Evaluation and optimization of cranberry seed oil extraction methods

Palaniappan Thyagarajan

Department of Bioresource Engineering

McGill University

Montreal, Quebec

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ABSTRACT

Cranberry seed oil is a fine quality oil which can be marketed for its many beneficial functions to human health due to the presence of a unique combination of omega- 3, 6 and 9 fatty acids and the high concentration of antioxidants. Although there is no available clear data about the oil percentage of cranberry seeds in literature, an experiment was done to analyze the oil yields obtained through different processes such as heat reflux, microwave and ultrasound, both quantitatively and qualitatively. During preliminary experiments, better oil yields were obtained with ground seeds (10.11 ± 0.2%) in comparison with unground seeds (8.17 ± 0.3%) , and hence the seeds were made into a fine powder using a blender and the fine powders were separated by sieve (1mm). Hexane (10.2 ± 0.2%) was also found to be more efficient in oil extraction than Hexane: Ethanol (7:3) (6.45 ± 0.1%). The oil extraction process was based on four major operating factors namely the sample/solvent ratio, temperature, time and applied power for the heat reflux, microwave and ultrasound extraction. The results showed that the oil yield increased with an increase in sample/solvent ratio at 10g/100ml of hexane, temperature of 70°C in heat reflux which provided an oil yield about 11.19 ± 0.1% while in the case of microwave, best conditions were for a sample/solvent ratio of 5g/30ml of hexane, power at 125W, which gave an oil yield of 24.15 ± 0.3% maintaining the temperature at 66°C. In comparison, with ultrasound extraction at a sample/ solvent ratio of 5g/30ml of hexane, and a power at 150W, the oil yield was 32.35 ± 0.3%. The best yield results obtained for the tested methods for extracting oil from seeds were found and compared as: Heat reflux < Microwave <Ultrasound. The advantage of ultrasound was a relatively high yield over other methods with a short process time and lower process heat from the applied power. Analysis of quality in oil particularly the concentration of α - tocopherol was estimated by spectrophotometry at 520 nm and results found that α - tocopherol concentration was greatly affected by temperature at 70°C in case of heat reflux which retained around 0.266 ± 0.02 µg while in the case of microwave at power 125W and ratio 5g/30ml of

hexane the tocopherol retained was around 0.346 ± 0.007 µg whereas in ultrasound the tocopherol was retained upto 0.428 ± 0.01 µg on applying power at 100W. The solvent extraction with hexane coupled with microwave or ultrasound energy released a greater amount of α - tocopherol from the plant matrix than for cold pressed method. For conventional cranberry seed oil extraction the cold pressed method was a better option than heat-reflux method in terms of oil quality, otherwise newer techniques such as MAE and UAE are highly recommended both in terms of increased oil yields and increased tocopherol content.

Évaluation et optimisation de méthodes d'extraction de l'huile de graines de canneberges

Résumé

L'huile de graines de canneberges est une huile de qualité qui peut être mise en marché pour ses propriétés fonctionnelles "santé" issues de sa composition particulière en acides gras oméga 3, 6 et 9 et de sa forte concentration en antioxydants. Il existe peu ou pas d'information sur la teneur en huile de la graine de canneberge, cependant l'industrie alimentaire s'intéresse à l'extraction de cette huile, qu'elle effectue présentement par pressage à froid, malgré un faible rendement. Afin de palier à ce problème, une étude a débuté afin d'analyser les rendements en huile obtenus par différents procédés d'extraction dont l'ébullition à reflux, les microondes et les ultrasons. L'ébullition à reflux améliore l'extraction en améliorant la diffusion du solvant par la chaleur, tandis que dans le cas des microondes et des ultrasons, c'est plutôt la particularité des ondes qui influencent l'extraction. Les objectifs de cette recherche ont donc visé à obtenir de bons rendements en huile avec une forte teneur en α - tocophérol. Lors des essais préliminaires, de meilleurs rendements ont été obtenus avec des graines moulues (10.11 ± 0.2%) en comparaison avec des graines non moulues (8.17 ± 0.3%), ainsi tous les essais d'extraction ont par la suite été faits avec des graines finement moulues et tamisées (1mm).L'hexane (10.2 ± 0.2%) s' est avéré plus efficace pour l'extraction qu'un mélange d'hexane: éthanol (7:3) (6.45 ± 0.1%). L'étude de l'extraction de l'huile s'est concentrée sur quatre facteurs opérationnels soit le ratio d'échantillon/solvant, la température, le temps et la puissance appliquée pour l'ébullition à reflux, et l'extraction microonde et par ultrasons. Nos résultats ont démontré une augmentation du rendement en huile avec une augmentation du ratio échantillon/solvant à 10g/100 ml d'hexane, à une température de 70°C pour l' ébullition à reflux avec un rendement de 11.19 ± 0.1% tandis qu'avec l'extraction microonde, les meilleures conditions furent pour un ratio d'échantillon/solvant de 5g/30ml d'hexane, et une puissance de 125W, donnant une rendement en huile de 24.15 ± 0.3% à une température de 66°C. En comparaison, l'extraction ultrason, pour un ratio d'échantillon/solvant de 5g/30ml d'hexane, à une intensité de 150W, le rendement en huile a été de 32.35 ± 0.3%. Ainsi, les meilleurs résultats en terme de rendement en huile pour

les différentes méthodes d'extraction sont classés comme suit: Ébullition par reflux < Microonde < Ultrason. L'avantage de l'extraction par ultrasons fut son haut rendement en huile, pour un procédé rapide, à température peu élevée. Le rendement maximal en huile a été obtenu par l'extraction ultrason avec 1.61 g. L'analyse de la qualité de l'huile, en particulier la teneur en α - tocophérol a été effectuée par spectrophotométrie à 520 nm et les résultats ont démontrés que la teneur en α - tocophérol était grandement affectée par la température, à 70°C dans le cas de l'ébullition par reflux avec 0.266 ± 0.02 µg, tandis qu'avec l'extraction microonde à la puissance de 125W au ratio de 5g/30ml d'hexane, la teneur en tocophérol était de 0.346 ± 0.007 µg, tandis que pour les ultrasons, la teneur en tocophérol était de 0.428 ± 0.01 µg pour une puissance de 100W. L'extraction par solvant, jumelée avec les microondes ou les ultrasons, libère une plus grande concentration en α - tocophérol des cellules de la plante en comparaison avec la pression à froid. En conclusion, l'extraction par pression à froid obtient une huile de meilleure qualité que l'huile obtenue par ébullition par reflux, tandis que l'extraction par microondes et par ultrasons est fortement recommandée pour un meilleur rendement en huile et une plus haute teneur en tocophérol.

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NOMENCLATURE

α - tocopherol	**Alpha tocopherol**
^{o}C	**Degree Celcius (temperature)**
µg	**Micrograms (mass)**
µm	**micrometer**
µl	**micro liter**
DNA	**Deoxyribo Nucleic acid**
g	**Grams (mass)**
GHz	**Gigahertz (frequency)**
Ha	**Hectares (area)**
HPLC	**High Performance Liquid Chromatography**
IU	**International Unit for measuring biological effect**
Kcal	**Kilocalories (energy)**
Kg	**Kilogram (mass)**
kHz	**Kilohertz (ultrasonic frequency)**
MAE	**Microwave Assisted Extraction**
mg	**Milligrams (mass)**
MHz	**Megahertz (frequency)**

Min	**Minutes (time)**
ml	**millilitre (volume)**
mm	**Millimeter (pore size)**
nm	**nanometer (optical density)**
n mol	**Nano mole**
rpm	**revolutions per minute**
$\mathbf{s^{-1}min^{-1}}$	**/seconds/minute (mw irradiation)**
UAE	**Ultrasound Assisted Extraction**
W	**Watts (microwave power)**

CHAPTER I

GENERAL INTRODUCTION

1.1 Introduction

Cranberries are healthy fruits that contribute color, flavour, nutritional value, and functionality to a variety of food products. They are one of the few Native American fruits. The North American cranberry (*Vaccinium macrocarpon*) is recognized by the United States Department of Agriculture (USDA), as the common cultivar for fresh cranberries and cranberry juice cocktail. The European variety, grown in parts of central Europe, Finland, and Germany, is known as *Vaccinium oxycoccus*. This is a smaller fruit with anthocyanins and acid profiles slightly different to that of the North American variety (Girard and Sinha, 2006). Phenolic acids, flavonoids, and tannins are phytochemicals commonly encountered in foods of plant origin (Lugasi and Hovari, 2000). Their quantitative distribution can vary between different organs of the plants and within different populations of the same plant species and for this reason they are often used for plant taxonomical classification (Robards and Antolovich, 1997; Merken and Beecher, 2000a). The predominant bioactive compounds found in cranberries are the flavonols, the flavan-3-ols, the anthocyanins, the tannins (ellagitannins and proanthocyanidins), and the phenolic acid derivatives. These phytochemicals are commonly associated with the fruit's organoleptic (sensory) qualities and have also shown diverse biological properties and physiological activities in animals (Robards and Antolovich, 1997; Kren and Martinkove, 2001). Numerous clinical trials and epidemiological studies have established an inverse correlation between the intake of phenol-rich fruits and vegetables and the occurrence of diseases such as inflammation, cardiovascular disease, cancer, and aging-related disorders (Willet, 2001). Tocopherols and omega-3-fatty acids have also been isolated and identified in cranberry seed oil. The cranberry seed oil is known for its unique combination of different omega fatty acids.

Plant-based lipophilic compounds such as edible oils, phytochemicals, flavors, fragrances and colors are valuable products in the food, pharmaceutical and chemical industries. Extraction is one of the key processing steps in recovering and purifying lipophilic ingredients

contained in plant-based materials (Liu, 1999). Classical extraction technologies are based on the use of an appropriate solvent to remove lipophilic compounds from the interior of plant tissues. The choice of a suitable solvent in combination with sufficient mechanical agitation influences mass transport processes and subsequently the efficiency of the extraction. The most widely used solvent to extract edible oils from plant sources is hexane. Hexane is available at low cost and is efficient in terms of oil and solvent recovery (Mustakas, 1980; Serrato, 1981). More recently, the use of alternative solvents such as alcohols (isopropanol or ethanol) and supercritical carbon dioxide has increased due to environmental, health and safety concerns (Dunnuck, 1991). Alternative solvents may be less efficient due to a decreased molecular affinity between the solvent and solute and while the costs for solvent and process equipment can be higher (Baker and Sullivan, 1983; Freidrich and Pryde, 1984; Karnofsky, 1981). Attention is thus centered nowadays, by the food industry on the optimization of the extraction processes. In the current work, we are focusing on the study of the solvent extraction of oil from cranberry seeds exploring various extraction promoting technologies such as reflux heat, microwave assisted and ultrasound assisted extraction.

The determination of the content of either edible oil or total fat in cranberry seeds is of paramount importance to the processed cranberry industry, which produces a considerable quantity of seeds as a by-product of cranberry fruit processing (drying and juice making). The potential exploitation of the cranberry seed oil depends on its availability and quality as a commercial product. The most widely used procedures for oil extraction from a solid plant matrix is cold pressing with low yields and conventional Soxhlet extraction (on which official methods are based) which is straightforward and inexpensive but also slow and tedious. The most severe shortcomings of Soxhlet extraction are the long time involved and the large volumes of organic solvents that are released into the atmosphere; the Soxhlet procedure is thus far from clean. A great variety of new approaches based on different principles namely, supercritical fluid extraction (SFE), microwave assisted extraction (MAE), accelerated solvent extraction in closed systems at high temperature and pressure (ASE), etc., have been developed in the last few years to circumvent the shortcomings of conventional Soxhlet extraction.

Microwave-assisted extraction (MAE) is broadly used to extract valuable components from Chinese herbs, vegetables and plants (Bayramoglu *et al.,* 2008; Cravotto and Boffa, 2008; Du and Zhou, 2006; Mahesar *et al.,* 2008). It has proved to be effective, high yielding and energy

saving (Pan *et al.,* 2003; Shu *et al.,* 2003). The applicability of microwave energy for the extraction of various types of compounds from plant material, food and soil was first investigated by Ganzler *et al.,* (1986). The production of volatile material from plant exposed to microwave energy in an air stream has been discussed by Craveiro *et al.,* (1989). Chen and Spiro, (1994) studied the microwave heating characteristics for the extraction of rosemary and peppermint leaves suspended in hexane, ethanol and a mixture of hexane and ethanol. Paré, (1995), patented a general extraction method for biological matter using microwave energy and a microwave transparent solvent such as hexane or acetone. The water (polar substance) in the plant material selectively absorbs the microwave energy, dissipating it as heat, and the increasing vapour pressure within the cellular structure causes it to rupture. Thus, the plant compounds are exposed and collected by the microwave transparent solvent which acts as a cold environment to maintain the quality of thermosensitive compounds.

Another potential new technology that may improve extraction of lipophilic compounds from plants is ultrasound-assisted solvent extraction (UAE). High-intensity ultrasonication can accelerate heat and mass transport in a variety of food process operations and has been successfully used to improve drying, mixing, homogenization and extraction (Fairbanks, 2001; Mason, 1992; Mason *et al.,* 1996; Povey, 1998). Ultrasonication is the application of high-intensity, high-frequency sound waves and their interaction with materials (Luque-Garcia and Luque de Castro, 2003). The propagation and interaction of sound waves alters the physical and chemical properties of materials to which they are subjected (Mason and Lorimer, 1988). In the case of raw plant tissues, ultrasound has been suggested to disrupt plant cell walls thereby facilitating the release of extractable compounds and enhance the mass transport of solvent from the continuous phase into the plant cells (Vinatoru, 2001). Romdhane and Gourdon, (2002) investigated the extraction of pyrethrines from pyrethrum flowers and oil from woad seeds. In these cases, acceleration of extraction kinetics and increase in yield was observed, however less so in the case of woad seeds. Vinatoru *et al.,* (1997) showed improved yields of lipophilic compounds extracted with ultrasound from herbs such as coriander and fennel.

1.2 Hypothesis:

At present, cranberry seed oil is mainly extracted from cranberry seeds by cold- pressing extraction as to keep the quality of the thermolabile liphophilic compounds, however, the process

efficiency is low. There was therefore room to improve the yield and a study, to find a better extraction technique, was planned and is presented here. To date, novel extraction methods like MAE and UAE have not been used for extracting the oil from cranberry seeds. Our experiment was thus planned to test the hypothesis that microwave and ultrasound assisted extraction could be used to extract oil from cranberry seeds with greater yield efficiency than conventional heat-reflux techniques and to evaluate the residual α - tocopherol content in the oil by spectrophotometry.

1.3 Objectives:

The overall objective of this research was to optimize the extraction of oil from cranberry seeds using three different extraction methods. Specifically, the goal was to maximize cranberry seed oil yields without the loss of its naturally occurring antioxidant compounds. Specific objectives of this study were to:

1) Determine the optimal conditions required for microwave and ultrasound assisted extraction of oil from cranberry seeds and compare the results obtained through heat-reflux.

2) Investigate the effect of the three extraction methods on the α – tocopherol content in oil determined by spectrophotometry.

CHAPTER II

LITERATURE REVIEW

2.1 CRANBERRY-General introduction

Cranberries are a group of 400 shrubs or small trees worldwide which fall under the genus *Vaccinium.* Cranberries are low, creeping shrubs or vines up to 2 metres (7 ft) long and 5 to 20 centimetres (2 to 8 in) in height with slender, wiry stems that are not thickly woody with small evergreen leaves and found generally on acidic, sandy and organic soils. The flowers are dark pink, with very distinct reflexed petals, leaving the style and stamens fully exposed and pointing forward. They are pollinated by insects. The fruit is a berry that is larger than the leaves of the plant; it is initially white, but turns a deep red when fully ripe. It is edible, with an acidic and tart taste that can overwhelm its sweetness.

2.1.1 Species and description:

There are three main species of cranberry, classified as:

Subgenus *Oxycoccus*, sect. *Oxycoccus*

Vaccinium oxycoccos or *Oxycoccus palustris* (**Common Cranberry** or **Northern Cranberry**)

The common cranberry has trailing, wiry and woody vines; shoots from axillary buds, often ascending upto 1-3 cm high. Twigs are dark brown to red, glabrous to pubescent. Leaves are persistent, ovate, occasionally elliptical, 2-3 mm wide and 5-6 mm long; Flowers are borne singly in the axils of reduced leaves at the base of current shoots, but mostly, the leafy portion of the fertile shoot does not develop, giving the illusion that *V.oxycoccus* has an inflorescence comprised of a short rachis bearing 1-4 flowers on long slender pedicels. Berries are quite small compared with *V.macrocarpon* and fruiting is usually sparse. It can be seen in British Columbia and Alaska. (Vander Kloet, 1988).

Vaccinium macrocarpon or *Oxycoccus macrocarpus* (**Large cranberry**, **American Cranberry**, **Bearberry**)

The large American cranberry has trailing and woody vines; shoots from axillary buds, frequently erect or ascending upto 4-15 cm long. Leaves are persistent, narrowly elliptical or elliptical upto 3-4 mm wide, 7-10 mm long. Flowers are borne singly in the axils of reduced

leaves, which is at the base of current shoots. According to Camp (1944), the *V. oxycoccus*, is a derived species from the primitive one known as *V.macrocarpon.* It is normally seen from Newfoundland, west to central Minnesota, south to northern Illinois, northern Ohio and central Indiana, and in the Appalachian mountains to Tennessee. (Vander Kloet, 1988).

Subgenus *Oxycoccus*, sect. *Oxycoccoides. Vaccinium erythrocarpum* or *Oxycoccus erythrocarpus* (Southern Mountain Cranberry)

The southern mountain cranberry is a crown-forming shrub 80-150 cm high. Leaves are elliptical, ovate to oblong-lanceolate and deciduous about 18-29 mm wide and 43-63 mm long with glandular-pubescent beneath, green on both sides; Calyx and pedicel are usually glabrous having small calyx lobes with pedicel up to 1.5 cm long. The berries are usually insipid, rarely sweet or tart. It can be seen at high elevations from west Virginia to northern Georgia. (Vander Kloet, 1988).

2.1.2 Etymology and history:

"Craneberry", was first named by early European settlers to America who felt the expanding flower, stem, calyx, and petals resembled the neck, head, and bill of a crane. Except for the case of *V. arboreum,* all the berries produced by the genus are edible; the flavor of these berries varies from insipid to tart, but all are eaten by both birds and mammals. Martin *et al.,* (1951) cited that *Vaccinium* was the favourite food for 53 species of wildlife. *Vaccinium* berries were traditionally eaten by North American aboriginals. The Inuit preserved the *V.uliginosum* in seal oil and blubber (Holm *et al.,* 1912) or gathered in leather bags that were then stored in permafrost (Darrow and Camp, 1945).Taylor,(1974), has argued that cranberries (*V.oxycoccus*) were an important food in the Inuit diet, because it contains enough acid to balance their meat diet. The European settlers have a long history of cranberry usage (Hedrick, 1919; Fernald and Kinsey, 1943), but none were the most prominent consumers than those who settled in Newfoundland, where the location of *V.vitis-idaea* is still a closely guarded secret. Indeed, Newfoundland is the only North American jurisdiction to have an act for the protection of the partridgeberry (Torrey, 1914); only during open season may the berry be picked with impunity. (Vander Kloet, 1988).

2.1.3 Cranberry production in North America from past to present:

Vaccinium macrocarpon, the large or American cranberry, became the first species in the genus to be cultivated in Cape Cod, Massachusetts, with a few vines in 1816. But it was not until 1845 that the cranberry could be established as a marketable commodity (Hedrick, 1919; Peterson *et al.,* 1968). The crop is confined to cool, moist, natural or artificial bogs that can be flooded or drained as desired. These bogs may remain productive for many successive years; indeed, several bogs in New Jersey and Cape Cod have been productive for more than 75 years (McGregor, 1976). Although the centre of cranberry production and cultivation is still Massachusetts (Table 2.1), *V. macrocarpon* has been successfully introduced to the peatlands of British Columbia, Quebec, Washington, and Oregon. (Vander Kloet, 1988).

Table 2.1: Cranberry production in North America:-, the early years 1970-1979. (McGregor, 1976; Hall, 1978; Vander Kloet, 1988)

Place	Area Cultivated(ha)		Yield (Tonnes)	
	1970	**1979**	**1970**	**1979**
Massachusetts	4360	4480	47664	49076
Wisconsin	2280	2840	24925	40953
New Jersey	1240	1200	13556	11495
British Columbia	475	480	5900	6577
Washington	400	440	4373	6680
Oregon	298	320	3258	4295
Quebec	40	_	320	_
Nova Scotia	10	22	80	18

Due to the advancement in agricultural technology in the 20th century, commercial cranberry production went up to 170,000 tonnes annually from 9,000 ha under production primarily in Wisconsin, Massachusetts, New Jersey, Washington and Oregon along with British Columbia, Quebec and Nova Scotia (Dana, 1990). They are principally grown in constructed "bogs" surrounded by dikes (Dana,1990; Eck,1990).Water is pumped into the bogs to flood them each autumn-once to float the berries for harvesting and later, in colder production areas such as Wisconsin, to allow several inches of ice to form for winter protection of the plant. Almost all cranberries are processed into juice, sauce, preserves, jelly or other products for consumption

(Eck, 1990). Generally, the fruit is frozen in bulk following harvest, processed, and sold to the consumer. The most important cultivars in each production region are 'Early Black' and 'I Lowes' in Massachusetts and New Jersey; 'Searles', 'McFarlin', 'Stevens', and 'Ben Lear' in Wisconsin; and 'McFarlin', 'Stevens', and 'Crowley' in the Pacific Northwest (Galletta,1975; Dana,1983; Eck,1990). Over 100 wild cultivars have been reported (Chandler and Demoranville, 1961; Dana, 1983). Between 1929 and 1961, a cooperative breeding program between the U.S. Department of Agriculture and institutions in Wisconsin, Massachusetts, New Jersey and Washington produced thousands of seedlings from crosses among these native selections (Galletta,1975).This program existed for only one generation of hybridization and selection but demonstrated that breeding could definitely improve economically important traits such as yield, vigor, pigment content, flavor, disease and pest resistance and storage ability (Galletta, 1975; Moore and Ballington, 1991).

