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Identification and quantification of fatty acids in nut oils by GC-MS

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Abstract

The main objective of this thesis was to identify and quantitate the fatty acid composition in the following nine nut oils; almond (*Prunus dulcis*) oil, hazelnut (*Corylus avellana*) oil, peanut (*Archis hypogaea*) oil, walnut (*Juglans regia*) oil, macadamia (*Macadamia tetraphylla*) oil, pistachio (*Pistacia vera*) oil, kukui (*Aleurites moluccans*) oil, tamanu (*Calophyllum inophyllum*) oil, and argan (*Argania spinosa*) oil. Nuts contain carbohydrates, unsaturated fatty acids, vitamins, minerals, fibers, and antioxidants. Several studies associate nut consumption with lower cholesterol levels, hence reducing the risk of coronary heart diseases. Other studies have reported reduced risk of diabetes in women, and prostate cancer in men. Nuts have a beneficial fatty acid (FA) composition with more than 82% mono- and polyunsaturated fatty acids (MUFAs and PUFAs) and a low content of saturated fatty acid content (SFAs) at 18%. The FA composition in all nine nut oils was identified and quantified using a gas chromatograph (GC) coupled to a sector mass spectrometer (MS). The FAs in the nut oils were esterified into fatty acid methyl esters prior to the GC-MS analysis.

The unsaturated FAs content in the nut oils ranged from 71% in tamanu oil to 93% in almond oil, and the SFA content ranged between 7% in almond oil to 29% in tamanu oil. The MUFA C18:1n-9 was the most abundant FA in all nut oils, except for walnut- and kukui oil, where the essential PUFA, linoleic acid (LA, C18:2n-6) was the most abundant FA. Moreover walnut- and kukui oil had the highest content of the other essential FA alpha-linoleic acid (ALA, 18:3n-3) with the lowest n-6/n-3 ratio at 5 and 2. However, kukui oil is not edible so further refining and detoxification are needed prior to consumption. Consumption of walnuts or walnut oil can therefore contribute to higher n-3 PUFA content in the diet, and can therefore be argued that walnut oil is the most health promoting nut oil.

Sammendrag

Hovedmålet med denne oppgaven var å identifisere og kvantifisere fettsyreprofilene til nøtteoljene mandelolje (*Prunus dulcis*), hasselnøttolje (*Corylus avellana*), peanøttolje (*Archis hypogaea*), valnøttolje (*Juglans regia*), macadamiaolje (*Macadamia tetraphylla*), pistasjolje (*Pistacia vera*), kukuiolje (*Aleurites moluccans*), tamanuolje (*Calophyllum inophyllum*), og arganolje (*Argania spinosa*). Nøtter inneholder rikelig med næringsstoffer som karbohydrater, umettede fettsyrer, vitaminer, mineraler, fiber og antioksidanter. Den fordelaktige fettsyresammensetningen i nøtter består av over 82% en- og flerumettete fettsyrer og under 18% mettede fettsyrer. Tidligere studier indikerer en sammenheng mellom det å spise nøtter med et lavere kolesterol nivå, som kan være en innvirkende faktor for å redusere risikoen for å få hjerte- og karsykdommer. Andre studier i rapporter også redusert risiko for diabetes hos kvinner, og prostatakreft hos menn. Fettsyresammensetningen i alle ni nøtteoljene ble identifisere og kvantifisert ved bruk av en gasskromatograf (GC) koblet til ett sektor massespektrometer (MS). Fettsyrer i alle nøtteoljene ble esterifisert til fettsyremetylestere før GC-MS-analyse.

Det umettede fettsyreinnholdet i nøtteoljene var fra 71 % i tamanuolje til 93% i mandelolje, mens det mettede fettsyreinnholdet var fra 7% i mandelolje til 29% i tamanuolje. Den enumettede fettsyren C18:1n-9 var fettsyren som forekom mest i alle nøtteoljene, utenom i valnøtt- og kukuiolje. I disse oljene var det den essensielle flerumettete fettsyren linolsyre (LA, C18:2n-6) som var den fettsyren som forekom mest. Videre hadde valnøtt- og tamanuolje høyest innhold av den andre essensielle fettsyren α-linolensyre (ALA C18:3n-3) og disse nøtteoljene var oljene med lavest n-6/n-3 forhold på henholdsvis 5 og 2. Selv om kukuiolje ikke kan spises, kan oljen gjøres spiselig ved filtrering og detoksifikasjon. Å spise valnøtter vil bidra til et høyere n-3 flerumettete fettsyre innhold i kosten, og det kan derfor argumenteres for at valnøttolje er den mest helsebringende nøtteøljen.

Abbreviations

ALA	Alpha-linoleic acid
CHD	Coronary heart disease
DAG	Diacylglyceride
DHA	Docosahexaenoic acid
EI	Electron ionization
EFA	Essential fatty acid
EPA	Eicosapentaenoic acid
FA	Fatty acid
FFA	Free fatty acid
FAME	Fatty acid methyl ester
IS	Internal standard
LA	Linoleic acid
MAG	Monoacylglyceride
MS	Mass spectrometry
MUFA	Monounsaturated fatty acid
NL	Neutral lipid
GC	Gas chromatography
PL	Polar lipid
PUFA	Polyunsaturated fatty acid
SFA	Saturated fatty acid
TAG	Triacylglyceride
UFA	Unsaturated fatty acid

1. Introduction

Nuts are one of the most nutritionally dense foods available, and have been a part of the human diet since the stone age (Eaton & Konner, 1985; Kirbaslar et al., 2012; Ros & Mataix, 2006; Wilkinson, 2005). The general term 'nuts' have several definitions, a common one botanical definition, is that "nuts are a seed or a fruit with an edible kernel and a hard-shell" (Wilkinson, 2015). Common edible nuts are almonds, brazil nuts, cashew nuts, hazelnuts, macadamia nuts, pine nuts, pecan nut, pistachio nuts and walnuts. Consumers also regard the groundnut peanut as a nut even though it is actually a legume in the pea family (A.Britannica, 2017). Peanuts have the same nutrient profile as tree nuts and are in this study included in the term 'nuts' (Wang, 2018).

In general nuts contain nutrients such as carbohydrates, dietary fibers, proteins and unsaturated FAs (Brufau et al., 2006; Ros & Mataix, 2006; Salas-Salvadó et al., 2006; Wilkinson, 2005). Other minor nutrients are minerals, vitamins, phytosterols and antioxidants (Segura et al., 2006; Wilkinson, 2005). Nuts are a good source of lipids containing up to 75% of the weight (Miraliakbari & Shahidi, 2008). These lipids are 90% in triacylglycerides (TAGs), which are rich in esterified mono- and polyunsaturated fatty acids (MUFAs and PUFAs) , hence a low saturated fatty acids (SFA) content in nuts (Miraliakbari & Shahidi, 2008; Ros & Mataix, 2006). Although, mono- and diacylglycerides (MAGs and DAGs) and sterol esters are present in nuts, though in low quantities. Nuts are considered a good source of fats because of the high MUFA and PUFA (84-96%) content and low SFA content(Ros, 2010). Studies shows that substitution of SFAs in the diet with unsaturated FAs could decrease the cholesterol level (Ros & Mataix, 2006). While high intake of SFAs, meat and manufactured *trans*-FAs are increase cholesterol levels. Therefore, associated with the development of cardiovascular diseases (Ascherio et al., 1999; Ros & Mataix, 2006). The MUFA, oleic acid (18:1n-9), is the most abundant FA in most nuts, except for walnuts, pine nuts, and brazil nuts, where the PUFA linoleic acid (LA, C18:2n-6), is the most abundant FA. Additionally, walnuts are also rich in α-linolenic acid (ALA, C18:3n-3).

FAs are absorbed in humans, as non-esterified free fatty acids(FFAs) detached from the TAG structures as free fatty acid (FFAs), in addition MAGs could also be absorbed in the body (Christie, 2003; Gutnikov, 1995; Michalski et al., 2013; Quehenberger et al., 2011; Ros, 2010).

Oxidized FAs provides twice as much energy than polysaccharides and is therefore an efficient way to store energy in humans (Ros & Mataix, 2006). FAs have several important biological and functional roles in the human body. LA and ALA are the two essential fatty acids (EFAs) the human body cannot synthesize. LA acts as a precursor for arachidonic acid (AA, 20:4n-6) and ALA as a precursor for the two longer chained FAs; eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3) (Harris, 2005; Simopoulos, 1991). DHA are important for normal growth and development of the retina, and brain, while EPA lower cholesterol levels, and its antithrombic effects (blood clots) (Bang et al., 1976; O'Brien & Sampson, 1965; Simopoulos, 1991)

Nuts are either consumed raw or salted as snacks, or incorporated in food products such as ice cream, baked goods and sauces. In addition, nut oils are used as an additive in cosmetics (Geiselhart et al., 2018). Dietary guidelines including nuts and media promotion of the potential health benefits, have increased the worlds tree nut production by 24% in the last 10 years. nut consumption increases ((INC), 2018; Ros, 2010; Wien, 2017). Several epidemiological studies have shown health benefits from nut consumption, where frequent nut intake reduce the risk of coronary hearth diseases (CHD) (Ros, 2010; Ryan et al., 2006), prostate cancer (Jiang et al., 2002) and for women reduced risk of diabetes (Jain et al., 1999). Studies associate the vitamin E, antioxidants, fibers, minerals, folates, phytosterols and the FAs content to attribute to these health benefits (Ros, 2010; Wilkinson, 2005). Several studies have researched the nutrient profile in nuts to investigate potential health benefits (Ros, 2010). Though, this study will concentrate on the FA composition in commercially available nut oils by use of gas chromatography (GC) coupled with a mass spectrometer (MS).

There are three main extraction methods to extract the oils from nuts: expeller-, solvent- and enzymatic extraction (Atabani et al., 2013). The most conventional method is expeller extraction, where the oilseeds are pressed by a mechanical press or an electrical screw press. Solvent extraction removes the crude oil from the seed by using an organic liquid solvent, while enzymatic extraction utilizes enzymes to extract the crude oil. Then the crude oils are prepared by filtration, and refining or partial refining into final oils. Refined oils contains the oilseeds TAGs, while partially refined oils contain TAGs and phospholipids (Atabani et al., 2013; Michalski et

al., 2013; O'Brien, 2004). To which degree these final steps from crude oil to final oil are done depend on the oil's application as either food oil, cosmetic oil or in varnishes or even in biofuels (Atabani et al., 2013)

In this study the food oils hazelnut-, peanut-, walnut- and pistachio oil and the cosmetic oils tamanu-, kukui-, almond-, and argan oil are studied. Almond- and argan oil are available as food oils, while only small amounts of kukui oil could be used for food purposes. Regarding tamanu oil there are no food oil alternative available. In this study the FAs profile are investigated from a nutritional perspective.

1.1 Aim of the study

The aim of this study was to identify and quantify the FAs in the following commercially available nut oils: almond oil (*Prunus dulcis)*, hazelnut oil *(Corylus avellana)*, peanut oil (*Archis hypogaea*), walnut oil *(Juglans regia)*, macadamia oil *(Macadamia tetraphylla)*, pistachio oil *(Pistacia vera)*, kukui oil *(Aleurites moluccana)*, tamanu oil *(Calophyllum inophyllum)*, and argan oil *(Argania spinosa).* These profiles (obtained by GC-MS) are then consider the potential health benefits, with focus on MUFAs, PUFAs, and the amount of LA and ALA for each nut oil.

2. Theory

2.1 Lipids

Lipids are naturally occurring compounds soluble in organic solvents such as benzene and chloroform (Hart et al., 2011). However, in reality some lipids are often more soluble in water than organic solvents (Christie, 2003). A more accurate definition suggested by Christie (2003) is "that lipids are FAs and their naturally occurring derivatives (esters or amides), together with compounds closely related through biosynthetic pathways e.g. prostanoids and alcohols" (Christie, 2003). Lipids contribute to vital metabolic processes in living organisms, where phospholipids and sterols are important structural elements in cell membranes and the TAGs are usually stored as energy (Nelson & Cox, 2017). Fats and oils are characterized by their state at room temperature. Fats contain high amount of SFAs and hence they are solid at room temperature. Oils containing high amounts of MUFAs and PUFAs and hence they are liquids at room temperature, vegetable oils are normally derived from plants such as soybeans, olives, and nuts. Although coconut- and palm oil are vegetable oils, thus they are solid at room temperature, due to high contents of SFAs and *trans*-FAs (Srigley & Mossoba, 2016). Plant seeds rich in oils have TAGs as main storage reserves, because carbohydrates takes more space and contain less calories than fat (Bockisch, 1998). Plant seeds not rich in oils have starch as the major storage reserve instead. Lipids such as glycolipids and phospholipids are present only in low amounts in these starch rich plants. Sterols and ceramides are other lipids also present in plants (Christie, 2003). The three most abundant FAs in vegetable oils are palmitic acid (C16:0), oleic acid (C18:1n-9) and linoleic acid (LA, C18:2n-6) (Christie, 2003). In fact the MUFA, C18:1n-9 is the most abundant FA in most plants and animals, while LA is the most abundant PUFA (A.Gunstone et al., 2007; Rustan & Drevon, 2001).

2.1.1 Fatty acids

Fatty acids are hydrocarbon chains with a carboxylic group at one end (fig. 2.1). (Rustan & Drevon, 2001). The term "saturated fatty acids" is used when the hydrocarbon chains are without double bonds, whereas the term “unsaturated fatty acids is used when there are one or more double bonds. Additionally, the double bonds are mainly methylene interrupted (Christie, 2003;

Nelson & Cox, 2017). The most common FAs originating from plants and animals have a chain length of 16-22 and even numbered, usually with zero to six *cis* double bonds. but FAs. Though, FA up to 100 carbon atoms, *trans*-double bonds and odd numbered chains occur in nature, but are rare (Christie, 1998; Christie, 2003; Nelson & Cox, 2017). Overall, FAs vary in complexity from simple saturated short carbon chains to long complex unsaturated chains with different *cis/trans*- configuration- and different positions of the double bonds. In addition, the FAs may carry additional functional groups such as keto-, hydroxyl-, peroxy- and epoxy groups (Gutnikov, 1995; Quehenberger et al., 2011).

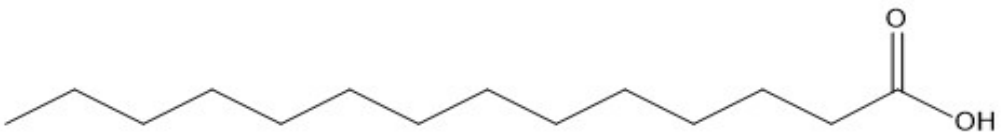


Fig. 2.1 The fatty acid myristic acid (C14:0) with a carboxylic group shown on the right.

