalene and sterols) as bottom product from this natural source. When the glycerides (in case of the esterification) or the FFAs from deodorizer distillates have been removed, then there is a feed material available for obtaining enriched fractions of tocochromanols and carotenes of much higher concentration. In this feed material, tocochromanols and carotenes (in case of palm oil) are the main components and have to be separated from other unsaponifiable substances present, such as squalene and sterols. Of these compounds, squalene has the highest solubility in SC-CO_2, all phytosterols have rather low solubility in CO_2 (and remain in the oil phase), and tocochromanols exhibit an intermediate solubility between the two. In a second separation step tocochromanols are separated from phytosterols. A further purification of these compounds is possible, e.g. with adsorptive or chromatographic techniques, again using supercritical fluids figure 1 [14-20].

Removal of Alcohol from Wine and Beer, and Related Applications

De-alcoholised wine or beer is achieved by removing ethanol from water. Distillation is well known for this purpose with the disadvantage that aroma compounds will also be removed. New techniques like membrane separation (pervaporation) emerge, and in between these is SFE with CO_2. Starting from an aqueous solution with about 10% (w/w) ethanol, ethanol can be removed by SC-CO_2 in a stripping column. The rate of ethanol removal depends strongly on temperature. Reducing the alcohol content to values well below 0.5% (w/w) requires about 2.5h at 45°C under non-optimized conditions. Much shorter times for the ethanol removal can be obtained if flow rates and mass transfer equipment are carefully selected. With the information available in the literature, for instance from, a column for de alcoholising aqueous solutions can be designed. Recovery of aroma compounds is achieved by a side column in which a separation from ethanol is carried out [21-25].

A related process that can be mentioned is the recovery of absolute alcohol. Many studies were carried out at conditions of complete miscibility of ethanol and CO_2 in order to get a high solubility of ethanol in the vapour phase. At these conditions, anhydrous ethanol cannot be produced. However, ethanol can be concentrated above azeotropic composition whenever the pressure in the ternary mixture CO_2 + ethanol + water is below the critical pressure of the binary mixtures CO_2 + ethanol [26].

Removal of Fat from Foods

Edible oils and their components has been the target of supercritical fluid processing since the early 70s. Although triacylglycerides are only fairly soluble in SC-CO_2, the advantages of organic solvent-free processing have stimulated research and development in various areas. One of these is the removal of fat from food. The process has been fully designed for commercial application, using the aforementioned standard design. The process has the advantage of producing fat-free or fat-reduced potato chips. According to the expected taste the amount of remaining fat in the potato chips can easily be controlled rather low solubility in CO_2 (and remain in the oil phase), and tocochromanols exhibit an intermediate solubility between the two. In a second separation step tocochromanols are separated from phytosterols. A further purification of these compounds is possible, e.g. with adsorptive or chromatographic techniques, again using supercritical fluids [27-30].

Application of SFE in Food Safety

At present, food safety includes many different issues such as detection of frauds, adulterations and contaminations. Among these topics, detection of food pollutants is important not only for consumers but also for administrations, control laboratories, and regulatory agencies. In order to protect consumers' health, regulations establish strict limits to the presence of pollutants in foods that must be carefully observed and determined. Generally, the analysis of food pollutants is linked to long extraction and cleanup procedures commonly based on the use of, e.g., Soxhlet and/or saponification. These procedures are laborious and time consuming and, besides, usually employ large volumes of toxic organic solvents. With the objective of reducing both, the sample preparation time and the massive use of organic solvents, techniques based on compressed fluids such as SFE have been developed. One of the main areas of application of SFE in the last few years has been in food pollutants analysis, mainly pesticide residues and environmental pollutants [31].

Several methods has been developed for the analysis of multiple pesticides (organochlorine, organophosphorus, organonitrogen and pyrethroid) in potatoes, tomatoes, apples, lettuces and honey with a single cleanup step using supercritical CO_2 modified with 10% of acetonitrile. Similar study have been carried out for the analysis of multiresidues of pesticides, using SFE as a cleanup step, in cereals, fish muscle, vegetable canned soups, vegetables or infant and diet foods. A common characteristic of these studies is the extremely high selectivity of SFE in the isolation of the low polarity pesticides; this fact makes SFE probably the technique of choice to isolate pesticides from low fat food. As mentioned in the introduction, to correctly asses the concentration of an analyte in a food sample, a quantitative recovery should be obtained that will mostly depend on the recovery of the analytes and not on the extraction itself. To improve the recovery of the pollutants, a common strategy is the use of solid traps. These traps consist on a solid phase compatible with the analyte and are flushed away with a compatible solvent. The trapping step is very important in SFE method development not only because its effect in the quantitative recovery of the analytes but also because an extra selectivity can easily be introduced, especially in the case of solid-phase trapping, avoiding the use of further post-extraction clean up. Supercritical carbon dioxide extraction can advantageously be used to extract non-polar pollutants, such as PAH from foods. Different extraction and cleanup methods have been used, but the extracting conditions turned to be very similar (around 300 bar and 100°C) to optimize the PAH extraction [32-35].

Supercritical drying

Supercritical drying goes beyond the critical point of the working fluid in order to avoid the direct liquid-gas transition seen in ordinary drying. It is a process to remove liquid in a precisely controlled way, similar to freeze drying. It is commonly used in the production of aero gel and in the preparation of biological specimens for scanning electron microscopy. As a substance crosses the boundary from liquid to gas (phase diagram), the substance volatilizes and so the volume of the liquid decreases. As this happens, surface tension at the solid-liquid interface pulls against any structures that the liquid is attached to. Delicate structures, like cell walls, the dendrites in silica gel and the tiny machinery of micro electro mechanical devices, tend to be broken apart by this surface tension as the interface moves by.

To avoid this, the sample can be brought from the liquid phase to the gas phase without crossing the liquid-gas boundary on the phase diagram; in freeze-drying, this means going around to the left (low temperature, low pressure). However, some structures are disrupted even by the solid-gas boundary. Supercritical drying, on the other hand, goes around the line to the right, on the high-temperature, high-pressure side. This route from liquid to gas does not cross any phase boundary, instead passing through the supercritical region, where the distinction between gas and liquid ceases to apply. Fluids suitable for supercritical drying include carbon dioxide and Freon. Nitrous oxide has similar physical behaviour to carbon dioxide, but is a powerful oxidizer in its supercritical state. Supercritical water is also a powerful oxidizer, partly because its critical point occurs at such a high temperature (374°C) and pressure (647°K and 22.064 MPa) [36].

Temperature control (table 1) was achieved by placing the extractor vessel and cyclone separator in separate convection ovens. The extraction oven was set to the required extraction temperature for 30 min to achieve temperature equilibration prior to initiating the static extraction period. Temperature was automatically controlled by reading the temperature of the loaded CO_2 stream leaving the extractor, and adjusting the oven heater. The collection oven was set at 60°C in all experiments [37].

For the static extraction period, the CO_2 pump was operated in the pressure control mode and the on/off valve at the exit of the extraction oven was maintained in the off position. During the dynamic extraction period, the main pump was set to the flow control mode, the aforementioned on/off valve was set to the on position and the extraction pressure was maintained by the back pressure regulator (BPR) located in the collection oven. After 90 min of dynamic extraction (in experiments of > 90 min duration), oil collection was switched from one cyclone separator to the other with a multi position valve located behind the BPR. In this way the researchers we obtained complete information and some duplicates on the effect of extraction temperature and pressure on oil collected during the 90 min extraction period [38].

Two similar systems were utilized for each replicate. One was located in the Extraction Laboratory of Biological Materials (LEMaB) of the main author (Replicate 1) in Santiago, Chile and the other at the facilities of Thar Designs in Pittsburgh, PA (Replicate 2). The main difference was that temperature control in Replicate 2 was achieved by circulating water from a thermo stated bath at the required extraction and collection temperature through heat jackets on extraction and cyclone vessels. Once the experiment was concluded, each cyclone separator was washed out with 10 ml hexane and the collected miscella was placed in a sealed vial and kept refrigerated up to the time of analysis. Just prior to quantization of recovered oil and color, hexane was removed by placing the vials on a vacuum oven set at *ca*. 60°C and 0.27 bar (absolute pressure) [39].

For oil comparison purposes, five additional batches of 250g flaked seeds were placed in a 500 mL jacketed extraction vessel and processed with 21g CO_2/min at 300 bar for 270 min. Oil was recovered in a 200 mL jacketed cyclone separator. The extraction vessel was heated by circulating water at 40°C from a thermo stated bath, whereas the cyclone vessel was tempered by circulating tap water (*ca*. 15°C) through their jackets [40].

Experiment #	Extraction Conditions			Yield (g oil/100g flaked seeds)		Photometric
	Tempareture (°C)	Pressure (bar)	Dynamic Time (min)	Recovered oil	Extracted oil	Color (Rep.1)
1	40	300	180	5.12 ± 0.15	5.51 ± 0.42	5.29
2	40	400	90	4.20 ± 0.11	4.65 ± 0.13	5.93
3	40	400	270	6.18 ± 0.05	6.56 ± 0.35	5.75
4	40	500	180	5.28 ± 0.16	5.75 ± 0.13	7.11
5	50	300	90	4.10 ± 0.25	4.74 ± 0.70	6.83
6	50	300	270	6.61 ± 0.53	6.87 ± 0.21	6.63
7	50	400	180	5.70 ± 0.04	6.07 ± 0.08	7.18

8	50	500	90	4.77 ± 0.36	5.04 ± 0.21	7.15
9	50	500	270	6.97 ± 0.06	7.00 ± 0.05	8.48
10	60	300	180	5.71 ± 0.36	5.96 ± 0.43	7.47
11	60	400	90	5.51 ± 0.03	5.71 ± 0.45	7.66
12	60	400	270	7.01 ± 0.10	7.09 ± 0.12	7.89
13	60	500	180	6.33 ± 0.10	6.68 ± 0.35	8.97

Table 1: *Experimental Designs & Results [37].*

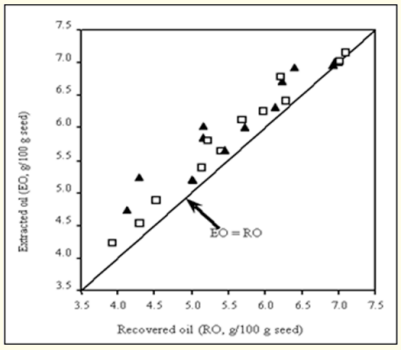

Figure 1: *Relationship between extracted oil (□) and recovered oil (▲) [37].*

The response surface is plotted in figure 2. It is clear that for relatively short extraction times of 1.5h, it is possible to increase yield by increasing extraction pressure or, more effectively, by increasing extraction temperature. These effects are not as clear for long extraction times of 4.5h when, independently of extraction conditions, yields are close to the maximal 7.12% value). In agreement with these plots, experimental values of yield ranged from 4.7% (1.5h extraction with SCO_2 at 40°C and 400 bar) to 7.1% (4.5 h extraction with SCO_2 at 60°C and 400 bar) (Table 1) [41-45].

