AN EVALUATION OF OIL EXTRACTION TECHNOLOGIES FOR SEA BUCKTHORN SEED AND PULP OILS

BY

RYAN W. YAKIMISHEN

A Thesis submitted to
the Faculty of Graduate Studies
In Partial Fulfillment of the Requirements for the Degree of

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Department of Biosystems Engineering
University of Manitoba
Winnipeg, Manitoba

The Faculty of Graduate Studies
500 University Centre, University of Manitoba
Winnipeg, Manitoba R3T 2N2

Phone: (204) 474-9377
Fax: (204) 474-7553
graduate_studies@umanitoba.ca

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ABSTRACT

Sea buckthorn has become recognized as a specialty crop, ideally suited for the Canadian prairies, having economical viability in the functional foods and nutraceutical markets. A real need exists for the determination of feasible oil extraction technologies for sea buckthorn, evaluating processing, component extraction efficiency, and oil quality in terms of nutritional composition as related to processing, extraction, and economic feasibility.

An evaluation of oil extraction technologies, namely supercritical fluid extraction (carbon dioxide) (SCFE CO_2), screw pressing, and an aqueous extraction technique was conducted, comparing oil recoveries and nutritional composition with a solvent extraction method employing petroleum ether. Sea buckthorn seed and pulp-flakes (cv. Indian-Summer) were prepared using a pilot process comprising steps of juice extraction (bladder press), drying (24 h at 50°C), and mechanical separation (sieving), which yielded 5 kg of seeds and 3 kg of pulp-flakes from 100 kg of thawed berries.

Oil contents ($\%_C$) of seeds (moisture content of 9.8% w.b.) and pulp-flakes (moisture content of 6.9% w.b.) were determined to be 8.2%$_C$ and 11.9%$_C$, respectively using the solvent extraction method. Seed oil recoveries were 65.1% and 41.2% for SCFE CO_2 and screw pressing, respectively. Pulp-flake oil recovery was 86.3% for SCFE CO_2. No oil was recovered from the pulp-flakes using a screw press. An oil recovery of 6% was determined by aqueous extraction for recovering pulp oil from whole thawed berries (oil content of 2.2%$_C$ determined by a chloroform/methanol procedure).

Supercritical fluid extraction using CO_2, recovered oils having favorable levels of fatty acids, tocopherols and tocotrienols, carotenoids, and sterols, components which are highly valued in the health food industry. It is suspected that the sale of by-products, the development of an efficient harvesting method (mechanical systems), and increased extraction efficiency of oils may contribute to lowering the price of the oils, necessary to recover the costs (namely the purchase an SCFE system, electricity, raw materials, and consumables) associated with SCFE CO_2 oil extraction.

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Sea buckthorn processing is like playing a piano,
there are many ways to strike the keys.

Dr. Karl Heilscher, Professor

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1. INTRODUCTION

1.1 Development of a nutraceutical industry in Canada

Optimum nutrition has been theorized as a concept to encompass the maximum nutritional value of foods to ensure optimal well-being and health, while minimizing the risk of disease throughout a lifespan (Roberfroid 2000). However, literature indicates that nutrition with respect to the optimization of health is still very primitive and loosely understood. Increased life expectancy, disease onset, and exponentially rising healthcare costs have forced societies to implement prevention strategies to control illness through improved nutrition practices focusing on the disease prevention. Attempts toward optimizing health through prevention have been made by the introduction of concentrated nutritional food products known as functional foods and nutraceuticals (Roberfroid 2000).

The functional food and nutraceutical markets, collectively estimated as a multi-billion dollar global industry (6 to 86 billion, 5 to 7.5% annual increase), have been gaining tremendous popularity (Oomah and Mazza 1999; Hardy 2000; Menrad 2003). Thousands of products have been developed for this market aimed at the health conscious consumer. However, there is an imminent concern that unprecedented growth rates are likely and have already attracted irresponsible market entrants distributing products that do not deliver on quality (Hardy 2000).

Only recently Canada, like many countries, has been confronted with an increasing elderly population, a consumer demand for healthier foods, and now more than ever stringent product regulations to assess product quality and

deliverance. In addition, Canada is a leading producer of agricultural products (60 Mt/yr) including grains, oilseeds, and specialty crops having enormous potential for being processed into functional foods and nutraceuticals (Oomah and Mazza 1999). Jointly, the demand for healthier foods and the availability of a ready supply of marketable materials have triggered enormous economical potential for value-added processing and extraction of nutritionally valuable components in Canada. In addition, there is a growing movement in the prairie provinces to diversify agriculture with the introduction of new specialty crops (Storey 2000). While the potential for specialty crops and value-added processing exists, reality governs that derived products must generate prices sufficient enough to cover industry production, processing, and marketing costs, while returning a profit to all vertical market segments. Currently 100 functional foods ranging from plant and animal sources native to Canada are being produced and marketed (Oomah and Mazza 1999).

1.2 Sea buckthorn as a nutraceutical product source

Imbedded in the functional food and nutraceutical market segment, though still virtually unknown in North America, is sea buckthorn. Sea buckthorn (*Hippophae rhamnoides* L.) coined as one of the most-vitamin-rich berry plants in the plant kingdom, carries the credentials for becoming highly valued for healthy living, improving well-being, enhancement of lifestyle, and the potential for disease prevention. Widely recognized in Northern regions of Europe and Asia, sea buckthorn has been used medicinally for thousands of years. Ancient Greek

writings list sea buckthorn as a remedy for horses. Leaves of young branches were incorporated in the diet to increase weight gain and produce shining coats. Hence *Hippophaë*, the latin name for sea buckthorn was derived, 'hippo' meaning horse, and 'phaos' meaning to shine (Li and Schroeder 1999).

Often referred to as a "miracle plant", sea buckthorn has been used extensively in eastern medicinal practices. Sea buckthorn berries have been used for many centuries in Europe and Asia as a nutritional food source. Examples of popular food items containing sea buckthorn include jams, jellies, juices, liquours, and wines. In addition to beverages, hundreds of other sea buckthorn products have been developed from extracts of the berries, leaves, and bark (Schroeder and Yao 1995). Ice-cream and energy snacks have been produced, though are less popular than the juices and juice blends. Sea buckthorn leaves are also just as valuable, and have been prepared for use in teas (Schoeder and Yao 1995). Thus, sea buckthorn has been used in its entirety, whole or as an ingredient in many different foods.

Sea buckthorn has become recognized as a specialty crop, ideally suited for the Canadian prairies, having economical viability in the functional foods and nutraceutical markets (Storey 2000). While a sea buckthorn industry is already established in China, Russia, and Germany, the industry in terms of growing and cultivation has only just begun in Canada, with mechanization of fruit and leaf harvesting being introduced, and processing quickly following close behind. Canada is currently faced with the development of an industry, having minimal previous experience and technical knowledge in this area. Much of the research

in terms of growing and cultivation, harvesting, and processing of sea buckthorn has been conducted in other countries making this information virtually inaccessible by language barriers. However, assuming that this information can be readily translated, it may not be directly applicable to Canada. Thus, a strong need exists for Canadian-specific sea buckthorn research, including studies ranging from growing to processing and evaluating these effects on Canadian sea buckthorn cultivars (J. Winniski, Pearl Creek Farms, Melville, SK, sea buckthorn grower/producer, personal communication, 2002). Canadian-specific research will help to validate data which has been collected in other countries and on other sea buckthorn species and cultivars.

Sea buckthorn seed and pulp oils are considered to be the most valuable components of the berries comprising fat-soluble vitamins and plant sterols (Yang and Kallio 2002a). Sea buckthorn oils have attracted considerable attention from researchers mainly for their nutritional and medicinal value which can be incorporated into several products having vast economic potential in the cosmetic, nutraceutical, and pharmaceutical industries (Li 1998). In North America, the nutritional and medicinal value of the oil is largely unknown and potential sea buckthorn oil markets remain untapped (Schroeder and Yao 1995). However, these markets are closely related to cosmetics, nutraceuticals, and pharmaceuticals which are very demanding and exacting, requiring products that must meet stringent criteria of performance and specifications (Kalustian 1985).

1.3 Processing research opportunities

Puuponen-Pimiä (2002) stated that processing including the extraction of oils should be designed to optimize or protect the nutritional components contained in plant oils. Moreover, there must be criteria for evaluating the effect of processing on physiological functions of nutritional components. Currently no evaluative studies have been conducted on the extraction of sea buckthorn oils (seed and pulp) to assess processing effects on final nutritional quality. Thus, a need exists for the determination of feasible oil extraction technologies for sea buckthorn; evaluation of processing by component extraction efficiency, oil quality in terms of nutritional composition as related to processing and extraction, and economic feasibility.

Information on marketing is critical to the growers and processors of sea buckthorn (Storey 2000). Specifically, processors are beginning to ask questions on operational costs of processing and the remaining size of profit margins. Experience indicates that without proper analysis of processing and feasibility of production, mistakes can be made (Storey 2000). Currently, no information is publicly available on the costs of processing sea buckthorn berries, information which is vitally important to potential investors seeking to move forward into primary and secondary processing. A need also exists to include information on alternative processing methods including supercritical fluid extraction using carbon dioxide (SCFE CO_2), hexane extraction, and cold pressing technologies (Storey 2000). In reference to pesticide analysis, Kim et al. (2002) stated that an ideal extraction method should yield a quantitative recovery of target analytes

without loss or degradation. This concept is also can directly applicable to the area of nutraceutical product development namely the extraction of sea buckthorn oils, targeting nutritional compounds with out inducing degradation of nutritional components.

1.4 Objectives of research work

The objectives of this research were:

1. To develop a pilot process to separate seeds and pulp-flakes from whole sea buckthorn berries (cv. Indian-Summer) as required for oil extraction;
2. To determine the oil content of sea buckthorn seeds and pulp-flakes (obtained from the pilot process by drying) using a solvent extraction technique employing petroleum ether as the extraction solvent;
3. To compare oil (seed and pulp-flake) recoveries by supercritical fluid extraction (carbon dioxide), screw pressing, and an aqueous extraction technique with the solvent extraction method. Oil recoveries were evaluated assuming solvent extraction represents 100% oil recovery;
4. To determine the nutritional quality of the extracted oils by quantifying fatty acids, tocopherols and tocotrienols, total carotenoids, and sterols, as these levels may be affected by the method of oil extraction;
5. And, to determine the cost of oil extraction associated with a potential oil extraction method.

2. REVIEW OF LITERATURE

The following review of literature presents background information on sea buckthorn as a native and domesticated shrub, and research in the area of edible oil extraction technologies. Sea buckthorn, though growing in popularity among the functional food and nutraceutical market, is still virtually unknown in Canada. Thus, section 2.1 is devoted to providing background information on sea buckthorn including its description and history, the chemical and nutritional composition of its harvestable components as well as the importance of these components in the area of functional foods and nutraceuticals, and its global presence including its current development and production in Canada. Section 2.2 discusses past and present methods for oil extraction, the importance of oil quality and its dependence on processing techniques employed, and the feasibility of oil extraction technologies for sea buckthorn.

2.1 Sea buckthorn

2.1.1 Description and history

Sea buckthorn (*Hippophaë rhamnoides* L., Sanddorn in German; Oblepikha in Russian, Rokitnik in Polish, and Tyrni in Finnish) is a dioecious, wind-pollinated shrub belonging to the Elaeagnaceae family (Jeppsson et al. 1999). It has been recognized that the species *rhamnoides* comprises 9 subspecies (ssp.) of which *sinensis*, *mongolica*, and *rhamnoides* are of commercial interest (Rousi 1971; Yang and Kallio 2001).

Obvious physical characteristics of sea buckthorn include abrasive terminal and lateral thorns and bright yellow, orange, or red edible fruit (berries) (approximately 4 to 60 g/100 berries) (Li and Schroeder 1999). The flavour of the berries, characterized as being astringent and sour, have been processed with sweeteners to produce pleasant tasting food products (Tang et al. 2001). Popular products include juices and liqueurs, candy, and ice-cream (Schroeder and Yao 1995). Sea buckthorn shrubs are extremely variable in height (0.5 to 20 m) and pruning is often required in orchard practices (Li and Schroeder 1999). Figure 2.1 shows a female (Fig. 2.1a) and male (Fig. 2.1b) sea buckthorn shrub. Male sea buckthorn shrubs (fruitless) are required for pollination.

(a) **(b)**

Fig. 2.1. a) A female sea buckthorn shrub with ripe clusters or "cobs" of berries. b) A male sea buckthorn shrub.

Berries begin to emerge in the fourth year after planting (S. McLoughlin, CEO Seabuckthorn International Inc. formerly known as Canada Seabuckthorn Enterprises Limited, Peachland, BC, sea buckthorn product distributor, personal communication, 2001).

Sea buckthorn, known for its visual attractiveness and productive food source has been in existence for centuries (Schroeder and Yao 1995). In a recent archaeological study, sea buckthorn was found to inhabit the Central Pyrénées, a mountain range bordering France and Spain, approximately 15,000 yr ago (Peñalba et al. 1997; Heinz and Barbaza 1998). The natural habitat of this shrub extends from China, Mongolia, Russia, and many parts of Europe (Li and Schroeder 1999). Sea buckthorn has also been known to flourish in mountainous areas including the Himalayas (Shinwari and Gilani 2003). In addition to these natural habitats, sea buckthorn is currently being grown and cultivated in other parts of the world, including Canada (Beveridge et al. 2002).

As a hardy, adaptable shrub, sea buckthorn can grow in arid to very wet conditions and can withstand temperatures from -43 to 40°C (Li and Schroeder 1999). In addition, its complex root system with nitrogen-fixing nodules is invaluable for preventing soil erosion while replenishing soil nitrogen and other essential soil nutrients (Li and Schroeder 1999; Tian 2002). For these reasons, sea buckthorn has been used extensively in shelterbelt, soil and water conservation, and reforestation management projects. Thus, sea buckthorn has been ideally suited for wildlife habitat enhancement (Li and Schroeder 1999). Shinwari and Gilani (2003) support the utilization of sea buckthorn as a land reclamation tool and concluded that sea buckthorn has been used for socio-economic improvement in many communities of Northern Pakistan. Other uses for sea buckthorn include ornamental berry decorations and home burglar deterrents, because of sharp thorns.

2.1.2 Nutritional composition

In spite of the effective use of sea buckthorn for ecological management, the discovery of its unique chemical and nutritional components has offered vast medicinal opportunities and enormous economic potential in the functional food and nutraceutical industries (Oomah and Mazza 1999; Storey 2000). Although the terms "functional food" and "nutraceutical" have often been used interchangeably, Health Canada (1998) has made a distinction between them. A "functional food" is defined as a food consumed as part of a usual diet, which demonstrates physiological benefits or reduces the risk of chronic disease beyond basic nutritional functions or both. In comparison, a "nutraceutical" is a product isolated from foods that is sold in medicinal forms, not usually associated with conventional foods. A nutraceutical also provides protection against chronic disease (Health Canada 1998). Bioactive components such as vitamins, fats, and other phytochemicals are the ingredients in functional foods and nutraceuticals which are responsible for this reduction and protection against chronic disease (Andlauer and Fürst 2002). Sea buckthorn, because of its many bioactive components, is well suited as a functional food and can be used as a source of nutraceuticals (Storey 2000).

Sea buckthorn is among the most nutritious and vitamin rich shrubs in the plant kingdom (Li and Schroeder 1999). The berries, being the most popular harvestable product are sought for their physiological effects. They contain many bioactive components including ascorbic acid (vitamin C), α-tocopherol (generically referred to as vitamin E), phenolic compounds (flavonoids and

carotenoids), and other phytochemicals (Oomah and Mazza 1999; Yang and Kallio 2002a).

Tang and Tigerstedt (2001) found vitamin C concentrations as high as 676.2 mg/100 g of berries for many Chinese subspecies namely, *sinensis.* Others have reported ranges between 360 (ssp. *rhamnoides*) and 2500 mg/100 g (ssp. *sinensis*) (Yao et al. 1992; Schroeder and Yao 1995; Li and Schroeder 1996). Table 2.1 shows a comparison of vitamin C concentrations in some fruit.

Table 2.1. A comparison of vitamin C concentrations (mg/100 g of fruit) in some fruit (adapted from Lee and Kader 2000).

Fruit	Vitamin C Concentration (mg/100 g of fruit)
Banana	19
Kiwifruit	65
Orange	83
Black current	92
Sea buckthorn	360 - 2500 (Li and Schroeder 1996)

It has been stated that the synergistic combination of vitamin C and E, in addition to being important antioxidants, makes sea buckthorn berries an optimal raw material in functional food or nutraceutical applications (Kallio et al. 2002a).

Tocopherols and tocotrienols, long recognized for their antioxidant activity and oil stabilizing properties are found in sea buckthorn berries (Dugan and Kraybill 1956; Kallio et al. 2002a and 2002b). Seeds (ssp. *mongolica* and *sinensis*) were found to contain α-, β-, γ-, and δ-tocopherols which constituted 93 to 98% (84 to 318 mg/kg) of the total tocopherol content, while α-tocopherol constituted 76 to 89% (56 to 140 mg/kg) in whole berries (Kallio et al. 2002b).

Phenolic compounds, also found in sea buckthorn berries have been reported as being important antioxidants in the food industry for improving the quality and nutritional value of foods (Kähkönen et al. 1999; Häkkinen et al. 1999b). Häkkinen et al. (1999a) reported that flavonols, a particular group of flavonoids, represented 87% of the phenolic compounds (kaempferol, quercetin, myricetin, *p*-coumaric acid, caffeic acid, ferulic acid, *p*-hydroxy-benzoic acid, and ellagic acid) identified in sea buckthorn compared to several other berries crops including raspberries, strawberries, blueberries, and cranberries.

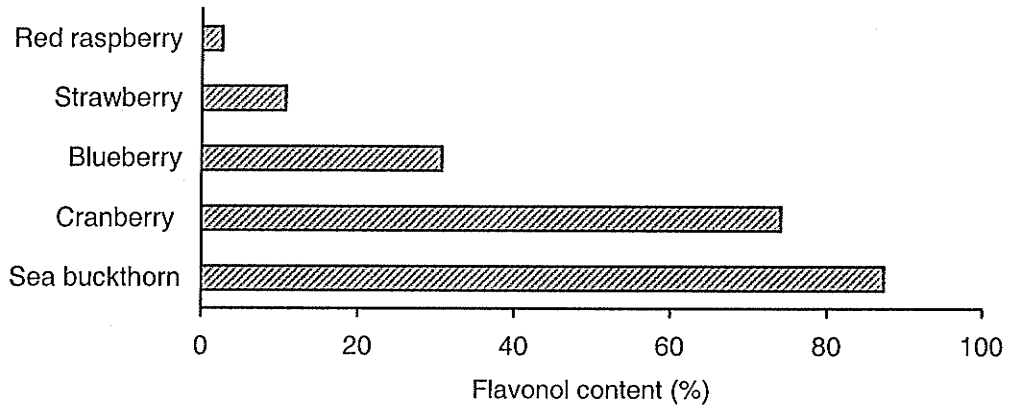


Fig. 2.2. Flavonols as a percentage of the total phenolic compounds (kaempferol, quercetin, myricetin, *p*-coumaric acid, caffeic acid, ferulic acid, *p*-hydroxy-benzoic acid, and ellagic acid) identified in some common berries (adapted from Häkkinen et al. 1999a).

Carotenoids are responsible for the colour of the oils and mainly exist in the mesocarp (fleshy fruit material or pulp). In addition, carotenoids also function as useful antioxidants. β-carotene being the most abundant in the pulp, has been reported to constitute 15 to 55% of total carotenoids. The concentrations of β-carotene are commonly 100 to 500 mg/100 g and 20 to 100 mg/100 g in pulp and seed oil, respectively (Yang and Kallio 2002a).

Plant sterols or phytosterols are another component of sea buckthorn berries which compliments this unique shrub for use in medicinal applications. However, little is known about the sterols present in sea buckthorn berries (Yang et al. 2001). Yang et al. (2001) reported total sterol content of seeds, pulp, and fresh whole berries (ssp. *rhamnoides* and *sinensis*) ranging from 1200 to 1800, 240 to 400, and 340 to 520 mg/kg, respectively. The lack of original mass spectrometric data makes an objective evaluation of sterol identification impossible. Thus, an urgent need exists for further study of sterols in sea buckthorn (Yang et al. 2001).

The seeds (or kernels; one per berry) and pulp of sea buckthorn berries are rich in oil (Yang and Kallio 2002a). The berries of ssp. *rhamnoides* contained a higher proportion of oil in seeds (11.3 vs. 7.3%), lyophilized pulp (18.9 vs. 8.0%), and lyophilized whole berries (16.6 vs. 7.9%) than berries of ssp. *sinensis* (Yang and Kallio 2001). Singh and Dogra (1996) reported seed oil contents of 8.9 to 11.7% and 8.4% for ssp. *turkestanica* and *sinensis*, respectively. All oil contents are expressed as a percentage (% w/w) of oil mass per mass of seeds, pulp, or whole berries and will be expressed in this fashion throughout this report unless otherwise stated.

In addition to oil richness, sea buckthorn berry oils are unique in that the compositions of the seed and pulp oils are distinctly different. The seed oil, defined as being highly unsaturated, comprises two essential fatty acids (EFAs), α-linolenic acid or "Omega-3" (18:3*n*-3) and linoleic acid or "Omega-6" (18:2*n*-6). The contribution of α-linolenic and linoleic acids of the total fatty acid composition

are commonly 20 to 35 and 30 to 40%, respectively (Yang and Kallio 2002a). These fatty acids are "essential" because they cannot be synthesized within the body and therefore must be consumed (Krause and Mahan 1979). In addition, palmitic (16:0), steric (18:0), oleic (18:1*n*-9), and vaccenic (18:1*n*-7, 11-octadecanoic) acids are also present in seed oil, though low amounts have been reported (Yang and Kallio 2002a). Palmitoleic (16:1*n*-7) acid is practically non-existent in seed oil (Yang and Kallio 2001). Oil from the pulp is characterized as being more saturated and comprises primarily palmitic and palmitoleic acids with lower levels of α-linolenic acid (Kallio et al. 2002b).

Data collected on the nutritional components previously discussed are extremely variable. Genetic factors, origin and growing environment, harvest times and maturity of berries, and method of oil isolation all contribute to this variability (Kallio et al. 2002a; Kallio et al. 2002b; Tang et al. 2001; Tang and Tigerstedt 2001; Yang and Kallio 2002a and 2002b; Yang and Kallio 2001; Yang et al. 2001; Gao et al. 2000).

In addition to the berries, the leaves and bark of sea buckthorn are becoming recognized as useful, harvestable components (Storey 2000; Mann et al. 2002). Leaves and bark contain many nutritional components making them worthy in functional food and nutraceutical products. Currently, there are limited papers reporting the use of sea buckthorn bark, however more information is available on the leaves. Phenolic compounds found in the leaves have been reported as useful antioxidants (Bandonienė et al. 2000; Vaher and Koel 2002).

2.1.3 Medicinal applications

Throughout history, sea buckthorn berries have been used in Tibetan and Mongolian medicines. The berries, being recognized as a medicinal ingredient were listed in the Chinese Pharmacopeia in 1977. Although sea buckthorn berries contain numerous nutritional components, many of the health benefits are attributed to the berry oils, which have demonstrated many pharmacological functions (Oomah and Mazza 1999).

Clinical investigations on the medicinal uses of sea buckthorn were first conducted in Russia during the 1950s (Li 1998). However, many publications are only case reports rather than scientific investigations and have been written in Russian and Chinese. For these reasons, validation research on health claims associated with the oils is needed. Currently, some health claims are being evaluated in Europe (Yang and Kallio 2002a).

Sea buckthorn oil treatments are vast and research on their medicinal uses is growing. Primary areas of treatment include and are not limited to antioxidation, skin and mucosa repair, cardiovascular disease prevention, immune system restoration, and anticancer applications (Yang and Kallio 2002a). Studies by Geetha et al. 2002, Süleyman et al. 2002, Cunshe 1995, and Shi et al. 1994, reported that components from sea buckthorn (fruit, seed, and leaf extracts) have shown to increase antioxidation for the prevention of disease.

Seed and pulp oils have long been known for their pharmacological functions in reference to the protection and regeneration of body tissues. The oils have been used to treat oral mucositis, various ulcers, and skin damage from

radiation exposure and burns. Studies done by Yang et al. (1999 and 2000) report positive effects on patients with atopic dermatitis, as characterized by a reduction in eczematous inflammations and other topical, dry-itchy lesions. In addition, seed and pulp oils have shown both preventive and curative functions against gastric ulcers in experimental rats (Xing et al. 2002; Süleyman et al. 2001) while phenolic compounds such as flavonoids, found in the berries have been linked to good stomach and intestinal health in humans (Puupponen-Pimiä 2002).

Declining intake of fruits and vegetable has contributed to a general increase in cardiovascular risk. Sea buckthorn seed and pulp oil have shown to decrease and inhibit platelet aggregation thereby reducing the risk of cardiovascular disease (Johansson et al. 2000; Eccleston et al. 2002). However, it has been suggested that further studies are required to assess dose-response effects in relation to the practical use of sea buckthorn oils as a potential treatment (Johansson et al. 2000).

Xu (1995) reported that pharmacological functions of sea buckthorn oils in the area of anticancer treatments is positive. Research conducted on laboratory animals treated with seed and pulp oils concluded an increase in immune-system functions and therefore tumor inhibition was evident (Zhang et al. 1989a). However, further research is required to validate and extend these and other findings to human medicine practices.

2.1.4 Current trends in sea buckthorn applications

In Europe and Asia, 10 different drugs manufactured from sea buckthorn components have been reported and are available in liquid, powder, paste, pill, and spray form (Li 1998). For example, popular pill products include vitamin C tablets because of the high vitamin C concentration in sea buckthorn berries. There are several other value-added products being manufactured including teas (from leaves) and animal feed (leaves, pulp, and seed residues) (Li 1998). Table 2.2 outlines product categories that have been developed from sea buckthorn components.

Table 2.2. Sea buckthorn components and product categories (Li 1998).

Components	Product categories			
Bark	Pharmaceuticals Cosmetics			
Leaves	Pharmaceuticals Cosmetics Tea Animal feed			
Fruit	Oil	Pharmaceuticals Drinks Food products Cosmetics		
	Juice	Sports drinks Health drinks		
		Pulp	Food Beverages Brewery	
			Oil	Pharmaceuticals Cosmetics
			Residues	Animal feed
Seeds	Oil	Pharmaceuticals Cosmetics		
	Residues	Animal feed		

Figure 2.3 shows a multitude of sea buckthorn products (juices, wine, liqueurs, tea, energy bars, and candy) manufactured in Germany.

Fig. 2.3. A variety of sea buckthorn products: (1) juices, (2) wine, (3) liqueurs, (4) tea, (5) energy bars, (6) candy, (7) other paraphernalia (tea mugs)) manufactured in Germany.

Sea buckthorn oils are used in the cosmetic industry because of the anti-aging properties they contain. Some of the cosmetic applications for the oils are facial creams, body lotions, and sunscreen products (Schroeder and Yao 1995). Cosmetic products including facial creams and lotions have been used and were reported, though unconfirmed, to have positive therapeutic effects on melanosis and wrinkles (Li 1998). Cosmonauts have used cream derived from sea buckthorn components for protection against cosmic radiation (Li and Schroeder 1999). Other specialty products derived from sea buckthorn oils

include skin creams, shampoos, lip balms, bath beads, and encapsulated edible oil tablets (Fig. 2.4). The encapsulation of sea buckthorn oils has been explored to improve oxidation stability and product shelf life (Partanen et al. 2002).

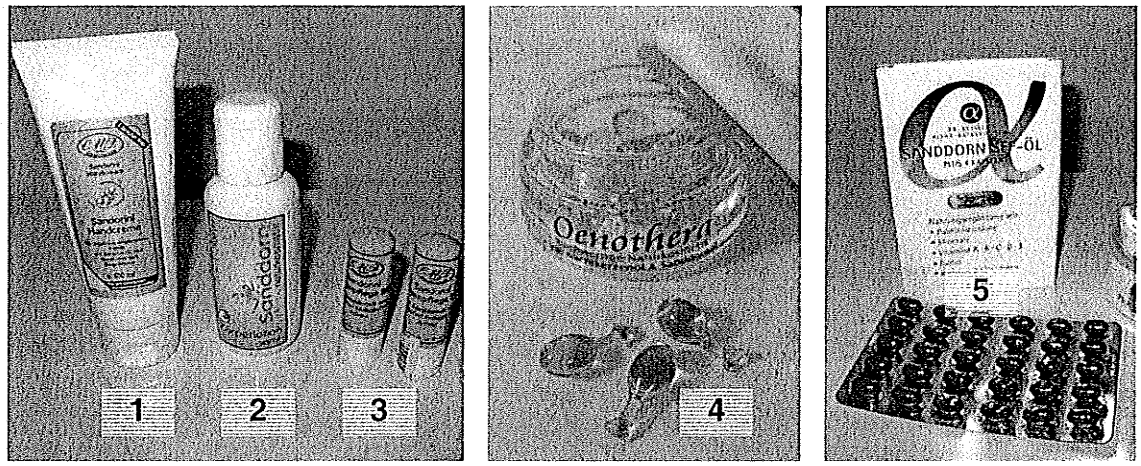


Fig. 2.4. Cosmetic products manufactured in Germany ((1) skin cream, (2) shampoo, (3) lip balm, (4) bath beads, and (5) encapsulated seed oil tablets) derived from sea buckthorn oils.

2.1.5 Global presence of sea buckthorn

The sea buckthorn industry is active primarily in Russia and China with considerable interest growing in Germany and other parts of the world. It has been estimated that with an average production of 200 kg/ha (berries), the world's annual yield is approximately 280,000 t of berries. The growing demand for health products and cosmetics has lead to extensive international research and commercialization of sea buckthorn. Recently, sea buckthorn oils have been increasing in popularity throughout Japan, Europe, and North America as a result of their nutritional effects being realized in western countries (Yang and Kallio 2002a).

Since the 1940s, commercialization in Russia has grown with the interest of bioactive components found in the berries, leaves, and bark (Li and Schroeder 1999). Currently, there are approximately 6,000 ha of sea buckthorn in Russia which are used for the production of nutritional and medicinal products including raw fruit, juices, aerosols, ointments, oils, and pills. Russia, known as a pioneer in the development of sea buckthorn cultivation is conducting research on the selection of new sea buckthorn varieties (Dillon et al. 2002).

In China, research and commercial production of sea buckthorn began in the 1980s and since then, over 300,000 ha have been planted. A current estimate indicates China having 90% of the world's sea buckthorn resource of 1.4 million ha (Yang and Kallio 2002a). There are approximately 150 processing factories producing over 200 sea buckthorn products (Li and Schroeder 1999). In addition, sea buckthorn has also been used extensively in ecological programs to control and reduce soil erosion in China (Yang and Kallio 2002a).

The sea buckthorn industry in Germany is considerably smaller than in Russia and China. Germany offers expertise in the area of sea buckthorn mechanical harvesting and processing technologies. There are currently 300 ha of sea buckthorn being processed into concentrated juices, purées, jams, and snacks. However, the majority of sea buckthorn products manufactured in Germany have recently targeted the supplementary health food market segment, with considerable interest in the manufacturing of oil products (Dillon et al. 2002).

2.1.6 Canada's sea buckthorn industry

An increased interest in alternative crops for nutraceutical and functional food production has introduced sea buckthorn as an interesting new crop for Canada. An unconfirmed estimated 500 ha of sea buckthorn, including producing and non-producing shrubs have been planted, however it has been estimated that only 60 ha have commercial producing potential (S. McLoughlin, CEO, Seabuckthorn International Inc., Peachland, BC, personal communication, 2002). The largest population of sea buckthorn in North America is in Manitoba and Saskatchewan, planted primarily in field shelterbelt settings. Currently, the Canadian sea buckthorn industry is in the infant stages of production, and processing is limited. However, research is actively being pursued in the areas of plant breeding, harvesting, and processing (Dillon et al. 2002).

Russian sea buckthorn cultivars were imported and introduced to Canada in 1938. Initially grown as an ornament, it gained recognition as an effective shelterbelt management shrub. The Prairie Farm Rehabilitation Administration (PFRA) in Indian Head, Saskatchewan have been growing sea buckthorn for over 30 yr and have used this shrub in numerous prairie conservation programs including wildlife habitat enhancement, farmstead protection, erosion control, and mine land reclamation (Schroeder and Yao 1995). Ideally suited for the Canadian prairies, sea buckthorn has thrived in shelterbelts and fruit production in these settings has been recorded as being good to excellent (Li and Schroeder 1999).

"Indian-Summer" as the name suggests, is a seed strain of sea buckthorn that was developed jointly by researchers from the PFRA in Indian Head and the Pacific Agri-Food Research Centre in Summerland, British Columbia (Dr. T. Li, Horticulture and Environmental Studies, Summerland, BC, personal communication, 2002). This strain, which is generally accepted to be a cultivar (cv.) belonging to ssp. *mongolica*, has been used in prairie shelterbelts since the 1970s (Li and Schroeder 1999; PFRA Shelterbelt Center 2001). Research conducted at the PFRA Shelterbelt Centre showed cv. Indian-Summer to be the cultivar of choice based on higher than average berry yields and good fruit and seed quality for processing (PFRA Shelterbelt Center 2001).

Relying on previously completed research in Asia and Europe on the health benefits and growing practices of sea buckthorn, Canadian growers have become interested in this potentially profitable, value added crop. Recently, Canadian growers have planted sea buckthorn in orchard settings for future harvesting and processing (Anand 2002) (Fig.2.5). The majority of commercial orchards planted in Canada include cv. Indian-Summer and ssp. *sinensis*.

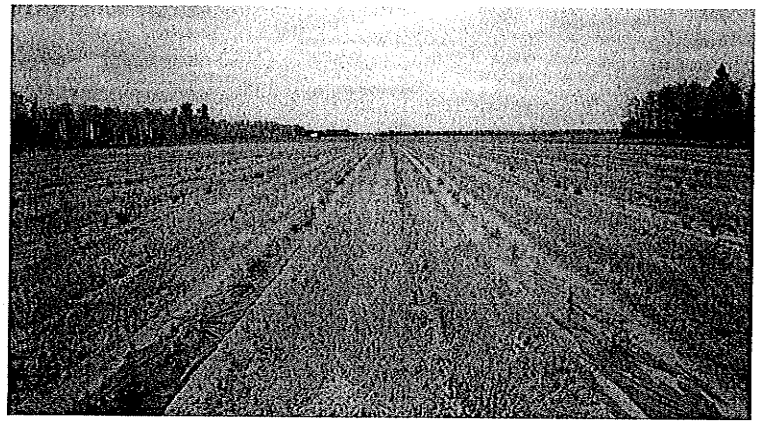


Fig. 2.5. A developing sea buckthorn orchard in Manitoba. Rows of sea buckthorn seedlings.

2.2 Oil extraction technologies

2.2.1 Introduction to oil extraction

For thousands of years, oils have been recovered from plant materials such as seeds, nuts, and fruit. Today, hundreds of plant varieties produce oils in sufficient quantities and/or with economic potential to warrant extraction and processing (Table 2.3). As a result, oil extraction technologies have been extensively studied to maximize oil yields and minimize the production of undesirable impurities during extraction (Carr 1997).

Table 2.3. Some major sources of plant oils (adapted form Johnson (1997) and Bockisch (1998, p. 174)).

Source	Oil content (% w/w)
Lentils, dried	1
Sea buckthorn berry (pulp)	**2.3 (Yang and Kallio 2001)***
Oat flakes	6.5
Sea buckthorn seed	**9.3 (Yang and Kallio 2001)***
Wheat germ	11
Cottonseed	19
Soybeans	20
Coconuts	34
Canola	42
Peanut (shelled)	47
Palm	47
Palm kernel	48
Almonds	54
Pecan nuts	71

* Variability of oil contents among sea buckthorn species.

However, research on the extraction of high-value oils from plants, namely those used in the nutraceutical and cosmetic industries, is limited. Although often rare,

these plants exhibit oils having unique compositions and properties that justify their exploitation and extraction for use in niche products.

In principle, there are two groups of plant tissues, namely seeds and pulp that have been targeted for their oils. Edible oil extraction first involves the organization of the raw oil-bearing material into solid and liquid components. It is from these components that oil can then be readily recovered. Dry extraction technologies such as pressing (screw presses), solvents, and supercritical fluids (SCFs) have been commonly employed to recover oil from dried solid components (moisture content 3 to 6% wet basis (w.b.)) such as seeds, nuts, and other dried plant tissues (Bockisch 1998, p. 432-433; Carr 1997; Taylor et al. 1993). A renewed interest in aqueous processes (or wet extraction technologies) using centrifugation techniques being used for the recovery of oils from liquid or solid-liquid mixtures such as oils from fruit juices or from cells of fruit mesocarp (Hagenmaier 1997). Thus, the oil-bearing material (seeds or pulp) strongly dictates the extraction technology to be employed. Extraction options may be limited to technologies such as SCFs and aqueous processes as industries seek high oil yields while trying to satisfy consumers who demand high quality products that have been "naturally" processed without toxic solvents.

A general overview of current oil extraction technologies including pressing, solvent extraction, supercritical fluid extraction (SCFE), and aqueous extraction will now be discussed, following a discussion on the preparation of oil-bearing material prior to extraction.

2.2.2 Preparation of oil-bearing materials

Preparatory steps of oil-bearing materials prior to extraction are discussed in this section. Several steps are recommended to provide maximum oil yields from oilseeds (Galloway 1976). These steps include decorticating, crushing, conditioning, and flaking. These preparation steps are commonly employed on oilseeds prior to pressing, solvent extraction, and SCFE operations. Oil fruits lend themselves to a series of different preparatory steps and will be specifically discussed in section 2.2.6.

2.2.2.1 Cleaning Cleaning of oil-bearing materials such as oilseeds is conducted so that the resulting oil is not contaminated with foreign material (Carr 1997). Foreign material such as sand, stones, mechanical parts from harvesting machines, weeds, leaves, and other plant materials may be introduced during the collection of oilseeds while harvesting and can disrupt subsequent processing (Bockicsh 1998, p. 363). Proper cleaning will increase processing capacity, reduce equipment maintenance, and improve oil and meal quality. Screens and aspirators are commonly employed in seed cleaning operations (Carr 1997).

2.2.2.2 Decorticating Decorticating or dehulling involves the removal of hulls to expose oil-containing meats. Roller mills are used to crack the hulls or seed coats by a gentle crushing action. Care must be taken not to break or crush the hulls too finely, which can make later separation of hulls from the meats difficult

or even impossible. Pneumatic systems and electroseparators have been used to successfully separate hulls from dehulled meats (Bockisch 1998, p.368; Galloway 1976). Some seeds such as peanuts can be shelled by hand, while others such as sunflower, cottonseed, and soybeans require a machine dehulling process (Galloway 1976). Extraction without dehulling is practiced for some crops such as canola and safflower. Dehulling presents a problem of disposal, unless a feasible by-product (boiler fuel) can be made from the hulls (Ward 1984). In most cases, dehulling is usually a recommended procedure since the hulls do not contain oil and can diminish extraction capacities (up to 20%) and increase equipment wear (Bredeson 1978; Ward 1984). During pressing, Carlson et al. (1985) indicated that the high hull content of crambe meal increased friction and caused scorching of the press cake.

2.2.2.3 Crushing After decorticating, hammer mills (hand or motor driven) are commonly employed to crush seeds to a coarse meal (Carr 1997). Crushing is conducted to rupture oil cell walls allowing for higher oil recovery (Galloway 1976; Bredeson 1978). For pressing operations it is important to avoid over crushing or excessive grinding as this operation can produce pieces which can contaminate the oil and are difficult to remove during subsequent filtration steps (Carr 1997).

2.2.2.4 Conditioning Conditioning of oil-bearing materials will vary according to the product and method of oil extraction employed (Galloway 1976). It involves the application of heat by cooking to break intercellular emulsions, rupture cell membranes (to destroy compartmentalization of oil trapped in cells), coagulate proteins to reduce foots (uncoagulated proteins or other small fractions of suspended solids) in the extracted oil, deactivate enzymes, and decrease oil viscosity to improve oil extraction yields. Improper cooking can lead to an accumulation of "foots", which can hinder filtering during final clarification of the extracted oil (Ward 1984). In addition, excessive cooking can reduce the quality of the oil and extracted meal and may reduce the capacity of some extraction systems, namely presses. In most cases, especially for new specialty oilseeds, cooking conditions have yet to be fully explored (Wiesenborn et al. 2002). Cooking temperatures and times can vary with the type of oil-bearing material and may range from 80 to 105°C and 15 to 20 min, respectively (Peterson et al. 1983). Longer cooking times contribute to protein degradation. Cooking also provides sterilization and can detoxify undesirable seed constituents such as moulds and other bacteria (Ward 1983; Carr 1997).

Conditioning is always coupled with the adjustment of moisture content. The desired effect is to give the seed the right elasticity for pressing or to avoid fine crumbling of seed flakes (during the flaking operation) at low humidity before solvent extraction (Bockisch 1998, p.379). Moisture content plays a critical role especially during pressing or pre-pressing operations (pre-pressing is discussed

in section 2.2.3.4). If the moisture content is too high or too low, excessive solids can be pressed out with the oil (Williams 1997).

2.2.2.5 Flaking Flaking is essentially a refined grinding process which produces a uniform pariticle size. It involves rolling the crushed oil-bearing materials into thin flakes approximately 0.20 to 0.35 mm thick. Flaking is required prior to solvent extraction which promotes good solvent contact (greater surface to volume ratio), resulting in higher oil yields (Bockisch 1998, p. 374-376). Flaking is also conducted prior to pressing, though to a lesser extent for reasons associated with excessive crushing.

2.2.3 Oil pressing

2.2.3.1 History and applications Pressing or "expression" (sometimes referred to as expelling) is the process of mechanically pressing liquid out of liquid-containing solids using high pressures (Gurnham and Masson 1946; Khan and Hanna 1983). Ancient Egyptian, Phoenician, and Chinese civilizations were among the first to employ this method for extracting crude oils from plant materials (Dunning 1953). Though widely recognized as inefficient, pressing methods have been experiencing a renewed interest because of increased environmental concerns raised from traditional oil recovery methods (Bockisch 1998, p. 380). In addition, pressed oils may be well suited to niche markets in the food industry, such as the "organic" or natural product sector (Wang and Johnson 2001).

Early methods of extraction involved filling a cloth bag with ground seeds. The bagged material was hung and the oil was allowed to freely drain. It was recognized that with the addition of pressure (usually by hand), the amount of oil recovered could be greatly increased. Large rocks and lever systems were often used to generate greater pressures up to 690 kPa. Advancements in pressing technology later developed these archaic methods into mechanical systems such as screw, ratchet, or hydraulic jacks, generating substantially higher extracting pressures (up to 6900 kPa) (Carr 1997).

Mechanical pressing is advantageous for oilseeds with higher oil contents (usually >20%) because it is commercially viable. Seeds such as soybeans, canola, and shelled peanuts are prime candidates for pressing (Carr 1997;

Johnson 1997) because of their high oil content. The efficiency of a mechanical oil press rarely exceeds 90% (Khan and Hanna 1983). Thus, if the oil content in the seed is low, the residual oil remaining in the meal or cake may account for a substantial portion of the total oil content (Bockisch 1998, p. 380).

Many of the early presses were classified as batch systems developed to process one batch of seeds at a time. Today, though often described as a practical and economic way to extract oil from locally grown seeds in remote areas, batch presses are very inefficient, requiring intensive labor for low oil yields. Other power-driven commercial presses have replaced smaller hand-operated systems and are capable of processing several tonnes of seed per day. While these systems are simpler to use, requiring minimal operator training and provide excellent yields, substantial machinery costs, long delivery times, availability of spare parts, and the necessity of electricity discourages remote area operations (Carr 1997). Most often, pressing is the technology of choice for many techno-economically challenged countries.

Continuous screw presses are employed in technologically advanced areas where a sufficient raw material supply justifies a continuous operation. Screw presses exert much greater pressures (137 to 300 MPa) than hydraulic batch presses resulting in a greater recovery of oil (Peterson et al. 1983; Bockisch 1998, p.383). In addition, screw presses have large processing capacities capable of handling 40 kg/h to 180 t/h of raw material (Carr 1997). Screw pressing has also been recognized as a suitable process for commercial production of organic edible oils from new oilseed crops (Singh et al. 2002a).

2.2.3.2 Theory In theory, a screw press can be divided into three operations including feeding, ramming, and plugging. A screw press is a continuous screw auger designed to meter seed or ground seed flakes (feeding) and subject these materials to gradually increasing pressures (ramming) through a barrel cage. Increasing the diameter of the screw shaft and/or decreasing the pitch in the screw flights increase the pressure along the length of the barrel cage. A plug of compressed meal then forms at the discharge end (plugging) by means of a choking device. Figure 2.6 shows a typical compression curve relating the volume (compression ratio = V_1/V_2) of material displaced along the distance of the barrel cage during pressing (Ward 1976).

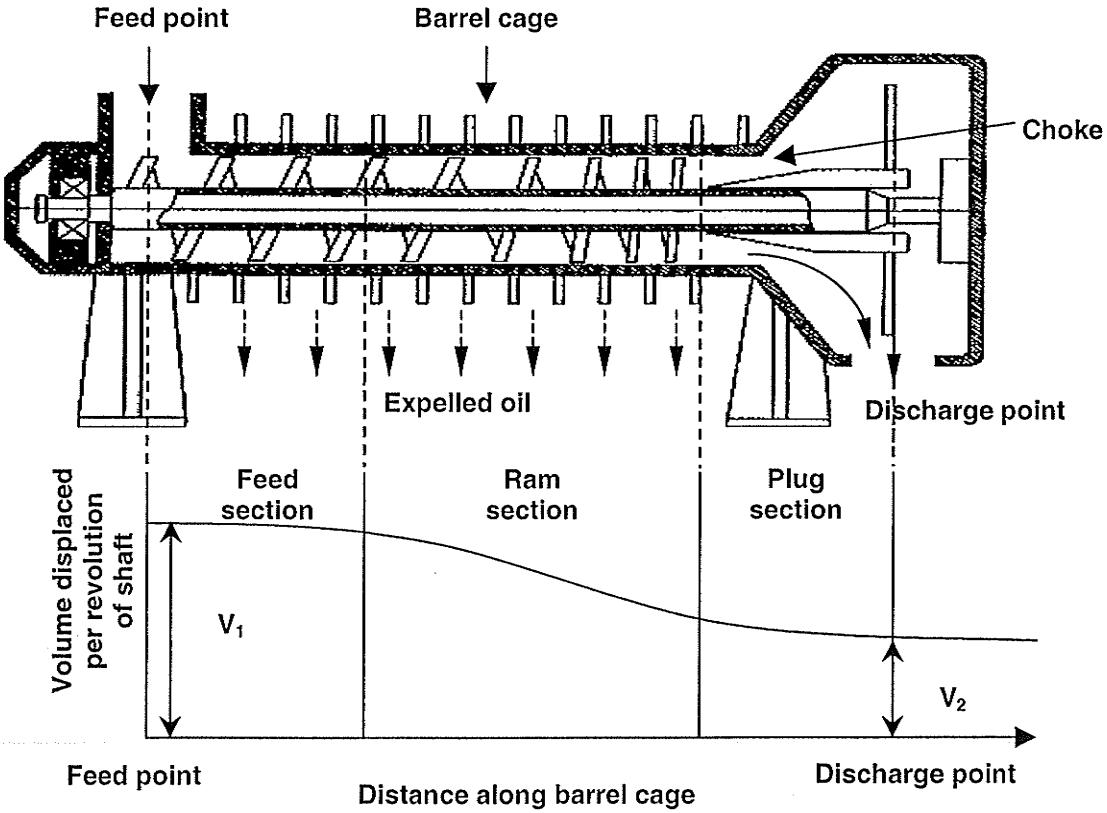


Fig 2.6. Compression curve relating the volume (compressing ratio = V_1/V_2) of material displaced along the distance of the barrel cage during screw pressing (adapted from and Hilton (1999) and Ward (1976)).

Choking, causing back pressure, contributes to a pressure increase on the meal. Back pressure can be controlled by varying the orifice size through which the meal is discharged. Throughout this continuous process, oil is collected from small openings (to restrict the passage of solids during pressing) along the barrel cage (Carr 1997).

2.2.3.3 Cold pressing Cold pressing involves the extraction of oil without external application or internal generation of heat. Oil having temperatures <60°C during pressing are classified as "cold pressed" (Bockisch 1998, p. 384). Most often, cold pressed oils are oils that have been extracted without a pre-cooking step. As previously discussed, cooking is a common pretreatment for oilseeds prior to expelling which utilizes heat to aid in the rupturing of oil-containing cells and promote coalescence of oil droplets by reducing the viscosity (Carr 1997). Even without the pre-cooking step, typical operating pressures (approximately 40 MPa) can elevate temperatures to 170°C (Bockisch 1998, p. 383). Although cooking and higher pressures can generate higher oil yields, heating can cause thermal degradation of heat-labile components. Thus, cold pressing is feasible only if much lower yields are acceptable. Cold pressed oils from fresh and good quality seeds often do not require further refining to remove cloudiness, colour, or flavour (Carr 1997). Today, cold pressing is a popular extraction method for the production of oils such as flax and hemp seed which have become popular products in the functional food and nutraceutical industry.

The production of excessive heat while pressing has long been recognized as a problematic area, which causes degradation of extracted oils (Dunning 1953). Various water-cooling systems such as water-cooled screws and barrel cages have been implemented to control high temperatures during pressing.

2.2.3.4 Pre-pressing Pre-pressing is commonly used as a preliminary extraction step. Mechanical screw pressing was the principal means of extracting vegetable oils until the 1950s, when the introduction of solvent extraction incorporated screw presses into a pre-pressing operation for use on high oil content seeds (Bredeson 1978). There is a general agreement that pre-pressing followed by solvent extraction gives better overall economy when high oil content seeds are processed. Typical processing involves feeding seed flakes to a series of screw presses to receive a mild pressing operation (3 to 4 MPa) that reduces the oil content below 20% (Bockisch 1998, p. 380-384). Thus, this step removes most of the oil while avoiding excessive pressure, power consumption, solvent use (as in solvent extraction), as well as temperature extremes. Some presses or "extruders" can be used to consolidate tiny flakes into large fragments for good solvent percolation during solvent extraction (Carr 1997). These presses have been designed to imitate an extrusion process, ultimately reducing the residual oil content in the cake (by 2%) while saving energy by the omission of pretreatment steps such as crushing, flaking, and

conditioning. However, power demand for these modified systems is approximately twice that of conventional presses (Bockisch 1998, p. 384-385).

2.2.3.5 Advantages and disadvantages of pressing Several advantages and disadvantages of batch and continuous pressing have been recognized. Batch systems with smaller capacities have socio-economic significance in that they are viewed as a valuable addition to cottage level operations and provide gainful employment (Singh and Bargale 2000). However, intensive labor demands and low oil yields render these presses as nothing more than that. Continuous screw pressing permits higher oil yields through substantially higher pressures than their smaller batch (hydraulic press) counterparts. When used as a pre-extraction operation they are positively viewed as an essential part of most of modern solvent extraction plants (Bockisch 1998, p. 389-390; Carr 1997). However, continuous systems require skilled mechanics for operation.

Efficient screw press operations often rely on the operator's skill, making automation and consistent performance difficult to achieve. In addition, trouble shooting and resetting for different oilseeds can be difficult especially if the operators are inexperienced. Screw presses are also heavy power consumers. Great care must be taken in the design of press configurations and screw assemblies to ensure that no power is wasted. For these reasons, operation and maintenance costs for pressing can be high (Stainsby 1988). Pressing is also ultimately self-defeating. During pressing, oil containing capillaries become narrowed and sheared, eventually sealing off oil flow, placing a limit on the

lowest residual oil content that can be obtained (Johnson 1997). In addition, pressing is not selective (discussed in section 2.2.5.2) and the expelled oil contains high levels of impurities (mostly foots) and must be heated and filtered for edible use (Carr 1997).

Regardless of whether batch or continuous systems are employed, the underlying operational simplicity renders pressing a preferred extraction technology in many technologically challenged areas (Bredeson 1978). In addition, mechanical presses yield a chemical-free, protein rich meal (Singh and Bargale 2000).

2.2.3.6 Research Mechanical pressing of oilseeds has long been studied and extensively evaluated. Research has been conducted on several common and niche oil crops such as soybeans, rapeseed, jojoba, raspberry seed, crambe seed, melon seed, conophor nut, and groundnut as well as several others, with oil contents ranging from 23 to 60% (Bargale et al. 1999; Singh and Bargale 2000; Abu-Arabi et al. 2000; Oomah et al. 2000; Singh et al. 2002a and 2002b; Ajibola et al. 1990; Fasina and Ajibola 1989; and Adeeko and Ajibola 1990). Using a laboratory hydraulic press, Koo (1942) and Khan and Hanna (1984) showed that moisture content, pressure, temperature, and pressing time are the primary factors affecting oil yield from seeds. Oil yields generally increased with increasing temperature, pressure, and pressing time.

Singh et al. (1984) indicated that moisture content had the greatest influence on oil yield using an expression technique. Data for several oilseeds

(cottonseed, rapeseed, peanut, tung nut, sesame seed) showed that an optimum moisture content range (2 to 13% w.b.) exists for achieving maximum oil yield (Dunning 1956; Koo 1942; Bockisch 1998, p. 381). Bockisch (1998, p. 381) stated that residual oil in extracted cake steeply increases for moisture contents <2.5% (w.b.) and is a result of loss in seed elasticity. Sivala et al. (1991) showed a decline in oil recovery (using the hydraulic press method) from rice bran for moisture contents >11% (w.b.). Singh et al. (2002a) showed that oil recovery with a screw press increased with decreasing seed moisture content to a high of 82.5% at 3.5% (w.b.). In another study, oil recovery from a screw press significantly increased from 69 to 80.9% and 67.7 to 78.9% for cooked and uncooked seeds, respectively, as moisture content decreased from 10.1 to 3.7 % (w.b.) (Singh et al. 2002b). Pressing rate was found to decrease from 5.81 to 5.17 kg/h and 6.09 to 5.19 kg/h for cooked and uncooked seeds, respectively, as the moisture content also decreased from 10.1 to 3.7 % (w.b.). In addition, a decrease in moisture content, increased sediment content in extracted oil from 0.9 to 7.8% and 1.1 to 5.4% (dry-solids mass per mass of unfiltered oil), for cooked and uncooked seeds, respectively.

Dedio and Dorrell (1977) extracted oil from whole flax seeds using a hydraulic press. Preliminary studies showed that pressures and pressing times of >88.5 MPa and 3 min, respectively, only slightly increased the amount of oil extracted. In a study with cottonseed, Hickox (1953) reported that additional cooking time and/or an increase in temperature above 15 min and 105°C, respectively, had little effect on the amount of residual cake oil using a hydraulic

press (provided that the temperature of the meats was raised to a point where the breaking down of cell walls occurred) (Hickox 1953). Singh et al. (2002a) showed that oil recovery increased with increasing cooking temperature and time to a maximum of 75.9% at 100°C and 12 min, versus 70.9% for uncooked seed and a low of 70.6% at 120°C and 20 min. Extrusion cooking prior to screw pressing provided a convenient method for heating and ultimately tissue disruption in a single step operation. In addition, 90% of the available oil could be recovered from screw pressed, extruded soy samples (Bargale et al. 1999).