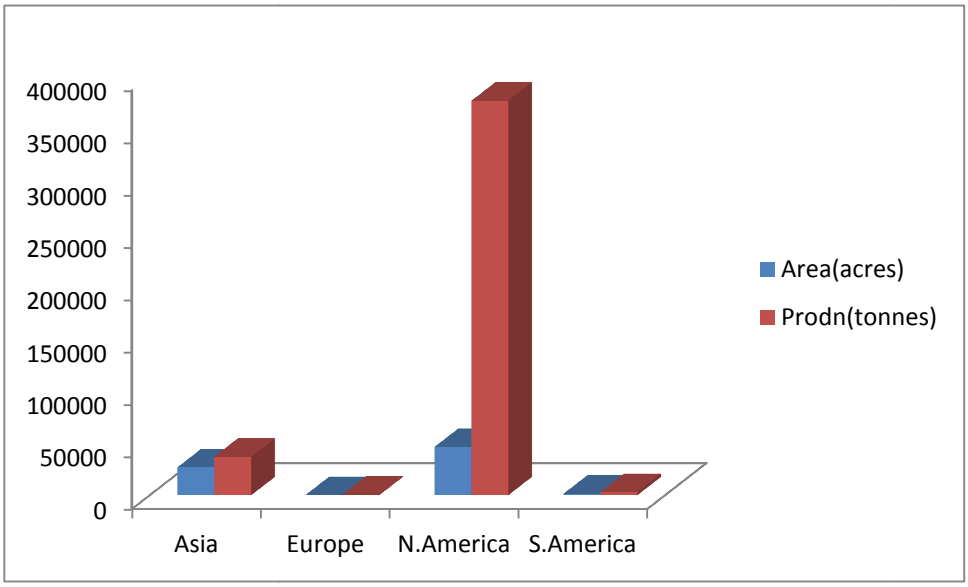

Fig 2.1: Cranberry production world wide in 2005 (Strik, 2007).

There were 74,679 acres of cranberries reported to be harvested worldwide in 2005 (Fig 2.1). The United states is the world's largest producer, with 39,200 acres of cranberry (*Vaccinium macrocarpon* Ait.) and 74% of total world production. In the United States, Wisconsin and Massachusetts have the largest cranberry acreage, accounting for 80% of the total. Other states producing cranberries are New Jersey, Oregon and Washington.

Belarus has the second largest area, with 19,768 acres, but the yield of cranberry (*Vaccinium oxycoccus* L.) is relatively low, with a total production of 25,353 tons. In comparison, Canada has the second highest production in the world, harvesting 68,556 tons of large-fruited cranberry from 7045 acres. The area in Europe is currently reported as 247 acres, all were focused in Romania; but,there are commercial cranberry plantings also in Ireland and Poland that are not yet reported in official statistics. Other countries producing the small-fruited cranberry are Ukraine, Latvia and the Republic of Azerbaijan in West Asia. In South America, the only country producing the large-fruited cranberry on established beds is Chile with 1500 acres.

The majority of the cranberry production worldwide is processed. In the United States, 94% of the total production was processed in 2004. Cranberries are processed for juice, sauces and also for sweetened dried berries. Chile exported 40 tons of fresh cranberries in 2004. Fresh cranberries are stored from harvest (September or October) until the time of sale during thanksgiving (October in Canada and November in the United States) and Christmas seasons (Strik, 2007).

Recently in 2007, after the advancement of harvesting and production techniques, Canada has become a world leader in Cranberry production as it became an exporter of the fresh berries (Table 2.2).

Table 2.2 : General Cranberry Production Information in Canada (Hughs, 2005)

Canadian Production	79,163 metric tonnes on 3,952 hectares
Farm gate value	$73.5 million
Domestic consumption	0.70 kg/person(fresh)
Export	$44.3 million
Import	$17.2 million

2.2 Oils:

Oils can be described as hydrophobic (water-insoluble) substances that are liquid at room temperature and comprise mostly triglycerides (Belitz *et al.,* 2009). In general, most oils are viscous liquids that are soluble in organic solvents. Often, rather than being called oils, they are placed along with fats, waxes, sterols and other similarly hydrophobic compounds into a class of

organic compounds called lipids. Fats are very similar to oils having a solid nature at ambient temperatures. Considering their biological origin, it is perhaps not surprising that almost 40% of our diet comprises of biological oils and fats (in their several different forms). Apart from our diet which comprises of important sources of nutritionally-significant *unsaturated fatty acids*, the oils and their sources are important agricultural commodities (Gunstone, 2011). Based on the source of origin, there are mainly two kinds of oils; *organic* and *essential* oils. *Organic* or *biological* oils are those produced by living beings (such as plants, animals, microorganisms, etc.) through biological process. Keeping in mind the variety of living organisms that are capable of producing or processing them, these oils are very diverse in source and composition. *Essential oils* can be broadly defined as volatile oils which differ fundamentally from the fixed fatty oils such as linseed, coconut and olive in being more mobile and volatile. Many essential oils, apart from being used for first aid medicines are also ideal for bath oils, perfumes or room fresheners. Even when they are used purely for aesthetic purposes, they are still fulfilling a positive preventive and therapeutic role (Lawless, 1997).

Fatty acids (FAs) are the most significant constituents of all vegetable, animal and marine (fish or algal) oils. They are an important class of aliphatic lipids comprised of an alkyl chain (carbon backbone) with a carboxylic acid group (-COOH) at one end and a methyl group (-CH3) at the other end. The length (i.e., the number of carbon atoms, including that of the carboxylic group) and the level of saturation (i.e., the number of double bonds and their location) in the alkyl chain are the two most important features that affect the physical and functional properties of FAs (Gurr *et al.,* 2002; Belitz *et al.,* 2009; Casal and Oliveira, 2007). Since the polar nature of the –COOH group is balanced out by the non-polar nature of the alkyl chain, FAs are neutral in polarity; hence, they are often classified as neutral lipids (Akoh and Min, 2008). The most prominent functional role of FAs is as a source of metabolic energy other than or in addition to the usual source – glucose. FAs also play several crucial biological roles including the formation and maintenance of cellular membranes, cell signalling, etc. For these reasons, they have a prominent presence and pivotal role in human and animal diet, health and nutrition (Belitz *et al.,* 2009).

The FAs that contain one or more double bonds in their alkyl chains are known as monounsaturated FAs (MUFAs) and the latter are called polyunsaturated FAs (PUFAs). In order to further differentiate unsaturated FAs from each other biologically, an additional *omega*

terminology is used. Nutritionally, ω-3 and ω-6 FAs are the most significant and abundant (Holme and Peck, 1998; Gurr *et al.,* 2002; Belitz *et al.,* 2009; Casal and Oliveira 2007). The presence of double bonds render these FAs with lower melting points than saturated FAs; a property responsible for the characteristic liquid nature (at room temperature) of most vegetable and fish oils (both of which are rich sources of unsaturated FAs) (Belitz *et al.,* 2009). In contrast, most fats of animal origin such as butter or lard are solid at room temperature as they are made up predominantly of saturated fatty acids. The *fluid property* of unsaturated FAs, particularly PUFAs is responsible for maintaining the structural integrity of cellular membranes and is of great biological significance (Gurr *et al.,* 2002). Besides being vital to membrane fluidity, PUFAs have been proven to possess remarkable health-promoting functions against a wide range of disorders, most notably cardiovascular diseases. The therapeutic effects of PUFAs on heart disease has been so well-documented and understood that the American Heart Association (AHA) recently published a set of recommendations for the use of ω -6 FAs (Kris-Etherton *et al.*, 2003). Other disorders that have been targeted by PUFAs include inflammatory bowel disease (Siguel and Lerman, 1996), rheumatoid arthritis (Zurier *et al.,* 2005), cancer (Caygill *et al.,* 1996) and even diabetes (Abete *et al.,* 2009).

2.2.1 Omega 3 fatty acids:

Cranberry oil has a high linolenic acid content (omega-3 fatty acids). Linolenic acid has been studied as a food additive and nutraceutical ingredient in preventing coronary heart disease and cancer. Cranberry oil has a high polyunsaturated: saturated fatty acid ratio in a neutral lipid fraction, of 10:1. This ratio is regarded as having value in reducing serum cholesterol, atherosclerosis and in preventing heart disease (Heeg *et al.,* 2002). All berry seed oils have in common a high content of PUFAs with essential fatty acids. These fatty acids cannot be synthesized within an organism from other components by any known chemical pathway and therefore must be obtained from the diet. The higher consumption level of n-6 fatty acids compared with n-3 fatty acids is considered in the prevention of cancers, heart diseases, hypertension and autoimmune disorders (Connor, 2000; Hung *et al.,* 2000; Aronson *et al.,* 2001). In the western diet the present n-6/n-3 ratio has been estimated to be between 10 and 25 to 1(Parry *et al.,* 2005; Bawa, 2008), while the recommended ratio is estimated to be 4 to 1 (Parry *et al.,* 2005).The n-6 to n-3 PUFA imbalance is due to the increase in usage of vegetable and seed oil, and a decreased intake of fish, fish oil and vegetable oils containing a high level of n-3 fatty acids.

Table 2.3: Fatty acid profile of cranberry seed oil (Liangli *et al.*, 2005)

Lipid number	Composition(g/100g fatty acids)of seed oil
12:0	Nd
14:0	Nd
16:0	5.0-6.0
16:1	Nd
18:0	1.0-2.0
18:1	20.0-25.0
18:2n-6	35-40
18:3n-3	30.0-35.0
20:0	0.1
20:2	Nd
20:5n-3	1.1
Sat	6.1-8.1
MUFA	20.0-25.0
PUFA	66.1-76.1
n-6	35.0-40.0
n-3	31.1-36.1
n-6/n-3 ratio	1.0-1.3

Note: Nd: Not detected.

Berry seed oils on the other hand have a remarkable n-6/n-3 FA ratio compared with some other vegetable oils (Parker *et al.,* 2003; Parry *et al.,* 2006). Moreover, these seed oils are also rich in various antioxidants, which are related to a protective effect against cardiovascular lipid oxidation and antitumor activities (Wang and Jiao, 2000; Van Hoed *et al.,* 2009). Typically, cranberry seed oil contains, by weight, approximately 30-35% of α - linolenic acid (omega-3), 35-40% linoleic acid (omega-6), along with 20-25% of oleic acid (omega-9)(Liangli *et al.,* 2005). Moreover cranberry seed oil is edible, having a pleasant flavour, and appears to have good oxidative stability. In contrast, other known sources of fatty acids from seeds lack these advantages. For example, flaxseed oil is not a readily edible oil, but can be used as a drying oil in

the paint industry. Oils from soybean, fish, rapeseed and canola are more or less neutral in flavor or lack the typical pleasant cranberry flavor and the presence of beneficial tocotrienols. In addition, fish oil lacks the stability against oxidation exhibited by cranberry oil. While these oils have omega-3 fatty acids, isolated cranberry seed oil also has both omega-6 and omega-9 fatty acids which play important roles in various health aspects (Nawar, 2004). The percentage of oil available in cranberry seeds is not cited in the literature, however the fatty acids composition in cold-pressed cranberry oil has been reported and is listed in Table 2.3

2.2.2 Bioactive compounds in cranberry oil:

Cranberries and cranberry products are known for their high concentrations of total polyphenols (Vinson *et al.,* 2008). More specifically, cranberry oils are rich in anthocyanins, tocopherols, tocotrienols, tannins (ellagitannins and proanthocyanidins), and have significant concentrations of the flavonoids-flavonols and flavan-3-ols (Neto, 2007; Ruel and Couillard, 2007; USDA, 2004; 2007). Recently, many authors have reported the presence of certain phenolic acid derivatives in cranberries such as hydroxycinnamic acid (HCA), and hydroxybenzoic acid (HBA) (Neto, 2007; Ruel and Couillard, 2007). However these phenolic acids occur naturally in their free forms, they are considered as structural moieties in polyphenol compounds (Seeram and Heber, 2007). Flavonoids are defined as naturally-occurring organic species that possess two six-carbon aromatic centers, called the A and B rings, and a three-carbon bridge, called the C ring which forms a phenol bridge with oxygen (Robards and Antolovich, 1997; Haslam, 1998; Gee and Johnson, 2001). Flavonoids are further classified based on the degree of unsaturation and oxidation of their three-carbon heterocycle segment (Fig.2.2).

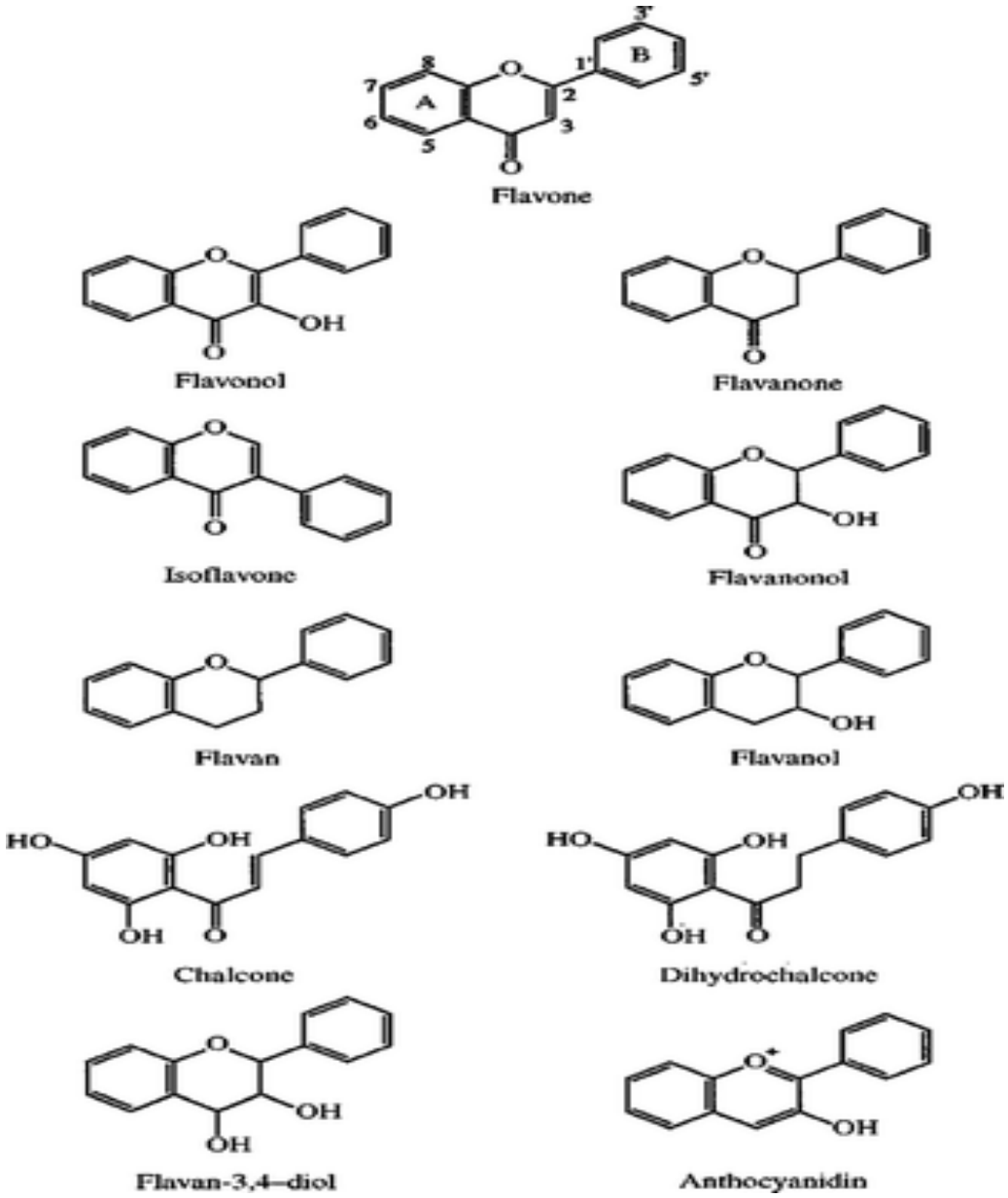

Fig 2.2: Structure of the various classes of flavonoids (Robards and Antolovich, 1997).

Antioxidants are generally applied to those compounds that interrupt the free radical chain reactions involved in lipid oxidation (Gelmez, 2008). Knowledge about the influence of oxidative stress and the role of vitamins on human health has gained much attention in the last few decades. Oxidative stress can be defined as an imbalance between oxygen free-radicals formation and their scavenging by antioxidants, playing an important contributory role in the pathogenesis of numerous degenerative (McCall and Frei , 1999) or chronic diseases, such as arteriosclerosis, allergy or cancer (Wittenstein *et al.,* 2002). Humans and other biological

organisms have evolved a variety of mechanisms to protect themselves from the potentially toxic effects of reactive molecules (McCall and Frei, 1999).The so-called antioxidation complex includes some notable enzymes, such as catalase and superoxide dismutases, repair enzymes, such as DNA glycosylases as well as water and lipid soluble vitamins, such as ascorbic acid (vitamin C), tocopherol (vitamin E), carotenoids, and retinol (vitamin A) (McCall and Frei, 1999). Hence, Vitamins A and E are essential to human health for defense mechanisms. Both groups have free-radical scavenging properties that function as physiologic antioxidants and also play an important role in the prevention of neuromuscular deficits by maintaining a proper blood red-cell lifetime, and preventing abnormal platelets activity (Morris *et al.,* 1994; Amir *et al.,* 2009). Vitamin E is a term used to designate a family of related compounds (tocopherols) which has a common structure as shown in Fig 2.3, with a chromanol head and a phytyl tail (Hong *et al.,* 2009). These compounds are classified as a vitamin, because they cannot be synthesized by humans, and therefore must be obtained from the diet (Ruperez *et al.,* 2001).

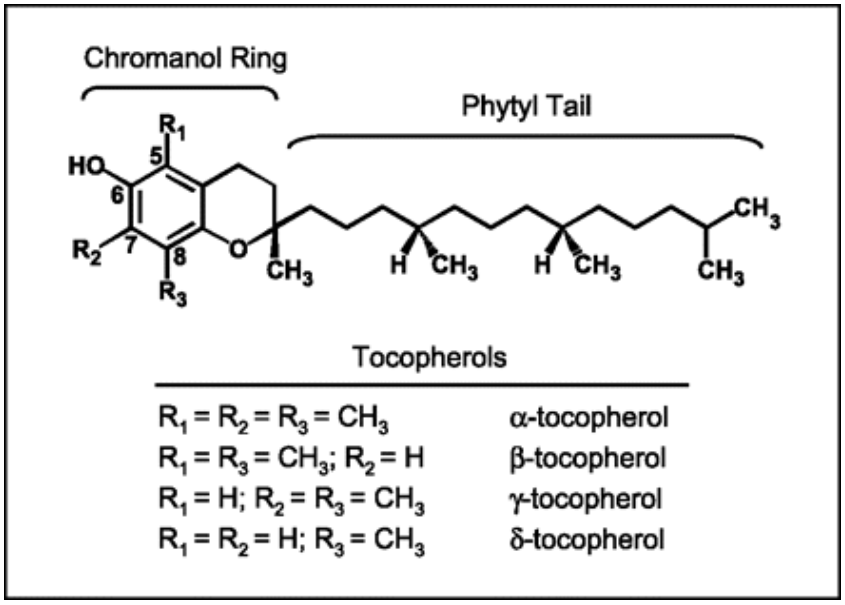

Fig 2.3: Structure of Tocopherol (Hong *et al.*, 2009).

Vitamin E is found mainly in fat products such as vegetable and seed oils (Ruperez *et al.,* 2001), and it is present in cranberry oil in abundance. Tocopherols play an important role in preventing the oxidation of biological material and lipoproteins caused by free radicals (Stone *et al.,* 2003). Clinical trials have shown that mixed tocopherols can inhibit platelet aggregation in humans and can reduce lipoprotein oxidation which causes atherosclerosis (Liu *et al.,* 2003).

Normal physiological processes such as aerobic respiration and metabolism produce free radicals. Some of the free radicals formed by the body include superoxide anion and hydroxyl radical. These free radicals are extremely reactive and attack damaged body cells. Many antioxidant compounds can react with these free radicals thereby protecting the body cells from oxidation. In this way, tocopherols have been studied and found to help in the inhibition of certain cancers and reduced risk of atherosclerosis (McCall and Frei, 1999; Wittenstein *et al.,* 2002).The existing spectrophotometric methods for vitamin E determination make use of the oxidizing ability of the 6-hydroxychroman ring of α-tocopherol to the corresponding quinine, i.e., α - tocopherylquinone, with an oxidizing reaction finally giving coloured products. The official method utilizes the Fe (III)-bipyridyl (Meites, 1982) or bathophenanthroline (Helrich, 1990) complex reagent as the oxidizing agent. The Fe (III) in these reagents is reduced by vitamin E to the corresponding Fe (II) complex, the absorbance of which is measured at 520 nm. (Esma *et al.,* 1997).

Table 2.4: Composition of tocopherols and tocotrienols in mg/100g of cold-pressed cranberry seed oil (Stone and Papas, 2003; Papas, 1999; Yang, 2003).