2.1.1.1 Nomenclature of fatty acids

Systematically FAs are named with the IUPAC-system as carboxylic acid derivatives, where the chain is numbered from the carboxylic carbon. The name originates from the saturated hydrocarbon with the same number of carbons, but the -e endings are replaced with -oic (Christie, 2003). If one double bond is present the *cis*/*trans*- configuration and position of the double bond is written before the hydrocarbon name. When, two or more double bonds are present the -oic ending changes to -dienoc, -trienoic, and -tetraenoic etc. (for two, three, and four double bonds, respectively). Consistenly, the *cis/trans*-configuration are implemented in the name such as in figure 2.2. Trivial names were used before the systematic nomenclature was introduced, and is still widely used in literature. The names usually reflect common or early source of the acids, but does not give any structural information. Though, shorthand names provide information about the structure, where the number before the colon represent the number of carbons in the chain and the number after the colon represent the number of double bonds. The shorthand name C18:2, therefore indicates a chain length of 18 carbons atoms with two double bonds. However, the shorthand name does not explain, where these double bonds are, or the *cis/trans*-configuration. More accurately the shorthand name C18:2 could be written C18:2n-6c, where the position of

double bond is denoted by 'n-x' (or with the Greek 'ω-x'), and x is number of carbon atoms from the double bond to the methyl end (also called omega). If two double bonds are present, it is recommended to add the *cis/trans*-configuration as a 't' for *trans* or 'c' for *cis* such as in figure 2.2. (Christie, 2003; Scrimgeour, C. M. & Harwood, J. L., 2007). An overview of the three nomenclatures for FAs is illustrated in figure 2.2.

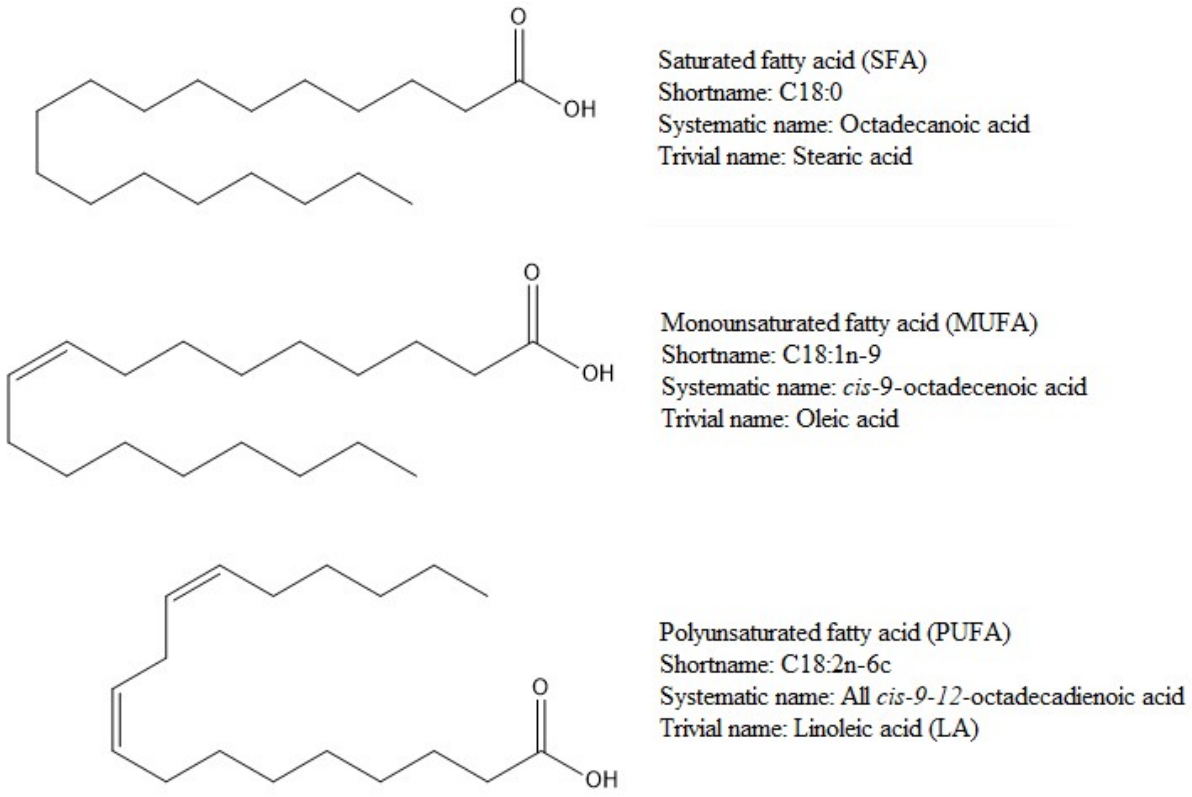


Fig. 2.2 Nomenclature of three FAs common in nuts. On top stearic acid, also known as C18:0 and octadecanoic acid. In the middle oleic acid, also known as C18:1n-9 and *cis*-9-octadecenoic acid. At the bottom linoleic acid, LA, also known as C18:2-6c and *all cis-9-12*-octadecadienoic acid.

2.1.1.2 Acylglycerides

The human body absorbs non-esterified FFAs on demand, which are released from TAGs from the glycerol backbone (Michalski et al., 2013; Quehenberger et al., 2011). To distinguish the stereochemistry of the acylglycerides the 'sn' prefix refers to the glycerol backbone at vertical position, where sn-1 is the upper carbon, sn-2 is the middle carbon and sn-3 the lower carbon (C-1, C-2, and C-3, respectively) as in figure 2.3. When the prefix is skipped, or the 'x' prefix is used, the stereochemistry is unknown or unspecified. The 'rac' prefix indicates racemic (equal amount of the isomers) stereochemistry (A.Gunstone et al., 2007; Christie, 2003).

MAGs are monoesters with a glycerol backbone (propane-1,2,3-triol) where one of the hydroxyl groups is esterified with a FA. MAGs occur in two isomers 1-MAG and 2-MAG (fig 2.3) depending on where the ester bond is positioned on the sn-1 or the sn-2. DAGs are diesters with a glycerol backbone, where two of the hydroxyl groups are esterified with FAs. DAGs exists in three structural isomers sn-1,2-DAG, or sn-2,3-DAG and sn-1,3-DAG depending on which carbons the ester bonds are positioned (Goñi & Alonso, 1999). In TAGs all three hydroxyl groups are esterified with FAs, where all FAs could be the same or two or three FAs, could be different (fig 2.3) (A.Gunstone et al., 2007; Christie, 2003).

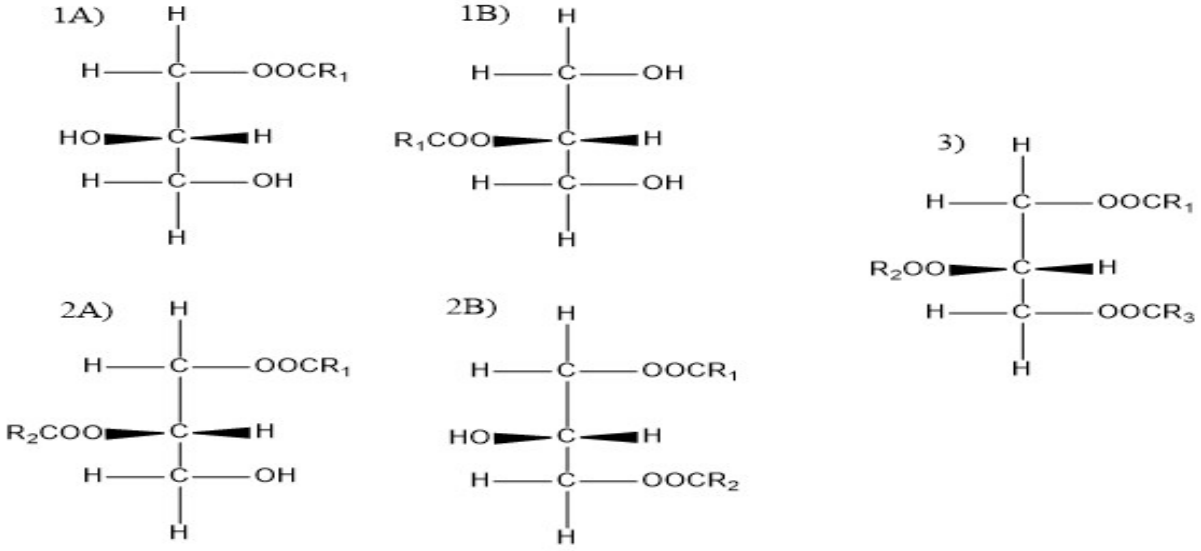


Fig 2.3 Fischer projections illustrates isomers of MAGs, DAGs and TAG. 1A) is sn-1 MAG and 1B) is sn-2 MAG. 2A) is sn-1,2-DAG or sn-2,3 MAG and 2B is sn-1,3-DAG. A 3) Illustrates TAGs where two esterified FAs are on the same side and the third at the opposite side. Based on illustration from Scrimgeour, C.M. and Harwood, J.L. (2007).

2.1.1.2 Saturated, monounsaturated and polyunsaturated fatty acids

The FAs are classified into SFAs, MUFAs and PUFAs based on the number of double bonds. The most common plant SFAs have a chain length 14, 16 and 18 carbon atoms as the most common in plants (Rustan & Drevon, 2001; Scrimgeour, C. M. & Harwood, J. L., 2007). The most common MUFAs have one *cis*-double bond and a chain length of 16-22 carbon atoms. Positional isomers and MUFAs with *trans*-double bond are found in nature and are therefore present in a biological lipid sample. PUFAs have two or more double bonds in the carbon atom chain, where the first doubled bond usually is between the third and fourth carbon atom from the methyl end (Rustan & Drevon, 2001).

2.1.1 Fatty acids and potential health benefits

n-6 (omega-6) PUFAs have the first double bond between the sixth and seventh carbon atom from the methyl end. While n-3 PUFAs (omega-3) have the first double bond between the third and fourth carbon atom from the methyl end. The n-6 FAs are represented by EFA, LA (C18:2n-6) and n-3 FAs are represented by EFA, ALA (C18:3n-3), and these two must be obtained through the diet (de Lorgeril & Salen, 2004; Simopoulos, 1991). Since animal enzymes cannot introduce double bonds before the sixth carbon. In plants LA(C18:2n-6) is more abundant than ALA because LA(C18:2n-6) is found in most plant seeds (Christie, 2003; Simopoulos, 1991). While, ALA(C18:3n-3) on the other hand is found in chloroplast on green leafy vegetables (Simopoulos, 1991), baked beans, walnuts, flaxseed oil and soybean oil (Li et al., 2002 referred in Li et al., 2006). EFAs obtained through the diet are added extra double bond and the chain lengths are increased to metabolize LA (C18:2n-6) into AA (C20:4n-6) and ALA (C18:3n-3) into EPA (C20:5n-3) and DHA (C22:6n-3) humans and animals except carnivores by increasing the chain length and adding extra double bonds (De Gómez Dumm & Brenner, 1975; Gerster, 1998; Simopoulos, 1991). EPA and DHA are mainly found in fish oils, while ARA is found in the phospholipids of grain-fed animals. Bang and Dyerberg in the 1970s emphasized the EPA's contribution in the prevention of heart attacks in Eskimos. Since EPA lowered cholesterol levels, increased bleeding time, and had antithrombotic effects (reducing the formation of blood clots) (Bang & Dyerberg, 1972; Bang et al., 1976). DHA is important for the normal development of

the brain and retina, since DHA is the most abundant FA in the structural lipids in brain and the retina (Anderson, 1970; O'Brien & Sampson, 1965).

In Simopoulos (1991) review on omega-3 FA in health and disease emphasize the n-3 FAs importance for growth and development, throughout the life cycle of humans. N-3 FAs replace n-6 FAs in the cell membranes, decrease TAGs in the human body and modulate prostaglandin metabolism. Additionally, the n-3 FAs from several studies have shown n-3 FAs contribution on reduced of CHD's (Simopoulos, 1991). Humans evolved on a diet with low SFAs a balanced n-6 and n-3 ratio of ~1 from vegetable sources and animal sources. In 1987, the western countries diets had an imbalance of n-6 and n-3, where the ratios were between ~10 and ~ 20-25:1 from vegetable and animal sources. Bjerve et al. (1989) recommended 450-650 mg ALA, and 250-450 mg EPA + DHA in a normal 2000 kcal diet. Whereas, a newer study from de Lorgeril and Salen (2004) recommends a dietary intake of ALA to be 2 g per day or 0.6-1% of the total energy intake. Since plants cannot convert ALA to EPA and DHA, vegans are dependent on ALA rich plant-based diets to metabolize EPA and DHA (Gerster, 1998).

Increased n-3 FAs consumption would not prevent CHD, but by including n-3 FAs in the diet may help the prevention of CHD (Simopoulos, 1991). In addition to help prevent of other nutritional related diseases, such as diabetes (Tapsell et al., 2004).
Studies has shown that n-3 PUFAs would improve inflammatory conditions such as rheumatoid arthritis (Simopoulos, 1991). Other studies showed that symptoms of depression could be improved by n-3 FAs (Husted & Bouzinova, 2016; Li & Sinclair, 2002). Diets rich in SFAs increase the cholesterol levels, while n-3 FAs decrease the cholesterol levels and TAGs. High amount cholesterol in the blood veins may to plaque formation and potential blood clots (Mathews et al., 2000). Nutritionists have therefore emphasized adding fish or fish oils rich in n-3 FAs in Western diets to obtain a normal balance between n-3/n-6 FAs (Mathews et al., 2000). Tapsell et al. (2004) stated that 30g walnuts per day should give enough PUFAs to improve lipid profile for people with diabetes. Another study reports that almond consumption could reduce colon cancer risk (Davis & Iwahashi, 2001).

2.2 Nut production and nut oils

The USA, China and Turkey contributes with 57% of the worlds tree nu production, where almond, pistachios and walnuts are the main produced nuts ((INC), 2018). Additionally, China is the main producer of peanuts followed by India, the USA and Nigeria. In high economic countries such as the USA, and most of the Western European countries almond, walnut, cashews and pistachios are the most consumed nuts (39%, 18%, 17%, 11%, and 8%, respectively) ((INC), 2018). In middle economic countries such as China, India, Turkey and Vietnam the most consumed nuts are: walnuts, cashew, pistachio, almond and hazelnuts (29%, 22%, 19%, and 5%, respectively) (INC, 2018). Promotion of potential health benefits from nut consumption, as well as improved economy scale have led attraction to nut production as an investment (Wilkinson, 2005).

Almond oil (*Prunus dulcis*)

Almond oil is extracted from *Prunus dulcis* or *Prunus amygdalus* nuts. Almonds are belived to originate from Asia, and from there traded to the rest of the world (Janick & Paull, 2008). Sweet edible almonds (fig 2.4) are produced for oil, eaten as snacks, or used as an ingredient in several dishes, and food products (Geiselhart et al., 2018; Wilkinson, 2005). Non-edible bitter almonds for soaps and perfumes (Wilkinson, 2005). The high C18:1n-9 content in almond oil is preferable in massage oils and in skin-care products. Studies associate almond consumption with lower cholesterol levels, and may reduce the risk of CHD's (Scrimgeour, C. M. & Harwood, J. L., 2007).