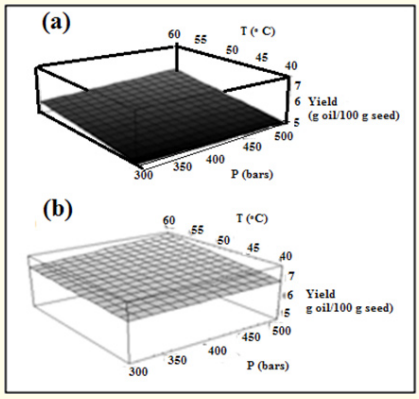

Figure 2: *Response surface for changes in extracted oil as a function of extraction temperature & pressure for (a) 1.5 h & (b) 4.5 h as extraction dynamic time [37].*

However, pseudo solubility values for equivalent experimental conditions decreased when they were evaluated based on oil recoveries and volumes of CO_2 used up in longer extractions (data not shown). The reduction in pseudo solubility was between 31.3 and 40.4% (average of 35.3%) after 180 min of extraction and between 42.4 and 54.5% (average of 48.7%) after 270 min of extraction. This indicates a reduction in extraction rate after 90 min, probably due to depletion of surface oil on flaked rosehip seeds. However, since no data was collected for very short extraction times, this depletion of surface oil may have occurred in less than 1.5 h, specially when using SCO_2 with high solvent power (high density, high temperature). This hypothesis may be confirmed by comparing our pseudo solubility values with true triglyceride solubility values for oilseeds, in general or rosehip seed, in particular. It is relevant to point out that del Valle's and Aguilera's formula appears to be valid in the 20-80°C and 150-680 bar region, whereas that of Illés and co-authors was generated using solubility data measured at 35 and 55°C and several pressures between 100 and 400 bars [41-45].

Extraction Conditions			Solubility Values (c, g oil/L SCo_2)		
Temperature (T, °C)	**Pressure (P, bar)**	**SCo_2 density (g/L)**	**This Study[1]**	**Del Valle and Aguilera (1988)**	**IIIes., *et al.* (1997)**
40	300	912.4	1.94	6.95	6.50
40	400	958.7	2.18 (0.02)	11.82	12.46
40	500	996.3	2.28	17.87	20.66
50	300	874.1	1.83 (0.03)	7.16	6.22
50	400	926.3	2.19	13.35	13.34
50	500	965.8	2.33 (0.03)	20.89	23.09
60	300	832.3	2.22	6.98	5.33
60	400	891.9	2.45 (0.04)	14.65	13.22
60	500	935.3	2.69	24.40	24.71
[1]Pseudosolubility Values after 90 min extraction.					
$^2c = \rho^{10.724}.\exp\left(40.361 - \frac{18708}{T+273.2} - \frac{2186840}{(T+273.2)^2}\right)$					
$^3c = \rho^{13.138}.\exp\left(-70.864 - \frac{5266}{T+273.2}\right)$					

Table 2: *Comparison of oil pseudo & true solubility values under different extraction conditions [37].*

The effect of several variables on extraction rates (table 2) were reviewed by Eggers (1996). He has claimed that extraction rates are determined by equilibrium restraints only during the initial stages of the extraction process. In the case of rapeseed extracted with SCO_2 at 40°C and 300 bar, Eggers (1996) reported that for mechanically de oiled seed containing 24.8% residual oil, the amount of SCO_2 required to extract 55% of the initial oil content (the percentage that was removed in our case under equivalent extraction conditions) was between 136 and 152% of that required if conditions of loading to saturation were achieved. The actual SCO_2 requirement depended on whether the press cake was extracted as is or following additional flaking treatment (which caused pseudo solubility values to be 26.5-34.2% smaller than true solubility values). It is conceivable that this percentage would increase for seeds containing less oil initially, or oil that is more tightly held (less intensive micro structural destruction as a result of seed pre-treatment). The solvent flow rate also has some effect on solvent requirements for extraction. In the case of milled corn germ (•23.4% oil; Christianson., *et al*. 1984) treated at 50°C and 500 bar, the SCO_2 requirement for extracting up to a residual content of 2.57% oil (the residual content in flaked rosehip seeds following 90 min extraction under equivalent conditions) was 114, 125 and 181% of the theoretical value (solvent loaded to saturation) when using 21.7, 33.3 and 66.7g CO_2/100g substrate/min, respectively. This would cause a decrease in pseudo solubility of between 12.3 and 44.8% of the true solubility values. It is interesting to point out that in our case we utilized 21.0

g CO_2/100 g substrate/min, whereas Illés., *et al*. (1997) presumably utilized 4.8g CO_2/100 g substrate/min in their solubility determinations (this value was estimated based on the assumption that their 114 mL extraction vessel was loaded with 0.5 g/mL of hip rose seed, the packing utilized in our studies). Eggers (1996) recommends using 16.6-83.3 g CO_2/100 g substrate/min, which corresponds to superficial solvent velocities of between 1 and 5 mm/s. Illés., *et al*. (1997) claimed that they could obtain solubility values at the outlet of their extractor if they used •12.9 g CO_2/100 g substrate/min [41-45].

Supercritical Fluid Extraction in Bioprocess Technology

Recent investigations on the applications of SCE from post fermentation biomass or in situ extraction of inhibitory fermentation products as a promising method for increasing yield are reviewed (Khosravi-Darani and Vasheghani-Farahani 2005). Although SC-CO_2 is unfriendly and toxic, for some living cells, which precludes direct fermentation in dense CO_2, it does not rule out other useful applications for in situ extraction of inhibitory fermentation products and fractional extraction of biomass constituents due to the potential of system modification by physical parameters and addition of co-solvents to selectively extract compounds of varying polarity, volatility and hydrophilicity with no contamination [41-45].

The advantages of utilizing SCE have been well documented. The application of SCF is simple, inexpensive, non- injurious to the structure and function of some enzymes and protein activities. Nowadays, SCE is a well-known unit operation, with some industrial as well as many lab and pilot scale applications. Introduction of SC-CO_2 to fermentation broth decreases the overall viscosity, facilitates the handling of the broth and enhances mass transfer from the liquid to the SC-phase. Randolph has summarized special advantages of SCE, especially for the biotechnology industries (1990) [41-45].

1. High diffusivity reduces mass transfer limitations from porous solid matrices
2. Low surface tension allows penetration and wetting of pores to extract from cell
3. Selectivity of extraction due to sensitivity of solubility to changes in P and T
4. Manipulating crystal size of solid compounds produced from SCFs by change in P and T
5. Separating of compounds that cannot be distilled, owing to their thermal instability.
6. Increased enhancement factors (ratio of actual solubility to ideal gas solubility)
7. Low reactivity and toxicity of SC-CO_2 or ethane, and their gaseous state

The main disadvantages of SCE processes include low solubility of bio molecules in SCF and high capital costs. Furthermore, insufficient data exist on the physical properties of many bio-molecules, making prediction of phase behaviour difficult. The addition of co-solvents may obviate the advantage of minimal solvent residues in the final product [41-45].

In Situ Extraction from the Biomass of Microbial Fermentation

In situ product removal is the fast removal of product from a producing cell thereby preventing its subsequent interference with cellular or medium components. Freeman and co-workers indicated future directions including application in situ extraction to a wider range of products and the developed methodologies, applicable under sterile conditions in the immediate vicinity of the producing cells. End-product inhibition occurs in many fermentation processes and in situ removal of them typically enhances product formation rates, yields, and specificity. Techniques that have been employed for in situ removal of fermentation products include liquid-liquid extractive fermentation, use of selective membranes, cell recycling, adsorption, microcapsule application and vacuum fermentation. However, the intimate contact of an organic phase with the broth implies that the organic components of this phase may be present in the aqueous phase at saturation levels. The disadvantage of liquid-liquid extraction is the residual of toxic solvent, which presents significant separation, purification, and environmental challenges. Also membrane fermentation and adsorption vacuum fermentation are not cost-effective. Guvenc., *et al*. (1998) demonstrated the feasibility of ethanol extraction from a post–fermentation broth using SC-CO_2. However, application of SC-CO_2 for in situ extractive fermentation has been limited by its inhibitory effect on the metabolism of a variety of yeasts and bacteria. This toxicity is attributed, in part, to the acidic pH that results from the increased solubility of CO_2 at

high partial Ps. By buffering the medium and carefully controlling the compression and expansion conditions, the survival rate of cells increases. Van Eijs., *et al*. developed an extraction procedure in which the *Lactobacillus plantarum* cell death was minimized [41-45].

Useful Compounds from Japanese Citrus

Citrus by products contain compounds such as polyphenols, limonoids, carotenoids and essential oils that are considered useful for the application in pharmaceutical, cosmetic and food industries. One of the promising techniques to recover these compounds is by supercritical carbon dioxide extraction. Taking sudachi residues as a representative sample, supercritical carbon dioxide extraction experiments were carried out in the temperature range of 40 to 80°C and pressure range of 10 to 30 MPa. The effect of addition of ethanol (EtOH), as entrainer, was also studied. Results indicated that the yield increased with increasing pressure. Addition of 5mol% EtOH as entrainer increased the yield by more than three times of that without the entrainer (EtOH). Based on gas chromatography mass spectrometry (GCMS) analyses, the extracts consisted mainly of limonene, terpenes, some fatty acids and its esters The amount of polyphenols in the extracts, estimated using the Folin Ciocalteau method increased with increasing amount of EtOH Experiments conducted on samples other than sudachi residues also showed promising [41-45].

Armando T. Quitain reported the results of extraction experiments at 40°C, at pressure range of 10-30 MPa. In a total of 4 runs, equivalent to about 20 h of continuous flow of carbon dioxide gas at a rate of 2L/min the extraction yield at 10 MPa was 545 mg/100 g sample. The cumulative yield increased with increasing pressure, obtaining up to 773 mg/100-sample at 30 MPa. At 20 MPa, addition of 5 mol% EtOH significantly increased the yield by more than three times of that with no EtOH addition. This increase in the extraction yield with the addition of EtOH could be attributed to the enhanced extraction of the oil components, as observed in their our previous studies on okara and co extraction of some polar compounds like polyphenols [41-45] .