Tocopherols (mg/100g)			**Tocotrienols (mg/100g)**	
α	**γ**	**Total**	**γ**	**Total**
20	**10**	**30**	**150**	**150**

Recently the beneficial effects of minor components in vegetable and seed oils have become a subject of interest (Shahidi, 2000). Therefore studies have been conducted on these oils obtained by cold pressed methods for their PUFA and antioxidant properties (Van Hoed *et al.,* 2009). Since the oil obtained from the berry seeds by cold pressing does not have standard extraction parameters, the stability of oil against external factors like oxygen and light is a major concern. Therefore in the case of berry seed oils, cold pressing is carried out between 40° C and 60° C depending on the composition of the seeds (Van Hoed *et al.,* 2009). Solvent extraction by hexane was reported to yield maximum amount of tocopherol content in oil (Oomah *et al.,* 2000; Parry and Yu, 2004) but has the drawback of possible solvent residues in the oil. Hence cold

pressing extraction maintaining room temperatures seems to retain the natural antioxidants and provide nutritional quality, desirable flavor and oil stability (Van Hoed *et al.,* 2009).

2.2.3 Health properties of cranberry bioactive compounds:

Cranberries (*Vaccinium macrocarpon*) have been known as one of the earliest functional foods used, in the early processed food items such as pemmican (a dried meat and berry mixture) and sauces, principally used for medicinal purposes. Native New Englanders treated wounds and blood poisoning using cranberries in poultices and also for treating urinary disorders, diarrhea and diabetes. Cranberries were consumed by long ocean voyagers to remain unaffected from scurvy (Eck, 1990; Henig and Leahy, 2000). Today, cranberries are known to contain many biologically active components. Currently, between the University of Illinois NAPRALERT ^{SM} database (maintained by the program for collaborative research in the pharmaceutical sciences (PCRPS), college of pharmacy, University of Illinois at Chicago, 833 South wood street (M/C 877), Chicago, IL) and Dr. Duke's Phytochemical and ethnobotanical databases (available at http://www.ars-grin.gov/duke),120 compounds with over 700 biological activities have been reported for *Vaccinium* species in the literature. For instance there are 40 compounds with 130 effects associated with anticancer activity, 35 compounds with 108 effects associated with antioxidant activity and 25 compounds with 45 effects associated with anti-inflammatory activity. Only a few of these references are specific to *V.macrocarpon*, but this is probably due more to a lack of research with the species, than to a lack of biologically active compounds. It may be assumed that in the *Vaccinium* genus, biological activities exist such as bacterial anti-adhesion bioactivity due to the high content of proanthocyanidin and acid found.(Howell *et al.,* 1998; Shahidi and Weerasinghe, 2004).

Cranberry has been found to fight against bacterial infections such as urinary tract disorders, dental decay and stomach ulcers (Heinonen, 2007). Although berry phenolics are potent in vitro antioxidants, they exert in vivo biological activities beyond antioxidation and can have complementary and overlapping mechanisms of action (Seeram and Heber, 2007). In in-vitro experimental studies the berries in addition to possessing phenolics, antioxidant and free radical-scavenging activities, also exhibit metal chelation, antiproliferative, anticarcinogenic, antibacterial, anti-inflammatory, antiallergenic, and antiviral properties (Lee, 2000;Merken and Beecher, 2000a and 2000b; Nijveldt *et al.,* 2001; Higdon, 2007). This would explain how strong

the berries fight against infections, allergies, inflammation, hypertension and arthritis (Robards and Antolovich, 1997; Merken and Beecher, 2000a).

There are many reported additional health beneficial compounds in cranberries. Pectin is a source of digestible fiber, which has been reported to have anticancer and cholesterol lowering activity, and has been used as an important ingredient in the processing of cranberries into cranberry sauce, for its gelling property. Ellagic acid, a potential antioxidant, with anti-mutagen, anti-carcinogen and anti-viral activities has been reported in cranberry at 120 mg/kg, on a dry weight basis (Daniel *et al.,* 1989). Resveratrol, another antioxidant reported in red wine which is good for cardiovascular health is found in cranberries with concentrations similar to that of grape juice at 1.07 and 1.56 n mol/g, respectively (Wang *et al.,* 2002). Likewise, tocotrienols (Table 2.4) and omega-3 fatty acids have also been isolated and identified in cranberry seed oil and found to be exhibiting the same beneficial effect as ellagic acid and resveratrol (Nawar, 2000; Shahidi and Weerasinghe, 2004).

2.3 Cranberry seed oil extraction:

The lipids present in biological materials are held within tissues by various interactive forces like van der waals, electrostatic, hydrogen and covalent bonding (Shahidi and Wanasundara, 2002). Adequate energy input by chemical or physical means is needed to overcome these forces to separate/extract the lipids from the tightly held biological matrix. Whereas many oil extraction methods have been employed such as room temperature, solvent extraction, Soxhlet extraction, super- critical fluid extraction, ultra-sound assisted extraction, mechanical pressing, aqueous extraction, and so forth (Martinez and Maestri, 2008; Sheibani and Ghaziaskar, 2008; Lee and Lin, 2007; Luthria *et al*., 2007; Sanagi *et al*., 2005; Rostagno *et al*., 2004; Kwaku and Ohta, 1997), some of them are time consuming (Soxhlet extraction, ultra-sonic extraction), have low recovery (mechanical pressing), require large amounts of solvent (conventional solvent extraction), are expensive (super-critical extraction) or energy intensive (pressurized liquid extraction) (Martinez and Maestri, 2008; Grigonis *et al*., 2005; Huie, 2002). Oil expellers based on mechanical pressing are generally used for oilseeds having very high oil content above 20%. Expelling is used as the first step to reduce the oil content of these seeds which are then further extracted by solvent extraction as the final and finishing process of oil

extraction (Goss, 1946). For oilseed feedstocks with relatively low oil content less than 18-20%, direct solvent extraction is utilized for better oil recovery (Gunstone *et al.,* 1986; Bernardini, 1976).

Nowadays cranberry seed oil has become of general interest to the food industry as it contains a well balanced fatty acid profile along with plant phytochemicals of interest for their dietary, technical and health properties. Furthermore, with the increased production of cranberries, locally and worldwide, and the significant proportion of the cranberries being processed, there is a large production of cranberry seed by-products from which oil can be extracted. Such oils are usually only available in limited quantities and, if they are to be marketed, it is essential to ensure that the sources located will provide a reliable and adequate supply of good quality material. If the oils are to be used as dietary supplements, as health foods, as gourmet oils, or in the cosmetics industry, it is important that the seeds be handled, transported, and stored under conditions that maintain quality. Many fruits are now processed at centralised facilities. This means that larger quantities of "waste products" are available at one centre and can be more easily treated to recover oil and other valuable by-products. This is particularly relevant in the fruit industry where pips, stones, and kernels are available in large quantities. (Gunstone and Harwood, 2007).

For cranberry seed oil, the fatty acid composition has been reported and information about the minor components (tocopherols and tocotrienols) is available (Tables 2.3 and 2.4). Based on this, claims are frequently made for the superior properties of cranberry oil. These may be valid, but there are few, if any, where tests have been carried out to support the claims. Most vegetable oils contain only three fatty acids at levels exceeding 10% [palmitic (16:0), oleic (18:1), and linoleic (18:2)] and these three frequently having a combined level of 90% or more. This means that other acids, such as Δ-9-hexadecenoic, stearic, or linolenic acid are generally present at low levels, if at all. The many edible oils can be subdivided into those in which oleic acid dominates, those in which linoleic dominates, and those in which these two acids are present at similarly high levels. Palmitic acid, though always present, is seldom the dominant component. Beyond these, however, are some oils with less-common acids, sometimes at a quite high concentration (Gunstone and Harwood, 2007). These speciality oils are sold on the basis of their particular fatty acid profile and their specific content in phytochemicals. In the case of

cranberry seed oil, it is marketed for its balance of omega-9, omega-6 and omega-3 acids and its high content of tocopherols and tocotrienols.

Extraction can be carried out by cold pressing at temperatures not exceeding 45°C, pressing at higher temperatures, and/or solvent extraction. Solvent extraction is often not favoured for berry oils as they are often marketed as natural/speciality products. Supercritical fluid extraction with carbon dioxide is an acceptable possibility, but only limited use is made of this method due to the high initial cost of the equipment and operating cost. A further possibility is to use enzymes to break down cell walls followed by extraction under the mildest possible conditions. Some specialty oils, such as walnut, virgin olive, hazelnut, and pistachio, can be used as expressed, merely after limited filtering, but for others some further refining is necessary. If the oil has a characteristic flavour of its own, it may be desirable to retain this, and high-temperature deodorisation must then be excluded or operated at the lowest possible temperature. Once obtained in its final form, the oil must be protected from deterioration particularly from oxidation. This necessitates the use of stainless steel equipment, blanketing with nitrogen, and avoiding unnecessary exposure to heat, air and light. Depending on the market application and customer acceptance, a natural and/or synthetic antioxidant can be added to provide further protection (Gunstone and Harwood, 2007). But in the case of cranberry seed oil the innate longevity of the oil is one of its unique characteristic feature as it does not deteriorate easily (Nawar, 2004).

2.3.1 Solvent extraction of oil:

Solvent extraction consists of liberating plant compounds, including oil, into a recipient solvent. The choice of the solvent type depends on the solubility of the solute in the solvent and its capacity to drive the extraction process and ensure maximum yield. It has been demonstrated that solvent extraction has the advantage of attaining much lower residual content of oil in the solid matrix as compared to pressing alone. Solvent extraction is the most efficient method for extracting oil from plant matrices from the standpoint of oil recovery (Goss, 1946) and has been widely employed for extraction of oil from common oilseeds. Grinding, cooking, pressing or flaking are the preliminary operations performed prior to solvent extraction to rupture the oil bearing cells, making them readily available during solvent extraction. During solvent extraction, two processes are responsible for removing oil from the seeds. In the first step, which is

relatively faster, oil extraction occurs mainly by diffusion. During the contact between oilseed and solvent, the solvent diffuses through the seed mass and extracts the readily available oil from the ruptured cells. Whereas, in the second process, removal of the residual amount of oil occurs by osmosis or leaching which is time consuming (Bernardini, 1976; Goss, 1946).

2.3.2 Microwave assisted extraction of oil:

Microwaves are electromagnetic radiations with a frequency from 0.3 to 300 GHz (Camel, 2001) (Fig 2.4). In order to avoid interferences with radio communications, domestic and industrial applications, microwave heating generally operate at 2.45 GHz. Owing to their electromagnetic nature, microwaves possess electric and magnetic fields which are perpendicular to each other. The electric field causes heating via two simultaneous mechanisms, namely, dipolar rotation and ionic conduction (Thuery, 1992; Demesmey and Olle, 1993; Sinquin *et al*., 1993). Dipolar rotation is due to the alignment with the electric field of the molecules possessing a dipole moment (either permanent or induced by the electric field) in both the solvent and the solid sample. This oscillation produces collisions with surrounding molecules and thus the liberation of thermal energy into the medium. Microwave-assisted extraction (MAE) is broadly used to extract valuable components from medicinal herbs, vegetables and plants (Bayramoglu *et al.,* 2008; Cravotto and Boffa, 2008; Du and Zhou, 2006; Mahesar *et al.,* 2008; Nemes and Orsat, 2011). It has proved to be effective, high yielding and energy saving (Pan *et al.,* 2003; Shu *et al.,* 2003).

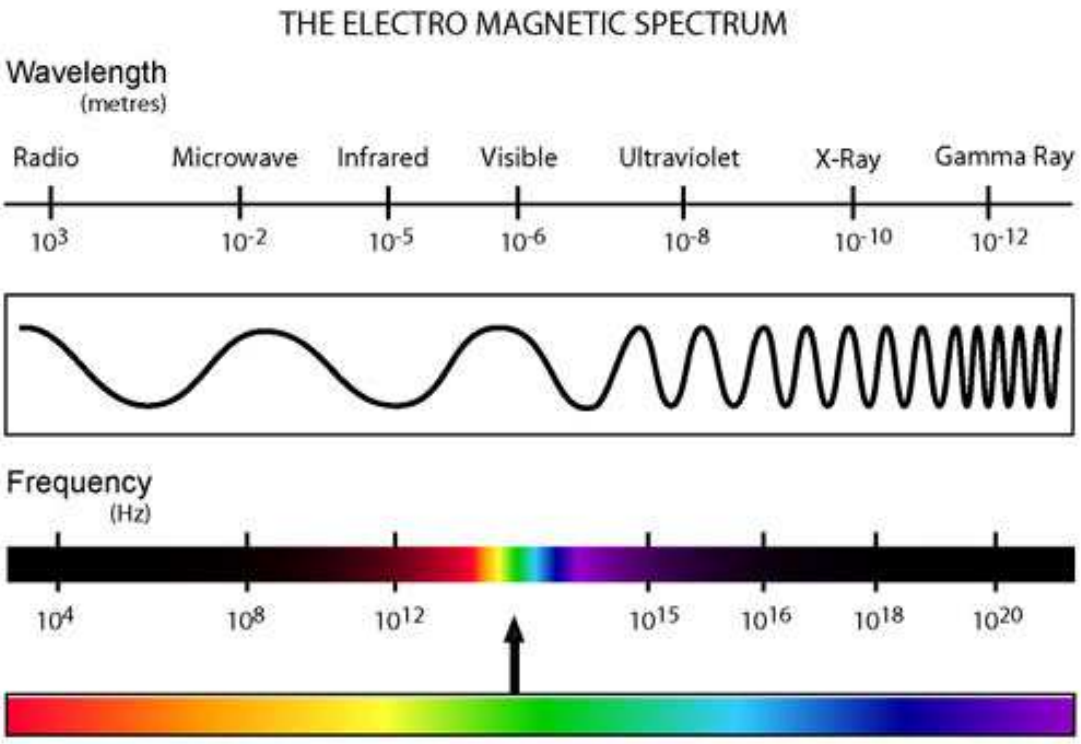

Fig 2.4: The Electromagnetic spectrum (Thomas Publishing Company 2012).

Consequently, unlike classical conductive heating methods, microwaves heat the whole sample simultaneously (assuming of course that the whole sample has the right combinations of dielectric properties to absorb the microwave energy and dissipate it as heat). In the case of extraction, the advantage of microwave heating is the disruption of weak hydrogen bounds promoted by the dipole rotation of the molecules. A higher viscosity of the medium lowers this mechanism by affecting molecular rotation (Sinquin *et al*., 1993; Camel and Bermond, 1999). Furthermore, the migration of dissolved ions increases solvent penetration into the matrix and thus facilitates the solvation of the analyte (Ganzler *et al*., 1990). Ionic currents are also induced in the solution by the electric field. As the medium resists these currents, friction occurs and heat is liberated by the Joule effect. This phenomenon depends on the size and charge of the ions present in the solution. The effect of microwave energy is strongly dependent on the nature of both the solvent and the solid matrix. Solvents generally used cover a wide range of polarities, from heptane to water. Most of the time, the chosen solvent possesses a high dielectric constant and strongly absorbs microwave energy (such as water), however, the extracting selectivity and the ability of the medium to interact with microwaves can be modulated by using mixtures of

solvents (Renoe, 1994). In some cases, the matrix itself interacts with microwaves while the surrounding solvent possesses a low dielectric constant and thus remains colder as is the case with hexane (Jassie *et al*., 1997). This latter situation presents some obvious advantages in the case of thermosensitive compounds and has been successfully used for the extraction of essential oils (Paré, 1990 and, 1991; Chen and Spiro, 1994; Romele and Polesello, 1997). When using a microwave transparent solvent, the microwaves interact selectively with the polar molecules present in the glands, trichomes or vascular tissues of the plant material. Localised heating leads to the expansion and rupture of cell walls and is followed by the liberation of essential oils and speciality compounds into the solvent (Paré and Bélanger, 1994; Jassie *et al*., 1997; Majors, 1998). This situation can also be obtained when a dry sample has been re-hydrated before extraction (Paré, 1990; Paré and Bélanger, 1994; Majors, 1998). In fact, moisture content is essential in MAE because water locally superheats and promotes the liberation of analytes into the surrounding medium (Onuska and Terry, 1993; Budzinski *et al*., 1996; Jassie *et al*., 1997; Kaufmann and Christen, 2002).

Recently, microwave assisted extraction has been gaining in popularity for the extraction of a variety of compounds due to the environmentally friendly and economical traits of the technique (Mahesar *et al*., 2008; Pan *et al*., 2002). A general extraction method for biological matter using microwave energy and a microwave-transparent extractant was patented by Paré, (1991). In general, with microwave assisted extraction (MAE), rapid generation of heat and pressure within the biological system forces out compounds from the biological matrix, producing good quality extracts with better target compound recovery (Hemwimon *et al.,* 2007). The microwaves also cause efficient internal (volumetric) heating of the target material by interacting with the matrix at the molecular level. This interaction, thus leads to volumetric temperature rise of the entire mixture as opposed to with conventional heating where only the mixture near the walls of the vessel effectively gets heated (Kappe *et al*., 2009). The rapid heating leads to localized high temperature and pressure gradients which assist in cellular wall degradation and enhanced mass transfer rates. Additionally, enhanced bioactivity of certain compounds can be obtained (i.e. antioxidant activity of anthraquinones obtained from *Morinda citrifolia*) using MAE when compared to ultra-sound assisted and maceration extraction processes (Hemwimon *et al*., 2007). The efficiency of the MAE process depends on time,

temperature, solid-liquid ratio, type and composition of solvent used (Hemwimon *et al*., 2007; Grigonis *et al*., 2005; Kwon *et al*., 2003; Pan *et al*., 2002; Paré and Bélanger, 1994).

Selection of appropriate solvent influences the extraction yield during MAE process, as the solvent acts as a conduit for energy coupling, mass transfer and exerting pressure on the biological matrix (Rostango *et al*., 2007). The polarity of the solvent and the target compound to be extracted from the plant material is critical for maximum yield. For instance, using polar solvents to extract polar compounds will result in maximum recovery of the compounds from the substrate. While using relatively non-polar solvents like hexane and toluene during MAE reduces the microwave energy transfer, and sometimes the addition of a certain percentage of water (about 10%) can help in improving the extraction yields (Wang and Weller, 2006).

Microwave technology has also been used for extraction of essential oils. Essential oils are a class of volatile aromatic oils obtained by extraction from plant material. These oils are rich in volatile fractions that contain monoterpenes, sesquiterpenes and oxygenated derivatives. These compounds are degraded by action of heat, light and oxygen due to their higher degree of unsaturation. By employing microwave extraction for extracting these sensitive oils, the thermolytic and hydrolytic effects of some of the conventional methods (steam distillation, hydrodistillation) are diminished. Several researchers have shown that microwave assisted extraction of these oils offers possibility for better reproduction of the natural aroma of the essential oil as compared to other methods (Ferhat and Meklati, 2007). MAE of essential oils has been patented by Paré (1991). For this developed process, the samples were suspended in hexane and the microwaves concentrated on the inner glandular and vascular systems of the plant material. Owing to the high moisture content of these structures they were heated almost specifically and this promoted disruption of cell membranes releasing the analytes into the solvent. The same author also patented the extraction of volatiles from biological material (Paré, 1990); the method involved the exposure of oil-containing cellular matter or glandular systems to microwaves and the dissolution of essential oil in a suitable organic solvent. Another patent has been deposited by Paré (1994) concerning the extraction of volatile oils from plants and biological material. This procedure was applied by Chen and Spiro (1994) for the extraction of essential oils from rosemary and peppermint leaves suspended in hexane, ethanol or mixtures of the two solvents. Scanning electron micrographs of the leaves showed structural changes in the

oil-containing glands after microwave extraction: some of them were found to be collapsed and others completely disintegrated. Two distinct extraction mechanisms are plausible, one involving diffusion of the essential oil across the gland wall and the other involving rupture of the gland and liberation of the constituents into the solvent. Either of the two phenomena may be prominent according to the different maturation stages of the glands. MAE has also been compared to classical hydrodistillation for the extraction of essential oils from 10 different plant species using a domestic microwave oven (Collin *et al*., 1991). The yields were generally similar, but the chromatographic profiles varied dramatically, especially with respect to the ratios between the different substances. So far MAE has not been used for extracting the oil from berry seeds. Also, no comparative study on the effect of microwave on the extraction efficiency from the berry seeds under different operating conditions was found in the literature.

2.3.3 Ultrasound assisted solvent extraction of oil:

Another method of extraction, known as *sonication-assisted* or ultrasound-assisted solvent extraction. Based on the level of intensity, ultrasonic treatment could be of two types; high intensity (also called *power ultrasound*) or low intensity. The former makes use of low frequencies (20-100 kHz) and the latter employs much higher frequencies (2-10 MHz). Both techniques have been utilized for a variety of applications in the food processing industry and *high intensity* ultrasound has been proven to be the preferred method for cell disruption and extraction (Piyasena *et al.,* 2003; Zenker *et al.,* 2003; Knorr *et al.,* 2004; Condón and Raso, 2005; Feng *et al.,* 2009; Demirdöven and Baysal, 2009).