Fig. 2.4 Picture of almonds. Photo by Tina Øvrebø.

Argan oil (*Argania spinosa*)

Argan trees are important for the ecosystem in Morocco to prevent desertification (A.Gunstone et al., 2007; Janick & Paull, 2008). Argan tree nuts are known for the high-quality oil, which Moroccan rural people substitute for olive oil. Argan oil is used in pharmacological industry and in skin-care applications. The oil has gone from being a valuable oil for the people of Morocco to becoming a high-priced oil worldwide (Janick & Paull, 2008). Nuts from argan trees are inedible for humans, so the kernels (fig 2.5) are utilized for oil production only. FAs are 99% of the oil contain FAs, and the last 1% is tocopherols (vitamin E compounds). The FAs in argan oil is 80% unsaturated FA with C18:1n-9 as predominant MUFA, and linoleic acid as the predominant PUFA (Janick & Paull, 2008).

Fig. 2.5 Argan fruit and kernel (Roger Culos).

Kukui oil *(Aleurites moluccans*)

The kukui tree have several names, candlenut, candleberry, Indian walnut and Varnish tree (fig 2.6) (Phytexence, 2015). The tree are native to Indonesia, Malaysia and Hawaii, and are today widespread in the tropic regions (Elevitch & Manner, 2006; Janick & Paull, 2008). The kukui nut is toxic due to high iodine values, in addition may contain high amounts of cyanide (A.Gunstone et al., 2007; Phytexence, 2015). Furthermore, the nut seed can contain toxic compounds which can cause vomiting, and diarrhea (Gonzalez-Stuart & Rivera, 2017). Today kukui oil is used in the cosmetic industry, as well as a treatment for burns, eczema, itches and dry skin (A.Gunstone et al., 2007). The oil is rich in PUFAs, especially (18:2n-6) and (C18:3n-3) (Ako et al., 2005).

Fig. 2.6 Kukuinut tree with flowers and kukui fruits with seed inside. ©Elevitch

Hazelnut oil (*Corylus avellana*)

Hazelnut is the third most produced tree nut after almond and walnut. The nuts are grown in Turkey, Italy, Australia, New Zealand, USA and Spain. Hazelnut kernels are eaten as snacks, used in chocolate products and in baked goods (fig. 2.7). The FA composition of hazelnut oils are similar to olive oil (Janick & Paull, 2008). The kernels contain roughly 60% oil, with oleic acid (18:1n-9) and linoleic acid (18:2n-6) as the major FAs (A.Gunstone et al., 2007). Hazelnuts are also a rich source of vitamin E, and antioxidants (Geiselhart et al., 2018; Janick & Paull, 2008).

Fig. 2.7 Hazelnuts kernels. Photo by Tina Øvrebø.

Macadamia oil (*Macadamia. tetraphylla*)

Two species of macadamia are commercially grown to produce gourmet oil and skin-products: Smooth shelled *Macadamia integrifolia* and rough shelled *M. Tetraphylla* (B.Gunstone & Harwood, 2007). Macadamia originate from Australia, and are mainly produced in Australia and Hawaii. The kernels are either roasted (fig. 2.9), incorporated in bakery goods or pressed for the high-quality oil Used in both cooking and skin-products (Janick & Paull, 2008). The macadamia nut consumption has increased due to scientific evidence associating macadamia consumption to lower risk of CHD's. Macadamia oil is popular in skin-care products, since the oil contain high levels of SFAs (B.Gunstone & Harwood, 2007).

Fig. 2.8 Picture of macadamia kernels photo by Tina Øvrebø

Pistachio oil (*Pistachia* vera)

Pistachios originate from Asia and *Pistachia vera* is the only pistachio specie which is edible, it is grown commercially in Iran, California, Turkey, Greece, and Italy (B.Gunstone & Harwood, 2007; Janick & Paull, 2008). Pistachios are mostly eaten fresh or roasted with or without salt, it is also and used in baked goods, candy, sausages and as a flavor in e.g. ice cream (fig. 2.9). Only a small amount of the produced pistachios is pressed for oil because of its low-fat content, around 55%, which is lower than other tree nuts and peanuts. The oil is mainly produced for food and cosmetic purposes (B.Gunstone & Harwood, 2007).

Fig. 2.9 Picture of pistachios kernels inside their shells. Photo by Tina Øvrebø

Peanut oil (*Arachis hypogea)*

Peanuts are called groundnuts or earthnuts, since they grow in the ground. Peanuts are a legume, where, the nuts are enclosed in a shell that is attached to the roots underground (A.Gunstone et al., 2007). Peanuts originates from the South American continent and the *A*rachis *hypogea* specie is commercial produced. China, India, Nigeria, and the USA are the main peanut producers. The FA composition in peanuts are similar to olives and in China 50% of peanuts produced are used for oil extraction, 30% for food, 7% is exported, and 8% is used for seed preserving. The peanut kernels contain roughly 45 % oil, rich in C18:1n-9, and LA, C18:2n-6, hence low SFA content (A.Gunstone et al., 2007; Wang, 2018).

Fig. 2.10 Photo of peanuts with two kernels inside the shells. Photo by Tina Øvrebø

Tamanu oil (*Calophyllum inophyllum*)

Tamanu trees are also called kamani, alexandrian laurel, beach mahogany, beauty leaf and oil nut tree (Friday & Okano, 2006). The tamanu tree is produced in the South-Pacific (Islandtrend, 2017). The oil in the kernels comes from two weeks dehydration from the sun on the ground. Though, tamanu oil is not edible, but could be after refinement and detoxification (Lim, 2012). However, the oil is mostly used as a treatment for burns, skin-diseases, wounds, in oil lamps, varnishes, soaps, skin moisturizers, and could even be used as biodiesel since the FAs composition meets the biodiesel requirements in the USA, and EU (Azam et al., 2005; Lim, 2012). The oil contain mostly MUFAs, then similar amounts of PUFAs and SFAs (B.Gunstone & Harwood, 2007).

Fig. 2.11 Tamanu fruit with one large seed kernel inside. Photo by Friday and Okano (2006)

Walnut oil (*Juglans regia)*

Walnut (*Juglans regia*) is called English- or Persian walnut and originates from Iran and its native habitat ranges from Iran to Turkey, China, and Himalaya (Janick & Paull, 2008; Wilkinson, 2005). China, France and India are the main producers of walnuts. Walnuts are consumed as snacks or dessert, or incorporated in baked goods (Janick & Paull, 2008). Walnuts are regarded as health promoting because of low the n-6/n-3 ratio of 5 and studies reports lower cholesterol levels from frequent walnut consumption (Li et al., 2006; Tapsell et al., 2004; Vingering et al., 2010). Walnut oil is rich in PUFAs phytochemicals, antioxidants and low in SFAs (B.Gunstone & Harwood, 2007).

Fig. 2.12 Three walnuts with shell. Photo by Tina Øvrebø

2.3 Analysis

2.3.1 Internal standard

An internal standard (IS) is commonly used for quantitative analysis in chromatography. Where a known amount of IS with similar concentration as the analytes is added to the sample before sample preparations. The IS could then cancel out minor potential data variations, obtained during the sample preparations procedures. The use of IS do not require equal sample volumes or response factors. Though, the selected IS should never be a component in the sample and never overlap any sample peaks. Additionally, the IS should elute near the analytes, be chemically similar and well resolved from the analytes. Lastly the IS should have high purity. Several ISs may be applied if several analytes with different chemical properties are to be determined (McNair & Miller, 2009).

2.3.2 Extraction

The most common sample preparation methods s are extraction and derivatization to prepare the analytes prior to liquid injection on the GC (McNair & Miller, 2009). The extraction method a dissolved chemical X from a liquid phase A, by bringing the solution in contact with a liquid B, where liquids A and B are immiscible (Wells, 2003). Liquid-liquid extraction (LLE) moves the analytes from a aqueous liquid phase (raffinate) into an organic solvent phase (extraction phase), based on the analytes and the two immiscible liquid solubility (Li et al., 2013; McNair & Miller, 2009; Miller, 2005). The opposite is also used, where the raffinate is organic and extraction solvent is aqueous liquid. Agitation is essential to ensure that all the analytes encounters with the extraction solvent for the analytes to migrate over to the extraction solvent. An equilibrium occur since the analytes may be present in both liquid phases. The equilibrium is explained by the partition coefficient K_p (Eq 2.1), explaining the analytes[A] concentration ratio between the liquid phases.

$$[A]_{raffinate} \underset{\rightleftharpoons}{K_p} [A]_{extraction\ solvent} \qquad \text{Eq} \quad 2.1$$

High K_p value indicate that all, or almost all, analytes of interests have migrated to the extraction

solvent (Simpson, 2000). The K_p is affected by several factors, such as temperature, pH and agitation speed (McNair & Miller, 2009; Miller, 2005).

Solid-phase extraction (SPE) is another a simple and a rapid extraction technique, where the extraction solvent is a solid phase rather than a liquid. In general, analytes distribute between the liquid sample and the solid stationary phase surface. By changing the solvents composition, the interferences are washed away. So that the analytes of interest later can be are eluted out of the SPE cartridge/column. The analytes [A] distribution between the solid phase and the liquid phase are explained by the distribution coefficient K_D (Eq 2.2).

$$K_D = \frac{[A]_{liquid\ sample}}{[A]_{extraction\ solve}} \qquad \text{Eq. 2.2}$$

Figure 2.13 explains the four steps of SPE: conditioning, load of sample, wash and elution. Conditioning activates the sorbent surface inside the SPE cartridge/column to ensure reproducible retention of the analytes of interest, and may remove impurities already in the cartridge (Wells, 2003) . The second step is to load the sample onto the column. Then at the next step undesired matrix components are removed from the sorbent. The final step is adding elution solvents in order to recover the analytes of interest from the solid phase (Simpson, 2000).

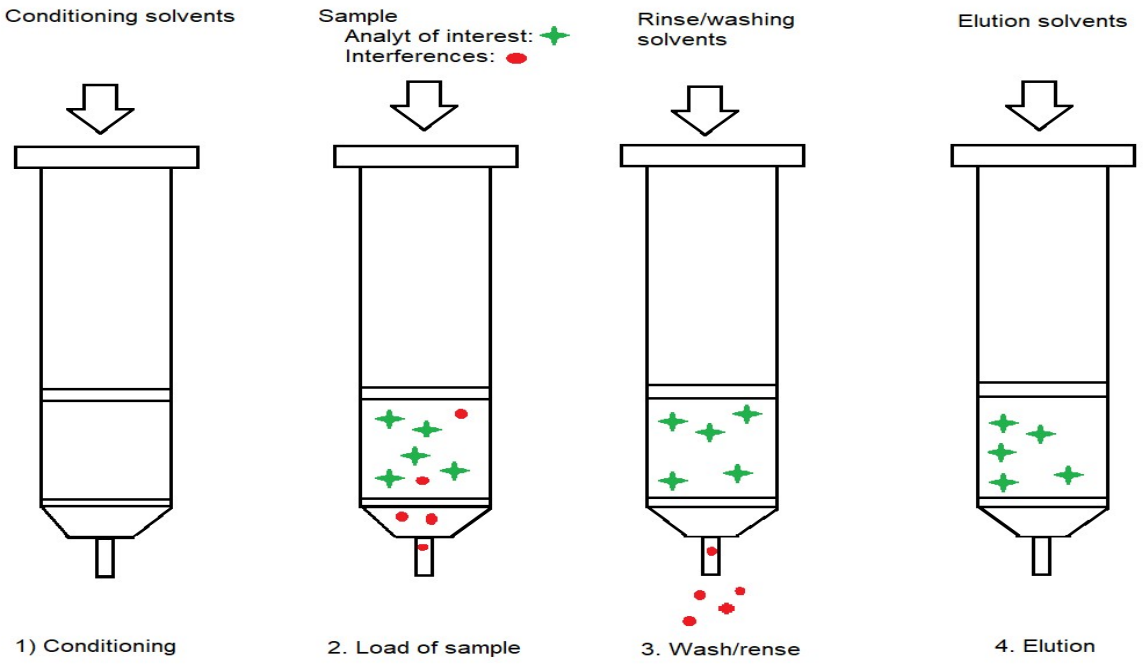
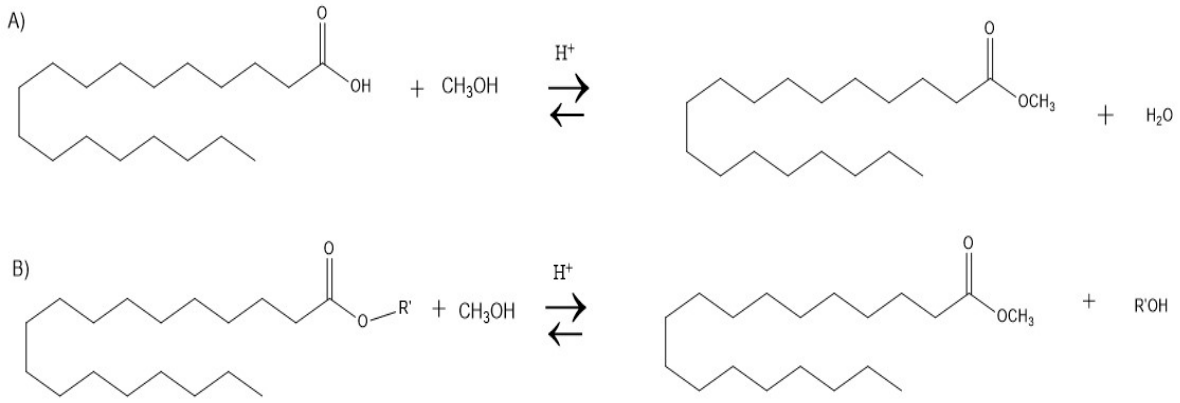


Fig. 2.13 Illustration of the four steps of solid-phase extraction: 1. Condition activate the sorbent in the SPE column. 2. The sample is added. 3. Washing and rinsing the column for remaining interferences and unwanted compounds from the solvent. 4. Elution solvents are added to elute the analyte of interest.

2.3.3 Esterification

Esterification is one of the most common derivatization techniques in which non-volatile compounds are converted into more volatile and thermally stable compounds suitable for GC analysis (Gutnikov, 1995; McNair & Miller, 2009). Transesterification detach FAs from the TAG glycerol back bone, and convert them into FAMEs, which are less polar than and have lower boiling points than esterified-FAs. (Christie, 2003; Eder, 1995). Two common esterification methods are acid-catalyzed and base-catalyzed. In acid-catalyzed the esterified FAs are transesterified into FAMEs, while FFAs are esterified (Scheme 1).