The authors identified the compounds by GC-MS were sub divided into three main groups of limonene, terpenes and fatty acids. Based on the total peak areas of these groups of compounds, the effect of extraction conditions on the relative composition of the extracts was investigated. Limonene was obtained predominantly at 10 and 20 MPa, but at a higher pressure of 30 MPa, terpenes and fatty acids were mostly obtained over limonene. Continuous extraction on the same sample with the addition of EtOH at different concentration (in mol %) yielded the composition behaviour of the extracts. Limonene was obtained initially with pure supercritical carbon dioxide, but it was not detected in the succeeding runs with the addition of EtOH as entrainer. The general trend for all the pressures studied shows a diminishing amount of terpenes, while the relative composition of fatty acids and some other polar compounds increased.

Nautiyal and Tiwari in the year 2011 (Table 3) in their study have varied pressure in between 8-25 MPa, at 55°C, 5kg h^{-1} flow rate of SC-CO_2 and 2 hour batch extraction time. These data are presented in table 3. With an increase in pressure at constant temperature the yield of the oil was found to increase till 150 bars. Above 150 bars decrease in the degree of extraction was noticed specifically at 20 and 25 MPa. Value addition components get extracted in the pressures range of 8-15 MPa. This pressure onwards no increase in the yield of the oil was found. Anomalous behaviour of decrease in yield at high pressures at 55 bar may be due to the obstruction for the flow of SC-CO_2 because of decrease in void space of the packed bed. Temperature was varied between 28°C (subcritical) and 60°C, the pressure was 15MPa, batch time was 2 hour and flow rate of the SFE was 5 kg h^{-1}. It was found that at constant pressure with increase in temperature the yield of orange oil was found to increase till 55°C. At 60°C the extraction of orange oil was found to decrease slightly. Major constituents of the oil, d-limonene was found to increase up to 89.28% at 60°C. α-pinene, terpinolene, myrecene that is 2.5-3%, the rest of the constituents was less than 1% each [41-45].

Extraction pressure: 150 bar Flow rate of CO_2: 5 kg/hour Batch time: 2 hours Ground orange peel charged: 184 gm	
Extraction temperature °C	**Yield of oil (wt % of peels)**
28	0.68
35	0.93
45	1.45
55	2.22

Table 3: *Effect of SFE temperature on the extraction of Orange oil (Nautiyal & Tiwari 2011).*

Decaffeinating Coffee

This is as invented by Kurt Zosel; Coffee is mixed with pure water. (Figure 3)

1. When the coffee absorbs the water the grains expand, their pores get opened and the caffeine molecules become mobile.
2. At this point carbon dioxide is added (A 100% Natural Element) at a 100 atmospheres pressure to the pure water.
3. Basically the water and the carbon dioxide are mixed to create the sparkling water.
4. The carbon dioxide acts like a magnet and attracts all the caffeine molecules that became movable.
5. When the caffeine is captured by the carbon dioxide, this is removed.
6. The carbon dioxide is very selective and it doesn't touch the carbohydrates and proteins of the coffee beans.
7. The carbohydrates and the proteins are the ones that give the coffee the flavour and smell after it is made.
8. When the carbon dioxide has finished removing the caffeine, the coffee seeds are dried naturally
9. Carbon dioxide is then recycled and caffeine is sold for other commercial uses.

Soaking green coffee beans in water expands their size, and thus allowing the caffeine to dissolve into water inside the bean [46-48].

Caffeine removal occurs in an extraction vessel, which may be 70 feet high and 10 feet in diameter, suffused with carbon dioxide at roughly 200 degrees Fahrenheit and 250 atmospheres. Caffeine diffuses into this supercritical carbon dioxide, along with some water. Beans enter at the top of the chamber and move toward the bottom over five hours. To extract the caffeine continuously, the beans lower in the column are exposed to fresher carbon dioxide, which ensures that the caffeine concentration inside beans is always higher than in the surrounding solvent. Caffeine therefore always diffuses out of the beans [46-48].

Decaffeinated beans at the bottom of the vessel are removed, dried and roasted.

Recovery of dissolved caffeine occurs in an absorption chamber. A shower of water droplets leaches the caffeine out of the supercritical carbon dioxide. The caffeine in this aqueous extract is then often sold to soft-drink manufacturers and drug companies. The purified carbon dioxide is recirculated for further use.

Caffeine is a small, (figure 3) bitter-tasting alkaloid. High-quality Arabica coffee beans (the source of most specialty coffees) are typically 1 percent caffeine by weight, whereas cheaper and bitterer Robusta beans have twice that amount [46-48].

It is spurred by the belief that excessive coffee drinking had poisoned his father, the German chemist Ludwig Roselius, in about 1900, found a number of compounds that dissolved the natural caffeine in coffee beans without ruining the drink's taste. Chloroform and benzene did the job but were toxic, so for 70 years methylene chloride became the solvent of choice [46-48].

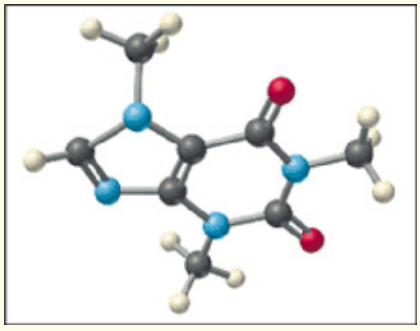

Figure 3: *Caffeine.*

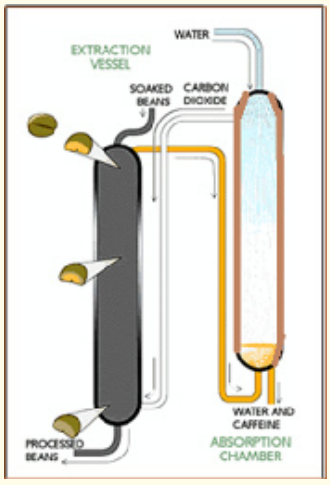

Figure 4: *Supercritical CO_2 processing for decaffeination.*

When it was discovered in the 1980s to be a suspected carcinogen, the chemical was abandoned by all the big U.S. coffee labels. The Food and Drug Administration continues to permit the use of methylene chloride if the residues in the coffee are below 10 parts per million. Processing for specialty decafs still often uses it because it perturbs other flavorings so little. Many other solvents can serve to debuzz coffee. An "all-natural" label may mean that ethyl acetate is the solvent in use, because that chemical occurs naturally in fruit. Water also works as a means of decaffeination. (Figure 4) The so-called Swiss water process soaks green coffee beans in a solution that contains the chemical components of beans dissolved from a previous batch, except for the caffeine. Because the water is already saturated with sugars and peptides, only the caffeine passes from the beans into the water [46-48].

Another process, illustrated here, uses supercritical carbon dioxide as a solvent; in this state, the carbon dioxide is intermediate between a gas and a liquid. The variety of caffeine extraction methods demonstrates that a lot of sleepless nights have gone into helping the world get a good night's rest.

Benefits of This Method

a. Extracts Caffeine, effectively. Is a direct contact method but does not use chemicals.
b. Doesn't chemically affect proteins or carbohydrates of the coffee beans.
c. The by-products are natural and a 100% recyclable.

Mango peel waste (*Mangifera indica* L.) was extracted by Maria del P., *et al*. in the year 2013 employing SC-CO_2, wherein they have claimed that Processes using supercritical fluid extraction of active compounds represent a great potential for applications in agri-food and pharmaceutical industries. The aim of their study was to exploit the mango peel waste (*Mangifera indica* L.) for obtaining bioactive extracts. Mango skin waste was submitted to extraction process with two sequential steps: supercritical CO_2 extraction ($scCO_2$) followed by pressurized ethanol (PE) from the residue of first stage, extraction operated at 300 bar and 40°C, conventional ethanol extraction (CE) was done. The extracts obtained were evaluated by spectrophotometric method in terms of Total Carotenoids Content (TCC) (µg carotenoids/g db), Total Phenolic Content (TPC) (mg GAE/g db), Total Flavonoids Content (TFC) (mg CE/g db); and Antioxidant Activity (%AA) by DPPH method. All experiments were performed in duplicate, an ANOVA and Tukey test was performed ($p < 0.05$) (SAS, version 9.1.3). The results demonstrated that TCC value is dependent on the solvent, being high for $ScCO_2$ with carotenoids contents of 5604.60 ± 0.51 µg in contrast to PE, 359.45 ± 0.35 and CE, 704.4 ± 0.9 µg carotenoids/g db. TCC results follow the trend $ScCO_2$ > CE > PE mean while for TPC and %AA trend was CE > PE > $ScCO_2$. The results obtained showed that the combination of extraction methods achieves appreciable results and suggests the potential of this agro-industrial residue for obtaining active compounds [46-48].

The authors have observed in the second extraction stage (PE), had the highest overall extraction yield, this behaviour is due to the use of high pressures in organic solvents for extraction processes promotes mass transfer of solute to the solvent, improving extraction yield as suggested by Mustafa., *et al*. However, it is important to note that polar nature of the solvent also has a positive influence on the compounds that are present in the matrix indicating a greater presence of polar substances in mango peel extracts, this influence in the global extraction yields was reported by Martinez-Correa., *et al*. Extraction yield was higher than $scCO_2$ this behaviour was reported in several studies, which also obtained global yields for the conventional processes higher than supercritical extraction.

They observed that total flavonoids had significant differences in conventional ethanol (CE) extraction shown the higher contents of flavonoids (10.5 ± 0.02 mg CE/g db). This value was within the range of as reported by Abdul., *et al*. for mango peel extraction. Therefore it was considered that SC-CO_2 was efficient to extract the low polarity fractions of flavonoids.

The authors concluded in their study that the combination of extraction methods achieves appreciable results in terms of phenolic content in pressurized ethanol. Flavonoids and total carotenoids in Mango mesocarp (*Mangifera indica* L.) are low polarity and were preferentially extracted with supercritical carbon dioxide. Mango peel waste represents a valuable source of natural antioxidants and represents potentiality for processes of extraction of value-added compounds with important biological activity [46-48].

Bioactive Compounds from *Garcinia mangostana L.*

Zarena A. S. in the year 2011 characterized the bioactive compounds from *Garcinia mangostana* L. employing supercritical CO_2. Mangos teen fruits usually grown in Southeast Asia, have found international market in recent years because of their growing knowledge in the pharmaceutical and food industry. To sum up, this work highlights the importance of mangosteen pericarp which have been traditionally used as an indigenous medicine as a rich source of health benefits. Supercritical carbon dioxide (SC-CO_2) extractions were carried with and without ethanol as modifier. The use of ethanol as an entrainer in SC-CO_2 increased the overall yield and the xanthone recovery, comparing well with the Soxtec™ method. Response surface methodology (RSM) proved to be extremely useful in predictive modelling and optimization of extraction conditions such as pressure, temperature, solvent to material ratio and time on the extracts yield. Analytical tools such as RPHPLC- DAD, LC-ESI-MS and 1H, 13C NMR spectral techniques were useful in screening quantification and identification of xanthones, phenolic acids, flavonoids and anthocyanin compounds. A one-step enzymatic glycosylation of α-mangostin in $SCCO_2$ was successfully employed for the synthesis of α-mangostin-D-glucoside using amyloglucosidase [46-48].