The basic principle behind the use of ultrasonic waves is *elastic deformation* in *piezoelectric* materials as a result of the application of a high frequency (50/60 Hz) electric field. The deformation in the piezoelectric *transducer* is then converted to mechanical vibrations and amplified before being transmitted to a resonating *probe* or *sonotrode* which is in contact with the processing medium (Raichel, 2006). Sound waves propagate through a medium by alternating *compression* and *expansion* (or *rarefaction*) cycles longitudinally and/or transversely; hence they are in effect mechanical waves of alternating high and low pressure (from an equilibrium pressure) respectively. These vibrations (or oscillations) have a mechanical effect on the molecules or particles in the medium. When ultrasonic waves pass through a liquid medium,

in the expansion cycle, the pressure becomes so low that the intermolecular forces (that keep the molecules of the medium together) are overcome and small gas-filled bubbles or *cavities* are created. During the consecutive compression cycle, these cavities contract, followed by expansion in the next cycle and so on. In course of time, the cavities expand to their limit imploding on themselves (Fig 2.5). This implosion is called cavitation and is the chief mechanism behind the mechanical effect of ultrasonic waves (Suslick, 1990). Low intensity ultrasound causes stable cavitation wherein the stable cavities produced oscillate and remain in the medium for extended cycles or time periods. High intensity ultrasound creates cavities that continue to increase in size over consecutive expansion cycles, eventually imploding after just a few cycles (typically over a few microseconds); this is called transient cavitation. The implosion creates a hot spot in the immediate vicinity, where high temperature, high shear forces and free radicals work together to bring about the cell disruption of biological cells in the medium. This kind of cavitation has a rapid mechanical or shear effect (as a result of temperature and pressure increase) on the molecules in its immediate vicinity. This exciting phenomenon has been very well-studied and has been manipulated to serve a great variety of purposes (Suslick, 1988; Suslick, 1990; Condón and Raso, 2005; Feng *et al.,* 2009). For example, if the medium contains biological material, which are made up of cells, this mechanical effect is so efficient that the cell membranes are ruptured leading to the transfer of the intra-cellular material into the medium. When the medium is made up of solvents (i.e. liquids with specific affinities for dissolving specific compounds or molecules only), the resulting selective dissolution process becomes the principle for ultrasound-assisted solvent extraction (Wang and Weller, 2006).

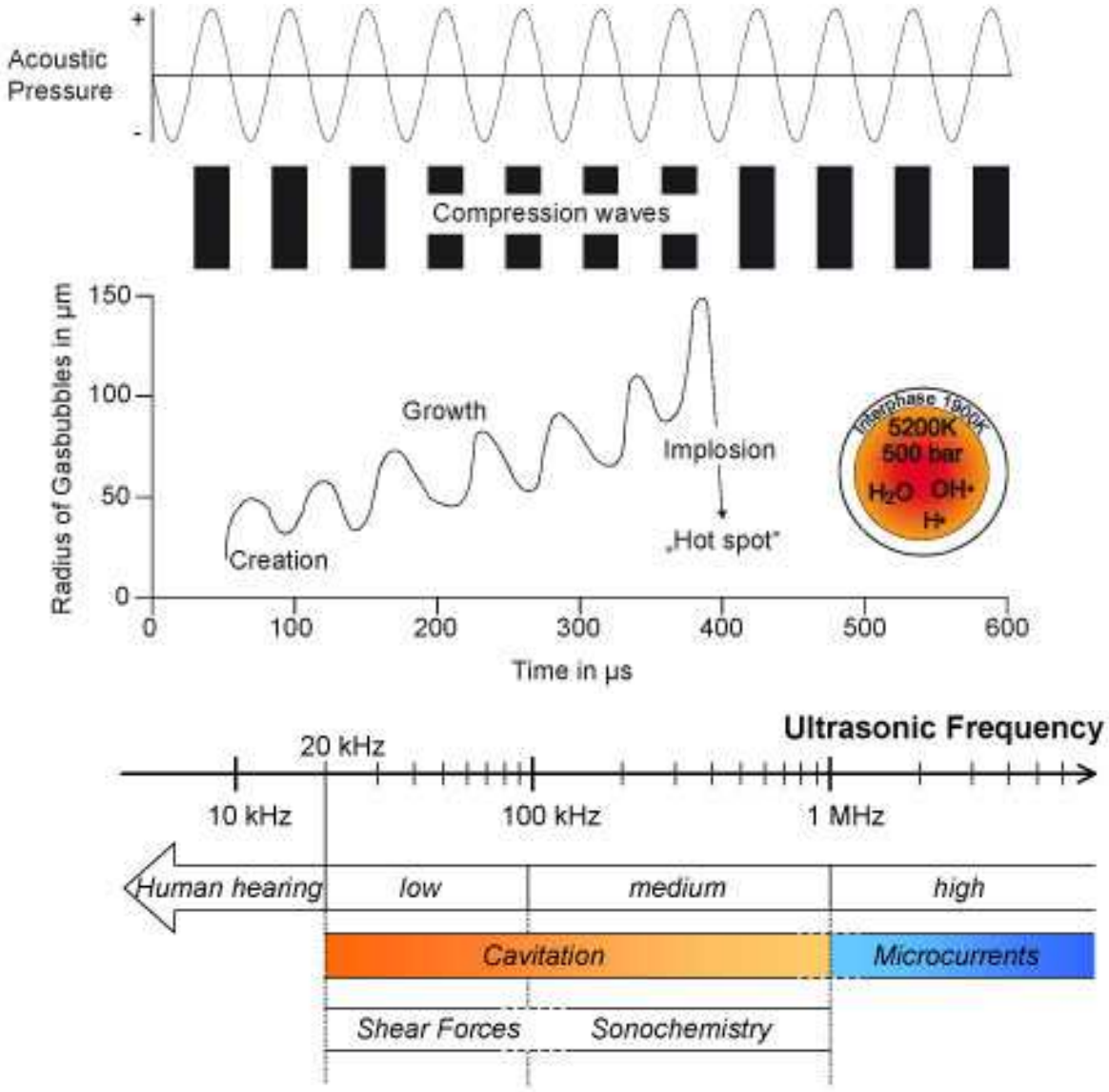

Fig 2.5: Principles of ultrasonic cavitation and cell disruption. (Sonotronic Nagel GmbH, 2011).

Of the many factors that determine the power output of an ultrasonic probe, resonance amplitude and ultrasonic wave intensity (UWI) are the most important. Resonance (or vibrational) amplitude is the vertical (up-and-down) distance (measured in microns) travelled by the probe when it vibrates. Ultrasonic wave intensity (UWI) or power intensity, on the other hand, is the amount of power transmitted to the medium (in watts), per unit cross-sectional area of the ultrasound probe. Although both variables have been used to characterize ultrasonic treatments in scientific literature, resonance amplitude has been found to be a more exact and stable parameter than power output for estimating cavitational cell disruption, especially in

microbial cells. Factors that affect the ultrasonic power output include frequency, resonance amplitude, hydrostatic pressure and temperature of the processing medium (Tsukamoto *et al.,* 2004; Bermúdez-Aguirre *et al.,* 2011). Ultrasonic technology has been applied widely in the extraction of components from many materials. The application of ultrasound techniques in solvent extraction has attracted increasing attention. It has been widely applied in extracting oil, pigment, proteins and flavonoids from plant material (Han *et al.,* 2004; Li *et al.,* 2003; Li *et al.,* 2004a, b; Shi *et al.,* 2004). Research showed that this technology has great efficiency, uses less energy and does not destroy the effective component of the materials. Romdhane and Gourdon (2002) investigated extraction of pyrethrines from pyrethrum flowers and oil from woad seeds. In both cases, acceleration of extraction kinetics and increase in yield was observed, however less so in the case of woad seeds. Vinatoru *et al.,* (1997) showed improved yields of lipophilic compounds extracted from herbs such as coriander and fennel. So far, UAE has not been used for extracting oil from berry seeds. Also, no comparative study on the effect of ultrasound on the extraction efficiency from the berry seeds under different operating conditions was found in the literature. But this technique has already been applied for extracting the oil from some other seeds such as fennel (*Foeniculum vulgare* L.), peganum (*Peganum harmala* L.)(Toma *et al.,* 2001), woad (*Isatis tinctoria* L.)(Romdhane and Gourdon, 2002) rose hip (*Rosa canina* L.) (Szentmihalyi *et al.,* 2002) as well as sunflower (*Helianthus annuus* L.), soybean (*Glycine max* L.) and rape (*Brassica napus* L.) (Luque-Garcia and Luque de Castro, 2004). In these studies ground seeds were used, except in the case of peganum (Toma *et al.,* 2001), although powerful sonication could itself serve to crush the plant material (Vinatoru, 2001). The strong mechanical effect of a 20 kHz ultrasonic vibration during extraction, leading to an enhanced milling process, was demonstrated in the case of marigold (*Calendula officinalis* L.) leaves (Luque-Garcia and Luque de Castro, 2004). However, ultrasound greatly affected the eugenol extraction only from the milled clove (*Syzygium aromaticum* L.) flowers (Vinatoru, 2001). n-Hexane was used to extract oil (Toma *et al.,* 2001; Romdhane and Gourdon, 2002; Szentmihalyi *et al.,* 2002; Luque-Garcia and Luque de Castro, 2004) from a variety of seeds with the help of ultrasonic energy. In the reported studies, the extraction efficiency of ultrasound-assisted solvent extraction (SE) of oil from oleaginous seeds was equal or better than that of the conventional SE but with a drastic reduction of the extraction time (Luque-Garcia and Luque de Castro, 2004).

The extraction efficiency of bioactive substances from plants and seeds has been found to be significantly improved by the utilization of ultrasound waves (Cravotto and Boffa, 2008). Sonication has been used successfully to isolate pharmaceutically active components from *Salvia officinalis* and has also been used to increase the yield of xylem from corn hulls (Ebringerova and Hromadova, 1997). Cravotto and Boffa (2008), reported that in the extraction of soybean-germ oil, the Soxhlet method gave a yield of only 8.6%, while using ultrasonic extraction (UE) resulted in a yield of 17.7%. Furthermore, UE has been recognized for its application in the vegetable oil industry to reduce the extraction time and thus improve production efficiency (Babaei *et al.,* 2006).

Based on the review of the prior art in the presented studies, it can be hypothesized that application of novel methods like MAE and UAE may improve extraction of oil from cranberry seeds. The objective of this study was thus to test this hypothesis by determining the influence of microwave and ultrasonic power and intensity on the efficiency of oil extraction from cranberry seeds as compared to reflux heating extraction.

2.4 Conclusions:

Cranberry seed oil has become of general interest to the food industry for its particular fatty acid profile along with its content in other components (phyto-compounds) that give the oil interesting dietary, technical and health functional properties. If these oils are to be used as cosmetic ingredients, as dietary supplements, as health or speciality foods, it is important that the seeds be handled, transported, stored and processed adequately as to maintain their quality. With the growth of the cranberry industry worldwide, there are larger quantities of “waste products”, principally solid cakes and seeds, which are available following the principal cranberry processing into juice and dried products. This availability should be addressed and the waste products used to recover the seed oil and other valuable by-products.

CHAPTER III

MATERIALS AND METHODS

3.1 Materials and reagents:

Cranberry seeds (*Vaccinium macrocarpon*) were obtained from *Fruit d'or*, a company in Notre-Dame-de-Lourdes, Quebec, Canada. This variety of berries is widely grown in both Canada and the USA and particularly in the province of Quebec. The solvents and chemicals such as hexane, ethanol, α - tocopherol, xylene, 2,2-dipyridyl and ferric chloride that were used were all of HPLC grade obtained from Fisher Scientific and Sigma-Aldrich, Canada.

3.2 Determination of moisture content:

The moisture content in cranberry seeds was determined by the oven method. A known weight of seeds (5 ± 0.05 g) was kept in a hot air oven for 24 hr at 107°C for drying. The experiments were done in triplicates and the final weight of the sample was determined.

The wet basis moisture content was calculated by:

$$\frac{Initial\ mass \quad - \quad Final\ mass}{Initial\ mass} \times 100 \qquad (3.1)$$

3.3 Sample preparation:

Cranberry seeds of different mass (g ±0.01 g) were weighed using an electronic balance (Model APX-200, USA) and were powdered using a domestic blender (Model BA-880, China) for 2 min, then made to pass through a standard 1 mm sieve, thus ensuring uniformity of particle size in the sample.

3.4 Preliminary experiments:

Preliminary trial experiments were carried out in triplicates for reflux method using whole seeds and powdered seeds in order to explore the oil yield and the necessity for a grinding pretreatment to ensure cellular breakdown for the oil release. The tested extraction parameters were sample/solvent ratios ranging between 2.5 to 10 g of cranberry seeds/100ml hexane, over

the range of temperature of 62-70°C and time of 3 to 12 hrs. Results were found to be not impressive in case of whole seeds in terms of the oil yields (8.17 ± 0.3%) as they were comparatively less than for powdered seeds (10.11 ± 0.2%). This is due to the fact that the powdered seeds have more interaction with solvents as the cells have already been ruptured which facilitated the oil release. This leads to the conclusion that a grinding step is required in the preparation of the seeds for subsequent effective oil extraction. Another set of experiments was carried out in triplicates to roughly study the choice of solvent. In this experiment, pure hexane was compared with a combination of both hexane and ethanol (in a 7:3 ratio) and the combination solvent had a significant reduction in obtained oil yields (6.45 ± 0.1%) when compared with the yield obtained using hexane alone as the solvent which gave higher yields (10.2 ± 0.2%).This preliminary experiment concluded that the extraction efficiency of hexane was better compared with a combination of both hexane and ethanol leading to an experimental design limited to hexane as the solvent.

3.5 Heat reflux method:

Heat reflux method was performed as a conventional method for comparing the oil yield results with the microwave and ultrasound assisted extraction methods. The heat reflux experimental setup was placed in a controlled temperature water bath (Lab line Instruments, Inc.,) set at various temperatures from 62° to 70° C. A 500 ml conical flask was securely attached to a stand and connected to a fitted condenser to capture the solvents. Solvent was composed of pure hexane. 100 ml of the solvent was added for all experiments and the reflux extraction was performed separately with different sample ratios (2.5g ± 0.03 to 10g ± 0.03 /100ml of solvent) of powdered cranberry seeds for different time periods from 3 to 12 hrs. The extracted oil thus obtained was separated from the solid sample using vacuum filtration with (55mm Whatman filter paper). To ensure complete separation of oil from the solid sample, it was further rinsed with hexane of HPLC grade. The oil was then separated from the solvents using a Rotavapor (BUCHI R-205) fitted with a controlled temperature water bath (BUCHI B-490). The percentage of oil yield was determined gravimetrically as:

$$\text{Oil Yield (\%)} = (m_0/m_s)*100 \qquad (3.2)$$

Where m_s is the mass of ground cranberry seeds (g) and m_0 the mass of extracted oil (g). A central composite design was formulated for the fixed parameters and the oil yield obtained were used to generate a response surface model using JMP software by SAS.

3.6 Microwave assisted extraction:

Based on the results obtained from preliminary studies (poor results on using a 7:3 hexane to ethanol ratio) and the literature review (which reports mostly the use of hexane in oil extraction for best results), hexane was selected for extracting oil from cranberry seeds in microwave assisted extraction. Powdered cranberry seeds (2.5g ± 0.03 to 10g ± 0.03 /30ml of hexane) were placed in a 250 ml quartz vessel. The volume of hexane was limited to thirty milliliters for extraction due to the smaller size of the reaction vessel (250 ml). The quartz vessel was introduced into the microwave cavity and fitted with a condenser. A focused type, open-vessel microwave system (Star system 2, CEM Matthews, USA) operating at 800 W maximum power with a frequency of 2,450 MHz was used. The microwave power applied was in the range between 100-225 W for 5 to 20 min. The power levels used for the experiments were expressed as a percentage of the power supplied within the microwave cavity as per the cavity's calibration. The maximum microwave output power in the cavity was calibrated using the protocol developed by Cheng *et al*., (2006). The regression equation (Eq. 3.3) obtained from the calibration was used to convert % power level into applied Watts.

Watts = (% Power level *8.27) – 19.2 (3.3)

The mode of microwave power applied was intermittent with power on for $30s\ min^{-1}$.

The extracted oil thus obtained was separated from the sample using vacuum filtration with (55mm Whatman filter paper). To ensure complete separation of the oil from the seed sample, the sample was further rinsed with hexane of HPLC grade. Finally, the oil was separated from the solvents using a Rotavapor (BUCHI R-205) fitted to a controlled temperature water bath (BUCHI B-490). A central composite design was formulated for the fixed extraction parameters and the oil yield obtained were used to generate a response surface model using JMP software from SAS.

3.7 Ultrasound assisted extraction:

Based on the results obtained from preliminary studies (poor results on using hexane: ethanol-7:3 ratio) and the literature review (which reports the use of hexane in oil extraction for best results) hexane was also selected for extracting oil from cranberry seeds in ultrasound assisted extraction. Powdered cranberry seeds (2.5g ± 0.03 to 10g ± 0.03 /30ml of hexane) were used in this extraction method. A high intensity ultrasonic processor (Vibra-Cell TM VCX-500, Sonics & Materials, CT, USA) equipped with an ultrasonic horn transducer and a tuned titanium alloy probe (or horn) resonating at a frequency of 20 kHz and maximum amplitude of 124 μm was employed. The processor was designed to maintain constant amplitude by continuously adjusting the power (or energy) output which ranges from 100 to 200 W for 5 to 30 min from the probe in response to the load (suspension). The ultrasonic processor used in the current study was designed to automatically adjust the power output to the probe load (suspension) so as to deliver constant resonance amplitude. The oil thus obtained was separated from the sample using vacuum filtration with (55mm Whatman filter paper). To ensure complete separation of oil from the sample, it was rinsed with hexane of HPLC grade. The oil was then separated from the solvents using a Rotavapor (BUCHI R-205) fitted to a temperature controlled water bath (BUCHI B-490). A central composite design was formulated for the fixed extraction parameters and the oil yield obtained were used to generate a response surface model using JMP software from SAS.

3.8 Experimental design:

Face centered central composite design (CCD) was used to determine the optimal conditions of operation for heat-reflux, MAE and UAE and their effect on oil yield and oil quality from powdered cranberry seeds. A central composite design with uniform precision is highly efficient and provides sufficient information on the effects of process variables for resourceful optimization with a reduced number of total experimental runs. The three independent factors studied in the case of heat reflux were the extraction time, X_1 (3, 7.5 and 12 hrs), temperature, X_2 (62, 66 and 70°C) and sample ratio, X_3 (2.5, 6.25 and 10g/100ml of hexane). In microwave extraction, the factors were the extraction time, X_1 (10, 12.5 and 15 min), microwave power, X_2 (100, 112.5 and 125 W) and sample ratio, X_3 (3, 4 and 5g/30ml of hexane). For ultrasound extraction, the factors were the extraction time, X_1 (10, 12.5 and 15 min), ultrasonic

power, X_2 (100, 125 and 150 W) and sample ratio, X_3 (3, 4 and 5g/30ml of hexane). The oil from cranberry seeds showed good results on the above factors only. Changing values in these factors does not significantly change the yield of oil. Therefore inorder to obtain good quality of oil without the loss of phytonutrients these values were maintained throughout the experiments. These were coded at three levels as shown in Tables 3.1 to 3.6.

Table 3.1: Independent variables in CCRD for optimization of oil yield from powdered cranberry seeds through heat reflux

Independent variables	Coded levels		
	-1	**0**	**1**
Extraction time (hr) X_1	3	7.5	12
Temperature (°C) X_2	62	66	70
Sample ratio (g/100ml of hexane) X_3	2.5	6.25	10

Table 3.2: Face-centered central composite design (CCD) followed to study the oil yield from heat reflux extraction of cranberry seeds

Pattern	Time(hr)	Temperature (°C)	Ratio (g/100ml of hexane)
+-+	720	62	10
+--	720	62	2.5
00A	450	66	10
+++	720	70	10
000	450	66	6.25
A00	720	66	6.25
000	450	66	6.25
000	450	66	6.25
000	450	66	6.25
0a0	450	62	6.25
++-	720	70	2.5
-++	180	70	10
000	450	66	6.25
---	180	62	2.5
000	450	66	6.25
-+-	180	70	2.5
00a	450	66	2.5
a00	180	66	6.25
--+	180	62	10
0A0	450	70	6.25

Table 3.3: Independent variables in CCRD for optimization of oil yield from powdered cranberry seeds through microwave assisted extraction

Independent variables	Coded levels		
	-1	0	1
Extraction time (min)X_1	10	12.5	15
Microwave power (W) X_2	100	112.5	125
Sample ratio (g/30ml of hexane) X_3	3	4	5

Table 3.4: Face-centered central composite design (CCD) to study the oil yield from microwave extraction of cranberry seeds

Pattern	Time (min)	Power (W)	Ratio (g/30ml of hexane)
000	12.5	112.5	4
A00	15	112.5	4
000	12.5	112.5	4
-+-	10	125	3
000	12.5	112.5	4
-++	10	125	5
+--	15	100	3
000	12.5	112.5	4
--+	10	100	5
000	12.5	112.5	4
00a	12.5	112.5	3
00A	12.5	112.5	5
a00	10	112.5	4
+-+	15	100	5
+++	15	125	5
---	10	100	3
++-	15	125	3
0A0	12.5	125	4
0a0	12.5	100	4
000	12.5	112.5	4

Table 3.5: Independent variables in CCRD for optimization of oil yield from powdered cranberry seeds through ultrasound assisted extraction

Independent variables	Coded levels		
	-1	0	1
Extraction time (min)X_1	10	12.5	15
Ultrasonic power (W) X_2	100	125	150
Sample ratio (g/30ml of hexane) X_3	3	4	5

Table 3.6: Face-centered central composite design (CCD) to study the oil yield from ultrasound extraction of cranberry seeds

Pattern	Time (min)	Power (W)	Ratio (g/30ml of hexane)
0A0	12.5	150	4
-+-	10	150	3
+-+	15	100	5
000	12.5	125	4
--+	10	100	5
++-	15	150	3
000	12.5	125	4
0a0	12.5	100	4
000	12.5	125	4
000	12.5	125	4
-++	10	150	5
00A	12.5	125	5
+++	15	150	5
A00	15	125	4
+--	15	100	3
000	12.5	125	4
---	10	100	3
000	12.5	125	4
00a	12.5	125	3
a00	10	125	4

The CCD included eight factorial points, six axial points and six center points, which totals 20 experimental runs, and was used to fit the second-order polynomial model similar to that shown in Eq. 3.4 (Haaland, 1989; Abdeshahian *et al*., 2010).

$$Y = \beta_0 + \sum_{i=1}^{3} \beta_i x_i + \sum_{i=1}^{3} \beta_{ii} x_i^2 + \sum_{i<j=1}^{3} \sum \beta_{ij} x_i x_j \quad (3.4)$$

Where Y is the predicted response, $_0$ is the regression coefficient for the intercept (a constant), $_i$is the coefficient for the linear effect, $_{ii}$ is the coefficient for the quadratic effect, $_{ij}$ is the coefficient for the interaction effect, of variables i and j, x_i and x_j are independent variables. The JMP software Version 8 (SAS Institute Inc., Cary, NC, USA) was used for the statistical analysis and to generate the response surface plots for the quadratic models.