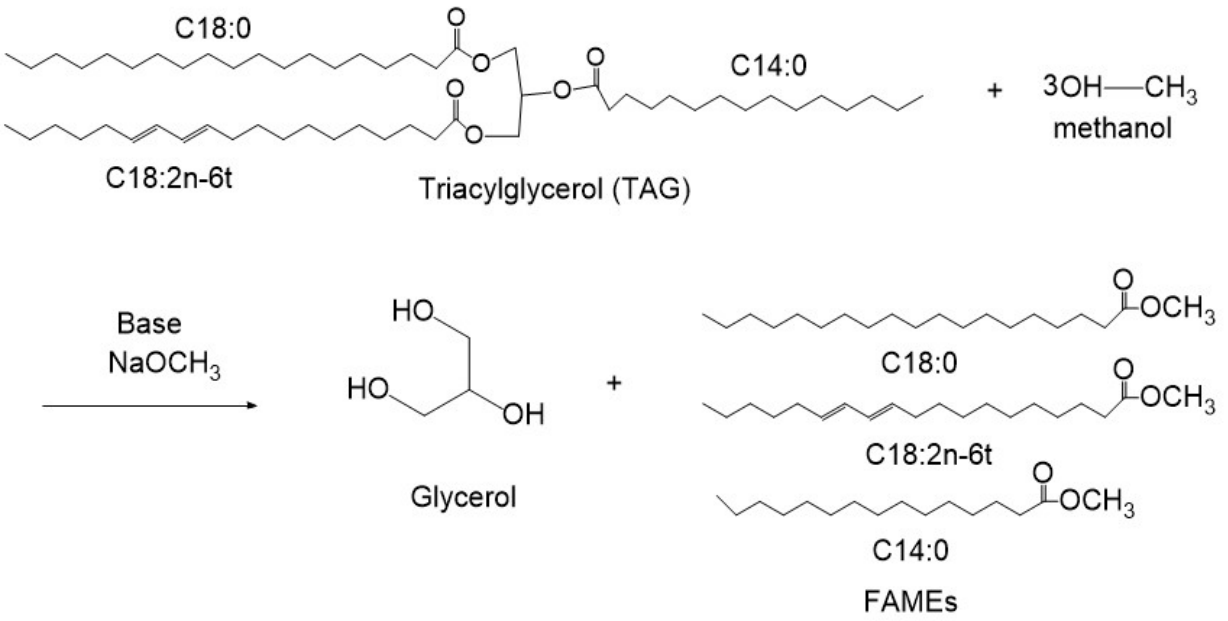


Scheme 1. A) Free fatty acid (FFAs) are esterified by anhydrous methanol in the presence of an acid catalyst (H^+). B) Esterified-FA re-esterified into fatty acid methyl ester (FAME).

The samples are heated with an excess amount of anhydrous methanol together in a solution of an acid catalyst often BF_3 (boron trifluoride). However, water may prevent the reaction from occurring. The acid-based esterification and transesterification method has the broadest applicability, but require heating and may oxidize unsaturated FAs (Christie, 2003). As well as, during heating with an acid-catalyst, solvent evaporation and extracting FAMEs up to C_{14} can be lost (Eder, 1995).

Base-catalyzed transesterification is rapid and occur at room temperature, in addition to not degrade the FAs or isomerize the double bonds (Eder, 1995; Gutnikov, 1995). The most common base catalyst is metallic sodium methoxide, but metallic potassium methoxide is also

often used. The metallic anhydrous methanol in the presence of base catalysts reacts with O-acyl groups and substitute them with a methyl group. FAMEs and glycerol are the end products (Scheme 2.) (Christie, 2003).



Scheme 2. The esterified FAs on the glycerol backbone on TAG in excess amount of anhydrous methanol reacts with the sodium methoxide for formation of the glycerol and FAMEs. Scheme based on equation 2 in (Christie, 2003).

In base- catalyzed esterification FFAs are not esterified since water is absent and anhydrous methanol does not esterify them (Eder, 1995). Overall, the FAMEs derivatives have lower boiling points than FAs, and non-polar and volatile enough to analyzed by GC (Christie, 2003).

2.4 Fatty acid analysis

There are several analytical techniques available for lipid analysis and FAs determination. In fact, lipid analysts lead the development of chromatographic techniques 40 years ago, especially in gas-liquid chromatography (GLC), GC and thin layer chromatography (TLC)(Christie, 2003). High performance liquid chromatography (HPLC), super-critical fluid chromatography (SFC), and capillary electrophoresis (CE) are other method applied in lipid analysis (Li et al., 2013). TLC is "low-tech", simple and a rapid method. Since TLC provides identification on site it is most often used for analytical preparations and in organic chemistry (Christie, 2003; Li et al., 2013; McNair & Miller, 2009).

Chromatographic techniques are often coupled with spectrometric detectors to provide more information. Mass spectrometers (MS) are common detectors for FA analysis, which provide extensive information about the unknown sample, such as the structure, elemental composition, and molecular weight (Gutnikov, 1995; McNair & Miller, 2009). In addition, flame ionization detector is another commonly used detector, though the detector's selectivity limits the detectors applicability for complicated matrixes. Since the flame ionization detector provide information about the retention time and instrument response. Besides any structural information about the analytes (Dodds et al., 2005; Gutnikov, 1995). However, for routine analysis where you are looking for specific analytes and have standards. Then GC coupled to a flame ionization detector is a good alternative, and also since it provides good sensitivity, and simple to operate (Christie, 2003). In identification and quantification of FA a GC-MS is applied, since this method provide high resolution, good sensitivity, as well as structural information about the analysis. Although, GC does require volatile analytes, hence sample preparation is often required (McNair & Miller, 2009).

2.4.1 Principles in gas chromatography and mass spectrometry

The retention time in GC can be used to indicate a chosen analyte if the column variables such as the column (length, stationary phase, thickness), and instrument variables temperature, and pressure are kept constant. In reality, the retention times are not unique for each individual analyte hence GC cannot be used for qualitative confirmation, without standards for every analyte (McNair & Miller, 2009). As the MS provides extensive information about each analyte, a GC-MS provide both quantitative and qualitative information for each analyte.

GC utilize a gas mobile phase, usually N_2, He, or H_2, with the stationary phase within the column. The analytes interaction with the stationary phase and their chemical characteristics (e.g. boiling point, polarity) and molecular weight affects the time through the column. In general, low molecular weight analytes have shorter retention time through the column, than analytes with high molecular weight. There are two major types of columns; packed columns and capillary open tubular columns. Open tubular columns are further divided into wall coated open tubular, porous layer open tubular and support coated open tubular columns. Packed columns are filled with silica stationary phase particles and in open tubular columns the stationary phase is coated on the inside. Wall coated open tubular columns are most used column today, since it provides high resolution, sensitivity, but it has low capacity. While the packed columns offer higher capacity, but with lower resolution, and therefore seldom used (Harris, 2010). Volatile analytes or volatile analyte-derivatives are injected with a needle through a septum into the injector port with a mobile phase referred to as carrier gas (Fig 2.14). To evaporate the analytes rapidly the injector port is heated. A carrier gas transfers the vapor through a heated column with the stationary phase for separation. Then the separated analytes flow through a detector, for example MS, for detection (Harris, 2010).

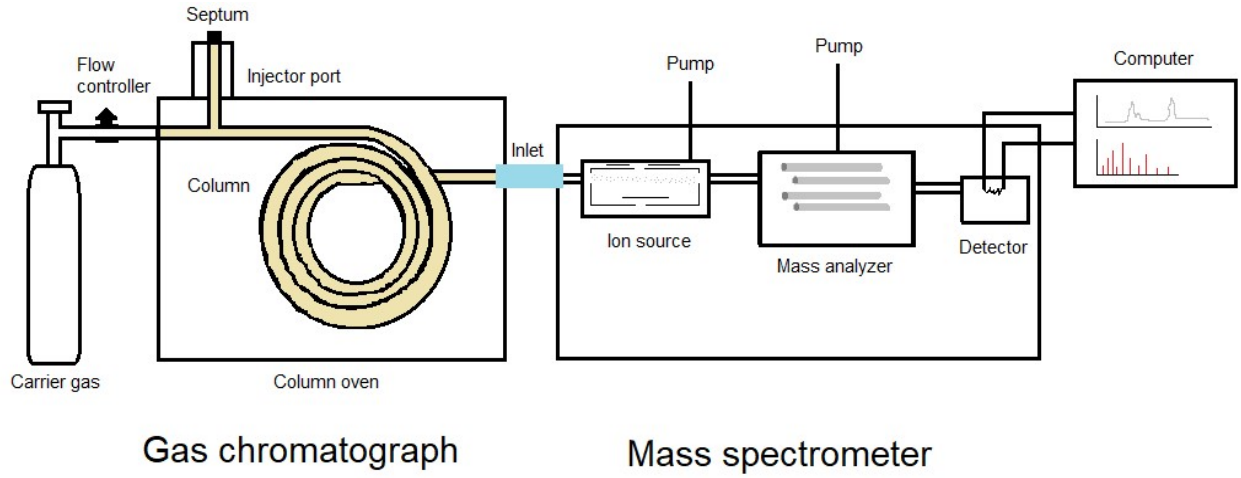


Fig. 2.14 Illustration of a GC-MS with EI ion source and a quadruple as mass analyzer. The analytes are injected into the injector port and vaporized, before the carrier gas transports the analytes through the column for separation. The analytes are transported further into the ion source for ionization. Then the analytes flow into a mass analyzer and separated according to their *m/z* ratio. At last the ions are sent to the detector for detection. Made in paint by Tina Øvrebø.

By coupling the GC to a MS, the instrumentation can be used to obtain qualitative and quantitative information since a mass spectrum is characteristic for each analyte. Mass spectrometry is a method to study the fragment- and molecule ions aided by mass-to-charge ratio (*m/z*). Gas phase molecules from the GC are ionized into ions by the ion source, before separation in the mass analyzer (Fig. 2.14). The separated gas phase ions are sent to the detector (e.g. electron multiplier or photo multiplier) for recording and to be reported to a computer (Fig. 2.14).

Electron ionization

Electronic ionization (EI, also called electron impact) and chemical ionization are the two most common ion sources for GC. EI is the oldest and simplest ionization technique and is often used together with GC-MS. Figure 2.15 illustrates an EI ion source block which is heated and in vacuum. A filament emits highly energic electrons (70eV), through the source block chamber to the electron trap on the other side (Fig. 2.15).

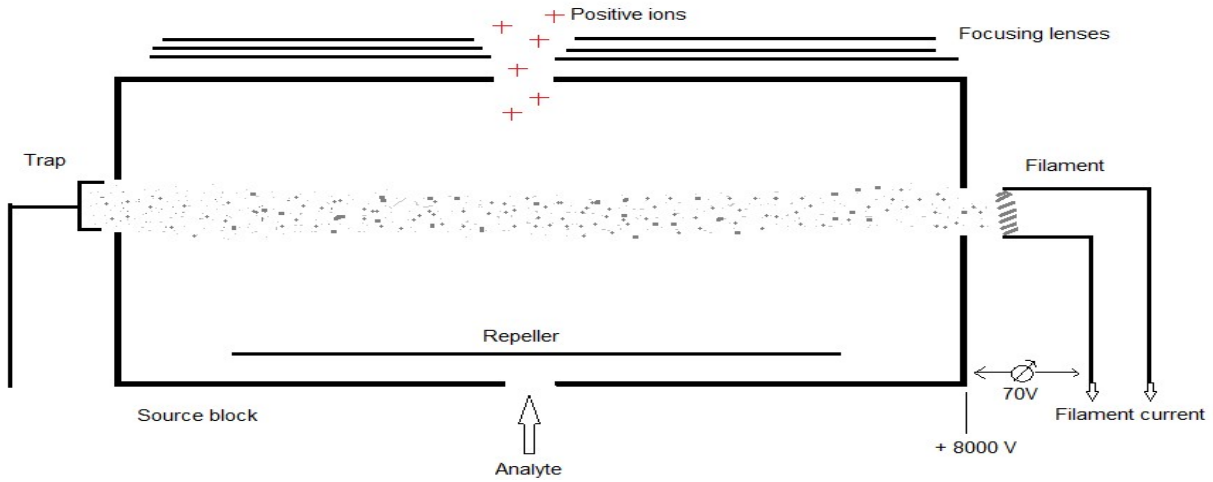


Fig. 2.15 An electron ionization source block where the analyte ions comes in the source block beneath the repeller. The ions are ionized when they collide with the electrons (70 eV) moving from the filament towards the ion trap at the opposite side.

An effluent of gas phase molecules passes through the chamber are ionized when they collide with the highly energetic electrons. The gas phase molecules outer valence electrons are ejected leaving a radical-cation ($M^{+\cdot}$) and electrons (Eq 2.3). The internal energy of the molecular ion is high enough to cause fragments with different *m/z* – ratio (Harris, 2010; McNair & Miller, 2009). Ionized molecules, mostly cations, are repelled by a repeller (with a small positive charge) towards charged lenses. Then imparted with high velocity before going into the mass analyzer (Fleming & Williams, 2007).

$$M + e^- (70eV) \rightarrow M^{\cdot +} + 2e^- \qquad \text{Eq 2.3}$$

Mass analyzer

Mass analyzers separate the gas phase ions exiting from the ion source based on their mass-to-charge ratio *(m/z)* with electrical and/or magnetic fields (Harris, 2010). In a magnetic sector the incoming ions are exposed to a magnetic field (B) which changes the ions path and only ions with chosen *m/z* ratio goes through to the detector at the end. In electric sectors (E) the ions are exposed to an electric field and only the ions with chosen kinetic energy goes through (fig 2.16). These sectors are usually connected to combine the strength of each analyzer, while avoiding their weaknesses e.g. EB, BE, BEB, and EBE combinations (de Hoffmann & Stroobant, 2007).

There are several types of mass analyzers available e.g. magnetic sector and/or electric sector instruments, quadrupoles, time of flight (TOF), ion trap and orbitrap (de Hoffmann & Stroobant, 2007; Harris, 2010; Miller, 2005).