The conversion yield was optimized using central composite rotatable design. The results on long-term stabilities of mangos teen extract in oil-in water (MIO/W) emulsions have shown to possess important implications for the design of whey protein concentrate stabilized emulsions for development of functionally bioactive compounds for health benefits. An overall result of the investigation are highly encouraging and adds to the current knowledge in the pharmaceutical and food industries for the possible commercial application of SC-CO_2 in the extraction of bioactive compounds from mangosteen pericarp. There is an increasing public awareness

of the health, environment safety and hazards associated with the use of organic solvents in food processing and the possible solvent contamination of the final products [46-48].

The high cost of organic solvents and the increasingly stringent environmental regulations together with the new requirements of the pharmaceutical and food industries for ultra-pure and high added value products have emphasized the need for the development of new and clean technologies for the processing of food products. Supercritical carbon dioxide (SC-CO_2) as a solvent has provided an excellent alternative to chemical solvents. Over the past three decades, $SCCO_2$ has been used for the extraction and isolation of valuable compounds from natural products [46-48].

Carbon dioxide is particularly suitable solvent for food processing applications, because it's moderate critical temperature (31.1°C) and critical pressure (7.38 MPa) enables the extraction of thermally labile food compounds in near natural form. Additionally, it is non-toxic, environmentally acceptable and relatively inexpensive. Compared with conventional solvents CO_2 does not leave any harmful solvent residue after extraction. Mangosteen (*Garcinia mangostana* L.) belongs to the family of Guttiferae and is named ''the queen of fruits", it is a slow-growing tropical evergreen tree mainly found in India, Myanmar, Sri Lanka and Thailand. It bears dark red to purple rounded fruits of 5-7 cm in diameter. The edible portion of fruit (aril) is white, soft with a slightly sour taste. The pericarp of mangosteen fruit is 6-10 mm in thickness and has been used in Thai indigenous medicine for the treatment of skin infections, wounds and diarrhea for many years. The major secondary metabolites of mangosteen have found to be prenylated xanthone and oxygenated xanthones, tannin, isoflavone, flavones and other bioactive substances. Xanthones have a variety of biological activity, for example antioxidant, antibacterial, antifungal, antitumor, antiplatelet aggregation, antithrombotic, prevention of oxidative damage of LDL, and inhibition of HIV-1 protease [46-48].

Extraction and Fractionation of Corn Bran Oil

Scott L Taylor and Jerry W. King studied the extraction and fractionation of Corn Bran oil (table 4) exploiting analytical supercritical fluid instrumentation. Two-step fractionation processes, such as those just proposed, require considerable effort to optimize. The cost of materials, time and labour, and equipment can be substantial in screening for the best conditions for either extraction or fractionation (chromatography). With the advent of modern, automated analytical SFE instrumentation, such modules can be used to address the optimization of such separation processes. With slight modification, the described equipment can be changed into a small-scale preparative chromatograph employing lower cost sorbent material in place of the sample matrix used in traditional analytical SFE. Such units can be used to optimize the SFE stage that is conducted prior to SFC.

Recently, it has been reported that the hexane-derived extract from corn bran contains high levels of ferulate-phytosterol esters (FPE) similar in composition and function to oryzanol, an ingredient with nutritional functionality that is found in rice bran and rice bran oil. Oryzanol has been shown to lower the levels of serum cholesterol in laboratory animals and humans. Recently, ferulate phytosterol esters, and in particular the sitostanyl ester, have also been shown to have similar cholesterol lowering activity [46-48].

Corn bran and corn fibre are obtained as by-products from the dry- and wet-milling of corn, respectively processes that are used in converting corn into numerous products. Moreau., *et al*. reported that both corn bran and corn fibre yield oils that contain FPE. Moreau., *et al*. performed hexane and SC-CO_2 extractions and found that the corn fibre oil contained higher percentages (3-6%, w) of FPE than corn bran oil (1.5%, w). They also noted that corn fibre, which contains similar sterol components, produced lower levels of oil than corn bran. Unfortunately, in both cases, the nutraceutically-active components are at low levels in a predominately triglyceride-containing oil extract. Enrichment of these active components in an extract or concentrate would be an appreciable effort in continuation [46-48].

Supercritical fluid fractionation (SFF) studies were also performed with an Isco model SFX 3560 automated extractor. The sorbents tested for the SFF were as follows: silica gel (60-200 mesh, J.T. Baker Chemical, Phillipsburg, NJ), amino-propyl bonded silica (40 µm, Varian Associates, Harbor City, CA), neutral alumina (60-325 mesh, Fisher Scientific, Fair Lawn, NJ), and diol-modified silica (37-55 µm,

Millipore Corporation/Waters Chromatography, Milford, MA). The sorbents were added to a 10-mL extraction vessel, and corn bran oil (~0.4 g) was manually applied to the top of the sorbent bed. The extraction/fractionation procedure was then commenced with fractions collected at the timed intervals. The first fraction was intended to remove most of the triglyceride-based oil. The parameters for subsequent fractions were designed to fractionate and enrich the collection of FPE [46-48].

	Pressure	Temperature	Time	Flow rate	Solvent
Fraction 1	69.0 MPa	80°C	60 min	2 mL/mi	CO_2
Fraction 2	34.5 MPa	40°C	60 min	2 mL/min	1% EtOH–CO_2
Fraction 3	34.5 MPa	40°C	60 min	2 mL/min	2% EtOH–CO_2
Fraction 4	34.5 MPa	40°C	60 min	2 mL/min	3% EtOH–CO_2
Fraction 5	34.5 MPa	40°C	60 min	2 mL/min	5% EtOH–CO_2
Fraction 6	34.5 MPa	40°C	60 min	2 mL/min	7% EtOH–CO_2
Fraction 7	34.5 MPa	40°C	60 min	2 mL/min	10% EtOH–CO_2
Fraction 8	34.5 MPa	40°C	60 min	2 mL/min	15% EtOH–CO_2
Fraction 9	34.5 MPa	40°C	30 min	2 mL/min	20% EtOH–CO_2

Table 4: *Parameters for Corn Bran Oil Extraction.*

The authors reported that the first fraction contained the majority of the fatty acid-phytosterol esters and triglycerides and averaged 89.2% (w) of the mass for the five SFF runs. The fourth fraction mainly consisted of free fatty acids, free sterols, diglycerides, and ferulate-phytosterol esters and averaged 9.2% (w) of the mass for the five SFF runs. The FPEs were collected in the third (2% EtOH–CO_2) and fourth (10% EtOH–CO_2) fractions. However, they were concentrated in the latter, where on average, 99.6% (w) were present. The FPE composed on average 14.5% (w) of the fourth fraction for the five SFF runs, whereas they only represented 1.29% (w) of the starting corn bran oil. Thus, an enrichment factor of 11.24 can be attained from this fractionation/enrichment process. (Table 4) [46-48].

In their findings the individual runs showed a small but noticeable decline in mass recovery for the fifth SFF run, and this may indicate a slight decline in sorbent efficiency. However, the individual components of the corn bran oil showed approximately 100% recoveries, except for the free fatty acids, free sterols, and 1, 3 diglycerides. In retrospect, the decrease in mass recovery may be due to an instrumentation error in collection efficiency. An instrumentation error is proposed, because worn extraction chamber seals were replaced after the fifth run and a small but noticeable amount of oil was found when the extraction chamber was cleaned, thus indicating a small loss of sample. The high recoveries associated with the free sterols and 1, 3 diglycerides warrant further investigation. This could possibly be related to the analytical chromatography system, which is not specifically optimized for their analysis. The peaks tended to be broad and had shoulder peaks and thus were not the ideal peak shape for chromatographic analysis. Likewise, the low recoveries of free fatty acids are still unaccounted for. It was noted earlier that a sorbent reconditioning step was carried out between each SFF experiment. Fractions collected during these reconditioning runs yielded an average of 0.46 mg. Therefore, carry over from one run to the next does not seem to be problematic [46-48].

Wheat germ oil extraction

Shao., et al. investigated the supercritical extraction of wheat germ with carbon dioxide as a solvent. Supercritical CO_2 extraction was carried out using a pilot plant extraction system. Thermostatic baths were switched on to reach the operating temperature required for extraction. Gaseous CO_2 was introduced into a compressor. The extraction vessel was 1000 ml volume capable of operating up to 500 bars and 75°C with the circulation of heated water. The independent variables were temperature (40, 50 and 60°C), pressure (200, 275 and 350 bar) and flow rate (15, 20 and 25 L/h), Table 1. After 200g sample was placed in extraction vessel, the extraction temperature, pressure and flow rates were controlled automatically and maintained for 60 min. When the desired pressure, temperature and flow rate were reached, the extraction was started. The oil dissolved in the supercritical CO_2 was separated from the carbon

dioxide and collected in the separator. Conventional extraction was carried out using hexane in a Soxhlet apparatus for 20h (with a fraction wheat germ size of 0.75 mm and humidity less than 0.35%) to ensure maximum extraction efficiency [46-48].

The authors considered these values very important to establish an indisputable basis for comparison to the high-pressure process. Many parameters can influence the separation performance of wheat germ oil extraction. It was shown that wheat germ oil yield has a complex relationship with independent variables that encompass both first and second-order polynomials and may have more than one maximum point. The best way of expressing the effect of any parameter on the yield within the experimental space under investigated was to generate response surface plots of the equation. The three-dimensional response surfaces were plotted as a function of the interactions of any two of the variables by holding the other one at a middle value. Contour plot and response surface curve showing predicted response surface of oil yield as a function of pressure and flow rate. It showed that at temperature 50°C the oil yield of wheat germ increased with increase in pressure. The oil yield increased from about 7.13% to 10.00% as the pressure was increased from 200 to 350 bars. The optimum pressure for the maximum yield of oil was around 350 bars. At lower pressure, the solubility of oil affected by vapour pressure of the oil, apparently at this stage CO_2 relatively act as an ideal gas that does not have any special characteristic of a solvent [46-48].