3.9 Determination of alpha-tocopherols in cranberry seed oil:

α-tocopherol was estimated in the cranberry oil by the Emmerie-Engel (1938) reaction as reported by Rosenberg (1992) and results were obtained for the oil yields from the different extraction methods. The Emmerie -Engel reaction is based on the reduction of ferric to ferrous ions by tocopherols, which, with 2, 2'-dipyridyl, forms a red colour. Tocopherols are first extracted with xylene and read with a spectrophotometer at 520nm following addition of ferric chloride. Oil extracted from powdered cranberry seeds following different treatment combinations with Reflux, MAE and UAE was tested for the determination of the α - tocopherol content.

3.9.1 Reagents and materials:

Pure α-tocopherol solution purchased from Sigma Aldrich was used as a standard. The other reagents such as Xylene, 2,2-dipyridyl and the ferric chloride solution were also purchased from Sigma-Aldrich. A 20-200µl micropipette of Nichipet EX (Japan) was used for adding samples, standards and reagents. A centrifuge (IEC, Spinette, USA) was used for the centrifugation of the samples. Disposable plastic cuvettes (Fisher Scientific, Canada) were used in this experiment for the spectrophotometric analysis.

3.9.2 Standard and sample preparation:

The standard obtained from Sigma-Aldrich contained 100mg of α- tocopherol. Dilution was carried out by accurately weighing 1 mg of α- tocopherol dissolved in 1000 ml of ethanol. Aliquots of α - tocopherol (0.12, 0.16, 0.25 and 0.5 ml) were taken having the corresponding concentrations of (0.1 to 0.5 µg). Similarly 0.6 g of 2, 2-dipyridyl and ferric chloride were taken and dissolved in 500ml of 2-propanol and ethanol respectively. For the oil samples about 0.2 ml was used for all the tocopherol determination from heat reflux, MAE and UAE oils.

3.9.3 Procedure:

A Biochrom Ultrospec 1000 model spectrophotometer was used. Aliquots (0.12, 0.16, 0.25, and 0.50 ml) of a 1 mg/l solution of standard α- tocopherol in ethanol were transferred in a cuvette. To each of these solutions 0.3 ml of 2, 2-dipyridyl reagent were added into the cuvette and mixed. 0.20 ml of ferric chloride reagent was also added and mixed well. The absorbance of the final mixture was read at 520 nm to establish its optical density (OD). Then, the standard curve was drawn (Fig. 3.1). The above-described procedure was followed using 0.2 ml of oil sample solutions obtained by the three tested extraction methods. To this, 0.4 ml of xylene and 0.4 ml of ethanol were added and kept for centrifugation at 3300 rpm for 3 min. The upper xylene layer was transferred into another tube followed by addition of 0.3 ml of 2, 2-dipyridyl and 0.2 ml of ferric chloride reagent. The mixture was well mixed and transferred to a cuvette to be read at 520 nm. The α - tocopherol content in the extracts was calculated from the regression equation of the standard curve.

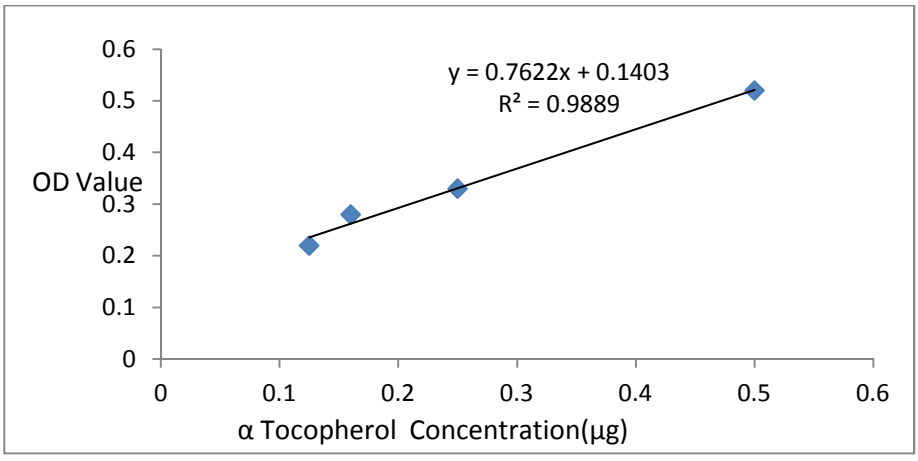

Fig 3.1: Representation of the standard curve of α-tocopherol concentration vs OD values at 520 nm.

3.10 Optimization:

Statistical analysis was performed using the JMP 8 software (SAS Institute Inc., Cary, NC, USA). The data were analyzed by analysis of variance (ANOVA) and the adequacy of the response surface model was determined by evaluating the lack of fit and coefficient of determination (R^2). The statistical significance of the model and its variables were determined at 5% probability level ($p<0.05$). The optimal conditions for heat reflux, MAE and UAE of powdered cranberry seed affecting oil yields and α - tocopherol concentrations were obtained based on the modeling and desirability function that could be visually illustrated in terms of three-dimensional response surface plots. Model parameters are estimated by least square regressions. The information about the statistical fit of the model, the contribution of each factor variable to the statistical fit, the residual error and lack-of-fit are provided in the analysis of variance. The lack-of-fit test gives information about the adequacy of the quadratic response surface (RS) model, by comparing the variation around the model with the "pure" variation within the replicated observations at the center points. The lack - of - fit test decomposes the residual error in "pure" error and "bias" error. The "pure" error is obtained from the variation of the center points replicates around their mean value. The "bias" error is obtained from the variation of mean values around the model prediction. If the model is adequate, the lack-of-fit test is not significant (SAS, 2003; Nemes and Orsat, 2010).

3.10.1 Response surface methodology:

Response surface (RSM) methodologies are used in process development for optimisation of one or more response variables as a function of several quantitative factors. A quadratic RS model is estimated by least square regression. The model describes the RS within the experimental range. Therefore, it can be used to determine the factor levels that optimise the response variable. RS studies often start with two-level factorial or fractional factorial designs to identify the factors that significantly influence the response. Then, the designs are augmented to central composite designs by addition of center points and axial points. The levels of the factors are coded (-1, 0, +1) denoting the low, central, and high levels respectively. For the axial points, each factor is set in turn, at a distance of ± α from the center (level 0) while the other factors are kept at the center. In the case of face centered central composite designs (CCD), α=1. For the center points, all factors are kept at their central level (Lundstedt *et al*., 1998; Mason *et al*., 2003; Myers and Montgomery, 2002; SAS, 2003; Nemes and Orsat, 2010).

CHAPTER IV

RESULTS AND DISCUSSION

4.1 Determination of moisture content:

The mean moisture content of triplicate measurements of the cranberry seeds, on wet basis, was found to be 6.32 ± 0.2%.

4.2 Heat reflux oil extraction yields:

Table 4.1: Face-centered central composite design (CCD) with observed response for oil yield from heat reflux extraction of cranberry seeds

Pattern	Time (min)	Temperature (°C)	Ratio (g/100ml)	Oil yield (%)		
				Actual	Predicted	Std. error
+-+	720	62	10	10.23	10.29	±0.14
+--	720	62	2.5	8.14	8.21	±0.14
00A	450	66	10	10.52	10.54	±0.11
+++	720	70	10	11.19	11.12	±0.14
000	450	66	6.25	9.22	9.19	±0.05
A00	720	66	6.25	9.3	9.32	±0.11
000	450	66	6.25	9.19	9.19	±0.05
000	450	66	6.25	9.23	9.19	±0.05
000	450	66	6.25	9.27	9.19	±0.05
0a0	450	62	6.25	9.17	8.91	±0.11
++-	720	70	2.5	8.85	8.74	±0.14
-++	180	70	10	11.10	11	±0.14
000	450	66	6.25	9.22	9.19	±0.05
---	180	62	2.5	8.18	8.21	±0.14
000	450	66	6.25	9.23	9.19	±0.05
-+-	180	70	2.5	8.73	8.64	±0.14
00a	450	66	2.5	8.25	8.32	±0.11
a00	180	66	6.25	9.2	9.26	±0.11
--+	180	62	10	10.2	10.27	±0.14
0A0	450	70	6.25	9.31	9.54	±0.11

Note: The actual values obtained from these patterns are the mean of triplicates except the central pattern (000).

The obtained oil yield with heat reflux ranged between 8.14 ± 0.1 % to 11.19 ± 0.1 % depending of the operating conditions for extraction. The studied factors were temperature between 62-70°C, time between 3 to 12 hr and sample/solvent ratios between 2.5 to 10 g/100ml of hexane. The results are presented according to the response obtained in the CCD experiments for the overall design presented in Table 4.1. Responses consisted of the percentage of oil extracted relative to powdered cranberry seeds. The maximum oil yield obtained by heat reflux extraction of powdered cranberry seeds occurred at a treatment temperature of 70°C, for a treatment duration of 12 hr and a sample size ratio of 10g/100ml of hexane. The most important treatment factors were temperature and sample size ratio whereas the duration was not as important.

Response surface regression models were fitted to the experimental data. The ANOVA of the quadratic regression model for oil yield by heat reflux extraction expressed in percentage are given in Table 4.2. The model obtained for percentage oil yield was significant with p value ≤ 0.0001, with R^2 value of 0.9871. It was found that there is a reasonable agreement with the adjusted R^2 of 0.9755 in the model. The lack of fit was not significant (p>0.05), suggesting that the model was well fitted and could be used to predict the oil yield from powdered cranberry seeds by heat-reflux method. This model indicated that the effect of sample/solvent ratio (g/100ml of hexane) and temperature had the greatest significance on oil yield from powdered seeds. Values of (p) less than 0.05 indicated the significant terms in the model, based on which, the linear term such as sample/solvent ratio (g/100ml hexane) and temperature were found to be significant. The predictive, second-order polynomial model for oil yield in heat reflux extraction method from powdered cranberry seeds, in terms of coded factors levels is presented in Eq 4.1 with a $R^2 = 0.99$.

Oil Yield (%) =

$$+9.204+0.03(Time)+1.109(Ratio)+0.326(Temp)+$$

$$0.005(Time*Ratio)+0.0275(Time*Temp)+0.075(Ratio*Temp)$$
$$+0.08(Time^2)+0.07(Temp^2)+0.215(Ratio^2) \quad (4.1)$$

Table 4.2: ANOVA for the effect on sample size (ratio), time and temperature on oil yield from heat reflux extraction

Oil yield(%) by heat-reflux extraction					
Source	**SS**	**DF**	**MS**	**F-Value**	***p*-value**
Model	13.936	9	1.548	85.21	<0.0001*
Lack of Fit	0.178	5	0.035	53.51	0.0002*
Error	0.181	10	0.018		
Term	**Estimate**	**Std. Error**	***t-ratio***	**F-Value**	***p*-value**
Intercept	9.204	0.0463	198.61		<0.0001*
Time	0.03	0.0426	0.70	2.420	0.4976
Ratio	1.109	0.0426	26.02	439.14	<0.0001*
Temp	0.326	0.0426	7.65	2.276	<0.0001*
Time*Ratio	0.005	0.0476	0.10	4.118	0.9185
Time*Temp	0.0275	0.0476	0.58	2.584	0.5767
Ratio*Temp	0.075	0.0476	1.57	2.101	0.1466
Time*Time	0.08	0.0812	0.98	0.673	0.3482
Ratio*Ratio	0.215	0.0812	2.64	4.876	0.0245*
Temp*Temp	0.07	0.0812	0.86	0.673	0.4093
R^2	0.9871				
Adjusted R^2	0.9755				

*Significant

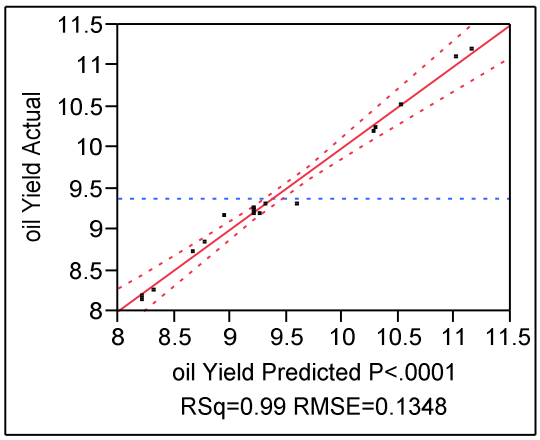

Fig 4.1: Predicted (%) vs. measured (%) of oil through heat reflux of powdered cranberry seeds.

The Pearson's correlation coefficient for actual vs. predictive value is r=0.99 in the case of heat reflux which gave significant values of $p \leq 0.0001$ and RMSE=0.1348 respectively. A plot of actual by predicted values of oil yield shows the close agreement between these values, suggesting that the response surface model can be used to predict oil yield under different experimental conditions as shown in Fig. 4.1.

4.2.1 Optimum conditions for maximum extraction of oil from powdered cranberry seeds through heat reflux:

The most important and widely used methodology for the optimization of process parameters is the Derringer function or desirability function (d) (Bezerra *et al.,* 2008). In order to maximize the response of the dependent variables, a numerical optimization was carried by statistical means which provided the overall optimum conditions of extraction of oil from cranberry seeds at 720 min with sample ratio of 10g/100ml of hexane around 70°C. The desirability factor of 0.88 was obtained at these conditions at the maximum yield of 11.14 ± 0.2% as shown in Fig 4.2.

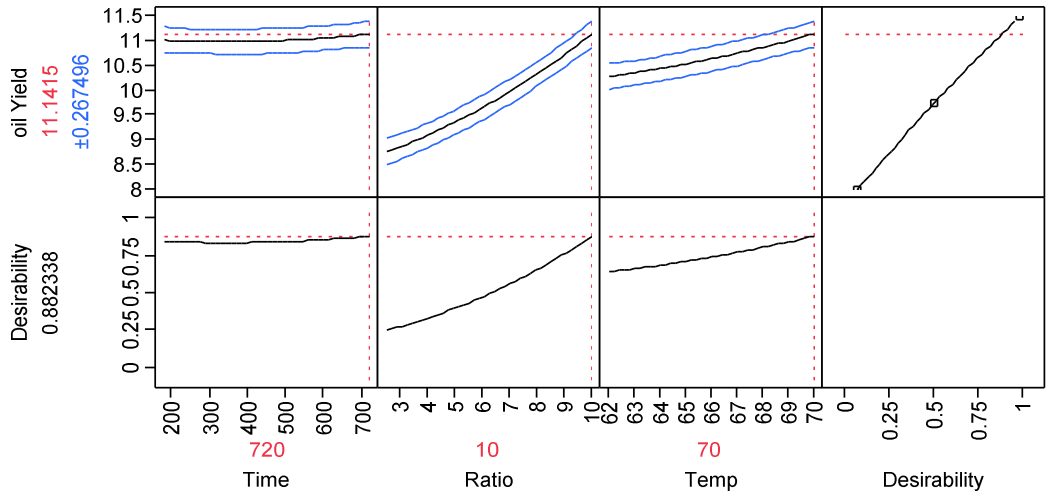

Fig 4.2: Prediction profiler for cranberry oil extraction by heat reflux

4.2.2 Effect of process variables on oil yield:

In heat reflux extraction, the extraction time did not affect the oil yield and thus time is not a factor presented in the response surface graph. The relationship between independent and dependent variables can be well illustrated with a three-dimensional response surface plot. So far no literature has been found to report on the heat reflux extraction of berry seed oil. Fig 4.3 presents the response surface plot for the oil yield as a function of the interaction of sample size ratio and temperature. It was found that as the sample size ratio (2.5 to 10g/100ml of hexane) increases, there is a steady increase in the oil yield. The maximum oil yield was obtained at the sample ratio of 10g/100ml of hexane providing the yield of 1.11 g (11.19 ± 0.1%) after 12 hr at 70°C. In heat reflux extraction, time did not affect the oil yield. It can also be found in Fig 4.3 that, the temperature effect was significant, where the oil yield increased with the gradual increase in temperature. Since hexane has a boiling point ranging between 65-70 degrees, its use as an effective solvent employed to extract oil from the seeds is supported as the oil yields peaked at 70 degrees giving the maximum yield of 1.11g (11.19 ± 0.1%) after 12 hr of extraction in the solvent (hexane) maintained at its boiling point.

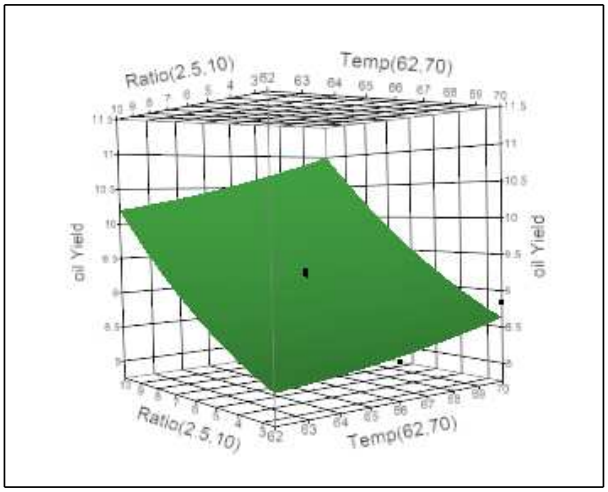

Fig 4.3: Response surface plot of the significant factor in heat reflux; effect of sample size and temperature on the oil yield of cranberry seed.

4.3 Microwave assisted oil extraction:

The obtained oil yield with microwave ranged between 15.73 ± 0.3% to 24.15 ± 0.3% depending of the operating conditions for extraction. The tested factors were for power between 100 and 125 W, time varying between 10 to 15 min and sample/solvent ratios between 3 to 5 g / 30 ml of hexane at a controlled temperature of 66°C. The results are presented for the response obtained in the CCD experiments and the overall design is presented in Table 4.3. Responses consisted of the percentage of oil extracted relative to powdered cranberry seeds. At first glance we can see from the table that the most influencing parameters on extraction yield are the sample size ratio and the microwave power. The best tested condition for extracting cranberry oil from powdered seeds by microwave was experienced at power 125 W, extracting time of 15 min and a sample size ratio of 5g/30ml of hexane. Inspite of employing different extraction durations the oil yield was not significantly affected by it. The temperature was not a factor as it was maintained constant at 66°C for all microwave extraction combinations.

Table 4.3: Face-centered central composite design (CCD) with observed response for oil yield from microwave extraction of cranberry seeds

Pattern	Time (min)	Power (W)	Ratio (g/30ml)	Oil yield (%)		
				Actual	Predicted	Std. error
000	12.5	112.5	4	20.83	20.540	±0.117
A00	15	112.5	4	21.62	20.939	±0.240
000	12.5	112.5	4	20.33	20.540	±0.117
-+-	10	125	3	22.32	22.187	±0.305
000	12.5	112.5	4	20.42	20.540	±0.117
-++	10	125	5	24.09	23.922	±0.305
+--	15	100	3	15.73	15.938	±0.305
000	12.5	112.5	4	20.51	20.540	±0.117
--+	10	100	5	18.5	18.352	±0.305
000	12.5	112.5	4	20.48	20.540	±0.117
00a	12.5	112.5	3	19.47	19.271	±0.240
00A	12.5	112.5	5	21.28	21.311	±0.240
a00	10	112.5	4	20.26	20.773	±0.240
+-+	15	100	5	18.11	18.283	±0.305
+++	15	125	5	24.15	24.258	±0.305
---	10	100	3	16.01	15.942	±0.305
++-	15	125	3	22.4	22.588	±0.305
0A0	12.5	125	4	23.17	23.171	±0.240
0a0	12.5	100	4	17.23	17.061	±0.240
000	12.5	112.5	4	20.34	20.540	±0.117

Note: The actual values obtained from these patterns are the mean of triplicates except the central pattern (000).

Response surface regression models were fitted to the experimental data. The ANOVA of the quadratic regression model for oil yield by microwave extraction expressed in percentage are given in Table 4.4. The model obtained for percentage oil yield was significant with p value ≤ 0.0001, with R^2 value of 0.9889. It was found that there is a reasonable agreement with the adjusted R^2 of 0.9789 in the model. The lack of fit was not significant (p>0.05), suggesting that the model was well fitted and could be used to predict the oil yield from powdered cranberry seeds by microwave extraction. This model indicated that the linear effects of power and

sample/solvent ratio (g/30ml of hexane) had the greatest significance on oil yield from powdered seeds. Values of (p) less than 0.05 indicate the significant terms in the model, based on which the linear terms such as power, sample/solvent ratio (g/30ml hexane) were found to be significant. The predictive, second-order, polynomial model for oil yield in microwave extraction from powdered cranberry seeds, in terms of coded factors levels is shown in Eq. 4.2 with a $R^2 = 0.99$.