Pressing (with no preparation of the oil-bearing material) of cold, whole seeds (sunflower and rapeseed) using small cottage-level units produced cakes having 13 to 18% residual oil (Ward 1984). Particle size has also been known to affect oil recovery. High extraction yields were noted from coarsely ground sunflower seed (particle size of 0.8814 mm) over whole, dehulled, or finely ground samples pressed in a barrel and plunger system (Singh et al. 1984).

Hutchens (1949), Bernardini (1976), and Bredeson (1978) suggest pre-pressing as an effective preliminary extraction procedure for seeds with intermediate to high oil contents such as flaxseed, sesame seed, and peanuts. Bredeson (1983) stated that a pre-pressing operation for soybeans can increase the capacity of a given solvent extraction plant from 50 to 100% because of the increased density and porosity of extruded material. In a pilot-scale pre-pressing operation, Carlson et al. (1993) subjected conditioned cuphea seed (approximately 79 to 93°C and 2.9% w.b.) with a pressure in the range of 4.83 to 5.87 MPa. Reducing the material flow rate from 75 to 65 and then to 22 kg/h of

seed (slowest speed), yielded cake with residual oil contents of 17.1, 13.8, and 8.5% (d.b.), respectively. Nearly 20% of the oil was recovered using a pre-pressing operation (Carlson et al. 1993).

Wang and Johnson (2001) investigated pressing and solvent extraction (hexane) for soybeans and found that the extracted oils were significantly different in that the pressed oils contained less tocopherols and were more oxidized during subsequent refining. Oomah et al. (2000) also found lower tocopherol contents for cold pressed oils, versus hexane extraction. Although the reason for this difference was unclear, it was stated that the presence of non-lipid material in the cold pressed oil might have contributed to the dilution of the concentration of tocopherols.

2.2.3.7 Sea buckthorn applications Research on the recovery of oils from sea buckthorn using pressing technologies is virtually non-existent. However, Yang and Kallio (2002a) suggested that pressing is not a suitable method of isolating oil from the seeds due to low yield and high price of the raw material.

2.2.4 Solvent extraction

2.2.4.1 History and applications Solvent extraction is the process of separating liquid from a solid-liquid system using a solvent (Gurnham and Masson 1946). Plant oils can be separated from proteins and carbohydrates using a solvent. Hexane has commercially been used as the solvent of choice, however, other solvents such as ethanol, isopropanol, and methylene chloride have shown great promise as alternative solvents (Johnson and Lusas 1983).

Solvent extraction is generally employed when a residual oil content of <2% is desired (Bockisch 1998, p. 389). As a rule of thumb, materials with low oil contents (<20%) such as soybean, cottonseed, grapeseed, and rice bran are subject to batch or continuous solvent-extraction processes. Materials containing higher oil contents (>20%) such as canola, peanuts, and sunflower seed are usually processed in two-stages, beginning with initial pre-pressing operations followed by solvent extraction (Bernardini 1976). Although solvent extraction can be directly applied to higher oil content materials, it is usually more economical to first reduce the oil content below 20% using a pre-pressing operation (Bockisch 1998, p. 389).

2.2.4.2 Theory Solvent extraction is explained by dissolution theory and is based on the laws of thermodynamics. Dissolution during extraction, involves two endothermic and one exothermic processes. Energy is first required to break the solute (oil) into isolated molecules. The amount of energy is small when the solute molecules are non-polar. In addition, energy is also required to break

solvent intermolecular bonds to accommodate the solute. Finally, an exothermic reaction results from solute-solvent interaction causing solution equilibrium. Thus, solvent extraction is a chemical process aimed to recover oil using a solvent (Johnson and Lusas 1983).

The dissolution of materials can be predicted by the nature of the solute and solvent. In principle, "like dissolves like". Non-polar solutes are generally more soluble in non-polar solvents (Johnson and Lusas 1983). Thus, edible oils which are non-polar, are readily solubilized by non-polar solvents such as hexane and petroleum ether. In contrast, it is well known that water a polar molecule does not dissolve oil.

Fundamentally, solvent extraction involves introducing an oil-bearing material into a bath of solvent (Kemper 1997). The solvent is then allowed to soak into the structure of the material to bind with the oil. Upon concentration of the oil in the solvent, forming a solution referred to as miscella, the oil migrates out of the material into the solvent bath. This process continues until the oil and solvent come into equilibrium within the particle and the surrounding miscella.

Solvent extraction is carried out by either an immersion or percolation process (Bockisch 1998, p. 399-400). These processes are capable of processing anywhere from 100 to 4000 t/d of seed (Bockisch 1998, p. 412-413). Immersion involves dipping the oil-bearing material into a solvent (Fig. 2.7). Frequent agitation, often carried out using a screw conveyor, is required to ensure that locally concentrated solvent (containing oil) is replaced. However, agitation may cause a reduction in the material particle size which can later

cause an increase in oil refining costs as the fine particles have to be removed from the miscella. The immersion process is usually conducted on materials with high fiber content and low oil concentration. Also, immersion is ideally suited for materials which are difficult to prepare or for materials that are in a comminuted form (fine particles or powder). Percolation involves the permanent wetting of the oil-bearing material with a flowing solvent (usually under the influence of gravity). This process ensures that locally saturated solvent is continuously replaced with new, oil free solvent. An advantage of percolation is that the oil-bearing material does not require agitation. However, powdered materials cannot be handled in percolation extractors since the fines can hinder solvent flow. Pelletizing of the fines is sometimes practiced, though is often ineffective and very expensive (Bernardini 1976).

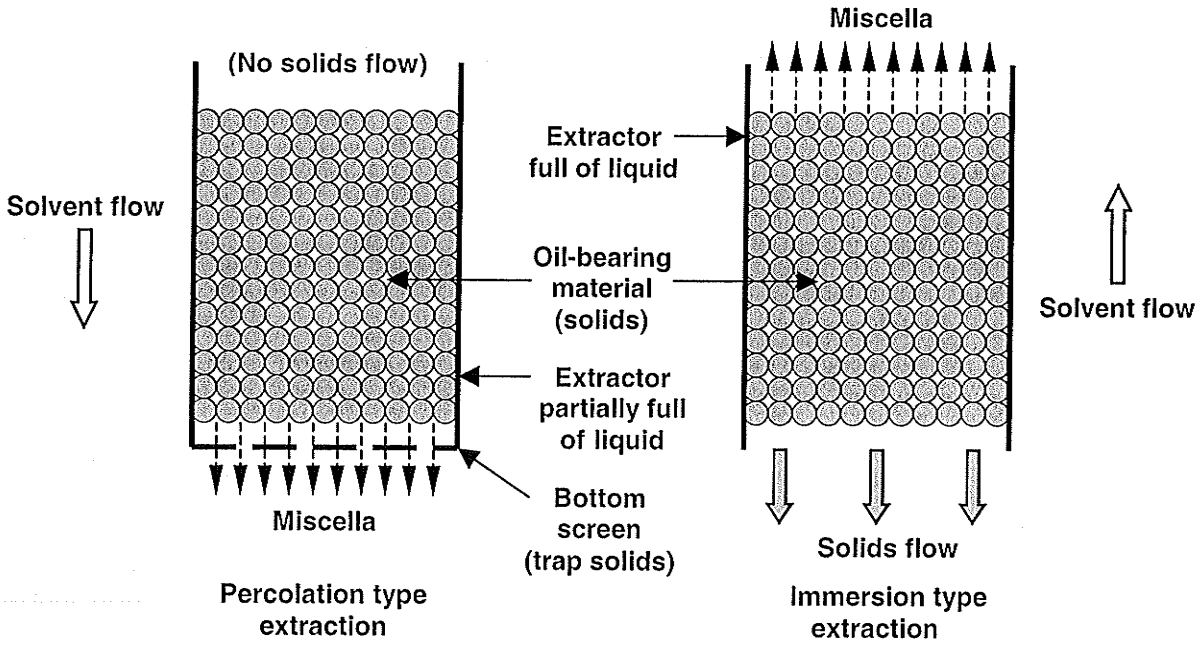


Fig. 2.7. A schematic drawing of solvent flow in a percolation versus immersion extractor (adapted from Milligan 1976).

As previously discussed, seeds are prepared for solvent extraction combining processing such as cracking, heating, and flaking or by conditioning, pre-pressing (screw), grinding, and flaking. Although these processes are designed to distort and rupture oil-containing cells to ensure maximum oil yield, they play an important role in allowing rapid percolation (or immersion) and draining of solvent (Bredeson 1983). Factors such as uniformity of particle size, presence of whole seeds or large particles of uncrushed seed, oil and moisture content of the cake, and cake density and porosity all influence the flow of solvent through the meal (Ward 1984).

After extraction, the oil is recovered from the miscella by distillation. The miscella and extracted meal are carefully desolventized to yield an edible grade oil, suitable feed material, and reuseable solvent. Although solvent recovery is important from an economic standpoint, health concerns raised from the use of solvents warrant extracted oils and meal to be free of toxic chemicals (Myers 1983). The percolation and immersion miscella contains considerable amounts of solvent, approximately 70 to 80% and 87 to 93%, respectively. Thus, distillation is an energy intensive process (Bockisch 1998, p. 420).

Refining of solvent extracted oils is a major consideration. Some oils such as those extracted from soybean and canola contain 2 to 3% "gums" (non-fatty materials) which are mainly phospholipids (phosphatides). Gums can settle out in storage tanks, causing high refining losses and therefore must be removed. Degumming is a process which converts phosphatides to hydrated gums, which are insoluble in water and can be separated by centrifugation (Carr 1997).

2.2.4.3 Solvent selection Many solvents are available for extracting oils from plant materials, however some are more suitable than others. Several points should be considered when selecting a solvent (Johnson and Lusas 1983):

i) The ability of a solvent to be used in existing equipment with low retrofitting costs and operating profitability;

ii) The use of high power solvents effective at low operating temperatures especially for the recovery heat-sensitive components, such as oils;

iii) Solvents must be non-toxic to plant workers and consumers (humans and animals) when the oil or meal is used as food or feed;

iv) The selectivity of the solvent. It is desirable to use a solvent that extracts only triglycerides (targeted oil), devoid of undesirable components such as phosphatides, free fatty acids (FFAs), waxes, and pigments. When selectivity is low, refining costs can be substantial;

v) Non-flammability to reduce the hazard potential of fires and explosions;

vi) The solvents should be stable to heat, light, and water, and should not react with the oil, meal, or equipment as these reactions can cause toxicity problems;

vii) High purity solvents should be used to ensure uniform operation and extractions;

viii) Low solubility of water with the solvent is desired since steam is commonly employed to strip trace solvent residuals from the oil and meal. In addition, if solubility is low separation of water from the solvent can be enhanced;

ix) Volatility of the solvent should not be too high as it can contributed to extensive evaporative losses;

x) And, the solvent must be economically available in adequate supply.

Several factors influence solvent extraction efficiency. Bockisch (1998, p. 400-406) summarized some of the major factors contributing to oil recovery, such as the extraction temperature, extraction time, particle (oil-bearing material) moisture content, and the particle size. A higher temperature is essential for extraction because it can decrease viscosity and increase solubility of the oil. The addition of heat can increase oil recovery of olive husk from 78% at 20°C to almost 90% at 50°C. Extraction time is also important because the dissolution of oil and diffusion of solvent is not instantaneous. Since many solvent extractors have no mechanical means of making oil available, time is required to allow the solvent to leach the oil or allow the oil to bind to the solvent (Christensen 1983). At lower oil concentrations, longer residence time (solvent to particle contact) is required (Tranchino and Melle 1984). As previously stated, conditioning is used to adjust the seed moisture content. High moisture contents (water being polar) hinder wetting of the particles and penetration of the solvent. Low moisture contents induce fine crumbling of the particles, inhibiting solvent percolation. Karlovic et al. (1992) showed that oil recoveries doubled when the moisture content of corn germ decreased from 12 to 8% (w.b.). Finally, particle size influences the success of extraction. The particles must allow for adequate solvent percolation while minimizing solvent diffusion. The reduction of particle size increases surface area, decreases solvent penetration path lengths,

ultimately resulting in increased oil transfer rates into the solvent (Nieh and Snyder 1991a). Others have found that solvent grinding, a method whereby solvent is added to seeds during grinding, improves overall extraction efficiency (Diosady et al. 1983).

2.2.4.4 Advantages and disadvantages of solvent extraction Although solvent extraction has been used for producing mass quantities of oil, several disadvantages have been addressed (Anand et al. 2000). Solvent extraction typically requires the use of toxic, expensive, and harmful chemicals (hexane), which can be left behind in the extracted oil. In addition, high processing temperatures can lead to degradation of the extracted oil. Separation of the oil and recovery of the solvent by distillation is an energy intensive process and extensive refining (degumming to remove gums) is often required to produce marketable end-products. Finally, solvent extraction, like pressing, is not selective.

2.2.4.5 Research Literature has shown that solvent extraction has been widely recognized in industry as an efficient and economical technology for recovering oils from oilseeds. Some traditional oilseeds that have been processed using this technology are soybeans, cottonseed, sunflower seed, groundnuts, palmkernels, canola, and crambe seed (Hutchins 1976; Stein and Glaser 1976; Carlson et al. 1985). In addition, this technology has been extensively used in the research sector for analytically determining oil content in seeds (Taylor et al.

1993). Several analytical methods have been reported, however the most common ones are based on a Soxhlet extraction (percolation of solvent through a bed of oil-bearing material) of dried samples using petroleum ether, hexane, or diethyl ether as the extraction solvent (Sahasrabudhe and Smallbone 1983; Brühl and Matthäus 1999; Tulbentçi 1986; Lyon and Becker 1987). This method is preferred because of its simplicity and few non-lipid components are extracted. Other advantages include complete elimination of emulsion problems and reduced time for oil content determination (a range of 16 to 22 h for Soxhlet extraction down to 4 h for Goldfisch extractor, a modified Soxhlet extraction system) (Cross 1990; Lyon and Becker 1987; Labconco Corporation 1997).

Hexane has long been used as the solvent of choice for extracting oil from oilseeds. However, due to recent health and environmental concerns, hexane has been experiencing increased government scrutiny (Conkerton et al. 1995). Other extraction solvents such as heptane and isohexane have shown promise and are likely replacements for hexane. Wan et al. (1995a) indicated that although 100% of oil was recovered from cottonseed using hexane at 55°C, heptane was competitive in recovering 95.9% of the oil under similar operating conditions. Extraction of cottonseed oil using isohexane increased daily throughputs by 20% while plant energy savings increased by 40%, as compared to hexane (Wan et al. 1995b). In addition, Abu-Arabi et al. (2000) noted that petroleum ether was economically attractive since it was comparatively cheaper ($16/L) than hexane ($36/L) for extracting jojoba oil. Other solvents such as

ethanol and isopropanol have been explored, however, neither is presently economically feasible as a suitable replacement (Wan et al. 1995a).

It should be stated that different solvents have different solvent capacities, dictating the amount of non-fatty materials that are extracted with the oil (Attah and Ibemesi 1990). Unlike hexane, ethanol has shown to be effective in removing toxic compounds such as gossypol (a toxic compound to monogastric animals) and aflatoxin (a toxin produced from the mold of *Aspergillus flavus* L.) from oil-bearing materials (Abraham et al. 1993, Johnson et al. 1986; Hron et al. 1992 and 1994). In addition, ethanol extraction was used to produce stabilized brown rice products yielding oil of good quality (Champagne et al. 1992). Ethanol extraction can remove FFAs and lipid oxidation products, thus eliminating off-odours and off-flavours. Aqueous ethanol has the tendency to extract additional non-triglyceride materials such as phospholipids from the soybean flour meal (Nieh and Snyder 1991b). In addition to the extraction of non-oil components, Kwiatkowski (2002) found that water was also extracted from fresh corn. During oil extraction, Abraham et al. (1993) and Baker and Sullivan (1983) stated that for moisture contents >3%, ethanol extracts moisture from cottonseed and soy flakes, respectively. This in turn, decreases oil solubility in the solvent and decreases overall oil extraction efficiency (Abraham et al. 1993).

2.2.4.6 Sea buckthorn applications Information on solvent extraction of sea buckthorn oils is scarce. Soxhlet extraction with petroleum ether was reported as an analytical method for the determination of oil content in sea buckthorn berries (Berezhnaya et al. 1989). Mamedov et al. (1981) reported an oil content range 17.0 to 21.8% (w/w) of air dried berries using petroleum ether. Oil content of dried pulp residues (dried at 50 to 60°C) after juice extraction (by pressing) was found to be 22.6% (w/w) also using petroleum ether (Aslanov and Novruzov 1976). In many cases, the petroleum ether was distilled off in a rotary evaporator with a water bath temperature of 40 to 50°C (Mamedov et al. 1981). Yang and Kallio (2002a) indicated that solvent extraction is not a suitable method mainly because harmful solvent residues can be left behind in the extracted oil and adds to environmental pollution.

2.2.5 Supercritical fluid extraction (SCFE)

2.2.5.1 History and applications Supercritical fluid extraction (SCFE or sometime abbreviated as SFE) technology is a separation process which utilizes the properties of supercritical fluids (SCF) to extract valuable compounds or remove impurities from raw materials. Conventional applications include the extraction of caffeine from coffee, nicotine from tobacco, and essential flavours and aromas from hops, fruits, and spices (Johnson 1997).

The major components of a SCFE system include a pressure-rated extraction vessel, a pressure reduction valve, a separator, and a compressor or pump. Extractions are carried out in the extraction vessel whereby a solvent, while in contact with a solute matrix (oil-bearing material), is compressed and maintained at a high pressure. A reduction in the system pressure causes the dissolved solute (oil) to precipitate and be separated from the solvent (McHugh and Krukonis 1986, p. 98-99). The decompressed solvent is rerouted back to the compressor for reuse. Figure 2.8 shows the basic stages involved in a typical SCFE system.

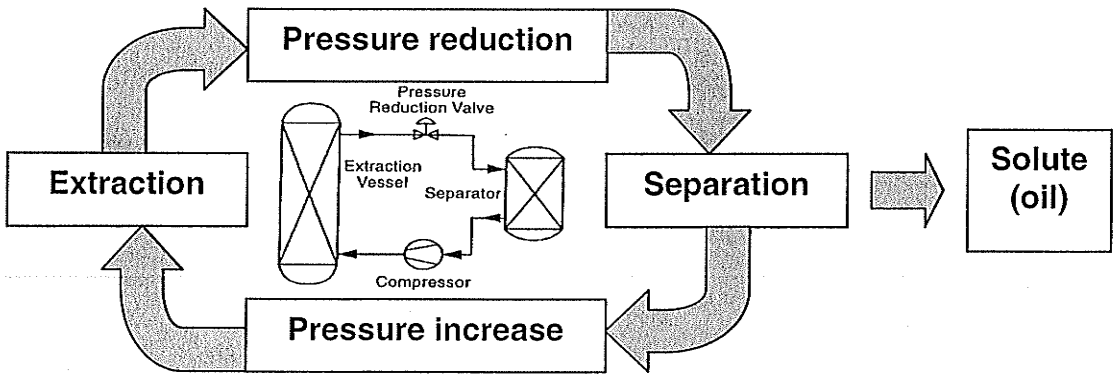


Fig. 2.8. Process stages of a typical SCFE system (adapted from King 1997 and McHugh and Krukonis 1986, p.98).

2.2.5.2 Theory Supercritical fluid extraction is similar to conventional solvent extraction in that oil can be "washed" from oil-bearing materials using a solvent. However, SCFE differs from conventional solvent extraction in that the solvent is a fluid above its critical point (Bulley et al. 1984). The critical point is defined by the critical pressure (P_c) and temperature (T_c) at which, a single substance is no longer in its liquid or gas state. At this point, the supercritical state is achieved and the substance takes on supercritical properties. Figure 2.9 shows a phase diagram and supercritical region for a single substance.

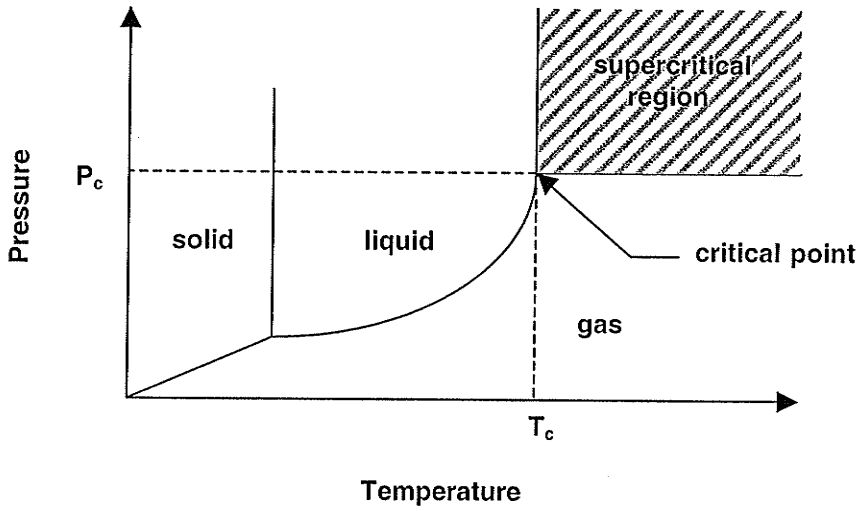


Fig. 2.9. A phase diagram showing the supercritical region for a single substance (adapted from Sihvonen et al. (1999)).

When a substance has been compressed and heated above its critical point, the resulting fluid can solubilize substantial quantities of oil that would otherwise be insoluble in the same substance if it were in its ordinary liquid or gas state (Johnson 1997). Table 2.7 shows a list of critical temperatures and pressures for some fluids used in SCFE applications (Mchugh and Krukonis 1986, p. 4).

Table 2.4. Critical points of some fluids used in SCFE applications (Mchugh and Krukonis 1986, p. 4).

Fluid	Critical Temperature (°C)	Critical Pressure (MPa)
Ethylene	9.3	5.0
Carbon dioxide	31.1	7.4
Ethane	32.2	4.9
Propane	96.7	4.2
Ammonia	132.5	11.3
Benzene	289.0	4.9
Toluene	318.6	4.1
Water	374.2	22.0

Density, diffusivity, and viscosity are the major physiochemical properties contributing to extraction efficiency of SCFs. Supercritical fluids have the densities of their liquids forms, but the diffusivites and viscosities of their gas forms (Rizvi et al. 1986) (Table 2.4).

Table 2.5. Physiochemcial properties of various fluids at different states (Rizvi et al. 1986).

State of fluid	Density (g/cm^3)	Diffusivity (cm^2/s)	Viscosity ($g/cm \cdot s$)
Gas			
P = 0.1013 MPa, T = 15-30°C	$(0.6 - 2) \times 10^{-3}$	0.1 - 0.4	$(1 - 3) \times 10^{-4}$
Liquid			
P = 0.1013 MPa, T = 15-30°C	0.6 - 1.6	$(0.2 - 2) \times 10^{-5}$	$(0.2 - 3) \times 10^{-2}$
Supercritical			
$P = P_c$, $T = T_c$	0.2 - 0.5	0.7×10^{-3}	$(1 - 3) \times 10^{-4}$
$P = 4P_c$, $T = 4T_c$	0.4 - 0.9	0.2×10^{-3}	$(3 - 9) \times 10^{-4}$

The solvating power of a SCF can be related to its density in the region near the critical point and is pressure dependent. Small increases in pressure above the critical point, greatly increases the density of a SCF and can be correlated to its solvating power (McHugh and Krukonis 1986, p. 3-7). Thus, when a fluid is in a state of high compression, it takes on a correspondingly high density, similar to that of liquid solvents (Table 2.4). Having a liquid-like density, SCFs have the capability of solubilizing a variety of materials just as liquids (King 1997). The solvating power of a SCF also increases with increasing temperature, though at much higher pressures. Unlike liquid solvents, SCFs have diffusivities closer to gases, facilitating rapid mass transfer of solutes (oil) from a solute matrix (oil-bearing material). In addition, gas-like viscosities can be achieved which provide appreciable penetrating power of the SCF into a solute matrix, also increasing extraction efficiency (Rizvi et al. 1986).

Altering density, similar to changing liquid solvents in conventional extraction, changes the selectivity of a SCF. Selectivity refers to the ability of a solvent to remove only desirable components from a solute matrix. Thus, if oil is the target material, it is desirable to extract only triglycerides and leave undesirable components such as phosphatides, FFAs, waxes, and pigments in the meal. The density of a SCF is pressure dependent, thus by changing pressure, selective extractions can be obtained (Fig. 2.10). An extraction condition of 60°C/30 MPa yields oil comprising essential oils, oil esters, free fatty acids, other fatty oils, waxes/resins, and pigments (all components to the left of the temperature and pressure line) while an extraction condition of 60°C/10 MPa

yields only essential oils. When selectivity is low, inherent in pressing operations and often the case with conventional solvent extraction (the use of a single solvent incapable of property alterations), the cost of further refining is high (Johnson 1997).

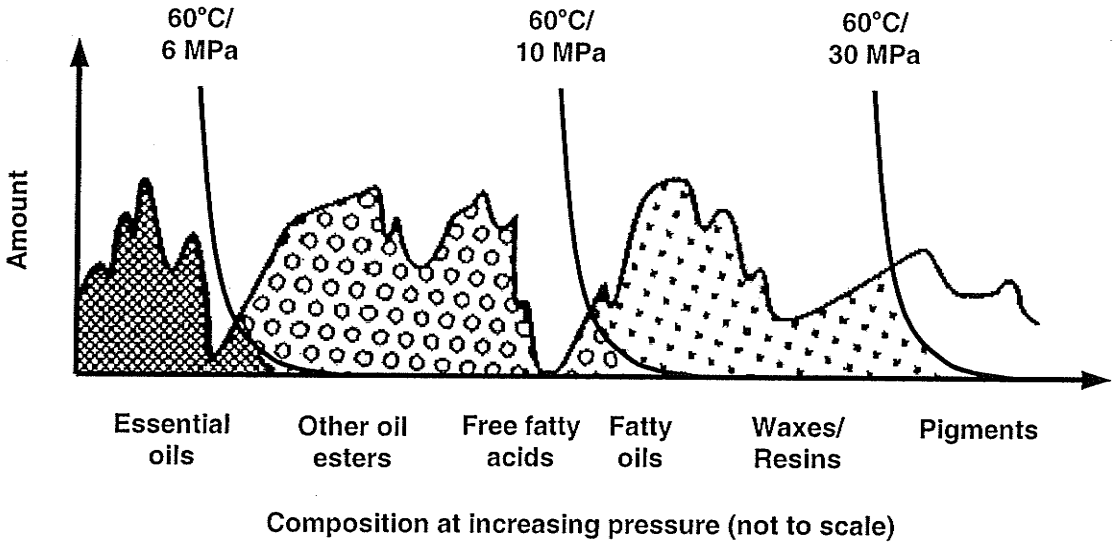


Fig. 2.10. Cummulative composition and amounts of oil-containing components recovered by SCFE CO_2 at increasing pressures (adapted from Brogle 1982).

Entrainers and particle size manipulation have also been shown to affect extraction efficiency. The solvating power of a supercritical fluid can be increased with the addition of entrainers, also called co-solvents or modifiers. Ethanol is a common entrainer used in SCFE applications (Montanari et al. 1999). Entrainers can decrease extraction times and increase selectivity and yields, while using milder extracting conditions (lower pressures and temperatures). However, the incorporation of entrainers leads to increased capital costs (Sihvonen et al. 1999) associated with additional equipment

requirements. Efficiency of extraction also depends on the contact time between the supercritical fluid and oil-bearing particles (Bulley et al. 1984). Particle size reduction by milling or grinding, increases oil extraction yields by increasing the surface area-to-volume ratio, thereby increasing the contact area of the supercritical fluid with the oil-bearing material (del Valle and Uquiche 2002).

2.2.5.3 Advantages and disadvantages of SCFE Several advantages of the SCFE process have been summarized by Anand et al. (2000). First, the ability to use non-toxic solvents makes SCFE the technology of choice for the food industry. Low operating temperatures prevent quality degradation of thermally sensitive bio-based materials. In addition, an oxygen-free environment maintained during extraction prevents the extracted material from becoming oxidized (Chen and Ling 2000). With minor changes in pressure and/or temperature, solvents can be easily and economically recovered making SCFE energy efficient. Unlike pressing or conventional solvent extraction, selectivity can be controlled for targeted extractions. High purity products can be achieved with little refining. As an environmentally friendly process, SCFE is free of waste disposal hazards. Given less labour, time, solvent use, space, and therefore operation costs required for extractions, SCFE offers a the potential to be competitive with conventional solvent extraction operations in certain applications (Johnson 1997). Manninen et al. (1997) indicated that the high pressures (30 MPa) associated with SCFE process can be used to manufacture edible oil

products free of microorganisms and spores, another major advantage especially for industries controlled by strict regulations and guidelines in food processing.

A disadvantage limiting the use of SCFE is its high capital cost. Expensive high-pressure equipment (estimate in 1987: $100,000 for pilot scale; >$1,000,000 for commercial systems) is the major deterrent for commercial use (Friedrich and Pryde 1984; Swientek 1987; Sihvonen et al. 1999). In addition, much of the research with SCFE has been kept proprietary and discrete, hindering its acceptance in the food processing industry (Swientek 1987). In addition, most SCFE systems are small-scale batch operations. A major question in SCFE is how to continuously feed and discharge (oil and extracted meal) while operating at high pressures. This problem challenges whether SCFE systems are feasible for scale-up extractions to be used as a competitive technology for oil extraction (Johnson 1997).

2.2.5.4 Research Although the theory of the supercritical state has been know for more than 150 yr, its application in extraction applications has only been recently explored over the last three decades (Sihvonen et al. 1999). Even more recently, the application of SCFE technology has only just begun to be explored in the area of oil seeds and other plant-based materials (Johnson 1997). The motivation for the development of SCFE technology has been a result of increased energy costs for traditional extraction technologies, increased government scrutiny and regulations for industrial solvents (hexane), stringent pollution-control legislation, demand for improved waste disposal practices, and

increased performance demand and returns from materials which traditional processing techniques may not meet (McHugh and Krukonis 1986, p. 1-2).

Supercritical fluid extraction technology has been broadly applied (Anand et al. 2000). However, recent research activities have been concentrated in the food and agriculture sector on the extraction and analysis of pesticides and lipids (oils and fats) (Rozzi and Singh 2002). Recently, SCFE has become an important extraction technology because of the requirements of the medical and food industries for ultra-pure and natural products. In addition, an increased worldwide interest in eastern medicines has led to the demand for "naturally" prepared products (Chen and Ling 2000). Thus, an increasing number of industries have become interested in supercritical techniques (Sihvonen et al. 1999).

Many compounds have been used in supercritical processes, however carbon dioxide (CO_2) is the most widely used extraction solvent (Sihvonen et al. 1999). Viewed positively by the pharmaceutical, nutraceutical, and food industries, CO_2, a non-polar molecule, is abundant, inexpensive, renewable, non-toxic, non-corrosive, non-flammable, non-explosive, environmentally friendly, and has a low critical temperature which makes it suitable for extracting thermally sensitive compounds, such as edible oils (Sihvonen et al. 1999; Rozzi and Singh 2002, Chiu et al. 2002).

Numerous publications exist on the use of SCFE CO_2 for the extraction of edible oils and other components from plant materials. Extracted oils include pecan oil (Maness et al. 1995), olive husk oil (de Lucas et al. 2002), walnut oil

(Oliveira et al. 2002), flaxseed oil (Bozan and Temelli 2002), millet bran oil (Devittori et al. 2000), rice bran oil (Kuk and Dowd 1998; Xu and Godber 2000); celery seed oil (Papamichail et al. 2000), grape seed oil (Gómez et al. 1996; Lee et al. 2000), cloudberry seed oil (Manninen et al. 1997), *Nigella damascena* L. or "Love in a Mist" seed oil (Daukšas et al. 2002), *Dimorphotheca pluvialis* L. or "Munch" seed oil (Muuse et al. 1994), perilla seed oil (Kim et al. 1996; Kim et al. 1998); nutmeg seed oil (Spricigo et al. 1999); peanut oil (Goodrum and Kilgo 1987), pistachio nut oil (Palazoğlu and Balaban 1998), oil from soy flakes, canola seed, and corn germ (Taylor et al. 1993), spearmint oil (Platin et al.1994), cotton seed oil (List et al. 1984; Kuk and Hron 1994), soybean oil (Mangold 1983; Friedrich and Pryde 1984; Montanari et al. 1999), sunflower seed oil (Kiriamiti et al. 2001), rosehip seed oil (del Valle and Uquiche 2002; Szentmihályi et al. 2002), black pepper oil (Ferreira and Meireles 2002), oil from wheat germ and borage seed (Gómez and de la Ossa 2000 and 2002), and ginseng root hair oil (Wang et al. 2001). Other plant components such as extracts from sage (Djarmati et al. 1991), flavonoids from ginkgo leaves (Chiu et al. 2002), and lycopene from tomatoes (Rozzi et al. 2002) have also been recovered using SCFE CO_2.

Several of these studies have attained oil yield similar or higher than that of solvent extraction employing hexane, petroleum ether or diethyl ether as the extraction solvent (Maness et al. 1995; Gómez et al. 1996; Kuk and Hron 1994; Gómez and de la Ossa 2000 and 2002). Others reported that higher oil yields could be obtained with the use of entrainers, such as ethanol (Daukšas et al.

2002; Chiu et al. 2002; Wang et al. 2001). Pressure had the greatest influenced on oil yield, while temperature had little or no effect (Lee et al. 2000; Daukšas et al. 2002; Palazoğlu and Balaban 1998). In addition, decrease of partical size by milling increased oil yields (Kim et al. 1996; Spricigo et al. 1999; Goodrum and Kilgo 1987). SCFE extracted oils were usually of "refined" quality, thus further refining was often not required (Gómez et al. 1996; List et al. 1984). Oils were reported to be clear with light color, devoid of phosphatides, and have high tocopherol and α-linolenic levels (Kuk and Dowd 1998; Oliveira et al. 2002; Devittori et al. 2000; Montanari et al. 1999; Brühl and Matthäus 1999; Kim et al. 1996; Bozan and Temelli 2002). Ibáñez et al. (2002) indicated that supercritical fluid extraction was effective in isolating and concentrating high value nutraceutical compounds, such as sterols and tocopherols, from low quality olive oil. In addition, SCFE was reported to be a suitable replacement for traditional extractions utilizing organic solvents, in that costs associated with solvent disposal and the exposure of laboratory personnel to toxic and flammable solvents could be eliminated (Friedrich and Pryde 1984).

While the majority of supercritical fluid extraction patents are concentrated on the food, pharmaceutical, and chemical industry, supercritical technology has been applied successfully in other areas such as environmental protection (Sihvonen et al. 1999). In a review of recent research articles between 1999 and 2000, Rozzi and Singh (2002) showed that pesticides (followed by lipids) were the leading analytes extracted using supercritical fluid techniques. Several studies have been conducted to isolate pesticides and other chemical residues

from contaminated fruits, vegetables, soils, biological tissues, and other materials (Motohashi et al. 2000; Norman and Panton 2001; Prados-Rosales et al. 2003; Halvosen et al. 2000; Ghassempour et al. 2002; Tavlarides et al. 2000; Kreuzig et al. 2000; Nerín et al. 2002). Lang and Wai (2001) also indicated that in addition to extracting desired compounds from plants, SCFE has been used to extract pesticides and residue contaminants from natural products.

2.2.5.5 Sea buckthorn applications Supercritical fluid extraction has been utilized in several parts of the world for the recovery of sea buckthorn oils, however limited research is available on the determination of extraction parameters (pressure, temperature, particle size, etc.) used for supplying these oils. In addition, little information is known about the effects the operating parameters have on yield and nutritional quality of sea buckthorn oils. Adding to this information gap, research in this area has been conducted in other countries and in various languages, increasing the level of difficulty for the international sharing of this knowledge.

Štastová et al. (1996) studied the rate of oil extraction using SCFE CO_2 from dried seeds and pulp of three varieties of sea buckthorn. Operating pressures and temperatures were in the ranges of 9.6 to 27 MPa and 25 to 60°C, respectively. Base oil levels were determined by a Soxhlet extraction procedure using hexane. Optimum solubilities were reported for seed (6.5 to 7.4 mg oil/g CO_2 or 0.65 to 0.74%) and pulp (7.9 to 8.6 mg oil/g CO_2 or 0.79 to 0.86%) oils for an operating pressure and temperature of 27 MPa and 40°C,

respectively. No marked change in oil composition was observed during the course of extractions.

Shaftan et al. (1979) showed that oil could be recovered from air-dried sea buckthorn pulp by SCFE CO_2, operating at a pressure and temperature of 5.7 MPa and 20°C (subcritical extraction), respectively. A low yield of CO_2 extract was attained (7.5% w/w). Higher levels of EFAs and tocopherols were found. In addition, Shaftan et al. (1986) reported oil extractions from a dried, ground mixture of sea buckthorn seeds and pulp (cited by Štastová et al. (1996)). Oil yield range was 4 to 8% (w/w) after 3 to 3.5 h, with the same operating pressure and temperature. Others have reported extracting sea buckthorn pulp oil in a pilot-scale system whereby extractions were conducted over a 1.5 h period, with an operating pressure of 30 MPa and temperatures ranging from 40 to 60°C (Manninen et al. 1997). No oil yields were documented. Supercritical fluid extraction has also been used to extract and prepare oils for clinical studies, but again, further report on extraction parameters and oil yields were not stated (Yang et al. 1999; Xing et al. 2002).

FLAVEX Naturextrakte GmbH (Rehlingen, Germany), a company specializing in the extraction of herbal active components, extracted sea buckthorn oil at a pressure of 35 MPa and temperature of 40°C (Štastová et al. 1996). Oil yield was 16.5% (w/w) though failed to state what part (from seeds or pulp) the oil was extracted from. The oils were then analyzed using supercritical fluid chromatography. The extract contained high levels of palmitoleic acid, which may indicate that the oil was extracted from pulp and not

seeds. Another study conducted by FLAVEX indicated seed and pulp oil (whole dried berries) contents of 8 and 12.5%, respectively, extracted at 45°C and 32 MPa. Seed oils comprised high levels of linoleic and linolenic acids, while pulp oils contained higher levels of palmitoleic acid (Quirin and Gerard 1995). An unpleasant odour was noted from the pulp oil, however, because of its stability, it can be deodorized without a loss in quality. Quirin and Gerard (1995) also indicated that the CO_2 extracted pulp oil contained pesticide residues. Though the residues were found to be present in minute amounts, there is a concern that some pesticides can be found everywhere today.

It has been suggested that upon SCFE optimization, aseptic oil and sufficient yield could be obtained (Yang and Kallio 2002). Other benefits include the ability of adjusting the composition of the extracted oils by the careful selection of operating parameters of the extraction process. While SCFE CO_2 is the common method of extraction of sea buckthorn oils, the high cost of the raw material and the high production cost of the SCFE process places a high price ($160 to $300/kg) on the oils, seed oil being more expensive than the pulp oil.

2.2.6 Aqueous extraction

2.2.6.1 History and applications Aqueous extraction (wet extraction) involves the use of water as a processing aid for the physical separation of oil from plant materials (Hagenmaier 1997). Unlike dry processes such as solvent or SCFE technology, aqueous extraction is based on the insolubility rather than the dissolution of oil. Although oil can be extracted from oilseeds using aqueous processes, the extraction of oil from oil fruits such as palms, olives, and coconuts is more common (Hagenmaier 1997; Bockisch 1998, p. 346). Thus, aqueous extraction has been widely used for recovering oils from materials that are wet (high water contents >50%), eliminating an initial drying operation (Hagenmaier 1997). Aqueous extraction predates modern oil-recovery processes like pressing and solvent extraction. One of the oldest examples (over 2000 yr old) is the wet processing of soybeans to make tofu (Hagenmaier 1997).

Unlike oilseeds which can be stored and transported easily, pulp oils must be produced close to the production of the fruit. For this reason, in addition to having a completely different matrix compared with oilseeds, pulp oil production has developed as an independent technology (Hagenmaier 1997). Olive oil production will now be discussed for the purpose of explaining the processes involved with aqueous extraction.

Olives, similar to sea buckthorn, contain oil in the pulp, as well as in the kernel. However, the processing of olives is primarily conducted for the extraction of the pulp oil after the kernels have been removed. Traditionally, the fruit was first crushed in mills and placed into bags or frames for pressing. The

pressed must (oil-containing juice) was allowed to settle under gravity and oil was then skimmed or decanted from the top. Today, high-powered hydraulic presses and centrifugal separators have drastically improved oil processing capacities (Bockisch 1998, p.354).

2.2.6.2 Theory Currently, there are two methods for extracting olive oil, namely pressing and separation via centrifuges. Pressing is done in batches, up to 250 kg/batch over 2 h. Centrifuges offer an advantage over pressing in that it is a continuous operation. In addition, they allow for larger centrifugal accelerations to be reached, greatly increasing the rate of oil recovery over early density or gravity-type separators (Bockisch 1998, p. 353-354). Some centrifuges such as decanters, can extract oil from wet pulp (feed) in one step (Fig. 2.11).

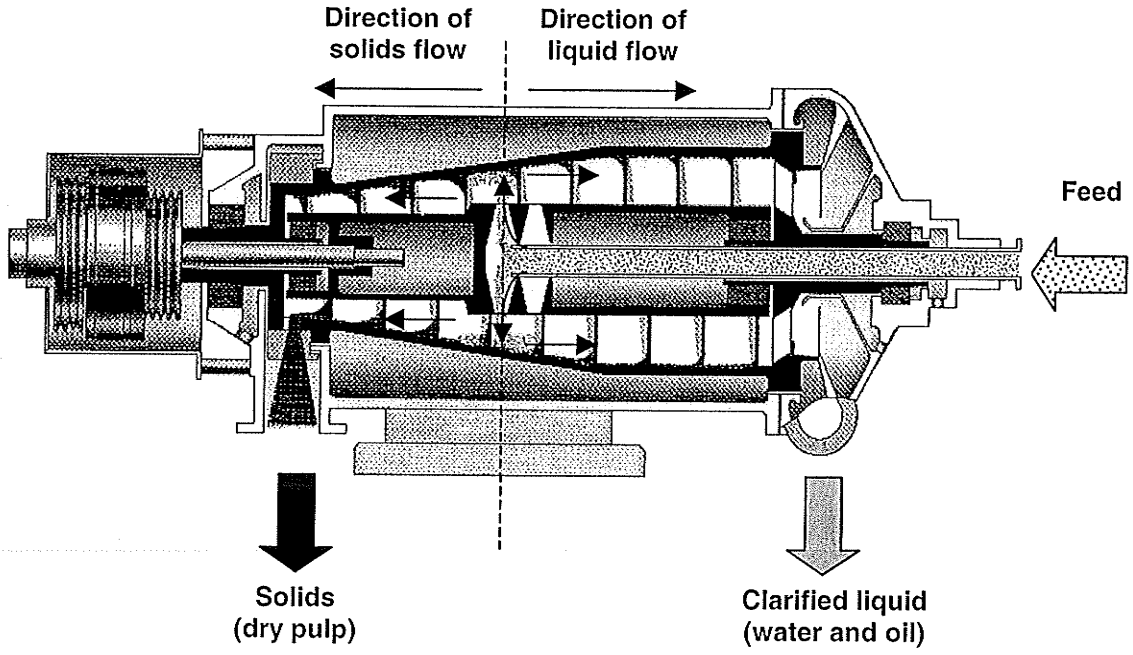


Fig. 2.11. Separation of solid and liquid fractions using a decanter centrifuge (adapted from Bott and Schöttler 1989).

Such machines can directly extract oil without a pre-pressing operation. In addition, some decanters can separate the feed material into three phases including dry pulp, water, and oil. Clarification using a separator centrifuge can be used to polish the oil, removing residual solids and water (Fig. 2.12).

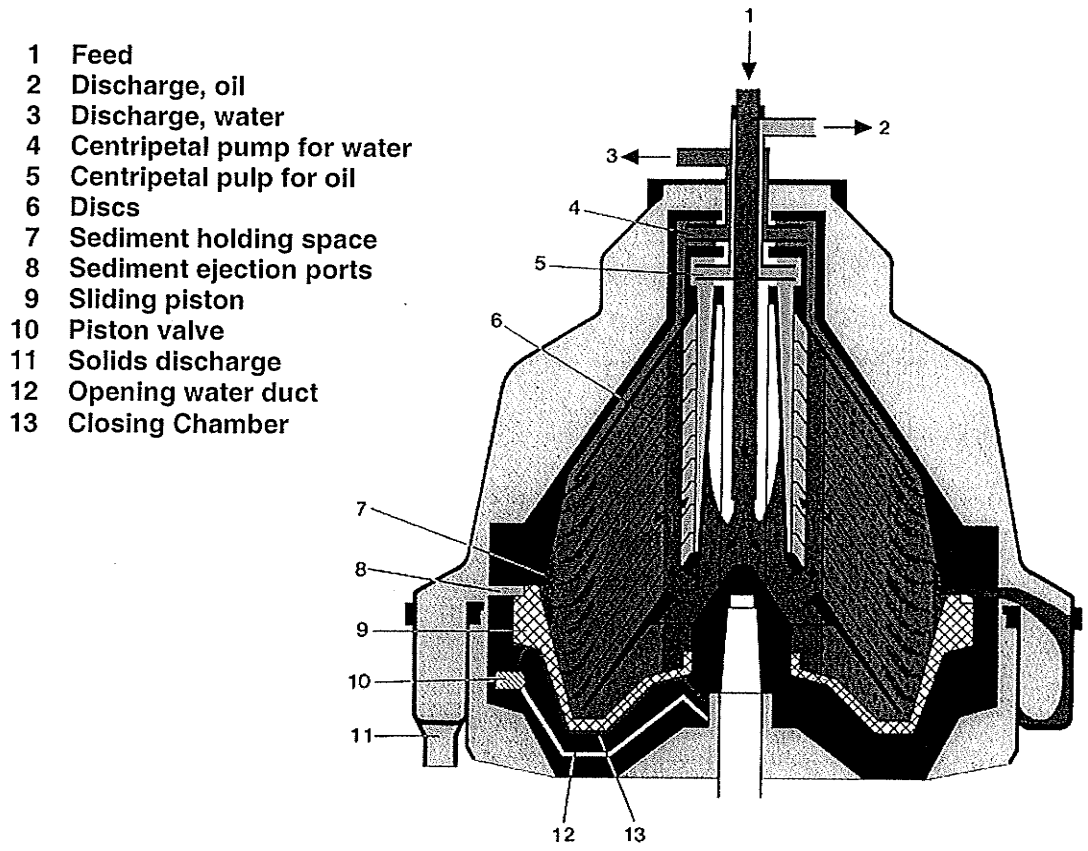


Fig. 2.12. Clarification of oil (removal of water and residual solids) by a centrifugal separator (Bott and Schöttler 1989).

If extraction is done using centrifuges (whereby decanters and separators are used jointly), processing capacities of over 32,500 kg/h of raw material can be achieved (Bockisch 1998, p. 354).

2.2.6.3 Advantages and disadvantages of aqueous extraction Oxidation of oil is a major concern in aqueous processing. Enzymes such as lipases and lipoxygenases which are present in the raw material are released during crushing and cause hydrolysis (of glycerides) and oxidation, respectively. Another disadvantage of aqueous processing is the disposal of the various waste streams, incomplete oil recovery, and the high cost of sanitary equipment required when producing food products. However, several benefits accompany this process. Aqueous processing has long been well suited for small-scale, low-technology operations. Unlike many solvents, water has the advantage of being non-toxic and non-flammable. The aqueous process can be adapted to accommodate many complex bioprocess operations such as fermentation, pasteurization, and the use of enzymes to aid oil recovery (Hagenmaier 1997).

2.2.6.4 FRIOLEX® FRIOLEX® (FResh OIL EXtraction) is a patented oil recovery process which employs solvents to enhance oil recovery. Developed and patented in Germany by Dr. Frische GmbH, this process is currently being exclusively commercialized and marketed by Westfalia Separator AG. FRIOLEX® is an aqueous extraction process involving the physical separation of oil from oil-bearing materials using a decanter centrifuge in conjunction with alcohol (ethanol), a food-grade extraction agent (Schmulgen 2000). Residual impurities present in the decanted raw oil are removed using centrifugal separators. Ethanol is used as an emulsion breaker to enhance oil recovery. Thus, this process is advantageous, since no harmful solvents, such as hexane

are used. FRIOLEX® is suitable for the extraction of high value oils such as those from seeds, nuts, and other sources (Table 2.5). To date, sea buckthorn oils have not been extracted using the FRIOLEX® process.

Table 2.6. Some oil-bearing materials tested using the FRIOLEX® process (adapted from Schmulgen 2000).

Seeds	Nuts	Other sources
Blue poppy	Almond	Apple skin
Borage*	Brazil nut	Castor
Cocoa*	Cashew nut and shell	Cod liver
Canola	Hazel nut	Maize germ*
Coriander	Peanut	Egg yolk*
Crambe	Purging croton	Hops
Euphobia lathyris	Shea butter nut	Jojoba*
Flax*	Walnut	Lupin*
Grape*	Cedar nut	Olive*
Grapefruit		Rice husk*
Hemp*		Salmon
Kiwi		Wheat germ
Gold of pleasure		
Melon seed		
Neem*		
Evening primrose*		
Pumkin*		
Rapeseed*		
Rasberry		
Rosehip*		
High-oleic sunflower*		
Mustard*		
Soybean		

* Materials tested on pilot or industrial scale systems.

The FRIOLEX® process can be broken down into the following unit operations (Fig. 2.13):

i) Grinding or fine disintegration of the raw material;

ii) Addition and mixing of water and alcohol (ethanol);

iii) Reaction time allowed for oil to be extracted from the cells of the raw material and coalesce into larger droplets;

iv) Oil separation using a decanter centrifuge;

v) Oil polishing and oil drying;

vi) And, drying of solids (recovery of water and alcohol).

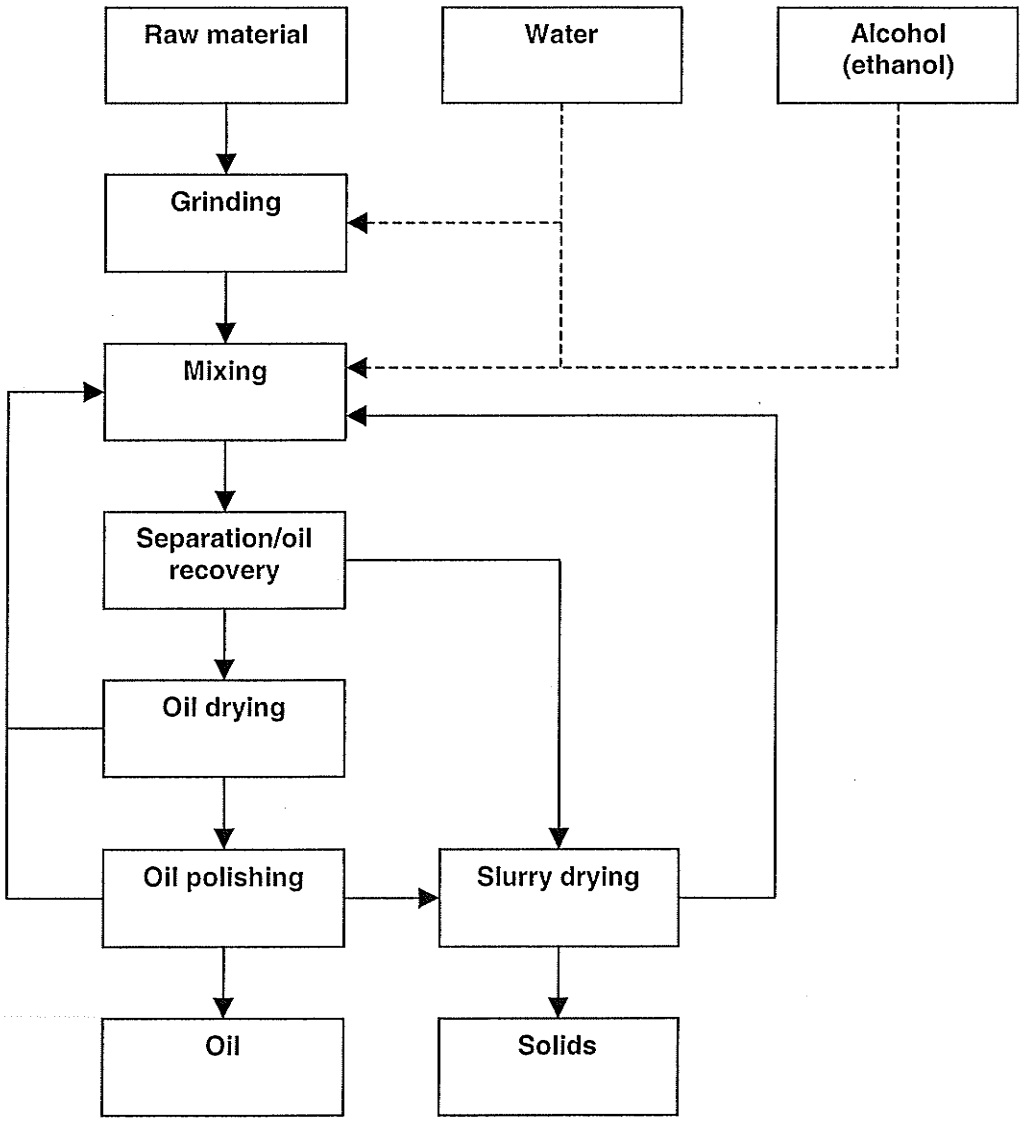


Fig. 2.13. Unit operation flow diagram of the FRIOLEX® process (adapted from Hruschka 2000).

Several advantages of the FRIOLEX® process have been recognized. Most importantly, high oil recoveries can be obtained (Schmulgen 2000). Oil recoveries range from 90 to 99% (w/w) of the oil content, depending on the product. Pilot scale tests showed that oil recovery from hemp was approximately 90% versus 65% for cold pressing. Although yields are higher than cold pressing, yields are not as high as solvent-based processes. FRIOLEX® has been positioned as a process which can achieve oil yields similar to that of conventional solvent extraction systems (hexane), producing an oil having similar quality to that of cold-pressed oils. Figure 2.13 shows the positioning of the FRIOLEX® process in relation to other common oil extraction technologies.

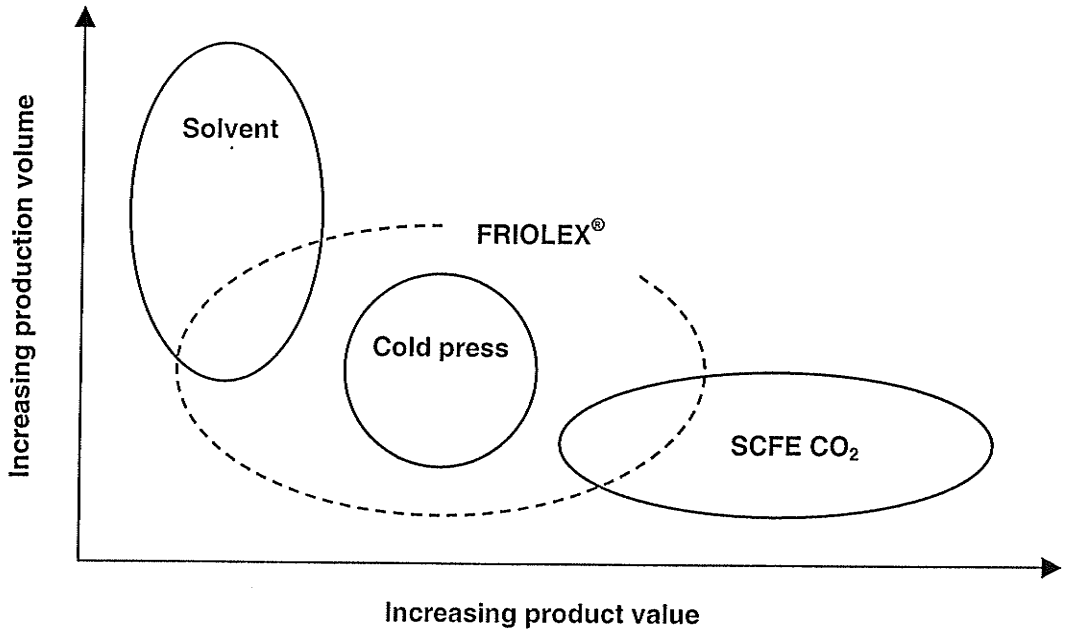


Fig. 2.14. Relative position of the FRIOLEX® process versus other common oil extraction technologies (adapted from Schmulgen 2000).