However, at high pressures, the solubility of the oil increased due to the increase in density of CO_2. As the density increases, the distance between molecules decreases and the interaction between oil and CO_2 increases, leading to greater oil solubility in CO_2. Piras., *et al*. Explored the SC-CO_2 of wheat germ oil. The effects of pressure (200-300 bars at 40°C) and extraction time on the oil quality/quantity of feed were studied. A comparison was also made between the relative qualities of material obtained by SC-CO_2 extraction and by organic solvent extraction. The extracts were analyzed for α-tocopherol and polyunsaturated fatty acid content. The maximum wheat germ oil yield at about 9% was obtained with SC-CO_2 carbon dioxide extraction at 300 bars, while fatty acid and α-tocopherol composition of the extracts was not remarkable affected by either pressure or the extraction method. The SC-CO_2 gives CO_2 extraction yield not significantly that of different from organic solvent extraction. A comparison is made between the relative qualities of the oils produced by SC-CO_2 and by organic solvent extraction (hexane, methanol, chloroform-methanol 2:1 mixtures) in terms of a much lower selectivity. CO_2 yielded oil contained as undesirable compounds. The effect of the specific mass of solvent, q (kg CO_2/kg feed) on the global extraction yield Y (%) for all the seed oils investigated [46-48].

In general, the increase of the temperature resulted in the decrease of the extraction yield, due to the decrease of the solvents density, whose effect seems to have dominated over the increase of the solute vapour pressure. In some of the cases the temperature had a positive effect on the extraction yield and, oppositely, a negative effect on the extraction rate. At higher pressure (300 bars) it was shown that the temperature had no influence on the total extraction yield and extraction rate, and the highest yields were obtained [46-48].

In the most of the systems investigated solvent flow rate did not influence the extraction yield. From the experimental results for the system almond fruits oil - CO_2 it is evident that oil yield, at the initial stage of extraction, increased increasing CO_2 flow rate, with constant pressure and constant temperature. On the other hand, in the system borage seed - CO_2, the investigation of the effect of solvent flow rate on the extraction yield showed that the lower flow rate test resulted in a slightly higher oil load, but the higher flow rate lead to a shorter extraction time to reach the same yield as the lower flow rate test. The extraction yield increases by decreasing the particle size of the ground materials.

It is due to the higher amount of oil released as the substrate cells are destroyed by milling, and this because of the amount of oil is easily extracted for direct exposure to the supercritical CO_2. Moreover, shorter diffusion paths in the milled solid matrix result in a smaller intraparticle resistance to diffusion. The moisture containing pre-treatment conditioning did not affect the oil quality as measured by free fatty acid content. Increasing seed moisture content from 2.5% to 7.5% increased the extraction yield [46-48].

In two of the systems, the addition of entrainers was achieved with the aim of enhancement the extract, efficiency were added with aim to achieve enhancement SC-CO_2 extraction process. Caprylic acid methyl ester as an entrainer in the extraction process of borage seed was added. The highest solubility of pure caprylic acid methyl ester in dense CO_2 was determined at 100 and 300 bar. The addition of this entrainer increased the yield of pure extract at the pressures investigated. Due to the high solubility of caprylic acid methyl ester at the lower pressure it was easy to separate entrainer, which constituted only 4.22% of the total borage seed extract. In the extraction of corn germ laboratory experiments were carried out at constant pressure (300 bars) and temperature (42°C) with amount of ethyl alcohol (entrainer) ranging from 0% to 10% by weight in CO_2. The alcohol content in the solvent had a strong influence on the rate of the extraction. With increasing the amount of alcohol in the fluid results in decrease the extraction time and the amount of CO_2 consumption. Supercritical fluid extraction (SFE) using carbon dioxide (CO_2) is the answer to the growing demand for pure and natural substances for applications in the food, beverage, pharmaceuticals, fragrance and cosmetic industries [46-48].

SFE is not only the gentlest extraction method for sensitive feed stocks; it also delivers a number of additional, compelling advantages:

a. 100% natural, safe, inexpensive and environmentally responsible solvent
b. Residue-free end-products
c. Selective process control
d. Improved quality and yield
e. Fully scalable process

Besides being the method of choice for applications designed to extract flavors and aromas, CO_2-SFE is also gaining momentum in the high-growth sector of plant substances (Nutraceutical, botanicals, and food supplements). Here, requirements for naturalness and purity are very high and conventional methods simply are not up to the challenge. Supercritical fluid extraction using CO_2 meets the toughest process standards and delivers results, including higher yields considerations that make CO_2-SFE an alternative well worth considering.

Evonik has been working with SFE since 1980, continuously improving on the process and the technology to either optimize existing products or develop completely new, high quality solutions for various end markets. Here, the combination of extensive know-how and advanced technology translates into shorter turn-around times and tailored solutions for optimum, cost-effective results that give our customers the edge. Evonik operates six extraction facilities of varying scope and size, for projects ranging from rapid and flexible development all the way to volume production, allowing for throughputs ranging from one kilogram to thousands of tons [46-48].

Cocoa Defatting

Conventional cocoa defatting processes are mechanical, using either presses or expellers, and they hit a wall at approximately 10% of residual fat content. Further defatting is just not feasible by mechanical means. CO_2-SFE, however, further reduces fat content down to < 0.5% with no negative impact on polyphenol content or quality. In addition, this comprehensive defatting method also meets current regulation and ordinance standards regarding cocoa processing [46-48].

Truly fat free cocoa powder opens a completely new spectrum of applications, including sweets, fat free creams, fillings and baked goods.

The solubility of lipophilic substances also facilitates the extraction of valuable fats and oils. Here, the oil seed is directly extracted after milling, for instance to isolate fatty acids without damaging them.

The selectivity and modulability of CO_2 solvent power versus sterins and other vegetable active ingredients can also be applied for scrubbing purposes. Here, active ingredient feedstock is freed of triglycerides (de oiled), and can thus be concentrated and stabilized. Naturally, off flavor components resulting from the process itself can be easily extracted as well.

The advantages of cocoa defatting/deoiling using supercritical fluid extraction with carbon dioxide:

a. The cocoa butter is completely removed from the cocoa powder with no loss of polyphenole content or quality
b. New applications for fat free powder
c. 100% compliant with ordinances and regulations for cocoa processing
d. Efficient extraction of valuable fats and oils from oilseeds
e. Scrubs/deoils vegetable active ingredients

No process-related solvent residue inherent to CO_2 extraction technology and method [46-48].

Phytochemical Extraction

There is a solvent free alternative, however: supercritical fluid extraction (SFE) using carbon dioxide (CO_2) delivers a higher quality product with a much lower risk of damaging the source material. This method preserves sensitive active ingredients because no oxygen is fed into the process and operating temperatures can be kept low. The net result: higher ingredient yields and consistently high quality levels. Applying supercritical CO_2 also eliminates the need for expensive and time-consuming process stages (such as distillates) common to conventional methods. Supercritical fluid extraction using carbon dioxide not only makes the process more economical, but also delivers better results.

The advantages of obtaining plant extract using supercritical fluid extraction with carbon dioxide:

a. Gentle extraction of active plant extracts under inert process conditions
b. No oxidative changes
c. Preserves active ingredients and their efficacy
d. No solvent residue, i.e. no process-related solvent residue that could lead to use restrictions

Sterilization in the foods processing

High microbial content in foods and health care products represents a serious risk to human health and is simply no longer admissible in today's spirit of health conscious diets and an absolutely safe food supply. In addition to these self-evident concerns, microorganisms pose a significant economic threat that can lead to total write-offs of expensive raw materials.

Germ reduction is therefore understandably a crucial preoccupation. The most common methods involve thermal processing, e.g. pasteurization. Gas, radiation and chemicals offer effective alternatives, yet all known methods come at a price: degraded quality (denatured proteins, mineral and vitamin depletion, sensorial alterations) and/or heavy restrictions. There are innovative approaches to germ reduction out there that work with hydrostatic pressure up to several thousand bars, but the cost will still remain prohibitive for the foreseeable future [46-48].

Sterilization method, on the other hand, builds on moderate pressures up to 400 bar (approx. 5800 psi) and is inspired by supercritical fluid extraction technology. Here, water activity, particle size, germ height and desired microbial reduction still remain vital considerations as are the qualitative requirements and ultimate purpose of the goods to be treated. Here, spore content is a critical aspect because pressure-based sterilization only deals with vegetative microbes (bacteria, yeasts, fungi), but not spores. The latter have to be addressed with additional measures such as germ induction and pressure cycles. Besides effectively sterilizing the treated substance, the process can also be adjusted to affect other characteristics requiring extractive measures this in addition to being much gentler on your feedstock than with conventional methods.

The bottom line: A process that not only effectively deals with a problem, but also adds value by enabling you to obtain other extracts or to remove pollutants or to enhance the quality and marketability of your end-product.

The advantages of sterilization using supercritical fluid treatment with carbon dioxide:

a. Gentler process than conventional thermal methods
b. Can be combined with other extractive applications

c. Not restricted in use

d. No process-related solvent residue inherent to CO_2 extraction process and technology

Bioactive of Ginger and gingirol extraction

Ginger (*Zingiber officinale Roscoe*) is one of the most widely used herbs that contains several interesting bioactive constituents and possesses health promoting properties. 6-gingerol, a major pungent ingredient of ginger, also has potent antioxidant activity. Monitoring of 6-gingerol content during the drying process, ginger extraction with supercritical CO_2 and bioactive properties analysis of extracts were performed. Fresh mature ginger rhizomes with 94.17 ± 0.16% moisture content were dried using a rotary air dryer at 55 ±2°C for 11 hours to achieve a moisture content of 11.54 ± 0.29%. After the drying process, 6-gingerol content of the ginger rhizomes were reduced from 21.15 ± 0.13 to 18.81 ± 0.15 mg/g dry weight basis. Dried ginger was then pulverized to coarse powder, approximately 0.5 mm in diameter, prior to extraction. The supercritical CO2 extraction of ginger was undertaken with two conditions of 200 bars at 35°C and 230 bars at 40°C. The results showed that the extracts from both conditions had gingerol contents of 238.94 ± 0.79 and 170.50 ± 0.45 mg/g extract, total phenolic contents of 183.96 ± 1.25 and 126.04 ± 0.72 mg gallic acid/g extract, respectively. In addition, the ginger extracts showed antioxidant activities using DPPH (1, 1-Diphenyl-2-picrylhydrazyl) radical scavenging assay, compared with BHT standard, expressed as EC_{50}, that were 13.09 ± 1.77 and 26.68 ± 1.76 µg/ml, respectively. The antioxidant activities using ABTS (2, 2′-azinobis [3 ethylbenzothiazoline-6-sulfonic acids] radical cation scavenging assay were 813.33 ± 6.67 and 724.44 ± 7.70 µmol Trolox/g extract, respectively [48-50].