Table 4.4: ANOVA for the effect on sample size (ratio) and microwave power on oil yield from microwave assisted extraction

Oil yield(%) by microwave assisted extraction					
Source	**SS**	**DF**	**MS**	**F-Value**	***p*-value**
Model	105.17	9	11.68	99.41	<0.0001*
Lack of Fit	1.006	5	0.2013	5.95	0.0362*
Error	1.17	10	0.1176		
Term	**Estimate**	**Std. Error**	***t-ratio***	**F-Value**	***p*-value**
Intercept	20.540	0.117	174.27		<0.0001*
Time	0.083	0.108	0.77	0.5860	0.4616
Power	3.055	0.108	28.18	793.93	<0.0001*
Ratio	1.02	0.108	9.41	88.50	<0.0001*
Time*Power	0.10125	0.121	0.84	0.6977	0.4231
Time*Ratio	-0.0162	0.121	-0.13	0.0180	0.8960
Power*Ratio	-0.16875	0.121	-1.39	1.9379	0.1941
Time*Time	0.31636	0.206	1.53	2.3414	0.1570
Power*Power	-0.4236	0.206	-2.05	4.1984	0.0676
Ratio*Ratio	-0.2486	0.206	-1.20	1.4462	0.2568
R^2	0.9889				
Adjusted R^2	0.9789				

*Significant

Oil Yield (%) =

+20.540+0.083(*Time*)+3.055(*Power*)+1.02(*Ratio*)+0.10125(*Time*Power*)-

0.0162(*Time*Ratio*)-0.16875(*Ratio*Power*)+0.3163($Time^2$)-

0.4236($Power^2$)-0.2486($Ratio^2$) (4.2)

In the case of microwave, the Pearson's correlation coefficient for actual vs. predictive value is r=0.99 which gave significant values of p≤0.0001and RMSE=0.3429 respectively. A plot of actual by predicted values of oil yield shows the close agreement between these values, suggesting that the response surface model can be used to predict oil yield under different experimental conditions as shown in Fig. 4.4.

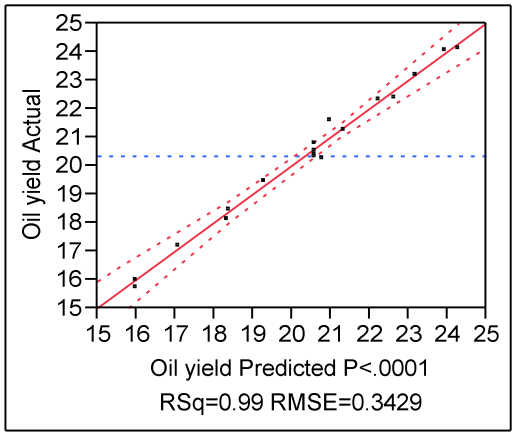

Fig 4.4: Predicted (%) vs. measured (%) of oil yield obtained from microwave extraction of powdered cranberry seeds

4.3.1 Optimum conditions for maximum extraction of oil from powdered cranberry seeds through microwave:

Derringer function (d) (Bezerra *et al*., 2008) is the currently used methodology for the optimization of process parameters. A numerical optimization was carried out by statistical means which provided the overall optimum conditions for extraction of oil from cranberry seeds at 10 min with sample size ratio of 5g/30ml of hexane and microwave power 125 W at a controlled temperature of 66 °C to maximize the response of the dependent variables. The

desirability factor of 0.87 was obtained at these conditions at the maximum oil yield of 23.92 ±0.6% as shown in Fig 4.5.

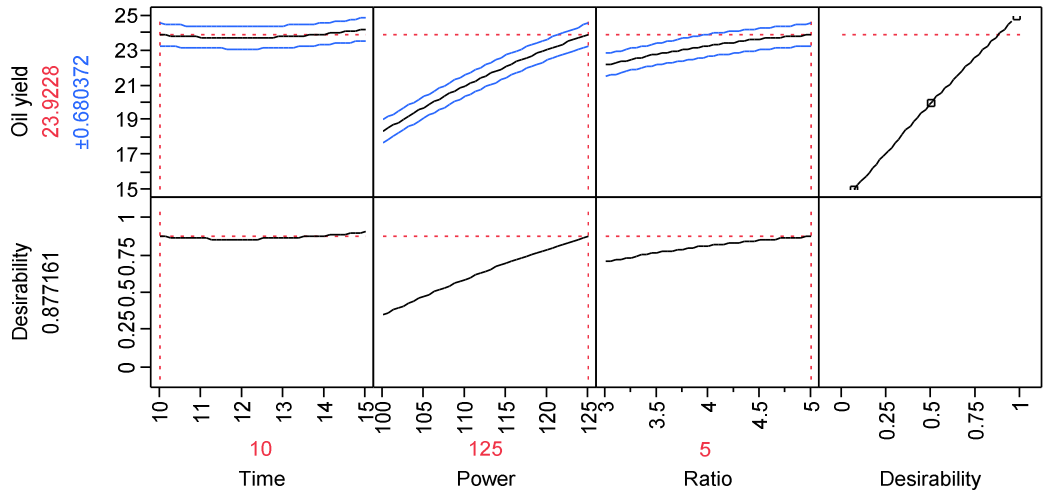

Fig 4.5: Prediction profiler for microwave assisted extraction

4.3.2 Effect of process variables on oil yield:

In microwave assisted extraction, time does not significantly affect the oil yield ($p=0.4616$), hence this factor was not presented in the response surface graph. The relationship between independent and dependent variables can be well illustrated with a three-dimensional response surface plot. Fig 4.6 presents the percentage oil yield as a function of processing parameters with significant interaction, namely sample size ratio and microwave power between 100 to 125W for a controlled temperature at 66°C. It was found that the oil yield increases as the mass of sample used increases from 3 to 5 g/30 ml of hexane. A maximum yield of 1.20 g (24.15 ± 0.3%) was obtained with the sample size ratio of 5 g /30ml of hexane at 66°C and maximum applied power. Indeed, it was found that power was significant ($p<0.0001$) and that as the microwave power increases, there is an increase in the oil yield. The oil yield increased as the applied power increased from 100 to 125 W and reached the maximum of 1.20 g (24.15 ± 0.3%) in 5 g /30ml of hexane at the applied microwave power of 125 W. With the applied microwave power there is a steady increase in temperature and pressure inside the cellular structures leading to volume swelling of the cells, and subsequent cell walls rupture which results in rapid release of the components into the surrounding solvent (Chemat *et al*., 2005). Li *et al*. (2004a) obtained similar results in a study on oil extracted from soybean flour using microwave pre-treatment.

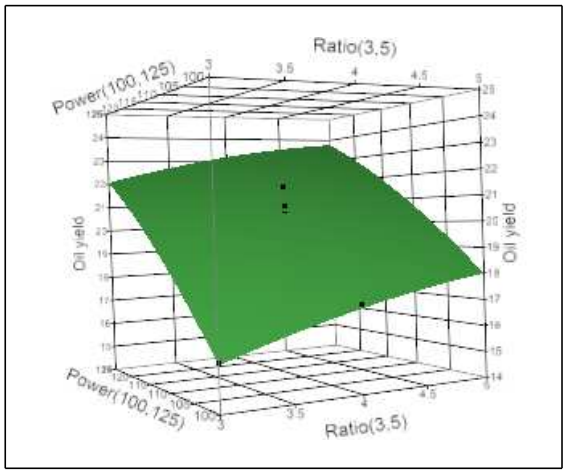

Fig 4.6: Response surface plot of the significant factors in microwave assisted extraction; effect of sample size and power on the oil yield of cranberry seed.

4.4 Ultrasound oil extraction yields:

The obtained oil yield with ultrasound ranged between 24.21 ± 0.3% to 32.35 ± 0.3% depending on the operating conditions for extraction. The tested factors were power ranging between 100 and 150 W, time varying between 10 to 15 min and sample/solvent ratio ranging between 3 to 5 g / 30 ml of hexane at the controlled temperature of 66°C. The results are presented as per the response obtained in the CCD experiments and the overall design is presented in Table 4.5. Responses consisted of the percentage of oil extracted relative to powdered cranberry seeds. At first glance we can see from the table that the most important parameters influencing the extraction yield are the sample size ratio and the ultrasonic power. The best tested condition for extracting cranberry oil from powdered seeds by ultrasound was for a power of 150 W, an extracting time of 15 min and a sample size ratio of 5g/30ml of hexane. Inspite of employing different extraction durations the oil yield was not significantly affected by it. The temperature was not a tested factor as the temperature was maintained for all ultrasound extraction combinations at 66°C.

Table 4.5: Face-centered central composite design (CCD) with observed response for oil yield from ultrasound extraction of cranberry seeds

Pattern	Time (min)	Power (W)	Ratio (g/30ml)	Oil yield (%)		
				Actual	Predicted	Std. error
0A0	12.5	150	4	31.12	31.462	±0.23
-+-	10	150	3	30.39	30.258	±0.29
+-+	15	100	5	26.72	26.806	±0.29
000	12.5	125	4	28.8	28.520	±0.11
--+	10	100	5	26.38	26.537	±0.29
++-	15	150	3	30.92	30.717	±0.29
000	12.5	125	4	28.79	28.520	±0.11
0a0	12.5	100	4	25.76	25.594	±0.23
000	12.5	125	4	28.75	28.520	±0.11
000	12.5	125	4	28.88	28.520	±0.11
-++	10	150	5	32.16	32.100	±0.29
00A	12.5	125	5	29.86	29.624	±0.23
+++	15	150	5	32.35	32.399	±0.29
A00	15	125	4	28.48	28.530	±0.23
+--	15	100	3	24.53	24.544	±0.29
000	12.5	125	4	28.12	28.520	±0.11
---	10	100	3	24.21	24.115	±0.29
000	12.5	125	4	28.14	28.520	±0.11
00a	12.5	125	3	27.16	27.572	±0.23
a00	10	125	4	28.04	28.166	±0.23

Note: The actual values obtained from these patterns are the mean of triplicates except the central pattern (000).

Response surface regression models were fitted to the experimental data. The ANOVA of the quadratic regression model for percentage oil yield by ultrasound extraction is presented in Table 4.6. The model obtained for oil yield was significant with p value ≤ 0.0001, with R^2 value of 0.9884. It was found that there is a reasonable agreement with the adjusted R^2 of 0.9781 in the model. The lack of fit was not significant ($p>0.05$), suggesting that the model was well fitted and could be used to predict the oil yield from powdered cranberry seeds by ultrasonic extraction. This model indicated that the linear effects of power and sample/solvent ratio (g/30ml of hexane) had the greatest significance on oil yield from the cranberry seeds. Values of (p) less than 0.05

indicate the significant terms in the model, based on which the linear terms such as power, sample/solvent ratio (g/30ml hexane) was found to be significant. The predictive, second-order, polynomial model for oil yield with ultrasound extraction from powdered cranberry seeds, in terms of coded factors levels is presented in Eq. 4.3 with a $R^2 = 0.99$.

Table 4.6: ANOVA for the effect on sample size (ratio) and ultrasonic power on oil yield during ultrasound assisted extraction

Oil yield (%) Ultrasound assisted extraction					
Source	**SS**	**DF**	**MS**	**F-Value**	***p*-value**
Model	97.21	9	10.80	95.37	<0.0001*
Lack of Fit	0.516	5	0.103	0.8368	0.5751
Error	1.132	10	0.113		
Term	**Estimate**	**Std. Error**	***t-ratio***	**F-Value**	***p*-value**
Intercept	28.520	0.115	*246.52*		<0.0001*
Time	0.182	0.106	1.71	2.92	0.1180
Power	2.934	0.106	27.57	760.05	<0.0001*
Ratio	1.026	0.106	9.64	92.94	<0.0001*
Time*Power	0.0075	0.118	0.06	0.0040	0.9510
Time*Ratio	-0.04	0.118	-0.34	0.1130	0.7437
Power*Ratio	-0.145	0.118	-1.22	1.48	0.2509
Time*Time	-0.1718	0.202	-0.85	0.7168	0.4170
Power*Power	0.00818	0.202	0.04	0.0016	0.9686
Ratio*Ratio	0.07818	0.202	0.39	0.1484	0.7081
R^2	0.9884				
Adjusted R^2	0.9781				

*Significant

Oil Yield (%) =

+28.520+0.182(*Time*)+2.934(*Power*)+

1.026(*Ratio*)+0.0075(*Time*Power*)-0.04(*Time*Ratio*)-0.145

(*Ratio*Power*)-0.1718($Time^2$)+0.00818($Power^2$)+0.07818($Ratio^2$) (4.3)

The Pearson's correlation coefficient for actual vs. predictive value is r=0.99 in the case of ultrasound assisted extraction which gave significant values of p≤0.0001 and RMSE=0.3365 respectively. A plot of actual by predicted values of oil yield shows the close agreement between these values, suggesting that the response surface model can be used to predict oil yield under different experimental conditions as shown in Fig. 4.7.

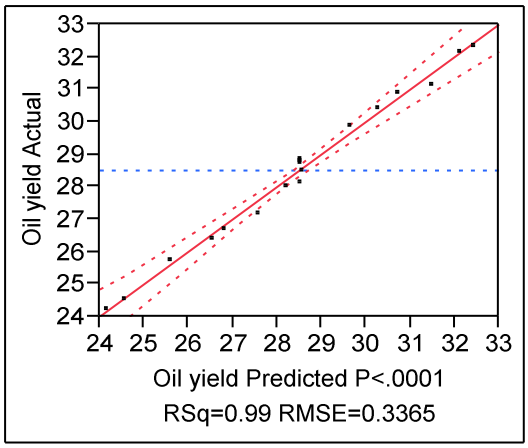

Fig 4.7: Predicted (%) vs. measured (%) of oil yields from ultrasound extraction from powdered cranberry seeds

4.4.1 Optimum conditions for maximum extraction of oil from powdered cranberry seeds through ultrasound:

The most widely used methodology in process optimization is the Derringer function or desirability function (d) (Bezerra *et al*., 2008). In order to maximize the response of the dependent variables, a numerical optimization was carried by statistical means which provided the overall optimum conditions of extraction of oil from cranberry seeds at 11 min with sample size ratio of 5g/30ml of hexane and ultrasound power of 150 W at 66°C. The desirability factor of 0.90 was obtained at these conditions at the maximum oil yield of 32.29 ± 0.6% as shown in Fig. 4.8.

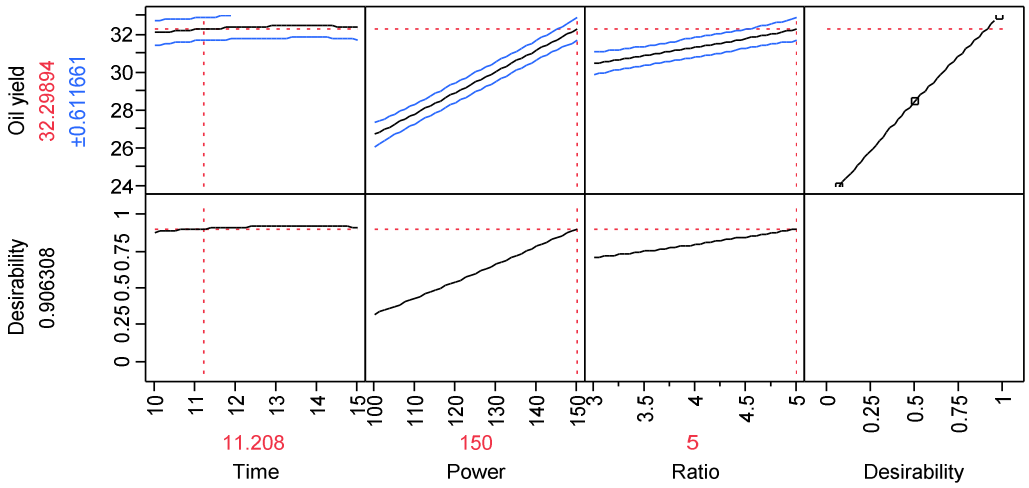

Fig 4.8 Prediction profiler for ultrasound extraction

4.4.2 Effect of process variables on oil yield:

In ultrasound assisted extraction, time does not significantly affect the oil yield, hence time was not presented in the response surface graph. The relationship between independent and dependent variables can be well illustrated with a three -dimensional response surface plots. Fig 4.9 presents the oil yield as affected by the interaction of sample size ratio and ultrasonic power at a temperature of 66°C. It was found that the oil yield increased as power increased from 100 to 150 W providing the maximum yield of 1.61g (32.35 ± 0.3%) at 150 W, for 10 min at 66°C and a 5 g sample. With the increasing power of ultrasound waves, a higher amplitude is created which leads to the creation and collapse of numerous bubbles in a short period of time (Hemwimol *et al*., 2006) causing high pressure and shock wave generation, leading to the enhancement and penetration of the solvent into the cell tissues thus accelerating the intracellular product release into the solvent through the disrupted cell walls. Similar results were obtained by Li *et al*. (2004b) and Sivakumar *et al*. (2007), in the ultrasound-assisted extraction of oil from soybean. The results indicate that the extraction yield increased as the sample ratio increased from 3 to 5 g/30ml of hexane, providing the yield of 1.61g (32.35 ± 0.3%) at 5g/30ml of hexane in 10 min at 66°C. The effect of ultrasound on extraction is due to the vibration occurring in the interfaces between solid matrix and the solvent caused by the ultrasonic waves (Ai-jun *et al*., 2007). For a given medium the vibration is proportional to the ultrasonic power. The higher the

ultrasonic power, the greater the intensity of the vibration will be, which potentially leads to an increase in extracting yield.

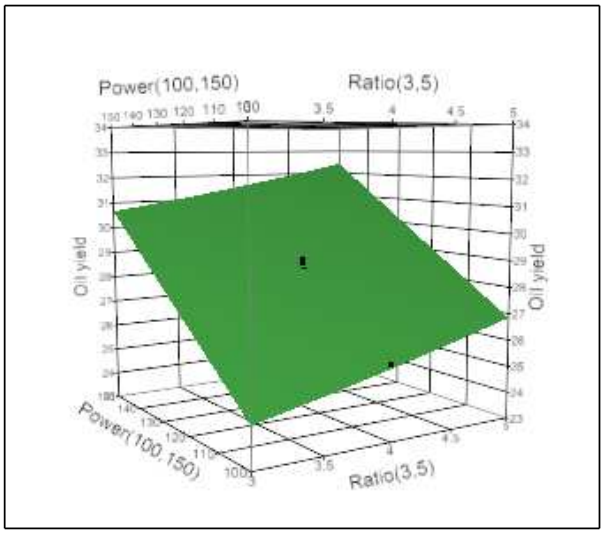

Fig 4.9: Response surface plot of the significant factor in ultrasound assisted extraction; effect of power and sample size on the oil yield of cranberry seed.

4.5 Comparison of extraction methods:

The maximum yields (10.23 ± 0.1%),(10.52 ± 0.1%) and (11.19 ± 0.1%) obtained by heat reflux at varying temperatures such as 62°C, 66°C and 70°C with the tested parameters of 12 hr,10g/100ml of hexane; 7.5 hr, 10g/100ml of hexane and 12 hr,10g/100ml of hexane were significantly lower than the maximum yields (24.15 ± 0.3%) obtained by MAE at 15 min, 125 W and 5g/30ml of hexane at 66°C and (32.35 ± 0.3 %) by UAE at 15 min, 150 W and 5g/30ml of hexane at 66°C as shown in Fig 4.10. The most important factor to consider in this context is the time and the temperature combination. In heat reflux the time taken is longer and the temperature employed were very different which may sometimes decrease the oil quality and deteriorate the phytonutrients present. In the case of microwave, both the time and the temperature are minimized potentially leading to a better oil quality and thereby retaining the phytonutrients. Considering the significant reduction in process time of the MAE process compared to reflux extraction, it becomes an attractive alternative with better yield and with potential higher quality. The significant yield within a short process time obtained by microwave extraction as compared to heat reflux extraction is explained from the ability of microwaves to interact with the sample

matrix at the molecular level and the potential for inducing significant physical disruptions into the matrix. The greater microstructure disruption in the MAE vs heat reflux extraction results in greater porosity and higher yields associated with MAE. In case of microwave assisted extraction, the presence of water in the sample a polar solvent, influences both the amount and the rate of heating produced. When the samples are treated with microwaves, water molecules are initially targeted and they evaporate rapidly, increasing pressure on the cell wall structure. The rapid dehydration alters the cell wall structure and changes its permeability, resulting in intra-cellular contents expelled out thus enhancing the mass transfer rate (Chemat *et al*., 2005). Due to the disruption of the oil bearing structures present in the seeds, there is an increase in the amount of oil extracted with microwave as compared to conventional extraction. Saoud *et al.* (2005) obtained similar results of improved yield in a study on oil extracted from eucalyptus leaves using a microwave treatment.

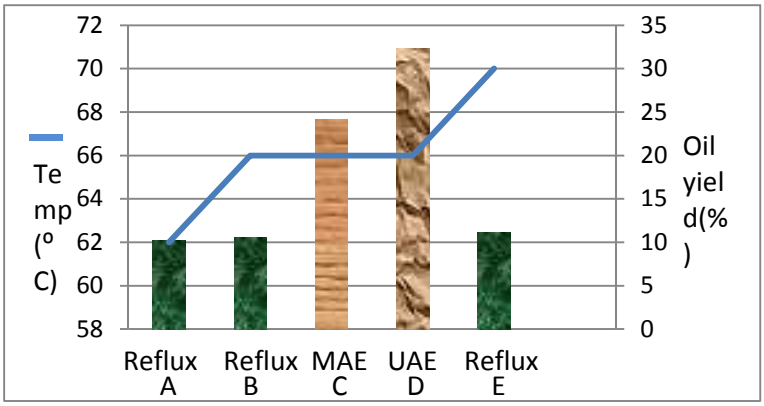

Fig 4.10: Comparison of extraction methods on oil yield.

Note: Reflux-Heat Reflux method; MAE-Microwave assisted Extraction; UAE-Ultrasound assisted Extraction. A-Reflux: 12hr; 10g/100ml of hexane at 62 °C .B-Reflux: 7.5 hr; 10g/100ml of hexane at 66°C.C-MAE: 15 min; 125W and 5g/30ml of hexane at 66°C.D-UAE: 15 min; 150W and 5g/30ml of hexane at 66°C.E-Reflux: 12 hr; 10g/100ml of hexane at 70°C.