FRIOLEX® can also be used on oil-bearing materials with low oil contents (>12%) and no pre-drying of the materials is necessary. In addition, processing temperatures are low (45 to 65°C) and no harmful solvents such as hexane are used, making this technology suitable for the extraction of high value oils. Claims have been made, indicating that the oil products are highly stable (oxidation is avoided by nitrogen blanketing during extraction), emulsion free, free of vitamin degradation, and low in phosphatide content. In addition, higher value meal and meal derivatives are possible (Schmulgen 2000). Finally, the FRIOLEX® process is relatively inexpensive for high production throughputs (1000 kg/h of raw material).

Research has shown that the reflux with solvents, in combination with centrifugation improves oil recovery (Sahasrabudhe and Smallbone 1983). Nieh and Snyder (1991b) showed that hexane miscella, when washed with ethanol, enhanced separation of defatted meal during centrifugation. Altering pH has also been shown to improve oil recovery. During centrifugation, Bizimana et al. (1993) reported an oil recovery increase from 65.77 to 71.45% when changing the pH of a water/avocado mixture from 4.0 to 5.5.

2.2.6.5 Sea buckthorn applications Information on centrifugation and decanting methods for the recovery of sea buckthorn oils is not well documented. However, Yang and Kallio (2002a) indicated that centrifugation and decanting are efficient methods for separating oil from the juice fraction of sea buckthorn berries. In addition, Beveridge et al. (1999) suggested that decanter centrifuges

could be used to simultaneously remove suspended solids and oil from the pressed, aqueous phase of sea buckthorn juice.

2.2.7 Enzyme-assisted extraction

Conventional oilseed processing involves flaking, cooking, and crushing to rupture cell walls causing oil to be more readily released during extraction. However, the most efficient way to rupture cell walls at the cellular level is to employ enzymes (Owusu-Ansah 1997).

2.2.7.1 Enzyme-assisted pressing Enzymes are utilized in the pretreatment process prior to oil-pressing. After flaking, enzymes are added which hydrolyze the cell walls of the oil-bearing material causing the cell walls to become more porous. As a result, enzymes have been exploited to improve oil-pressing operations. Temperatures associated with enzyme-assisted pressing are lower than those of conventional pressing operations resulting in oil with better quality characteristics. However, the cost of enzyme production and the long incubation periods during extraction are discouraging limitations (Owusu-Ansah 1997).

2.2.7.2 Enzyme-assisted solvent extraction Enzymes are added to the hydrated, flaked oil-bearing material. After the desired reaction time (determined experimentally), the flakes are then dried to a desired moisture content and the oil is extracted using solvents. The primary advantage of enzymes in solvent extraction is to increase oil recovery yield and reduce the amount of solvent used during extraction. Again, long incubation periods, high cost of enzymes, and additional energy required for drying are some deterrents of this technology (Owusu-Ansah 1997).

2.2.7.3 Enzyme-assisted aqueous extraction This process involves the addition of enzymes to isolate oil from finely ground materials in water. Centrifugation is then employed to separate oil from an aqueous and solid phase. For enzyme-assisted extraction of canola seed oil, the concentration of some undesirable compounds (glucosinolates, tannins, sinapine, and phytic acids) in the extracted meal is reduced. Thus, greater quantities of the meal can be used for feed applications. The primary limitation of this technology is lower oil yields. Commonly, 18 to 25% of the available oil remains bound in the fine proteinaceous part upon final centrifugal clarification of the oil (Owusu-Ansah 1997).

2.2.7.4 Research Several studies have highlighted the use of enzymes in the processing melon seeds, olives, and peanuts (Fullbrook 1983; Neidleman and Geigert 1984; James 1985; Sharma et al. 2002). Sosulski and Sosulski (1993) reported a residual oil content in press cake from enzyme-treated canola seeds of 7.4% compared to a cold-pressed control of 16.8%. Oil quality was inferior to cold-pressed, however better than solvent-extracted oil. Enzymatic pre-treatment of Chilean hazelnut prior to cold pressing decreased residual meal oil by 9.5%. Enzyme assisted processing is costly due to the production of the biocatalysts, limiting this technology application to the production of valuable oils such as those used in cosmetics and pharmaceuticals (Zúñiga et al. 2003). Enzyme-assisted aqueous extraction was carried out on coconut, rice bran, and peanuts (Man et al. 1997; Hanmoungjai et al. 2001; Sharma et al. 2002). These

reports concluded improved oil recovery yields. Beveridge et al. (2002) indicated the use of enzymes during the extraction of juice from sea buckthorn (cv. Indian-Summer) berries. In addition, several companies in Germany have been using enzymes pre-treatments to improve extraction yields of sea buckthorn oils (Utioh 2002).

2.3 Oil Quality

The process used to separate oil from oil-bearing materials has a direct effect on the extractability and quality of oil (Bargale et al. 1999). It is important to avoid deleterious factors such as long processing times, contact with oxygen, high temperature, light, and other oxidation catalysts if high quality oils are to be obtained. In addition, the initial quality of oilseeds should be very high and processing should be continuous and rapid (Ohlson 1976). Processing as related to palm oil extraction comprises a wet rendering process (aqueous processing) involving steps of sterilization, digestion, extraction, clarification, and final purification of the oil. As a result, an oil loss of 5 to 10% (w/w) can be expected with the quality of oil suffering depending on harvesting and processing conditions (George and Arumughan 1992). Thus, processing and extraction conditions play a major role in extraction efficiency and final oil quality.

Oil quality is also affected by the contamination of other oils and foreign material, colour fixation from increased temperatures, increases in FFAs, and oxidation (Burkhalter 1976). The amount of FFAs is a measure of the quality of unrefined and refined oil. If it is too high, inedible uses for the oil should be sought (Cowan 1976). Ohlson (1976) indicated that there was a difference in oil quality and composition when white mustard seed oil was extracted using different solvents. Thus, oil quality and composition can vary depending on the extraction method used.

2.3.1 Research

Several studies have indicated the impacts associated with processing and extraction of fruit and seed oils, on oil quality (Roden and Ullyot 1985; Sarojini et al. 1985; Eng and Tat 1985; Gordon and Rahman 1991; Giovacchino et al. 1994; Chu 1995; Kiritsakis et al. 1998; Oomah and Mazza 1998; Morales and Aparicio 1999). While increased temperatures can increase oil yield, high processing temperatures can cause degradation in oil quality. Yoon et al. (1987) indicated a color change of light yellow to dark brown after rice bran oil and palm oil were heated at 180°C for 50 h. In addition, a higher reduction in polyunsaturated fatty acid (PUFA) content (linoleic acid) was reported compared with monounsaturated fatty acid (MUFA) content (oleic acid) in both oils. Hénon et al. (1997) also showed the degradation of α-linolenic acid though at much harsher conditions (210°C for 86 h). High temperatures during oil processing are uncommon though have been used in deodorization steps conducted under vacuum and nitrogen.

Tocopherols such as vitamin E can limit the availability of oxidants that decompose PUFAs, thus have served to increase the stability of some oils, such as soybean oil (Almonor et al. 1998). Tocopherols are unstable antioxidants which are extracted under mild conditions and for this reason they are good indicators for possible alterations of extracted oils (Brühl and Mathäus 1999) Jung et al. (1989) reported that the refining of soybean oil removed 32% of tocopherols (primarily γ- and δ-tocopherol) indicating that crude unrefined oil was more stable to oxidation than refined oil. Similar results were concluded from a

study conducted by Gordon and Rahman (1991) on coconut oil. However, although tocopherols play a major role in oil stabilization, increasing the levels of tocopherols above those naturally occurring in the crude oil did not guarantee additional stabilization.

Currently, there is a growing concern of processing effects on nutraceutical compounds. Studies have been conducted to determine the damage induced during the processing and extraction of nutraceutical components. Lycopene a nutraceutical compound found in tomatoes, is positively associated with cancer risk reduction (Zanoni et al. 1999). Studies aim to prevent lycopene degradation during tomato storage, processing, and oxygen and high temperature exposure (Zanoni et al. 1999; Lewicki et al. 2002). Carotenoids are other important compounds which have been targeted for measurement during processing because of their antioxidant activity and responsibility for long term stability of oils (Szentmihályi et al. 2002). Isoflavones, phenolic compounds found mainly in soybeans have also been accredited with health promoting functions and a need exists to protect these components during processing (Jackson et al. 2002). Marín et al. (2002) studied the effect of processing on changes in the nutraceutical composition of lemon juices from different extraction systems. Flavonoids in lemon juice, targeted for their functional properties, were found to have varying concentrations depending on the extraction technology employed (Marín et al. 2002).

2.3.2 Sea buckthorn applications

Several parameters have been used to quantify extracted oil quality such as peroxide value, iodine value, moisture content, specific gravity, refractive index, and viscosity. However, sea buckthorn oil quality has been primarily evaluated on nutritional composition such as total carotenoid content and fatty acid composition. The carotenoid concentration depends on the plant variety and growing conditions, as well as the influence of temperature, light, and storage time of the berries. While the majority of biologically active substances in the oils are fatty acids, it has been suggested that the fatty acid composition of sea buckthorn oils might be a useful characteristic for control during processing (Mogilevskaya et al. 1979). In addition, antioxidants such as α-tocopherol (vitamin E) and β-carotene found in sea buckthorn pulp and seed oil are worthy of measurement because of their role in protection against cancer and heart disease (Wang et al. 1996).

2.4 An overview of sea buckthorn seed and pulp preparation

Various laboratory and industrial-scale methods have been utilized for the separation of seeds from pulp of sea buckthorn prior to cultivation or analytical experimentation. Li and Schoeder (1999) reported a laboratory method of seed separation by macerating thawed berries in a household blender. The macerated mixture was diluted with water and poured through a series of screens to collect seeds. Food processors and mortars have also been used to crush thawed berries before pressing to improve juice extraction (Tang et al. 2001; Süleyman et al. 2001). Kallio et al. (2002b) reported another method of separating seeds from pulp by pressing thawed berries. After juice extraction, the press cake (containing seeds and pulp) was then rinsed with distilled water to break the seeds away from the pulp. The seeds and pulp were then dried at room temperature and separated mechanically. It should be noted that in the literature, dried seeds and pulp were often separated mechanically. However, many of the papers failed to indicate and elaborate on the type or technique used for mechanical separation.

Beveridge (1999), Zhang et al. (1989b), and Liu and Liu (1989) outlined potential industrial-scale methods of converting thawed berries to dried seeds and pulp. These methods included unit operations of juice extraction (by conventional rack and cloth pressing at pressures in the range of 3.7 to 5.3 kPa) immediately followed by separation of the press cake into seeds and pulp using a finisher. A finisher can be described as a cylindrical screen (mesh opening size < seed size) through which pulp of fruit can pass and seeds cannot

(Fig. 2.15). Rubber paddles rotate against the cylindrical screen aiding in pulp removal. Often, water is added to the press cake inside the finisher to aid in the separation of seeds from pulp. Other methods of separation include rough filtration using a series of screens (to remove seeds and pulp) in combination with a decanter centrifuge (to remove residual pulp). The seeds and pulp could then be dried and separated mechanically. Again, these processes require the input material to be wet, either naturally (by the juice contained inside the berries) or with the addition of water.

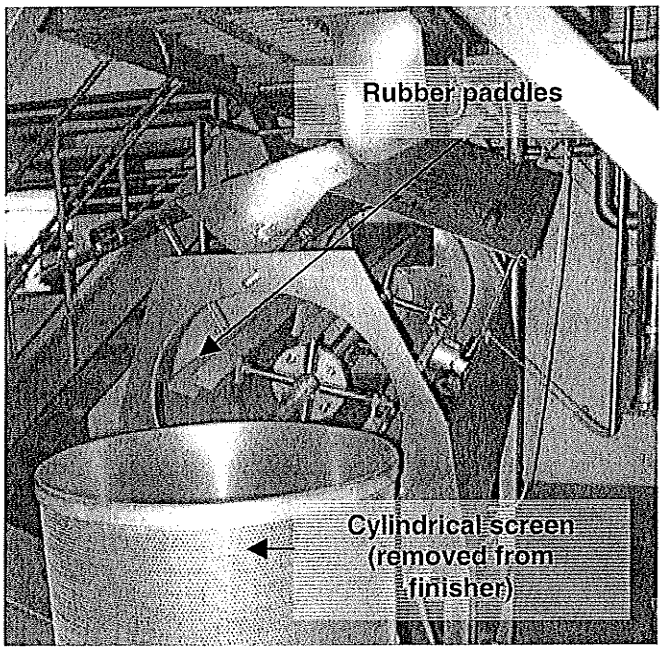


Fig. 2.15. A finisher with cylindrical screen removed to show the rubber paddles for seed/pulp separation.

Other methods of separating press cake into seed and pulp fractions include initial room temperature or low-temperature (50 to 60°C) drying followed by mechanical separation or wind screening (Aslanov and Novruzov 1976; Štastová et al. 1996; Manninen et al. 1997; Yang and Kallio 2001 and 2002b).

The type of mechanical separation was not elaborated on. Wind screening provided a reduction (was not quantified) in pulp from the initially separated seed fraction, however was less effective in removing seeds from the pulp fraction. Alternatively, Berezhnaya et al. (1989) reported a method for seed extraction from whole berries using a berry blade. A slit was made along the berry, whereby the seed could then be manually retrieved. This method suited small-scale, laboratory testing procedures such as analytical evaluation.

2.4.1 Laboratory oil extraction method

Solvent extraction has been considered the most critical step for the analysis of total fat (lipids or oils), neutral and polar lipids, and fatty acid composition (Sahasrabudhe and Smallbone 1983). Polar solvent mixtures such as chloroform and methanol are exhaustive oil extracting chemicals, and have been used extensively where knowledge of total lipid composition is required (Folch et al. 1957; Bligh and Dyer 1959; Sahasrabudhe and Smallbone 1983; Khor and Chan 1985). While practically, sea buckthorn oils have been extracted using SCFE CO_2, these oils have commonly been extracted for analytical purposes by a modified procedure outlined by Folch et al. (1957), utilizing a chloroform/methanol solvent mixture (Yang et al. 1999; Yang and Kallio 2002a).

3. MATERIALS AND METHODS

3.1 Collection of sea buckthorn berries

Berries from the cultivar Indian-Summer were selected as the experimental material because of their availability and volume at which they could be harvested to supply this research. Initially, ssp. *sinensis* berries were considered for comparison with the cultivar Indian-Summer, however were not economically available in the quantity required for experimentation at that time.

Berries were collected from mature shrubs (a 15 year old orchard) at Pearl Creek Farms (a fruit tree nursery at Melville, SK). Frozen berries were manually harvested in November 2001 and again in November 2002. Immediately after harvesting, the berries were hand cleaned to remove visible debris (dried leaves, branches, and damaged berries) induced by harvesting. The lighter debris was removed by wind screening. Cleaned berries were then bagged in 50 kg portions (double bagged to prevent leakage during storage and transport) and were packaged in cardboard boxes. The berries remained frozen (approximately -15°C) from the time they were harvested to the time they arrived at the University of Manitoba, Winnipeg, Manitoba (approximately 2 wk later), via bus (approximately 10 h in transport). The berries were then stored in a walk-in freezer at -25°C to avoid desiccation and external moisture condensation and were processed approximately 2 to 3 mo later.

3.2 Preparation of experimental material

Whole frozen berries were prepared based on the extraction technology under evaluation including solvent extraction, supercritical fluid extraction with carbon dioxide (SCFE CO_2), screw pressing, and aqueous extraction. Solvent extraction, supercritical fluid extraction with carbon dioxide (SCFE CO_2), and screw pressing required dried seeds and dried pulp for the extraction of the seed and pulp oils, respectively (pulp oil is collectively defined as pulp and peel oil, combined). Thus, a process was developed to separate and collect dried seeds and pulp. In addition, the nature of the aqueous extraction process required that the starting material be wet (whole thawed berries).

3.2.1 Laboratory preparation of seeds and pulp for oil extraction

A method for the production of seeds and pulp was developed based on trial and error modifications of previously conducted research with sea buckthorn as discussed in the literature review (section 2.4). The use of a finisher and decanter centrifuge for the production of seeds and pulp was quickly discounted due to their high throughput requirements (t/h), which could not be supplied. In addition, the laboratory method outlined by Li and Schroeder (1999) and Kallio et al. (2002b) for separating of seeds and pulp was also eliminated because of the necessary addition of water. Adding water increased the complexity of documenting a mass balance throughout the separation process. Thus, the addition of water was avoided to simplify processing and mass balance

calculations. In addition, for process simplification at the laboratory level, the step of crushing or maceration of the berries was not conducted.

The critical steps extricated from the literature for separating seeds from pulp included thawing of berries, juice extraction, drying of press cake, threshing, and mechanical separation of dried seeds and pulp. A laboratory setup allowed for trial and error approach to assimilate these critical steps on bench-scale equipment. Berries from the November 2001 harvest were used in the development of the laboratory bench-scale process.

3.2.1.1 Juice extraction Small quantities of berries (approximately 10 kg/batch) from the November 2001 harvest were removed as needed from a walk-in freezer (-25°C). Batches of 0.3 to 0.5 kg of berries were allowed to thaw (single layer of berries) at room temperature for approximately 0.5 h on aluminum trays (30 x 20 x 2 cm, length x width x depth). A simple berry press was assembled to extract juice from the berries. Components of the press included a juice container, a cheese mold, supporting rack, and plunger (Fig. 3.1).

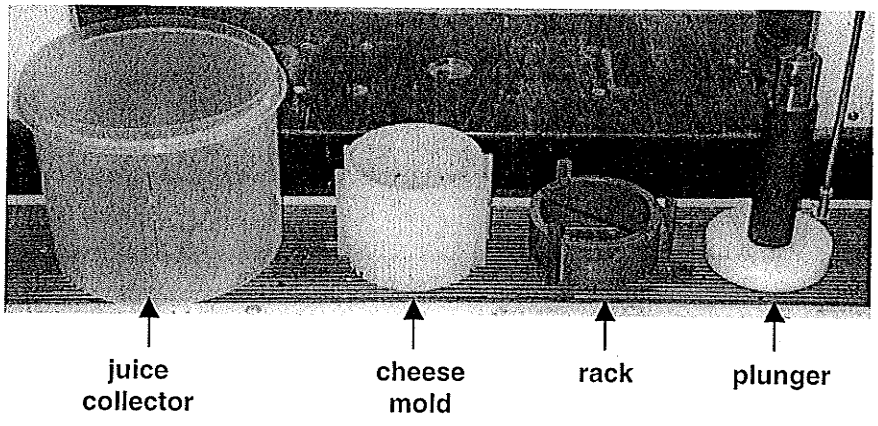
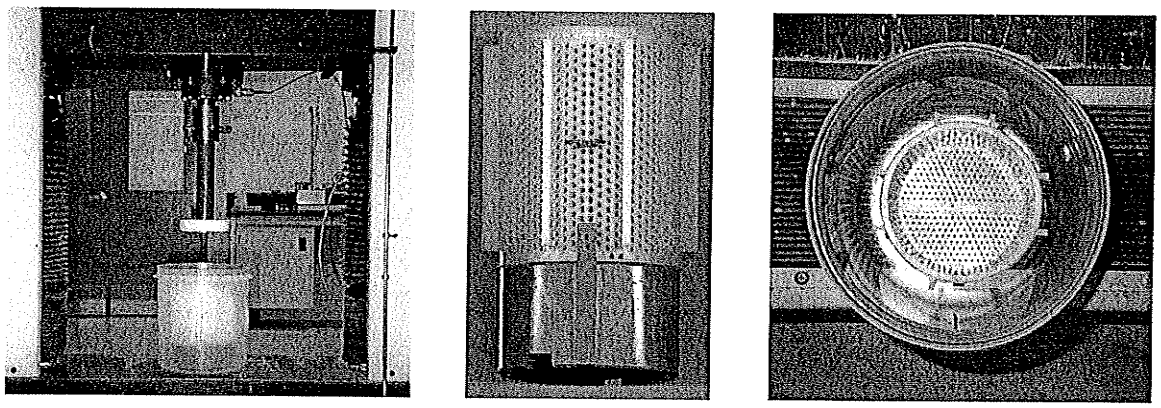


Fig. 3.1. Components of the press used for extracting juice from sea buckthorn berries.

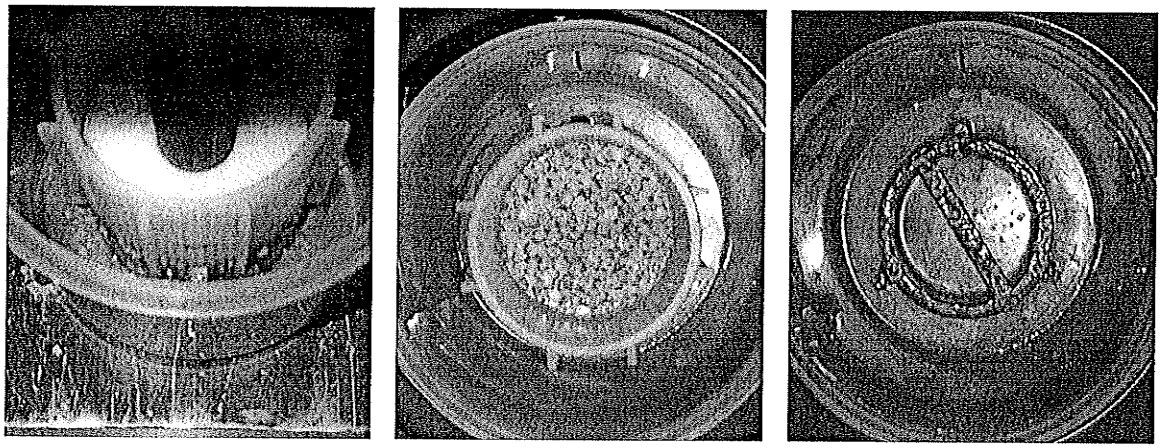
A tall, semi-transparent plastic container (0.20 m height, 0.19 m diameter) enabled the juice extraction to be easily viewed while maintaining sufficient capacity to collect and prevent the juice from splattering. A long, narrow slit (approximately 100 x 3 mm, respectively) was cut along the side of the container to view the plunger depth. The cheese mold (110 mm inside depth and 105 mm inside diameter) was ideally suited to house the thawed berries during pressing. In addition, the mold filtered the juice through 1 mm openings to retain seeds and pulp. During pressing, a rack was used to support and elevate the mold above the juice. The mold and rack assembly were placed inside the juice container. The plunger (100 mm diameter) was used to compress the berries and was operated by a universal compression machine (ATS Universal Testing Machine, model 1410CC, capacity 10,000 lb (4536 kg), Applied Test Systems Incorporated, Butler, PA). Figure 3.2 shows the berry press assembly.



(a) (b) (c)

Fig. 3.2. (a) Berry press assembly with mold and rack. (b) Mold and rack assembly. (c) Top view of mold and rack assembly inside the juice container.

Figure 3.3a shows the berry press in operation with the press cake inside the mold (Fig. 3.3b) and the juice collected inside the juice container after pressing (Fig. 3.3c).



(a) (b) (c)

Fig. 3.3. (a) Berry press during juice extraction operation. (b) Press cake inside mold after pressing. (c) Collection of juice inside the juice container.

3.2.1.2 Drying The press cake was then collected and crumbled onto a cookie tray (0.38 x 0.25 m) giving a drying layer of approximately 20 mm. The press cake was then oven-dried at 50°C for 24 h (A. Anand, Process Development Consultant, Food Development Centre (FDC), Portage la Prairie, MB, personal communication, 2003). Hereafter, the press cake (containing a mixture of seeds and pulp) recovered after juice extraction and the press cake after drying will be referred to as the "wet" and "dry" cake, respectively.

3.2.1.3 Separation The dry cake was removed from the cookie tray and emptied into a blender (Osterizer, model LR47897, 120 V, 60 Hz, Sunbeam Corporation, Delray Beach, FL) in approximately 20 g batches. The blender served as a threshing device to gently break seeds from pulp and remove the white seed membranes or skins encapsulating the seeds. To ensure that seeds did not become damaged during threshing, the blades of the blender were covered with short sections (1.5 cm) of surgical tubing (Nalgene lab/food grade tubing, 8007 non-toxic autoclavable, i.d. = 32 mm, o.d. = 64 mm). In addition, the blender was operated in "stir" mode (slowest RPM setting, though exact RPM was not specified by the manufacturer) for short time intervals (1 to 3 s), repeatedly for 10 to 15 cycles. A periodic visual inspection of the threshed mixture was conducted to prevent possible seed damage (cracked or dehulled seeds) as well as the degree of separation of seeds from pulp.

A series of standard testing sieves were used for separating seeds from pulp. By trial and error, sieves were arranged (top and bottom layer screen openings of 9-mesh (or 2.00 mm) and 12-mesh (or 1.52 mm), respectively) to collect three fractions including, (1) pulp, debris, and some seeds (above the top screen), (2) pulp with some seeds (above the bottom screen), and (3) pulp (bottom collecting tray). Fractions (1) and (2) were emptied into the blender and threshed again. The mixture was again separated in the sieves to produce cleaner fractions (seeds without pulp and pulp without seeds). The combination of threshing and sieving was repeated 3 to 4 times. By the physical nature of the dried-pulp, this fraction will be referred to as the pulp-flake fraction.

3.2.2 Development of a pilot process

A pilot process for the production of seeds and pulp-flakes was developed from scaled-up laboratory methods and was necessary for the production of greater quantities of material (dried seeds and pulp) required for experimentation. The pilot process included similar unit operations as outlined in the laboratory methods, which included steps in the order of maceration, juice extraction, drying, and sieving (Fig. 3.4) (Appendix A1).

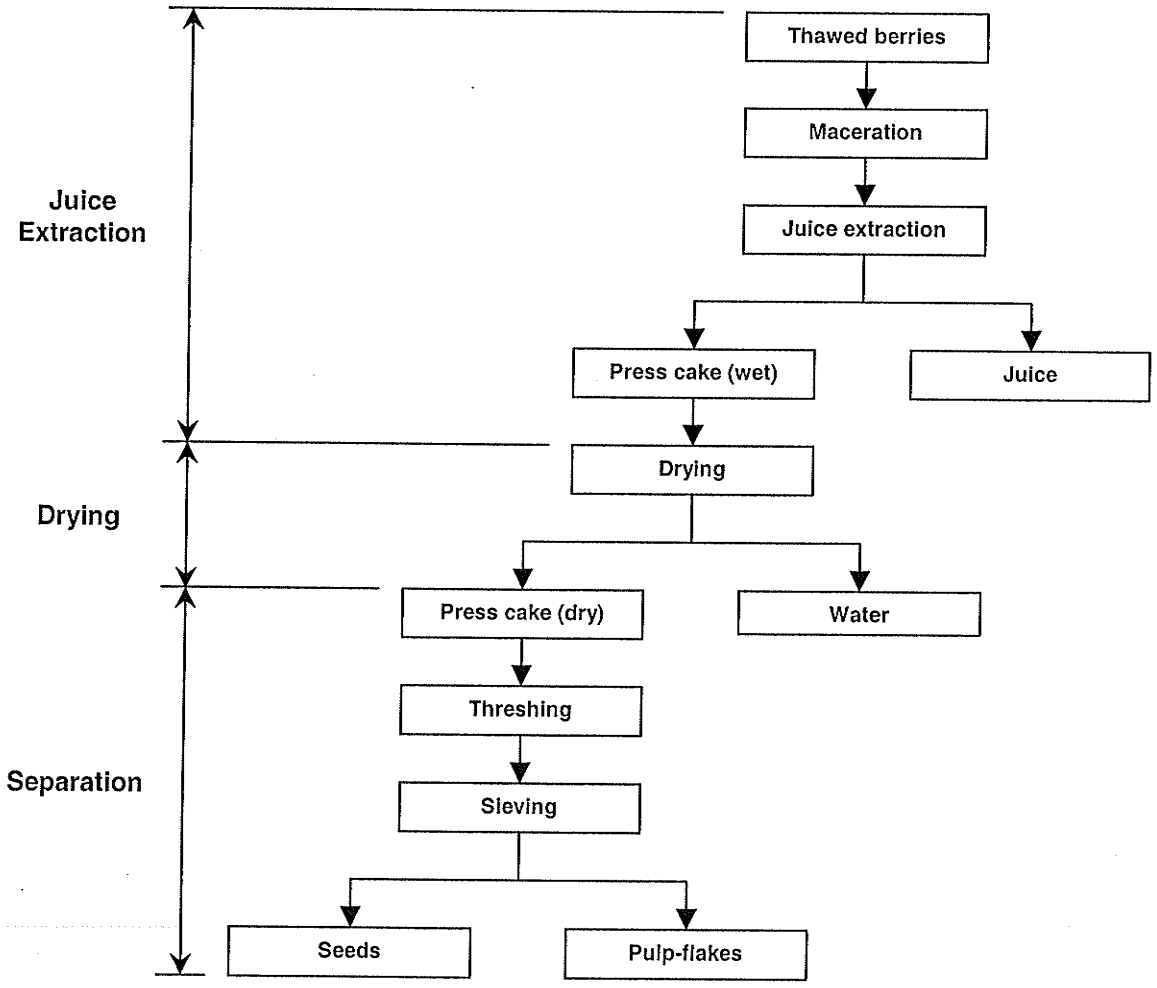


Fig. 3.4. Pilot process including stages of juice extraction, drying, and separation for the production of seeds and pulp-flakes.

All oil extraction trials were conducted on experimental material obtained from the pilot process. The Food Development Centre in Portage la Prairie, MB provided the necessary space and equipment for the pilot processing of the sea buckthorn berries.

3.2.2.1 Juice extraction The berries of the 2002 harvest year were utilized in the preparation of experimental material for oil extraction. All berries were initially thawed for 48 h to room temperature (approximately 20°C). Thawed berries were then emptied into a large stainless steel container where they continued to thaw for approximately 2 h before being processed. While in the container, berries were manually mixed for approximately 15 to 20 min to ensure sample homogeneity and assist with thawing. Mixing was also conducted periodically until the stainless steel container had been completely emptied. Berries were inspected to ensure thawing was complete by gently bursting the berries in the fingers.

After thawing, the berries were transferred to an industrial mixer (Hobart Cutter Mixer, model HCM300, 1140 RPM single speed, Hobart Corporation, Troy, OH) equipped with a plastic, two-blade (knead/mix) attachment. Plastic blades were used to gently macerate the berries and prevent seed damage. Conducted in batches (5 to 7 kg of berries), the mixer macerated the thawed berries for approximately 30 s. The purpose of this step was to burst the berries to aid juice extraction. A visual inspection of the macerated berries was completed to assess the effectiveness of the mixer and degree of maceration.

The macerated berries were then emptied from the mixer into a 15 L plastic pail, and were transferred to a fruit bladder press (Willmes Bladder Fruit Press, model WP60/30, 60 L, Koch Stainless Steel Products Corporation, Winnipeg, MB) to press and recover juice. Operating pressure of the bladder press was in the range of 0.6 to 0.8 MPa. Pressing was stopped after 2 to 5 min when little or no dripping from the bladder press was observed. A stainless steel cage (1.5 x 10.0 mm openings) lined with a 120-mesh (or 0.12 mm) cloth filter held back the wet press cake inside the bladder press. The juice was filtered through a course cone screen (2.5 mm openings) and collected in 15 L pails (approximately 15 kg, filled) and stored at -25°C. Residual seeds and pulp which passed through the press cage and filter was collected from the cone screen and added to the back to the bladder press to recover additional juice. The bladder press was filled twice with macerated berries before removing the wet press cake. Due to the capacities of the equipment, bladder pressing was done in small batches (5 to 7 kg) to ensure adequate juice extraction. Emptying and cleaning of the bladder press was done manually, and the wet press cake was collected into plastic tubs.

Considerable attention was given to the cleaning of the processing equipment (stainless steel containers, mixer, and bladder press) to conserve juice and prevent wet press cake losses. Industrial kitchen utensils including rubber spatulas and stainless steel spoons were used. In addition, great care was taken during the transfer of material from the mixer to the bladder press to avoid sample spillage.

3.2.2.2 Drying Wet press cake collected from the bladder press was crumbled onto several (3 to 5) perforated drying trays (0.75 x 0.50 x 0.05 m, long x wide x deep with a perforation size of 10-mesh or 1.91 mm) giving a drying layer of approximately 20 mm. Thorough mixing ensured that each tray contained a homogenous portion of the total wet press cake collected from all juice extraction batches. The wet press cake was dried at 50°C for 24 h in a ventilated drying oven (Gas Fired Variable Circulation Laboratory Dryer, 1.8 kW, 20-tray, Proctor & Schwartz Corporation, Philadelphia, PA) to remove moisture (a requirement for solvent extraction, SCFE CO_2, and cold pressing) and assist with separation.

3.2.2.3 Separation The dry cake was carefully removed from the drying trays and emptied into the industrial mixer in approximately 2 kg batches. The industrial mixer served as a threshing unit to gently break seeds from pulp and remove the white seed skins encapsulating the seeds (using the plastic, two-blade knead/mix attachment). The mixer was operated for short time intervals (approximately 3 s), repeatedly for 10 to 15 cycles. This was done to ensure that seeds did not become damaged during threshing. A periodic visual inspection of the threshed mixture was conducted to assess possible seed damage (cracked or dehulled seeds) seeds as well as the degree of separation of seeds from pulp.

A vibratory screen separator (SWECO, model LS24S444, 1200 RPM, Sweco Canada, Toronto, ON) equipped with a stackable arrangement of two screens was assembled for the separation of seeds from pulp-flakes. Screens were arranged (top and bottom layer screen openings of 6-mesh (or 3.35 mm)

and 10-mesh (or 1.91 mm), respectively) to collect three fractions including, debris with pulp (small branches and large pulp flakes above the top screen), seeds with pulp (above the bottom screen), and pulp (visibly free of seeds and debris). The two fractions including the debris with pulp and seeds with pulp were emptied into the mixer and threshed again. The mixture was again separated with the vibratory separator to produce cleaner fractions or debris and seeds free of pulp. The combination of threshing and separating was repeated 3 to 4 times.

3.2.2.4 Classification of separated fractions A representative sample from each fraction (4 fractions) was removed (approximately 20 g), comprising two visibly distinct components in each fraction, namely seeds with some pulp, pulp with some seeds, debris with some pulp, and an inseparable seeds and pulp mixture. Classifications of fractions were assigned by calculating the percentages (mass-based) of the two visible components in each fraction. Thus, a component >50% (major component) was assigned to describe the fraction. Similarly, a component <50% was considered the minor component. Thus, the final fractions collected after separation were classified as "seeds", "pulp" (referred throughout this report as pulp-flakes), "inseparable seeds and pulp", and "debris". Only the seeds and pulp fractions were used in solvent extraction, SCFE CO_2, and screw pressing trials. The inseparable mixture and the debris fraction were excluded from experimentation and were considered as process losses.

The seed fraction was further cleaned by compressed air to remove residual pulp-flakes that were not removed by mechanical separation. Further cleaning of the pulp-flakes to remove residual seeds was not conducted since this fraction was visibly clean (free of seeds) after separation. The seeds and pulp-flake fractions were then stored at -5°C until they were used in the oil extraction experiments, approximately 1 to 4 mo later.

3.2.2.5 Mass balance calculation A mass balance, including material losses, was calculated and documented throughout the preparation of experimental material during pilot processing. The mass balance was expressed as a mass percentage of processed materials in relation to the mass of initial starting material (whole thawed berries).

3.2.2.6 Moisture content determination Moisture contents were determined gravimetrically by oven-drying. Following the American Society of Agricultural Engineers (ASAE) standard S352.2 (2002) for moisture measurement of unground grain and seeds, sea buckthorn seeds were dried at 130°C for 4 h. The wet press cake, dry press cake, pulp-flake, and whole berry moisture contents were determined after drying at 105°C for 24 h, a method adopted form Tang and Tigerstedt (2001) for the determination of moisture content of whole sea buckthorn berries. A 5 g sample of material (seeds, pulp-flakes, wet and dry press cake, and whole berries) was weighed and placed in a circular aluminum dish (55 mm diameter, 15 mm deep). After drying, the samples were removed

and placed in a desiccater to cool. A wet basis moisture content (w.b. expressed as a percentage) was then calculated by dividing the weight loss (sample water content) by the original weight. Moisture contents of the seeds and pulp were checked again prior to solvent extraction, SCFE CO_2, and screw pressing trials. All moisture contents were conducted in triplicates.

3.2.2.7 Particle size analysis of experimental material Particle distribution of the experimental material including seeds (whole and ground) and pulp-flakes was determined by a system of sieves following the American Association of Cereal Chemists method 66-20 (AACC 2000). Canadian Standard Sieves (CAN/CGSB-8.1, 20.3 cm diameter, W.S. Tyler, St. Catherines, ON) were arranged (No. 6, 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 45, or 3.35, 2.36, 2.00, 1.70, 1.40, 1.18, 1.00, 0.850, 0.710, 0.600, 0.500, 0.425, 0.325 mm aperture, respectively) to collect the particle sizes. The sieves were loaded into a vibratory sieve shaker (RO-TAP, model RX-29, 1725 RPM, 110V, W.S. Tyler, Mentor, OH) and approximately 50 g of seeds (whole or ground) and 100 g of pulp were placed on top of the top sieve (No. 6). All samples were sieved for 5 min, trials conducted in triplicate.

3.2.2.8 Sampling and sample reduction Experimental material used for moisture content determinations and extraction trials were sampled according to Association of Official Analytical Chemists method 965.16 (AOAC 1999). Representative samples were taken from larger volumes of experimental material

by emptying the total volume (seeds, pulp-flakes, wet and dry cake, and whole berries) onto a clean, large stainless steel tray (0.70 x 0.40 x 0.02 m). The material was then thoroughly mixed, coned, and divided into quarters. Diagonal quarters were discarded and the remainder of the material was mixed again. This was repeated until a desired sample size was obtained.

3.3 Oil extraction experiments

3.3.1 Solvent extraction

Solvent extraction trials were carried out on sea buckthorn seeds and pulp-flakes using a Goldfisch laboratory oil extraction apparatus (6-unit Labconco Goldfisch Fat Extraction Apparatus, model 35001, 115 V, 50/60 Hz, 5.2 A, 100 W/heater, Labconco Corporation, Kansas City, MI) (Fig. 3.5).

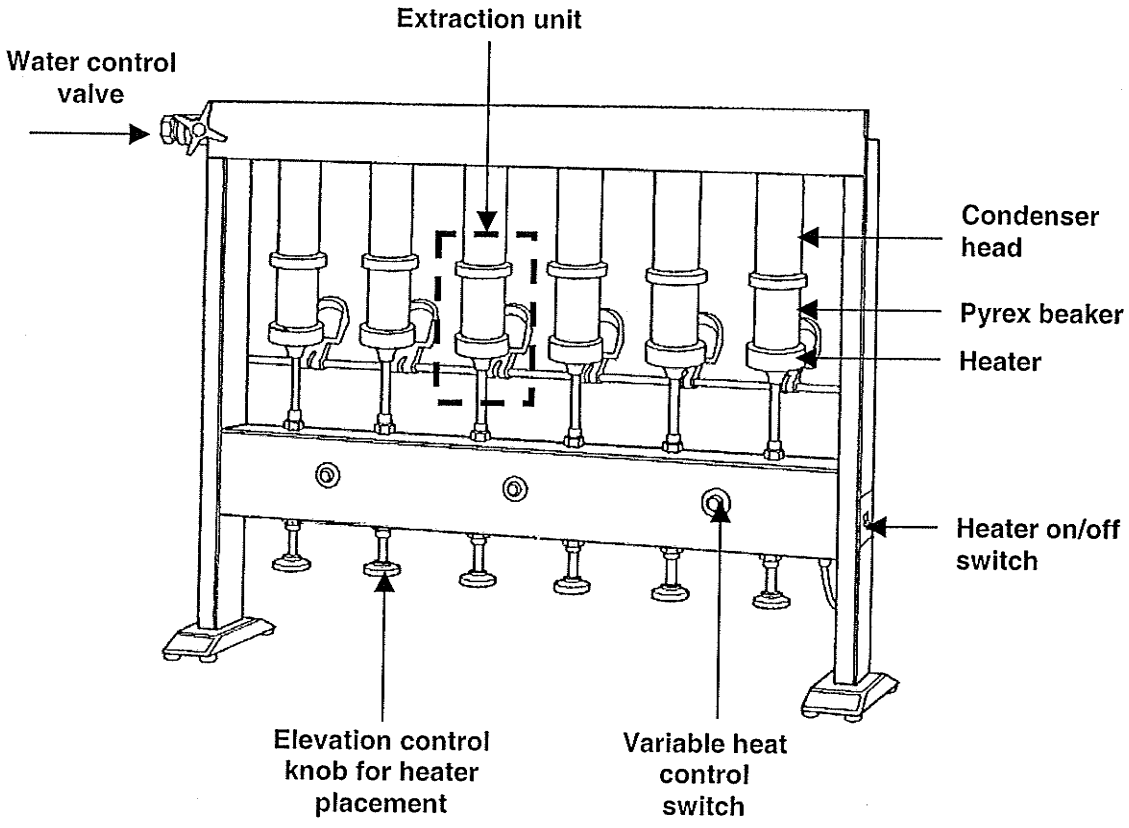


Fig. 3.5. A Goldfisch oil extraction apparatus (adapted from Labconco Corporation 1997).

Extractions were conducted using a series of extraction units comprising Pyrex beakers as the solvent chambers. Figure. 3.6 shows an expanded view of a single extraction unit. Samples of seeds and pulp-flakes were placed inside sample thimbles and were inserted into sample tubes. The samples were positioned between the boiling solvent (contained inside the Pyrex beakers) and the condenser head. During heating, the solvent vaporized and condensed on the inside of the condenser head allowing the condensate (miscella) to percolate down through the sample material and be collected inside the Pyrex beaker.

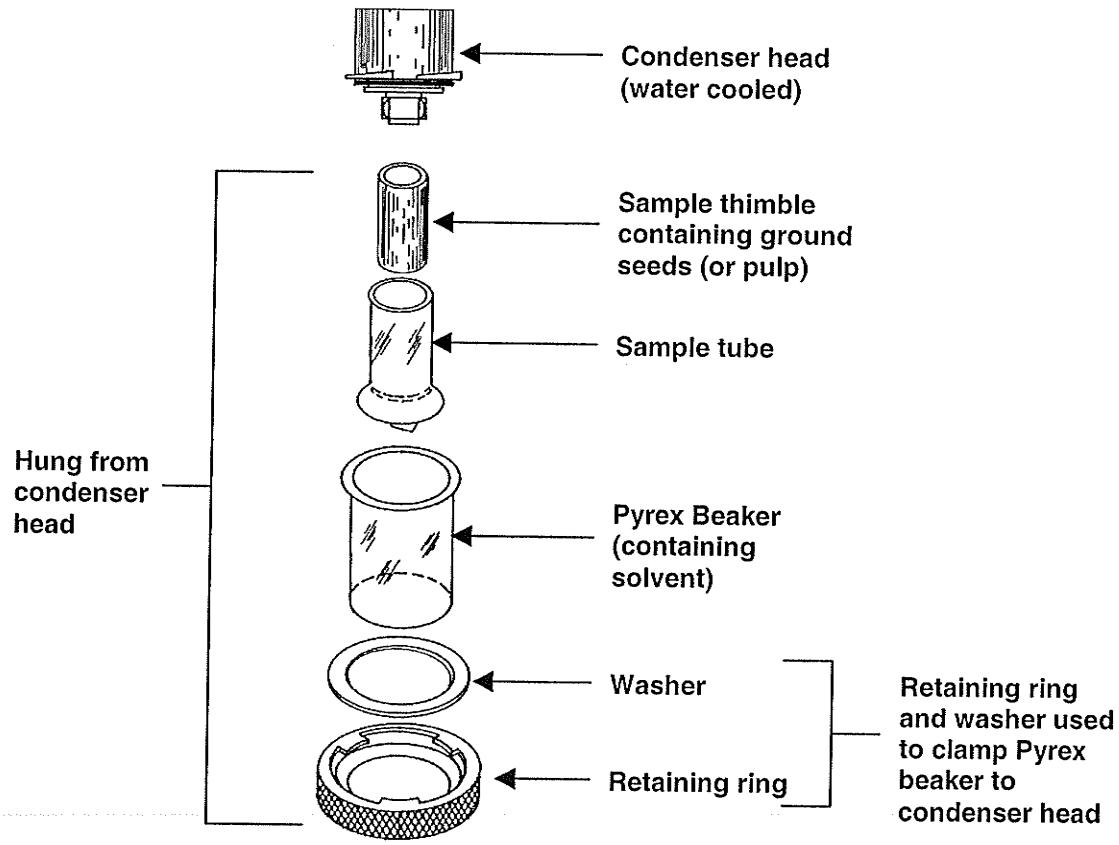


Fig. 3.6. An expanded view of a single extraction unit (adapted from Labconco Corporation 1997).

Extractions were conducted following the American Association of Cereal Chemists method 02-01A (AACC 2000). Approximately 50 g of sample were ground (only seeds) in a rotary mill (Stein Mill, model M-2, F. Stein Labs Incorporated, Atchison, KS). Grinding was carried out in 2-15 s cycles (15 s time break between grind cycles) to avoid sample heating and ensure adequate particle size reduction for solvent percolation. A 5 g sample (seeds or pulp-flakes) was placed in a coned sheet of Whatman no. 5 filter paper (110 mm diameter). The sample was then capped with a second sheet of coned filter paper and inserted into the sample thimble.

Petroleum ether (laboratory grade, boiling range 40 to 60°C) as the extraction solvent (35 mL) was added to each Pyrex beaker. The thimble and beakers were then placed in the Goldfisch extractor. Extractions were conducted over a 6 h period, after which, miscella from each beaker was emptied into a round evaporation flask. The evaporating flask was then connected to an all-glass rotary vacuum evaporator (Büchi Rotavapor, model EL-130, and Büchi Water Bath, model 461, Büchi Laboratories, Flawil, Switzerland) to recover the petroleum ether from the oil. Evaporation was conducted for 20 min at 50°C, after which the remaining oil mass was recorded. Percent oil extracted (S_P) using petroleum ether was calculated using equation 3.1.

$$S_P = \left(\frac{O_S}{M_S}\right) \cdot 100 \qquad \textbf{(3.1)}$$

where:

S_P = percent oil extracted by solvent extraction (petroleum ether), %
O_S = mass of oil extracted by solvent (petroleum ether), g
M_S = mass of the oil-bearing (ground seeds or pulp-flakes) material before solvent extraction, kg

Separate experiments were conducted to measure the temperature of the miscella (oil and petroleum ether mixture) during the extraction of seed and pulp oil. Extraction temperatures were measured using a T-type thermocouple connected to an Omega microprocessor thermometer (model HH23, Stamford, CT). After 2 h of percolation, the thermocouple was inserted into the pyrex beaker (containing the miscella) which was disconnected from the condenser head. The beaker was placed on the heater during temperature recording. All temperatures were measured in triplicate.

3.3.2 Supercritical fluid extraction

Supercritical fluid extraction trials were conducted at the FDC in Portage la Prairie using a supercritical fluid extraction system (Supercritical Fluid Extraction Screening System, 220 V, 20 A, three phase 60 Hz, Newport Scientific Inc., Jessup, MD). This system was a complete turnkey assembly for extractions up to 68.9 MPa gauge (10 000 psig). The major components of the SCFE system included a carbon dioxide (CO_2) source (compressed cylinder of liquefied carbon dioxide gas, 99% pure), a continuous compressor (10,000 psig single-ended diaphragm type), a cylindrical extraction vessel (300 mL capacity, stainless steel), a temperature controlled heating-pad (silicone-rubber type, 305 x 152 mm, 240V, 180 W) external to the extraction vessel, a stainless steel cylindrical oil collection vessel (125 mL capacity), and a flow-rate indicator and flow totalizer (Fig. 3.7) (see also Appendix A2).

Seeds were prepared following the method used prior to solvent extraction trials. In addition, an alternate grinding time of 2-5 s grinding cycles was used to prepare the seed samples. A sample of 140 g of ground seeds and 70 g of pulp-flakes (masses of samples were limited by the capacity of the extraction vessel) were loaded into a high-pressure extraction vessel (300 series stainless steel, corrosion resistant, 381 mm inside diameter, 267 mm inside depth) (Fig. 3.8) (Appendix A2). Steel wool (grade #2, medium-coarse) was placed at the bottom and top of the extraction vessel to prevent the sample from entering and clogging the gas inlet (supercritical CO_2 flow) and outlet (supercritical CO_2 and dissolved oil flow) of the extraction vessel during operation. Steel wool was also inserted at

a midway point between two equal layers of the sample to prevent channeling of the supercritical CO_2.

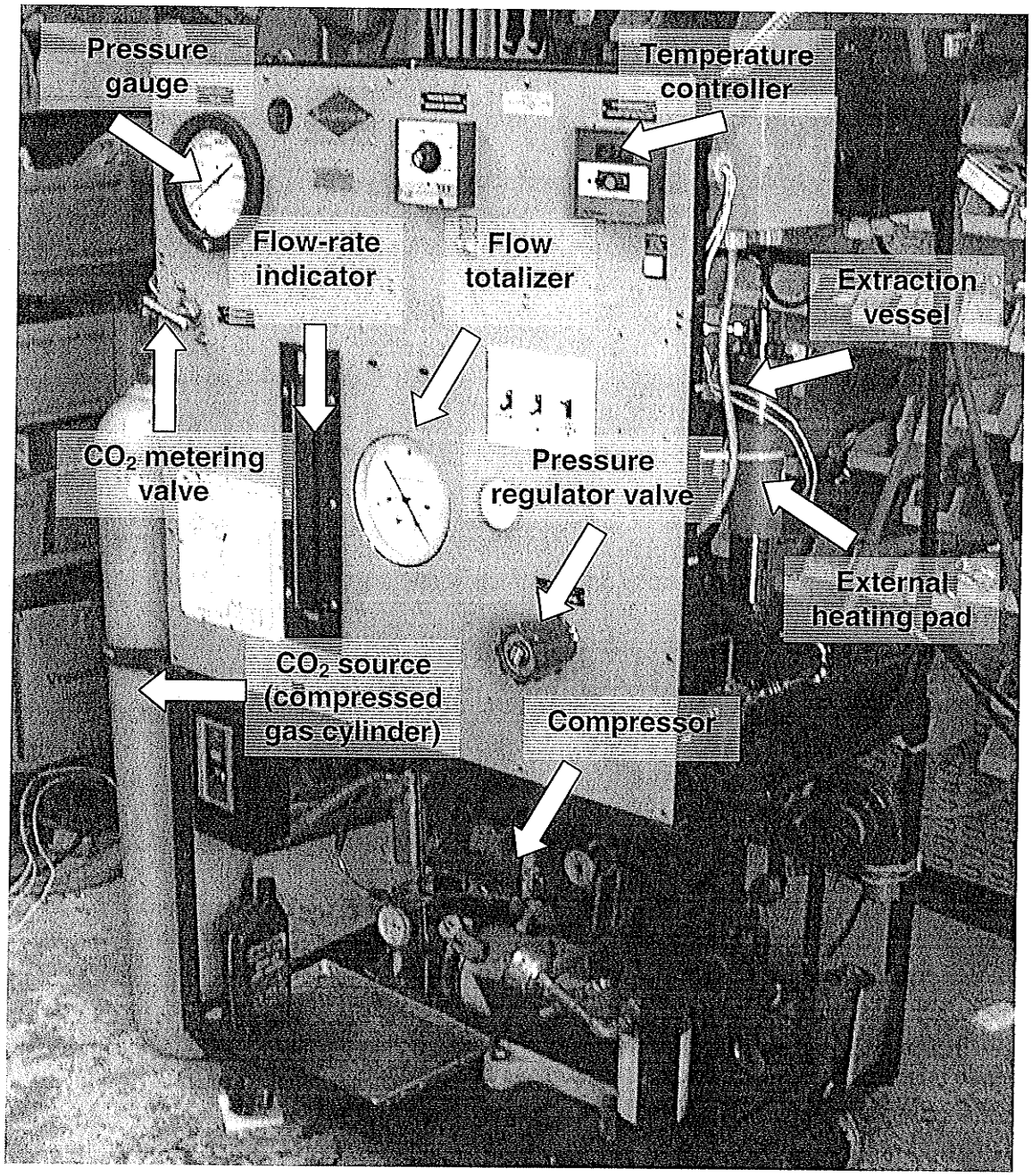


Fig. 3.7. Supercritcal fluid extraction system and major components (oil collection vessel not shown).

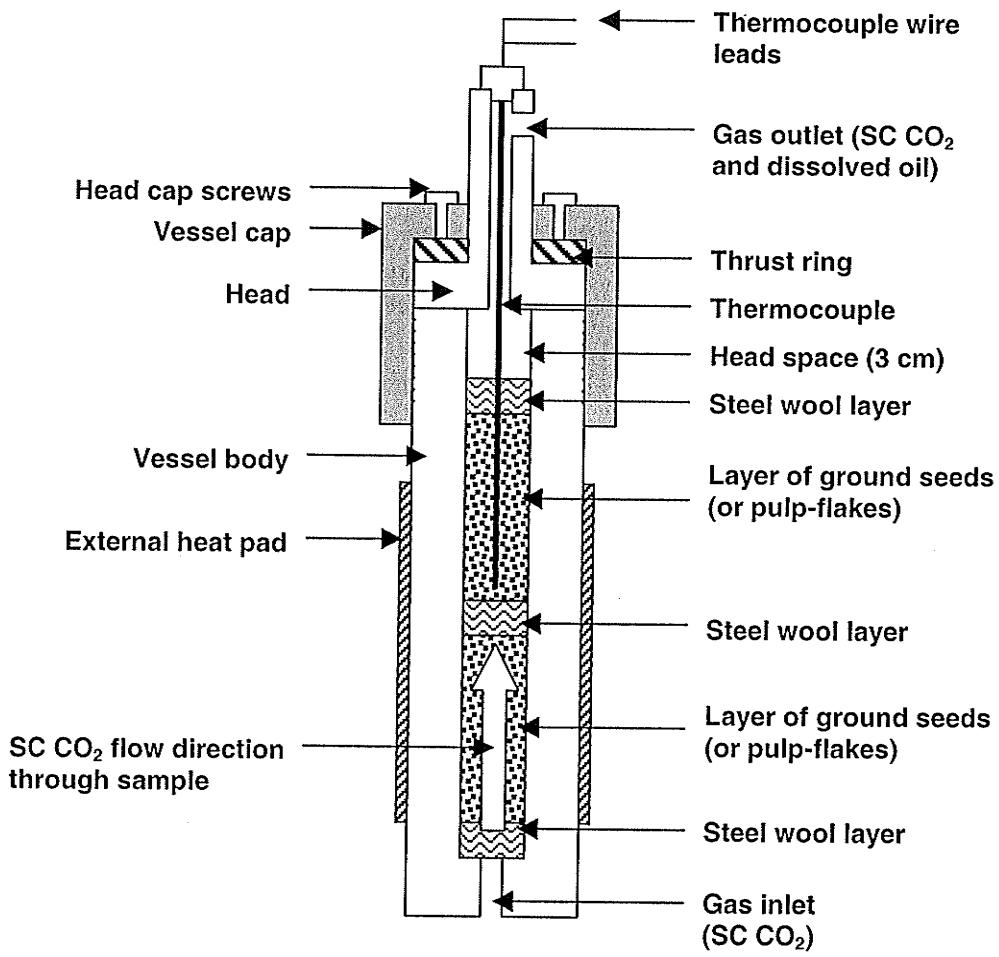


Fig. 3.8. Placement of sample in the extraction vessel.