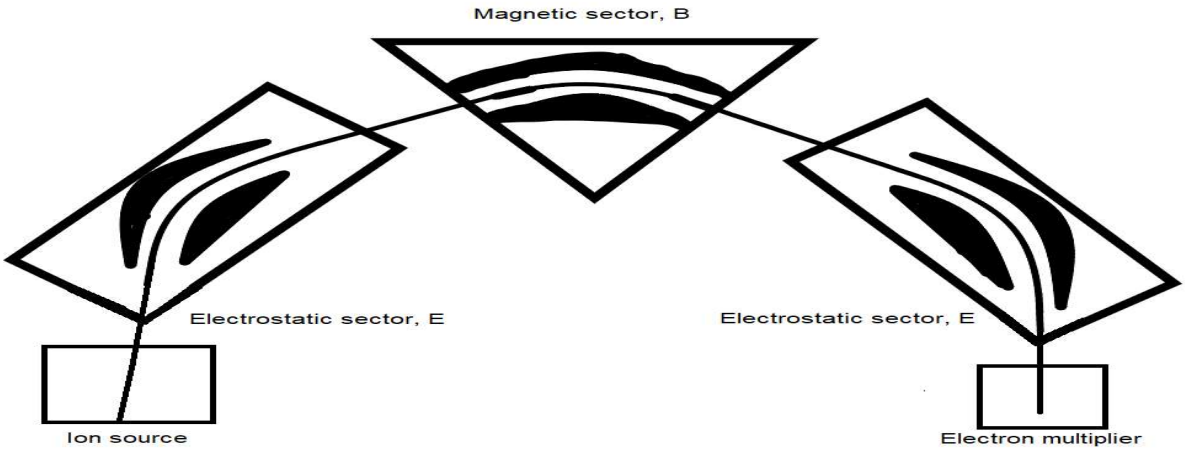


Fig. 2.16 Tri-sector Autospec instrument with EBE geometry and a photo multiplier. Inspired from Bateman (2015) fig. 7.

Electron multiplier

After the mass analyzer the fragment- and molecular ions continue to an electron multiplier detector, which counts the ions to provide a mass spectrum (fig. 2.14). In electron multipliers the ions strike a semi-conductive surface to produce a cascade of electrons accelerated by a potential difference to the next semi conductive surface to produce a larger cascade of ions, and so on (fig. 2.17). This is repeated until the original input of ions are magnified 1 millionfold. The produced mass spectrum is the ion abundance as a function of m/z, which can be used to calculate the molecular weight and to predict the structure. With controlled conditions each mass spectrum is characteristic for each compound (McNair & Miller, 2009).

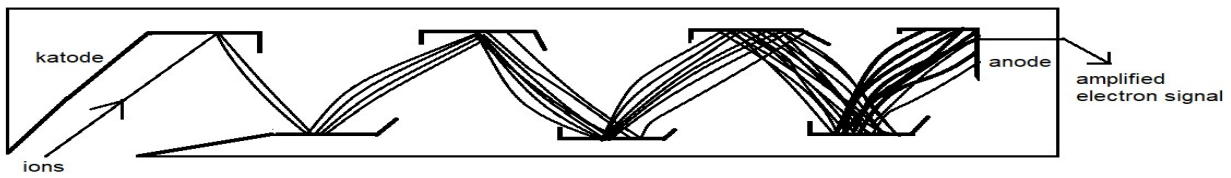


Fig. 2.17 An illustration of a electron multiplier, where ions strike a surface to produce a larger cascade of ions, then transported to a new surface, hence larger cascades are produced. Until enough ions are magnified. Illustration is inspired from a sketch from McNair and Miller (2009).

3.Method

3.1 Chemicals

The chemicals quality, and supplier used in this study are listed in table 3.1. Additionally, the GC-vials, and Pasteur pipettes used were delivered by VWR Chemicals, part of AVANTOR, Radnor, PA, USA.

Table 3.1 Chemicals used in this study with quality and supplier.

Chemicals	Quality	Supplier
Chloroform	HiPerSolv CHROMANORM	VWR Chemicals, part of AVANTOR, Radnor, PA, USA
n-heptane	ANALR NORMAPUR Quality	Sigma-Aldrich, now Merck KPGaA, Darmstadt, Germany
Methanol	HiPerSolv CHROMANORM	VWR Chemicals part of AVANTOR, Radnor, PA, USA
BF_3-MeOH in 14% methanol		Sigma-Aldrich, now Merck KPGaA, Darmstadt, Germany
Sodium (s)	Purum	Merck, Darmstadt, Germany

3.2 Standards

A triacylglyceride (TAG) C19:0 (Larodan AB, Solna, Sweden) was used as internal standard (IS), in order to quantify the FAs. Chloroform was used to dissolve three GC-vials with 10 mg IS until the end concentrations were 10 mg/mL. The dissolve IS were then stored in a freezer at - 20 ºC prior to transesterification and GC-MS analysis. Restek Food Industry FAME mix (Restek food industries, Restek, Bellefone, Pa, USA) was used to identify the FAs in the oil. In addition, other individual FAME standards C16:1n-7, C18:1n-7c, C18:1n-7t, C18:1n-6 and C26:0 were also used for identification of the FAMEs (Larodan AB, Solna, Sweden)

3.2.1 Esterification of a fatty acid standard

To prepare the FA standard solution of C18:1n-7 (Larodan, Solna, Sweden) in ethanol for acid-catalyzed esterification into FAME prior to GC-MS analysis. A Pasteur pipette was used to transfer the FA solution to a 1.5 mL GC-vial, and the sample was evaporated with N_2(g) (AGA AS, Melbourne, Australia) to remove ethanol. The FA was re-dissolved in 1 mL heptane and shaken 40 seconds at a vortex mixer (YellowLine TTS 2, IKA Werke GmbH & CO. KG, Straufen im Breisgrau, Germany), then transferred to a Duran ® GL14 culture tubes and evaporated with N_2(g) for 30 minutes. 1 mL boron trifluoride 14% in methanol solution (BF_3-MeOH) was added to the culture tube before it was put in a water bath at 70ºC for 5 minutes. 1 mL heptane was added, and the sample was shaken with a vortex mixer before being allowed to settle for around 2minutes. The heptane phase was transferred to a GC vial and kept in a freezer -20 ºC until analyzed.

3.3 Sample preparation and standards

Hazelnut oil, walnut oil, and peanut oil were bought at the local food store and almond oil at the pharmacy (fig 3.1). Pistachio- and argan oil were bought online at Bigbuy UK (Bigbuy UK Ltd, Birmingham, England), while macadamia oil, tamanu oil, and kukui oil all were delivered by Telemark Urtebrænderi AS (Porsgrunn, Norway) (fig 3.1). All oils were stored dry at room temperature in the dark.

Fig 3.1 Nut oils used in this thesis. From the left; walnut-, hazelnut-, peanut-, pistachio-, macadamia-, kukui-, tamanu-, argan-, and almond oil. Photo by Tina Øvrebø.

A stock solution was made with 37.2 – 54.5 mg of each nut oil was dissolved with n-heptane until end concentration of 2 mg/mL prior to esterification. An overview of the nut oils used in this study with their extraction methods, country of origin, and the suppliers are seen in table. 3.2.

Table 3.2 Overview of the extraction methods, the origin, and the suppliers of the nut oils.

Oil	Extraction	Origin	Oil type	Supplier
Almond	Unsaponifiable [a)]	Unsure	Cosmetic	"Apotek 1" and PharmaQ, APRO,
Argan	Cold pressed	Morocco	Cosmetic	Telemark urtebrænderi AS
Hazelnut	Expeller pressed	Unsure	Food	International collections AS
Kukui	Cold pressed [b)]	Hawaii	Cosmetic	Telemark urtebrænderi AS
Macadamia	Cold pressed [b)]	Australia	Cosmetic	Telemark urtebrænderi AS
Pistachio	Roasted, expeller pressed and filtered	California	Food	La Tourangelle, AS
Peanut	Expeller pressed	Unsure	Food	International collection AS
Tamanu	Cold pressed [b)]	Madagascar	Cosmetic	Telemark urtebrænderi AS
Walnut	Expeller pressed	Unsure	Food	International collection AS

a) The oil is the remaining components after alkaline hydrolysis (saponification), which is soluble in organic solvents. Meaning the lipid fraction that cannot be transformed into soap (Nichols et al., 2011).

b) Assumed expeller pressed

3.3.1 Sample derivatization

Five replicates from each individual nut oil stock solution was esterified. A Hamilton® syringe was used to transfer 2 mL of nut oil stock solution to Duran® GL14 culture tubes, then 50 µL IS was added. A 3.3 mg/mL sodium methoxide solution was prepared by dissolving metallic sodium in methanol. 1.5 mL of the 3.3 mg/mL sodium methoxide solution was transferred to the culture tubes with a Hamilton® syringe. Five replicates of each nut oil were prepared. They were shaken horizontally (horizontal shaker PSU-10i, BIOSAN, Riga, Latvia) for 30 minutes at 350 rpm and left to settle vertically for 10 minutes. The heptane phases (top layer) were transferred to 1.5 mL GC vials with Pasteur pipettes and evaporated under N_2(g) at 40 ºC (AGA, Melbourne, Australia) until dryness, before being re-dissolved in 1.0 mL heptane.

Almond oil, hazelnut oil, peanut oil, and walnut oil samples were analyzed with GC-MS directly after esterification. While the argan oil, kukui oil, tamanu oil, macadamia oil, and pistachio oil

replicates had precipitation in the vials, due to too much lipids relative to heptane. In order to centrifuge the samples, the heptane phases were transferred to separate microtubes (MCT-150-C Axygen® a Corning brand, Corning, NY, USA) before 10 minutes centrifugation at 148000 rpm on a Sigma 1-14 (Osterode am Harz, Germany). Then the liquid and not the supernatant was transferred by Pasteur pipettes to GC vials before analysis with GC-MS.

3.3.2 Solid-phase extraction of nut oils

The preparation of the three fractions neutral lipids (NL), polar lipids (PL) and FFAs for all nut oils were done by the master students Ingrid Hausberg and Stine Marie Fykse Haraldsen. Where the PL and FFA fractions was used further in this study. The PL fractions from all nut oils were first transferred from SPE tubes to Duran® GL 14 culture tubes by Pasteur pipettes and evaporated under N_2(g) at 40 ºC (AGA, Melbourne, Australia) until dryness. Then the samples were dissolved in 2 mL heptane and esterified as described 3.3.1. Note that these samples were not centrifuged. The FFAs fractions were transferred from SPE tubes to Duran® GL 14 culture tubes by Pasteur pipettes. The samples were then evaporated under N_2(g) at 40 ºC (AGA, Melbourne, Australia) until dryness, and added 1 mL boron trifluoride as described in section 3.2.1. The oils were fractionated with SPE without any IS added, and with only one replicate. Since the SPE machine required a few weeks maintenance.

3.3 GC-MS

To identify and quantify FAMEs in the nut oils an Agilent 6890N Series Gas Chromatograph (GC, Agilent Technology, Wilmington, DE, USA) coupled with a three-sector EBE geometry instrument MS AutoSpec - Ultima mass spectrometer M629 (MS, Miromass Ltd, Manchester, England) with an EI as ion source was used. The MS was tuned to 40-600 *m/z* range and a resolution of 1000. A CTC PAL autosampler (CTC 257 Analytics, AG, Zwingen, Switzerland) was used to inject the sample into an injection chamber with helium as carrier gas (99,99990 %, Yara, Rjukan, Norway), 1.0 µL of sample was injected with a split ratio of 1:10.The carrier gas had a 1 mL/minutes constant flow and was kept at a constant pressure of 95 kPa. The EI ion source was set at 250 ºC and produced 70 eV electrons. A 60 m Restek column (Rtx®-2330) with 0.25 mm ID, and a 0,2 µm film thickness of fused silica 90%biscyanopropyl/10% phenylcyanopropyl polysiloxane stationary phase (Restek Corporation, 256 Bellefonte, PA, USA) was used for separation. Fig 3.2 illustrates the GC oven temperature program with a total analysis time of 92 minutes. The software u MassLynx 4.0 (Waters, Milford, MA, USA) and a NIST 08 Mass Spectral library (Gaithersburg, MD, USA) was used for identification and quantification of the FAs.

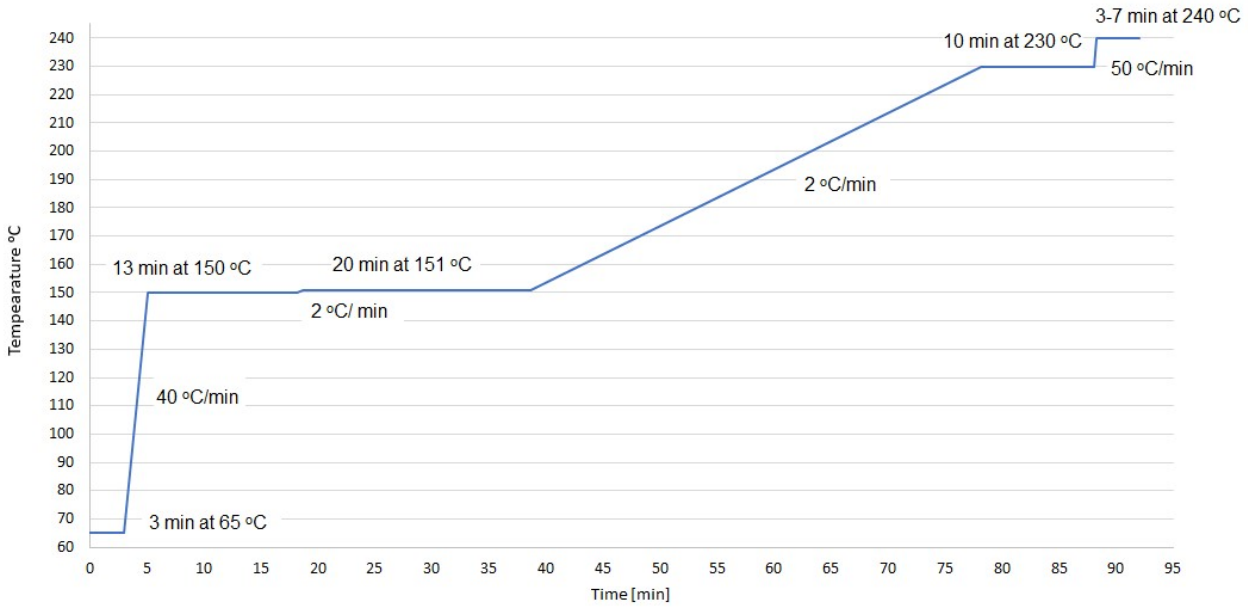


Fig. 3.2 Illustrates the GC oven temperature program utilized for elution of FAMEs.