As mentioned earlier in the discussion and as presented in Fig 4.10, the maximum yield (32.35 ± 0.3 %) obtained by UAE at 66°C with an extraction time of 15 min, 150W and 5g/30ml of hexane, was significantly greater than the maximum extraction yield (10.23 ± 0.1%),(10.52 ± 0.1%) and (11.19 ± 0.1%) obtained by heat reflux at 12 hr,10g/100ml of hexane at 62° C; 7.5 hr, 10g/100ml of hexane at 66° C and 12 hr,10g/100ml of hexane at 70° C respectively. Here again the most important factor to consider in this context is the time and the temperature combination. In heat reflux the time taken was greater and the temperatures employed were very different

which may sometimes decrease the oil quality and deteriorate the phytonutrients present. In the case of ultrasound, both the time and the temperature are minimized potentially leading to a better oil quality with the retention of the phytonutrients. Considering the significant reduction in process time of the UAE, it becomes an attractive alternative with better yield and with potential higher quality. The significant yield in a short process time obtained by ultrasound extraction as compared to heat reflux extraction comes from the ultrasound themselves. Greater ultrasonic waves are created when the power increases which leads to the creation and collapse of bubbles in a short period of time (Hemwimol *et al*., 2006) causing high pressure and shock wave generation, leading to the enhancement of penetration of the solvent into the cell tissues and the acceleration of the intracellular product release into the solvent by disrupting the cell walls. Similar results were obtained by Qing *et al.* (2009), in the ultrasound assisted extraction of oil from almond powder.

The maximum yield (32.35 ± 0.3 %) obtained using UAE at 66°C with an extraction time of 15 min, 150W and 5g/30ml of hexane is significantly greater than the value (24.15 ± 0.3%) obtained using MAE at 66°C with an extraction time of 15 min, 125 W and 5g/30ml of hexane as shown in Fig 4.10. The most important factor to consider in this context is the difference in the process parameters and mechanisms of oil extraction. In the case of microwave assisted extraction, it consists of microwave heating the sample in contact with the solvent by means of direct electromagnetic energy transfer (Venkatesh and Raghavan, 2004; Nemes and Orsat, 2010). Microwave heating is known to be volumetric in nature so microwave irradiation produces efficient internal heating by coupling microwaves with polar components inside the sample and solvent. This process involves disruption of hydrogen bonds, as a result of microwave-induced dipole rotation of molecules, and migration of the ions, which enhance penetration of the solvent into the matrix, allowing dissolution of the components to be extracted (Hudaib *et al*., 2003). In the case of ultrasound extraction, oil yields may be explained in terms of cavitational effects caused by the application of high-intensity ultrasound. As large amplitude ultrasound waves travel through a mass medium, they cause compression and shearing of solvent molecules resulting in localized changes in density of solvent (Price *et al.,* 1995). As a consequence, the initially sinusoidal compression and shear waves at a finite distance from the ultrasonic transducer will be distorted into shock waves. This abrupt decrease in pressure at the edge of the saw tooth shaped ultrasonic wave in the negative pressure cycle generates small bubbles. These

bubbles collapse in the positive pressure cycle and produce turbulent flow conditions associated with high pressures and temperatures (Mason, 1997; Mason and Cordmas, 1996; Mason, 1992; Price, 1990, 1993). Since formation and collapse of bubbles occurs over very short periods of time, typically a few microseconds (Hardcastle *et al*., 2000), heat transfer from cavitational bubbles to the medium is small causing only gradual temperature rise in the medium which leads to the release of components to be extracted. Hence both ultrasound and microwave-assisted methods would significantly improve extraction yield of cranberry seed oil, with higher efficiency, reduced extraction time and sample size than conventional solvent extraction methods.

4.6 Determination of α-tocopherol in cranberry seed oil:

Tocopherol was estimated in the cranberry oil by the Emmerie-Engel reaction as reported by Rosenberg, (1992) and results were obtained for the oil extracted using different extraction methods. The spectrophotometric study of the α - tocopherol standard permitted the establishment of a standard curve presenting the spectrophotometric optical density (OD) value at 520 nm as a function of the α - tocopherol concentration. The standard curve is presented in Fig. 4.11.

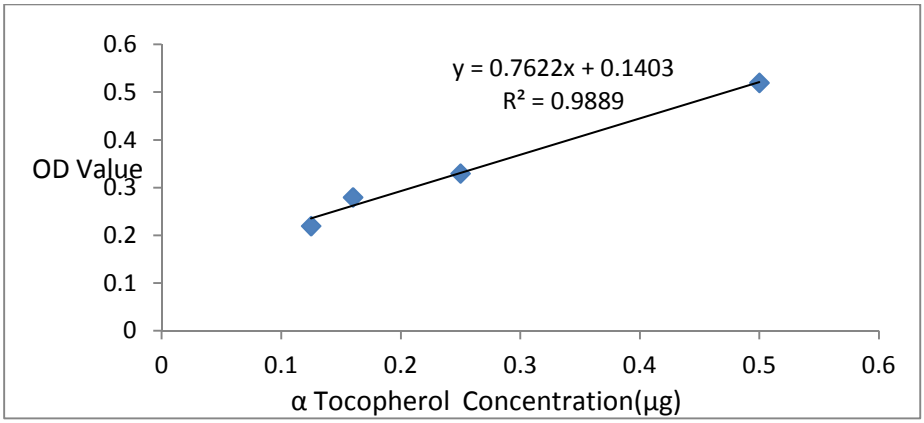

Fig 4.11: Representation of the standard curve of α - tocopherol concentration vs OD values at 520 nm.

4.6.1 Effect of process variables on α - tocopherol concentration in oil obtained from heat reflux extraction:

The values and ANOVA for α - tocopherol concentration in oil obtained by heat reflux are presented in Tables 4.7 and 4.8.

The obtained α - tocopherol concentration with heat reflux ranged between 0.005 ± 0.02µg to 0.266 ± 0.02µg depending of the operating conditions of cranberry oil extraction. The results are presented as per the response obtained in the CCD experiments and the overall design is presented in Table 4.7. Responses consisted of the concentration of α - tocopherol relative to OD values of the oil samples. At first glance, from this table it appears that the most influencing parameter on α - tocopherol concentration is the temperature. The best tested conditions for the α - tocopherol content was found in oil obtained by heat reflux at a temperature of 70°C, extracting time of 3 hr and a sample size ratio of 10g/100ml of hexane.

Table 4.7: Concentration of α - tocopherol in heat -reflux extraction

Time (hr)	Ratio (g/100ml)	Temp (° C)	O.D Value	α - tocopherol(µg)	Predicted value (µg)	Std. error
7.5	6.25	66	0.217	0.144	0.149	±0.008
7.5	6.25	66	0.229	0.160	0.149	±0.008
12	6.25	66	0.237	0.171	0.134	±0.018
12	10	62	0.133	0.034	0.038	±0.023
3	2.5	62	0.111	0.005	0.016	±0.023
3	10	70	0.310	0.266	0.258	±0.023
3	6.25	66	0.193	0.113	0.133	±0.018
3	10	62	0.135	0.037	0.045	±0.023
7.5	6.25	66	0.207	0.131	0.149	±0.008
3	2.5	70	0.274	0.219	0.218	±0.023
7.5	6.25	70	0.293	0.244	0.231	±0.018
7.5	10	66	0.266	0.209	0.177	±0.018
7.5	6.25	66	0.202	0.125	0.149	±0.008
7.5	6.25	66	0.242	0.177	0.149	±0.008
12	2.5	62	0.118	0.014	0.024	±0.023
7.5	6.25	66	0.205	0.129	0.149	±0.008
7.5	2.5	66	0.21	0.135	0.150	±0.018
7.5	6.25	62	0.127	0.026	0.023	±0.018
12	10	70	0.256	0.195	0.220	±0.023
12	2.5	70	0.284	0.232	0.227	±0.023

Response surface regression models were fitted to the experimental data. The ANOVA of the quadratic regression model for the concentration of α - tocopherol by heat reflux extraction expressed in micrograms is given in Table 4.8.

Table 4.8: ANOVA for the effect on temperature (°C) of heat reflux on α - tocopherol concentration

α - tocopherol Concentration in oil from Heat reflux					
Source	**SS**	**DF**	**MS**	**F-Value**	***p*-value**
Model	0.1152	9	0.0128	19.023	<0.0001*
Lack of Fit	0.0046	5	0.0009	2.2068	0.2027
Error	0.0067	10	0.0006		
Term	**Estimate**	**Std. Error**	***t-ratio***	**F-Value**	***p*-value**
Intercept	0.149	0.00892	16.75		<0.0001*
Time	0.0006	0.00820	0.07	0.0053	0.9431
Ratio	0.0136	0.00820	1.66	2.7474	0.1284
Temp	0.104	0.00820	12.68	160.66	<0.0001*
Time*Ratio	-0.012	0.00917	-1.31	1.7112	0.2201
Time*Temp	-0.008	0.00917	-0.87	0.7605	0.4036
Temp*Ratio	-0.0052	0.00917	-0.57	0.3275	0.5798
Time*Time	-0.0150	0.01564	-0.96	0.9303	0.3575
Ratio*Ratio	0.0149	0.01564	0.95	0.9080	0.3631
Temp*Temp	-0.0220	0.01564	-1.41	1.9934	0.1883
R^2	0.9448				
Adjusted R^2	0.8951				

*Significant

The model obtained for α - tocopherol concentration in terms of micrograms was significant with p value ≤ 0.0001, with R^2 value of 0.9448. It was found that there is a reasonable agreement with the adjusted R^2 of 0.8951 in the model. The lack of fit was not significant (p>0.05),

suggesting that the model was well fitted and could be used to predict the concentration of α - tocopherol in oil extracted from powdered cranberry seeds by heat-reflux. This model indicated that the linear effect of extraction temperature (°C) had the greatest significance on the α - tocopherol concentration in the oil. Values of (p) less than 0.05 indicate the significant terms in the model, based on which the linear term such as temperature (°C) was found to be significant.The predictive, second-order, polynomial model for tocopherol concentration in oil obtained by heat reflux extraction from powdered cranberry seeds, in terms of coded factors levels is shown in Eq 4.4 with a R^2=0.94.

Concentration of α – tocopherol =

=+0.149+0.0006(*Time*)+0.0136(*Ratio*)+0.104(*Temp*)-0.012(*Time*Ratio*)-

0.008(*Time*Temp*)-0.0052(*Ratio*Temp*)-0.0150($Time^2$)-

0.0220($Temp^2$)+0.0149($Ratio^2$) (4.4)

The relationship between independent and dependent variables can be well illustrated with a three-dimensional response surface. Fig 4.12 gives the interaction effect of temperature on the concentration of α - tocopherol. It was found that as the temperature (62 to 70°C) increased there was a steady increase in the concentration of α - tocopherol which peaked at 70°C. During the extraction process the application of elevated temperatures facilitates the extraction of compounds trapped within the plant matrix such as α- tocopherol along with the oil. Certain plant compounds are however sensitive to thermodegradation. It appears that within the temperature range tested, there was no detrimental thermal effect on the α - tocopherol content. The maximum concentration of α - tocopherol was obtained at the sample size of 10g/100ml of hexane providing the value of 0.266 ± 0.02 µg following 3 hr of extraction at 70°C. With this extraction method, neither sample size nor time had an effect on the concentration of α-tocopherol in the extracted oil.

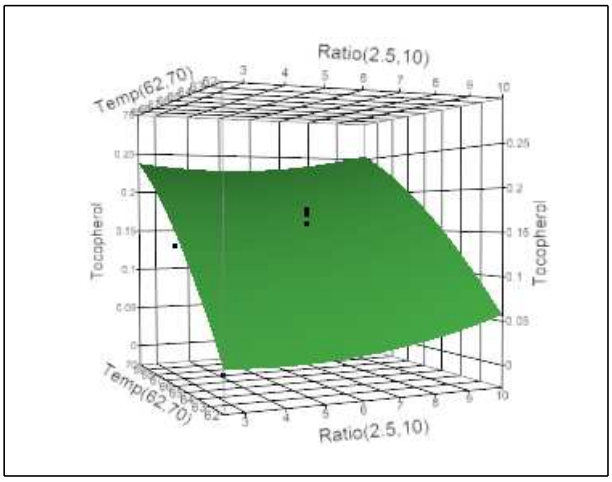

Fig 4.12: Response surface plot of the significant factor in heat reflux; effect of temperature on α tocopherol concentration in cranberry seed oil.

4.6.2 Effect of process variables on α - tocopherol concentration in microwave assisted extracted oil:

The values and the ANOVA for the α - tocopherol concentrations in oil obtained by MAE are presented in Tables 4.9 and 4.10.

The obtained α - tocopherol concentration with microwave ranged between 0.131 ± 0.007µg to 0.346 ± 0.007µg depending of the operating conditions of cranberry oil extraction. The results are presented as per the response obtained in the CCD experiments and the overall design presented in Table 4.9. Responses consisted of the concentration of α - tocopherol relative to OD values of the oil samples. From this table, one can see that the most influencing parameters on α - tocopherol concentration are the power and the sample size ratio. The best tested condition in which the α - tocopherol was found in oil by microwave was for power 125 W, extracting time 15 min and a sample size ratio 5g/30ml of hexane. Inspite of employing different extraction durations, the α - tocopherol concentration was not affected by treatment time.

Table 4.9: Concentration of α-tocopherol in oil from microwave assisted extraction

Time (Min)	Power (Watts)	Ratio (g/30 ml)	O.D Value	α – tocopherol (µg)	Predicted Value (µg)	Std. error
12.5	100	4	0.215	0.142	0.146	±0.005
10	100	5	0.223	0.152	0.151	±0.007
10	125	3	0.316	0.274	0.273	±0.007
12.5	112.5	5	0.293	0.244	0.231	±0.005
15	125	5	0.371	0.346	0.351	±0.007
12.5	112.5	4	0.269	0.213	0.213	±0.002
12.5	112.5	4	0.264	0.206	0.213	±0.002
12.5	112.5	3	0.251	0.189	0.191	±0.005
12.5	112.5	4	0.263	0.205	0.213	±0.002
15	100	5	0.218	0.146	0.148	±0.007
10	100	3	0.207	0.131	0.127	±0.007
10	112.5	4	0.264	0.206	0.204	±0.005
12.5	112.5	4	0.27	0.214	0.213	±0.002
10	125	5	0.362	0.335	0.340	±0.007
15	125	3	0.33	0.293	0.296	±0.007
15	112.5	4	0.277	0.223	0.214	±0.005
15	100	3	0.214	0.140	0.136	±0.007
12.5	112.5	4	0.266	0.209	0.213	±0.002
12.5	112.5	4	0.27	0.214	0.213	±0.002
12.5	125	4	0.363	0.336	0.321	±0.005

Response surface regression models were fitted to the experimental data. The ANOVA of the quadratic regression model for tocopherol concentration in oil obtained by microwave assisted extraction is given in Table 4.10. The model obtained for tocopherol concentration in terms of micrograms was significant with p value ≤ 0.0001, with R^2 value of 0.9912. It was found that there is a reasonable agreement with the adjusted R^2 of 0.9833 in the model. The lack of fit was not significant ($p>0.05$), suggesting that the model was well fitted and could be used to

predict the concentration of α- tocopherol from powdered cranberry seeds by microwave extraction. This model indicated that the linear effect of sample/solvent ratio (g/30ml of hexane) and power (W) had the greatest significance on tocopherol concentration for the MAE production of oil from powdered seeds. Values of (p) less than 0.05 indicate the significant terms in the model, based on which the linear term such as sample/solvent ratio (g/30ml of hexane) and power (W) were found to be significant.

Table 4.10: ANOVA for the effect of power (W) and sample size ratio during microwave assisted extraction on the α- tocopherol concentration

α -Tocopherol Concentration by Microwave assisted Extraction Method					
Source	**SS**	**DF**	**MS**	**F-Value**	***p*-value**
Model	0.0829	9	0.0092	125.83	<0.0001*
Lack of Fit	0.0006	5	0.0001	0.00013	0.0206*
Error	0.0007	10	0.00007		
Term	**Estimate**	**Std. Error**	***t-ratio***	**F-Value**	***p*-value**
Intercept	0.2134	0.0029	72.54		<0.0001*
Time	0.005	0.0027	1.85	3.4127	0.0945
Power	0.0873	0.0027	32.25	1040.36	<0.0001*
Ratio	0.0196	0.0027	7.24	52.44	<0.0001*
Time*Power	0.0033	0.0030	1.12	1.243	0.2908
Time*Ratio	-0.0028	0.0030	-0.95	0.9027	0.3645
Power*Ratio	0.0108	0.0030	3.59	12.91	0.0049*
Time*Time	-0.0038	0.0051	-0.75	0.560	0.4713
Power*Power	0.0206	0.0051	4.00	15.98	0.0025*
Ratio*Ratio	-0.0018	0.0051	-0.36	0.130	0.7256
R^2	0.9912				
Adj.R^2	0.9833				

*Significant

The predictive, second-order, polynomial model for α - tocopherol concentration in microwave assisted extraction method from powdered cranberry seeds, in terms of coded factors levels is shown in Eq. 4.5 with a R^2= 0.99.

Concentration of α – tocopherol =

+0.2134+0.05(*Time*)+0.0873(*Power*)+

0.0196(*Ratio*)+0.0033(*Time*Power*)-0.0028(*Time*Ratio*)+0.0108(*Ratio*Power*)

-0.0038($Time^2$)+0.0206($Power^2$)-0.0018($Ratio^2$) (4.5)

The relationship between independent and dependent variables can be well illustrated with a three-dimensional response surface plot. Fig 4.13 presents the interaction of microwave power and sample size on the concentration of α - tocopherol. It was found that as the microwave power increases (100 to 125 W) there is a steady increase in the concentration of α - tocopherol which peaks at 125W. This can be explained by the fact that as the power (W) increases, the dissipated microwave energy may help release some forms of tocopherol as was reported by Imola, (2006) for the esterified forms of tocopherol in rice bran oil extracted by microwave. The maximum concentration of α - tocopherol was obtained at the sample ratio of 5g/30ml of hexane and 125W providing the value of 0.346 ± 0.007 µg following 15 min treatment at 66°C. In microwave assisted extraction, treatment time did not affect the concentration of tocopherol.

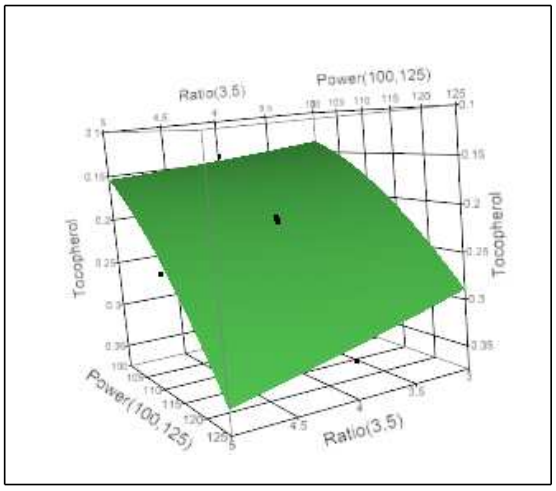

Fig 4.13: Response surface plot of the significant factor in microwave assisted extraction; effect of power (W) and sample size ratio (R) on α - tocopherol concentration of cranberry seed oil.

4.6.3 Effect of process variables on α- tocopherol concentration in ultrasound assisted extracted oil:

The values and ANOVA for α - tocopherol concentrations in oil obtained by UAE are shown in Tables 4.11 and 4.12.

Table 4.11: Concentration of α - tocopherol in ultrasound assisted extraction

Time (Min)	Power (Watts)	Ratio (g/30 ml)	O.D Value	α Tocopherol (µg)	Predicted value(µg)	Std. error
15	100	5	0.376	0.353	0.361	±0.01
12.5	125	4	0.35	0.319	0.312	±0.005
10	100	5	0.38	0.358	0.355	±0.01
10	150	5	0.203	0.126	0.132	±0.01
12.5	125	4	0.351	0.320	0.312	±0.005
12.5	125	4	0.352	0.321	0.312	±0.005
10	125	4	0.331	0.294	0.302	±0.01
12.5	125	5	0.33	0.293	0.287	±0.01
10	150	3	0.253	0.192	0.178	±0.01
12.5	125	4	0.35	0.319	0.312	±0.005
12.5	125	4	0.348	0.316	0.312	±0.005
15	125	4	0.337	0.302	0.315	±0.01
12.5	100	4	0.399	0.383	0.386	±0.01
15	150	5	0.221	0.150	0.142	±0.01
12.5	125	3	0.345	0.312	0.338	±0.01
12.5	125	4	0.351	0.320	0.312	±0.005
10	100	3	0.411	0.399	0.400	±0.01
15	100	3	0.433	0.428	0.416	±0.01
12.5	150	4	0.219	0.147	0.165	±0.01
15	150	3	0.261	0.202	0.198	±0.01

The obtained α - tocopherol concentrations with ultrasound ranged between 0.126 ± 0.01µg to 0.428 ± 0.01µg depending of the operating conditions of cranberry oil UAE extraction. The results are presented as per the response obtained in the CCD experiments and the overall design is presented in Table 4.11. Responses consisted of the concentration of α - tocopherol

relative to OD values of the oil samples. As can be seen in this table, the most influencing parameters on α - tocopherol concentration are the power and the sample size ratio. The best tested condition in which the α - tocopherol was found in oil by ultrasound was for a power of 100W, extracting time of 15 min and a sample size ratio of 3g/30ml of hexane. Inspite of employing different extraction durations the α - tocopherol concentration was not affected by this factor.