All samples (ground seeds and pulp-flakes) were gently compacted inside the extraction vessel to 0.6 kPa. A steel rod (378 mm outside diameter) was inserted into the extraction vessel and calibration weights were placed on top. Gentle compaction ensured maximum sample capacity and therefore efficient use of the vessel volume. After compaction, a 3-cm headspace allowance ensured that fibers of the steel wool did not interfere with the critical seal between the head and vessel body. A food grade sealant was applied along the rims of the head and vessel body to prevent gas leakage.

Tightening head cap screws to 60 Nm using a torque wrench, connected and sealed the head to the vessel body. The head of the extraction vessel was fitted with a J-type thermocouple to monitor the extraction temperature. The extraction vessel was then placed and connected inline to the SCFE system using quick-release couplings (Appendix A2).

Figure 3.9 shows a schematic drawing of the SCFE system components. All CO_2 gas exiting from the compressed gas cylinder was filtered before entering the compressor. After compression, the CO_2 gas entered the heated extraction vessel (now a supercritical fluid or SC CO_2), saturating the sample for approximately 10 to 15 min. It should be noted that no fluid was vented from the extraction vessel during the saturation period, referred to as the static extraction mode. An indicating temperature controller monitored the extraction vessel temperature which was fitted with a J-type thermocouple. The fluid flow was achieved by directing the excess compressed fluid (CO_2) back into the compressor's suction end. Manual adjustments of the back pressure regulator valve (varying the amount of feed back fluid) controlled the extraction vessel pressure. The system was equipped with a rupture disc assembly which provided over-pressure protection for the extraction vessel and extraction vessel pressure gauge.

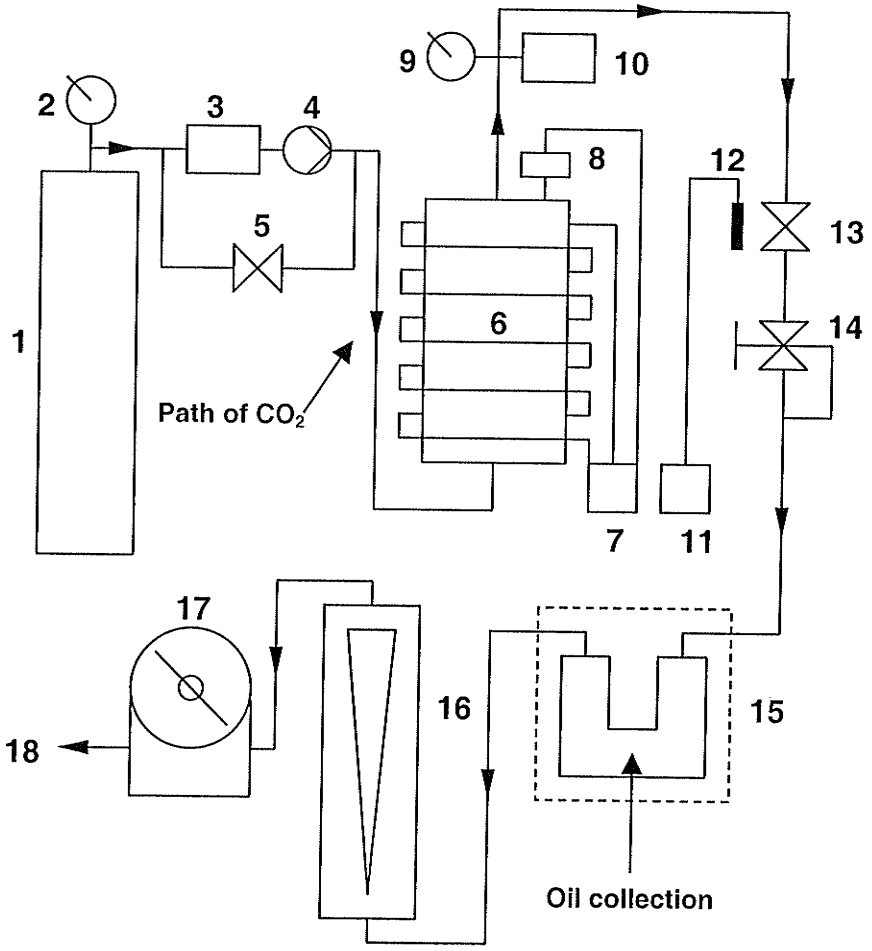


Fig. 3.9. Schematic drawing of the SCFE system used for the experimental extractions of sea buckthorn seed and pulp oils. System components: 1) compressed carbon dioxide gas, 2) and 9) pressure gauges, 3) filter, 4) diaphragm compressor, 5) back pressure regulator, 6) extraction vessel, 7) indicating temperature controller, 8) thermocouple, 10) rupture disc, 11) non-indicating temperature controller, 12) heater, 13) metering valve, 14) relief valve, 15) cold trap (oil collection vessel), 16) flow-rate indicator, 17) flow totalizer, and 18) CO_2 vent.

After the target temperature and pressure was reached (45°C and 35 MPa gauge or 5000 psig, respectively), the fluid was allowed to flow through the extraction vessel, referred to as the dynamic extraction mode. The fluid flow rate was controlled by a metering valve (solid stem needle type) and was heated to

prevent freezing. Flow rate of CO_2 through the sample in the extraction vessel (dynamic extraction mode) was maintained at approximately 4.5 L/min (10% of maximum flow of 38.2 L/min). Over-pressure protection was provided by a 34.5 kPa gauge (5 psig) relief valve connected in series with the metering valve.

Oil was released from the SC CO_2 and collected in a collection vessel exposed to atmospheric pressure (cold trap) (Appendix A2). The CO_2 gas (oil free) was directed through a gas flow-rate indicator which measured fluid flow rate (% of maximum flow of 38.2 L/min) and then through a flow totalizer which measured the SCFE system's total gas flow (10 L per dial revolution) upon being vented from the system.

Equation 3.2 was used to represent the percentage of oil recovered using SCFE CO_2 ($SCFE_P$) compared to the petroleum ether extraction. The amount of oil extracted was determined by taking the difference between the mass of the collection vessel before and after each extraction (after 3 and 6 h experiments or measured at 1 h increments).

$$SCFE_P = \left(\frac{\left(\frac{O_{SCFE}}{M_{SCFE}} \right) \cdot 100}{S_P} \right) \cdot 100 \qquad \textbf{(3.2)}$$

where:

$SCFE_P$ = percent oil extracted by SCFE CO_2, %
O_{SCFE} = mass of oil extracted by SCFE CO_2, g
M_{SCFE} = mass of the oil-bearing (ground seeds or pulp-flakes) material before extraction employing SCFE CO_2, kg
S_P = percent oil extracted by solvent extraction (petroleum ether), %

The amount of oil was then divided by the initial mass of the starting material (ground seeds or pulp-flakes), to determine the percentage of oil extracted. This percentage was then divided by the percent oil content (S_P) determined by solvent extraction using petroleum ether (equation 3.2).

3.3.3 Screw pressing

Oil pressing trials were conducted using a Täby oil screw press (model Type-20, Skeppsta Maskin AB, Örebro, Sweden), attached to and powered by an Electrolux Assistent mixer base (model DLX 2000, 120 V, 60 Hz, 450 W, Magic Mill International Headquarters, Monsey, NY) (Appendix A3). A computer and data acquisition system (Omega MultiScan 1200, Stamford, CT) was used to monitor and record screw press and extracted oil temperatures during pressing. Figure 3.10 shows the screw press (attached to mixer base), computer, and data acquisition system used during screw pressing trials.

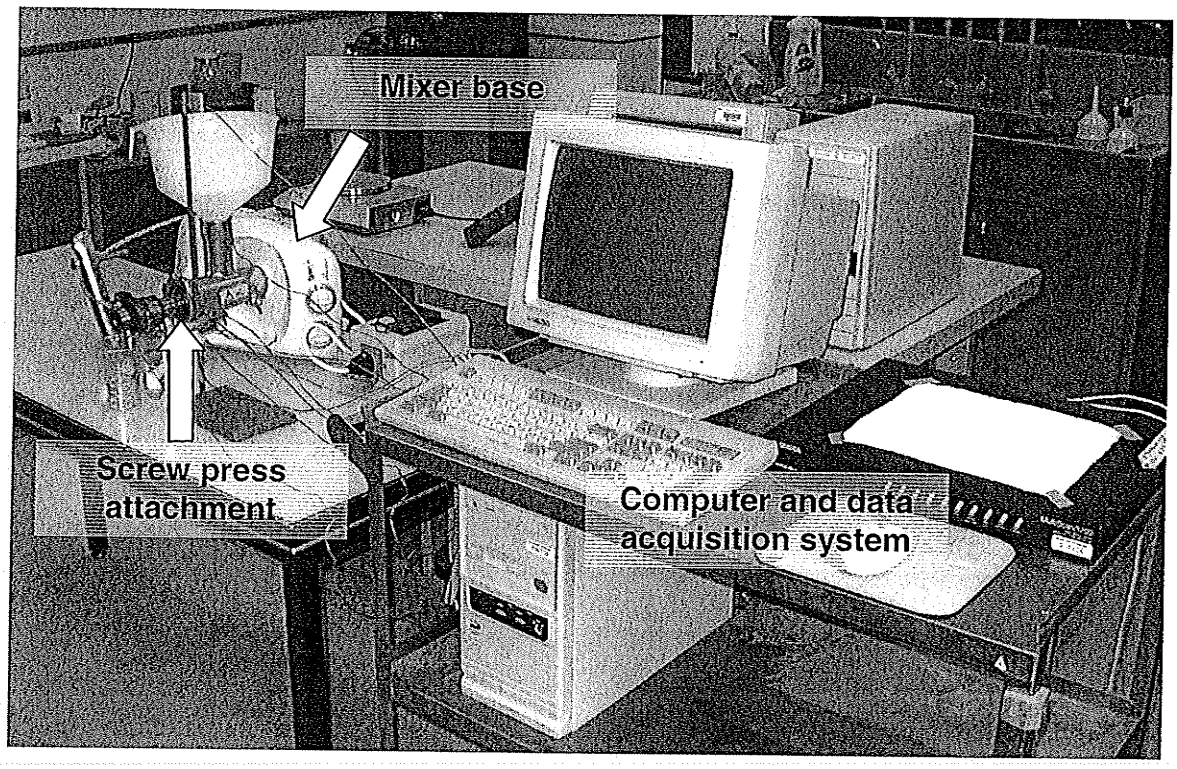


Fig. 3.10. Screw press (attached to mixer base), computer, and data acquisition systems used in screw pressing trials.

A method adopted from Singh et al. (2002) was used for oil pressing trials. Prior to pressing, the screw press was first allowed to heat for 20 min via an electrical-resistance heating ring (110 V, 120 W) attached around the press head, to raise the press head temperature to 95°C ± 5°C. The temperature of the heating ring was thermostatically controlled using a 100°C thermostat (model 2455R-L100C) (Fig. 3.11).

During pressing, T-type thermocouples measured the feed inlet, extracted-oil stream, collected oil, screw press barrel, heating ring, press cake outlet, and ambient temperature (Fig. 3.11) (Appendix A3). The feed inlet temperature was measured by inserting a thermocouple in the flow of seeds and pulp-flakes. A thermocouple was placed in the extracted oil stream to measure the temperature that the oil was exposed to during pressing. In addition, a thermocouple was inserted into the oil which was collected into a test tube. The screw press barrel temperature was measured by inserting a thermocouple into an oil outlet port. A thermocouple was mounted on the restriction die (6 mm) to measure the temperature of the press cake. The heating ring temperature was monitored by a thermocouple placed between the heating ring and press head.

After heating, whole untreated seeds and pulp-flakes (200 g) were pressed for 4 min to achieve steady flow of press cake and oil. Upon achieving steady flow, 300 g of seeds and pulp-flakes were fed into the screw press. Collection of the press cake and crude oil (unfiltered oil directly obtained from pressing) was initiated approximately 1 min after sample introduction (again to ensure steady flow) and stopped when the feed inlet was empty. Sample

collection time was determined using a stopwatch. The oil and press cake were collected in a 25 mL test tube and a 500 mL beaker, respectively after 8 min of pressing. The mass of the crude oil and cake were recorded and the press rate (PR = capacity of screw press) was calculated using equation 3.2 (Singh et al. 2002).

$$PR = \frac{O_C + C}{t} \qquad \textbf{(3.3)}$$

where:

PR = pressing rate or capacity of screw press, g/s
O_C = mass of crude oil, g
C = mass of cake, g
t = time, s
$O_C + C$ = assumed mass of processed material (seeds or pulp-flakes), g

Equation 3.4 was used to represent the percentage of oil recovered using a laboratory screw press (SP_P) compared to the petroleum ether extraction. The solids content of the pressed oil was determined following the method by Singh et al. (2002).

$$SP_P = \left(\frac{\left(\frac{O_{SP}}{M_{SP}} \right) \cdot 100}{S_P} \right) \cdot 100 \qquad \textbf{(3.4)}$$

where:

SP_P = percent oil extracted by screw press, %
O_{SP} = mass of oil extracted by screw press, g
M_{SP} = mass of the oil-bearing (ground seeds or pulp-flakes) material before screw pressing, kg
S_P = percent oil extracted by solvent extraction (petroleum ether), %

Pressed oil was vacuum filtered over a Buchner using a Whatman No. 4 filter paper. The filtered solids were rinsed with 150 mL of petroleum ether and allowed to dry under vacuum. The filter paper was weighed before and after filtration and drying, and the dry solids mass was calculated by the difference. Solids content was defined as dry solids mass per mass of unfiltered oil (equation 3.4).

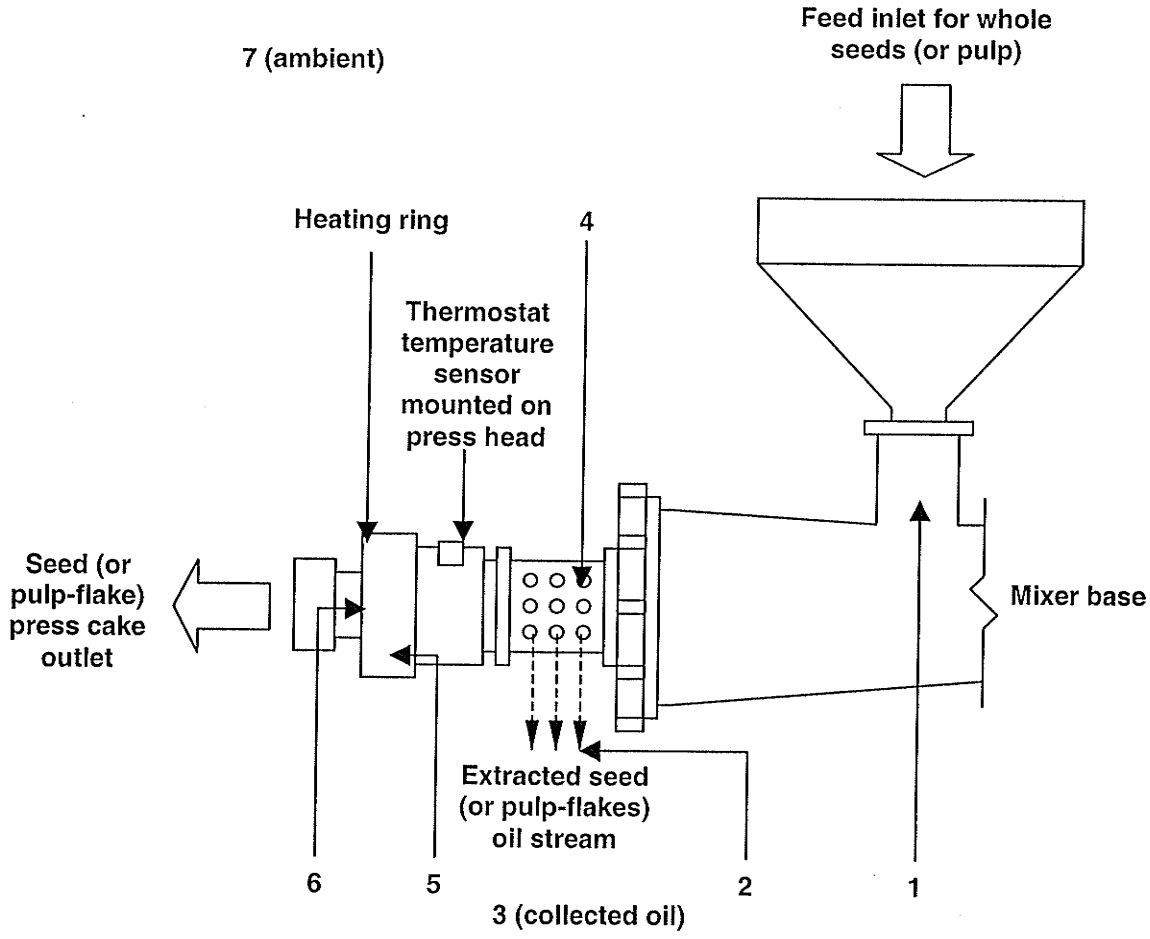


Fig. 3.11. A schematic drawing of the screw press and thermocouple placement for temperature monitoring. Thermocouples: (1) seed and pulp-flake feed inlet, (2) extracted oil stream, (3) collected oil, (4) screw press barrel, (5) heater ring, (6) press cake, (7) ambient.

3.3.4 Aqueous extraction

The methodology for the extraction of sea buckthorn seed and whole berry pulp oil was derived from preliminary experiments conducted at Westfalia Separator AG (Oelde, Germany) in September 2002 on ssp. *sinensis* seeds (obtained from Seabuckthorn International Inc., Peachland, BC, 2000 harvest) and cv. Indian-Summer berries (November 2001 harvest). Trials were conducted to evaluate an aqueous extraction process similar to the FRIOLEX® process as a potential technology for extracting sea buckthorn seed and pulp oil. Proprietary restriction did not allow for replication of "true" FRIOLEX® trial.

Seeds (cv. Indian-Summer) were ground as per solvent extraction and SCFE CO_2 trials. Distilled water was added to dilute 99% pure ethanol (99% EtOH) to 40% in a ratio of 3:2 (by volume, v/v), respectively. The amount of 40% EtOH added to ground seeds was calculated by multiplying 2.5 g of 40% EtOH by the mass of ground seeds used in the extraction. The slurry of ground seeds and ethanol was heated in a water bath for 2 h at 70°C, stirring periodically. The slurry was then centrifuged in 500 mL bottles for 10 min at 8275 x g (7000 RPM, Sorval RC-5C plus, rotor GS-3, Sorval, Newton, CT) and 20°C. The liquid fraction (40% EtOH and seed oil) was decanted and centrifuged in 50 mL vials for 10 min at 17,200 x g (10,000 RPM, rotor SS-34) and 20°C, to recover seed oil.

A preliminary extraction trial to recover pulp oil from whole sea buckthorn berries (2002 harvest year) was conducted at the Food Development Centre (Portage la Prairie, MB) to replicate the results obtained at Westfalia Separator

AG, in Oelde, Germany. Approximately 2 kg of berries were macerated using a blender (Osterizer, model LR47897, 120 V, 60 Hz, Sunbeam Corporation, Delray Beach, FL) in approximately 100 g batches. Similar to seed/pulp-flake separation in section 3.2.1.3, the blades of the blender were covered with short sections of surgical tubing to prevent seed damage. The blender was operated in "stir" mode for short time intervals (5 to 10 s), repeatedly for 5 to 10 cycles. A periodic visual inspection of the slurry was conducted to assess degree of maceration and possible seed damage.

The slurry was then heated in a water bath for 1 h at 45°C and then centrifuged in 250 mL bottles for 5 min at 7425 x g (7000 RPM, Sorvall RC Superspeed, model SV728211, rotor SLA-1500, Sorvall, Newton, CT) and 45°C. Centrifugation produced a three-phase system containing a top cream layer (an emulsion of pulp oil and suspended solids), a middle juice layer, and solids layer (seeds and pulp fiber). The juice (containing the cream layers) was decanted and mixed with ethanol (95% pure EtOH) (2:1, v/v), respectively. The mixture was heated in a water bath for 2 h at 80°C, stirring periodically. Centrifugation of the mixture was conducted in 50 mL vials for 10 min at 7425 x g (7000 RPM) and 45°C on the juice/cream/ethanol mixture. A resulting oil layer was observed on top and was drawn from each vial using a Pasteur pipette. The mass of oil recovered by aqueous extraction (O_{AQ}) was recorded and related to the mass of starting material (whole berries).

An additional trial was conducted in duplicate using 99% EtOH. After maceration (conducted using the methodology described previously), the pulp

slurry was heated in a water bath for 1 h at 45°C, stirring periodically. The slurry was then centrifuged in 500 mL bottles for 10 min at 8275 x g (7000 RPM, Sorvall RC-5C plus, rotor GS-3, Sorvall, Newton, CT) and 20°C. The juice and cream layers were decanted and mixed with 99% EtOH (2:1, v/v), respectively. The mixture was heated in a water bath for 2 h at 70°C, stirring periodically. Centrifugation of the mixture was conducted in 50 mL vials for 10 min at 17,200 x g (10,000 RPM, rotor SS-34) and 20°C on the juice/cream/ethanol mixture. The resulting oil layer was drawn from each vial using a Pasteur pipette. The mass of oil recovered was recorded and related to the mass of starting material (whole berries). Equation 3.4 was used to represent the percentage of oil recovered using an aqueous extraction technique (AQ_P) compared to the petroleum ether extraction.

$$AQ_P = \left(\frac{\left(\frac{O_{AQ}}{M_{AQ}} \right) \cdot 100}{S_P} \right) \cdot 100 \qquad \textbf{(3.5)}$$

where:

AQ_P = percent oil extracted by aqueous extraction, %
O_{AQ} = mass of oil extracted by aqueous extraction, g
M_{AQ} = mass of the oil-bearing (ground seeds or whole thawed berries) material before aqueous extraction, g
S_P = percent oil extracted by solvent extraction (chloroform/methanol extraction), %

3.3.5 Containment and storage of extracted oils

All extracted oils were contained in 20 mL plastic sample vials and the vials were wrapped with aluminum foil as suggested by Kiritsakis et al. (1984). The wrapped vials were then bagged individually in ziplock bags and stored at -25°C until quality analysis, conducted approximately 2 to 4 mo later.

3.4 Oil quality analysis methods

An analytical chloroform/methanol oil extraction procedure was performed following a modified method developed by Folch et al. (1957), on seeds and whole berry pulp (with seeds removed) of whole thawed, unprocessed berries. This extraction procedure was also carried out on seeds, pulp-flakes, and juice obtained from the pilot process. Oils recovered by the chloroform/methanol method were used as the control group against oils extracted from the technologies under evaluation, namely solvent extraction using petroleum ether, screw pressing, SCFE CO_2, and aqueous extraction (Table 3.1).

Table 3.1. Summary of oil extraction methods and oil materials. '✓' indicates recovery of oil.

Extraction method	Extracted oils (cv. Indian-Summer)				
	Unprocessed berries		Pilot processed berries		
	seeds	pulp [a]	seeds	juice	pulp-flakes
Solvent extraction	n/a	n/a	✓	n/a	✓
SCFE CO_2	n/a	n/a	✓	n/a	✓
Screw press	n/a	n/a	✓	n/a	n/a
Aqueous extraction	n/a	✓	n/a	n/a	n/a
Chloroform/methanol	✓	✓	✓	✓	✓

[a] Seeds removed.
n/a = not applicable.

3.4.1 Isolation of seeds

Seeds were isolated from unprocessed berries by placing approximately 50 g of whole thawed berries and 50 g (approximately 400 mL) of methanol into a blender. Methanol was added to aid maceration and juice removal from the

berries. The blender was operated as per the laboratory separation procedure described in section 3.2.1.3. The macerated mixture was then strained to remove excess juice and methanol. Seeds were manually separated from the remaining berry pulp using forceps. The pulp was added back to the juice and methanol mixture to give the seedless, whole berry pulp fraction.

3.4.2 Chloroform-methanol oil extraction procedure

Prior to oil extractions, all seeds were ground using a coffee grinder (Kenmore coffee grinder - 1/3 cup capacity). All seed samples were ground for 1 min following a 6-cycle regime including 10 s of grinding followed by 10 s without grinding, to avoid sample heating. No preparatory measures were conducted on the whole berry flesh, pulp-flakes, and juice samples prior to oil extractions. A chloroform:methanol (1:1, v/v) solvent mixture was prepared daily in a volume sufficient to extract oils from a set of samples (seeds, whole berry flesh, pulp-flakes, and juice). A double extraction was preformed on each sample to ensure extraction of all oil. All extractions were performed in duplicate.

Separatory funnel(s) (500 mL) fitted with filtration funnel(s) using Whatman #4 filter paper were prepared to carry out the oil extractions. Approximately 10 g of sample (seeds, whole berry flesh, pulp-flakes, and juice) was combined with 50 mL of chloroform and 50 mL of methanol and were homogenized (Heidolph Diax Homogenizer, model 900, 115 V, 900 W, 50/60 Hz, 10F Probe, 8000-24,000 RPM) for 2 min at maximum speed (speed 6, 24,000 RPM). The homogenized mixture was added to the filtration funnel(s) inserted in

the top of the separatory funnel(s). The beaker containing the homogenized mixture was rinsed with 50 mL of chloroform and added to the filtration funnel to rinse the filter paper. After filtration (when the filters were dry or no dripping from the filter was observed), the samples were removed from the filter papers. Distilled water (50 mL) was then added to each separatory funnel and stoppered to allow for gentle mixing (turing up-side-down repeatedly for 2 to 3 cycles). Vigorous mixing was avoided to prevent the formation of an emulsion. Liquid inside the separatory funnels were left overnight (15 h) to fractionate.

The remaining solid residue collected from the filter papers was re-suspended in a fresh solvent chloroform/methanol mixture (1:1 v/v, 100 mL), homogenized (2 min, maximum speed), filtered again, and allowed to fractionate overnight in the in separatory funnels. This step was conducted to complete the double extraction, ensuring an exhaustive oil extraction.

Fractionation was observed when the filtrate, settling at the bottom of the separatory funnels, was transparent. The filtrates were transferred to a weighed, round evaporating flask. Solvent evaporation to dryness (5 to 10 min at 55°C) was conducted using a rotary vacuum evaporator, a method for solvent recovery described in section 3.3.1. Iso-propanol was later added (5 mL) to assist in removing water present in the extracted oils. Again, the iso-propanol was evaporated using a rotary vacuum evaporator.

Oil masses were recorded and oils were collected for analysis. Hexane was added in three portions (2 mL, 2mL, and 1 mL) enabling complete transfer of all oil from the round evaporation flasks to 10 mL glass sample containers. All oil

samples were stored in hexane at -15°C until being analyzed approximately 2 wk later.

3.4.3 Nutritional component analysis procedures

3.4.3.1 Fatty acid compositional analysis Fatty acid composition was determined following a method modified from Yang and Kallio (2001). All oils were esterified by the following method. Approximately 50 mg of each oil were weighed (with an accuracy of 0.0001 g) and placed into 20 mL screw-top test tubes. Oil samples that were stored in solvent were weighed out after the solvent was evaporated off using nitrogen. An internal standard of 1 mg of heptadecenoic acid (C17:1) was added in 1 mL iso-octane solution. The iso-octane/internal standard mixture, in the amount of 1 mL was added to each oil sample followed by mixing to obtain a monophase system. Added to the mixture was 12 mL of 2% sulfuric acid in methanol. Samples were thoroughly mixed on a vortex shaker (Vortex Maxi Mix I, model M16700, 120 V, 60 Hz, 0.5 A, Barnstead/Thermolyne, Dubuque, IA) and then placed in an oven for 1 h at 65°C. Samples were vortexed every 5 min for the first 20 min of heating. Mixing was stopped when a monophase system was achieved while heating continued for the remaining time. Samples were allowed to cool and 6 mL of iso-octane and 6 mL of distilled water were then added to the test-tubes. Samples were mixed by turning the test-tubes upside-down. Final settling of the mixture was necessary to produce a clear upper layer fraction (esterified sample).

Approximately 1 mL of esterified sample was transferred each into dry chromatographic vials.

The fatty acid esters were analyzed using a Hewlett Packard Gas Chromatograph (GC) system (model 5890, Palo Alto, CA) equipped with programmed split/splitless injector and flame ionization detector. A Silica GC capillary column DB-23 (L = 30 m; i.d. = 0.25 mm; d_f = 0.25 µm, J & W Scientific, Folsom, CA) was used for GC analysis. The linear velocity of the carrier gas, hydrogen, was 0.5 m/s (split valve ratio 1:80). The temperature program was 155°C held for 2 min, increased at a rate of 2°C/min to 215°C, and held for 1 min. The injector and detector temperatures were operated at 250°C. The fatty acid esters were identified by comparison with a standard mixture of known composition (461, NuChek Prep, Elysian, MN) and the fatty acid composition was expressed as a mass percentage (% w/w in g/g) of the total fatty acids (mass of total fatty acid comprising total oil mass).

3.4.3.2 Tocopherol and tocotrienol anaylsis Tocopherols and tocotrienols were analyzed following a method adapted from Kallio et al. 2002b. Oils (100 mg) were dissolved in 5 mL of hexane. Tocopherols and tocotrienols were analyzed using normal-phase High Performance Liquid Chromatography (HPLC) (Shimadzu 10AD with a Shimadzu SIL-10A autoinjector, and RF-10AXL flourescence detector, Shimadzu Corporation, Kyoto, Japan). The excitation and emission wavelengths were 290 and 335 nm, respectively. A Prodigy 5µ silica column (L = 250 mm; i.d. = 3.20 mm; d_f = 5 µm, Phenomenex, St. Torrance, CA)

was used in the HPLC system. The sample injection volume was 10 µL and carrier fluid flow was 0.8 mL/min of *tert*-butyl methyl ether in hexane. The identification of individual peaks was correlated to an external calibration conducted using α-, β-, γ-, and δ-tocopherol and α-, β-, γ-, and δ-tocotrienol. Tocopherols and tocotrienols were expressed as a percentage of total tocopherols and tocotrienols analyzed in a GC vial containing oil and hexane, and were also expressed in mg/100 g of oil.

3.4.3.3 Determination of total carotenoids Total carotenoids were determined following a method modified from Gao et al. (2000). Oil in the amount of 0.1 g/10 mL of hexane was transferred to cuvettes and total carotenoids were measure at 460 nm using a spectrophotometer (Spectronic, model 3000 ARRAY, Milton Roy, Ivyland, PA). β-carotene (type II: synthetic) was used as a standard and total carotenoids were expressed in mg/100 g of oil.

3.4.3.4 Identification of sterols Sterols were analyzed by gas chromatography following a method adapted from Yang et al. (2001). Saponification of the oils were conducted prior to sterol analysis following the described method. Oils (100 mg) were weighed into 10 mL screw-top test tubes and 100 µL of internal standard was added. A mixture of 2 mg of 5α-cholestane in 1 mL of methyl-*tert* butyl ether was prepared and added to the test tubes. Finally, 2 mL of 1.0 N-methanolic potassium hydroxide was added to the test-tubes, then capped with screw-tops and vortexed to achieve a monophase solution. Test tubes were

then stored in a dark room for 18 h. Samples were then removed and deionized water and hexane in the amounts of 2 mL and 5 mL, respectively, was added. Test tubes were capped and vortexed for 10 s and contents were allowed to separated into a two-phase system. The top layers (hexane) were removed using a Pasteur pipette and emptied into separate, 3 mL screw-top test tubes. Hexane (2 mL) was added to the remaining bottom layers in the original screw-top test tubes. These samples were vortexed again for 10 s and were allowed to stand to achieve a two-phase system. Thus completing a double extraction. The top layer was again removed, and emptied into the 3 mL screw-top test tubes. Powdered anhydrous sodium sulfate (0.5 g) was added to each of the test tubes (3 mL test tubes) to remove any water present. The anhydrous sodium sulfate was allowed to settle (10 min) and the liquid was removed from each of the test tubes and were transferred to 4 mL vials. Evaporation to dryness was conducted on the vial contents under nitrogen. Iso-octane (1 mL) was added to the vials, vortexed, and transferred to GC vials.

Sterols were analyzed using Shimadzu GC-17A gas chromatograph (Shimadzu Corporation, Kyoto, Japan) with a DB-5 capillary column (L = 30 m; i.d. = 0.25 mm ; d_f = 0.25 μm, Restek, Bellefonte, PA). The column temperature was held at 60°C for 1 min, increased at 40°C/min to 240°C, held for 1 min, and finally increased at 2°C/min to 300°C held for 2 min. Hydrogen (2.2 mL/min) was used as a carrier gas. The injector and detector temperatures were 275 and 320°C, respectively. Sterols were identified by an external calibration which was conducted using campesterol, sitosterol, stigmasterol, cholesterol, and

5α-cholestane. Sterols were expressed as a mass percentage of total sterols identified in a GC vial containing oil and hexane, and were also expressed in mg/100 g of oil.

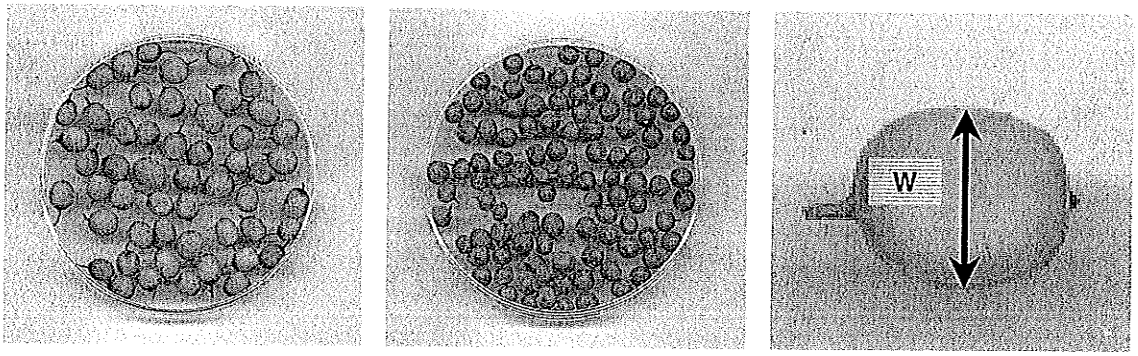
4. RESULTS AND DISCUSSION

4.1 Sea buckthorn as the experimental material

Sea buckthorn was selected as the experimental material for oil extraction trials since there has been considerable international interest in utilizing its oils (seed and pulp) in functional foods and nutraceuticals (Storey 2000). While the majority of the sea buckthorn trees in Canada are of the ssp. *sinensis* variety, at the time of this research, the trees were not mature and thus had a low berry production insufficient to fill our requirements. Thus, berries from the cv. Indian-Summer were selected because of their economic availability at the beginning of this research (starting in the Fall of 2001). *Sinensis* (ssp.) berries began to come available in the second year of this research (Fall of 2002) however, it was necessary to continue using the same materials to ensure uniformity among experimental trials.

Berries of cv. Indian-Summer (2001 harvest) were measured using a digital caliper to quantify size. Visually, cv. Indian-Summer berries tended to be oval or oblong and larger, compared with ssp. *sinensis* being more round and smaller in overall size (cv. Indian-Summer and ssp. *sinensis* berries were obtained from mature trees in November; ssp. *sinensis* berry samples were obtained from PFRA, Indian Head, Saskatchewan). The length of the minor axis or berry width (W) of cv. Indian-Summer and ssp. *sinensis* berries were measured (Fig. 4.1). A random sample of 100 berries from each variety were measured and a average berry width was determined to be 7.4 mm (S.D. = 0.7 mm) for cv. Indian-Summer and 7.1 mm (S.D. = 0.7) for ssp. *sinensis.* While the

difference in width between the two berry varieties was small (0.3 mm), ssp. *sinensis* berries were noticeably smaller in size due to their roundness. The mass of 100 berries was approximated from each variety: ssp. *sinensis* was 10 g/100 berries and cv. Indian-Summer was 20 g/100 berries. Both fell in the range of berry sizes of 4 to 60 g/100 berries indicated by Li and Schroeder (1999). The berries of cv. Indian-Summer appeared to be yellow/orange in color, while the orange color of ssp. *sinensis* was more pronounced.



(a) (b) (c)

Fig. 4.1. (a) Sample (approximately 10 g) of cv. Indian-Summer berries (2001 harvest year). (b) Sample (approximately 10 g) of ssp. *sinensis* berries (2001 harvest year). (c) Measured width, W, of cv. Indian-Summer berries.

4.2 Berry processing

4.2.1 Laboratory processing of sea buckthorn berries

Sea buckthorn berries were processed to generate seeds and pulp-flakes for oil extraction trials. A processing system extricated from the literature (see section 2.4) was evaluated and tested using laboratory scale equipment. Laboratory processing included a method for juice extraction using an ATS Universal Testing Machine equipped with a modified cheese press, dehydration using a laboratory-scale oven, and separation using a combination of threshing, sieving, and forceps to separate and recover seeds and pulp-flakes.

Juice extraction using a modified cheese press was effective in recovering juice from whole thawed berries. More than 15 preliminary juice extraction trials were run on 400 g batches of berries resulting in approximately 65 to 70% of the total mass (or approximately 500 mL) as unfiltered juice containing some residual solids in the form of pulp. An exact mass of pulp in the unfiltered juice was not quantified. No initial maceration of the berries was conducted for the preliminary laboratory juice recovery trials.

The extracted juice was opalescent/opaque, and bright orange in colour, as described in this fashion by Beveridge et al. (2002). Similar juice recovery results were obtained by Tang et al. (2001) from ssp. *sinensis*, *rhamnoides*, and other sea buckthorn hybrids, using 920 g batches, producing approximately 600 mL of filtered juice. Beveridge et al. (1999 and 2002) indicated juice yields of 55% (w/w), though yields of up to 68% (w/w) was suggested from cv. Indian-Summer, using rack and cloth or belt presses. Commercial processes of

sea buckthorn berries include methods for crushing whole berries using fruit mills prior to juice recovery (Beveridge et al. 1999). However, crushing was not conducted at the laboratory level, again to simplify processing and obtain an overall perspective for the performance of potential processing equipment. Beveridge et al. (2002) indicated that a combination of enzyme-pretreatment and maceration of berries can increase juice extraction up to 80% (w/w) however, enzyme-pretreatment was outside the scope of this project.

Press cake (pulp containing seeds after juice extraction) was manually recovered from the cheese press and dried at 50°C for 24 h. Drying parameters were established after running more than 15 drying trials. After separation using a modified blender (used for threshing) combined with sieving, the dried seed and pulp-flake moisture contents were determined to be approximately 10 and 8% (w.b.), respectively. After discussion with a process development consultant (A. Anand) at the Food Development Centre in Portage la Prairie, and a thorough review of oil extraction literature (namely pressing and solvent extraction technologies), these moisture contents fell within an acceptable range (3 to 13% w.b.) for oil extraction trials and was therefore assumed to be acceptable for sea buckthorn seeds and pulp-flakes. A low drying temperature of 50°C was selected to avoid degradation of heat labile components in the berry oils. Additional studies should be conducted to determine optimum drying temperatures and drying times to ensure optimum product quality of dehydrated seeds and pulp-flakes and moisture content range required for optimum oil recovery.

The laboratory processing procedure proved to be effective for the collection of seeds and pulp-flakes, however quantities were not sufficient for the production of materials required for oil extraction experiments. Laboratory processing losses in the form of material spillage and material waste was considered to be negligible because of the processing of small, manageable batches. However, the runs of small batch sizes (<500 g/batch) and manual separation proved to be extremely tedious, resulting in small production outputs. Manual methods of seed extraction from whole berries were explored by Berezhnaya et al. (1989), however these methods were suited for the processing and production of small quantities of materials (30 g/batch) as required by laboratory and analytical testing procedures such as providing material for the determination of fatty acid composition and lipid content.

4.2.2 Pilot process: laboratory scale-up results

A pilot process was developed based on a scale-up of the evaluated laboratory bench equipment. Sufficient quantities of material (seeds and pulp-flakes) were generated for oil extraction trials, a method which could be used for small production runs by cottage level operations. Indicated in the literature, berries are often washed prior to processing. In this research, the washing step was omitted to simplify the processing procedure and maintain better control of the mass balance of material throughout processing, a discussion to follow. Juice extraction capacity was greatly increased from 0.5 kg/batch at the laboratory level to 7 kg/batch at the pilot process level, resulting in the processing

of approximately 50 kg/h of thawed berries. Maceration of the berries was done at the pilot processing level to conform with industry convention, improving juice recovery.

Approximately 100 kg of frozen berries were used in each pilot processing batch. Two batch trials were conducted to generate sufficient experimental material. A mass balance was conducted to determine material flow through the pilot process and was reported as a mass percentage (% w/w) of the total starting material (thawed berries). Bladder pressing recovered 81.6% (standard deviation, S.D. = 2.3%) juice (containing suspended solids and pulp oil), 15.4% (S.D. = 0.9%) press cake (wet cake after juice pressing), with a 1.9% (S.D. = 1.4%) processing loss. Processing losses were quantified as unrecoverable material in the bladder press and accidental material spillage. The press cake was then dried, recovering 7.4% (S.D. = 0.5%) water, yielding 8.0% (S.D. = 0.4%) dried press cake. Mechanical separation of the dried press cake yielded 5.1% (S.D. = 0.1%) seeds, 2.6% (S.D. = 0.1%) pulp-flakes, with a 0.3% (S.D. = 0.1%) processing loss. In retrospect, 100 kg of frozen berries yielded approximately 5 kg of seeds and 3 kg of pulp-flakes.

The majority of the mass removed was in the form of moisture during juice extraction and drying operations. Yang and Kallio (2001) reported the mass percentages of seeds in frozen berries of ssp. *sinensis* and ssp. *rhamnoides* to be 6.1 and 5.9% respectively compared to 5.1% associated with cv. Indian-Summer and the pilot process employed. Storey (2000) reported a generic mass balance showing the mass percentage of seeds to be 4.2%. However, values

reported for wet pulp (69.0%) and juice (26.8%) were not in agreement with values obtained from the pilot process. This could be a result of the processing techniques employed, though were not stated in the report. Quirin and Gerard (1995) also reported a 50% (w/w) reduction in mass of press cake after drying. The ratio of dried seeds to pulp-flakes was reported to be 1.3 (Quirin and Gerard 1995) compared to 2.0 from the pilot process.

Processing losses were quantified as unrecoverable material after mechanical separation and accidental material spillage. Losses were also reported as "inseparable fractions" such as pulp-flakes containing debris (non seed and pulp materials) as well as a mixture of seeds and pulp, which could not be separated by the mechanical process. These losses were small and therefore have been reported accumulatively in the 0.3% loss associated with mechanical separation. The moisture content of the seeds and pulp-flakes after pilot processing were evaluated again and were found to be approximately 10 and 8% (w.b.), respectively, similar to the moisture content values determined after laboratory processing.

Separated seeds contained a visible portion of pulp-flakes, though was reported to comprise <0.5% of the total mass of the separated seeds fraction. Seeds comprised <1.5% of the total mass of the pulp-flake fraction. The separated seeds were refined further to remove residual pulp-flakes, using compressed air. This was done to ensure pulp-flake oil was not recovered during seed oil extraction trials. However, Manninen et al. (1997) reported using separated fractions seeds and pulp still containing minor components of pulp and

seeds, respectively as the starting material for SCFE CO_2 trials. The removal of seeds from the pulp-flake fraction was not necessary since all pulp-oil extraction trials were run on the pulp-flakes taken directly from the pilot process with no further grinding. Quirin and Gerard (1995) indicated that no oil can be extracted from seeds if they were not ground. Thus, it was assumed that throughout all extraction trials (namely solvent and SCFE CO_2) no oil from seeds would be extracted and mixed with oil obtained from pulp-flakes.

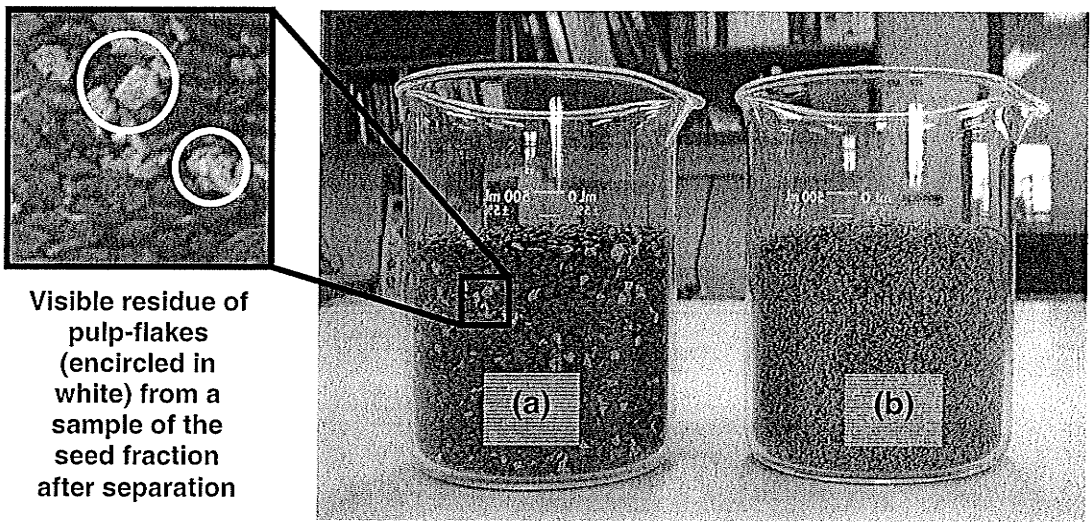


Fig. 4.2. Final samples obtained from the pilot process. (a) Seeds and (b) pulp-flakes.

4.2.3 Moisture contents of processed materials

Table 4.1 shows the moisture contents which were determined in triplicate on materials throughout the pilot process, namely thawed whole berries, press cake (wet material collected after juice was removed), and dried press cake (material collected after drying at 50°C for 24 h).

Table 4.1. Moisture contents (% wet basis, % w.b.) of selected materials during the pilot processing of sea buckthorn berries.

Batch (date)	Material	Moisture content (% w.b.)	S.D. [a] (% w.b.)
1 (February 2003)			
	Thawed berries	81.8	1.8
	Press cake	52.2	0.7
	Dried press cake	7.8	0.02
	Seeds	9.2	0.1
	Pulp-flakes	6.2	0.1
2 (May 2003)			
	Thawed berries	82.2	0.3
	Press cake	51.9	1.1
	Dried press cake	8.2	0.1
	Seeds	10.4	0.1
	Pulp-flakes	7.5	0.03

S.D. = standard deviation.
[a] Number of samples (n) = 3.

Moisture contents of seeds and pulp-flakes varied by 1.2% for seeds and 1.3% for pulp-flakes from batch 1 to batch 2, respectively. Higher moisture contents for the seeds and pulp-flakes in batch 2 may be attributed to high humidity levels inside the food processing facility at the Food Development Centre, where the pilot process was located at the time of processing (batch 1 conducted in February 2003, and batch 2 conducted in May 2003). Moisture contents were

periodically monitored throughout the course of oil extraction trials and varied approximately by ±1% for both seeds and pulp-flakes. Moisture contents of materials (seeds and pulp-flakes) were assumed to be constant during storage (approximately 1 to 4 months at -5°C) prior to oil extraction trials.

4.2.4 Particle size analysis of experimental materials

Particle sizes were quantified for pilot processed materials (100 g) including whole seeds (Fig. 4.3), pulp-flakes, seeds ground for 10 s, and seeds ground for 30 s (Table 4.2). The size of seeds and ground-seed particles were documented to provide additional descriptive information on the materials used prior to oil extractions. The majority of whole seeds (>99% w/w) fell between an aperture range of 2.00 to 3.35 mm (seed width W, or minor diameter; see Figure 4.3). Pulp-flakes (>59% w/w) fell between a size range of 0.850 to 2.00 mm, while 12% (w/w) fell between 0.425 to 0.500 mm. Seeds ground for 10 s fell between an aperture range of 0.500 to 2.360 mm (>57% w/w), while the seeds which were ground for 30 s fell between a smaller aperture range of 0.425 to 1.00 mm (>63% w/w).

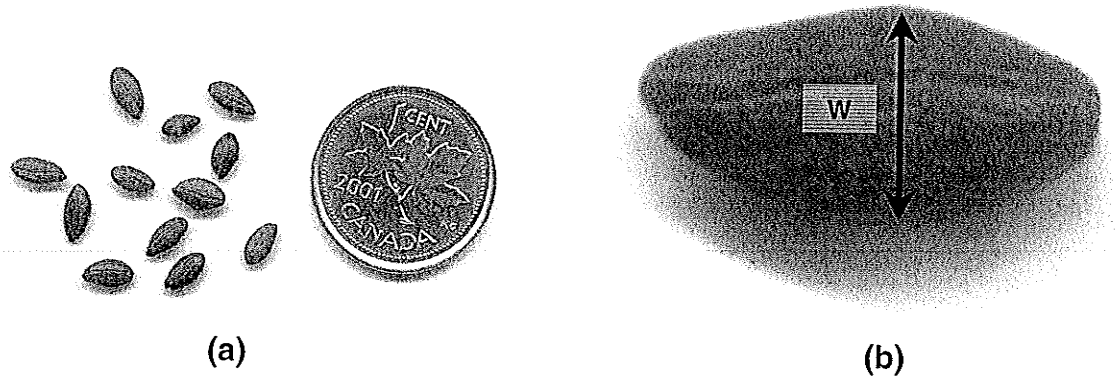


Fig. 4.3. (a) Relative size of cv. Indian-Summer seeds. (b) Measurement dimension of seeds .

Table 4.2. Particle size distribution of pilot processed materials. Means and standard deviations are presented as mass percentages (% w/w).

Aperture size (mm)	Whole seeds		Pulp-flakes		Seeds (10s grind)		Seeds (30s grind)	
	mean [b]	S.D. [a]	mean	S.D. [a]	mean	S.D. [a]	mean	S.D. [a]
3.35 - 2.36	54.2	1.4	1.4	0.1	1.8	0.1	0.0	0.0
2.36 - 2.00	45.4	1.4	4.3	0.1	5.8	0.7	0.0	0.0
2.00 - 1.70	0.4	0.04	11.6	0.1	6.5	0.3	0.9	0.1
1.70 - 1.40	0.0	0.0	15.8	0.3	10.5	0.4	1.5	0.2
1.40 - 1.18	0.0	0.0	11.6	0.3	10.9	0.3	3.3	0.1
1.18 - 1.00	0.0	0.0	11.3	0.2	14.6	0.3	8.4	0.4
1.00 - 0.850	0.0	0.0	8.9	0.1	11.0	0.5	10.8	0.6
0.850 - 0.710	0.0	0.0	6.5	0.3	10.7	0.5	15.3	0.2
0.719 - 0.600	0.0	0.0	5.3	0.1	7.5	0.1	13.9	0.1
0.600 - 0.500	0.0	0.0	6.3	0.1	6.0	0.2	12.4	0.3
0.500 - 0.425	0.0	0.0	12.4	0.4	4.3	0.3	10.7	1.3
0.425 - 0.355	0.0	0.0	3.6	0.2	2.6	0.3	4.9	0.5
<0.355	0.0	0.0	1.2	0.3	7.9	1.0	17.8	0.3

S.D. = standard deviation.

[a] n = 3.

[b] measurement of seed width, W (minor diameter).

4.3 Oil extraction trials

4.3.1 Material oil contents

Table 4.3 shows oil contents determined on seeds and pulp separated from thawed, unprocessed berries, and seeds, pulp-flakes, and juice from the pilot process, as determined by the chloroform/methanol oil extraction procedure. The chloroform/methanol extraction method accommodated both wet (whole berries) and dry (seed and pulp-flakes) materials for the determination of oil content. Pilot processed seeds and pulp-flakes were subjected to a drying operation (at 50°C for 24 h).

Table 4.3. Oil contents of seeds and pulp of thawed, unprocessed berries, and seeds, pulp-flakes, and juice from the pilot process, expressed in $\%_C$ (w/w) determined by a chloroform/methanol extraction.

Material		Harvest year			
		2001		2002	
		mean	S.D.	mean	S.D.
Thawed berries, $\%_C$					
	seeds	11.7	0.1	10.2	0.1
	pulp	2.7	0.1	2.2	0.04
Processed materials, $\%_C$ (Pilot process)					
	seeds	nda	nda	11.0	0.4
	pulp-flakes	nda	nda	19.7	2.3
	Juice [a]	nda	nda	2.0	0.3

S.D. = standard deviation.
[a] n = 3 (otherwise n = 2).
nda = no data available.

Oil contents ($\%_C$ w/w, subscript 'C' denoting oil content or total oil) of the materials were expressed as a mass percentage relating the total mass of oil (g) in the materials to the mass of the starting materials (kg). Seed oil content from

thawed berries was calculated based on g/kg of seeds. Pulp oil content was calculated based on g/kg of thawed berries. Seed, pulp-flake, and juice oil contents were calculated based on g/kg of seeds, pulp-flakes, and juice, respectively. Oil content determinations for the 2001 and 2002 harvest were conducted in September of 2003. All oil extraction trials including solvent extraction, SCFE CO_2, screw pressing, and aqueous extraction for seeds and pulp-flakes, were conducted in the summer of 2003 on berries from the 2002 harvest year.

Oil contents of the seeds and pulp appeared to be slightly higher for the 2001 harvest year than the 2002 harvest year. In the summer of 2002, drought conditions were present at the time of berry maturation, possibly contributing to the lower oil content in the seeds (1.5% lower) and pulp (0.5% lower). Frequently stated in the literature, varying oil content may be attributed to genetic factors, origin and growing environment, harvest times and maturity of berries, and method of oil isolation (Kallio et al. 2002a; Kallio et al. 2002b; Tang et al. 2001; Tang and Tigerstedt 2001; Yang and Kallio 2002a and 2002b; Yang and Kallio 2001; Yang et al. 2001; Gao et al. 2000). It appeared that the oil content of processed seeds was higher (0.8%) than that of seeds directly removed from thawed berries. However, since oil contents were expressed as the mass of oil to the mass of material and the seeds removed directly from the thawed berries were assumed to be at a higher moisture content than that of the dried seeds (approximately 10% w.b.). Thus, a direct comparison should not be made between oil contents of unprocessed and processed seeds due to differences in

moisture content. Moisture contents in this case should be compared on a dry basis, however, due to limited quantities of unprocessed seeds, moisture contents were not conducted.