Chairat Puengphian and Anchalee Sirichote (2008) had studied the extracts from the conditions at 200 bar and 35°C had significantly ($P \leq 0.05$) greater amounts of 6-gingerol (figure 5) than those at 230 bars and 40°C. The extraction conditions at 230 bars and 40°C also contributed to a decrease in the amounts of 6-gingerol and total phenolic content. The extracts obtained from 200 bars at 35°C and 230 bars at 40°C, had antioxidant activity (with DPPH method, expressed as EC50) of 13.09 ± 1.77 and 26.68 ± 1.76 µg/ml respectively compared to BHT standard 13.82 ± 0.38 µg/ml. The antioxidant activity with ABTS method, expressed as µmolTrolox were 813.33 ± 6.67 and 724.44 ±7.70 µ mol Trolox/g extract, respectively. This study found that the extracts retained high phenolics and high antioxidant activities. The ginger extracts produced from SC-CO_2 extraction were also free from chemical solvents, providing a significant advantage in their potential application as functional ingredients for the food industry [48-50].

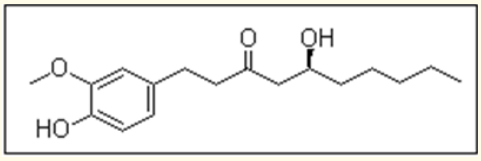

Figure 5: *(6)-Gingirol.*

Carotenoids from carrots and evaluation of products

Mei Sun and Feral Temelli reported that carotenoids are gaining growing interest in food industries due to their nutritional and colorant properties. The objectives of this study were to extract carotenoids from carrots with supercritical CO_2 (SC-CO_2) and to evaluate the antioxidant activity of the crude carrot oil extract and colour of the carrot residue. Crude carrot oil was extracted from freeze-dried carrots with SC-CO_2 at different temperature (40, 55, and 70°C) and pressure (27.6, 41.3, and 55.1 MPa) conditions for 4 h. Carotenoids in the crude carrot oil were identified and quantified by HPLC analysis. The antioxidant activity of the crude oil was determined using the ferricthiocyanate method. The colour parameters ('L', 'a', and 'b') of the carrot residue were determined by Hunter Lab spectrocolorimeter. Hue, chroma, and the overall colour change ΔE between feed material and residue were calculated. The main carotenoids in the crude carrot oil extract were α-carotene, β-carotene, and lutein. The extraction yield of α-carotene was137.8-330.3 µg/g feed material, β-carotene was 171.7-386.6 µg/g, lutein was 23.5-37.5 µg/g, and total carotenoids was 339.3-745.5 µg/g. The antioxidant activity of the SC-CO_2 extracted carrot crude oil ranged from 24.98 to 43.14, which was approximately half of that of butylated hydroxytoluene

BHT (75.04) measured at equal concentrations of crude oil and BHT, which is a commonly used synthetic antioxidant. The intensities of redness 'a' and yellowness 'b' of the carrot particles decreased and the brightness 'L' increased after extraction. Supercritical CO_2 extraction is an efficient technique to obtain natural food colorant with high antioxidant activity [48-50].

Authors have reported the extraction yields of α-carotene, β-carotene and total carotenoids increased dramatically with a temperature increase from 40 to 55°C, but only slightly with a further increase in temperature from 55 to 70°C at both 27.6 and 41.3 MPa. However, at 55.1 MPa, the extraction yields of α-carotene, β-carotene, and total carotenoids showed a drop with a temperature increase from 55 to 70°C following an increase from 40 to 55°C. The extraction yields of α-carotene, β-carotene and total carotenoids decreased with pressure from 27.6 to 41.3 MPa, but increased with an additional pressure increase from 41.3 to 55.1 MPa at temperatures of 40 and 55°C. At 70°C, however, the extraction yields of α-carotene, β-carotene and total carotenoids showed a steady decrease with pressure from 27.6 to 55.1 MPa. There was no clear trend for the change in the yield of lutein with temperature and pressure. Table 5 shows the yields of carotenoids obtained with SC-CO_2 [48-50].

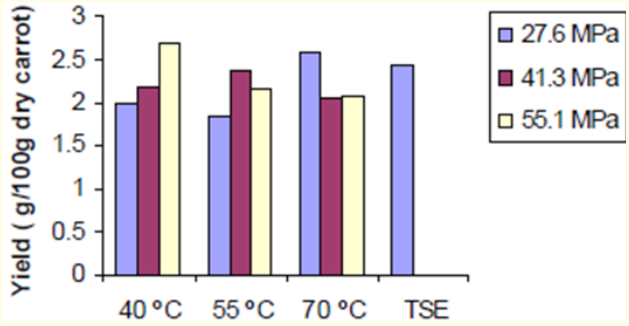

Figure 6: *Extraction yield of crude carrot oil with SFE (Mei Sun and Feral Temelli).*

T (°C)	P (MPa)	α-Carotene (µg/g feed)	β- Carotene (µg/g feed)	Lutein (µg/g feed)	Total Carotenoids (µg/g feed)
40	27.6	181.6	220.2	30.1	462.1
	41.3	137.8	171.7	29.7	339.3
	55.1	259.9	309.8	37.5	607.3
55	27.6	330.3	378.2	30.3	738.9
	41.3	246.6	299.5	34.5	580.6
	55.1	320.5	367.8	23.5	711.8
70	27.6	328.5	386.6	30.3	745.5
	41.3	269.9	308.5	27.2	605.8
	55.1	250.9	296.5	28.7	576.2

Table 5: *Yields of caretenoids obtained with SFE (Mei Sun and Feral Temelli).*

Authors extracted carotenoids Figure 6, table 5) from dried carrots using supercritical CO_2. Their findings showed that not only the hydrocarbon compounds such as α- and β-carotene but also the oxygenated carotenoids such as lutein were recovered with supercritical CO_2. The crude carrot oil extracted with SC-CO_2 had high antioxidant activity, which was approximately half of that of a synthetic antioxidant (BHT) measured at equal concentration. Colour analysis of carrot particles revealed that redness was removed to a greater extent than yellowness, especially at higher temperatures. In summary, SFE is an efficient extraction technique for the recovery of natural food colorants with high antioxidant activity [48-50].

When they investigated the antioxidant activity of SC-CO_2 extracted oil at equal concentration of 500 ppm with that of BHT, the values obtained for BHT was exactly the half of that obtained with SC-CO_2. This has clearly indicated that the shelf life of the crude oil of carrot obtained by SC-CO_2 was superior. The antioxidant activity of SC-CO_2 extracted carrot crude oil at different temperature and pressure conditions ranged from 24.98 to 43.14 which was approximately half of that obtained for BHT (75.04) [48-50].

Milan N. Sovilj in his critical review (2010) reported the SC-CO_2 extraction of selected seed oil (table 6) along with their feed in the table 44. He has also reviewed that wheat germ oil yield has a complex relationship with independent variables that encompass both first and second-order polynomials and may have more than one maximum point. The best way of expressing the effect of any parameter on the yield within the experimental space under investigated was to generate response surface plots of the equation. The three-dimensional response surfaces were plotted as a function of the interactions of any two of the variables by holding the other one at a middle value. Contour plot and response surface curve showing predicted response surface of oil yield as a function of pressure and flow rate [48-50].

Oil Seed	Amount of oil in the feed, %	Process parameter
Almond fruits *(Prinus duluis)*	15.5	P = 330 bar; T = 50°C; v = 20-40 Kg/h; w = 1.1-5.5%
	54.0	P = 350 bars; T = 40°C; V¬0.72-1.43 kg/h; d_{av} = 0.30-1.9 mm
	45.0-60.0	P = 350-550 bar; T = 35-50°C; V 10-30 kg/h; D_{av} 0.5 – 8.0 mm
Borago seed *(Borago officinalis L.)*	31.0	P = 200-300 bar; T = 10-55°C; V 7.5-12.0 kg/h; w– 6.6%
	31.0	P = 200-300 bar; T = 40-60°C; V 0.20 kg/h; d_{av} – 1.125 mm; W = 7.99%
	30.0	P = 100-350 bar; T = 40°C; V 30 L/h
Corn gern	45.0-55.0	P 300 bar; T – 42°C
Grape seed *(vitis vinifera L.)*	10.0	P = 280-550 bar; T = 40°C; d_{av} 0.39-0.97 mm v – 0.36 kg/h; W = 3.9%
	12.0	P = 100-500 bar; T = 40°C
Evening Primrose seed *(Oenothere biennis L.)*	28.0	P = 200-300 bar; T = 40-60°C; V – 0.17 kg/h; d_{av} – 0.63 mm
Hazelnut *(Corylus avellana L.)*	56.0-60.0	P – 150-600 bar; T – 40-60°C; V – 0.12 1/h; d_{ay} –1.0-2.0 mm
	60.0	P – 300-450 bar; T – 40-60°C; V = 0.06-0.30 kg/h; d_{ay} 0.85-1.00 mm
Linseed *(Linus usitatissimum L.)*	35.0-45.0	P – 300-500 bar; T – 47-52°C; V = 8.8 kg/h (kg h); day – 0.16-2.00 mm
Pumpkin seed *(cucurbita peppo)*	42.0-54.0	P = 150-300 bar; T = 40-60°C; V – 0.20 kg/h; d_{ay} – 0.50-0.63 mm
Walnut *(Juglans regia L.)*	65.0	P – 180-234 bar; T – 35-48°C; V = 4.0 L/h; d_{ay} – 0.01-0.50 mm
	74.0	P – 200-400 bar; T – 25-70°C; V = 10.5 kg/h; d_{ay} – 1.2-2.4 mm; W – 2.5-7.5%
Wheat germ	8.0-14.0	P – 200-350 bar; T – 40-60°C; V = 15-25 L/h
	11.0	P = 200-300 bar; T – 40°C

Table 6: *Supercritical CO_2 extraction of selected seed oil (Milan N. Sovilj).*

It showed that at temperature 50°C the oil yield of wheat germ increased with increase in pressure. The oil yield increased from about 7.13% to 10.00% as the pressure was increased from 200 to 350 bar. The optimum pressure for the maximum yield of oil was around 350 bars. At lower pressure, the solubility of oil affected by vapour pressure of the oil, apparently at this stage CO_2 relatively act as an ideal gas that does not have any special characteristic of a solvent. However, at high pressures, the solubility of the oil increased due to the increase in density of CO_2. As the density increases, the distance between molecules decreases and the interaction between oil and CO_2 increases, leading to greater oil solubility in CO_2. Piras., et al. explored the SC-CO_2 of wheat germ oil. The effects of pressure (200-300 bars at 40°C) and extraction time on the oil quality/quantity of feed were studied. A comparison was also made between the relative qualities of material obtained by SC-CO_2 extraction and by organic solvent extraction. The extracts were analyzed for α-tocopherol and polyunsaturated fatty acid content. The maximum wheat germ oil yield at about 9% was obtained with SC-CO_2 carbon dioxide extraction at 300 bar, while fatty acid and α-tocopherol composition of the extracts was not remarkable affected by either pressure or the extraction method. The SC-CO_2 gives a CO_2 extraction yield not significantly that of different from organic solvent extraction. A comparison is made between the relative qualities of the oils produced by SC-CO_2 and by organic solvent extraction (hexane, methanol, chloroform-methanol 2:1 mixture) in terms of a much lower selectivity. CO_2 yielded oil contained as undesirable compounds. The effect of the specific mass of solvent, q (kg CO_2/kg feed) on the global extraction yield Y (%) for all the seed oils investigated (figure 7) [48-50].