Table 4.12: ANOVA for the effect on power (W) and sample ratio of ultrasound assisted extraction on tocopherol concentration

Tocopherol concentration by ultrasound assisted extraction					
Source	**SS**	**DF**	**MS**	**F-Value**	***p*-value**
Model	0.1359	9	0.0151	70.96	<0.0001*
Lack of Fit	0.0021	5	0.0004	142.51	<0.0001*
Error	0.0021	10	0.0002		
Term	**Estimate**	**Std. Error**	***t-ratio***	**F-Value**	***p*-value**
Intercept	0.3120	0.0050	62.22		<0.0001*
Time	0.006	0.0046	1.43	2.0462	0.1831
Power	-0.1104	0.0046	-23.93	572.52	<0.0001*
Ratio	-0.0253	0.0046	-5.48	30.06	0.0003*
Time*Power	0.00125	0.0051	0.24	0.0587	0.8134
Time*Ratio	-0.0025	0.0051	-0.48	0.2349	0.6384
Power*Ratio	-0.0002	0.0051	-0.05	0.0023	0.9623
Time*Time	-0.0034	0.0087	-0.39	0.1501	0.7065
Power*Power	-0.0364	0.0087	-4.14	17.12	0.0020*
Ratio*Ratio	0.0010	0.0087	0.12	0.0154	0.9038
R^2	0.9845				
Adjusted R^2	0.9707				

*Significant

Response surface regression models were fitted to the experimental data. The ANOVA results of the quadratic regression model for α - tocopherol concentration in oil obtained by ultrasound assisted extraction expressed in micrograms are given in Table 4.12. The models obtained for tocopherol concentration in terms of micrograms were significant with p value ≤ 0.0001, with R^2 value of 0.9845. It was found that there is a reasonable agreement with the adjusted R^2 of 0.9707 in the model. The lack of fit was not significant (p>0.05), suggesting that the model was well fitted and could be used to predict the tocopherol concentration in oil obtained from powdered cranberry seeds by ultrasound assisted extraction. This model indicated that the linear effect of Power (W) and Sample size had the greatest significance on the tocopherol concentration in the extracted oil. Values of (p) less than 0.05 indicate the significant terms in the model, based on which the linear term such as power (W) and Sample size (R) were found to be significant.

The predictive, second-order, polynomial model for α - tocopherol concentration in oil obtained by ultrasound assisted extraction from powdered cranberry seeds, in terms of coded factors levels is shown in Eq 4.6 with a $R^2 = 0.98$.

Concentration of α – tocopherol =

$$=+0.3120+0.006(Time)-0.1104(Power)-0.0253(Ratio)$$

$$+0.00125(Time*Power)-0.0025(Time*Ratio)-0.0002(Ratio*Power)-$$

$$0.0034(Time^2)-0.0364(Power^2)+0.0010(Ratio^2) \quad (4.6)$$

The relationship between independent and dependent variables can be well illustrated with a three-dimensional response surface plot. Fig 4.14 presents the interaction of power with the concentration of α - tocopherol. It was found that as the ultrasonic power increases there is a decline in the concentration of α - tocopherol. This may be due to the formation of free radicals with the increase in pressure in the cell wall as the power increases at a given temperature which may partially consume the antioxidants released from the matrix as reported by Gelmez, (2008) in ultrasonic assisted and supercritical carbon dioxide extraction of antioxidants from roasted wheat germ and stated that supercritical carbon dioxide extraction performed better than UAE. The maximum concentration of α - tocopherol was obtained at the sample size of 3g/30ml of

hexane, 100 W giving the value of 0.428 ± 0.01 µg following 15 min of treatment at 66°C. In this method time did not affect the concentration of the α - tocopherol in the extracted oil.

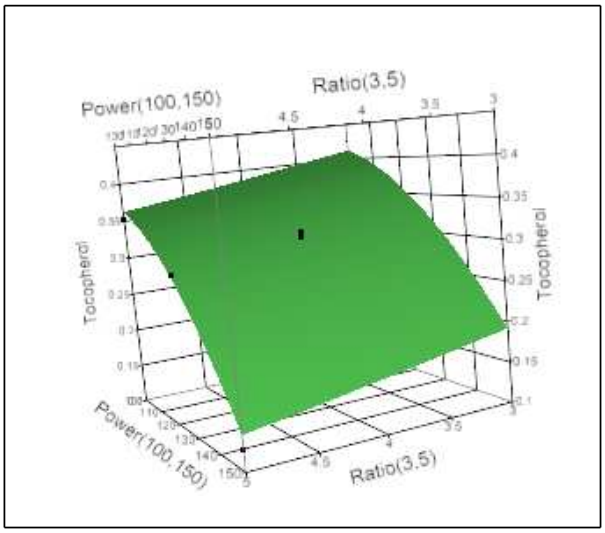

Fig 4.14: Response surface plot of the significant factor in ultrasound assisted extraction; effect of power (W) and sample size ratio (R) on α - tocopherol concentration of cranberry seed oil.

4.7 Comparison of reflux, microwave and ultrasound assisted extraction on the α - tocopherol content of extracted cranberry seed oil:

The maximum α - tocopherol concentrations (0.037 ± 0.02µg),(0.209 ± 0.02µg) and (0.266 ± 0. 02µg) obtained by heat reflux at varying temperatures of 62°C, 66°C and 70°C with the tested parameters of 3 hr, 10g/100ml of hexane; 7.5 hr, 10g/100ml of hexane and 3 hr, 10g/100ml of hexane, were significantly lower than the maximum value (0.346 ± 0.007µg) obtained by MAE at 15 min , 125 W and 5g/30ml of hexane at 66°C and (0.428 ± 0.01µg) by UAE at 15 min, 100 W and 3g/30ml of hexane at 66°C as shown in Fig 4.15. But the most important factor to consider in this context is the time and the temperature combination. In heat reflux the time taken and the temperature employed were very different which may sometimes decrease the phytonutrients and deteriorate the α - tocopherol present. In the case of microwave, both the time and the temperature are minimized thereby retaining the phytonutrients. Considering the significant reduction in process time of the MAE process compared to reflux extraction, it becomes an attractive alternative for the potential quality in oil and α - tocopherol concentration. This can be explained from the ability of microwaves to interact with the sample

matrix at the molecular level and the potential for inducing significant physical disruptions into the matrix. The greater microstructure disruption in the MAE vs heat reflux extraction results in greater porosity and higher yields associated with MAE. In case of microwave assisted extraction, the presence of water a polar solvent, influences both the amount and the rate of heating produced. When the samples are treated with microwaves, water molecules are initially targeted and they evaporate rapidly, increasing pressure on the cellwall structure. The rapid dehydration alters the cell wall structure and changes its permeability, resulting in intra-cellular contents expelled out thus enhancing the mass transfer rate (Chemat *et al*., 2005). Due to the disruption of the oil bearing structures present in the seeds, along with oil α - tocopherol extraction can be made possible using microwave as compared to conventional extraction. Oomah *et al.* (2002) obtained similar results of elevated tocopherol concentration of oil from hempseed using a microwave treatment.

As mentioned earlier in the discussion and in Fig 4.15, the maximum tocopherol yield (0.428 ± 0.01µg) obtained by UAE at 66°C with an extraction time of 15 min, 100W and 3g/30ml of hexane was significantly greater than the maximum extracted yield of tocopherol (0.037 ± 0.02µg),(0.209 ± 0.02µg) and (0.266 ± 0.02µg) obtained by heat reflux at 3 hr,10g/100ml of hexane at 62° C; 7.5 hr, 10g/100ml of hexane at 66° C and 3 hr,10g/100ml of hexane at 70° C. Here again, the most important factor to consider in this context is the time and the temperature combination. In heat reflux the time taken and the temperature employed were very different which may sometimes decrease phytonutrients and deteriorate the α - tocopherol present. In the case of ultrasound, both the time and the temperature are minimized potentially leading to a better oil quality with the retention of the phytonutrients.

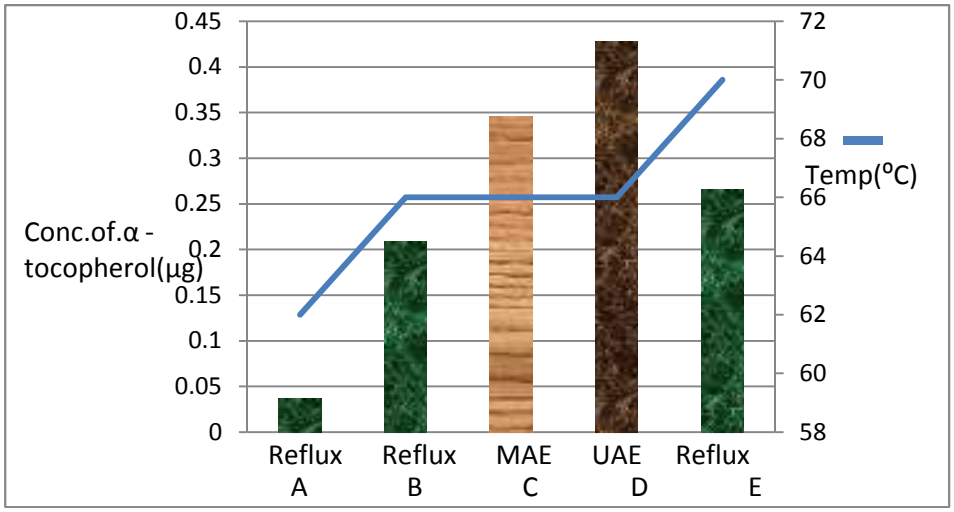

Fig 4.15: Comparison of extraction methods on α - tocopherol concentration.

Note: Reflux-Heat Reflux method; MAE-Microwave assisted Extraction; UAE-Ultrasound assisted Extraction. A-Reflux: 3hr ;10g/100ml of hexane at 62 °C .B-Reflux:7.5 hr;10g/100ml of hexane at 66°C.C-MAE:15 min;125W and 5g/30ml of hexane at 66°C.D-UAE:15 min;100W and 3g/30ml of hexane at 66°C.E-Reflux:3 hr;10g/100ml of hexane at 70°C.

Considering the significant reduction in process time of the UAE, it becomes an attractive alternative with better yield and with potential higher quality. The significant yield in a short process time obtained by ultrasound extraction as compared to heat reflux extraction comes from the ultrasound themselves. Greater ultrasonic waves are created when the power increases which leads to the creation and collapse of bubbles in a short period of time (Hemwimol *et al*., 2006) causing high pressure and shock wave generation, leading to the enhancement of penetration of the solvent into the cell tissues and the acceleration of the intracellular product release into the solvent by disrupting the cell walls. However although there is an increase in oil yield there could be also a reduction in α - tocopherol which may be due to the increase in power at a given temperature as reported by Suslick, (1998).

The maximum yield (0.428 ± 0.01µg) obtained using UAE at 66°C with an extraction time of 15 min, 100W and 3g/30ml of hexane is significantly greater than the value (0.346 ± 0.007µg) obtained using MAE at 66°C with an extraction time of 15 min, 125 W and 5g/30ml of hexane as shown in Fig 4.15. The most important factor to consider in this context is the difference in the process parameters and retention of antioxidants. In the case of microwave assisted extraction, it consists of microwave heating the sample in contact with the solvent by means of direct electromagnetic energy transfer (Venkatesh and Raghavan, 2004; Nemes and

Orsat, 2010). Microwave heating is known to be volumetric in nature so microwave irradiation produces efficient internal heating by coupling microwaves with polar components inside the solvent and the sample. This process involves disruption of hydrogen bonds, as a result of microwave-induced dipole rotation of molecules, and migration of the ions, which enhance penetration of the solvent into the matrix, allowing dissolution of the components to be extracted (Hudaib *et al*., 2003). In the case of ultrasound extraction, tocopherol's retention may be explained in terms of their release from the matrix by cavitational effects caused by the application of high-intensity ultrasound while not over processing. As large amplitude ultrasound waves travel through a mass medium, they cause compression and shearing of solvent molecules resulting in localized changes in density of solvent which impacts the plant matrix (Luque-Garcia and Luque de Castro 2003). As a consequence, an acoustic cavitation was created by these sinusoidal compression and shear waves that will improve the extraction process by releasing the product from plant matrix. (Rostagno *et al.,* 2003; Chemat *et al.,* 2004). This leads to better product recovery in reduced time and solvent usage (Wu *et al.,* 2001). Therefore in case of ultrasound-assisted extraction the α - tocopherol retention was good at lower power but decreases gradually due to pressure created in cell wall with increasing power by ultrasonic waves whereas in microwave-assisted extraction, with increasing power there is a steady retention of α - tocopherol of cranberry seed oil, as the compound is favourably released from the plant matrix without damage from heat.

4.8 Overview of maximum concentration of α – tocopherol in cranberry seed oil by various extraction processes:

Table 4.13 presents the concentration of α - tocopherol in oil obtained by four different methods (cold pressed, Heat-reflux, MAE and UAE). The results clearly indicate that the concentration of tocopherol obtained by cold pressed method (0.278 ± 0.02 μg) was greater than the concentration obtained by Heat-reflux method (0.266 ± 0.02 μg).

Table 4.13: Maximum concentration of α - tocopherol in cranberry seed oil by various extraction process (Composition of α - tocopherol in 100g of cold-pressed cranberry seed oil is 20mg as mentioned by Stone and Papas, 2003; Papas, 1999; Yang, 2003)

Cold Pressed method*	Heat-reflux method**	Microwave Assisted Extraction**	Ultrasound Assisted Extraction**
0.278 ± 0.02 µg	0.266 ± 0.02 µg	0.346 ± 0.007 µg	0.428 ± 0.01 µg

Note:* * *- Sample oil obtained from the company (Fruit d' or) for reference.* ** *- Oil extracted in the current experiment.

The difference in concentration of tocopherol can possibly be explained by the elevated temperature and long process time in heat reflux. In Heat - reflux method the temperature employed for extracting oil is between 62° C to 70° C, which may have affected the recovery of tocopherol whereas in cold pressed method the temperature used for oil extraction will be at room temperature or atmost between 40° C to 60° C, which facilitates the tocopherol extraction and hence the greater concentration of α - tocopherol by the cold pressed method. The results of tocopherol concentration obtained by MAE (0.346 ± 0.007 µg) and UAE (0.428 ± 0.01 µg) shown in Table 4.13 indicate that these values were greater than the concentration of tocopherol retained by cold pressed (0.278 ± 0.02 µg). This is because of the difference in methods of extraction i.e., microwave electromagnetic waves in the case of MAE and ultrasonic waves in UAE whereas in cold pressed, the oil extraction was performed by mechanical pressing. An important factor is the use of solvents. Oomah *et al.* (2000) and Parry and Yu, (2004) stated that solvent extraction by hexane was reported to yield maximum amount of tocopherol content in raspberry seed oil. Hence it can be stated that due to the advanced extraction technology (MAE and UAE) with a short period of time and the usage of the solvent hexane, the release of α – tocopherol from the plant matrix was higher than for cold pressed method. Morever since the availability of α - tocopherol concentration in cold pressed is greater than the heat- reflux, it can be recommended as a conventional cranberry seed oil extraction.

4.9 Conclusions:

This study indicates that microwave and ultrasound assisted extraction can be used as viable alternative extraction methods to cold-pressing or reflux heat oil extraction techniques for berry seed oils. Hexane was used as the solvent for oil extraction as it is a standard in various oil extraction applications. Initial trials with a mixture of solvents composed of Hexane:Ethanol (7:3) for the extraction lead to poor results in terms of oil yield. Powdered cranberry seeds were used throughout the experiments since preliminary extraction trials with whole seeds showed very poor oil yields.

Results indicate that in case of heat reflux, the oil yield value depends greatly on the sample/solvent ratio and temperature. The oil yield values obtained by microwave and ultrasound extraction are higher than those obtained by conventional reflux oil extraction under the same temperature condition. Maximum yield of 24.15 ± 0.3% was obtained by microwave extraction which was greater than heat-reflux extraction at 10.52 ± 0.1% under different process conditions and extraction time (heat reflux 7.5 hr; 10 g/100ml and 66°C; microwave 15 min; Power 125 W and 5 g/30 ml at 66°C). Microwave assisted extraction provided some advantages over conventional methods, such as minimised extraction time (minutes rather than hours); minimal use of solvent (30 ml rather than 100 ml) to reduce wastage of chemicals, and the quality of the extract can be retained with a reduction of phytonutrients loss in oil with controlled application of power and lower combination of time and temperature. Microwave assisted and ultrasound assisted extraction were compared for same applied power, temperature, sample size and the maximum yield of 32.35 ± 0.3% (Time-15 min; Power 150 W; Ratio-5 g/30 ml at 66°C and Ultrasonic frequency of 20,000 Hz), which was greater than the yield obtained by microwave at 24.15 ± 0.3% (15 min; Power 125 W and 5g/30 ml at 66°C). Yields obtained from conventional reflux method were also compared with ultrasound assisted extraction and the results showed that ultrasound yields 32.35 ± 0.3% (15 min; Power 150 W and 5g/30 ml at 66°C) were better than conventional method at 10.52 ± 0.1% (7.5 hr; 10 g/100ml and 66°C) which shows that ultrasonic extraction performed best as a fast, reliable and inexpensive technique.

Another set of experiments was carried out to estimate the α - tocopherol content by spectrophotometric method in the cranberry oil obtained by different methods of extraction. Results showed that UAE expressed greater values of α tocopherol concentration (0.428µg) in oil

at 100 W but gradually decreased on increasing the power whereas in other methods like MAE (0.346μg) and heat reflux extraction (0.266 μg), α - tocopherol concentrations although lower than UAE, increased gradually on increasing the intensity of the extraction process parameters. Optimum conditions for both oil yield and α tocopherol concentration were obtained from powdered cranberry seeds by heat reflux, MAE and UAE. For heat reflux the optimum process conditions are sample size ratio of 10g/100ml of hexane, 7.4 hr and 70°C which gave an oil yield of 10.99 ± 0.2% and tocopherol concentration of 0.255 ± 0.02 μg. In the case of MAE the optimized parameters were 15 min, power 125 W and sample size ratio of 5g/30ml of hexane which gave an oil yield of 24.25 ± 0.6% and tocopherol concentration of 0.351 ± 0.01 μg. In UAE, the optimized parameters were a sample size ratio of 4g/30ml of hexane, power 125 W and 15 min which gave an oil yield of 28.70 ± 0.6% and tocopherol concentration of 0.316 ± 0.02 μg. A final conclusion was made by comparing the recovery of α - tocopherol obtained using heat- reflux, MAE and UAE with cold pressed oil and found that the α - tocopherol concentration was significantly higher in case of cold pressed method than the heat-reflux method but lower than the MAE and UAE which shows that for conventional cranberry seed oil extraction the cold pressed method was the better option than heat-reflux method, otherwise newer techniques such as MAE and UAE are highly recommended.

CHAPTER V

SUMMARY AND CONCLUSION

5.1 Summary and conclusion:

Cranberries are small groups of shrubs found worldwide which constitute the genus *Vaccinium.* The North American cranberry (*Vaccinium macrocarpon*) is recognized by the United States Department of Agriculture (USDA), for its abundant source of predominant phenolic compounds and antioxidants. Cranberry oil has been extracted by cold pressing from the seeds and is known for its unique composition of tocopherols and omega fatty acids. Therefore the cranberry processing industries are focusing efforts to manufacture valuable products from cranberries.

The main objective of this research was to improve the yield and quality of oil rich in phytonutrients by different methods of extraction (heat reflux, MAE and UAE) as compared to the oil obtained through cold pressing in industry. Therefore this research was divided into two major objectives. The first objective was to extract and compare the oil yields obtained using heat-reflux, microwave and ultrasound-assisted extraction from cranberry seeds. Operational parameters such as solvent/solute ratio, extraction time, temperature and power level were investigated using central composite design. Optimum extraction conditions for heat reflux in which maximum oil yield (1.11g for a 10 g sample) was obtained are at 10g/100ml of hexane at 12 hr at 70°C. For microwave and ultrasound assisted extraction the temperature was maintained around 66° C. The optimum parameters for MAE are 125 W, 15 min and 5g/30ml of hexane and the yield of oil came around 1.20g (for a 5 g sample). In the case of UAE, the optimum extraction conditions were 150 W, 10min and 5g/30ml of hexane provided the oil yield of 1.61g (for a 5 g sample). Comparison with heat-reflux for cranberry oil from seeds confirmed the efficiency of MAE and UAE over heat reflux. The mechanisms of the wave propagation (both microwaves and ultrasounds) significantly acted on the release of the oil-based plant compounds into the solvent. Comparison was also done between Microwave and Ultrasound extraction and concluded that UAE oil yields were better than MAE because in UAE the employment of ultrasonic waves creates pressure within the cell structure rather than generation of heat

ultimately leading to greater product release. It was concluded that UAE can serve as a best option for extraction of cranberry seed oil.

Inspite of efficient extraction of oil from cranberry seeds by heat reflux, MAE and UAE, the quality of oil such as presence of phytonutrients should be examined and this was the purpose of the second objective of this research which determined the α – tocopherol content in oil through spectrophotometry. α -tocopherol is a form of vitamin E which acts as an antioxidant against free radicals. In the case of cranberry seed oil among other tocopherols present such as (gamma and delta), alpha tocopherol represents a considerable percentage composition. Calculated results revealed that extraction temperature plays an important role in the composition of tocopherol (0.266 ± 0.02 µg) in oil extracted through heat reflux but in the case of MAE the microwave power and ratio played an important role which yielded 0.346 ± 0.007 µg tocopherol, whereas in UAE, 0.428 ± 0.01 µg tocopherol was obtained which was greatly affected by the level of ultrasonic power. An overall comparison of the quality of the oil based on the α -tocopherol concentration obtained from these extraction methods to the cold pressed oil obtained from the company *Fruit d'or* revealed that the tocopherol concentration of the cranberry oil obtained through cold pressed method is found to be (0.278 ± 0.02 µg) which is slightly greater than (0.266 ± 0.02 µg), the concentration of tocopherol retained by heat reflux but less than the concentration of tocopherol retained through MAE (0.346 ± 0.007 µg) and UAE (0.428 ± 0.01 µg) respectively.

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