Kallio et al. (2002b) determined the seed content of frozen berries in g/kg of material or % (w/w) and oil contents (%$_C$ w/w) of seeds and pulp of whole, frozen berries from ssp. *sinensis* and ssp. *mongolica*, and cv. Indian-Summer (Table 4.4). Seed content in cv. Indian-Summer did not appear to be different among harvest year, however seed contents of cv. Indian-Summer appeared to be lesser than ssp. *sinensis* (approximately 2.9%) and greater than ssp. *mongolica* (approximately 1.7%).

Table 4.4. Comparison of seed content of frozen berries (% w/w) and oil contents (%$_C$ w/w) in seeds and pulp from whole, frozen berries from ssp. *sinensis*, ssp. *mongolica*, and cv. Indian-Summer.

Variety	Harvest Year	Seed content in berries		Oil content in seeds		Oil content in pulp	
		mean [c]	S.D.[c]	mean	S.D.	mean	S.D.
Ssp. *sinensis*	1997 [a]	8.5	2.9	9.7	0.2	4.1	2.8
Ssp. *mongolica*	1997 [a]	4.0	1.2	12.6	2.3	5.9	2.7
cv. Indian-Summer	2001 [b]	5.7	0.2	11.7	0.1	2.7	0.1
	2002 [b]	5.6	0.5	10.2	0.1	2.2	0.04

S.D. = standard deviation.
[a] Results by Kallio et al. (2002b), n = 5.
[b] n = 2.
[c] mean and standard deviation in % w/w (otherwise %$_C$ w/w).

Oil content of the seeds of cv. Indian-Summer appeared to be greater (2% for 2001 and 0.5% for 2002) when compared to ssp. *sinensis*, and only slightly lower (0.9% for 2001 and 2.4% for 2002) than ssp. *mongolica*. No trend was apparent

between the seed content and seed oil content for the listed varieties. Pulp oil content was approximately 50% greater in ssp. *sinensis* and ssp. *mongolica* when compared to cv. Indian-Summer. Results listed in Table 4.4 only represent guide values for seed and oil contents among the varieties since moisture contents of the materials (namely, ssp. *sinensis* and ssp. *mongolica*) were not known. Oil contents for cv. Indian-Summer seeds in relation to frozen berry mass was 0.2%$_C$ and 0.1%$_C$ for 2001 and 2002 harvest years, respectively.

In addition to quantifying the seed content in the berries, pulp content (% w/w) was determined during the isolation of seeds prior to chloroform/methanol extractions. The remaining fraction of pulp and juice (pulp and juice content, % w/w) after seeds were removed was 94.4% (w/w). The juice fraction was identified to represent 81.6% (w/w) of whole berries (determined by the pilot process). Thus, the pulp fraction was 12.8% (w/w). The oil content of the pulp and juice fractions was 2.2 and 2.0%, respectively. Thus, the total pulp oil content (% w/w of whole berries) from pulp and juice fractions was 4.4%$_C$.

An important distinction should be noted between the definition of oil content and oil recovery as related to the following sections on solvent extraction, SCFE CO_2, screw pressing, and aqueous extraction trials. Oil content was described as the total amount of oil contained in a material (%$_C$ w/w). Oil recovery represents the amount of oil recovered (%) in relation to the oil content (%$_C$). Again, assuming that moisture contents of materials were held constant over the course of extraction experiments, comparison of oil recoveries from the extraction technologies under evaluation can be made.

4.3.2 Solvent extraction

Oil recoveries were expressed as mass of oil extracted per mass of processed material (seeds or pulp-flakes were used as the material for solvent extraction trials) (g/kg or % w/w). The amount of oil extracted from the seeds and pulp-flakes of cv. Indian-Summer (in triplicate) was found to be 8.2%$_C$ (S.D. = 0.4%) and 11.9%$_C$ (S.D. = 0.1%), respectively. During extraction, miscella temperatures of the seed and pulp oils were measured and ranged from 43 to 44°C and 44 to 45°C, respectively. The seed oil was bright yellow and liquid at room temperature (20°C) while the pulp oil was red and began to solidify immediately when disconnected from the rotary vacuum evaporator operated at 50°C. Seed and pulp oils contained no visible solids. Based on the review of literature, solvent extraction is an exhaustive oil recovery method, often exceeding 99% recovery of oil from materials. Solvent extraction using petroleum ether was chosen to represent ideal recoveries (assumed 100% extraction) relative to the other technologies under evaluation, namely SCFE CO_2 and screw pressing. Aqueous extraction oil recoveries were compared to the chloroform/methanol oil extraction procedure due to the nature of the starting material (whole berries).

4.3.3 Supercritical fluid extraction

Figure 4.4 shows the maximum oil recovered using SCFE CO_2 compared to conventional solvent extraction using petroleum ether. Extracted oil was expressed as the mass of oil extracted per mass of processed material (seeds or pulp-flakes) (g/kg or % w/w). Extraction trials were conducted on seeds, ground for 10 s and 30 s. After 6 h, the amount of oil recovered from seeds ground for 30 s (65.1%, S.D. = 1.2%) nearly doubled the oil recovered from seeds ground for 10 s (37.0%, S.D. = 5.0%). Evident from the review of literature, the length of grinding time had a dramatic effect on increasing the amount of oil extracted from the seeds. Longer grinding times reduced particle size, thereby increasing solvent (CO_2) contact with the oil-bearing material, consequently increasing oil recoveries (del Valle and Uquiche 2002). Pulp oil was recovered more effectively, extracting 86.3% (S.D. = 13.6%) of the available oil, which was comparable to a petroleum ether extraction assumed to recover 100%. While SCFE CO_2 can be more selective than conventional solvents (such as hexane and petroleum ether) in recovering oils, additional compounds such as waxes may have been recovered under the harsh conditions employed during the SCFE CO_2 trials (namely high extraction pressures exceeding 30 MPa) (Brogle 1982). In this research, the amount of oil extracted by SCFE CO_2 may reflect an overestimate of the amount of oil that the SCFE technology recovered.

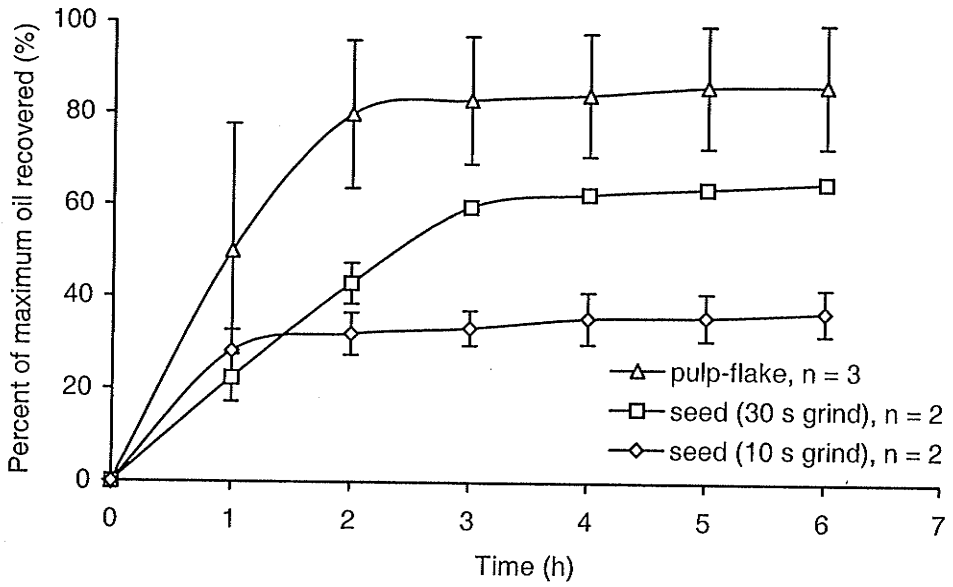


Fig. 4.4. Percent of maximum oil recovered from sea buckthorn seeds and pulp-flakes using a SCFE CO_2 system operating for 6 h (measurements taken at 1 h increments). A maximum of 100% represents solvent extraction using petroleum ether. Note: standard deviations for the curve "seeds (30 s grind)" are smaller than the data points shown for 3, 4, 5, and 6 h.

Results also indicated that a 3 h extraction was sufficient in reaching greater than 90% of the maximum oil extraction limit determined at 6 h (for seeds (10 s and 30 s grind) and pulp-flakes (37.0%, 65.1%, and 86.3%, respectively)) using SCFE CO_2 (Fig. 4.4). Four additional trials were conducted over 3 h, each for seeds (30 s grind) and pulp-flakes. The purpose of these additional trials was to ensure enough oil had been collected for future quality analysis. From these trials, the maximum oil recoveries from seeds (30 s grind) and pulp-flakes were 51.4% (S.D. = 9.9%) and 83.7% (S.D. = 10.5%), respectively. Thus, SCFE CO_2 oil recovery ranges for seeds (30 s grind) and pulp-flakes were 51.4 to 65.1% and 83.7% to 86.3%.

Reducing the extraction time from 6 h to 3 h also reduced CO_2 consumption. Vented volume of CO_2 (CO_2 consumption in litres) was measured during the extraction of oil from seeds (10 s and 30 s grind) and pulp-flakes over a 6 h and 3 h extraction period (Table 4.5). Vented volume of CO_2 was also measured during the 4 additional oil extraction trials for seeds (30 s grind) and pulp-flakes. Reducing the extraction time from 6 h to 3 h resulted in approximately 50% savings of CO_2 by volume. A 3 h extraction time was also noted to be reasonable according to industrial practices for the recovery of seed and pulp oil from sea buckthorn (Dr. P. May, FLAVEX Naturextrakte GmbH, Rehlingen, Germany, personal communication).

Table 4.5. Measured volume of CO_2 consumed in liters (vented CO_2) after 6 h and 3 h of supercritical fluid extraction using carbon dioxide.

Material	Time length of extraction (h)			
	6 h		3 h	
	mean	S.D.	mean	S.D.
Seeds (10 s grind) [a], L	1334	69	830	76
Seeds (30 s grind) [a], L	1091	76	494	99
Pulp-flakes [b], L	2300	356	986	284
Seeds (30 s grind) [c], L	n/a	n/a	438	66
Pulp-flakes [c], L	n/a	n/a	975	291

S.D. = standard deviation.
n/a = not applicable.
[a] n = 2.
[b] n = 3.
[c] n = 4.

Large fluctuations in gas flow were encountered during extraction of oils from both seed and pulp-flakes. This was largely attributed to the insufficient pressurization of the extraction vessel in the static extraction mode (A. Anand,

Process Development Consultant, Food Development Centre (FDC), Portage la Prairie, MB, personal communication). In addition, steady flow rates were difficult to achieve due to high sensitivity of the needle control valve. Fluctuations in gas flow resulted in a large variation of total CO_2 consumption (146 to 383 L/h) and corresponding standard deviations.

Solubilities were calculated by first converting the measured CO_2 consumption (L) to a mass (kg) (see Table 4.6) using the Ideal Gas Law equation (4.1).

$$M = \frac{n}{W_m} \cdot 100 = \frac{P \cdot V}{R \cdot T} \qquad \textbf{(4.1)}$$

where:

M = mass of CO_2 gas consumed by extraction, kg
N = number of moles, mol
W_m = molecular weight of CO_2, 44 g/mol
P = atmospheric pressure, assumed at 1 atm
V = volume of CO_2 gas consumed by extraction, L
R = universal gas constant, 0.0805 L· atm · mol^{-1} · K^{-1}
T = temperature, assumed at 294 K

The mass of extracted oil (kg) was then divided by the mass of CO_2 consumed by extraction. The solubilities of seed and pulp oil in CO_2 are presented in Table 4.7.

Table 4.6. Calculated mass of CO_2 consumed in kilograms (vented CO_2) after 6 h and 3 h of supercritical fluid extraction using carbon dioxide.

Material	Time length of extraction (h)			
	6 h		3 h	
	mean	S.D.	mean	S.D.
Seeds (10 s grind) [a], kg	2.5	0.1	1.5	0.1
Seeds (30 s grind) [a], kg	2.0	0.1	0.9	0.2
Pulp-flakes [b], kg	4.3	0.7	1.8	0.5
Seeds (30 s grind) [c], kg	n/a	n/a	0.8	0.1
Pulp-flakes [c], kg	n/a	n/a	1.8	0.5

S.D. = standard deviation.
n/a = not applicable.
[a] n = 2.
[b] n = 3
[c] n = 4.

Similarly, a 50% mass savings of CO_2 gas was evident when extraction durations were decreased from 6 to 3 h. The scale method of determining the mass of CO_2 was of low accuracy (0.2 kg error) and instability of the cylinder on the scale caused fluctuations in measurement, thus only providing some indication of the amount of CO_2 consumed during an extraction. It was assumed that the measured volume of CO_2 was accurate and the conversion of volume into mass was comparable to the measured mass determined from the scale method.

Solubility of seed oil ranged from 0.17% (10 s grind) to 0.37% (30 s grind) for a 6 h extraction and ranged from 0.27% (10 s grind) to 0.83% (30 s grind) for a 3 h extraction (Table 4.7). Solubilities tended to double when extraction durations decreased from 6 to 3 h and with increased grinding from 10 to 30 s. Solubility of pulp oil was approximately 0.40%. Quirin and Gerard (1995) reported seed and pulp-flake oil solubilities of 0.62 and 0.76% (extraction

conditions of 45°C and 32 MPa; sea buckthorn ssp. or cv. was not indicated; berries harvested in Lithuania).

Table 4.7. Solubilities of seed and pulp oils expressed as % (w/w).

Material	Time length of extraction (h)			
	6 h		3 h	
	mean	S.D.	mean	S.D.
Seeds (10 s grind) [a], %	0.17	0.03	0.27	0.01
Seeds (30 s grind) [a], %	0.37	0.02	0.83	0.18
Pulp-flakes [b], %	0.17	0.001	0.41	0.08
Seeds (30 s grind) [c], %	n/a	n/a	0.72	0.08
Pulp-flakes [c], %	n/a	n/a	0.40	0.08

S.D. = standard deviation.
n/a = not applicable.
[a] n = 2.
[b] n = 3.
[c] n = 4.

Šťastová et al. (1996) reported slightly higher solubilities of seed and pulp oil in the range of 0.65 to 0.74% and 0.79 to 0.86%, respectively (at optimal extraction conditions of 40°C and 27 MPa compared to 25°C/27 MPa and 40°C/17.4 MPa; again sea buckthorn ssp. or cv. was not indicated; berries harvested in the Czech Republic). It is suspected that solubilities >0.40% can be achieved through particle size manipulation by grinding (a grinding operation was not performed on the pulp-flakes). Grade of grinding (reduction of material size) was reported to have the largest effect on solubility and ultimately oil recovery from seeds and dried pulp (Šťastová et al. 1996).

Quirin and Gerard (1995) indicated that the pulp oil of dried sea buckthorn pulp was red and a semisolid. In addition, temperatures above 30°C returned the

oil back into liquid form. Seed oil was of a clear yellow-brown colour, liquid at room temperature. Similar oil characteristics were observed for cv. Indian-Summer seed and pulp oil.

4.3.4 Screw pressing

Screw pressing was conducted on seeds and pulp-flakes produced from the pilot process. Table 4.8 summarizes screw pressing trials highlighting material flow rates and maximum oil recoveries (%).

Table 4.8. Data summary of screw pressing trials on seeds.

Trial	Flow rate of seeds (kg/h)	Flow rate of unfiltered oil (g/h)	Oil sediment (g)	Flow rate of filtered oil (g/h)	Oil recovered (% w/w)
1	2.07	73.8	0.44	70.80	41.7
2	2.10	71.4	0.29	69.60	40.4
3	2.01	70.2	0.27	68.40	41.5
4 [a]	1.92	72.0	nda	nda	45.7
mean	2.06	71.8	0.33	69.60	41.2
S.D.	0.05	1.8	0.09	1.20	0.7

S.D. = standard deviation.
[a] Trial 4 not included in the mean and S.D.

The extracted, unfiltered oil was of a yellow/brown color and cloudy due to extracted foots (sediment) in the oil. Screw pressing proved to be inadequate for recovering sufficient quantities of oil from sea buckthorn seeds. A maximum of 41.7% of oil was recovered in relation to solvent extraction using petroleum ether. Screw pressing is generally considered as an unfeasible method for recovering oil from seeds having low oil contents (<20%$_C$). Bockisch (1998, p.

380) stated that the residual oil remaining in the meal or cake after screw pressing may account for a substantial portion of the total oil content, if the seed oil content is low. No oil was recovered from pulp-flakes by screw pressing. Literature describes screw pressing as a process primarily used for expelling oil from seeds or other granular based materials (Bargale et al. 1999; Singh and Bargale 2000; Abu-Arabi et al. 2000; Oomah et al. 2000; Singh et al. 2002a and 2002b; Ajibola et al. 1990; Fasina and Ajibola 1989; and Adeeko and Ajibola 1990). Pulp-flakes did not provide the resistance necessary during screw pressing to reach sufficient pressures required to extract the oil.

Table 4.9 shows temperatures at various locations (Fig 4.5) on the screw press including extracted oil temperatures. Temperature was monitored at 5 s intervals during screw pressing.

Table 4.9. Extraction temperatures of oil at various of locations on the screw press.

Thermocouple location	Temperature (°C)							
	Trial 1		Trial 2		Trail 3		Trail 4	
	mean	S.D. [a]	mean	S.D. [a]	mean	S.D. [a]	mean	S.D. [a]
1 Feed inlet	25.8	5.0	27.1	5.6	26.2	7.0	29.0	3.6
2 Extracted oil stream	63.0	7.4	65.3	5.1	66.9	5.8	69.6	3.8
3 Collected oil	26.6	0.6	28.7	0.7	30.0	0.6	33.4	0.8
4 Screw press barrel	55.1	2.3	58.1	3.4	56.0	3.1	47.3	1.5
5 Heater ring	107.0	18.2	108.3	15.9	112.5	17.0	102.3	14.0
6 Press cake outlet	97.9	8.4	103.0	8.5	101.5	9.0	100.7	8.8
7 Ambient	25.9	0.3	26.0	0.4	26.4	0.4	nda	nda

S.D. = standard deviation.
[a] n = 127 (127 readings at 5 s intervals).

The highest oil temperatures were evident at the oil outlet point (extracted oil stream, number 2), ranging from 63 to 70°C. These temperatures fell outside the

limit for classification of cold pressed oil (<60°C) as described by Bockisch (1998, p.384). Extraction trials were conducted with and without operation of the heater ring, though pressing without heat resulted in clogging of the screw press and thus no oil could be extracted.

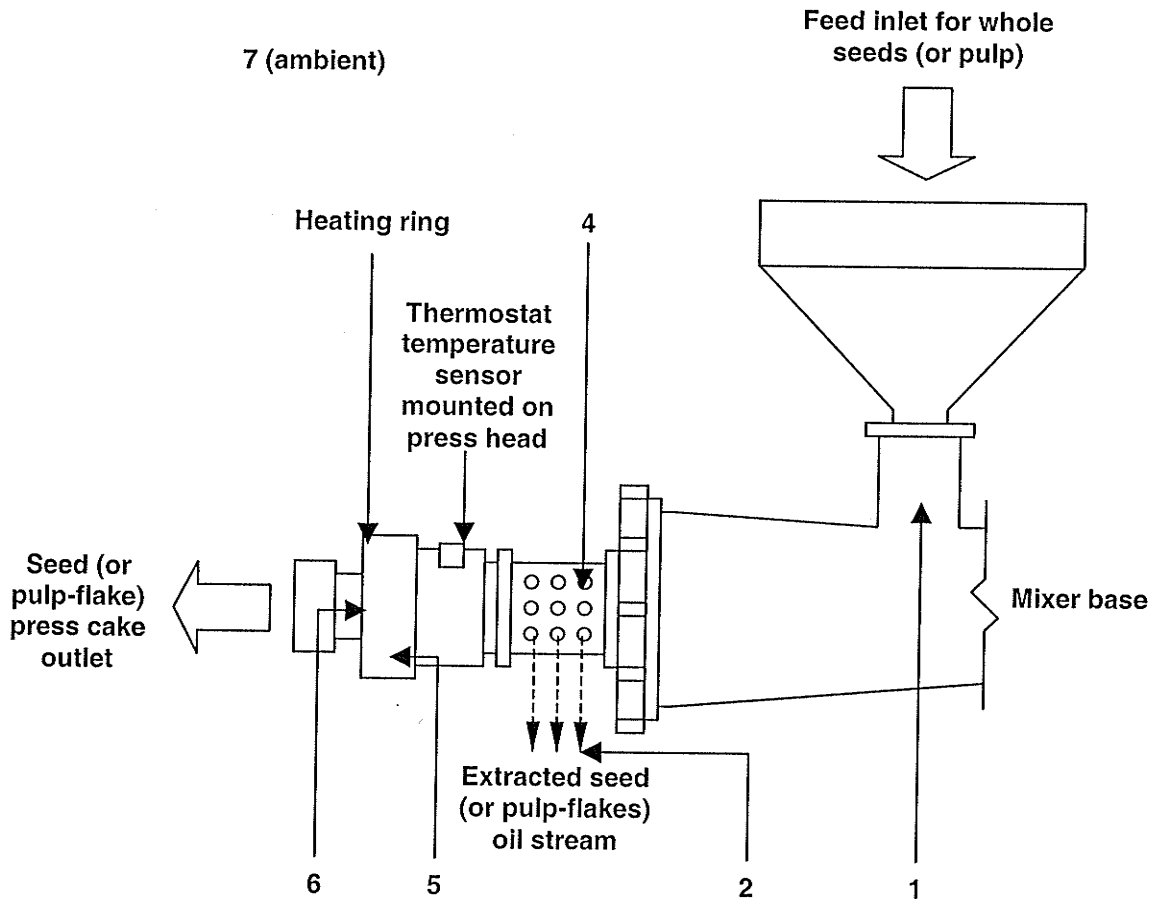


Fig. 4.5. Thermocouple locations for temperature monitoring during screw pressing. (1) Seed and pulp-flake feed inlet, (2) extracted oil stream, (3) collected oil, (4) screw press barrel, (5) heater ring, (6) press cake, (7) ambient.

4.3.5 Aqueous extraction

A preliminary extraction trial was conducted at Westfalia Separator AG (Oelde, Germany) to develop a water-based process which could be used to recover oil from sea buckthorn seeds and berry pulp. The extraction process used to recover these oils simulated a generic aqueous extraction process, not the patented FRIOLEX® process since process details were not disclosed for proprietary reasons. Oil recoveries and oil quality obtained from the simulated aqueous extraction process do not necessarily reflect oil recoveries and oil quality of the FRIOLEX® process. Table 4.10 highlights separated material fractions during a laboratory aqueous extraction technique.

Table 4.10. Material fractions and oil recoveries from sea buckthorn berries (expressed as % w/w) from an aqueous oil extraction procedure.

Trial	Material fractions			
	Solids	Juice [a]	Cream layer	
			Residual solids	Pulp oil
1 [b]	~30	~55	~12	>2
2	29.1	59.3	11.4	0.20
3	26.3	57.4	16.2	0.10
4	23.7	62.3	13.9	0.10
Mean [c]	26.4	59.7	13.8	0.13
S.D. [c]	2.7	2.5	2.4	0.06

[a] Oil and solids removed.
[b] Trial conducted at Westfalia Separator AG, Oelde, Germany on 2001 berry harvest; all other trials (2 to 4) conducted on 2002 berry harvest.
[c] On trials 2 to 4 (n = 3).

Predominant material fractions were identified, namely solids (26.4%), juice (59.7%, oil and solids removed), and a cream layer comprised of residual solids (13.8%) and pulp oil (0.13%). It was assumed that the majority of pulp oil and

suspended solids were removed from the juice after centrifuge operations. Beveridge et al. (1999 and 2002) also described the cream layer as a solidified floating layer containing a mixture of suspended solids and pulp oil. Results obtained from an aqueous extraction process at Westfalia indicated a recovery of approximately 75% (2001 harvest year) compared to the maximum oil content of 2.7%$_C$ (2002 harvest year, see Table 4.4) determined from a chloroform/methanol extraction from whole, thawed berries. Only one trial was carried out at Westfalia (using 95% EtOH) and was conducted primarily to determine the recovery of sea buckthorn seed and pulp oil using a process similar to FRIOLEX®. However, the oil recovery and materials obtained is of low confidence. Numbers shown are only approximate values and should only be used as evidence that the aqueous process was effective in removing oil from whole berries. Aqueous trials were replicated (3 trials in total) at the Food Development Centre (1 trial, using 95% EtOH) and at the University of Manitoba (2 additional trials, using 99% EtOH). In relation to the oil content determined on whole berry pulp from a chloroform/methanol extraction (2.2%$_C$, see Table 4.4), an average of 6% of oil was recovered, representing a substantial difference compared to the amount of oil recovered at Westfalia (75%).

Low oil recoveries obtained from the 3 trials conducted in Manitoba, indicate that the process conditions were not closely replicated. Processing parameters such as extraction temperature and centrifuge speed may need to be optimized to achieve an oil recovery achieved at Westfalia. Moisture content differences and variation in oil contents among berry batches (harvest years)

used at Wesfalia and trials run at the Food Development Centre may also be factors which contributed to the discrepancies in the amounts of oil extracted. However, in all aqueous extraction trials, the pulp oil was characterized as having a visually attractive dark-red color, clear, a pleasant mild fruity smell, and has remained as a liquid at room temperature (stored for 15 mo in a clear glass vial, nitrogen filled).

Seeds of the ssp. *sinensis* having an oil content of approximately 8.5%$_C$ (12% w.b.) were used as preliminary material for seed oil aqueous extraction trials since seeds from cv. Indian-Summer berries were presently not available from the pilot process. No oil was recovered from the seeds at Westfalia, Food Development Centre, or at the University of Manitoba. Westfalia commented that the particle size of ground seeds may need to be optimized to recover oil. FRIOLEX® has been shown to be effective on materials with oil contents >12%, which may be reflective of why seed and pulp oils were not effectively recovered using an aqueous extraction process.

4.3.1 Oil recovery summary

Table 4.11 summarizes oil recoveries for the extraction technologies under evaluation. As noted in the table, 'n/a' represents trials which were not conducted (a function of technology limitations for processing and oil extraction on the specified materials) and 'n/o' represents trials which were conducted though no oil was recovered.

Table 4.11. Summary of oil recovery data from solvent extraction, SCFE CO_2, screw pressing, and aqueous extraction trials.

Extraction method	Oil recovery (%)			
	Unprocessed berries		Pilot processed berries	
	seeds	pulp	seeds	pulp-flakes
Solvent extraction	n/a	n/a	100	100
SCFE CO_2	n/a	n/a	65.1	86.3
Screw press	n/a	n/a	41.2	n/o
Aqueous extraction	n/o	6 [a]	n/a	n/a

[a] Oil recovery based on chloroform/methanol oil content determination.
n/a = not applicable.
n/o = no oil recovered.

Oil recoveries of SCFE CO_2 and screw pressing trials were compared with solvent extraction trails being a common exhaustive method for oil recovery. Aqueous extraction trials were compared with the oil content determined by a chloroform/methanol extraction. The reason for not comparing aqueous recoveries with solvent extraction trials can be explained by the limitations of the technologies. The solvent extraction technique was limited to dried materials (approximately 10% w.b.). At best, results obtained from the chloroform/methanol determination of oil content was used to provide an indication of the effectiveness of the aqueous extraction technique on the extraction of pulp oil. While solvent extraction employing petroleum ether has been recognized as an acceptable method for oil content measurement, a direct comparison should not be made to that of the chloroform/methanol extraction method for oil content determination. Non-polar lipids such as triacylglycerols are more soluble in non-polar solvents such as petroleum ether. Chloroform, being a polar solvent has the tendency to dissolve polar lipids such as

phosopholipids. Thus, oil content can be determined, employing either petroleum ether or chloroform as the extraction solvent, however the components of the extracted oil can greatly differ (Johnson and Lusas 1983). Oil recoveries from the extraction technologies under evaluation represent unrefined oil. Extracted oils may contain unrelated oil components or impurities which may enhance oil recovery values.

Based solely on the mass of oil recovered, SCFE CO_2 has shown to clearly be the preferred technology for extracting oil from dried materials of sea buckthorn, namely seeds (65.1%) and pulp flakes (86.3%). Screw pressing was effective for recovering seed oil, however, at lower recoveries (approximately 40%) than that of SCFE CO_2. Higher extraction temperatures (>60%) during screw pressing may have destroyed heat sensitive, nutritional components in the oil. Daukšas et al. (2002) reported oil recoveries from *Nigella damascena* L. seed using cold press, Soxhlet extraction (diethyl ether) and SCFE CO_2 (40°C and 35 MPa). Assuming 100% recovery of oil from a soxhlet extraction, cold press and SCFE CO_2 oil recoveries were reported as 23 and 91%, respectively. Low oil recoveries from cold pressing was attributed to low efficiency of the mechanical press. Muuse et al. (1994) reported an oil recovery of 40%, oil pressed directly from whole *Dimorphotheca pluvialis* L. seeds using a screw press.

4.4 Oil quality

Oil quality was evaluated for cv. Indian-Summer berry oils (November 2001 and November 2002 harvest) in September 2003, indicating a storage period of 23 and 11 mo, respectively before quality analysis. The discussion on oil quality will be limited to selected components of fatty acids having concentrations >1% (<1% are trace amounts and have been placed in Appendix A5), tocopherols and tocotrienols having concentrations >1% (<1% are trace amounts and have been placed in Appendix A6), total carotenoids (Appendix A7) and sterols (Appendix A8). Refer to Appendix A4 to A8 for a complete listing of oil quality components (see Appendix A9 for a list of oil quality components for selected oil crops). Only 1 sample was analyzed (in duplicate) for each harvest year (2001 and 2002), material (seeds, pulp, and pulp-flakes), and oil extraction technology (solvent extraction, SCFE CO_2, screw pressing, and aqueous extraction). Oil availability, cost of quality analysis testing, and testing duration limited the number of samples which could be analyzed.

4.4.1 Fatty acids

Major fatty acids and their concentrations (>98%) in seed oil obtained from a chloroform/methanol extraction are presented in Table 4.12. Concentrations are expressed as a mass percentage (%, w/w in g/g) of total fatty acids (Appendix A5). Linoleic (18:2*n*-6) and linolenic acid (18:3*n*-3) were the predominant fatty acids found in the seed oil, with average concentrations of 35

and 36%, respectively. Literature indicated similar fatty acids concentrations among those presented in Table 4.12 (Yang and Kallio 2002a).

Harvest year (2001 and 2002) had some effect on fatty acid concentrations. Seeds (oil) from the 2002 harvest year had lower concentrations of oleic (18:1*n*-9) (13.5%) and linolenic acid (35.5%) and a higher concentration of linoleic acid (36.2%), compared to the 2001 harvest year. Processing (drying of seeds at 50°C for 24 h) had little or no effect on fatty acid concentrations.

Table 4.12. Major fatty acids and concentration levels in seed oil expressed as a mass percentage (% w/w) of total fatty acids.

Fatty acid (Common name)	Chloroform/methanol extraction					
	Seed (2001)		Seed (2002)		Processed seed (2002)	
	mean	S.D. [a]	mean	S.D. [a]	mean	S.D. [a]
16:0 (Palmitic)	7.8	0.1	7.5	0.1	7.5	0.1
18:0 (Stearic)	3.2	0.01	2.9	0.03	2.8	0.01
18:1*n*-9 (Oleic)	15.0	0.1	13.5	0.001	13.4	0.03
18:1*n*-7 (11-Octadecanoic)	2.3	0.03	2.3	0.01	2.3	0.02
18:2*n*-6 (Linoleic)	33.4	0.2	36.2	0.2	36.3	0.2
18:3*n*-3 (Linolenic)	36.3	0.004	35.5	0.03	35.9	0.2

S.D. = standard deviation.
[a] n = 2.

All standard deviations presented represent repeatability of the analysis method only, unless otherwise stated. While descriptive comments can be made on the effect of harvest year and processing on fatty acid concentrations, definitive conclusions should not be drawn. Analysis was conducted on single samples replicated for confirmation of measurement repeatability, and samples do not necessarily represent the total population.

Table 4.13 shows the major fatty acids (>98%) in seed oil from solvent extraction, SCFE CO_2, and screw pressing trials. No dramatic differences in fatty acid concentrations were apparent among extraction methods. Screw pressing produced an oil having a lower concentration of palmitic acid (16:0) (6.7%) and a higher concentration of linolenic acid (38.5%) compared to solvent extraction and SCFE CO_2 trials.

Table 4.13. Major fatty acids and concentrations in seed oil from solvent extraction, SCFE CO_2, and screw press trials (expressed as a mass percentage (% w/w) of total fatty acids).

Fatty acid (Common name)	Seed (2002)					
	Solvent extraction		SCFE CO_2 [a]		Screw press	
	mean	S.D. [b]	mean	S.D. [b]	mean	S.D. [b]
16:0 (Palmitic)	7.0	0.1	7.2	0.3	6.7	0.2
18:0 (Stearic)	2.6	0.04	2.4	0.1	2.5	0.01
18:1*n*-9 (Oleic)	13.6	0.02	13.0	0.3	13.6	0.04
18:1*n*-7 (11-Octadecanoic)	2.1	0.1	1.9	0.01	1.9	0.05
18:2*n*-6 (Linoleic)	35.5	0.2	35.9	0.1	35.3	0.1
18:3*n*-3 (Linolenic)	37.4	0.1	37.9	0.1	38.5	0.2

S.D. = standard deviation.
[a] A selected trial of 3 h extraction, 30 s grind (similar results were found for 6 h / 10 s and 30 s grind).
[b] n = 2.

There was no difference in fatty acid composition of seed oil, between SCFE CO_2 trials conducted at 3 and 6 h extraction durations. In addition, there was no difference in fatty acid composition as related to the grind times (10 and 30 s). Optimal grind times are required to minimize undesirable effects such as the production of free fatty acids (FFAs) which can be increased as high as 40 to 50% by grinding (Daukšas et al. 2002). Free fatty acids were not measured in this research.

Variation among data presented in Table 4.13 may be attributed to length of storage time of berries, processed materials, and oil prior to fatty acids determination. In addition, the fatty acid compositions are merely descriptive and do not necessarily reflect extraction technology performance.

Major fatty acids and their concentrations (>98%) were determined from pulp oil extracted by chloroform/methanol (Table 4.14). Palmitic and palmitoleic acid (16:1) were the predominant fatty acids found in the pulp and pulp-flake oil, with average concentration levels of 35 and 36%, respectively. Literature indicated similar fatty acid concentrations among those presented in Table 4.14 (Yang and Kallio 2002b). Harvest (2001 and 2002) year had some effect on fatty acid concentrations of unprocessed pulp from whole thawed berries (see Table 4.14).

Table 4.14. Major fatty acids and concentration levels in pulp oil expressed as a mass percentage (% w/w) of total fatty acids.

Fatty acid (Common name)	Chloroform/methanol extraction							
	Pulp (2001)		Pulp (2002)		Pulp-flakes (2002)		Juice (2002)	
	mean	S.D.	mean	S.D.	mean	S.D.	mean	S.D. [a]
16:0 (Palmitic)	39.8	0.3	34.4	0.8	34.8	0.3	34.4	0.8
16:1 (Palmitoleic)	35.8	0.1	37.5	0.7	34.4	0.01	38.4	0.4
18:0 (Stearic)	1.3	0.04	1.2	0.01	1.2	0.005	1.0	0.03
18:1*n*-9 (Oleic)	3.3	0.1	3.0	0.04	3.4	0.03	3.4	0.1
18:1*n*-7 (11-Octadecanoic)	5.9	0.1	7.4	0.2	7.1	0.03	7.4	0.2
18:2*n*-6 (Linoleic)	11.0	0.04	13.2	0.02	13.5	0.1	12.7	0.1
18:3*n*-3 (Linolenic)	1.0	0.04	1.4	0.01	2.0	0.2	1.3	0.03
24:1 (Nervonic)	0.2	0.001	0.1	0.02	1.1	0.01	0.02	0.03

S.D. = standard deviation.
[a] n = 3 (otherwise n = 2).

Processing (pulp-flake production by drying at 50°C for 24 h) reduced the concentration of palmitoleic acid (2002 harvest year) to 34.4% (from 37.5% for unprocessed pulp in the 2002 harvest year) and increased the concentration of nervonic acid (24:1) from an average of 0.2 to 1.1%. Again, variation among data presented in Table 4.14 may be attributed to length of storage time of berries, processed materials, and oil prior to fatty acids determination.

Fatty acid composition of juice oil was similar to that of pulp and pulp-flakes (2001 and 2002) indicating that juice and pulp oil are the same. Again, while descriptive comments can be made on the effect of harvest year and processing on fatty acid concentrations, definitive conclusions should not be drawn. Analysis was conducted on single samples replicated for confirmation of measurement repeatability only, and samples do not necessarily represent the total population.

Table 4.15 shows the major fatty acids and their concentrations (>98%) in pulp oil from solvent extraction (pulp-flakes), SCFE CO_2 (pulp-flakes), and aqueous extraction (thawed whole berries) trials. Overall, fatty acid concentrations in pulp oil did not change with extraction method or starting material (unprocessed pulp and pulp-flakes). Palmitoleic acid concentration was only slightly higher (approximately 2.9%) for aqueous extracted oil compared to solvent extraction and SCFE CO_2 trials. Aqueous extracted pulp oil (from whole thawed berries) had the lowest concentration of nervonic acid (0.05%). Again, there was no difference in fatty acid composition in pulp oil, between SCFE CO_2

trials conducted at 3 and 6 h durations (a grinding operation was not conducted on pulp-flakes).

Table 4.15. Major fatty acid and concentrations in pulp oil from solvent extraction, SCFE CO_2, and aqueous extraction trials (expressed as a mass percentage (% w/w) of total fatty acids).

Fatty acid (Common name)	Pulp (2002)					
	Solvent extraction		SCFE CO_2 [a]		Aqueous extraction	
	mean	S.D. [b]	mean	S.D. [b]	mean	S.D. [b]
16:0 (Palmitic)	35.2	0.1	35.5	0.01	34.4	0.004
16:1 (Palmitoleic)	35.0	0.03	36.3	0.1	38.5	0.01
18:0 (Stearic)	1.2	0.01	1.1	0.0	1.1	0.00
18:1*n*-9 (Oleic)	3.3	0.1	3.5	0.03	3.2	0.01
18:1*n*-7 (11-Octadecanoic)	6.9	0.01	6.9	0.05	7.3	0.02
18:2*n*-6 (Linoleic)	12.8	0.01	12.4	0.1	13.0	0.1
18:3*n*-3 (Linolenic)	1.5	0.01	1.2	0.005	1.1	0.01
24:1 (Nervonic)	1.3	0.1	0.9	0.02	0.05	0.01

S.D. = standard deviation.
[a] 3 h extraction, 30 s grind.
[b] n = 2.

While data presented on seed and pulp oil was consistent with harvest years and effect of processing, variation may be attributed to length of storage time of berries, processed materials, and oil prior to fatty acid determination. In addition, the fatty acid compositions listed in Table 4.15 are merely descriptive and do not necessarily reflect extraction technology performance.

4.4.2 Tocopherols and Tocotrienols

Concentrations (>98%) of tocopherols and tocotrienols in seed oil, extracted by chloroform/methanol, are shown in Table 4.16. Concentrations of total carotenoids were expressed in mg/100 g of oil and as a mass percentage (%) of the total tocopherols (Appendix A6). Seed oil was comprised primarily of α-tocopherol (vitamin E) and γ-tocopherol, resulting in >90% of total tocopherols and tocotrienols. This trend was also evident in the literature reviewed (Kallio et al. 2002b).

Table 4.16. Major tocopherols and tocotrienols and levels in seed oil expressed in mg/100 g of oil.

Tocopherol/ tocotrienol	Chloroform/methanol extraction					
	Seed (2001)		Seed (2002)		Processed seed (2002)	
	mean	S.D.	mean	S.D.	mean	S.D.
α-tocopherol	142.4	17.0	80.3	13.3	121.0	6.2
β-tocopherol	8.4	0.0001	8.8	0.05	9.5	0.6
γ-tocopherol	111.5	0.8	115.6	4.0	130.0	5.4
δ-tocopherol	4.7	0.2	5.9	0.2	6.4	0.9
β-tocotrienol	4.6	0.4	6.2	0.2	6.7	0.3
Plastochromanol-8	4.5	0.4	2.8	0.5	2.9	0.2

S.D. = standard deviation.
n = 2.

There were differences in the levels of tocopherols (α-tocopherol, β-tocopherol, γ-tocopherol, and δ-tocopherol) and tocotrienols (β-tocotrienol and plastochromanol-8, a derivative of γ-tocotrienol) among harvest year (2001 and 2002), with major differences in α-tocopherol levels for the harvest year 2001 (142.4 mg/100 g oil) and 2002 (80.3 mg/100 g oil). However, definitive conclusions should not be drawn based on harvest year as the sole factor

contributing to the differences in tocopherol and tocotrienol levels. Berries of the 2001 harvest year were in storage 12 mo longer than the berries of the 2002 harvest year. Beveridge (2003) indicated means and standard deviations of α-, β-, γ-, and δ-tocopherol levels in cv. Indian-Summer seed oil of 155 (S.D. = 7), 16.4 (S.D. = 1.7), 134.9 (S.D. = 2.8), and 11.3 (S.D. = 1.4) in mg/100 g oil.

While descriptive comments can be made on the effect of harvest year and processing on tocopherol/tocotrienol levels, definitive conclusions should not be drawn since analysis testing was conducted from single samples. In general, higher levels of tocopherols and tocotrienols (see Table 4.16) were evident in the processed seeds, namely α- and γ-tocopherol. Tocopherol and tocotrienol analysis for seed oil in berries harvested in November 2002 was conducted in September 2003 at which time the seed oil was extracted. In addition, tocopherol and tocotrienol analysis was conducted in September 2003 on seeds which were processed 4 mo prior, in May 2003. While it remains unclear why the processed seeds contained higher levels of tocopherols and tocotrienols compared to unprocessed seeds from the same harvest year, a storage period of 11 mo (November 2002 to September 2003) and method of storage may be possible contributing factors explaining this discrepancy. Duration and method of storage can alter the rate of oxidation, leading to degradation of tocopherols and tocotrienols (Beveridge 2003). Bulk berries were kept frozen (-25°C) in unsealed plastic bags while the processed seeds were immediately sealed in zip-lock bags and were kept refrigerated at -5°C, until analysis.

Table 4.17 shows the major tocopherols and tocotrienols comprising >98% of the total tocopherols and tocotrienols in seed oil from solvent extraction, SCFE CO_2, and screw press trials.

Table 4.17. Major tocopherols and tocotrienols and levels in seed oil related to solvent extraction, SCFE CO_2, and screw press trials (expressed in mg/100 g oil).

Tocopherol/ tocotrienol	Seed (2002)									
	Solvent extraction		SCFE CO_2						Screw press	
			3 h / 30 s		6 h / 10 s		6 h / 30 s			
	mean	S.D.	mean	S.D.	mean	S.D.	mean	S.D.	mean	S.D.
α-tocopherol	223.4	11.8	170.5	36.9	308.7	17.3	196.7	18.3	147.8	4.4
β-tocopherol	11.8	0.1	11.2	0.3	15.7	0.2	12.1	0.3	8.1	0.02
γ-tocopherol	177.4	4.5	154.2	18.4	228.3	4.8	176.0	10.2	127.0	4.1
δ-tocopherol	8.0	0.1	8.8	0.3	12.6	1.1	8.6	0.2	5.3	0.1
β-tocotrienol	9.7	0.3	7.6	1.5	11.4	0.4	9.1	0.6	7.2	0.4

S.D. = standard deviation.
n = 2.

Levels of α-tocopherol, the predominant tocopherol in the pulp oil, changed with extraction method, namely solvent extraction (223.4 mg/100 g oil), SCFE CO_2 (170.5 to 308.7 mg/100 g oil), and screw pressing (147.8 mg/100 g oil). Predominant changes occurred with α-tocopherol and γ-tocopherol levels increasing with extraction duration (3 to 6 h) and decreasing with increasing grind times (10 to 30s) (see Table 4.17). Longer extractions increased the amount of tocopherols extracted while the generation of heat during extended grinding (10 to 30 s) may have caused tocopherol and tocotrienol levels to decrease. Levels of α-tocopherol and γ-tocopherol in the extracted seed oil were lower for screw pressed oil compared with solvent extraction and SCFE CO_2 (6 h / 10 s) extracted oils. Generation of friction and ultimately heat resulting in temperatures

>60°C may have caused thermal degradation of α-tocopherol and γ-tocopherol. Plastochromanol-8 was reported to be <0.5%, among solvent extraction, SCFE CO_2, and screw press trials and was not presented in Table 4.17. The tocopherols and tocotrienols listed in Table 4.17 are merely descriptive and do not necessarily reflect extraction technology performance.

Oomah (2003) summarized tocopherol compositions from seed oil by various seed oil extraction methods. Levels of α-tocopherol for solvent extraction (hexane), SCFE CO_2 (technique not described), and pressing (technique not described), were 46.7, 46.7, and 42.2 mg/100 g oil compared to 223.4 (solvent extraction using petroleum ether), 196.7 (SCFE CO_2), and 147.8 mg/100 g oil (screw press) for the current research. However, vitamin E levels were listed as 215.7, 190.1, and 158.4 mg/100 g oil for hexane extraction, SCFE CO_2, and pressing, respectively. These values are remarkably close to the values determined for α-tocopherol as determined in this project. While large and small differences in the levels of α-tocopherol may be attributed to plant subpecies or cultivar, and growing conditions, pressing has been shown to produce seed oil having the lowest level of α-tocopherol.

Major tocopherols and tocotrienols were identified and their concentrations (>98%) were determined for pulp oil extracted by chloroform/methanol (Table 4.18). The predominant tocopherols found in the pulp oil were α-tocopherol and β-tocopherol, with α-tocopherol constituting 79 to 85% of the total tocopherols and tocotrienols identified. Kallio et al. (2002b) reported a slightly wider range for α-tocopherol having values falling between 76 and 89%. Kallio et al. (2002b)

found the levels of α-tocopherol in pulp oil of ssp. *sinensis* and ssp. *mongolica* to be 194 and 136 mg/100 g of oil, approximately 50% of the α-tocopherol levels found in cv. Indian-Summer pulp oil.

Table 4.18. Major tocopherols and tocotrienols and concentration levels in pulp oil expressed in mg/100 g oil.

Tocopherol/ tocotrienol	Chloroform/methanol extraction							
	Pulp (2001)		Pulp (2002)		Pulp-flakes (2002)		Juice (2002)	
	mean	S.D.	mean	S.D.	mean	S.D.	mean	S.D. [a]
α-tocopherol	345.0	20.3	281.9	21.4	220.8	0.0	110.4	156.1
β-tocopherol	23.5	1.28	20.6	2.8	21.1	0.2	15.6	1.1
γ-tocopherol	5.9	0.6	9.7	2.7	11.1	0.3	6.9	0.5
δ-tocopherol	2.7	0.2	5.9	1.0	6.5	0.3	1.5	0.4
γ-tocotrienol	6.5	0.28	9.0	5.8	1.7	1.2	3.9	0.8
Plastochromanol-8	20.8	0.6	16.2	1.2	13.2	0.15	12.9	0.1

S.D. = standard deviation.
[a] n = 3 (otherwise n = 2).

There were differences in tocopherol and tocotrienol levels with harvest year (2001 and 2002) and processing. Taking into account 23 mo of storage at -25°C, pulp oil from thawed berries of 2001 had a higher level of α-tocopherol (345.0 mg/100 g oil) than the pulp oil from thawed berries of 2002 (281.9 mg/100g oil) after 11 mo of storage at the same storage temperature. Processing had a logical effect, decreasing α-tocopherol levels in pulp-fakes which can be attributed to drying of the pulp-flakes, handling, and storage. A lower value of α-tocopherol (110.4 mg/100 g oil) was reported in the juice.

Solvent extracted pulp oil contained the highest level of α-tocopherol (143.7 mg/100 g oil), followed by aqueous extracted pulp oil (138.4 mg/100 g oil), and finally SCFE CO_2 pulp oil (101.1 to 113.0 mg/100 g oil) (Table 4.19). While

grinding time was not applicable for preparation of pulp-flakes prior to extraction, longer extraction durations (from 3 to 6 h) associated with SCFE CO_2 increased the level of α-tocopherol from 101.1 to 113.0 mg/100 g oil, a trend which was noted with the extraction of seed oil as previously discussed.

Table 4.19. Major tocopherols and tocotrienols and levels in pulp oil related to solvent extraction, SCFE CO_2, and aqueous extraction trials (expressed in mg/100 g oil).

Tocopherol/ tocotrienol	Pulp (2002)							
	Solvent extraction		SCFE CO_2				Aqueous extraction	
			3 h		6 h			
	mean	S.D.	mean	S.D.	mean	S.D.	mean	S.D.
α-tocopherol	143.7	7.9	101.1	16.2	113.0	12.1	138.4	11.4
β-tocopherol	14.5	0.1	11.3	0.01	12.6	0.5	9.4	1.5
γ-tocopherol	7.2	0.4	6.7	0.1	7.0	0.2	3.0	0.21
δ-tocopherol	5.3	1.2	6.0	0.03	6.2	0.2	n/d	n/d
γ-tocotrienol	2.3	0.6	2.3	0.003	2.5	1.0	2.9	0.2
Plastochromanol-8	8.1	0.2	1.6	0.4	1.9	1.1	8.8	1.0
δ-tocotrienol	4.4	0.3	6.1	1.1	5.9	0.7	n/d	n/d

S.D. = standard deviation.
n = 2.
n/d = not detected.

It is interesting to note that while solvent extracted pulp oil contained the highest level of α-tocopherol, the concentration of the total tocopherols and tocotrienols was lower (77.3%) than that of aqueous extracted pulp oil (84.5%) (Table 4.20). Again, tocopherol or tocotrienol concentration was expressed as the mass percentage (%) of the total tocopherols and tocotrienols in the pulp oil.

Finally, variation among data may be attributed to length of storage time of berries, processed materials, and oil prior to total tocopherol and tocotrienol determination. In addition, the analysis of tocopherol and tocotrienol levels (in

mg/100 g oil or %) are merely descriptive and do not necessarily reflect extraction technology performance.

Table 4.20. Major tocopherols and tocotrienols and concentrations in pulp oil related to solvent extraction, SCFE CO_2, and aqueous extraction trials (expressed as the mass percentage (% w/w) of the total tocopherols and tocotrienols).

Tocopherol/ tocotrienol	Pulp (2002)							
	Solvent		SCFE CO_2				Aqueous	
			3 h		6 h			
	mean	S.D.	mean	S.D.	mean	S.D.	mean	S.D.
α-tocopherol	77.3	0.02	74.2	2.6	75.2	0.1	84.5	0.4
β-tocopherol	7.8	0.4	8.4	1.1	8.4	0.6	5.7	0.4
γ-tocopherol	3.9	0.02	5.0	0.7	4.7	0.4	1.8	0.03
δ-tocopherol	2.9	0.5	4.5	0.5	4.2	0.3	n/d	n/d
γ-tocotrienol	1.2	0.2	1.7	0.2	1.6	0.5	1.8	0.1
Plastochromanol-8	4.4	0.1	1.2	0.4	1.2	0.6	5.3	0.1
δ-tocotrienol	2.4	0.05	4.4	0.2	3.9	0.05	n/d	n/d

S.D. = standard deviation.
n = 2.

4.4.3 Total carotenoids

Carotenoids are largely responsible for the red and yellow color pigment in vegetables (Krause and Mahan 1979). Thus, carotenoid levels can be used indirectly as an indicator of color intensity with higher values indicating greater color intensities. Total carotenoids in chloroform/methanol extracted seed oil, are shown in Table 4.21 (see also Appendix A7).

Table 4.21. Total carotenoids in seed oil expressed in mg/100 g of oil.

Chloroform/methanol extraction					
Seed (2001)		Seed (2002)		Processed seed (2002)	
mean	S.D.	mean	S.D.	mean	S.D.
21.0	1.6	22.5	0.2	17.2	0.2

S.D. = standard deviation.
n = 2.

A maximum total carotenoid level of 22.5 mg/100 g oil was noted in the seed oil of the 2002 harvest year. Beveridge et al. (1999) indicated trace amounts of total carotenoids in seed oil of some sea buckthorn varieties and higher ranges, namely 50 to 85 mg/100 g oil, in others. Beveridge (2003) indicated a total carotenoid level of 41.1 mg/100 g oil (S.D. = 13.4 mg/100 g oil) in seed oil of cv. Indian-Summer (method of extraction was not indicated).

There was a small difference in total carotenoids between harvest years (2001 and 2002). Seeds from the 2002 harvest year contained a slightly higher level of total carotenoids (an additional 1.5 mg/100 g oil). However, length of storage (23 mo) of the seeds (contained in frozen berries) from the 2001 harvest was greater than the length of storage (11 mo) of seeds (contained in frozen berries). This may suggest the deterioration of total carotenoids with increased storage time rather than a harvest year effect. Processing lowered the amount of total carotenoids by approximately 5 mg/100 g oil, a result expected by degradation of carotenoids (highly prone degradation caused by oxidation, heat, and light) by heat during drying and oxidation during general material handling and processing.

Again, definitive conclusions should not be drawn from the results provided on levels of total carotenoids in seed oil on harvest year and processing. Single samples were analyzed indicating results of a descriptive rather than statistical nature.

Total carotenoids in seed oil from solvent extraction, SCFE CO_2, and screw press trials are shown in Table 4.22. There were large fluctuations in total carotenoid content between solvent extraction (22.2 mg/100 g oil) SCFE CO_2 (6.2 to 28.4 mg/100 g oil), and screw press trials (15.3 mg/100 g oil).

Table 4.22. Total carotenoids in seed oil related to solvent extraction, SCFE CO_2, and screw press trials (expressed in mg/100 g of oil).

Seed (2002)									
Solvent extraction		SCFE CO_2						Screw press	
		3 h / 30 s		6 h / 10 s		6 h / 30 s			
mean	S.D.	mean	S.D.	mean	S.D.	mean	S.D.	mean	S.D.
22.2	0.7	6.2	2.6	28.4	2.1	11.7	0.1	15.3	1.2

S.D. = standard deviation.
n = 2.

A similar trend associated with extraction duration and grind times was noted with total carotenoid levels in seed oil compared to fluctuations in tocopherol and tocotrienol levels. Level of total carotenoids increased with extraction duration (3 to 6 h) and decrease with increasing grind times (10 to 30 s). Additional heat generated by longer grinding times may have caused thermal degradation of carotenoids. Again, due to the generation of friction and heat, screw pressed oil contained the lowest level of total carotenoids compared to solvent extracted and SCFE CO_2 (6 h / 10 s) seed oil.

It should be noted that the levels of total carotenoids are merely descriptive and do not necessarily reflect extraction technology performance.

Total carotenoids in chloroform/methanol extracted pulp oil, are shown in Table 4.23. Harvest year had effect on the level of total carotenoids in pulp (from whole thawed berries), with lower total carotenoid levels in 2001 (see Table 4.23). Again as previously discussed, it remains unclear whether this discrepancy is caused by a harvest year or length of storage effect.

Processing had some effect on the level of total carotenoids in pulp-flakes. Drying during processing may have reduced the level of total carotenoids from 382.8 mg/100 g oil from unprocessed pulp to 347.1 mg/100 g oil from processed pulp (pulp-flakes), though method of storage and storage time should not be ruled out. The level of total carotenoids in juice was comparable to those in pulp-flakes. Beveridge (2003) indicated a range of total carotenoids from 330 to 1000 mg/100 g oil, depending on plant subspecies or cultivar.