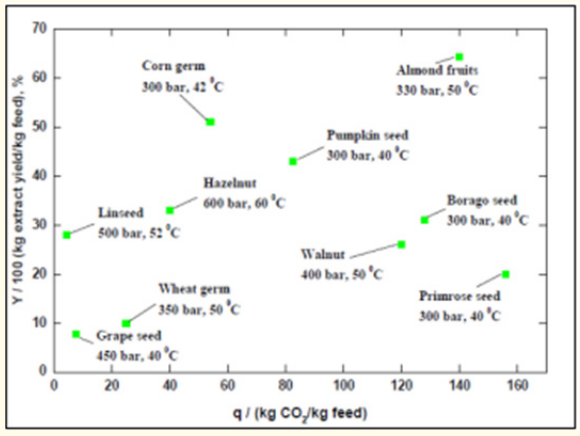

Figure 7: *Effcet of the specific solvent flow on oil seed yield (Milan N. Sovilj).*

Extraction of Rambutan Seed Oil with Response Surface Methodology

N. Yoswathana in the year 2013 has studied the extraction of Rambutan seed oil by SC-CO_2 (figure 8-9) extraction using response surface technology. She has also undertaken the conventional solvents for comparing the yields of the oil. Seed of rambutan as a waste of products from the canned fruit industry and was extracted by supercritical carbon dioxide ($ScCO_2$) using CO_2 as a solvent, maceration and Soxhlet extraction using ethanol as the solvent. An optimization study of $ScCO_2$ extraction using response surface methodology was performed and 3D response surfaces were plotted from the mathematical models. The optimal conditions based on combination responses were: pressure (X1) at 34.8 MPa, temperature (X2) at 56.7°C, the amount of ethanol (X3) in volume 14.5 ml. These optimum conditions of percent oil yield of 30.38. Therefore, it is considered that the $ScCO_2$ extraction is competitive with conventional extraction as shorter extracting times, high percent oil yield, less organic solvent and eco-environmental friendly. The extracted oil could be used in the cosmetic and food industry [48-50].

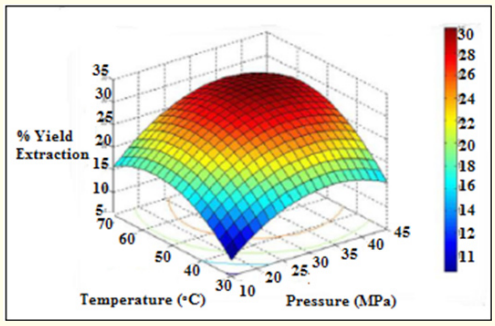

Figure 8: *Surface plot of temperature & pressure at 95% ethanol with 10 ml for percent oil yield from rambutan seeds (Nuttawan Yoswathana 2013).*

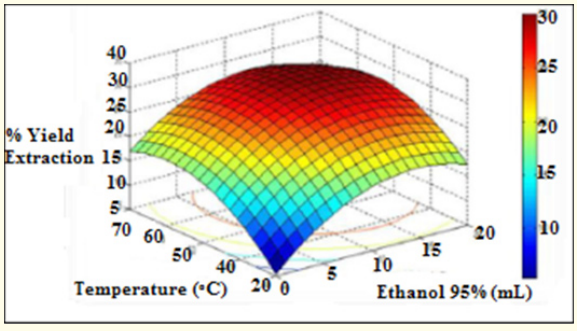

Figure 9: *Surface plot of volume of 95% ethanol and temperature at pressure 30 MPa for percent oil yield from ramubtan seeds (Nuttawan Yoswathana 2013).*

In this study, she gave her finding that increasing temperature lead to increase oil yield from 30 to 50°C. After increasing temperature up to 70°C, it was inversely with increasing temperature. This occurrence was supposed to be due to the applied high temperature, which led decomposition characteristic of oil. Pressure and temperature have a significant effect on oil yield extraction from rambutan seeds. According to Wei., *et al*. (2009), the higher temperature and pressure would cause softening of the plant tissue, and increasing the solubility of oil from rambutan seeds, which improves the rate of diffusion, thus giving a higher rate of extraction. Also, the experiment should be operated at the top conditions of 3D-plotted curve for optimum conditions for oil extraction (Figure 9) [48-50].

The surface plot in Figure 9 revealed that the amounts of extracted oil occurred increase from without ethanol to 10 ml of 95% ethanol. Percent oil yields decreased inversely with amount of 95% ethanol more than 10 ml. As a result, propose statistical model is adequate for predicting percent oil yield from rambutan seeds using $ScCO_2$, it was calculated from the equation (1) and gave the highest oil yield 30.38 at pressure 34.8 MPa, temperature 56.7°C, and using 14.5 ml of 95% ethanol at 2h extraction time as shown in Figurer 5. From experiment, the result reported that the highest oil yield was 28.42 at pressure 30 MPa, temperature 50°C, and using 10 ml of 95% ethanol at 2h extraction time. Also, the predicted and experimental values were not significantly different. Therefore, the $ScCO_2$ extraction using RSM is an appropriate method for oil extraction from plant as shown below in the equation 1 [48-50].

$$Y = -69.421 + 1.9480x_1 + 1.7429x_2 + 2.7173x_3 -$$
$$0.0268x_1 2 - 0.015x_2 2 - 0.0663x_2 2 + 0.0028x_1x_2 - 0.0914x_1x_3 - 0.0069x_2x_3$$

According to author response surface methodology was a good tool for optimization of extraction conditions. The experimental data of percent oil yield from rambutan seed using $ScCO_2$ were used to calculate the coefficients of the second- order polynomial in equation (159). The coefficient of determining (R_2) was 0.895, indicating adequate accuracy. The application of RSM yielded the regression which was an empirical relationship between percent oil yield extraction (Y) and the test variables in code units i.e.x_1,x_2 and x_3 were pressure (MPa), temperature (°C) and amount of co-solvent (ethanol) (ml), respectively.

Palm Oil Kernel Extraction

Mohammed Jahurul Haque Akanda in 2012 investigated a pressurization-depressurization SC-CO_2 extraction technique (pressure swing technique) was used to separate PKO from undehulled ground palm kernel. The PKO yields obtained by using combined pressure swing (PS) and continuous extraction are shown in Table 5. The authors compared these results with those of continuous SC-CO_2 extraction and combined PS extraction. Pressure swing extraction gave higher yield compared to continuous extraction at all pressures (Table 8). Moreover, they reported that the yield was doubled when PKO was extracted using a combined PS process for any given amount of CO_2 used. They also claimed that, almost all of the oil from palm kernel particles extracted with combined pressure swing and continuous extraction with the use of a low amount of CO_2. The highest total oil yield 46.9% was reported for the combined PS technique at 25 MPa with CO_2 flow rate of 2.49 g min^{-1}. In comparison, the yield of the continuous extraction was lower (34.9%) than the combined PS extraction at same pressure and carbon dioxide used [48-50].

Step	Time (min)	Yields (%)							
		Pressure swing extraction, pressure (MPa)				Continuous extractions, pressure (MPa)			
		10	15	20	25	10	15	20	25
1	10	1.6	2.8	4.5	9.9	0.8	1.2	2.4	3.9
2	10	1.7	3.8	5.0	12.0	0.9	1.2	2.5	3.9
3	10	1.7	2.7	3.3	4.6	1.0	1.4	2.4	4.1
4	120	13.8	19.8	24.2	20.4	8.2	12.9	19.5	23.0
Total	150	18.8	29.1	37.0	46.9	10.9	16.7	26.8	34.9

Table 7: *Yield of both combined pressure swing (PS) extraction & continuous extraction of palm kernel oil.*

They have reported that selective extraction of low vapour pressure oils can be done using supercritical fluids (table 7) which is not easily attainable by distillation. These oils cannot be fractionated by distilling because of the presence of impurities with equal volatility as the main components, thus failing to achieve good fractionation. Fractionation using supercritical fluid with respect to chemical composition to produce oil fractions with different carbon lengths and saturations has been investigated. Hassan., *et al.* fractionated PKO through supercritical carbon dioxide extraction. For the extraction and fractionation of PKO, the SC-CO_2 was used as a solvent in the pressure range 20.7-48.3 MPa and temperatures between 40 and 80°C. At lower pressures 20.7 and 27.6 MPa, the solubility of PKO in SC-CO_2 decreased with temperature while at higher pressures of 34.5, 41.4 and 48.3 MPa, the solubility increased with temperature. The authors found that the earlier fractions rich in short-chain triglycerides, while the later fractions were rich in longer chain triglycerides and unsaturated triglycerides. The authors also reported that the short chain fatty acids contained in PKO are easily soluble in SC-CO_2.

The objectives that are of significance in the palm oil industry, to decision makers are to maximize crude palm oil and palm kernel oil production and to minimize losses of palm oil and palm kernel during processing and also to bring production costs to the lowest possible level. SCF technology has a number of advantages over the conventional processing methods for the extraction, purification and fractionation of palm oil. These include low-temperature operation, selective separation, inert solvent, little wastewater and the extraction of a high-value product or a new product with improved functional or nutritional properties. Considerable attention has been given to the development of SCF processing in the oil and fat industry. However, use of SC-CO_2 for the extraction of palm oil from its fruits has been found to be relatively rare as compared to SC-CO_2 extraction of other vegetable oils [48-51].