3.4 Quantification

A previously established method from Devle et al. (2009) for qualitative and quantitative determination of FAs with GC-MS was used. This method considered as a routine analysis at the organic analytical chemistry group at NMBU. The method validation was carried out by Devle et al. (2009) and the acquisition modes of full-scan, selected ion monitoring (SIM) and reconstructed ion chromatogram (RIC) were compared on EBE sector instrument. All acquisition modes had satisfying results for the parameters LOD (ng/mL), LOQ (ng/mL), reproducibility, and linearity (Devle et al., 2009). To quantify the amount of each FA a C19:0 TAG was used as an IS, together with relative response factors (RRF) using equation 4.1 (Devle et al., 2009):

$$mass_{FA} = \left(\frac{Area_{FA} \times C_{mole\ IS}}{Area_{IS} \times RRF}\right) \times Molecular\ weight_{FA} \quad (4.1)$$

Previously determined RRF-values from Devle et al. (2009) can be seen in appendix II. The FAMEs in Restek Food Industry FAME mix standard are listed in appendix I. Regarding the FAs found in the nut oils not represented in the Restek mix were assigned the RRFs to the FAMEs they were most alike (Appendix II). The Restek Food Industry FAME mix was used together with NIST 08 library search for identification of the FAs in the nut oils. According to NIST user's guide "a match factor of 900 or more is a perfect match, while 800-900 is a good match, and less than 600 is a poor match" (NIST, 2017). Detected FAs not represented in Restek FAME mix were identified by other standards. To identify C18:1n-7c the C18:1n-7t ME (methyl ester), C18:1n-7c ME, C18:1n-6c ME and C18:1n-5c ME were analyzed and the C18:1n-7c matched in retention time and had similar MS-spectra. All standards were made by Aanrud (2016), except C18:1n-6c which was bought from Larodan (Solna, Sweden) as a FFA then esterified prior to analysis to GC-MS as described in section 3.2.1.

Remarks on the method

For argan oil, kukui oil, tamanu oil, macadamia oil, and pistachio oils the samples were after esterification were centrifuged, due to precipitation, and only the liquid heptane phases were analyzed further on the GC-MS. Though, some FAs would in theory be in the supernatant the IS would correct for it. Since a known amount of IS was added. However, the pistachio oil replicates were too different for the IS. To prevent precipitation in the new round of pistachio oil the method was done with a 1 mg / mL oil in heptane rather than 2 mg / mL. This round of pistachio oil did not precipitate, so a new esterification round for all nut oils were done, but due to lack of time these results were not analyzed except for pistachio oil.

In addition, the stock solutions were weighed out in media bottles (Duran ®, 50 mL), which weighs 45 g, meaning that the oil weighed out is 0.07 % - 0.10 % of the bottles weight. A more suitable way would be using micro weighing dishes which weighs 4 mg.

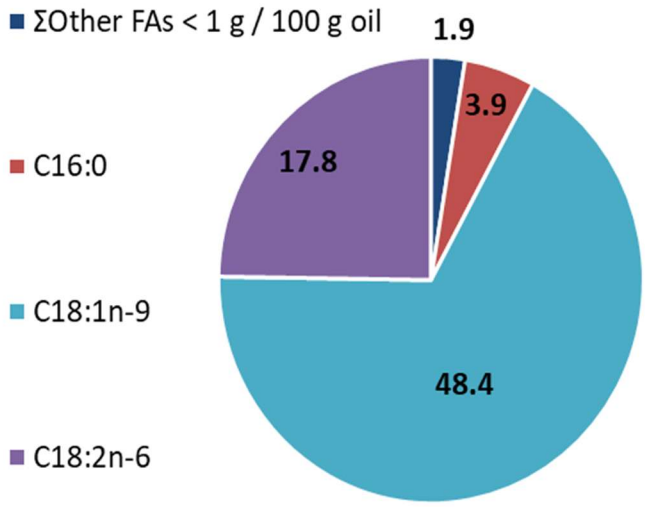
4. Results & discussion

In this study 14 to 19 FAs were identified and quantified in each of the nine nut oils Results from each nut oil are shown in section 4.1 and compared to literature, moreover the FA composition of all nut oils are compared in section 4.2 in grams FA per 100 g oil.

4.1 The fatty acid composition in nut oils

4.1.1 Almond oil

A total of 15 FAs were identified and quantified in almond oil, in addition three isomers were found which could not be identified (Appendix III). As seen clearly in figure 4.1 C18:1n-9 is the most abundant FA, then C18:2n-6, C16:0. These three FAs were the only FAs with a g FA/100 g oil amount above 1 g/100 g oil, hence the 12 other FAs were summed together in the "other FAs" slice to show their contribution (fig. 4.1).



Fig, 4.1 Illustrates almond oil FAs distribution were the values are given in g/100 g oil and FAs, where FAs with amount lower than 1 g / 100 g oil are summed together in "other FAs" (n=5).

The FA profile found in almond oil was similar to reported FA profiles in other studies (Čolić et al., 2017; Kirbaslar et al., 2012; Li & Hu, 2011; Venkatachalam & Sathe, 2006). Though, the C21:0 and C18:1n-7 FAs found in this study were not reported by previous studies (Kirbaslar et al., 2012; Miraliakbari & Shahidi, 2008; Venkatachalam & Sathe, 2006). On the other hand, C23:0 and C24:0 were not found in this study but have been reported by Čolić et al. (2017). The n-6/n-3 ratio in almond oil was 276, which is similar to the earlier reported n-6/n-3 ratios of 199, and 260 (Li et al., 2006; Rueda et al., 2014). Two n-6 FAs C20:2n-6 and C22:6n-6 were not found in this study, but have been previously reported (Čolić et al., 2017; Kirbaslar et al., 2012; Miraliakbari & Shahidi, 2008).

Almond oil had a low SFA content contributing 7% of the total FAs, while the MUFAs and PUFAs contributed 68% and 25%, respectively (table 4.1). Overall, the MUFA-, PUFA- and SFA distributions in almond oil were consistent, but lower than reported in literature (Li & Hu, 2011; USDA, 2018; Venkatachalam & Sathe, 2006). Though, Venkatachalam and Sathe (2006) reported lower amount of MUFA (61.6 g/100 g lipid), due to higher PUFA and SFA amount (29.3, and 9.1 g /100 g lipid, respectively) corresponding with the MUFA, PUFA, and SFA findings, 60.4-, 27.1- and 8.6 % of total FAs in Li et al. (2006).

Table 4.1. Tot. FAs, SFA, MUFA, PUFA, n-6, n-3, n-6/n-3 ratio, and the MUFA/SFA ratio in almond oil given in g/ 100 g oil (n=5 ± 1 SD)

Almond oil	**g/100 g oil**
Tot. FAs	71.97 ± 1.44
SFA	4.77 ± 0.34
MUFA	49.27 ± 1.17
PUFA	17.93 ± 0.31
n-6	17.82 ± 0.30
n-3	0.06 ± <0.01
n-6/n-3 ratio	275.51 ± 5.75
MUFA/SFA	10.36 ± 0.67

4.1.2 Argan oil

A total of 17 FAs were identified and quantified in Moroccan argan oil, in addition to one FA under the quantification limit (Appendix III). The MUFAs were the predominant FAs with C18:1n-9 as the most abundant FA (fig 4.2). The C18:2n-6 was the second most abundant FA, followed by C16:0 and C18:0 (fig 4.2). Though two of the four most abundant FAs were SFAs, the overall SFA contribution was lower than PUFA and MUFA (fig 4.2). As seen in figure 4.2 the remaining 13 FAs in argan oil were summed together in "other FAs", to indicate their low share of total FAs. All of these "other FAs" had previously been reported, except for the FAs C15:0, C17:1n-7 and C21:0, which were found in this study.

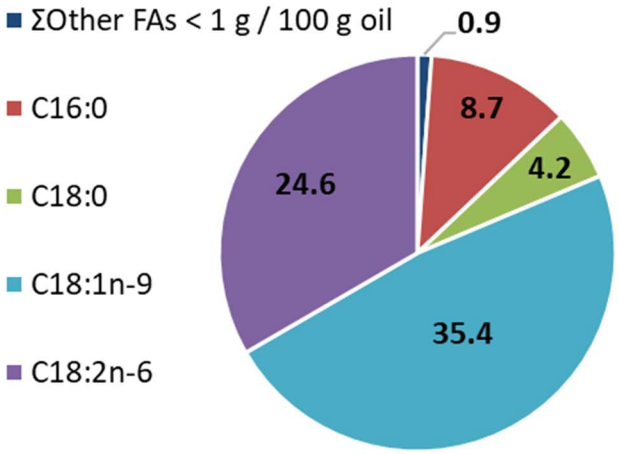


Fig 4.2 Illustrates argan oil FAs distribution were the values are given in g/100 g oil and FAs, where FAs with amount lower than 1 g / 100 g oil are summed together in "other FAs" (n=5).

Table 4.2 shows that argan oil had a high n-6/n-3 ratio of 668 compared to 256 and 117 earlier reported (Rueda et al., 2014; Vingering et al., 2010), this is due to a poorly quantified C18:3n-3 amount. Overall, the FA composition in argan oil was similar to previous literature (Charrouf & Guillaume, 2008; Janick & Paull, 2008), though slightly lower than reported in Rueda et al. (2014) and Vingering et al. (2010).

Table. 4.2 Total. FAs, SFA, MUFA, PUFA, n-6, n-3, n-6/n-3 ratio, and the MUFA/SFA ratio in argan oil given in g/ 100 g oil (n=5 ± 1 SD)

Argan oil	g / 100 g oil
Tot. FAs	73.83 ± 3.68
SFA	13.38 ± 0.74
MUFA	35.78 ± 1.43
PUFA	24.67 ± 1.85
n-6	24.63 ± 1.85
n-3	0.04 ± 0.01
n-6/n-3 ratio	668.32 ± 145.80
MUFA/SFA	2.67 ± 0.10

4.1.3 Hazelnut oil

In Hazelnut oil a total of 17 FAs were identified and quantified, in addition to two unknown isomers (Appendix III). Similar to argan oil the four most abundant FAs were C18:1n-9, C18:2n-6, C16:0 and C18:0 (fig 4.3). In figure 4.3 the 13 remaining FAs identified and quantified were summed into "other FAs" to indicate their relative low contribution compared to C18:1n-6, C18:2n-6 and C16:0.

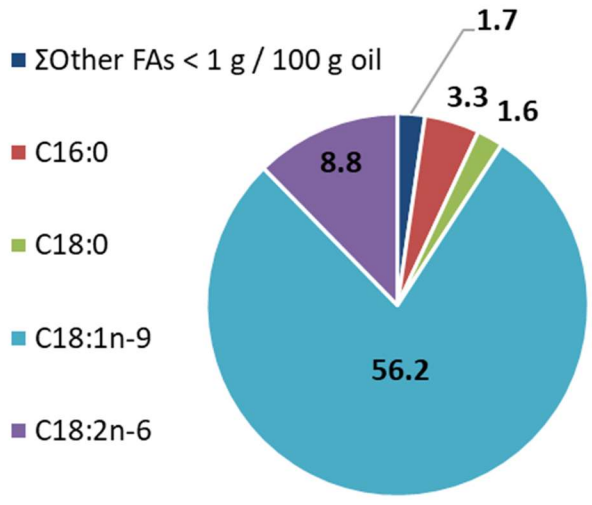


Fig 4.3 Illustrates hazelnut oil FAs distribution were the values are given in g/100 g oil and FAs, where FAs with amount lower than 1 g / 100 g oil are summed together in "other FAs" (n=5).

Table 4.3 show the high MUFA content in hazelnut oil compared to PUFA and SFA. The MUFA, PUFA and SFA ratios were similar to previous findings (B.Alasavar et al., 2008; Kirbaslar et al., 2012; Venkatachalam & Sathe, 2006; Vingering et al., 2010). Alasavar and Shahidi (2009) reported a total of 18 FAs in total from several hazelnut varieties, which agreed with this study. The FAs C18:1n-11, C22:1n-9 and C24:1 were not found in this study or by Kirbaslar et al. (2012), Venkatachalam and Sathe (2006); Vingering et al. (2010). On the other hand, the FAs C21:0, C23:0 and C24:0 found in this study, were not previously reported in literature. The quantified amounts of these three FAs were relatively small, 0.1-0.01 g FA/100 g oil (Appendix III). The hazelnut oil had a very high n-6/n-3 ratio of 253 (table 4.3) compared to 35.9 reported in Vingering et al., (2010). Vingering et al., (2010) reported 0.4 g /100 g oil C18:3n-3, and 12.9 g /100g oil C18:2n-6. While in this study 0.03 g /100g oil C18:3n-3 and 8.83 g /100 g oil (table 4.3). Therefore, in this study the high n-3/n-6 ratio is due to a poorly quantified C18:3n-3 amount.

Table 4.3 Total. FAs, SFA, MUFA, PUFA, n-6, n-3, n-6/n-3 ratio, and the MUFA/SFA ratio in hazelnut oil given in g/100 g oil (n=5).

Hazelnut oil	**g /100 g oil**
Tot. FAs	71.63 ± 1.39
SFA	5.55 ± 0.13
MUFA	57.21 ± 0.99
PUFA	8.86 ± 0.06
n-6	8.83 ± 0.06
n-3	0.03 ± <0.01
n-6/n-3 ratio	253.12 ± 4.84
MUFA/SFA	10.31 ± 0.06

4.1.4 Kukui oil

Only 14 FAs were identified and quantified in kukui oil, together with one unknown isomer (Appendix III). Kukui oil had the highest content of C18:3n-3 in all oils, and was one of the two nut oils with the C18:2n-6 as the most abundant FA (fig 4.4). Second most abundant FA was the C18:1n-9, then C18:3n-3, C16:0 and C18:0. The remaining FAs had a quantified amount below 1 g /100 g oil and were therefore summed in "other FAs" to indicate their contribution in figure 4.4.

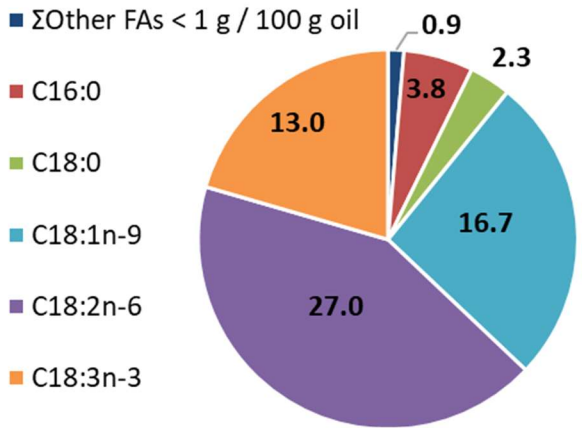


Fig 4.4 Illustrates kukui oil FAs distribution were the values are given in g/100 g oil and FAs, where FAs with amount lower than 1 g / 100 g oil are summed together in "other FAs" (n=5).

Since the oil has a high amount of C18:3n-3 the n-6/n-3 ratio is 2.71, which is the lowest of all the nut oils in this study (table 4.4 and appendix III).