Table 4.23. Total carotenoids in pulp oil expressed in mg/100 g of oil.

Chloroform/methanol extraction							
Pulp (2001)		Pulp (2002)		Pulp-flakes (2002)		Juice (2002)	
mean	S.D.	mean	S.D.	mean	S.D.	mean	S.D. [a]
290.4	2.0	382.8	1.5	347.1	48.2	345.5	17.6

S.D. = standard deviation.
[a] n = 3 (otherwise n = 2).

Table 4.24 lists total carotenoid levels of pulp oil extracted by solvent, SCFE CO_2 and an aqueous method. Solvent extraction and SCFE CO_2 trials on pulp-flakes were both carried out in July 2003, approximately 2 to 3 mo after pilot

processing conducted in May 2003. Solvent extracted oil had the highest level of total carotenoids (527.8 mg/100 g oil) compared to SCFE CO_2 extracted oil (122.3 to 148.4 mg/100 g oil). Solvent extracted pulp-flake oil had a higher level of total carotenoids than that determined from oil extracted by the chloroform/methanol extraction (382.8 mg/100 g oil). Storage method and storage time are possible explanations for this discrepancy. Solvent extracted pulp-flake oil was stored at -25°C for 3 mo (July 2003 to September 2003) before total carotenoids were determined. Pulp-flake oil extracted by chloroform/methanol was conducted on pulp-flakes which were stored at -5°C for 5 mo (May 2003 to September 2003). A question should be raised as to how the level of total carotenoids behave in a particular material state, namely unprocessed pulp and processed pulp-flakes. This may have a dramatic effect on the levels of total carotenoids.

Table 4.24. Total carotenoids in pulp oil related to solvent extraction, SCFE CO_2, and aqueous trials (expressed in mg/100 g of oil).

Pulp (2002)							
Solvent extraction		SCFE CO_2				Aqueous extraction	
		3 h		6 h			
mean	S.D.	mean	S.D.	mean	S.D.	mean	S.D.
527.8	14.9	122.3	3.7	148.4	11.7	292.4	16.6

S.D. = standard deviation.
n = 2.

The level of total carotenoids from SCFE CO_2 pulp-flake oil increased with extraction duration from 122.3 mg/100 g after 3 h to 148.4 mg/100 g oil after 6 h. Aqueous extraction trials were conducted on thawed whole berries in August

2003 (berries in storage for 10 mo from November 2002 to August 2003), after which, the oil was stored at -25°C for 1 mo until analysis. Total carotenoids in pulp oil (2002) extracted by chloroform methanol were higher (382.8 mg/100 g oil) than that of aqueous extracted oil (292.4 mg/100 g oil). Again, processing, storage, and extraction technique are possible factors explaining the lower levels of total carotenoids associated with aqueous extracted pulp oil compared with oil extracted by chloroform/methanol. The possibility of chloroform/methanol extracting oil containing higher levels of total carotenoids compared to aqueous or SCFE CO_2, should not be ruled out.

In summary, variation among data may be attributed to length of storage time of berries, processed materials, and oil prior to total carotenoid determination. In addition, the analysis of total carotenoids and levels are merely descriptive (conducted from single samples) and do not necessarily reflect extraction technology performance.

4.4.4 Sterols

Selected sterol concentrations of cholesterol, campesterol, stigmasterol, and β-sitosterol in seed oil extracted by a chloroform/methanol procedure are shown in Table 4.25. Concentrations were expressed in mg/100 g of oil and as a mass percentage (%) of the sterols identified (Appendix A8). Seed oil was comprised primarily of β-sitosterol (97%) with trace amounts of campesterol (2%). Cholesterol and stigmasterol were not detected. Yang et al. (2001) also reported β-sitosterol as the major sterol found in seeds of ssp. *sinensis*. Harvest

year had some effect on sterol levels. Seed oil of the 2001 harvest year had a β-sitosterol level of 541.3 mg/100 g oil compared to seed oil of the 2002 harvest year having a level of 599.6 mg/100 g oil. Processing had no effect on sterol levels (see Table 4.25).

Table 4.25. Identified sterols and levels in seed oil expressed in mg/100 g of oil.

Sterol	Chloroform/methanol extraction					
	Seed (2001)		Seed (2002)		Processed seed (2002)	
	mean	S.D.	mean	S.D.	mean	S.D.
Cholesterol	n/d	n/d	n/d	n/d	n/d	n/d
Campesterol	14.9	0.1	16.3	0.4	17.2	0.5
Stigmasterol	n/d	n/d	n/d	n/d	n/d	n/d
β-sitosterol	541.3	0.2	599.6	10.4	598.9	6.3

S.D. = standard deviation.
n = 2.
n/d = not detected.

The level of β-sitosterol seed oil changed with extraction method, namely solvent extraction (746.3 mg/100 g oil), SCFE CO_2 (667.8 to 910.0 mg/100 g oil), and screw pressing (635.0 mg/100 g oil). A similar trend was evident with campesterol levels. β-sitosterol and campesterol levels increased with extraction duration (3 to 6 h) and decreased with increasing grind times (10 to 30s) (see Table 4.26). Generation of heat during extended grinding (10 to 30 s) may have caused the levels of these compounds to decrease. β-sitosterol and campesterol levels in the extracted seed oil were lower for screw pressed oil compared to solvent extraction and SCFE CO_2 (6 h / 10 s) extracted oils. Generation of friction and ultimately heat resulting in temperatures >60°C may have caused thermal degradation of these compounds. Cholesterol was evident in the solvent

extracted seed oil (3.7 mg/100 g oil). Again, the sterol levels listed in Table 4.26 are merely descriptive and do not necessarily reflect extraction technology performance. More samples should be analyzed to confirm the trends which have been discussed. Beveridge (2003) reported total sterol levels of seed oil obtained by pressing, hexane extraction, and SCFE CO_2 to be 193.6, 1298.4, and 1217.1 mg/100 g oil, respectively.

Table 4.26. Identified sterols and levels in seed oil related to solvent extraction, SCFE CO_2, and screw press trials (expressed in mg/100 g oil).

Sterol	Seed (2002)									
	Solvent extraction		SCFE CO_2						Screw press	
			3 h / 30 s		6 h / 10 s		6 h / 30 s			
	mean	S.D.	mean	S.D.	mean	S.D.	mean	S.D.	mean	S.D.
Cholesterol	3.7	1.0	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d
Campesterol	22.4	0.5	19.9	0.1	26.3	0.9	22.5	0.7	18.0	10.7
Stigmasterol	n/d	n/d	n/d	n/d	2.7	0.0 [a]	n/d	n/d	2.7	0.0 [a]
β-sitosterol	746.3	22.8	667.8	20.8	910.0	45.0	748.1	5.1	635.0	343.9

S.D. = standard deviation.
[a] n = 1 (otherwise n = 2).
n/d = not detected.

Selected sterol concentrations of cholesterol, campesterol, stigmasterol, and β-sitosterol in pulp oil extracted by chloroform/methanol are shown in Table 4.27. Concentrations were expressed in mg/100 g of oil and as a mass percentage (%) of the sterols identified (Appendix A8). Campesterol, stigmasterol, and β-sitosterol were present in the pulp oil with β-sitosterol having the highest level (97%). There was no effect of harvest year on the level β-sitosterol, however harvest year had some effect on the level of campesterol and stigmasterol (seed Table 4.27). Processing of pulp-flakes had an effect on

increasing the level of β-sitosterol by 123.4 mg/100g oil. Juice had the lowest level of β-sitosterol (324.7 mg/100 g oil). Beveridge (2003) reported a total sterol content in fruit oil (pulp oil) of 770.6 mg/100 g oil.

Table 4.27. Identified sterols and levels in pulp oil expressed in mg/100 g oil.

Sterol	Chloroform/methanol extraction							
	Pulp (2001)		Pulp (2002)		Pulp-flakes (2002)		Juice (2002)	
	mean	S.D.	mean	S.D.	mean	S.D.	mean	S.D. [a]
Cholesterol	n/d	n/d	n/d	n/d	4.6	0.0 [b]	n/d	n/d
Campesterol	10.3	1.3	8.9	0.4	9.7	0.1	7.1	1.1
Stigmasterol	3.8	5.4	n/d	n/d	n/d	n/d	n/d	n/d
β-sitosterol	399.2	44.0	398.6	17.5	522.0	6.8	324.7	51.6

S.D. = standard deviation.
[a] n = 3 (otherwise n = 2).
[b] n = 1.
n/d = not detected.

Solvent extracted pulp oil contained the highest levels of cholesterol (4.5 mg/100 g oil), campesterol (12.4 mg/100 g oil), stigmasterol (6.6 mg/100 g oil), and β-sitosterol (576.9 mg/100 g oil) compared to SCFE CO_2 (525.4 mg/100 g oil) and aqueous extraction (288.6 mg/100 g oil) (Table 4.28). SCFE CO_2 extraction duration of had no effect on cholesterol, campesterol, and β-sitosterol levels. However, the level of stigmasterol increased to a level of 10.8 mg/100 g oil for a 6 h extraction. Cholesterol and stigmasterol were not detected in aqueous extracted pulp oil. The sterol levels listed in Table 4.28 are merely descriptive and do not necessarily reflect extraction technology performance. Again, more samples should be analyzed to confirm the trends which have been discussed.

Table 4.28. Identified sterols and levels in pulp oil related to solvent extraction, SCFE CO_2, and aqueous extraction trials (expressed in mg/100 g oil).

Sterol	Pulp (2002)							
	Solvent extraction		SCFE CO_2				Aqueous extraction	
			3 h		6 h			
	mean	S.D.	mean	S.D.	mean	S.D.	mean	S.D.
Cholesterol	4.5	1.4	n/d	n/d	n/d	n/d	n/d	n/d
Campesterol	12.4	0.6	10.9	0.04	10.9	0.2	6.6	0.6
Stigmasterol	6.6	2.5	n/d	n/d	10.8	3.4	n/d	n/d
β-sitosterol	576.9	32.3	525.0	13.5	525.7	5.2	288.6	8.6

S.D. = standard deviation.
n = 2.
n/d = not detected.

In summary, the information gathered in this research on the nutritional levels (fatty acids, tocopherols and tocotrienols, total carotenoids, and sterols) of sea buckthorn oils is important, contributing to the understanding of processing and extraction effects for utilization of these oils in functional foods and nutraceuticals. Nutritional profiles as those presented, are useful for characterizing sea buckthorn and detecting adulterations of these valuable oils (Yang et al. 2001).

4.4.5 Oil quality summary

Table 4.29 qualitatively indicates relative concentrations of fatty acids, tocopherols and tocotrienols, total carotenoids, and sterols in seed and pulp oils extracted by the methods under evaluation. Based solely on concentrations of selected quality parameters, solvent extraction was shown to yield oil having high concentrations of all parameters, followed by SCFE CO_2 having the highest concentrations in some of the selected parameters, and finally aqueous extraction and screw pressing having low to high concentrations of fewer quality parameters. Fatty acid concentrations were independent of the extraction method employed.

Table 4.29. Qualitative assessment of oil quality parameters relative to extraction method employed (order of increasing concentration: low < high < highest).

Oil components	Extraction method			
	Solvent Extraction	SCFE CO_2	Screw press	Aqueous extraction
Seed oil major fatty acids	similar concentrations for most fatty acids			
Pulp oil major fatty acids	similar concentrations for most fatty acids			
Seed oil major tocopherols and tocotrienols	high	highest	low	n/a
Pulp oil major tocopherols and tocotrienols	high	low	n/a	high
Seed oil total carotenoids	high	highest	low	n/a
Pulp oil total carotenoids	highest	low	n/a	high
Seed oil major sterols	high	highest	high	n/a
Pulp oil major sterols	highest	high	n/a	low

n/a = not applicable.

The solvent extraction method recovered oil (seed and pulp) with high concentrations of tocopherols and tocotrienols, total carotenoids, and sterols compared to screw press and aqueous extraction methods. Higher

concentrations of tocopherols and tocotrienols, total carotenoids, and sterols in seed oil were noted for SCFE CO_2 compared to solvent extraction. However, lower concentrations of pulp oil tocopherols and tocotrienols, pulp oil total carotenoids, and pulp oil sterols were noted for SCFE CO_2 compared to solvent extraction. This may be a function of extraction condition (extraction temperature and pressure), which was used during SCFE CO_2 extraction trials. It is suspected after having reviewed the literature that optimization of temperature and pressure conditions (though pressure may have a more dramatic effect) may enhance tocopherol and tocotrienol, total carotenoid, and sterol concentrations. Aqueous extracted pulp oil had high levels of tocopherols and tocotrienols which was similar to solvent extracted pulp oil. However, aqueous extracted pulp oil had low levels of major sterols when compared with solvent extracted pulp oil. Screw press seed oil contained low levels of tocopherols and tocotrienols when compared with solvent extraction and SCFE CO_2 methods. Concentrations of seed oil major sterols were consistent with seed oil concentrations from solvent extraction and SCFE CO_2 methods.

The information presented on the quality of sea buckthorn seed and pulp oils (cv. Indian-Summer) is merely a list of some of the popular nutritional components which have been addressed by the functional food and nutraceutical industry. While the market for sea buckthorn in Canada has not been solidified, the intent has been to provide data on the nutritional levels associated with a Canadian sea buckthorn cultivar such that it may be used as a guide to suggest directions for processing.

4.5 Economics of extractions

Supercritical fluid extraction has been adopted in many cases as the technology of choice for extracting high value components for functional food and nutraceutical end uses. Within this research, SCFE CO_2 was also shown to be the best overall choice for sea buckthorn, producing oil having favourable levels of fatty acids, tocopherols and tocotrienols, carotenoids, and sterols, components which are highly valued in the health food industry. In addition, though oil recoveries were lower than that of solvent extraction using petroleum ether (seed and pulp-flake oil recovery of 65.1% and 86.3%, respectively), recoveries were higher than that of screw pressing (for the extraction of seed oil) and aqueous extraction (for the recovery of pulp oil). While it has been shown that the extraction of sea buckthorn oils is technologically feasible, economic feasibility of oil extraction using SCFE CO_2 as an extraction is addressed.

Economic feasibility was assessed based the use of SCFE technology and on Manitoba's level of yearly berry production (16 ha or 40 acres at 2 t/acre of berries = 80,000 kg), assuming that each province has processing capabilities to minimize cost of transporting frozen berries. From the research, it was found that 100 kg could be processed into 81.6 kg of juice, 5.1 kg of seed, and 2.6 kg of pulp-flakes giving approximately 0.3 kg of seed oil and 0.3 kg of pulp oil (Table 4.30). Yang and Kallio (2002a) indicated a market price of 160 to $300/kg (European prices associated with SCFE CO_2 extraction) for sea buckthorn oils, the seed oil being more expensive than the pulp oil (compared to canola oil at approximately $5/kg).

Table 4.30. Analysis of press cake.

Material	Material mass (kg)	Oil content (% w/w)	SCFE CO_2 yield (% w/w)	Oil mass (kg)
seeds	5.1	8.2	65.1	0.27
pulp flakes	2.6	11.9	86.3	0.27
defatted meal total	7.2			

Thus, a return of $1.38/kg of berries from the sale of oil (assuming seed price and pulp oil price of 300 and $160/kg) could be expected. However, a current market price (2003) in Canada of $3.85/kg for berries (berries purchased for research) indicates that an additional return of $2.47/kg on input cost is required to break even without considering costs of conversion though processing. It is suspected that the current market price of $3.85/kg is in reference to manual harvesting, a method which has been deemed unfavourable due to intensive labour requirements compounded by the thorny nature of the plant. Additional returns need to be achieved from the sale of by-products such as juice and defatted material (seed and pulp-flakes) after processing and extraction operations, respectively.

The following calculation proposes a Canadian based oil price for seed and pulp oils, assuming that the sale of the oils would be solely responsible for covering the cost of purchasing a suitably sized SCFE system ($1,000,000 for a commercial unit), and raw material ($3.85/kg of berries). All expenses are current as of 2003. Assuming SCFE system capital cost of $1,000,000, 10 yr equipment life, and 10% cost of money, the necessary annual payment for capital translates to approximately $162,700 (based on calculated present value

interest factor for annuity of 6.14). It should be noted that a $1,000,000 SCFE system (100 L batch capacity: approximately 45 kg of seeds/batch and 21 kg of pulp-flakes/batch) has the potential of extracting Manitoba's annual production of sea buckthorn oil from processed seed and pulp-flakes (from 80,000 kg of berries) in 6 mo (A. Anand, Process Development Consultant, Food Development Centre (FDC), Portage la Prairie, MB, personal communication, 2004). The cost of raw materials (Manitoba's potential yearly production of 80,000 kg assuming no additional planting) at $3.85/kg is $308,000. The cost of CO_2 consumed was $3.19/kg of seed oil and $5.75/kg of pulp oil based on calculated solubilities and a current price of CO_2 gas at $2.30/kg. The consumption of CO_2 would increase yearly costs by approximately $700 for the extraction of seed oil (216 kg of seed oil from 80,000 kg of berries) and $1300 for the extraction of pulp oil (216 kg of pulp oil from 80,000 kg of berries). In terms of CO_2 cost, it was noted that 36% of the extraction cost was associated with the extraction of seed oil and 64% of the extraction cost was associated with the extraction of pulp oil. Thus, the price of seed and pulp oil required to break even is $390/kg and $700/kg, respectively. These prices are dramatically higher than the reported European prices.

An exercise was conducted to estimate juice and defatted meal value to cover berry cost, which could be used to offset seed and pulp oil prices (European prices were used) (Table 4.31). An estimate of $3.10/kg for juice and $1/kg was chosen arbitrarily as a potential base market price, which is reflective of prices established in Germany. Thus, using a potential market price of

$3.10/kg for juice and $1/kg for the defatted meal along with the European prices established for seed and pulp oils, the cost of berries could be covered. However, processing and extraction costs have yet been considered.

Table 4.31. Potential market prices of sea buckthorn by-products to estimate juice and defatted meal value.

Material	Mass [a] (kg)	Potential market price ($/kg)	Contribution ($)
Juice	81.6	3.10	252.96
Seed oil	0.27	300.00	81.00
Pulp oil	0.27	160.00	43.20
Defatted meal	7.2	1.00	7.20
Total			384.36
Cost of berries	100.0	3.85	385.00

[a] Material break down from 100 kg of berries (starting mass). Residual mass in the form of moisture and process losses is not shown.

Four case scenarios were formulated to show the effect of increasing the value of key sea buckthorn by-products to cover solely the cost of purchasing an SCFE system for extraction and to offset the price of oil (Table 4.32). The cost of CO_2 was considered negligible in relation to the material and extraction costs and was not factored into the determination of potential market prices. In addition, CO_2 can be recycled, suggesting further reduction in cost. Case I, indicates that an increase in juice value by 81% can contribute to the capital cost of an SCFE system. The large juice to oil ratio stresses the importance of juice as the key marketable item for covering the cost of an SCFE system as well as the cost of material conversion through processing prior to extraction.

Table 4.32. Proposed potential market prices for sea buckthorn materials to cover SCFE system purchase.

Case I: increasing juice value.

Material	Mass [a] (kg)	Potential market price ($/kg)	Contribution ($)
Juice	81.6	**5.60 (from 3.10)**	456.96
Seed oil	0.27	300.00	81.00
Pulp oil	0.27	160.00	43.20
Defatted meal	7.2	1.00	7.20
Total			588.36
Estimated cost of berries			385.00
Additional value to cover capital			204.00
Increase juice value by			**81%**

Case II: increasing seed oil value.

Material	Mass [a] (kg)	Potential market price ($/kg)	Contribution ($)
Juice	81.6	3.10	252.96
Seed oil	0.27	**1056.00 (from 300.00)**	285.12
Pulp oil	0.27	160.00	43.20
Defatted meal	7.2	1.00	7.20
Total			588.48
Estimated cost of berries			385.00
Additional value to cover capital			204.12
Increase seed oil value by			**252%**

Case III: increasing pulp oil value.

Material	Mass [a] (kg)	Potential market price ($/kg)	Contribution ($)
Juice	81.6	3.10	252.96
Seed oil	0.27	300.00	81.00
Pulp oil	0.27	**916.00 (from 160.00)**	247.32
Defatted meal	7.2	1.00	7.20
Total			588.48
Estimated cost of berries			385.00
Additional value to cover capital			204.12
Increase pulp oil value by			**473%**

[a] Material break down from 100 kg of berries (starting mass). Residual mass in the form of moisture and process losses is not shown.

Seed oil is of next importance followed by pulp oil, requiring an increase in value of 252% (Case II) and 473% (Case III), respectively (Table 4.32) to individually cover the cost of and SCFE system. It is speculated that these large value increases associated with seed and pulp oil to cover extraction and processing costs may hinder market entrance of the oils and ultimately deter consumer acceptance due to a high price.

Case IV includes balancing a combination of sea buckthorn by-products values to accommodate an SCFE system, suggesting more realistic market prices (Table 4.33).

Table 4.33. Case IV: potential market prices established from a combination of sea buckthorn materials to cover SCFE system purchase.

Material	Mass [a] (kg)	Potential market price ($/kg)	Contribution ($)
Juice	81.6	5.20	424.32
Seed Oil	0.27	360.00	97.20
Pulp Oil	0.27	195.00	52.65
Defatted meal	7.2	2.00	14.40
Total			588.57
Estimated cost of berries			385.00
Additional value to cover capital			204.00
Increase juice value by			**68% (down from 81%)**
Increase seed oil value by			**20% (down from 252%)**
Increase pulp oil value by			**22% (down from 473%)**
Increase defatted meal by			**100%**

[a] Material break down from 100 kg of berries (starting mass). Residual mass in the form of moisture and process losses is not shown.

Slight increases in the value of seed (20%) and pulp (22%) oil can reduce the amount of required value for the juice by 13%. In addition, it can be shown that if useful end uses can be found for the defatted meal, the value of juice, seed oil,

and pulp oil can be lowered or the additional contribution can be made towards CO_2, processing equipment (juice extraction ($25,000 for a bladder press) and drying equipment ($100,000 for an industrial drier)), electricity, labor, and facility infrastructure costs). The sale of pulp oil extracted from the juice (oil content in juice was found to be 2%$_C$ by the chloroform/methanol procedure) could also be used to offset seed and pulp oil prices and contribute to processing costs.

Sale of by-products, the development of an efficient harvesting method (mechanical methods), and research to increase extraction efficiency of oils using SCFE technology may contribute to optimizing market prices of sea buckthorn products to recover cost. Given that the current Manitoba sea buckthorn berry production would not accommodate year round processing (only 6 mo), co-processing with other crops would have to be incorporated to reduce capital contribution. However, it is still very premature to make any concrete statements regarding the sale and price of sea buckthorn products since the market for sea buckthorn products in Canada is not currently established.

5. CONCLUSIONS

An evaluation of oil extraction technologies for recovering seed and pulp oils from cv. Indian-Summer sea buckthorn berries was conducted based on oil recovery and nutritional quality of the oils. The following conclusions were made from this research:

1. An effective pilot process was developed from laboratory bench-scale equipment and was up-scaled to comprise a juice extraction procedure to remove juice from whole thawed berries using a bladder press followed by low temperature drying (24 h at 50°C) of the wet press cake containing seeds and pulp, and finally a separation process using mechanical sieving to segregate seeds from pulp-flakes. Processing of 100 kg of berries yielded 5 kg of seeds and 3 kg of pulp-flakes having a moisture contents of 9.8 and 6.9% (w.b.), respectively. Processing losses were found to be <0.3% of the total starting mass of thawed berries (100 kg).

2. Oil contents of seeds (moisture content of 9.8% w.b.) and pulp-flakes (moisture content of 6.9% w.b.) of cv. Indian-Summer were determined to be 8.2%$_C$ and 11.9%$_C$, respectively.

3. A 3 h supercritical fluid extraction conducted at a 45°C and 35 MPa using CO_2 was sufficient in reaching greater than 90% of the maximum oil

extraction limit determined at a 6 h extraction, resulting in oil recoveries of 37.0, 65.1, and 86.3%, for seeds of 10 s and 30 s grind and pulp-flakes, respectively. Reducing the extraction time from 6 h to 3 h resulted in a 50% mass savings of CO_2 (2 to 1 kg for ground seeds and 4 to 2 kg for pulp-flakes). Screw pressing resulted in an oil recovery of 41.2% from whole seeds with extracted oil temperatures reaching 70°C. No oil was extracted from pulp-flakes by screw pressing. In relation to the oil content determined on whole berry pulp from a chloroform/methanol extraction ($2.2\%_C$), an average of 6% of oil was recovered using an aqueous oil extraction technique, a process that was developed at Westfalia Separator AG (Oelde, Germany). This process does not imply true replication of the FRIOLEX® oil recovery process, process details withheld by the company for proprietary reasons.

4. The nutritional quality of the extracted oils was determined by quantifying fatty acids, tocopherols and tocotrienols, total carotenoids, and sterols levels of seed and pulp oils after oil extraction trials:

    a) Linoleic and linolenic acid were the predominant fatty acids found in the seed oil, having concentrations of 35 and 36%, respectively. Palmitic and palmitoleic acid were the predominant fatty acids found in the pulp oil, having concentrations of 35 and 36% respectively. There was no

apparent changes in fatty acid levels as related to extraction method, however, harvest year had some effect.

b) Seed oil was comprised primarily of α-tocopherol and γ-tocopherol, resulting in >90% of total tocopherols and tocotrienols. α-tocopherol levels in pulp appeared to change with extraction method, namely solvent extraction (223.4 mg/100 g oil), SCFE CO_2 (170.5 to 308.7 mg/100 g oil), and screw pressing (147.8 mg/100 g oil). α-tocopherol and β-tocopherol were the predominant tocopherols found in the pulp oil, with α-tocopherol constituting 79 to 85% of the total tocopherols and tocotrienols identified. Solvent extracted pulp oil contained the highest level of α-tocopherol (143.7 mg/100 g oil), followed by aqueous extracted pulp oil (138.4 mg/100 g oil), and finally SCFE CO_2 pulp oil (101.1 to 113.0 mg/100 g oil).

c) There appeared to be large fluctuations of total carotenoid content in seed oil between solvent extraction (22.2 mg/100 g oil) SCFE CO_2 (6.2 to 28.4 mg/100 g oil), and screw press trials (15.3 mg/100 g oil). Solvent extracted pulp oil had a higher level of total carotenoids (527.8 mg/100 g oil) compared to aqueous extracted oil (292.4 mg/100 g oil).

d) β-sitosterol in seed oil changed with extraction method, namely solvent extraction (746.3 mg/100 g oil), SCFE CO_2 (667.8 to 910.0 mg/100 g oil), and screw pressing (635.0 mg/100 g oil).

Solvent extracted pulp oil contained the highest levels of cholesterol (4.5 mg/100 g oil), campesterol (12.4 mg/100 g oil), stigmasterol (6.6 mg/100 g oil), and β-sitosterol (576.9 mg/100 g oil) compared to SCFE CO_2 (525.4 mg/100 g oil) and aqueous extraction (288.6 mg/100 g oil).

Levels of fatty acids, tocopherols and tocotrienols, total carotenoids, and sterols may also be dependent on berry maturity, harvest year, length of storage time before berry processing, and length of storage time of oil before oil quality analysis. In addition, statistics provided on nutritional quality levels are representative of the precision of the testing methods only and do not necessarily reflect a true sample of a total population.

5. Supercritical fluid extraction using carbon dioxide served as a potential method for extracting oil from both seeds and pulp. This observation was determined based on high oil recoveries yielding oils of good nutritional quality.

6. An estimated price of seed and pulp oil required to break even using SCFE CO_2 as a potential method for oil recovery is $390/kg and $700/kg, respectively, considerably higher than reported European prices. It is suspected that the sale of by-products (namely juice), the development of an efficient harvesting method (mechanical systems), and increased

extraction efficiency of oils may contribute to greater contributions to offset, seed oil and pulp oil prices, as well as capital and operational costs (namely the purchase an SCFE system, raw material, and consumables) associated with SCFE CO_2 extraction.

6. RECOMMENDATIONS FOR FUTURE RESEARCH

1. While the pilot process proved to be effective in isolating seeds from pulp-flakes, batch processing was tedious and labor intensive. A continuous system should be developed to increase processing capacities yielding seeds and pulp-flakes in sufficient quantities to extract oil from these low oil content materials.

2. Additional research must be conducted to learn more about the drying process (drying characteristics) for improving processing. Drying was identified as a critical processing step required for seed and pulp-flake separation and for extracting oil using solvents, SCFE, and screw pressing methods.

3. Further research should be conducted in the area of material preparation prior to oil extraction. Conditioning, in terms of optimizing material moisture contents and particle size (flaking) was not extensively studied and may play a major role in increasing oil yields as indicated in the literature.

4. Additional SCFE CO_2 trials should be conducted at alternate temperatures and pressures (lower pressures) to optimize the extraction of target analytes allowing for greater control over nutritional quality.

5. Additional trials are required to optimize an aqueous process to adequately evaluate this technology as a potential oil extraction method for sea buckthorn seed and pulp oil.

6. The use of enzymes has been introduced to increase oil yields from sea buckthorn seeds and pulp oil, however little literature is available. The use of enzymes was not studied in this project though is growing in popularity in the extraction of compounds in the functional food and nutraceutical industry.

7. Harvest date (berry maturity), storage time, and cultivar or subspecies (cv. Indian-Summer vs. ssp. *sinensis* - Canadian grown sea buckthorn varieties) should be studied to show these effects as they relate to the nutritional quality of the oils (fatty acid, tocopherol/tocotrienol, total carotenoids, and sterol levels).

8. Until the market has been established for sea buckthorn products in Canada, it is difficult to predict industrial viability (growing and cultivation, harvesting method, processing, component extraction, product manufacturing, and retail sales). There is a strong need for sea buckthorn awareness, balanced with the task of controlling product demand to accommodate this developing industry.

7. REFERENCES

AACC. 2000. 02-01A. Fat acidity - general method. In *2000 Approved Methods of the American Association of Cereal Chemists.* 10th ed, pp. 1-2. St. Paul, MN: AACC.

AOAC. 1990. 965.16. Sampling of animal feed. In *Official Methods of Analysis of the Association of Official Analytical Chemists.* 15th ed, p. 69. Arlington, VA: AOAC.

ASAE. 2002. 352.2. Moisture measurement - unground grain and seeds. In *2002 ASAE Standards.* 49th ed, p. 589. St. Joseph, MI: ASAE.

Abu-Arabi, M.K., M.A. Allawzi, H.S. Al-Zoubi and A. Tamimi. 2000. Extraction of jojoba oil by pressing and leaching. *The Chemical Engineering Journal* 76:61-65.

Abraham, G., R.J. Hron, Sr., M.S. Kuk and P.J. Wan. 1993. Water accumulation in the alcohol extraction of cottonseed. *Journal of the American Oil Chemists Society* 70:207-208.

Adeeko, K.A. and O.O. Ajibola. 1990. Processing factors affecting yield and quality of mechanically expressed groundnut oil. *Journal of Agricultural Engineering Research* 45:31-43.

Ajibola, O.O., S.E. Eniyemo, O.O. Fasina and K.A. Adeeko. 1990. Mechanical expression of oil from melon seeds. *Journal of Agricultural Engineering Research* 45:45-53.

Almonor, G.O., G.P. Fenner and R.F. Wilson. 1998. Temperature effects on tocopherol composition in soybeans with genetically improved oil quality. *Journal of the American Oil Chemists Society* 75:591-596.

Anand, A. 2002. Sea buckthorn: an investigation report. Food Development Centre, Box 1240, 810 Phillips St., Portage la Prairie, Manitoba, R1N 3J9. 9 p.

Anand, A., G. Leigh and A. Utioh. 2000. Supercritical fluid extraction technology - applications in the agri-food industry. Food Development Centre, Box 1240, 810 Phillips St., Portage la Prairie, MB, R1N 3J9. 28 p.

Andlauer, W. and P. Fürst. 2002. Nutraceuticals: a piece of history, present status and outlook. *Food Research international* 35:171-176.

ASAE. 2002. 352.2. Moisture measurement - unground grain and seeds. In *2002 ASAE Standards*. 49th ed, p. 589. St. Joseph, MI: ASAE.

Aslanov, S.M. and E.N. Novruzov. 1976. The oil from the pulp residues of *hippophaë rhamnoides*. *Chemistry of Natural Compounds* 12:584-585.

Attah, J.C. and J.A. Ibemesi. 1990. Solvent extraction of the oils of rubber, melon, pumpkin, and oilbean seeds. *Journal of the American Oil Chemists Society* 67:25-27.

Bandonienė, D., A. Pukalskas, P.R. Venskutonis and D. Gruzdienė. 2000. Preliminary screening of antioxidant activity of some plant extracts in rapeseed oil. *Food Research International* 33:785-791.

Bargale, P.C., R.J. Ford, F.W. Sosulski, D. Wulfsohn and J. Irudayaraj. 1999. Mechanical oil expression from extruded soybean samples. *Journal of the American Oil Chemists Society* 76:223-229.

Berezhnaya, G.A., I.P. Eliseev, V.D. Tsydendambaev and A.G. Vereshchagin. 1989. Fatty acid composition and quantitative lipid content in sea buckthorn fruit. *Applied Biochemistry and Microbiology* 24:471-475.

Bernardini, E. 1976. Batch and continuous solvent extraction. *Journal of the American Oil Chemists Society* 53:275-278.

Beveridge, T. 2003. Chemical composition and some physical properties. In *Sea Buckthorn (Hippophaë rhamnoides L.): Production and Utilization*, eds. T.S.C. Li and T. Beveridge, 79-88. Ottawa, Ontario: NRC Research Press.

Beveridge, T., T.S.C. Li, B.D. Oomah, and A. Smith. 1999. Sea buckthorn products: manufacture and composition. *Journal of Agriculture and Food Chemistry* 47:3480-3488.

Beveridge, T., J.E. Harrison and J. Drover. 2002. Processing effects on the composition of sea buckthorn juice from *Hippophaë rhamnoides* L. cv. Indian-Summer. *Journal of Agriculture and Food Chemistry* 50:113-116.

Bizimana, V., W.M. Breene and A.S. Csallany. 1993. Avocado oil extraction with appropriate technology for developing countries. *Journal of the American Oil Chemists Society* 70:821-822.

Bligh, E.G. and W.J. Dyer. 1959. A rapid method of total lipid extraction and purification. *Canadian Journal of Biochemistry and Physiology* 37:911-917.

Bockisch, M. 1998. *Fats and Oils Handbook.* Champaign, Illinois: AOCS Press.

Bott, E.W. and P. Schöttler. 1989. Centrifuges, decanters, and processing lines for the citrus industry. Technical-scientific documentation no. 14. Oelde, Germany: Westfalia Separator AG.

Bozan, B. and F. Temelli. 2002. Supercritical CO_2 extraction of flaxseed. *Journal of the American Oil Chemists Society* 79:231-235.

Bredeson, D.K. 1978. Mechanical extraction. *Journal of the American Oil Chemists Society* 55:762-764.

Bredeson, D.K. 1983. Mechanical oil extraction. *Journal of the American Oil Chemists Society* 60:163-165.

Brühl, L. and B. Matthäus. 1999. Extraction of oilseeds by SFE - a comparison with other methods for the determination of the oil content. *Fresenius Journal of Analytical Chemistry* 364:631-634.

Bulley, N.R., M. Fattori, A. Meisen and L. Moyls. 1984. Supercritical fluid extraction of vegetable oil seeds. *Journal of the American Oil Chemists Society* 61:1362-1365.

Burkhalter, J.P. 1976. Crude oil handling and storage. *Journal of the American Oil Chemists Society* 53:332-333.

Carlson, K.D., E.C. Baker and G.C. Mustakas. 1985. Processing of *Crambe abyssinica* seed in commercial extraction facilities. *Journal of the American Oil Chemists Society* 62:897-905.

Carlson, K.D., R. Kleiman and R.P. Pavlik. 1983. Prepress solvent extraction of cuphea seed. *Journal of the American Oil Chemists Society* 70:1269-1272.

Carr, R. 1997. Oilseed processing. In *Technology and Solvents for Extracting Oilseeds and Nonpetroleum Oils*, eds. P. Wan and P. Wakelyn, 101-120. Champaign, Illinois: AOCS Press.

Champagne, E.T., R.J. Hron, Sr. and G. Abraham. 1992. Utilizing ethanol to produce stabilized brown rice products. *Journal of the American Oil Chemists Society* 69:205-208.

Che Man, Y.B., M.I.B. Abdul Karim and C.T. Teng. 1997. Extraction of coconut oil with *Lactobacillus plantarum* 1041 IAM. *Journal of the American Oil Chemists society* 74:1115-1119.

Chen, Y.T. and Y.C. Ling. 2000. An overview of supercritical fluid extraction in Chinese herbal medicine: from preparation to analysis. *Journal of Food and Drug Analysis* 8:235-247.

Chiu, K.L., Y.C. Cheng, J.H. Chen, C.J. Chang and P.W. Yang. 2002. Supercritical fluids extraction of ginko ginkgolides and flavonoids. *Journal of Supercritical Fluids* 24:77-87.

Christensen, P.L. 1983. Solvent extraction: recent developments. *Journal of the American Oil Chemists Society* 60:214-223.

Chu, Y.H. 1995. Effects of soybean pretreatments on crude oil quality. *Journal of the American Oil Chemists Society* 72:177-181.

Conkerton, E.J., P.J. Wan and O.A. Richard. 1995. Hexane and heptane as extraction solvents for cottonseed: a laboratory-scale study. *Journal of the American Oil Chemists Society* 72:963-965.

Cowan, J.C. 1976. Degumming, refining, bleaching, and deodorization theory. *Journal of the American Oil Chemists Society* 53:344-346.

Cross, C.K. 1990. The determination of neutral oil in detergent products by solid phase extraction. *Journal of the American Oil Chemists Society* 67:142-143.

Cunshe, Y. 1995. A clinical study of reducing fat and anti-oxidation of dried *Hippophaë* emulsion. *Hippophaë* 8:33-35.

Daukšas, E., P.R. Venskutonis and B. Sivik. 2002. Comparison of oil from *Nigella damascena* seed recovered by pressing, conventional solvent extraction, and carbon dioxide extraction. *Journal of Food Science* 67:1021- 1024.

Dedio, W. and D.G. Dorrell. 1977. Factors affecting the pressure extraction of oil from flaxseed. *Journal of the American Oil Chemists Society* 54:313-315.

de Lucas, A., J. Rincón and I. Gracia. 2002. Influence of operating variables on yield and quality parameters of olive husk oil extracted with supercritical carbon dioxide. *Journal of the American Oil Chemists Society* 79:237-243.

del Valle, J.M. and E.L. Uquiche. 2002. Particle size effects on supercritical CO_2 extraction of oil-containing seeds. *Journal of the American Oil Chemists Society* 79:1261-1266.

Devittori, C., D. Gumy, A. Kusy, L. Colarow, C. Bertoli and P. Lambelet. 2000. Supercritical fluid extraction of oil from millet bran. *Journal of the American Oil Chemists Society* 77:573-579.

Dillon, R., P. Loder and D. King. 2002. Canadian sea buckthorn technology mission to Russia and Germany - final report. National Research Council Canada/Industrial Research Assistance Program (NRC/IRAP), Government of Canada. August 31 - September 14, 2002. p. 51.

Diosady, L.L., L.J. Rubin, N. Ting and O. Trass. 1983. Rapid extraction of canola oil. *Journal of the American Oil Chemists Society* 60:1658-1661.

Djarmati, Z., R.M. Jankov, E. Schwirtlich, B. Djulinac and A. Djordjevic. 1991. High antioxidant activity of extracts obtained from sage by supercritical CO_2 extraction. *Journal of the American Oil Chemists Society* 68:731-734.

Dugan, L.R., and H.R. Kraybill. 1956. Tocopherols as carry-through antioxidants. *Journal of the American Oil Chemists Society* 33:527-528.

Dunning, J.W. 1953. History and latest developments in expeller and screw press operations on cottonseed. *Journal of the American Oil Chemists Society* 30:486-492.

Dunning, J.W. 1956. Unit operations in a mechanical extraction mill. *Journal of the American Oil Chemists Society* 33:462-470.

Eccleston, C., B. Yang, R. Tahvonen, H.P. Kallio, G.H. Rimbach and A.M. Minihane. 2002. Effects of an antioxidant-rich juice (sea buckthorn) on risk factors for coronary heart disease in humans. *Journal of Nutritional Biochemistry* 13:346:354.

Eng, T.G. and M.M. Tat. 1985. Quality control in fruit processing. *Journal of the American Oil Chemists Society* 62274-282.

Fasina, O.O. and O.O. Ajibola. 1990. Mechanical expression of oil from conophor nut (*Tetracarpidium conophorum*). *Journal of Agricultural Engineering Research* 44:275-287.

Ferreira, S.R.S. and M.A.A. Meireles. 2002. Modeling the supercritical fluid extraction of black pepper (*Piper nigrum* L.) essential oil. *Journal of Food Engineering* 54:263-269.

Folch, J., M. Lees and G.H. Sloane-Stanley. 1957. A simple method for isolation and purification of total lipids in animal tissues. *Journal of Biological Chemistry* 226:497-509.

Friedrich, J.P. and E.H. Pryde. 1984. Supercritical CO_2 extraction of lipid-bearing materials and characterization of the products. *Journal of the American Oil Chemists Society* 61:223-228.

Fullbrook, P.D. 1983. The use of enzymes in the processing of oilseeds. *Journal of the American Oil Chemists Society* 60:476-478.

Galloway, J.P. 1976. Cleaning, cracking, dehulling, decorticating, and flaking of oil-bearing materials. *Journal of the American Oil Chemists Society* 53:271-274.

Gao, X., M. Ohlander, N. Jeppsson, L. Björk and V. Trajkovski. 2000. Changes in antioxidant effects and their relationship to phytonutrients in fruits of sea buckthorn (*Hippophaë rhamnoides* L.) during maturation. *Journal of Agricultural and Food Chemistry* 48:1485-1490.

Geetha, S., M.S. Ram, V. Singh, G. Ilavazhagan and R.C. Sawhney. 2002. Anti-oxidant and immunomodulatory properties of sea buckthorn (*Hippophaë rhamnoides*) - an in vitro study. *Journal of Ethnopharmacology* 79:373-378.

George, S. and C. Arumughan. Lipid profile of process streams of palm oil mill. *Journal of the American Oil Chemists Society* 69:283-287.

Ghassempour, A., A. Mohammadkhah, F. Najafi and M. Rajabzadeh. 2002. Monitoring of the pesticide diazinon in soil, stem, and surface water of rice fields. *Analytical Sciences* 18:779-783.

Giovacchino, L.D., M. Solinas and M. Miccoli. 1994. Effect of extraction systems on the quality of virgin olive oil. *Journal of the American Oil Chemists Society* 71:1189-1194.

Gómez, A.M. and E.M. de la Ossa. 2000. Quality of wheat germ oil extracted by liquid and supercritical carbon dioxide. *Journal of the American Oil Chemists Society* 77:969-974.

Gómez, A.M. and E.M. de la Ossa. 2002. Quality of borage seed oil extracted by liquid and supercritical carbon dioxide. *Chemical Engineering Journal* 88:103-109.

Gómez, A.M., C.P. López and E.M. de la Ossa. 1996. Recovery of grape seed oil by liquid and supercritical carbon dioxide extraction: a comparison with conventional solvent extraction. *The Chemical Engineering Journal* 61:227-231.

Goodrum, J.W. and M.B. Kilgo. 1987. Peanut oil extraction using compressed CO_2. *Energy in Agriculture* 6:265-271.

Gordon, M.H. and I.A. Rahman. 1991. Effect of processing on the compositional and oxidative stability of coconut oil. *Journal of the American Oil Chemists Society* 68:574-576.

Gurnham, C.F. and H.J. Masson. 1946. Expression of liquids from fibrous materials. *Industrial and Engineering Chemistry* 38:1309-1315.

Hagenmaier, R. 1997. Aqueous processing. In *Technology and Solvents for Extracting Oilseeds and Nonpetroleum Oils*, eds. P. Wan and P. Wakelyn, 311-322. Champaign, Illinois: AOCS Press.

Häkkinen, S., M. Heinonen, S. Kärenlampi, H. Mykkanen, J. Ruuskanen and R. Törrönen. 1999a. Screening of selected flavonoids and phenolic acids in 19 berries. *Food Research International* 32:345-353.

Häkkinen, S., S.O. Kärenlampi, I.M. Heinonen, H.M. Mykkänen and A.R. Törrönen. 1999b. Content of the flavonols quercetin, myricetin, and kaempferol in 25 edible berries. *Journal of Agricultural and Food Chemistry* 47:2274-2279.

Halvorsen, B.L., C. Thomsen, T. Greibrokk and E. Lundanes. 2000. Determination of fenpyroximate in apples by supercritical fluid extraction and packed capillary liquid chromatography with UV detection. *Journal of Chromatography* 880:121-128.

Hanmoungjai, P., D.L. Pyle and K. Niranjan. 2001. Enzymatic process for extracting oil and protein from rice bran. *Journal of the American Oil Chemists Society* 78:817-821.

Hardy, Gil. 2000. Nutraceuticals and functional foods: introduction and meaning. *Nutrition* 16:688-697.

Health Canada. 1998. Policy paper: nutraceuticals/functional foods and health claims on foods final policy. Therapeutic Products Programme and the Food Directorate from the Health Protection Branch. Government of Canada. November 2, 1998. 29 p.

Heinz, C. and M. Barbaza. 1998. Envioronmental changes during the late glacial and post-glacial in the Central Pyrénées (France): new charcoal analysis and archaeological data. *Review of Palaeobotany and Palynology* 104:1-17.

Hénon, G., Z. Kemény, K. Recseg, F. Zwobada and K. Kövári. 1997. Degradation of α-linolenic acid during heating. *Journal of the American Oil Chemists Society* 74:1615-1617.

Hickox, G.H. 1953. Some factors affecting the hydraulic extraction of cottonseed oil. *Journal of the American Oil Chemists Society* 30:481-486.

Hilton, D.J. 1999. Processing of fruit and vegetables. In *CIGR Handbook of Agricultural Engineering Volume IV - Agro-Processing Engineering*, ed. F.W. Bakker-Arkema, 362-380. St. Joseph, Michigan: ASAE.

Hron, R.J., Sr., G. Abraham, M.S. Kuk and G.S. Fisher. 1992. Acidic ethanol extraction of cottonseed. *Journal of the American Oil Chemists Society* 69:951-1002.

Hron, R.J., Sr., M.S. Kuk, G. Abraham, and P.J. Wan. 1994. Ethanol extraction of oil, gossypol and aflatoxin from cottonseed. *Journal of the American Oil Chemists Society* 71:417-421.

Hruschka, S.M. 2000. Current experience in the FRIOLEX® oils and fats recovery process. Presentation slides. GEA-Westfalia Separator, Mechanical Separation Division, 59302 Oelde, Germany.

Hutchins, R.P. 1949. Processing of oilseeds and nuts by hydraulic and mechanical screw press method. *Journal of the American Oil Chemists Society* 26:559-563.

Hutchins, R.P. 1976. Continuous solvent extraction of soybeans and cottonseed. *Journal of the American Oil Chemists Society* 53:279-282.

Ibáñez, E., A.M. Hurtado Benavides, F.J. Señoráns and G. Reglero. 2002. Concentration of sterols and tocopherols from olive oil with supercritical carbon dioxide. *Journal of the American Oil Chemists Society* 79:1255-1260.

James, A.T. 1985. The biotechnology of oilseed crops. *Journal of the American Oil Chemists Society* 62:204-206.

Jackson, C.-J.C., J.P. Dini, C. Lavandier, H.P.V. Rupasinghe, H. Faulkner, V. Poysa, D. Buzzell and S. DeGrandis. 2002. Effects of processing on the content and composition of isoflavones during manufacturing of soy beverage and tofu. *Process Biochemisty* 37:1117-1123.

Jeppsson, N., I.V. Bartish and H.A. Persson. 1999. DNA analysis as a tool in sea buckthorn breeding. In *Perspectives on New Crops and New Uses*, ed. J. Janick, 338-341. Alexandria, Virginia: ASHS Press.

Johansson, A.K., H. Korte, B. Yang., J.C. Stanley and H.P. Kallio. 2000. Sea buckthorn berry oil inhibits platelet aggregation. *Journal of Nutritional Biochemistry* 11:491-495.

Johnson, L. 1997. Theoretical, comparative, and historical analyses of alternative technologies for oilseeds extraction. In *Technology and Solvents for Extracting Oilseeds and Nonpetroleum Oils*, eds. P. Wan and P. Wakelyn, 4-47. Champaign, Illinois: AOCS Press.

Johnson, L.A., J.T. Farnsworth, N.Z. Sadek, N. Chamkasem, E.W. Lusas and B.L. Reid. Pilot plant studies on extracting cottonseed with methylene chloride. *Journal of the American Oil Chemists Society* 63:647-652.

Johnson, L.A. and E.W. Lusas. 1983. Comparison of alternative solvents for oils extraction. *Journal of the American Oil Chemists Society* 60:181-194.

Kähkönen, M.P., A.I. Hopia, H.J. Vuorela, J-.P. Rauha, K. Pihlaja, T.S. Kujala and Heinonen. Antioxidant activity of plant extracts containing phenolic compounds. *Journal of Agricultural and Food Chemistry* 47:3945-3962.

Karlovic, D., M. Solvilj and J. Turkulov. 1992. Kinetics of oil extraction from corn germ. *Journal of the American Oil Chemists Society* 69:471-476.

Kallio, H., B. Yang and P. Peippo. 2002a. Effects of different origins and harvesting time on vitamin C, tocopherols, and tocotrienols in sea buckthorn (*Hippophaë rhamnoides*) berries. *Journal of Agricultural and Food Chemistry* 50:6136-6142.

Kallio, H., B. Yang, P. Peippo, R. Tahvonen and R. Pan. 2002b. Triacylglycerols, glycerophospholipids, tocopherols, tocotrienols in berries and seeds of two sspecies (ssp. *sinensis* and *mongolica*) of sea buckthorn (*Hippophaë rhamnoides*). *Journal of Agriculture and Food Chemistry* 50:3004-3009.

Kalustian, P. 1985. Pharmaceutical and cosmetic used of palm and lauric products. *Journal of the American Oil Chemists Society* 62:431-435.

Kemper, T.G. 1997. Solvent recovery and loss management. In *Technology and Solvents for Extracting Oilseeds and Nonpetroleum Oils*, eds. P. Wan and P. Wakelyn, 148-152. Champaign, Illinois: AOCS Press.

Khan, L.M. and M.A. Hana. 1983. Expression of oil from oilseeds - a review. *Journal of Agriculture Engineering Research* 28:495-503.

Khan, L.M. and M.A. Hana. 1984. Expression of soybean oil. *Transactions of the ASAE* 27:190-194.

Khor, H.T. and S.L. Chan. 1985. Comparative studies of three solvent mixtures for the extraction of soybean lipids. *Journal of the American Oil Chemists Society* 62:98-99.

Kim, I.H., M.H. Kim, Y.E. Kim and Y.C. Lee. 1998. Oxidative stability and extraction of perilla seed oil with supercritical carbon dioxide. *Food Science and Biotechnology* 7:177-180.

Kim, B., K. Dal-Ho, E. Hwang, and S. Hun-Young. 2002. Comparison of sample extraction methods for the determination of bis (*p*-chlorophenyl) dichloroethlene (*p,p'*-DDE) in rice flour using isotope dilution mass spectrometry. *Bulletin of the Korean Chemical Society* 23:935-936.

Kim, S., W. Lee, S. Yeo and Y. Choi. 1996. Supercritical carbon dioxide extraction of perilla seed oil. *Foods and Biotechnology* 5:300-304.

King, J.W. 1997. Critical fluids for oil extraction. In *Technology and Solvents for Extracting Oilseeds and Nonpetroleum Oils*, eds. P. Wan and P. Wakelyn, 283-310. Champaign, Illinois: AOCS Press.

Kiriamiti, H.K., E. Rascol, A. Marty and J.S. Condoret. 2001. Extraction rates of oil from high oleic sunflower seeds with supercritical carbon dioxide. *Chemical Engineering and Processing* 41:711-718.

Kiritsakis, A.K. 1984. Effect of selected storage conditions and packaging materials on olive oil quality. Journal of the American Oil Chemists Society 61:1868-1870.

Kiritsakis, A.K., G.D. Nanos, Z. Polymenopoulos, T. Thomai and E.M. Sfakiotakis. 1998. Effect of fruit storage conditions on olive oil quality. *Journal of the American Oil Chemists Society* 75:721-724.

Koo, E.C. 1942. Expression of vegetable oil - a general equation on oil expression. *Journal of Industrial Engineering Chemistry* 34:342-345.

Krause, M.V. and L.K. Mahan. 1979. *Food, Nutrition and Diet Therapy*. 6th ed. Toronto, Ontario: W.B. Saunders Company.

Kreuzig, R., A. Koinecke and M. Bahadir. 2000. Use of supercritical fluid extraction in the analysis of pesticides in soil. *Journal of Biochemical and Biophysical Methods* 43:403-409.

Kuk, M.S. and M.K. Dowd. 1998. Supercritical CO_2 extraction of rice bran. *Journal of the American Oil Chemists Society* 75:623-628.

Kuk, M.S. and R.J. Hron, Sr. 1994. Supercritical carbon dioxide extraction of cottonseed with co-solvents. *Journal of the American Oil Chemists Society* 71:1353-1356.

Kwiatkowski, J.R. 2002. Ethanol extraction of oil from dry-milled corn: process optimization and rheological properties of the extract. ASAE Paper No. 026144. St. Joseph, MI: ASAE.

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

Labconco Corporation. 1997. Goldfische fat extraction apparatus model 35001 - instruction manual. 35181 Revision I / ECO 9286, Labconco Corporation, Kansas City, MO.

Lee, W.Y., Y.J. Cho, S.L. Oh, J.H. Park, W.S. Cha, J.Y. Jung and Y.H. Choi. 2000. Extraction of grape seed oil by supercritical CO_2 and ethanol modifier. *Food Science and Biotechnology* 9:174-178.

Lee, S.K. and A.A. Kader. 2000. Preharvest and postharvest factors influencing vitamin C content of horticultural crops. *Post Harvest Biology and Technology* 20:207-220.

Lewicki, P.P., H.V. Le and W. Pomarańska-Łazuka. 2002. Effect of pre-treatment on convective drying of tomatoes. *Journal of Food Engineering* 54:141-146.

Li, T.S.C. 1998. Nutraceuticals in sea buckthorn. Prairie Medicinal and Aromatic Plants Conference. Saskatoon, Saskatchewan, March 5-7, 1998. p. 10-44.

Li, T.S.C. and W.R. Schroeder. 1996. Sea buckthorn (*Hippophaë rhamnoides* L.): a multipurpose plant. *Hort Technology* 6:370-380.

Li, T.S.C and W.R. Schroeder. 1999. *A Grower's Guide to Sea Buckthorn.* Pacific Agri-Food Research Centre, Highway 97, 4200, Summerland, British Columbia, V0H 1Z0. Agriculture and Agri-Food Canada, Research Branch, Canada. 75 p.

List, G.R., J.P. Friedrich and J. Pominski. 1984. Characterization and processing of cottonseed oil obtained by extraction with supercritical carbon dioxide. *Journal of the American Oil Chemists Society* 61:1847-1848.

Liu, J. and Z. Liu. 1989. Research of processing technology for sea buckthorn concentrated juice. Proceedings of International Symposium on Sea Buckthorn (*Hippophaë rhamnoides* L.), Xian, China. p. 314-317.