Conclusions

Employing $SCCO_2$ extraction to bioactive is effective since no chemical is utilized. It does not chemically affect protein and carbohydrates of the coffee beans and the bye products are natural and 100% recyclable. The operation manipulation become convenient just by changing temperatures, pressures and flow rates. Batch time duration depends on the bulk density of the mass that is brought in contact with supercritical CO_2. Continuous extraction on the same sample with the addition of EtOH at different concentration (in mol %) yielded the composition behaviour of the extracts. Results indicated that the yield increased with increasing pressure. The feasibility of ethanol extraction from a post–fermentation broth using SC-CO_2, was compatible, however, application of SC-CO_2 for in situ extractive fermentation has been limited by its inhibitory effect on the metabolism of a variety of yeasts and bacteria. Recent investigations on the applications of SCE from post fermentation biomass or in situ extraction of inhibitory fermentation products found as a promising method for increasing yield. However, pseudo solubility values for equivalent experimental conditions decreased when they were evaluated based on oil recoveries and volumes of CO_2 used up in longer extractions. Supercritical drying goes beyond the critical point of the working fluid in order to avoid the direct liquid-gas transition seen in ordinary drying. SFE have several advantages for the enrichment of tocochromanols over conventional techniques such as vacuum distillation, in particular a lower operating temperature. Supercritical carbon dioxide can also be used as a more environmentally friendly solvent for dry cleaning as compared to more traditional solvents such as hydrocarbons and percholroethylene. However, use of SC-CO_2 for the extraction of palm oil from its fruits has been found to be relatively rare as compared to SC-CO_2 extraction of other vegetable oils.

Bibliography

1. Khosravi-Darani K., *et al*. "Solubility of poly (hydroxybutyrate) in supercritical carbon dioxide". *Journal of Chemical and Engineering Data* 48.4 (2003): 860-863.
2. Khosravi-Darani K and Vasheghani-Farahani E. "Application of supercritical fluid extraction in biotechnology". *Critical Reviews in Biotechnology* 25.4 (2005): 1-12.
3. Wells SL and DeSimone J. "CO_2 technology platform: an important tools for environmental problem solving". *Angewandte Chemie International Edition* 40.3 (2001): 518-527.
4. Hejazi P., *et al*. "Supercritical disruption of Ralstonia eutropha for recovery of poly (hydroxybutyrate)". *Biotechnology Progress* 19.5 (2003): 15-19.
5. Kashket ER. "Bioenergetics of lactic acid bacteria: cytoplasmic pH and osmotolerance". *FEMS Microbiology Reviews* 46.3 (1987): 223-244.
6. Mackey BM., "Factors affecting the resistance of Listeria monocytogenes to high hydrostatic pressure". *Food Biotechnology* 9.1-2 (1995): 1-11.
7. Castor TP Hong GT. "Supercritical Fluid Disruption of and Extraction from Microbial Cells". *US Patent* 5,380,826 (1995).
8. Athes V., *et al*. "Influence of polyols on the structural properties of Kluyveromyces lactis L-galactosidase under high hydrostatic pressure". *European Journal of Biochemistry* 255 (1998): 206-212.
9. Adams MW and Kelly R M. "Finding and using hyperthermophylic enzymes". *Trends in Biotechnology* 16.8 (1998): 329-332.
10. Jimenez-Carmona MM and Luque de Castro MD. "Reversemicelle formation: a strategy for enhancing CO_2 supercritical fluid extraction of polar analytes". *Analytica Chimica Acta* 358.1 (1998): 1-4.
11. Mozhaev VV., *et al*. "Exploiting the effects of high hydrostatic pressure in biotechnological applications". *Trends in Biotechnology* 12.12 (1994): 493-501.
12. Mozhaev V V., *et al*. "Application of high hydrostatic pressure for increasing activity and stability of enzymes". *Biotechnol Bioeng* 52.2 (1996): 320-331.
13. Nakamura K. "Biochemical reactions in supercritical fluids". *Trends in Biotechnology* 8 (1990): 288-292.
14. Noel Marie O M., *et al*. "Combined effects of pressure and temperature on enzyme stability". *High Pressure Research* 19.1-6 (2000): 317-322.

15. Frederiksen L., *et al*. "Preparation of liposomes encapsulating water-soluble compounds using supercritical carbon dioxide". *Journal of Pharmaceutical Sciences* 86.8 (1997): 921-928.
16. Lemay P. "The use of high pressure for separation and production of bioactive molecules". *(BBA) - Protein Structure and Molecular Enzymology* 1595.1-2 (2002): 357-366.
17. Jarzebski AB and Malinowski JJ. "Potentials and Prospects for Application of Supercritical Fluid Technology in Bioprocessing". *Process Biochemistry* 30.4 (1995): 343-352.
18. Zagrobelny J and Bright F V. "In situ studies of protein conformation in supercritical fluids: trypsin in carbon dioxide". *Biotechnology Progress* 8.5 (1992): 421-423.
19. Choi ES., *et al*. "Solubilities of o-, m- and p-coumaric acid isomers in carbon dioxide at 308.15–323.15K and 8.5–25 MPa". *Journal of Chemical & Engineering Data* 43.1 (1998): 6-8.
20. Stahl E., *et al*. "Extraction of Seed Oils with Liquid and Supercritical Carbon Dioxide". *Journal Agricultural and Food Chemistry* 28.6 (1980): 1153-1157.
21. Friedrich, JP and List GR. "Characterization of Soybean Oil Extraction by Supercritical Carbon Dioxide and Hexane". *Journal of Agricultural and Food Chemistry* 30.1 (1982): 192-193.
22. List GR., *et al*. "Properties and Processing of Corn Oils Obtained by Extraction with Supercritical Carbon Dioxide". *Journal of the American Oil Chemists Society* 61.12 (1984): 1849-1851.
23. Snyder JM., *et al*. "Effect of Moisture and Particle Size on the Extractability of Oils from Seeds with Supercritical CO_2". *Journal of the American Oil Chemists Society* 61.12 (1984): 1851-1856.
24. Halliwell B and Gutteridge JMC. "Free Radicals in Biology and Medicine, 3rd ed., Oxford, Oxford University Press. (1989).
25. Halliwell B. "Antioxidant characterization". *Biochemical Pharmacology* 49 (1995): 1341.
26. Willcox JK., *et al*. "Antioxidants and prevention of chronic disease". *Critical Reviews in Food Science and Nutrition* 44.4 (2004): 275-295.
27. Pokorny J and Korczak J. Preparation of natural antioxidant, in Antioxidants in Food: Practical Applications, 1st ed., Pokorny, J., Yanishlieva, N. and Gordon, M., Eds., Woodhead Publishing Limited, Abington, Cambridge, England (2001): 311-330.
28. Gordon MH. The development of oxidative rancidity in foods, in Antioxidants in Food: Practical Applications, 1st ed., Pokorny, J., Yanishlieva, N. and Gordon, M., Eds., Woodhead Publishing Limited, Abington, Cambridge, England (2001): 147-158.
29. Frankel EN and Meyer AS. "The problems of using one-dimensional methods to evaluate multifunctional food and biological antioxidants". *Journal of the Science of Food and Agriculture* 80.13 (2000): 1925-1941.
30. Yanishlieva NV.. Inhibition of oxidation, in Antioxidants in Food: Practical Applications, 1st ed., Pokorny, J., Yanishlieva, N. and Gordon, M., Eds., Wood head Publishing Limited, Abington, Cambridge, England (2001): 20-68.
31. Antolovich M. "Methods for testing antioxidant activity". *Analyst* 127.1 (2002): 183-198.
32. Roginsky V and Lissi EA. "Review of methods to determine chain-breaking antioxidant activity in food". *Food Chemistry* 92.2 (2005): 235-254.
33. Landvik SV., *et al*. "Efficacy of vitamin E in human health and disease, in Handbook of Antioxidants, 2nd ed., Cadenas, E. and Packer, L., Eds., Marcel Dekker, New York 75 (2002).
34. Sim´andi B., *et al*. "Supercritical fluid extraction of dandelion leaves". *The Journal of Supercritical Fluids* 23.2 (2002): 135-142.
35. Salda˜na MDA., *et al*. "Supercritical carbon dioxide extraction of methylxanthines from mat´e tea leaves". *Brazilian Journal of Chemical Engineering* 17.3 (2000): 251-259.
36. FP Schmitz and E Klesper. "Separation of styrene oligomers by Supercritical Fluid Chromatography (SFC) using a modified HPCL-instrument". *Polymer Bulletin* 5.11 (1981): 603-608.
37. ST Sie GWA and Rijnders. Separation Scientific 2 (1967): 755.
38. ST Sie., *et al*., "Microporous Polymers as Stationary Phases in Chromatography with High-Pressure Gases or Supercritical Fluids" C. L. A. Harbourn, Ed., Gus Chromatography-Z968, Elsevier, Amsterdam, the Netherlands, (1969).
39. JC Giddings., *et al*. *Science* 162.3849 (1968): 67.

40. JJ Czubryt., *et al. The Journal of Physical Chemistry* 74 (1970): 4260.
41. J. J. Czubryt, *et al. The Journal of Physical Chemistry* 74.4260 (1970).
42. FM Barton. Handbook of Solubility Parameters and Other Cohesion Purameters, CRC Press, Boca Raton, FL, (1983).
43. JW King J P and Friedrich. *Journal of Chromatography* 517. 449 (1990).
44. EJ Couch., *et al*. "Volumetric Behavior of Nitrous Oxide. Pressure-Volume Isotherms at High Pressures". *Journal of Chemical & Engineering Data* 6.229 (1961).
45. CR Yonker and RD Smith. "Solvatochromism: a dielectric continuum model applied to supercritical fluids". *The Journal of Physical Chemistry* 92.1 (1988): 235-238.
46. CR Yonker S L., *et al. The Journal of Physical Chemistry* 9$3022 (1986).
47. B Hemmaplardh and AD King Jr. *The Journal of Physical Chemistry* 76.2170 (1972).
48. JL Fulton., *et al*. "Inter- and intea-molecular hydrogen-bonding of polyethylene glycol dodecyl ethers in supercritical ethane". *The Journal of Supercritical Fluids* 3.4 (1990): 169-174.
49. E Stahl and E Willing. Pharm. Ind 42 1136 (1980).
50. JW Hellgeth., *et al*. "Supercritical Fluid Chromatography of Free Fatty Acids with On-Line FTIR Detection". *Journal of Chromatographic Science* 24.5 (1986): 183-188.
51. ML Lee and KE Markides. "Analytical Supercritical Fluid Chromatography and Extraction, Chromatography Conferences". *Provo* (1990): 97.
52. Moradas-Ferreira P., *et al*. "The molecular defenses against reactive oxygen species in yeast". *Mol Microbiol* 19.4 (1996): 651-658.

Volume 2 Issue 1 January 2016