Table 4.4 Total. FAs, SFA, MUFA, PUFA, n-6, n-3, n-6/n-3 ratio, and the MUFA/SFA ratio in kukui oil given in g/ 100 g oil (n=5)

Kukui oil	**g / 100 g oil**
Tot. FAs	63.43 ±7.60
SFA	6.32 ± 0.85
MUFA	17.11 ± 1.76
PUFA	40.00 ± 4.93
n-6	26.96 ± 3.09
n-3	13.04 ± 1.85
n-6/n-3 ratio	2.07 ± 0.08
MUFA/SFA	2.71 ± 0.11

This means that when considering the FA composition in a health perspective, kukui oil seems to be the most favorable oil to consume. Nevertheless, kukui oil is mainly used for cosmetic purposes and skin-care treatments. Because the kukui oil is considered to be toxic since the kukui plant contain toxic phorbol esters and saponins (Elevitch & Manner, 2006; Nelson et al., 2007). The use of kukui oil in cosmetic industry is favorable due to the moisturizing effects from the high PUFA content (Ako et al., 2005; Azam et al., 2005; B.Gunstone & Harwood, 2007; Martín et al., 2010). Lim (2012) reported that a in similar oil, the tamanu oil which is also non-edible, could be made edible after proper filtration and detoxification. If the same procedure has been attempted for kukui oil is not known. Since kukui oil is not edible and not suitable for cooking there is little research found regarding the FA composition (Atabani et al., 2013; PROTA, 2007). Henceforth, the FAs composition of 14 FAs is more comprehensive than the 9 FAs earlier determined in kukui oil (Ako et al., 2005; Martín et al., 2010; Pham et al., 2018).

4.1.5 Macadamia oil

A total of 16 FAs were quantified in macadamia oil from *M. tetraphylla* specie (Appendix III). Two unknown isomers were also found, in addition to one FA under the quantification limit. Macadamia oil had seven FAs with quantified amounts above 1 g/100g oil. The FAs C18:1n-9 and C16:1n-9 were the most abundant, and then the FA C16:0. In total, the MUFAs predominated, followed by SFAs and PUFAs, respectively (Table 4.5).

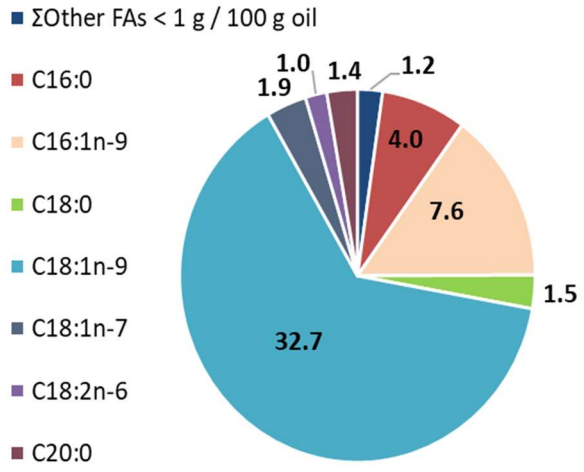


Fig 4.5 Illustrates macadamia oil FAs distribution were the values are given in g/100 g oil and FAs, where FAs with amount lower than 1 g / 100 g oil are summed together in "other FAs" (n=5).

Macadamia oil had a very low PUFA content, only represented by LA, C18:2n-6. The amount of ALA, C18:3n-3, was under the quantification limit (table 4.5). Since C18:3n-3 was the only n-3 FA found the n-6/n-3 ratio could not be determined for this oil (table 4.5). However, previous studies have reported n-6/n-3 ratios of 7.33, 8.33 and 10 (Li et al., 2006; Li & Hu, 2011; Maguire et al., 2004). The high MUFA content and low SFA content in macadamia nuts have led to studies evaluating the effect of macadamia nut consumption on health. These studies associated macadamia nut consumption and lower cholesterol levels (Garg et al., 2003; Griel et al., 2008; Hiraoka-Yamamoto et al., 2004). In addition the low PUFA content makes the macadamia oil more resistant against oxidation (Hsieh & Kinsella, 1989; Navarro & Rodrigues, 2016).

Table 4.5 Total. FAs, SFA, MUFA, PUFA, n-6, n-3, n-6/n-3 ratio, and the MUFA/SFA ratio in macadamia oil given in g/ 100 g oil (n=5 ± 1 SD)

Macadamia oil	**g / 100 g oil**
Tot. FAs	52.62 ± 6.67
SFA	7.78 ± 0.89
MUFA	43.98 ± 5.77
PUFA	1.01 ± 0.13
n-6	1.01 ± 0.13
n-3	-
n-6/n-3 ratio	-
MUFA/SFA	5.65 ± 0.31

The FA composition in this study was consistent with corresponding data found in literature regarding both *M. tetraphylla* and *M. integrifolia* (Janick & Paull, 2008; Kaijser et al., 2000; Li & Hu, 2011; Maguire et al., 2004; Venkatachalam & Sathe, 2006). Only one study reported the found C18:1n-7 in macadamia nuts, while the FAs C12:0, C17:0, and C17:1n-7 have not been previously reported in any other study (Kaijser et al., 2000; Li & Hu, 2011; Maguire et al., 2004; Venkatachalam & Sathe, 2006). Some variations in the FA composition may come from browning of the macadamia kernels. A study from Srichamnong and Srzednicki (2015) reported an correlation between FA compositions and browning of the macadamia nuts, the quantified FAs amount were almost twice as high in browned nuts compared to white non-discolored nuts (Srichamnong & Srzednicki, 2015).

4.1.6 Peanut oil

In peanut oil a total of 19 FAs were quantified, in addition to two unknown isomers (Appendix III). As in the other nut oils, the FA C18:1n-9 was the most abundant, followed by C18:2n-6 and the C16:0 (fig. 4.6). To see the remaining 14 FAs contribution compared to the five most abundant FAs, these were summed together in "other FAs" as seen in figure 4.6. The FA C22:0 was more abundant than C18:0 (Appendix III). Furthermore, peanut oil had the highest C22:0 quantified amount compared to all nut oils in this study (Appendix III).

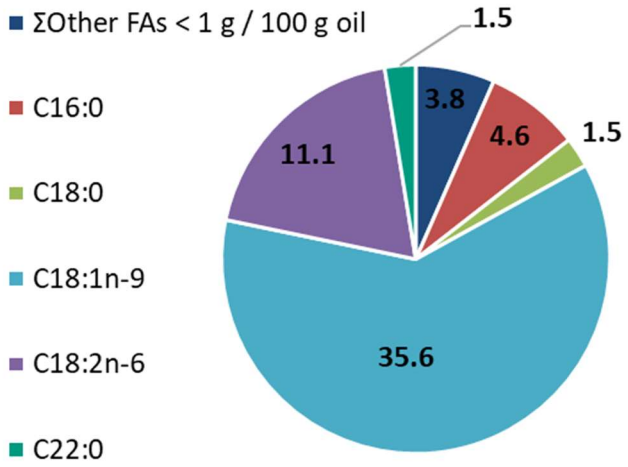


Fig 4.6 Illustrates peanut oil FAs distribution were the values are given in g/100 g oil and FAs, where FAs with amount lower than 1 g / 100 g oil are summed together in "other FAs" (n=5).

Our findings were in agreement with previous literature (B.Gunstone & Harwood, 2007; Kirbaslar et al., 2012; Maguire et al., 2004; Vingering et al., 2010; Wang, 2018). Except for the FAs C15:0, C21:0, and C26:0, found in this study have not been previously reported in literature. On the other hand, Kirbaslar et al. (2012) and Maguire et al. (2004) reported the FAs C20:1n-1, C20:2n-6 and C20:3n-6 not found in this study. The MUFA content in peanut oil was most abundant followed by PUFAs and SFA, respectively (table 4.6). The n-6/n-3 ratio in peanut was 119, which is lower than reported ratio of 131 found in Vingering et al. (2010) (table 4.6).

Table 4.5 Total. FAs, SFA, MUFA, PUFA, n-6, n-3, n-6/n-3 ratio, and the MUFA/SFA ratio in argan oil given in g/ 100 g oil (n=5)

Peanut oil	g / 100 g oil
Tot. FAs	57.27 ± 8.40
SFA	9.07 ± 1.48
MUFA	36.97 ± 4.60
PUFA	11.24 ± 1.61
n-6	11.14 ± 1.59
n-3	0.09 ± 0.02
n-6/n-3 ratio	119.11 ± 9.68
MUFA/SFA	4.08 ± 0.28

4.1.7 Pistachio oil

In this study 15 FAs were detected in pistachio oil, three unknown isomers were found, and one FA was under the quantification limit. The MUFA, C18:1n-9 was again the most abundant FA, followed by C18:2n-6, C16:0 and C18:1n-7 (fig 4.7). The remaining 11 other FAs had a quantified amount below 1 g /100 g oil, and therefore summed together as "other FAs" to show their contribution relative to the four most abundant FAs (fig. 4.7).

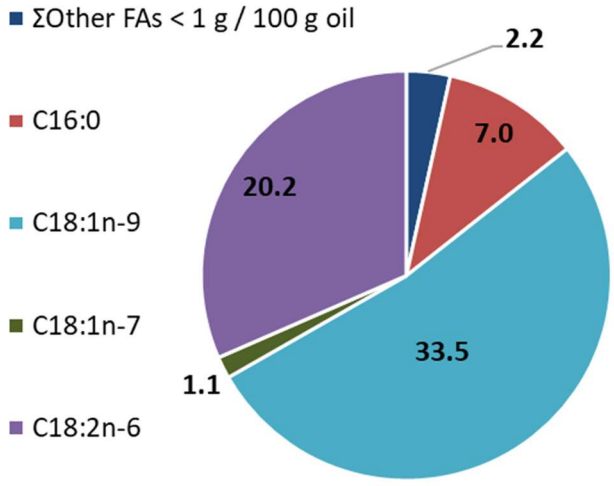


Fig 4.7 Illustrates pistachio oil FAs distribution were the values are given in g/100 g oil and FAs, where FAs with amount lower than 1 g / 100 g oil are summed together in "other FAs" (n=5).

The pistachio oil was rich in MUFAs contributing 55% of total FAs (table 4.7), which agreed with literature (Kirbaslar et al., 2012; Li & Hu, 2011; Venkatachalam & Sathe, 2006). Though, Kirbaslar et al. (2012) also reported C20:1n-1, C20:2n-6 and C20:3n-6, which were not found in this study. Furthermore, the FA C20:2n-6 was detected but not quantified. The shortest FA found in this study's pistachio oil was C14:0, while Venkatachalam and Sathe (2006) reported shorter chained FAs down to C6:0. Whereas Kirbaslar et al. (2012) reported C14:0 as shortest FA and Li and Hu (2011) reported C16:0 as shortest chained FA in pistachio oil.

Table 4.7 Total. FAs, SFA, MUFA, PUFA, n-6, n-3, n-6/n-3 ratio, and the MUFA/SFA ratio in pistachio oil given in g/ 100 g oil (n=5)

Pistachio oil	**g/100 g oil**
Tot. FAs	63.96 ± 3.45
SFA	7.98 ± 0.45
MUFA	35.35 ± 1.72
PUFA	20.63 ± 1.36
n-6	20.18 ± 1.34
n-3	0.43 ± 0.02
n-6/n-3 ratio	47.03 ± 1.78
MUFA/SFA	4.43 ± 0.12

4.1.8 Tamanu oil

A total of 15 FAs were found in tamanu oil, where the FA, C18:1n-9 was the most abundant FA, then C18:2n-6, C18:0 and C16:0 (fig. 4.8). The ratio between C18:1n-9 and C18:2n-6 quantified amount was smaller than in the other nut oils.

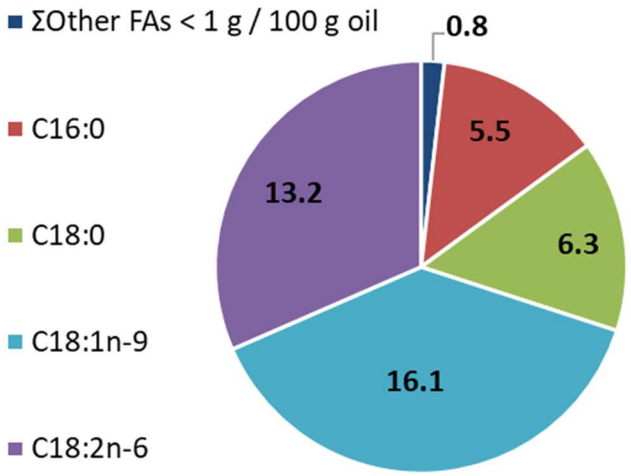


Fig 4.8 Illustrates tamanu oil FAs distribution were the values are given in g/100 g oil and FAs, where FAs with amount lower than 1 g / 100 g oil are summed together in "other FAs" (n=5).

The FA composition in tamanu oil was similar to previous findings (Atabani et al., 2013; B.Gunstone & Harwood, 2007; Crane et al., 2005; Léguillier et al., 2015). Though, the FAs C20:1n7, C20:3n-6, C22:1n-6 and C24:1n-9 have been found in tamanu oil (Crane et al., 2005; Léguillier et al., 2015), but were not found in this study. Instead in this study the FAs C17:0, C17:1n-7, C18:1n-7 and C21:0 in our study.

The tamanu oil is the second non-edible oil in this study in addition to kukui oil. Lim (2012) reported that proper filtration and detoxification might make tamanu oil edible. However, the tamanu oil FA composition with similar contents of MUFA, PUFA and SFA may not be a favorable composition regarding potential health benefits. Besides the tamanu oil is known for the medicinal uses, preserving timber, and its FAs composition is suitable for (Atabani et al., 2013).

Tamanu oil had relatively even contents of SFA, MUFA and PUFA (Table 4.8). The ratio between n-6 FAs and n-3 FAs in tamanu oil is 185, which is higher than the reported ratio of 99 in Léguillier et al. (2015).

Table 4.8 Total. FAs, SFA, MUFA, PUFA, n-6, n-3, n-6/n-3 ratio, and the MUFA/SFA ratio in tamanu oil given in g/ 100 g oil (n=5 ± 1 SD).

Tamanu oil	**g /100 g oil**
Tot. FAs	41.87 ± 3.47
SFA	12.18 ± 1.34
MUFA	16.42 ± 1.11
PUFA	13.27 ± 1.10
n-6	13.20 ± 1.10
n-3	0.07 ± 0.01
n-6/n-3 ratio	185.65 ± 19.63
MUFA/SFA	1.35 ± 0.07

4.1.9 Walnut oil

A total of 17 FAs were identified, and also three unknown FA isomers, were found in walnut oil. Walnut is the second oil after kukui oil with higher PUFA (78%) content than MUFA (14%) content and with a low SFA (10%) content (Table. 4.9). Notably due to the high ALA and LA content the n-6/n-3 ratio in walnut oil was 5.2. Which agrees with Vingering et al. (2010) and Li et al. (2006) that reported n-6/n-3 ratios of 4.9 and 5.3. Additionally, walnut oil was the only oil where a third PUFA C20:2n-6 was found in addition to C18:2n-6 and C18:3n-3, found in the other nut oils.

Table 4.9 of total. FAs, SFA, MUFA, PUFA, n-6, n-3, n-6/n-3 ratio, and the MUFA/SFA ratio in argan oil given in g/ 100 g oil (n=5).