Lyon, C.K. and R. Becker. 1987. Extraction and refining of oil from amaranth seed. *Journal of the American Oil Chemists Society* 64:233-236.

Mamedov, S.S., E.I. Gigienova, A.U. Umarov and S.M. Aslanov. 1981. Lipids of the oils of the fruit and leaves of *Hippophaë rhamnoides*. *Chemistry of Natural Compounds* 17:511-515.

Maness, N.O., D. Chrz, T. Pierce and G.H. Brusewitz. 1995. Quantitative extraction of pecan oil from small samples with supercritical carbon dioxide. *Journal of the American Oil Chemists Society* 72:665-669.

Mangold, H.K. 1983. Liquefied gases and supercritical fluids in oilseed extraction. *Journal of the American Oil Chemists Society* 60:226-228.

Mann, D.D., D.S. Petkau and T.G. Crowe. 2003. Evaluation of a prototype sea buckthorn leaf harvester. *Canadian Biosystems Engineering* 45:2.9-2.15.

Manninen, P., E. Häivälä, S. Sarimo and H. Kallio. 1997. Distribution of microbes in supercritical CO_2 extraction of sea buckthorn (*Hippophaë rhamnoides*) oils. *Z Lebensm Unters Forsch A* 204:202-205.

Manninen, P., J. Pakarinen and H. Kallio. 1997. Large-scale supercritical carbon dioxide extraction and supercritical carbon dioxide countercurrent extraction of cloudberry seed oil. *Journal of Agricultural and Food Chemistry* 45:2533-2538.

Marin, F.R., M. Martinez, T. Uribesalgo, S. Castillo and M.J. Frutos. 2002. Changes in nutraceutical composition of lemon juices according to different industrial extraction systems. *Food Chemistry* 78:319-324.

McHugh, M.A. and V.J. Krukonis. 1986. *Supercritical Fluid Extraction: Principles and Practice*. Stoneham, MA: Butterworths Publishers.

Menrad, K. 2003. Market and marketing of functional food in Europe. *Journal of Food Engineering* 56:181-188.

Milligan, E.D. 1976. Survey of current solvent extraction equipment. *Journal of the American Oil Chemists Society* 53:286-290.

Mogilevskaya, M.P., L.D. Ageeva, Y.A. Koshelev and M.T. Yanotovsky. 1978. Method of analysis of palmitoleic acid in sea buckthorn oil. *Khimiko Farmatsevticheskii Zhurnal* 12:143-146.

Montanari, L., P. Fantozzi, J.M. Snyder and J.W. King. 1999. Selective extraction of phospholipids from soybeans with supercritical carbon dioxide and ethanol. *Journal of Supercritical Fluids* 14:87-93.

Morales, M.T. and R. Aparicio. 1999. Effect of extraction conditions on sensory quality of virgin olive oil. *Journal of the American Oil Chemists Society* 76:295-300.

Motohashi, N., H. Nagashima and C. Párkányi. 2000. Supercritical fluid extraction for the analysis of pesticide residue in miscellaneous samples. *Journal of Biochemical and Biophysical Methods* 43:313-328.

Muuse, B.G., F.P. Cuperus and J.T.P. Derksen. 1994. Extraction and characterization of *Dimorphotheca pluvialis* seed oil. *Journal of the American Oil Chemists Society* 71:313-317.

Myers, N.W. 1983. Solvent recovery. *Journal of the American Oil Chemists Society* 60:224-225.

Neidleman, S.L. and J. Geigert. 1984. Biotechnology and oleochemicals: changing patters. *Journal of the American Oil Chemists Society* 61:290-297.

Nerín, C., R. Batlle, M. Sartaguda and C. Pedrocchi. 2002. Supercritical fluid extraction of organochlorine pesticides and some metabolites in frogs from national park of ordesa and monte perdido. *Analytica Chimica Acta* 464:303-312.

Nieh, C.D. and H.E. Snyder. 1991a. Solvent extraction of oil from soybean flour I – extraction rate, a countercurrent extraction system, and oil quality. *Journal of the American Oil Chemists Society* 68:246-249.

Nieh, C.D. and H.E. Snyder. 1991b. Solvent extraction of oil from soybean flour II - pilot plant and two-solvent extractions. *Journal of the American Oil Chemists Society* 68:250-253.

Norman, K.N.T., and S.H.W. Panton. 2001. Supercritical fluid extraction and quantitative determination of organophosphorus pesticide residues in wheat and maize using gas chromatography with flame photometric and mass spectrometric detection. *Journal of Chromatography* 907:247-255.

Ohlson, J.S.R. 1976. Processing effects on oil quality. *Journal of the American Oil Chemists Society* 53:299-301.

Oliveira, R., M.F. Rodrigues and M.G. Bernardo-Gil. 2002. Characterization and supercritical carbon dioxide extraction of walnut oil. *Journal of the American Oil Chemists Society* 79:225-230.

Oomah, B.D. 2003. Sea buckthorn lipids. In *Sea Buckthorn (Hippophaë rhamnoides L.): Production and Utilization*, eds. T.S.C. Li and T. Beveridge, 51-68. Ottawa, Ontario: NRC Research Press.

Oomah, B.D., S. Ladet, D.V. Godfrey, J. Liang and B. Girard. 2000. Characteristics of raspberry (*Rubus idaeus* L.) seed oil. *Food Chemistry* 69:187-193.

Oomah, B.D. and G. Mazza. 1998. Compositional changes during commercial processing of flaxseed. *Industrial Crops and Products* 9:29-37.

Oomah, B.D. and G. Mazza. 1999. Health benefits of phytochemicals from selected Canadian crops. *Trends in Food Science and Technology* 10:193-198.

Owusu-Ansah, Y.J. 1997. Enzyme-assited extractions. In *Technology and Solvents for Extracting Oilseeds and Nonpetroleum Oils*, eds. P. Wan and P. Wakelyn, 323-332. Champaign, Illinois: AOCS Press.

Palazoğlu, T.K. and M.O. Balaban. 1998. Supercritical CO_2 extraction of lipids from roasted pistachio nuts. *Transactions of the ASAE* 41:679-684.

Papamichail, I., V. Louli and K. Magoulas. 2000. Supercritical fluid extraction of celery seed oil. *Journal of Supercritical Fluids* 18:213-226.

Partanen, R., H. Yoshii, H. Kallio, B. Yang. and P. Forssell. 2002. Encapsulation of sea buckthorn kernel oil in modified starches. *Journal of the American Oil Chemists Society* 79:219-223.

Peñalba M.C., M. Arnold, J. Guiot, J.C. Duplessy and J.L. de Beaulieu. 1997. Termination of the last glaciation in the Iberian Peninsula inferred from the pollen sequence of Quintanar de la Sierra. *Quarternary Research* 48:205-214.

Peterson, C.L., D.L. Auld and J.C. Thompson. 1983. Experiments with vegetable oil expression. *Transactions of the ASAE* 26:1298-1302.

PFRA Shelterbelt Centre. 2001. Sea buckthorn a multipurpose crop. Agriculture and Agri-Food Canada. Prairie Farm Rehabilitation Administration (PFRA) Shelterbelt Centre, Agriculture and Agri-Food Canada, Indian Head, Saskatchewan, S0G 2K0. October, 2001. 4 p.

Platin, S., E.Ö. Özer, U. Akman and Ö. Hortaçsu. 1994. Equilibrium distributions of key components of spearmint oil in sub/supercritical carbon dioxide. *Journal of the American Oil Chemists Society* 71:833-837.

Prados-Rosales, R.C., J.L. Luque Garcia and M.D. Luque de Castro. 2003. Rapid analytical method for the determination of pesticide residues in sunflower seeds based on focused microwave-assisted soxhlet extraction prior to gas chromatography - tandem mass spectrometry. *Journal of Chromatography* 993:121-129.

Puupponen-Pimiä, R., A.-M. Aura, K.-M. Oksman-Caldentey, P. Myllärinen, M. Saarela, T. Mattila-Sandholm and K. Poutanen. 2002. Development of functional ingredients for gut health. *Trends in Food Science and Technology* 13:3-11.

Quirin, K.W. and D. Gerard. 1995. Sea buckthorn pulp and kernel oils: valuable lipids for the skin. *Cosmetics and Toiletries Manufacture Worldwide* 57:57-60.

Rizvi, S.S.H., A.L. Benado, J.A. Zollweg and J.A. Daniels. Supercritical fluid extraction: fundamental principles and modeling methods. *Food Technology* 40:55-65.

Roberfroid, M.B. 2000. A European consensus of scientific concepts of functional foods. *Nutrition* 16:689-691.

Roden, A. and G. Ullyot. 1984. Quality control in edible oil processing. *Journal of the American Oil Chemists Society* 61:1109-1111.

Rousi, A. 1971. The genus *Hippophaë* L. a taxonomic study. *Annales Botanici Fennici* 8:177-227.

Rozzi, N.L. and R.K. Singh. 2002. Supercritical fluids and the food industry. *Comprehensive Reviews in Food Science and Food Safety* 1:33-44.

Rozzi, N.L., R.K. Singh, R.A. Vierling and B.A. Watkins. 2002. Supercritical fluid extraction of lycopene from tomato processing byproducts. *Journal of Agriculture and Food Chemistry* 50:2638-2643.

Sahasrabudhe, M.R. and B.W. Smallbone. 1983. Comparative evaluation of solvent extraction methods for the determination of neutral and polar lipids in beef. *Journal of the American Oil Chemists Society* 60:801-805.

Sarojini, G., K.C. Rao, P.G. Tulpule and G. Lakshminarayana. 1985. Effects of processing on physicochemical properties and fatty acid composition of *Hibiscus sabdariffa* seed oil. *Journal of the American Oil Chemists Society* 62:728-730.

Schmulgen, M. 2000. FRIOLEX® oil extraction technology opportunity. Manitoba Agri-Food Technologies Committee. Food Development Centre, Box 1240, 810 Phillips St., Portage la Prairie, Manitoba, R1N 3J9. June 2000. 4 p.

Schroeder, W.R. and Y. Yao. 1995. Sea buckthorn: a promising multipurpose crop for Saskatchewan. Prairie Farm Rehabilitation Administration (PFRA), Agriculture and Agri-Food Canada. Supplementary report 95-2. 7 p.

Shaftan, E.A., N.S. Mikhailova, N.D. Gol'berg and A.V. Pekhov. 1978. An investigation of the chemical composition of a CO_2 carbon dioxide extract from a pulp of *Hippophaë rhamnoides*. *Chemistry of Natural Compounds* 14:560-561.

Sharma, A., S.K. Khare and M.N. Gupta. 2002. Ezyme-assited aqueous extraction of peanut oil. *Journal of the American Oil Chemists Society* 79:215-218.

Shi, H., H. Cai, X. Chen and C. Yang. 1994. Studies on the antioxidative effect of *Hippophae rhamnoides* L. seed oil. *Acta Nutrimenta Sinica* 16:292-295.

Shinwari, Z.K. and S.S. Gilani. 2003. Sustainable harvest of medicinal plants at Bulashbar Nullah, Astore (Northern Pakistan). *Journal of Ethnopharmacology* 84:289-298.

Sihvonen, M., E. Järvenpää, V. Hietaniemi and R. Huopalahti. 1999. Advances in supercritical carbon dioxide technologies. *Trends in Food Science and Technology* 10:217-222.

Singh, J. and P.C. Bargale. 2000. Development of a small capacity double stage compression screw press for oil expression. *Journal of Food Engineering* 43:75-82.

Singh, V. and K.K. Dogra. 1996. Charactaristics, distribution, utilization, regeneration, biomass, and nutritional values of sea buckthorn (*Hippophaë*). *Indian Forester* 122:486-491.

Singh, M.S., A. Farsaie, L.E. Stewart and L.W. Douglass. 1984. Development of mathematical models to predict sunflower oil expression. *Transactions of the ASAE* 27:1190-1194.

Singh, K.K., D. Wiesenborn, N. Kangas and K. Tostenson. 2002a. Characterization of preparation parameters for improved screw pressing of crambe seed. *Transactions of the ASAE* 45:1029-1035.

Singh, K.K., D.P. Wiesenborn, K. Tostenson, and N. Kangas. 2002b. Influence of moisture content and cooking on screw pressing of crambe seed. *Journal of the American Oil Chemists Society* 79:165-170.

Sivala, K., N.G. Bhole, and R.K. Mukherjee. 1991. Effect of moisture on rice bran oil expression. *Journal of Agriculture Engineering Research* 50:81-91.

Sosulski, K. and F.W. Sosulski. 1993. Enzyme-aided vs. two-stage processing of canola: technology, product quality, and cost evaluation. *Journal of the American Oil Chemists Society* 70:825-829.

Spricigo, C.B., L.T. Pinto, A. Bolzan and A.F. Novais. 1999. Extraction of essential oil and lipids from nutmeg by liquid carbon dioxide. *Journal of Supercritical Fluids* 15:253-259.

Süleyman, H., K. Gumustekin, S. Taysi, S. Keles, N. Oztasan, O. Aktas, K. Altinkaynak, H. Timur, F. Akcay, S. Akar, S. Dane and M. Gul. 2002. Beneficial effects of *Hippophaë rhamnoides* L. on nicotine induced oxidative stress in rat blood compared with vitamin E. *Biological and Pharmaceutical Bulletin* 25:1133-1136.

Süleyman, H., M.E. Büyükokuroglu, M. Koruk, F. Akçay, A. Kiziltunç and A. Gepdiremen. 2001. The effects of *Hippophaë rhamnoides* L. extract on ethanol-induced gastric lesion and gastric tissue glutathione level in rats: a comparative study with melatonin and omeprazole. *Indian Journal of Pharmacology* 33:77-81.

Stainsby, M.A. 1988. Developments in screw pressing. *Journal of the American Oil Chemists Society* 65:1413-1416.

Štastová, J., J. Jež, M. Bártlová and H. Sovová. 1996. Rate of the vegetable oil extraction with supercritical CO_2 – III extraction from sea buckthorn. *Chemical Engineering Science* 51:4347-4352.

Storey, G. 2000. Competitive market analysis of sea buckthorn in Saskatchewan. Department of Agricultural Economics, University of Saskatchewan, Saskatoon, Saskatchewan. Saskatchewan Governement Publication AFIF 19970307. 39 p.

Stein W., and F.W. Glaser. 1976. Continuous solvent extraction of sunflower seed, groundnuts, palmkernels, rapeseed, and copra. *Journal of the American Oil Chemists Society* 53:283-285.

Swientek, R.J. 1987. Supercritical fluid extraction separates components in foods. *Food Processing* 48:32-36.

Szentmihályi, K., P. Vinkler, B. Lakatos, V. Illés and M. Then. 2002. Rose hip (*Rosa canina* L.) oil obtained from waste hip seeds by different extraction methods. *Bioresource Technology* 82:195-201.

Tang, X., N. Kälviäinen and H. Tuorila. 2001. Sensory and hedonic characteristics of juice of sea buckthorn (*Hippophaë rhamnoides* L.) origins and hybrids. *Lebensmittel-Wissenschaft und-Technologie* 34:102-110.

Tang, X. and P.M.A. Tigerstedt. 2001. Variation of physical and chemical characters within an elite sea buckthorn (*Hippophaë rhamnoides* L.) breeding population. *Scientia Horticulturae* 88:203-214.

Tavlarides, L.L., W. Zhou and G. Anitescu. 2000. Supercritical fluid technology for remediation of PCB/PAH-contaminated soils/sediments. Proceedings of the 2000 Conference on Hazardous Waste Research, Syracuse, NY. p. 239-255.

Taylor, S., J. King and G. List. 1993. Determination of oil content in oilseeds by analytical supercritical fluid extraction. *Journal of the American Oil Chemists Society* 70:437-439.

Tian, C., X. He., Y. Zhong and J. Chen. 2002. Effects of VA mycorrhizae and *Frankia* dual inoculation on growth and nitrogen fixation of *Hippophaë tibetana*. *Forest Ecology and Management* 170:307-312.

Tranchino, L. and F. Melle. 1984. Evaluation of extractive contact units for oil extraction from dehulled sunflower seed. *Journal of the American Oil Chemists Society* 61:1258-1261.

Tulbentçi, H.S.G. 1986. Extraction of rapeseed, linseed, safflower, and tobaccoseed with a new laboratory extractor. *Journal of the American Oil Chemists Society* 63:1465-1469.

Utioh, A. 2002. Sea buckthorn technology mission to Russia and Germany, August 30 - September 14, 2002: a final report. Food Development Centre, Box 1240, 810 Phillips St., Portage la Prairie, Manitoba, R1N 3J9. 134 p.

Vaher, M. and M. Koel. 2003. Separation of polyphenolic compounds extracted from plant matrices using capillary electrophoresis. *Journal of Chromatography* 990:225-230.

Wan, P.J, D.R. Pakarinen, R.J. Hron, Sr., O.L. Richard and E.J. Conkerton. 1995a. Alternative hydrocarbon solvents for cottonseed extraction. *Journal of the American Oil Chemists Society* 72:653-659.

Wan, P.J, R.J. Hron, Sr., M.K. Dowd, M.S. Kuk and E.J. Conkerton. 1995b. Alternative hydrocarbon solvents for cottonseed extraction: plant trials. *Journal of the American Oil Chemists Society* 72:661-664.

Wang, H., G. Cao and R.L. Prior. 1996. Total antioxidant capacity of fruits. *Journal of Agricultural and Food Chemistry* 44:701-705.

Wang, H.C., C.R. Chen and C.J. Chang. 2001. Carbon dioxide extraction of ginseng root hair oil and ginsenosides. *Food Chemistry* 72:505-509.

Wang, T. and L.A. Johnson. 2001. Refining normal and genetically enhanced soybean oils obtained by various extraction methods. *Journal of the American Oil Chemists Society* 78:809-815.

Ward, J.A. 1976. Processing high oil content seeds in continuous screw presses. *Journal of the American Oil Chemists Society* 53:261-264.

Ward, J.A. 1984. Pre-pressing of oil from rapeseed and sunflower. *Journal of the American Oil Chemists Society* 61:1358-1361.

Wiesenborn, D., R. Doddapaneni, K. Tostenson and N. Kangas. 2002. Kinetic characterization of cooking-induced changes in crambe seed prepared for expelling. *Journal of Food Engineering* 55:157-163.

Williams, M.A. 1997. Preparation of oil-bearing materials for extraction. In *Technology and Solvents for Extracting Oilseeds and Nonpetroleum Oils*, eds. P. Wan and P. Wakelyn, 121-136. Champaign, Illinois: AOCS Press.

Xing, J., B. Yang, Y. Dong, B. Wang, J. Wang and H.P. Kallio. 2002. Effects of sea buckthorn (*Hippophaë rhamnoides* L.) seed and pulp oils on experimental models of gastric ulcer in rats. *Fitoterapia* 73:644-650.

Xu, M. 1995. Anticancer effects of and direction of research on *Hippophaë*. *Hippophaë* 7:41-43.

Xu, Z. and J.S. Godber. 2000. Comparison of supercritical fluid and solvent extraction methods in extracting γ-oryzanol from rice bran. *Journal of the American Oil Chemists Society* 77:547551.

Yang, B. and H. Kallio. 2001. Fatty acid composition of lipids in sea buckthorn (*Hippophaë rhamnoides* L.) berries of different origins. *Journal of Agricultural and Food Chemistry* 49:1939-1947.

Yang, B. and H. Kallio. 2002a. Composition and physiological effects of sea buckthorn (*Hippophaë*) lipids. *Trends in Food Science and Technology* 13:160-167.

Yang, B. and H. Kallio. 2002b. Effects of harvesting time on triacylglycerols and glycerophospholipids of sea buckthorn (*Hippophaë rhamnoides* L.) berries of different origins. *Journal of Food Composition and Analysis* 15:143-157.

Yang, B., K.O. Kalimo, L.M. Mattila, S.E. Kallio, J.K. Katajisto, O.J. Peltola and H.P. Kallio. 1999. Effects of dietary supplementation with sea buckthorn (*Hippophaë rhamnoides*) seed and pulp oils on atopic dermatitis. *Journal of Nutritional Biochemistry* 10:622-630.

Yang, B., K.O. Kalimo, R.L. Tahvonen, L.M. Mattila, J.K. Katajisto and H.P. Kallio. 2000. Effect of dietary supplementation with sea buckthorn (*Hippophaë rhamnoides*) seed and pulp oils on the fatty acid composition of skin glycerophospholipids of patients with atopic dermatitis. *Journal of Nutritional Biochemistry* 11:338-340.

Yang, B., R.M. Karlsson, P.H. Oksman and H.P. Kallio. 2001. Phytosterols in sea buckthorn (*Hippophaë rhamnoides* L.) berries: identification and effects of different origins and harvesting times. *Journal of Agricultural and Food Chemistry* 49:5620-5629.

Yao, Y., P.M.A. Tigerstedt and P. Joy. 1992. Variation of vitamin C concentration and character correlation between and within natural sea buckthorn (*Hippophaë rhamnoides* L.) populations. *Acta Agriculturea Scandinavica Section F-Soil and Plant Science* 42:12-17.

Yoon, S.H., S.K. Kim, K.H. Kim, T.W. Kwon and Y.K. Teah. 1987. Evaluation of physicochemical changes in cooking oil during heating. *Journal of the American Oil Chemists Society* 64:870-873.

Zanoni, B., C. Peri, R. Nani and V. Lavelli. 1999. Oxidative heat damage of tomato halves as affected by drying. *Food Research International* 31:395-401.

Zhang, P., X. Ding, L. Mao, D. Li and L. Li. 1989a. Anti-tumor effects of fruit juice and seed oil of *Hippophaë rhamnoides* and their influences on immune function. Proceedings of International Symposium on Sea Buckthorn (*Hippophaë Rhamnoides* L.), Xian, China. p. 373-381.

Zhang, Y., J. Xu. and N. Yu. 1989b. Research on the centrifuge technology of sea buckthorn juice and oil. Proceedings of International Symposium on Sea Buckthorn (*Hippophaë rhamnoides* L.), Xian, China. p. 307-310.

Zúñiga, M.E., C. Soto, A. Mora, R. Chamy, and J.M. Lema. Enzymatic pre-treatment of guevina avellana mol oil extraction by pressing. *Process Biochemistry* (In Press).

Appendices

Appendix A1

Pilot process for the preparation of seeds and pulp-flakes

Juice extraction:

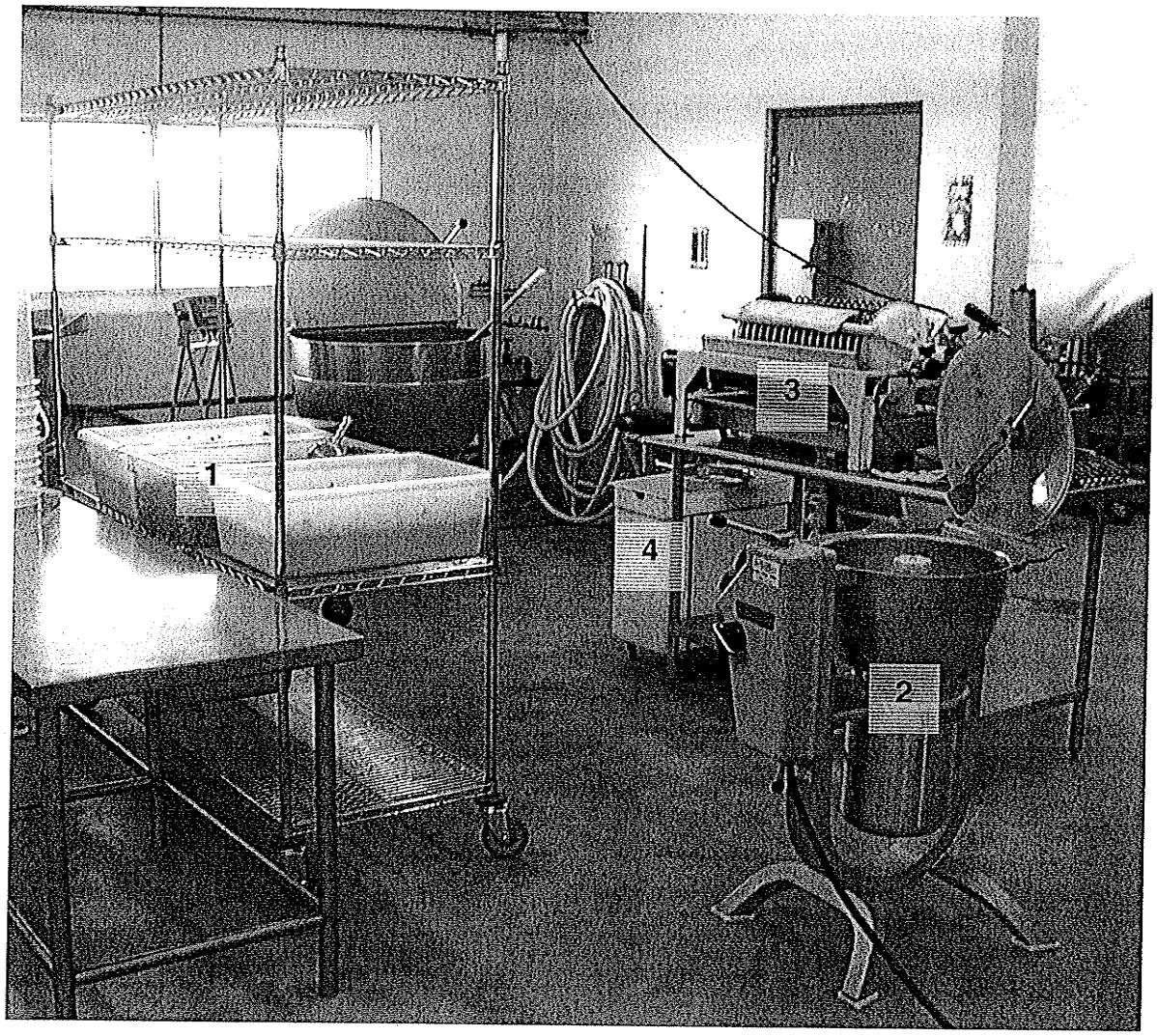
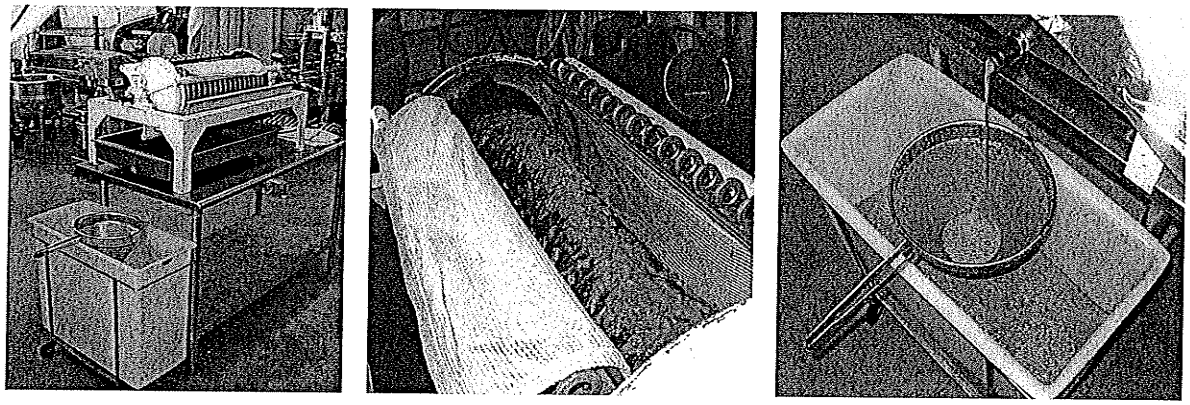


Fig. A1-1. Juice extraction pilot process. (1) Thawing of berries in plastic tubs. (2) Berry maceration using a cutter mixer. (3) Juice extraction using a fruit bladder press. (4) Juice filtering and collection.

(a) (b) (c)

Fig. A1-2. (a) Two-blade (knead/mix) attachment used inside the Hobart cutter mixer. (b) Berries being added to the cutter mixer. (c) Macerated berries ready to be transferred to the fruit press via 15 L plastic pails.



(a) (b) (c)

Fig. A1-3. (a) Fruit bladder press and collection tub. (b) Loading of macerated sea buckthorn berries into the bladder press. (c) Filtering and collection of sea buckthorn juice during pressing.

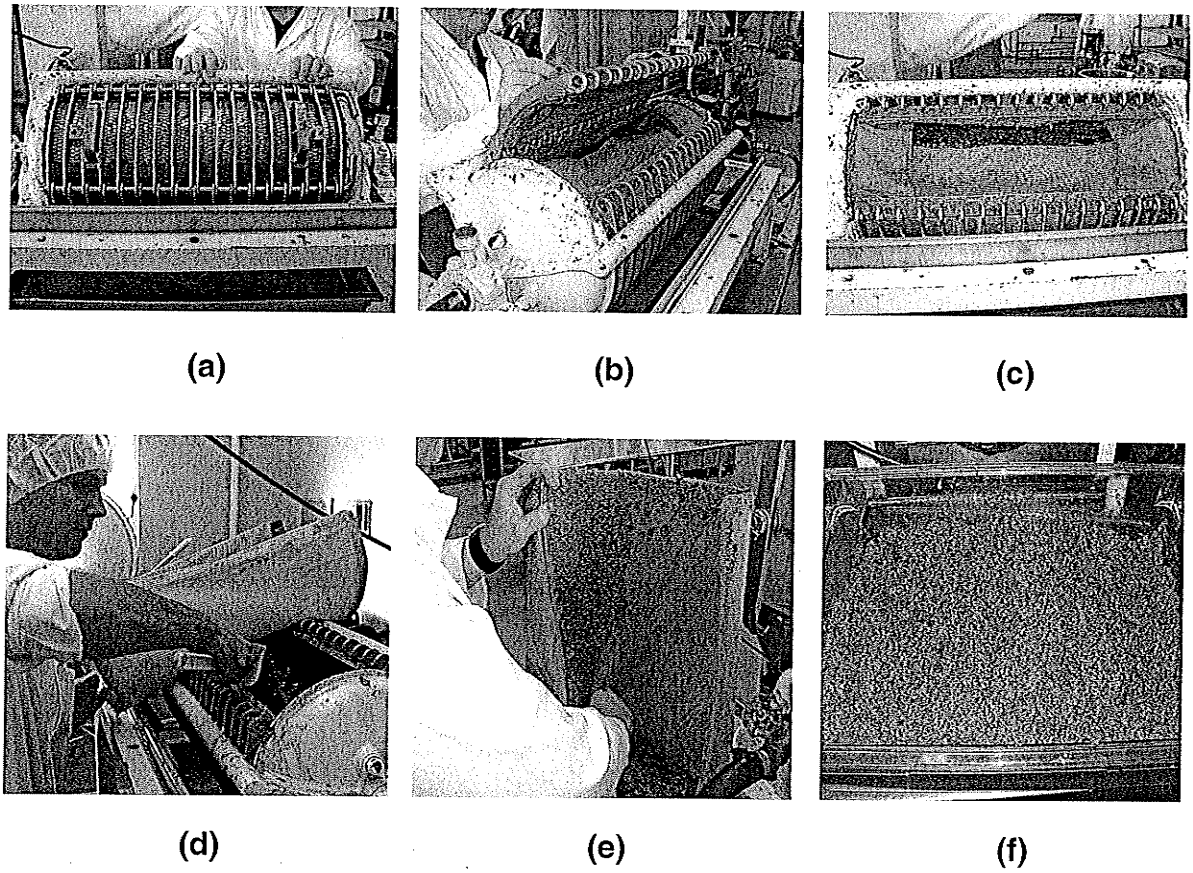
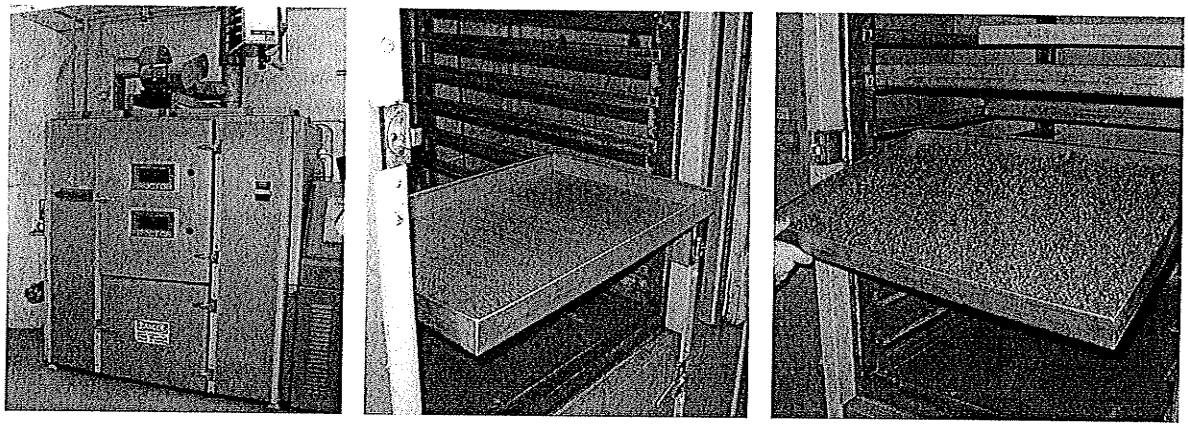


Fig. A1-4. (a) and (b) Opening of the bladder press. (c), (d) and (e) Removal and cleaning of the cloth filter to recover the wet cake. (f) Collection tub containing wet cake.

Drying:



(a) (b) (c)

Fig. A1-5. (a) A gas fired variable circulation laboratory dryer. (b) Stackable arrangement of drying trays. (c) Drying of wet cake.

Fig. A1-6. A tub of dry cake.

Separation:

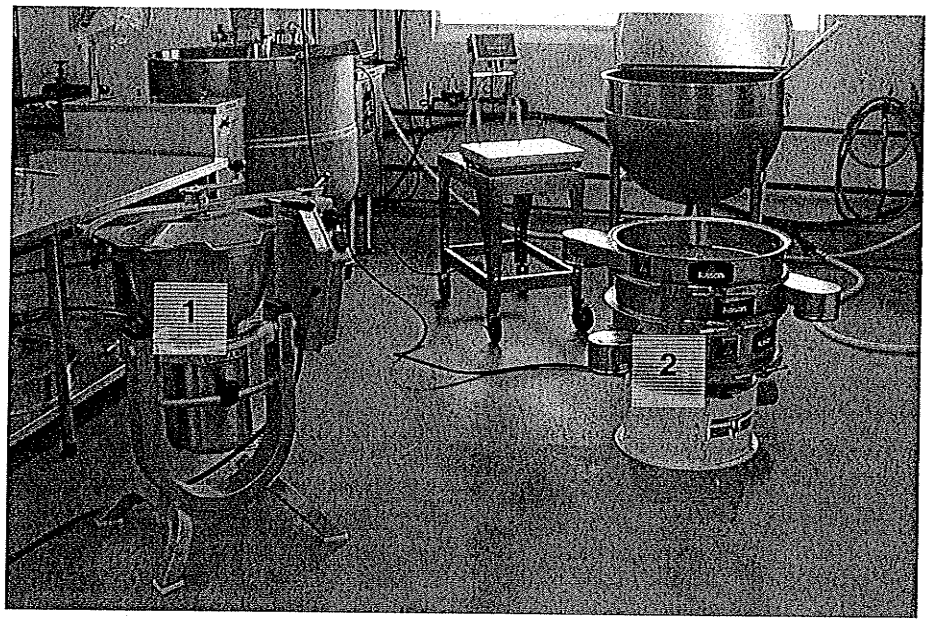
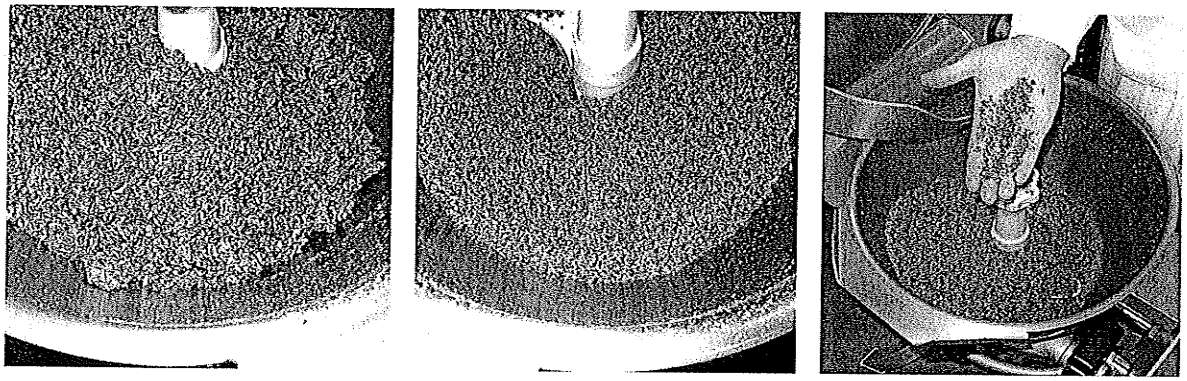
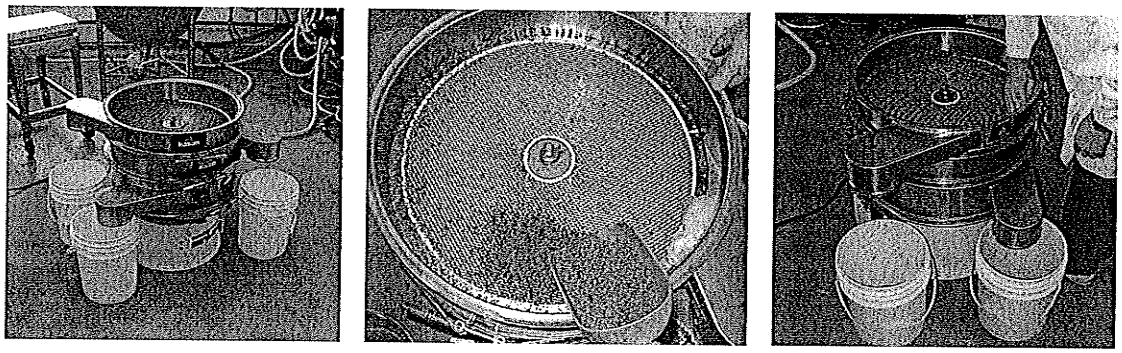


Fig. A1-7. Separation pilot process. (1) Hobart cutter mixer used to thresh the dry press cake. (2) Vibratory screen separator used to separate seeds from the pulp.



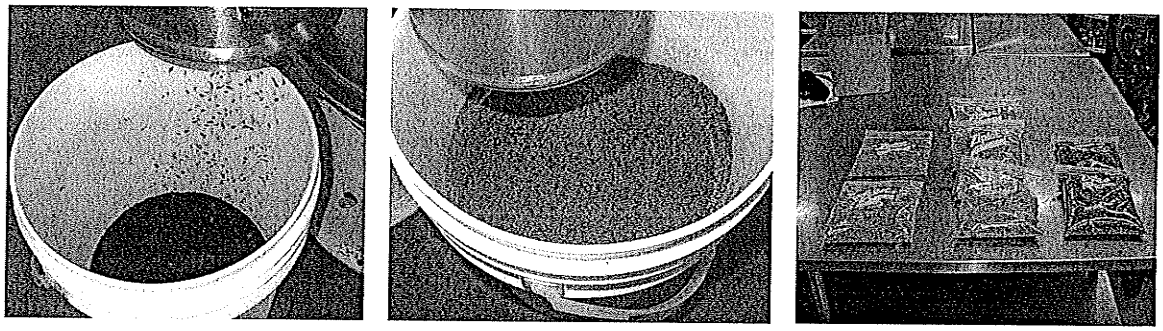
(a) (b) (c)

Fig. A1-8. (a) Dry cake before threshing. (b) and (c) Dry cake after threshing.



(a) (b) (c)

Fig. A1-9. (a) Arrangment of pails for the collection of seeds, pulp-flakes, and debris. (b) Adding threshed dry cake to the vibratory screen separator. (c) Separation and collection of seeds, pulp-flakes, and debris.



(a) (b) (c)

Fig. A1-10. (a) Collection of seeds. (b) Collection of pulp-flakes. (c) Bagged seeds and pulp-flakes for oil extraction trials.

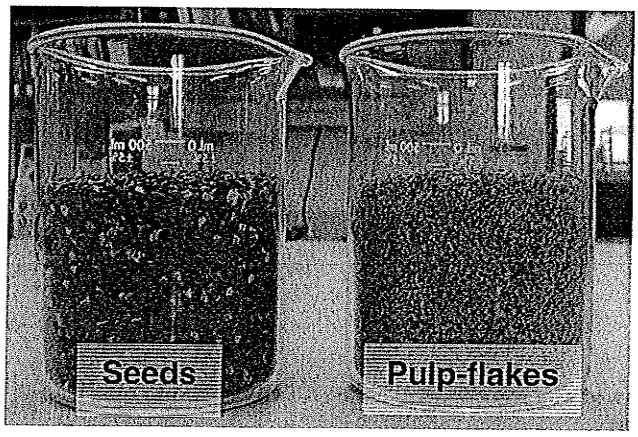


Fig. A1-11. A sample of seeds and pulp-flakes collected from the pilot process.

Appendix A2

Supercritical fluid extraction

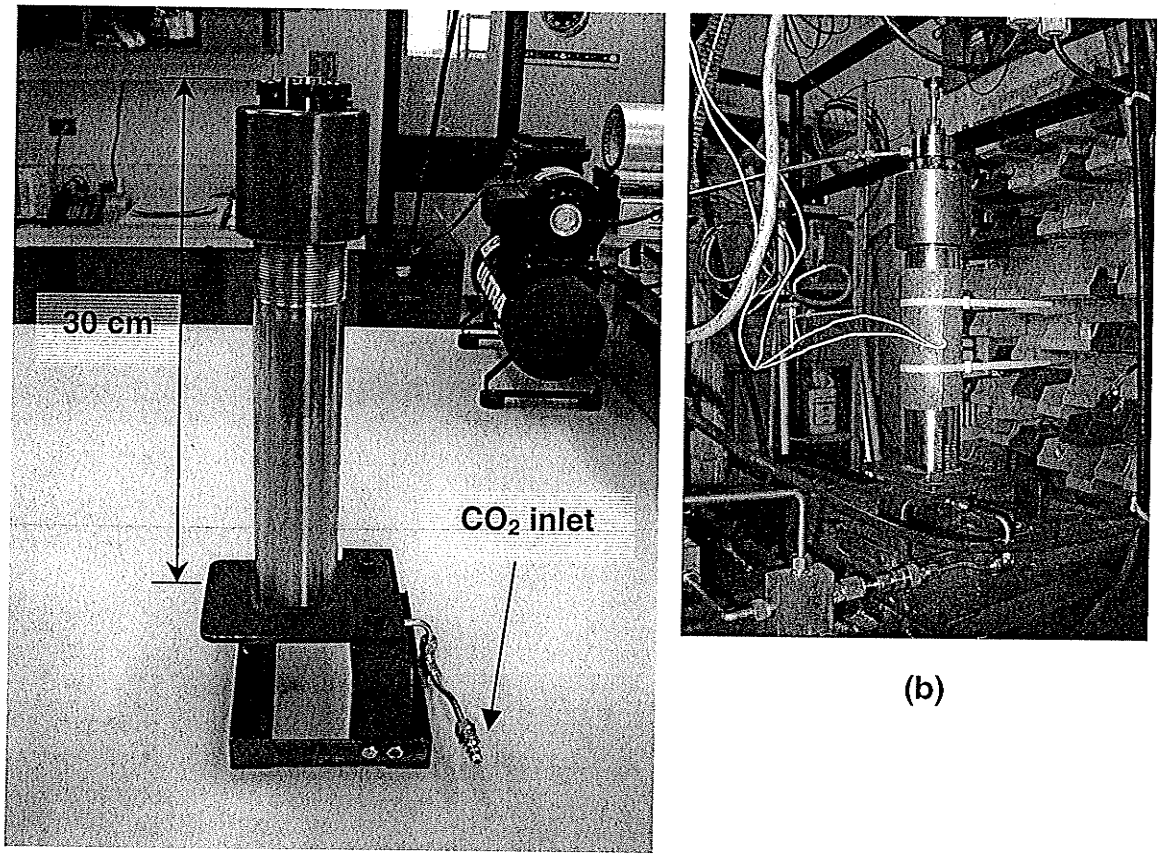
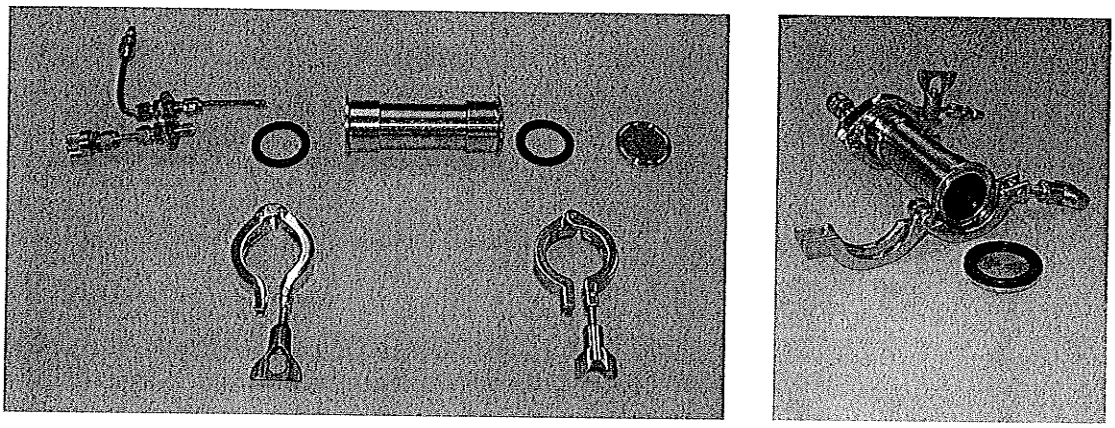
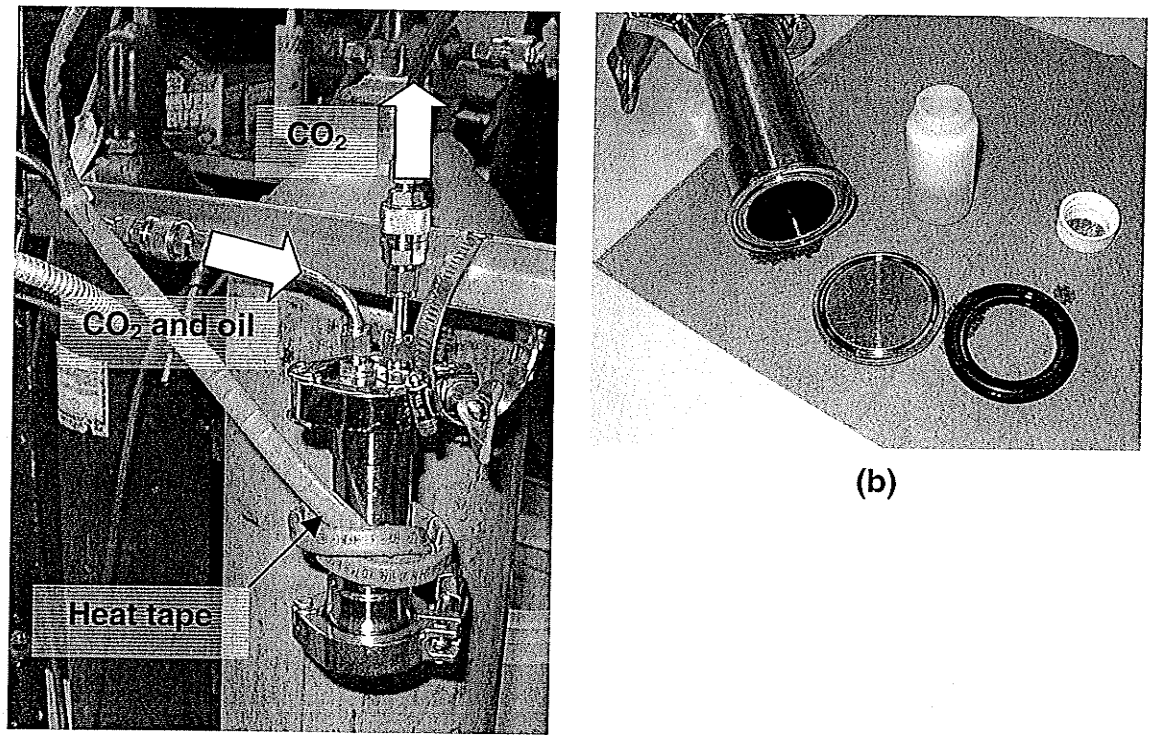


Fig. A2-1. (a) Extraction vessel. (b) Extraction vessel 'in-line', connected to CO_2 source and equipped with external heating pad.



(a) (b)

Fig. A2-2. (a) Disassembled oil collection vessel. (b) Assembled oil collection vessel with opening to retrieve oil.



(a) (b)

Fig. A2-3. (a) Oil collection vessel 'inline'. (b) Transferring of oil from collection vessel to oil container.

Appendix A3

Screw pressing

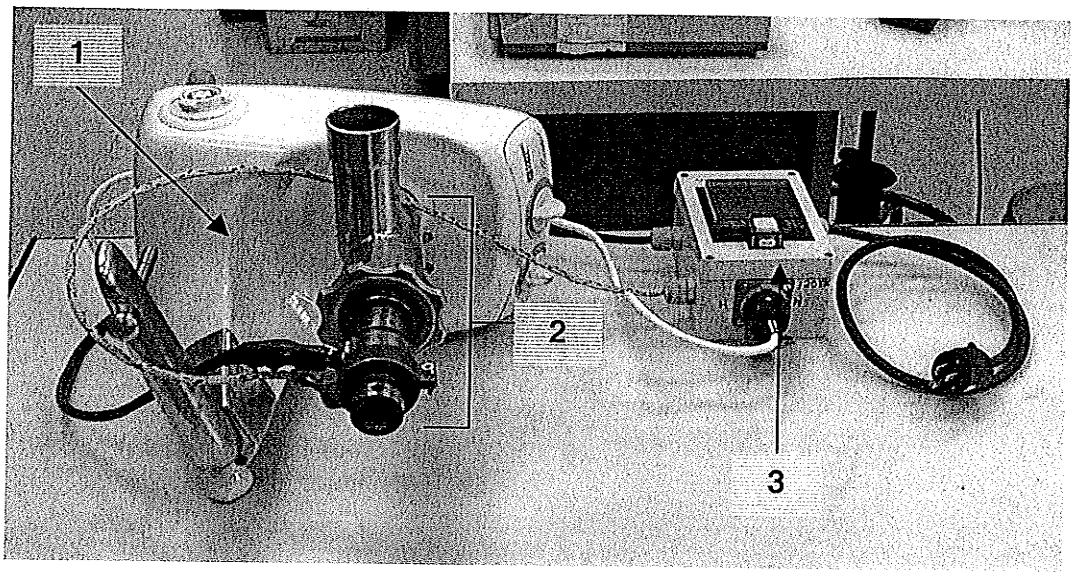


Fig. A3-1. Täby oil press used in oil pressing trials. (1) Mixer base. (2) Screw press attachment. (3) Fuse box housing the on/off switch for heat ring and mixer base.

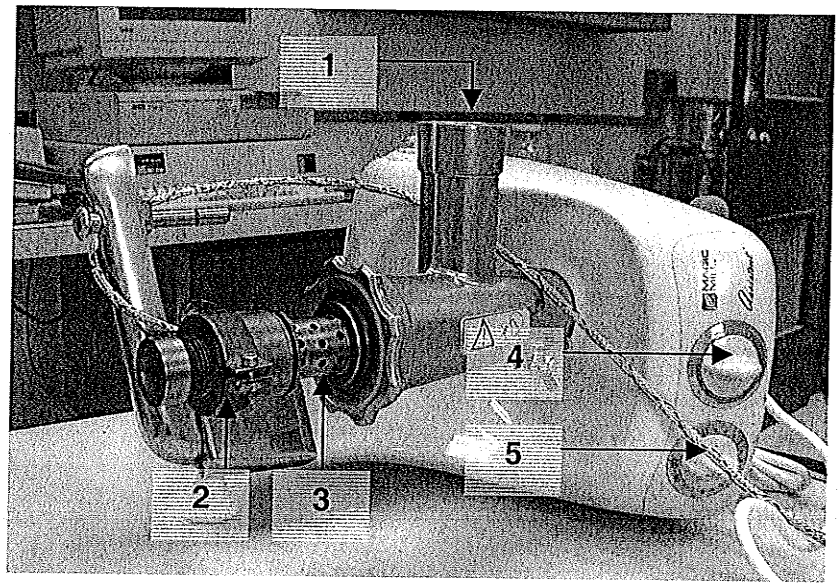


Fig. A3-2. Components of mixer base and screw press attachment: (1) feed inlet for seeds and pulp, (2) heating ring, (3) oil outlet port, (4) variable speed control, (5) screw press timer.

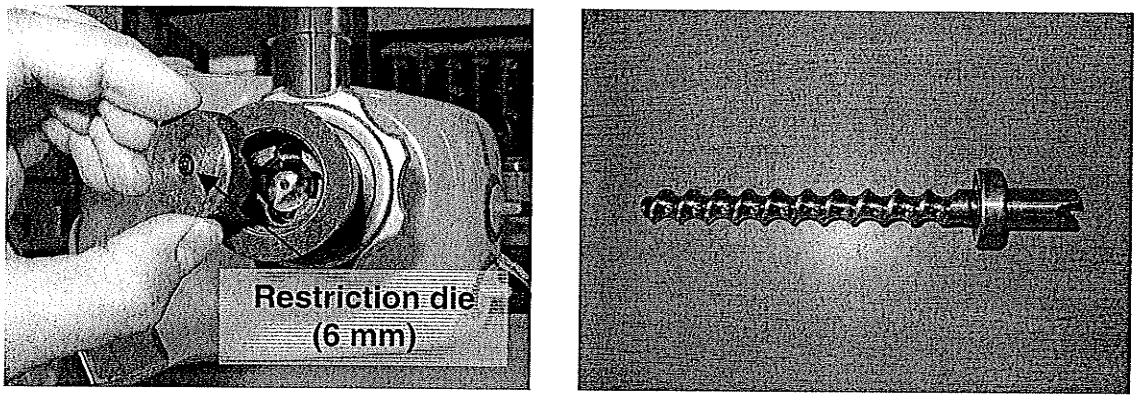


Fig. A3-3. A restriction die (6 mm) used for pressing seeds and pulp-flakes. Pressing screw (pitch = 15 mm, flight width = 2 mm, diameter = 18 mm).

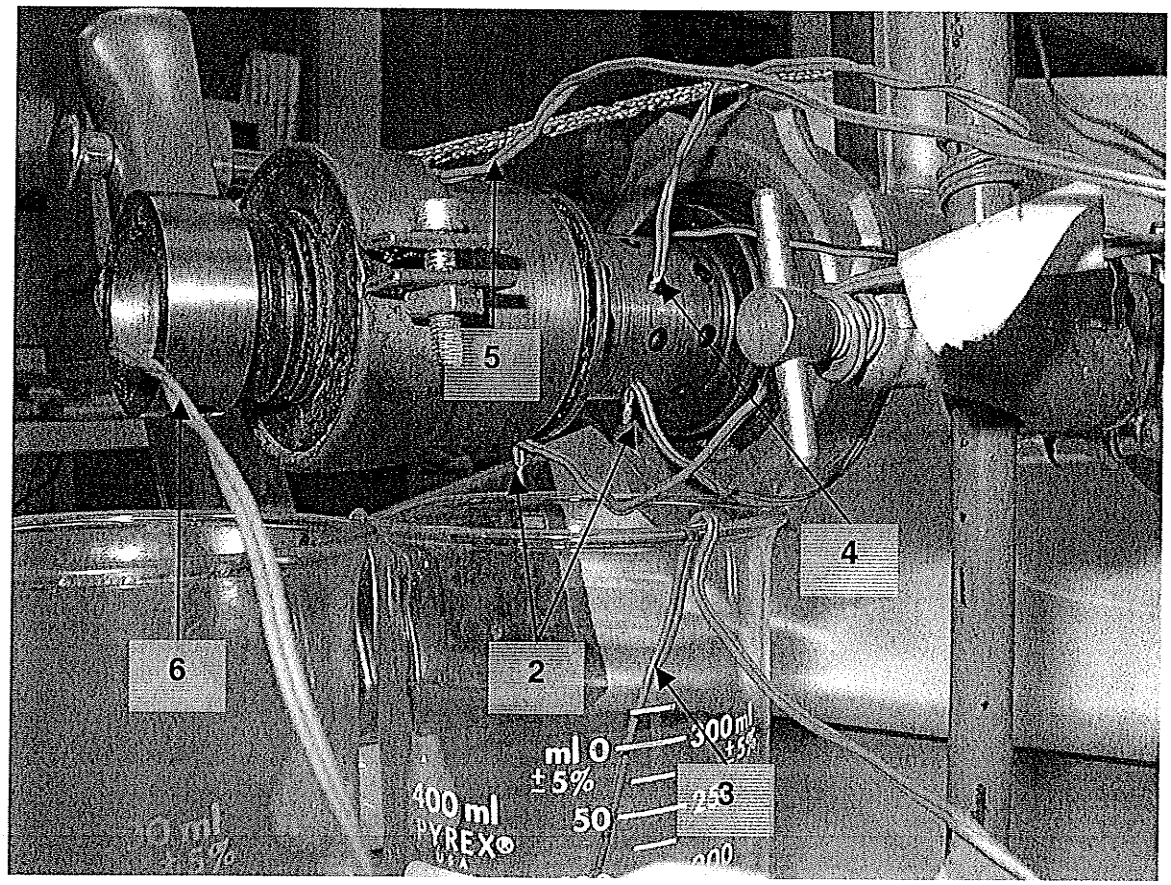


Fig. A3-4. Thermocouple placement for temperature measurement on screw press attachment: (2) extracted oil stream, (3) collected oil, (4) screw press barrel, (5) heater ring, and (6) press cake. (1) seed and pulp-flake feed inlet and (7) ambient are not shown.

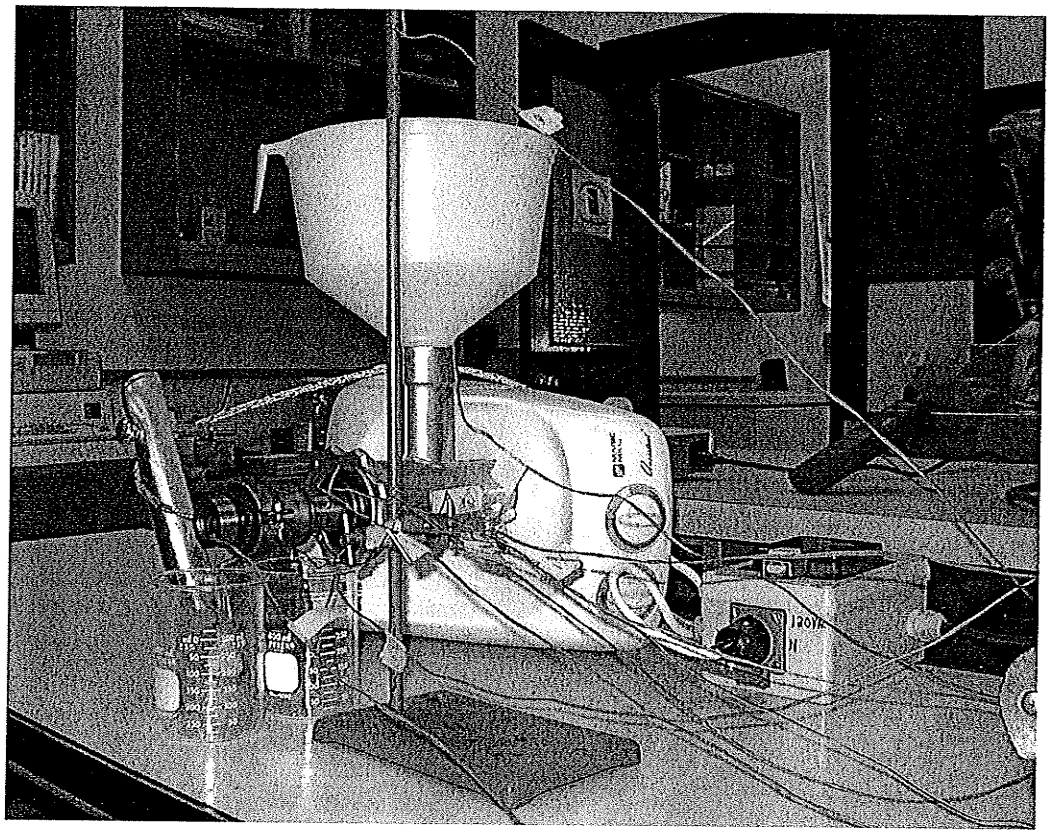


Fig. A3-5. Complete assembly of screw pressing apparatus including thermocouples and oil and cake collection beakers.

Appendix A4

Table of sample numbers for oil quality samples

Sample number	Oil source
1	processed seed / 2002 / solvent extraction
2	processed seed / 2002 / solvent extraction
3	pulp-flake / 2002 / solvent extraction
4	pulp-flake / 2002 / solvent extraction
5	processed seed / 2002 / screw press
6	processed seed / 2002 / screw press
7	processed seed / 2002 / SCFE CO_2 / 6 h / 10 s grind
8	processed seed / 2002 / SCFE CO_2 / 6 h / 10 s grind
9	processed seed / 2002 / SCFE CO_2 / 3 h / 30 s grind
10	processed seed / 2002 / SCFE CO_2 / 3 h / 30 s grind
11	processed seed / 2002 / SCFE CO_2 / 6 h / 30 s grind
12	processed seed / 2002 / SCFE CO_2 / 6 h / 30 s grind
13	pulp-flake / 2002 / SCFE CO_2 / 6 h / 30 s grind
14	pulp-flake / 2002 / SCFE CO_2 / 6 h / 30 s grind
15	pulp-flake / 2002 / SCFE CO_2 / 3 h / 30 s grind
16	pulp-flake / 2002 / SCFE CO_2 / 3 h / 30 s grind
17	pulp (of thawed whole berries) / 2002 / aqueous extraction
18	pulp (of thawed whole berries) / 2002 / aqueous extraction
19	pulp (of thawed whole berries) / 2001 / chloroform-methanol extraction
20	pulp (of thawed whole berries) / 2001 / chloroform-methanol extraction
21	pulp (of thawed whole berries) / 2002 / chloroform-methanol extraction
22	pulp (of thawed whole berries) / 2002 / chloroform-methanol extraction
23	unprocessed seed (of thawed whole berries) / 2001 / chloroform-methanol extraction
24	unprocessed seed (of thawed whole berries) / 2001 / chloroform-methanol extraction
25	unprocessed seed (of thawed whole berries) / 2002 / chloroform-methanol extraction
26	unprocessed seed (of thawed whole berries) / 2002 / chloroform-methanol extraction
27	processed seed / 2002 / chloroform-methanol extraction
28	processed seed / 2002 / chloroform-methanol extraction
29	pulp-flake / 2002 / chloroform-methanol extraction
30	pulp-flake / 2002 / chloroform-methanol extraction
31	juice / 2002 / chloroform-methanol extraction
32	juice / 2002 / chloroform-methanol extraction
33	juice / 2002 / chloroform-methanol extraction

Appendix A5

Table of fatty acids (% w/w)

Fatty acid	Sample number																
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%
C10:0	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
C12:0	0.000	0.000	0.128	0.127	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.098	0.081	0.087	0.094	0.068
C14:0	0.142	0.145	0.908	0.903	0.107	0.118	0.159	0.155	0.158	0.132	0.135	0.134	0.736	0.667	0.688	0.726	0.598
C14:1	0.042	0.046	0.084	0.000	0.000	0.000	0.046	0.000	0.047	0.000	0.000	0.037	0.070	0.070	0.067	0.069	0.062
C15:0	0.147	0.149	0.098	0.100	0.125	0.132	0.149	0.152	0.161	0.145	0.144	0.143	0.085	0.083	0.082	0.088	0.073
C16:0	6.885	7.035	35.159	35.270	6.554	6.839	7.349	7.294	7.445	6.980	6.939	6.940	35.581	35.614	35.502	35.486	34.387
C16:1	0.470	0.464	35.058	35.017	0.433	0.486	0.881	0.774	0.624	0.523	0.507	0.496	36.441	36.214	36.275	36.388	38.469
C17:0	0.066	0.031	0.090	0.096	0.060	0.000	0.068	0.069	0.065	0.000	0.067	0.062	0.082	0.079	0.082	0.091	0.073
C18:0	2.591	2.540	1.178	1.189	2.503	2.495	2.627	2.548	2.286	2.462	2.537	2.525	1.054	1.058	1.057	1.060	1.077
C18:1*n*-9	13.652	13.627	3.222	3.337	13.613	13.561	13.345	13.222	12.805	13.196	13.246	13.297	3.468	3.533	3.508	3.470	3.229
C18:1*n*-7	2.187	2.101	6.930	6.940	1.929	1.864	2.190	2.120	1.916	1.928	2.050	2.024	6.820	6.978	6.924	6.857	7.324
C18:2*n*-3	35.348	35.563	12.758	12.750	35.209	35.368	35.710	35.766	35.754	35.958	35.788	35.923	12.265	12.458	12.443	12.305	13.074
C18:3*n*-6	0.000	0.000	0.061	0.000	0.000	0.000	0.047	0.063	0.000	0.000	0.040	0.000	0.000	0.000	0.000	0.000	0.000
C18:3*n*-3	37.459	37.362	1.465	1.455	38.638	38.299	36.427	36.915	38.015	37.877	37.675	37.525	1.230	1.221	1.207	1.214	1.094
C20:0	0.467	0.440	0.330	0.337	0.399	0.402	0.447	0.438	0.338	0.390	0.415	0.415	0.279	0.283	0.303	0.292	0.219
C20:1	0.240	0.237	0.066	0.000	0.241	0.237	0.228	0.237	0.180	0.213	0.233	0.220	0.064	0.067	0.071	0.060	0.000
C20:2	0.053	0.050	0.096	0.000	0.053	0.057	0.054	0.054	0.046	0.050	0.051	0.054	0.000	0.000	0.000	0.046	0.000
C20:4	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
C20:3*n*-3	0.034	0.038	0.000	0.000	0.000	0.000	0.041	0.000	0.030	0.000	0.000	0.039	0.000	0.000	0.000	0.000	0.000
C20:3*n*-4	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
C22:0	0.142	0.118	0.421	0.412	0.095	0.099	0.161	0.135	0.095	0.104	0.125	0.114	0.303	0.277	0.318	0.331	0.052
C22:1	0.000	0.000	0.104	0.077	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.077	0.083	0.066	0.084	0.000
C22:2	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
C24:0/22:6	0.074	0.055	0.643	0.590	0.042	0.043	0.073	0.059	0.037	0.043	0.047	0.050	0.385	0.319	0.443	0.437	0.147
C24:1	0.000	0.000	1.201	1.398	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.961	0.914	0.876	0.902	0.055
Total	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0

Table of fatty acids (% w/w), continued

Fatty acid	Sample number															
	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33
	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%
C10:0	0.031	0.000	0.000	0.000	0.065	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
C12:0	0.068	0.115	0.105	0.092	0.094	0.000	0.000	0.000	0.000	0.000	0.000	0.125	0.128	0.069	0.070	0.075
C14:0	0.608	0.647	0.634	0.768	0.750	0.139	0.139	0.135	0.129	0.128	0.130	0.914	0.944	0.597	0.577	0.610
C14:1	0.057	0.068	0.065	0.070	0.069	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.066	0.059	0.065
C15:0	0.071	0.081	0.076	0.081	0.078	0.129	0.128	0.147	0.144	0.151	0.145	0.103	0.098	0.077	0.074	0.078
C16:0	34.382	39.649	40.026	33.862	34.929	7.728	7.853	7.595	7.474	7.510	7.436	35.065	34.584	34.179	35.223	33.652
C16:1	38.487	35.859	35.724	38.059	37.006	0.582	0.727	0.652	0.597	0.416	0.456	34.397	34.376	38.703	37.975	38.665
C17:0	0.070	0.095	0.094	0.084	0.076	0.073	0.071	0.071	0.077	0.079	0.078	0.095	0.092	0.084	0.077	0.083
C18:0	1.081	1.364	1.310	1.179	1.159	3.190	3.202	2.915	2.877	2.838	2.851	1.221	1.228	0.998	0.985	1.043
C18:1*n*-9	3.211	3.351	3.259	2.966	3.025	14.908	15.063	13.474	13.472	13.368	13.415	3.334	3.371	3.352	3.358	3.446
C18:1*n*-7	7.346	5.868	6.001	7.551	7.328	2.263	2.306	2.344	2.328	2.290	2.314	7.113	7.073	7.391	7.241	7.629
C18:2*n*-3	12.942	11.069	11.018	13.169	13.195	33.516	33.251	36.062	36.306	36.141	36.362	13.385	13.551	12.781	12.590	12.871
C18:3*n*-6	0.032	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
C18:3*n*-3	1.108	0.999	0.944	1.416	1.436	36.350	36.345	35.525	35.565	36.079	35.760	1.873	2.133	1.230	1.235	1.289
C20:0	0.222	0.280	0.278	0.269	0.261	0.535	0.554	0.535	0.505	0.498	0.503	0.378	0.339	0.232	0.214	0.234
C20:1	0.045	0.000	0.000	0.052	0.055	0.270	0.042	0.250	0.236	0.233	0.248	0.000	0.000	0.056	0.051	0.056
C20:2	0.023	0.097	0.000	0.000	0.000	0.061	0.057	0.060	0.057	0.067	0.064	0.000	0.000	0.000	0.000	0.000
C20:4	0.000	0.000	0.000	0.000	0.000	0.044	0.000	0.000	0.045	0.000	0.000	0.000	0.000	0.000	0.000	0.000
C20:3*n*-3	0.000	0.000	0.000	0.000	0.000	0.000	0.044	0.048	0.000	0.000	0.043	0.000	0.000	0.000	0.000	0.000
C20:3*n*-4	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
C22:0	0.059	0.114	0.111	0.094	0.114	0.147	0.148	0.128	0.125	0.131	0.131	0.362	0.358	0.063	0.065	0.064
C22:1	0.029	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.080	0.123	0.000	0.000	0.000
C22:2	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
C24:0/22:6	0.090	0.166	0.180	0.161	0.199	0.065	0.070	0.060	0.064	0.071	0.063	0.426	0.487	0.123	0.152	0.139
C24:1	0.038	0.177	0.175	0.127	0.161	0.000	0.000	0.000	0.000	0.000	0.000	1.130	1.115	0.000	0.053	0.000
Total	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0

Fatty acid types (% w/w)

Fatty acid types	Sample											
	1	2	3	4	5	6	7	8	9	10	11	12
Saturated fatty acids, %	26.35	26.24	49.11	49.30	25.43	25.55	26.57	26.19	25.30	25.38	25.71	25.71
Monousaturated fatty acids (MUFA), %	0.76	0.75	36.51	36.49	0.67	0.73	1.15	1.01	0.86	0.73	0.74	0.75
Polyunsaturated fatty acids (PUFA), %	72.89	73.01	14.38	14.21	73.90	73.72	72.28	72.80	73.84	73.89	73.55	73.54

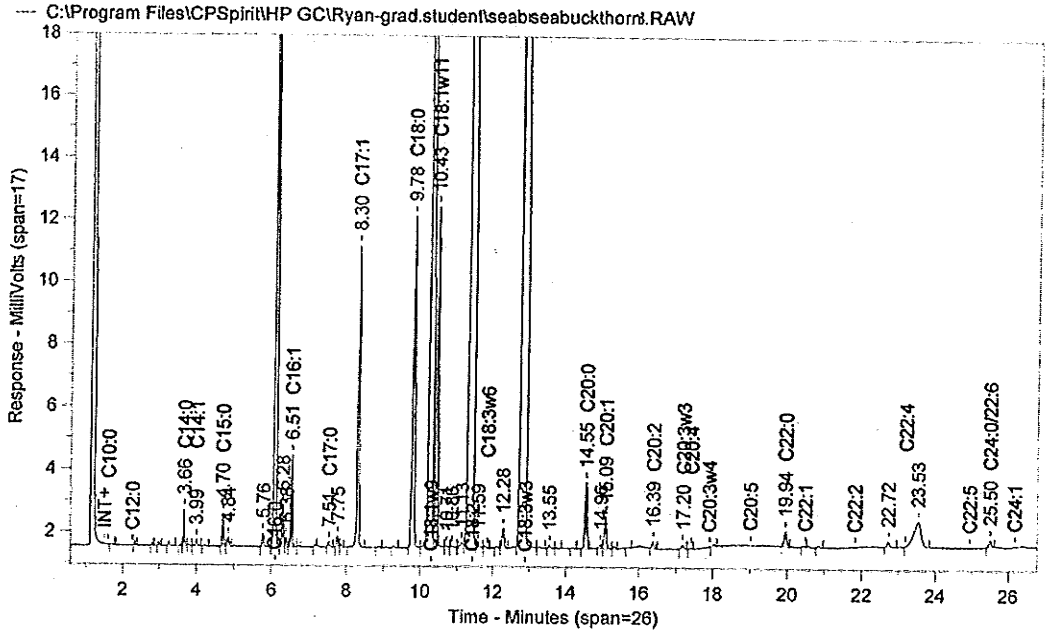
Fatty acid types	Sample											
	13	14	15	16	17	18	19	20	21	22	23	24
Saturated fatty acids, %	48.89	48.97	48.99	48.93	47.25	47.24	51.73	52.07	47.11	48.08	29.18	29.53
Monousaturated fatty acids (MUFA), %	37.62	37.35	37.36	37.50	38.58	38.65	36.11	35.97	38.31	37.29	0.85	0.77
Polyunsaturated fatty acids (PUFA), %	13.49	13.68	13.65	13.57	14.17	14.11	12.16	11.96	14.58	14.63	69.97	69.70

Fatty acid types	Sample								
	25	26	27	28	29	30	31	32	33
Saturated fatty acids, %	27.40	27.19	27.06	27.07	49.13	48.70	47.16	48.04	47.05
Monousaturated fatty acids (MUFA), %	0.91	0.84	0.65	0.70	35.61	35.62	38.83	38.14	38.79
Polyunsaturated fatty acids (PUFA), %	71.69	71.97	72.29	72.23	15.26	15.68	14.01	13.82	14.16

Sample of chromatogram report for fatty acids (example of sample number 2)

Chrom Perfect Chromatogram Report

--- C:\Program Files\CPSpirit\HP GC\Ryan-grad.student\seabseabuckthorn8.RAW



Instrument = HP
Heading 1 = Ryan - Oct.14,2003
Heading 2 = flow 40; 1 uL inj.; run time=33min. Mary's column

Sample Identification: 2
Fat (g) = _____
Wt.(mg) = 74.6mg
mg/mL = 10.7mg/mL

Today's Date = 10/15/03 Today's Time = 10:08:10 AM
Raw File Name = C:\Program Files\CPSpirit\HP GC\Ryan-grad.student\seabuckthorn.0008.RAW
Sample Name = seabuckthorn

Method File Name = C:\Program Files\CPSpirit\HP GC\FattyAcids2.met
Method Description = Flow=80; Inj 1 uL
Calibration File Name = C:\Program Files\CPSpirit\HP GC\FattyAcids2.cal
Run Time = 33

Peak #	Ret. Time	Peak Name	Amount	Amt %	Area	Area %	Group #
7	3.66	C14:0	0.080	0.145	2325	0.13	1
9	3.99	C14:1	0.026	0.046	746	0.04	2
11	4.70	C15:0	0.082	0.149	2440	0.14	1
12	4.84		0.000	0.000	824	0.05	0
13	5.76		0.000	0.000	1293	0.07	0
14	6.08	C16:0	3.890	7.035	118333	6.72	1
15	6.28		0.000	0.000	3559	0.20	0
16	6.38		0.000	0.000	808	0.05	0
17	6.51	C16:1	0.256	0.464	7689	0.44	2
20	7.51	C17:0	0.017	0.031	534	0.03	1
21	7.75		0.000	0.000	1102	0.06	0
23	8.30	C17:1	1.000	1.808	30757	1.75	2
26	9.78	C18:0	1.405	2.540	44567	2.53	1
27	10.29	C18:1w9	7.536	13.627	239000	13.57	1
28	10.43	C18:1w11	1.162	2.101	36930	2.10	1
29	10.71		0.000	0.000	513	0.03	0
30	10.86		0.000	0.000	513	0.03	0

internal standard - heptadecenoic acid (C17:1)

Printed on 10/15/03 10:08:20 AM

Page 1 of 2

Sample of chromatogram report for fatty acids (example of sample number 2), continued

31	11.13		0.000	0.000	1079	0.06	0
32	11.44	C18:2	19.667	35.563	625132	35.51	3
33	11.59		0.000	0.000	857	0.05	0
36	12.28		0.000	0.000	2719	0.15	0
37	12.88	C18:3w3	20.662	37.362	638378	36.26	3
39	13.55		0.000	0.000	506	0.03	0
42	14.55	C20:0	0.243	0.440	8290	0.47	1
43	14.96		0.000	0.000	620	0.04	0
44	15.09	C20:1	0.131	0.237	4343	0.25	2
47	16.39	C20:2	0.028	0.050	940	0.05	3
48	17.20	C20:3w3	0.021	0.038	682	0.04	3
51	19.94	C22:0	0.065	0.118	2245	0.13	1
54	22.72		0.000	0.000	842	0.05	0
55	23.53		0.000	0.000	11764	0.67	0
56	25.50	C24:0/22:6	0.030	0.055	1068	0.06	1

Total Area = 1760640

Total Amount = 55.30246

Checked by _____ Date _____

Group #	Name	Amount %
0		0.00
1	Saturated FA	26.24
2	MUFA	2.56
3	PUFA	73.01

Appendix A6

Table of tocopherols and tocotrienols (mg/100 g oil)

Tocopherol/Tocotrienol	Sample number									
	1		2		3		4		5	
	%	mg/100 g	%	mg/100g	%	mg/100g	%	mg/100 g	%	mg/100g
α-tocopherol	51.057	215.002	52.242	231.736	77.305	149.244	77.338	138.135	49.795	150.901
α-tocotrienol	0.000	0.000	0.000	0.000	0.000	0.000	0.373	0.067	0.000	0.000
β-tocopherol	2.831	11.921	2.654	11.770	7.542	14.559	8.065	14.404	2.653	8.041
β-tocotrienol	2.362	9.945	2.154	9.552	0.000	0.000	0.000	0.000	2.455	7.440
Plastochromanol-8	0.464	1.954	0.452	2.003	4.267	8.236	4.457	7.961	0.524	1.588
γ-tocopherol	41.376	174.235	40.715	180.600	3.847	7.426	3.869	6.910	42.843	129.835
γ-tocotrienol	0.000	0.000	0.000	0.000	1.402	2.707	1.052	1.878	0.000	0.000
δ-tocopherol	1.911	8.046	1.784	7.915	3.219	6.214	2.497	4.461	1.729	5.241
δ-tocotrienol	0.000	0.000	0.000	0.000	2.419	4.669	2.349	4.197	0.000	0.000
Total	100.0		100.0		100.0		100.0		100.0	

Tocopherol/Tocotrienol	Sample number									
	6		7		8		9		10	
	%	mg/100g	%	mg/100 g	%	mg/100g	%	mg/100g	%	mg/100 g
α-tocopherol	49.877	144.742	54.030	320.863	52.749	296.467	46.269	144.440	50.095	196.561
α-tocotrienol	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
β-tocopherol	2.781	8.070	2.614	15.524	2.818	15.838	3.530	11.020	2.901	11.383
β-tocotrienol	2.381	6.910	1.875	11.135	2.082	11.702	2.090	6.524	2.198	8.624
Plastochromanol-8	0.371	1.077	0.226	1.342	0.233	1.310	0.000	0.000	0.000	0.000
γ-tocopherol	42.752	124.065	39.004	231.630	40.015	224.897	45.212	141.140	42.608	167.183
γ-tocotrienol	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
δ-tocopherol	1.837	5.331	2.251	13.368	2.103	11.820	2.899	9.050	2.198	8.624
δ-tocotrienol	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Total	100.0		100.0		100.0		100.0		100.0	

Tocopherol/Tocotrienol	Sample number									
	11		12		13		14		15	
	%	mg/100g	%	mg/100g	%	mg/100 g	%	mg/100g	%	mg/100g
α-tocopherol	49.461	209.674	48.090	183.819	75.209	121.487	75.282	104.429	76.046	112.541
α-tocotrienol	0.000	0.000	0.000	0.000	0.818	1.321	0.563	0.781	0.765	1.132
β-tocopherol	2.921	12.383	3.117	11.914	8.012	12.942	8.833	12.253	7.605	11.255
β-tocotrienol	2.245	9.517	2.271	8.681	0.000	0.000	0.000	0.000	0.000	0.000
Plastochromanol-8	0.097	0.411	0.164	0.627	1.635	2.641	0.777	1.078	0.885	1.310
γ-tocopherol	43.221	183.221	44.151	168.763	4.447	7.183	4.977	6.904	4.447	6.581
γ-tocotrienol	0.000	0.000	0.000	0.000	1.975	3.190	1.315	1.824	1.564	2.315
δ-tocopherol	2.056	8.716	2.206	8.432	3.958	6.393	4.373	6.066	4.070	6.023
δ-tocotrienol	0.000	0.000	0.000	0.000	3.947	6.376	3.881	5.384	4.618	6.834
Total	100.0		100.0		100.0		100.0		100.0	

Tocopherol/Tocotrienol	Sample number									
	16		17		18		19		20	
	%	mg/100 g	%	mg/100g	%	mg/100g	%	mg/100 g	%	mg/100g
α-tocopherol	72.426	89.648	84.809	130.309	84.207	146.497	84.826	359.418	84.462	330.641
α-tocotrienol	0.528	0.654	0.849	1.304	0.797	1.387	0.753	3.191	0.846	3.312
β-tocopherol	9.104	11.269	5.443	8.363	6.016	10.466	5.755	24.385	5.766	22.572
β-tocotrienol	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Plastochromanol-8	1.491	1.846	5.247	8.062	5.440	9.464	5.003	21.198	5.211	20.399
γ-tocopherol	5.477	6.779	1.846	2.836	1.805	3.140	1.495	6.334	1.392	5.449
γ-tocotrienol	1.866	2.310	1.805	2.773	1.734	3.017	1.578	6.686	1.607	6.291
δ-tocopherol	4.837	5.987	0.000	0.000	0.000	0.000	0.591	2.504	0.715	2.799
δ-tocotrienol	4.270	5.285	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Total	100.0		100.0		100.0		100.0		100.0	

Table of tocopherols and tocotrienols (mg/100 g oil), continued

Tocopherol/Tocotrienol	Sample number									
	21		22		23		24		25	
	%	mg/100g	%	mg/100 g	%	mg/100g	%	mg/100g	%	mg/100 g
α-tocopherol	82.712	297.018	79.448	266.802	53.351	154.411	49.597	130.359	38.602	89.663
α-tocotrienol	0.366	1.314	1.542	5.178	0.000	0.000	0.000	0.000	0.000	0.000
β-tocopherol	6.297	22.612	5.559	18.668	2.906	8.411	3.200	8.411	3.822	8.878
β-tocotrienol	0.000	0.000	0.000	0.000	1.710	4.949	1.650	4.337	2.723	6.325
Plastochromanol-8	4.746	17.043	4.553	15.290	1.652	4.781	1.629	4.282	1.382	3.210
γ-tocopherol	2.165	7.774	3.456	11.606	38.721	112.068	42.192	110.896	51.009	118.481
γ-tocotrienol	1.366	4.905	3.907	13.120	0.000	0.000	0.000	0.000	0.000	0.000
δ-tocopherol	1.833	6.582	1.536	5.158	1.659	4.802	1.733	4.555	2.463	5.721
δ-tocotrienol	0.514	1.846	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Total	100.0		100.0		100.0		100.0		100.0	

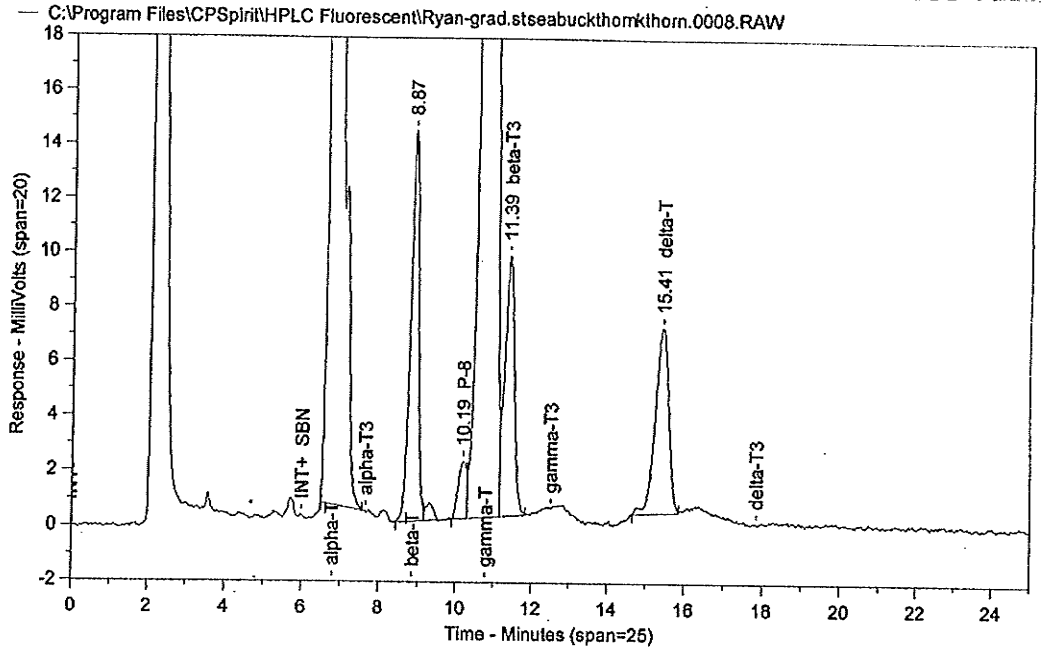
Tocopherol/Tocotrienol	Sample number									
	26		27		28		29		30	
	%	mg/100g	%	mg/100g	%	mg/100 g	%	mg/100g	%	mg/100g
α-tocopherol	34.223	70.840	43.815	125.342	43.686	116.637	78.615	220.773	79.195	220.814
α-tocotrienol	0.000	0.000	0.000	0.000	0.000	0.000	0.395	1.109	0.435	1.213
β-tocopherol	4.258	8.814	3.466	9.915	3.373	9.006	7.461	20.953	7.606	21.207
β-tocotrienol	2.943	6.092	2.412	6.900	2.448	6.536	0.000	0.000	0.000	0.000
Plastochromanol-8	1.180	2.443	1.073	3.070	1.060	2.830	4.738	13.306	4.696	13.094
γ-tocopherol	54.501	112.815	46.776	133.812	47.255	126.166	3.855	10.826	4.044	11.276
γ-tocotrienol	0.000	0.000	0.000	0.000	0.000	0.000	0.909	2.553	0.331	0.923
δ-tocopherol	2.895	5.993	2.458	7.032	2.177	5.812	2.394	6.723	2.243	6.254
δ-tocotrienol	0.000	0.000	0.000	0.000	0.000	0.000	1.633	4.586	1.450	4.043
Total	100.0		100.0		100.0		100.0		100.0	

Tocopherol/Tocotrienol	Sample number					
	31		32		33	
	%	mg/100 g	%	mg/100g	%	mg/100g
α-tocopherol	83.712	217.653	84.202	216.981	82.861	231.168
α-tocotrienol	0.982	2.553	0.785	2.023	1.032	2.879
β-tocopherol	5.812	15.111	5.797	14.938	6.052	16.884
β-tocotrienol	0.000	0.000	0.000	0.000	0.000	0.000
Plastochromanol-8	4.985	12.961	4.994	12.869	4.611	12.864
γ-tocopherol	2.535	6.591	2.536	6.535	2.677	7.468
γ-tocotrienol	1.415	3.679	1.251	3.224	1.710	4.771
δ-tocopherol	0.559	1.453	0.435	1.121	0.671	1.872
δ-tocotrienol	0.000	0.000	0.000	0.000	0.387	1.080
Total	100.0		100.0		100.0	

Sample of chromatogram report for tocopherols and tocotrienols (example of sample number 2)

Chrom Perfect Chromatogram Report

C:\Program Files\CPSpirit\HPLC Fluorescent\Ryan-grad.stseabuckthornkthorn.0008.RAW



Instrument = HPLC Fluorescent
Heading 1 = Ryan-seabuckthorn -oil tocopherol analysis
Heading 2 = Oct14/03; 10uL inj; 0.8mL/min

Sample Identification: 2
Wt. (mg) = 33.5 mg
mg/mL = 6.7mg/mL

Today's Date = 10/16/03 Today's Time = 3:21:31 PM
Raw File Name = C:\Program Files\CPSpirit\HPLC Fluorescent\Ryan-grad.student\seabuckthorn.0008.RAW
Sample Name = seabuckthorn

Method File Name = C:\Program Files\CPSpirit\HPLC Fluorescent\Tocopherol.MET
Method Description = Tocopherol Analysis - column: Phenomenex 250x3.2mm 5micron; 5u silicon
Calibration File Name = C:\Program Files\CPSpirit\HPLC Fluorescent\Tocopherol_T3.CAL
Run Time = 25

Peak #	Ret. Time	Peak Name	Amount	Amt %	Area	Area %
1	6.79	alpha-T	155.263	52.242	3133949	47.82
2	8.87	beta-T	7.886	2.654	209227	3.19
3	10.19	P-8	1.342	0.452	31540	0.48
4	10.79	gamma-T	121.002	40.715	2844147	43.40
5	11.39	beta-T3	6.400	2.154	169804	2.59
6	15.41	delta-T	5.303	1.784	164570	2.51

Total Area = 6553236 Total Amount = 297.1961

Checked by _____ Date _____

ug/g or ppm
alpha-T = 2317.36
beta-T = 117.70
P-8 = 20.03
gamma-T = 1806.00
delta-T = 79.15

beta-T3 = 95.52

Printed on 10/16/03 3:21:35 PM

Page 1 of 1

Appendix A7

Table of total carotenoids (mg/100 g of oil)

Sample number	Total carotenoids mg/100 g
1	22.7
2	21.6
3	538.3
4	517.3
5	14.5
6	16.2
7	29.9
8	27.0
9	4.3
10	8.0
11	11.6
12	11.8
13	156.7
14	140.1
15	119.7
16	124.9
17	280.7
18	304.1
19	291.8
20	289.0
21	383.9
22	381.7
23	19.9
24	22.1
25	22.4
26	22.6
27	17.4
28	17.1
29	313.0
30	381.2
31	359.0
32	325.5
33	351.9

Appendix A8

Table of sterols (mg/100 g oil)

Sterols	Sample number									
	1		2		3		4		5	
	%	mg/100 g	%	mg/100 g	%	mg/100 g	%	mg/100 g	%	mg/100 g
Cholesterol	0.562	4.435	0.402	3.036	0.873	5.471	0.598	3.434	0.000	0.000
Campesterol	2.883	22.763	2.912	21.990	2.048	12.826	2.087	11.988	2.654	11.756
Stigmasterol	0.000	0.000	0.000	0.000	1.338	8.384	0.000	4.846	0.000	0.000
β-sitosterol	96.555	762.382	96.686	730.138	95.741	599.719	96.471	554.085	97.346	431.248

Sterols	Sample number									
	6		7		8		9		10	
	%	mg/100 g	%	mg/100 g	%	mg/100 g	%	mg/100 g	%	mg/100 g
Cholesterol	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Campesterol	2.595	10.439	2.826	25.612	2.780	26.927	2.945	19.816	2.843	19.974
Stigmasterol	0.000	0.000	0.299	2.707	0.000	0.000	0.000	0.000	0.000	0.000
β-sitosterol	97.405	391.812	96.876	878.106	97.220	941.807	97.055	653.075	97.157	682.537

Sterols	Sample number									
	11		12		13		14		15	
	%	mg/100 g	%	mg/100 g	%	mg/100 g	%	mg/100 g	%	mg/100 g
Cholesterol	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Campesterol	2.970	23.009	2.865	21.962	1.995	11.045	1.998	10.810	2.067	10.882
Stigmasterol	0.000	0.000	0.000	0.000	2.387	13.215	0.000	8.342	0.000	0.000
β-sitosterol	97.030	751.696	97.135	744.546	95.618	529.328	96.461	521.982	97.933	515.512

Sterols	Sample number									
	16		17		18		19		20	
	%	mg/100 g	%	mg/100 g	%	mg/100 g	%	mg/100 g	%	mg/100 g
Cholesterol	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Campesterol	1.984	10.821	2.351	7.096	2.141	6.183	2.503	11.244	2.483	9.372
Stigmasterol	0.000	0.000	0.000	0.000	0.000	0.000	0.000	7.665	0.000	0.000
β-sitosterol	98.016	534.576	97.649	294.693	97.859	282.555	95.791	430.346	97.517	368.087

Table of sterols (mg/100 g oil), continued

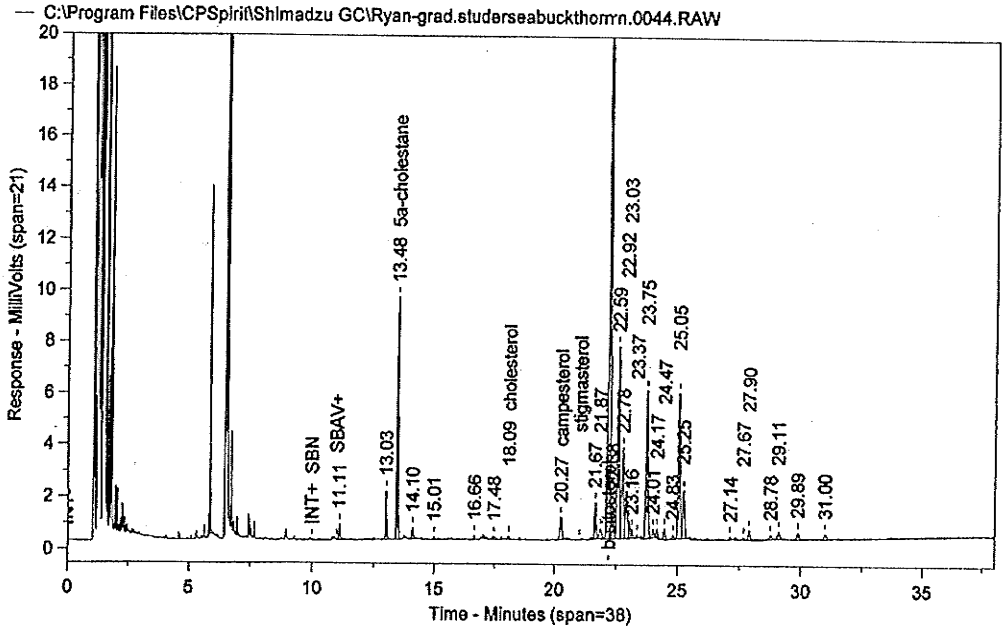
Sterols	Sample number									
	21		22		23		24		25	
	%	mg/100 g	%	mg/100 g	%	mg/100 g	%	mg/100 g	%	mg/100 g
Cholesterol	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Campesterol	2.179	9.154	2.169	8.560	2.685	14.931	2.665	14.827	2.656	16.558
Stigmasterol	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
β-sitosterol	97.821	410.946	97.831	386.168	97.315	541.091	97.335	541.444	97.344	606.957

Sterols	Sample number									
	26		27		28		29		30	
	%	mg/100 g	%	mg/100 g	%	mg/100 g	%	mg/100 g	%	mg/100 g
Cholesterol	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.874	4.647
Campesterol	2.636	16.037	2.828	17.563	2.748	16.799	1.789	9.597	1.837	9.767
Stigmasterol	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
β-sitosterol	97.364	592.256	97.172	603.386	97.252	594.408	98.211	526.776	97.288	517.132

Sterols	Sample number					
	31		32		33	
	%	mg/100 g	%	mg/100 g	%	mg/100 g
Cholesterol	0.000	0.000	0.000	0.000	0.000	0.000
Campesterol	2.147	6.436	2.142	6.491	2.143	8.416
Stigmasterol	0.000	0.000	0.000	0.000	0.000	0.000
β-sitosterol	97.853	293.345	97.858	296.506	97.857	384.306

Sample of chromatogram report for sterols (example of sample number 2)

Chrom Perfect Chromatogram Report



Instrument = Shimadzu
Heading 1 = sterols 1 uL injected; Oct22/03; split 40; total flow 93; col.flow 2.2
Heading 2 = 60C(1min) to 240C at 40deg/min hold 1 min; 2 deg/min to 300C hold 2min

Sample Identification: 2
Wt. (g) = _____

Today's Date = 12/8/03 Today's Time = 3:40:27 PM
Raw File Name = C:\Program Files\CPSpirit\Shimadzu GC\Ryan-grad.student\seabuckthorn.0044.RAW
Sample Name = seabuckthorn

Method File Name = C:\Program Files\CPSpirit\Shimadzu GC\Sterols.MET
Method Description = Sterol Analysis - column: DB-5; 30m;
Calibration File Name = C:\Program Files\CPSpirit\Shimadzu GC\Sterols.CAL
Run Time = 38.5

internal standard - 5α-cholestane

Peak #	Ret. Time	Peak Name	Amount	Amt %	Area	Area %	Group #
4	11.11		0.000	0.000	2275	0.61	0
7	13.03		0.000	0.000	6611	1.79	0
8	13.48	5a-cholestane	53.615	4.598	31976	8.63	0
11	14.10		0.000	0.000	2353	0.64	0
14	15.01		0.000	0.000	531	0.14	0
20	16.66		0.000	0.000	890	0.24	0
24	17.48		0.000	0.000	1171	0.32	0
26	18.09	cholesterol	3.227	0.277	614	0.17	0
29	20.27	campesterol	33.280	2.854	4448	1.20	0
35	21.67		0.000	0.000	6543	1.77	0
36	21.87		0.000	0.000	2654	0.72	0
37	22.21	b-sitosterol	1076.056	92.272	147686	39.88	0
38	22.38		0.000	0.000	6803	1.84	0
39	22.59		0.000	0.000	36613	9.89	0
40	22.78		0.000	0.000	17041	4.60	0
41	22.92		0.000	0.000	10023	2.71	0
42	23.03		0.000	0.000	3204	0.87	0

Sample of chromatogram report for sterols (example of sample number 2), continued

Chrom Perfect Chromatogram Report

43	23.16	0.000	0.000	1511	0.41	0
44	23.37	0.000	0.000	557	0.15	0
46	23.75	0.000	0.000	30394	8.21	0
47	24.01	0.000	0.000	1463	0.40	0
48	24.17	0.000	0.000	1592	0.43	0
49	24.47	0.000	0.000	2272	0.61	0
50	24.83	0.000	0.000	784	0.21	0
52	25.05	0.000	0.000	31135	8.41	0
53	25.25	0.000	0.000	9530	2.57	0
58	27.14	0.000	0.000	535	0.14	0
60	27.67	0.000	0.000	504	0.14	0
61	27.90	0.000	0.000	2111	0.57	0
63	28.78	0.000	0.000	1169	0.32	0
64	29.11	0.000	0.000	2030	0.55	0
65	29.89	0.000	0.000	1884	0.51	0
66	31.00	0.000	0.000	1421	0.38	0

Total Area = 370331.2

Total Amount = 1166.178

Checked by _____ Date _____

Appendix A9

Table of oil crops and selected nutritional components

Avocado (pulp) Oil (*Persea americana*)

Fatty acid	Concentration (%)	Tocopherol	Concentration (mg/100 g oil)	Sterol	Concentration (%)
16:0	9 - 18	α-tocopherol	6.4 - 10	Cholesterol	0 - 0.2
16:1	3 - 9	γ-tocopherol	0 - 1.9	Campesterol	6 - 8
18:0	0.4 - 1			Stigmasterol	0 - 2
18:1	56 - 74			β-sitosterol	89 - 92
18:2	10 - 17				
18:3	0 - 2				

Blackcurrant Oil (*Riber nigrum*)

Fatty acid	Concentration (%)	Tocopherol	Concentration (mg/100 g oil)	Sterol	Concentration (%)
14:0	0.1	α-tocopherol	32	Cholesterol	0.2 - 0.7
16:0	6 - 8	β-tocopherol	0.8	Campesterol	7.2 - 10.4
16:1	0 - 0.2	γ-tocopherol	64.7	Stigmasterol	0.5 - 1
18:0	1 - 2	δ-tocopherol	68	β-sitosterol	70 - 85
18:1	9 - 13				
18:2	45 - 50				
6c,9c,12c-18:3	14 - 20				
9c,12c,15c-18:3	12 - 15				
6c,9c,12c,15c-18:4	2 - 4				
20:0	0.2				
20:1	0.9 - 1				
22:0	0.1				
24:0	0.1				

Borage Oil (*Borrago officinalis*)

Fatty acid	Concentration (%)	Tocopherol	Concentration (mg/100 g oil)	Sterol	Concentration (%)
14:0	0.1	α-tocopherol	0 - 4.6	Campesterol	25 - 30
16:0	9.4 - 11.9	γ-tocopherol	3.3 - 27.2	β-sitosterol	22 - 42
16:1	0.4	δ-tocopherol	69.0 - 101.3		
18:0	2.6 - 5				
9c-18:1	14.6 - 21.3				
18:02	36.5 - 40.1				
6c,9c,12c-18:3	17.1 - 25.4				
9c,12c,15c-18:3	0.2				
6c,9c,12c,15c-18:4	0.2				
20:0	0.2				
20:1	2.9 - 4.1				
22:0	1.8 - 2.8				
24:0	1.2 - 4.5				

Table of oil crops and selected nutritional components, continued

Evening Primrose Oil (*Oenothera biennis*)

Fatty acid	Concentration (%)	Tocopherol	Concentration (mg/100 g oil)	Sterol	Concentration (%)
12:0	0.03	α-tocopherol	7.6 - 35.6	Campesterol	8 - 9
14:0	0.07	γ-tocopherol	18.7 - 35.8	β-sitosterol	87 - 90
16:0	6 - 10	δ-tocopherol	0 - 19		
16:1	0.04				
18:0	1.5 - 3.5				
18:1	5 - 12				
18:2	65 - 80				
6c,9c,12c-18:3	8 - 14				
9c,12c,15c-18:3	0.2				
20:0	0.3				
20:1	0.2				
22:0	0.1				
24:0	0.1				

Hempseed Oil (*Cannabis sativa*)

Fatty acid	Concentration (%)	Tocopherol	Concentration (mg/100 g oil)	Sterol	Concentration (%)
16:0	6 - 12	n/a	n/a	Campesterol	17
18:0	1 - 2			Stigmasterol	15
18:1	11 - 16			β-sitosterol	44
18:2	45 - 65				
18:3	15 - 30				
20:0	2				

Linseed Oil (Flax) (*Linum usitatissimum*)

Fatty acid	Concentration (%)	Tocopherol	Concentration (mg/100 g oil)	Sterol	Concentration (%)
16:0	5.7 - 7	α-tocopherol	0.5 - 1.0	Cholesterol	0 - 0.9
18:0	3 - 4	γ-tocopherol	43.0 - 57.5	Campesterol	25 - 31
18:1	20 - 20.3	δ-tocopherol	0.4 - 0.8	Stigmasterol	6 - 9
18:2	17 - 17.3			β-sitosterol	45 - 53
18:3	52 - 54				
20:0	0 - 0.1				

Table of oil crops and selected nutritional components, continued

Mango Pulp Oil

Fatty acid	Concentration (%)	Tocopherol	Concentration (mg/100 g oil)	Sterol	Concentration (%)
12:0	0.3 - 3	n/a	n/a	n/a	n/a
14:0	1 - 12				
16:0	22 - 30				
18:0	16 - 30				
18:1	1 - 2				
18:2	24 - 40				
18:3	3 - 10				
20:0	5 - 9				

Olive Oil (*Olea europaea*)

Fatty acid	Concentration (%)	Tocopherol	Concentration (mg/100 g oil)	Sterol	Concentration (%)
14:0	0 - 0.1	α-tocopherol	6.3 - 13.5	Cholesterol	0 - 0.5
16:0	7.5 - 20	γ-tocopherol	0.7 - 1.5	Campesterol	0 - 4
16:1	0.3 - 3.5			Stigmasterol	0 - 4
18:0	0.5 - 5			β-sitosterol	75 - 80
18:1	55 - 83				
18:2	3.5 - 21				
18:3	0 - 1.5				
20:0	0 - 0.8				
22:0	0 - 0.2				
24:0	0 - 1				

Palm Oil (*Elaeis guineensis*)

Fatty acid	Concentration (%)	Tocopherol	Concentration (mg/100 g oil)	Sterol	Concentration (%)
12:0	0 - 0.4	α-tocopherol	0.4 - 19.3	Cholesterol	2.6 - 6.7
14:0	0.5 - 2	β-tocopherol	0 - 23.4	Campesterol	18.7 - 27.5
16:0	40 - 48	γ-tocopherol	0 - 52.6	Stigmasterol	8.5 - 13.9
16:1	0 - 0.6	δ-tocopherol	0 - 12.3	β-sitosterol	50.2 - 62.1
18:0	3.5 - 6.5	α-tocotrienol	0.4 - 33.6		
18:1	36 - 44	γ-tocotrienol	1.4 - 71.0		
18:2	6.5 - 12	δ-tocotrienol	0 - 37.7		
18:3	0 - 0.5				
20:0	0 - 1				
20:1	0 - 0.2				
22:0	0 - 0.1				
24:0	0 - 0.2				

Table of oil crops and selected nutritional components, continued

Peanut / Groundnut Oil (*Arachis hypogaea*)

Fatty acid	Concentration (%)	Tocopherol	Concentration (mg/100 g oil)	Sterol	Concentration (%)
12:0	0 - 0.1	α-tocopherol	4.9- 37.3	Cholesterol	0 - 3.8
14:0	0 - 0.1	β-tocopherol	0 - 4.1	Campesterol	12 - 19.8
16:0	8.3 - 14	γ-tocopherol	8.8 - 39.0	Stigmasterol	5.4 - 13.2
16:1	0 - 0.2	δ-tocopherol	0 - 2.2	β-sitosterol	47.4 - 67.7
18:0	1.9 - 4.4				
18:1	36.4 - 67.1				
18:2	14 - 43				
18:3	0 - 0.1				
20:0	1.1 - 1.7				
20:1	0.7 - 1.7				
22:0	2.1 - 4.4				
22:1	0 - 0.3				
24:0	1.1 - 2.2				
24:1	0 - 0.3				

Rapeseed Oil (low erucic, Canola)

Fatty acid	Concentration (%)	Tocopherol	Concentration (mg/100 g oil)	Sterol	Concentration (%)
14:0	0 - 0.2	α-tocopherol	10.0 - 38.6	Cholesterol	0.5 - 1.3
16:0	3.3 - 6	β-tocopherol	0 - 14.0	Campesterol	24.7 - 38.6
16:1	0.1 - 0.6	γ-tocopherol	18.9 - 75.3	Stigmasterol	0 - 0.7
17:0	0.3	δ-tocopherol	0 - 2.2	β-sitosterol	45 - 58
18:0	1.1 - 2.5				
18:1	52 - 67				
18:2	16 - 25				
18:3	6 - 14				
20:0	0.2 - 0.8				
20:1	0.1 - 3.4				
20:2	0 - 0.1				
22:0	0 - 0.5				
22:1	0 - 4.7				
22:2	0 - 0.1				
24:0	0 - 0.2				
24:1	0 - 0.4				

Table of oil crops and selected nutritional components, continued

Soybean Oil

Fatty acid	Concentration (%)	Tocopherol	Concentration (mg/100 g oil)	Sterol	Concentration (%)
12:0	0 - 0.1	α-tocopherol	0.9 - 35.2	Cholesterol	0.6 - 1.4
14:0	0 - 0.2	β-tocopherol	0 - 4.0	Campesterol	15.8 - 24.2
16:0	9.7 - 13.3	γ-tocopherol	8.9 - 240.0	Stigmasterol	15.9 - 19.1
16:1	0 - 0.2	δ-tocopherol	15.0 - 93.2	β-sitosterol	51.7 - 57.6
18:0	3 - 5.4	α-tocotrienol	0 - 6.9		
18:1	17.7 - 28.5	γ-tocotrienol	0 - 10.3		
18:2	49.8 - 57.1				
18:3	5.5 - 9.5				
20:0	0.1 - 0.6				
20:1	0 - 0.3				
20:2	0 - 0.1				
22:0	0.3 - 0.7				
22:1	0 - 0.3				
24:0	0 - 0.4				

Sunflower Seed Oil (*Helianthus annuus*)

Fatty acid	Concentration (%)	Tocopherol	Concentration (mg/100 g oil)	Sterol	Concentration (%)
12:0	0 - 0.1	α-tocopherol	40.0 - 95.0	Cholesterol	0 - 0.7
14:0	0 - 0.2	β-tocopherol	0 - 5.0	Campesterol	7 - 13
16:0	5 - 8	γ-tocopherol	0 - 5.0	Stigmasterol	7 - 12
16:1	0 - 0.3	δ-tocopherol	0 - 10	β-sitosterol	56 - 65
18:0	2.5 - 7				
18:1	13 - 40				
18:2	48 - 74				
18:3	0 - 0.3				
20:0	0.2 - 0.5				
20:1	0 - 0.5				
22:0	0.5 - 1.3				
22:1	0 - 0.5				
24:0	0 - 0.4				

Table of oil crops and selected nutritional components, continued

Wheat Germ Oil (*Triticum aestinum* / durum)

Fatty acid	Concentration (%)	Tocopherol	Concentration (mg/100 g oil)	Sterol	Concentration (%)
14:0	0 - 0.2	α-tocopherol	45.0 - 310.0	Campesterol	19 - 29
16:0	12 - 20	β-tocopherol	20.0 - 115.0	Stigmasterol	0.3 - 4
16:1	0.2 - 0.5	γ-tocopherol	1.8 - 95.0	β-sitosterol	56 - 67
18:0	0.3 - 3	δ-tocopherol	2.0 - 10.0		
18:1	13 - 23	α-tocotrienol	1.0 - 20.0		
18:2	50 - 59	β-tocotrienol	1.0 - 20.0		
18:3	2 - 9				
20:0	0.3				
20:1	0.3				
22:0	0 - 0.1				
22:1	0.3				
24:0	0 - 1				

Source for "Table of oil crops and selected nutritional components" (Appendix A9):

Firestone, D. 1999. *Physical and Chemical Characteristics of Oils, Fats, and Waxes.* Champaign, Illinois: AOCS Press.