Walnut oil	g /100 g oil
Tot. FAs	78.94 ± 13.7
SFA	7.58 ± 1.28
MUFA	11.14 ± 1.84
PUFA	60.24 ± 10.63
n-6	50.09 ± 8.65
n-3	9.60 ± 1.88
n-6/n-3 ratio	5.24 ± 0.17
MUFA/SFA	1.47 ± 0.04

The FA C18:2n-6 is the most abundant FA, followed by similar amounts of C18:1n-9 and C18:3n-3 (fig. 4.9). These findings agree with literature (Kirbaslar et al., 2012; Li & Hu, 2011; Venkatachalam & Sathe, 2006; Vingering et al., 2010). The shortest FA found in this study was C12:0, while Venkatachalam and Sathe (2006) reported short chained FAs below 12 carbon atoms. However, other studies reported C14:0 as the shortest FA (Kirbaslar et al., 2012; Li & Hu, 2011; Miraliakbari & Shahidi, 2008). This study found C17:0 and C21:0 in walnut oil, while Li and Hu (2011) and Kirbaslar et al. (2012) did not report these two. Vingering et al. (2010) reported C17:0, while Venkatachalam and Sathe (2006) C17:0 and C21:0. Literature have reported several FAs not found in this study such as C20:1n-1, C20:3n-6, C22:1n-6 (Kirbaslar et al., 2012).

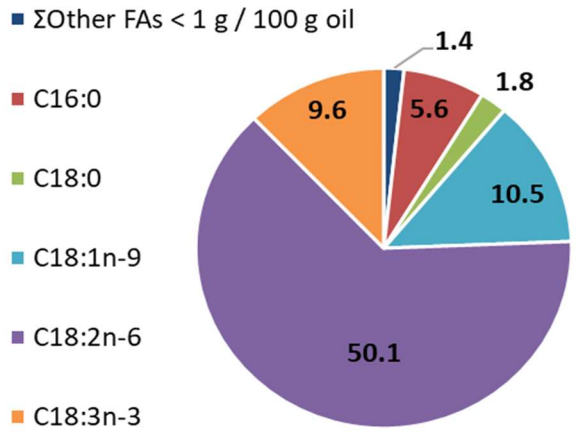


Fig 4.9 Illustrates walnut oil FAs distribution were the values are given in g/100 g oil and FAs, where FAs with amount lower than 1 g / 100 g oil are summed together in "other FAs" (n=5).

4.2 Comparison of the fatty acids in the nut oils

The total FA content in the nut oils ranged from 41.87 to 78.94 g/100 g oil (fig. 4.10). In this study the esterification method converts FAs into FAMEs from PL and NL (TAG, DAG, and MAG).

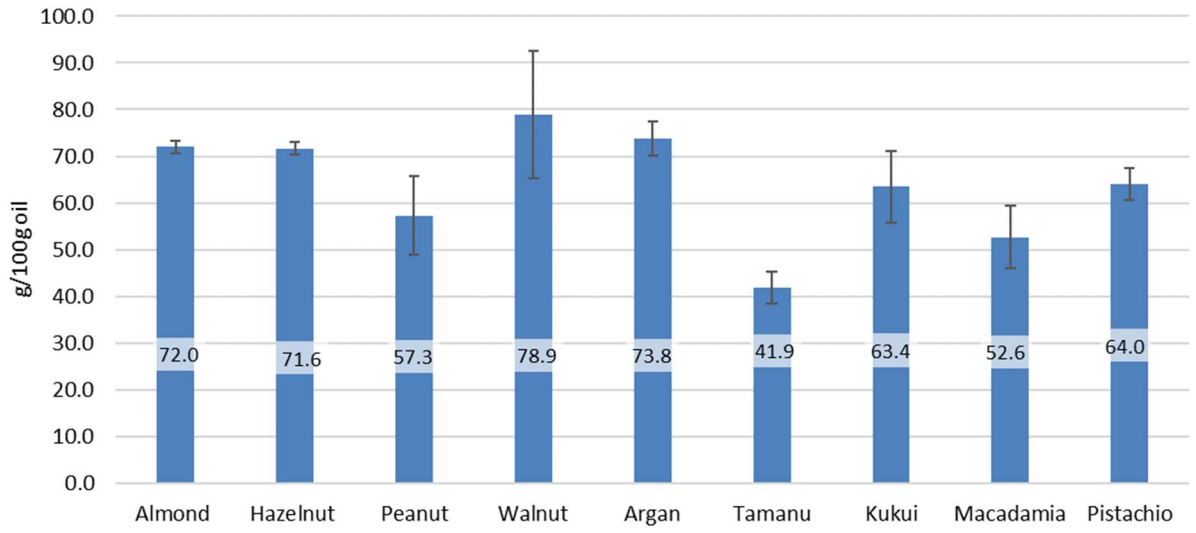


Fig. 4.10 Total FAs content in all nut oils given in average g / 100 g oil ± SD (n = 5).

Li et al. (2006) analyzed lipid content and reported total lipid content (g/100 g) composition in eight nuts including almond, walnut, macadamia and pistachio. The lipid content ranged from 53.5 g/100 g to 75.4 g/100 g. They reported that TAG was the predominant lipid class in the analyzed nut samples. Percentages of TAG ranged from 95.9% to 98.4% (Li et al., 2006). From this we can assume that the values of FA found in this study is valid as the esterification method used quantifies all FAs in NL and PL which includes TAG.

Some bigger differences were found between FA content in this study and lipid content in Li et al., (2006). However, this can be contributed to that Li et al., (2006) used the Macadamia specie, *M. integrifolia* rather than *M. tetraphylla* used in this study. In addition to the pistachio in this study was roasted and it is not specified in Li et al., (2006). If the pistachios were not roasted this could explain the higher values in this study as oil roasting increases the fat content by approximately 4% (Brufau et al., 2006).

Saturated, monounsaturated and polyunsaturated fatty acids in the nut oils

The analyzed nut oils in this study have a high unsaturated FA content ranging from 71% in tamanu oil to 93% in almond oil. MUFAs were the most abundant unsaturated FAs in all nut oils, except for walnut oil and kukui oil, where PUFAs were the most abundant. Overall, the SFA content in all oil were below 20%, except for tamanu oil which was below 30% (fig 4.11). Macadamia oil was the oil with the lowest PUFA content. In addition, the tamanu oil has the

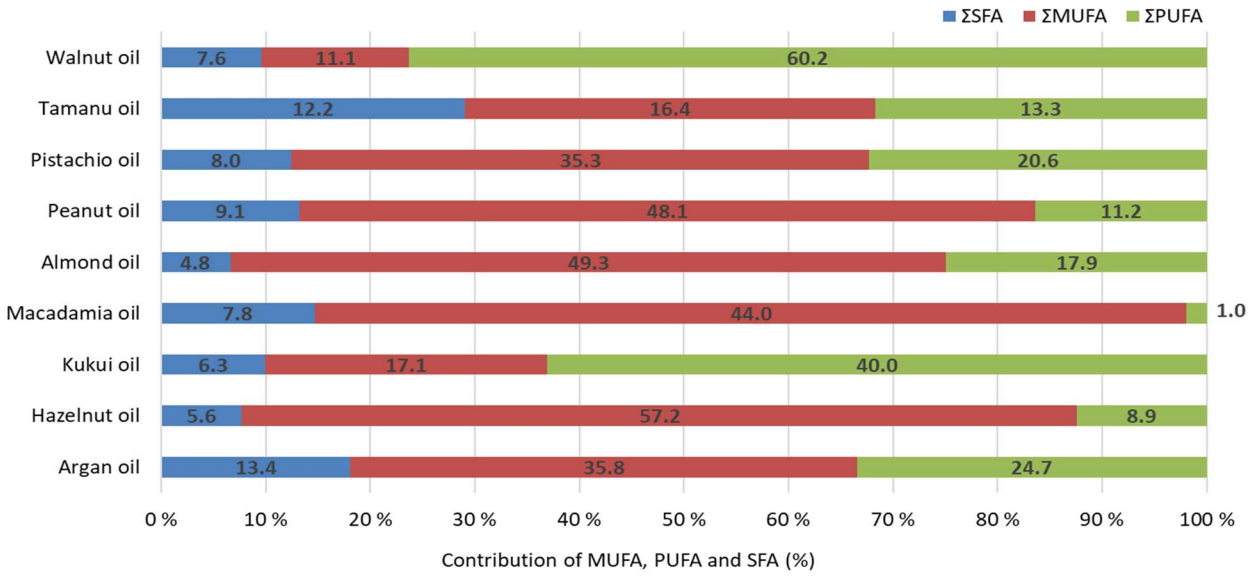


Fig 4.11 ΣSFA, ΣMUFA and ΣPUFA in all nut oils with their contribution in %, and the data labels given in g /100 g oil on the bars.

Walnut oil has the highest PUFA content of 60.2 g/100 g oil, the lowest MUFA content of 11.1 g/100 g oil and a SFA content around average, 7.6 g/100 g oil. Additionally, Kukui oil has the second lowest PUFA content of 40.0 g/100 g oil, third lowest MUFA content of 17.1, and an average SFA content of 6.3 g/100 g oil. Though, kukui oil had a favourable FA composition the plant is toxic and can't be eaten without detoxification. Because walnut- and kukui have oil had the highest PUFA contribution, they also have the highest quantified amount of LA, C18:2n-6 and ALA, C18:3n-3 (Appendix III). Walnuts are the whole foods with the highest ALA content in all edible plants (Hepburn et al., 1986, referred to in Ros., 2010). Sabate et al. (1993) indicated that a diet including moderate amounts of walnuts would lower the serum cholesterol levels. Other studies demonstrated reduced risk of type-2 diabetes in women by replacing SFAs and

trans-FAs with PUFAs (Meyer et al., 2001; Storlien et al., 1996). Overall, walnut oil had the highest PUFA content and is the associated lower cholesterol levels, therefore walnut oil nut consumption could contribute to potential health benefits.

Almond oil was the oil with the lowest SFA content, above average MUFA content, and average PUFA content (fig 4.11). Almonds as snacks in the diet for people with excess amount of lipids in the blood (hyperlipidemic) reduce coronary heart disease factor as a result of the MUFA content and non-fat contents protein and fiber (Jenkins et al., 2002). Ahmad (2010) reported that some properties of almond oil showed cardiovascular benefits such as lowering the LDL-cholesterol, while increasing HDL-cholesterol amongst others. In figure 4.11 hazelnut have the second lowest SFA content, with the highest quantified amount of MUFAs, though the second lowest PUFA amount. Hazelnut incorporated into the diet may prevent LDL cholesterol to oxidize and form plaque inside the blood veins (Orem et al., 2013). Del Gobbo et al. (2015) suggest that intake of tree nuts lowers the risk of cardiovascular disease by lowering LDL cholesterol, and triglycerides. However, the cholesterol lowering effect comes primarily from the quantity of nuts rather than the nut type (Del Gobbo et al., 2015).

The fatty acid compositions in nut oils

The most abundant FA in all nut oils was the MUFA, C18:1n-9, except for walnut oil and kukui oil, where the PUFA, C18:2n-6 was the most abundant (fig 4.12). This is in agreement with previous literature stating that C18:1n-9 is the most abundant FA in plants, together with C18:2n-6, C16:0, and C14:0 (A.Gunstone et al., 2007; Christie, 2003; Ros & Mataix, 2006). The remaining FAs in the nut oils were summed together in the "other FAs" fraction to demonstrate their contribution. Macadamia oil and kukui oil had the highest contribution of “other FAs” (fig 4.12). Where the high C18:3n-3 amount of in kukui oil with 13.04 ± 1.85 g / 100 g oil explains the high “other FAs” bar (Appendix III). In macadamia oil the high amount of C16:1n-7 (7.65 ± 1.07 g / 100 g oil) is the explanation of the high “other FAs” contribution. Kaijser et al. (2000) reported 17 – 34% C16:1n-7 contribution in MUFAs compared to 18% found in this study. Tamanu oil had the highest amount of the SFAs, C18:0, and average amount of C16:0, which together contributes 97% of the total SFA content.

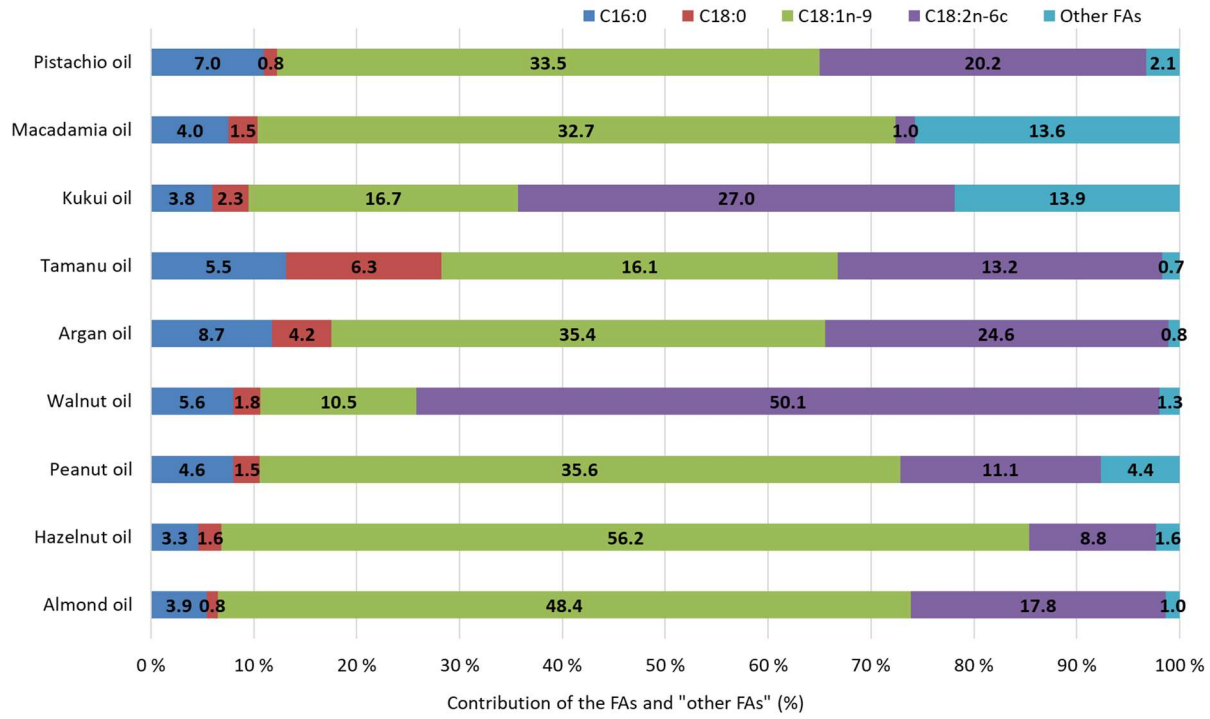


Fig 4.12 Contribution in percent of C16:0, C18:0, C18:1n-9, C18:2n-6 and Σ Other FAs in all nut oils with the quantified amount as data labels given in average g /100 g oil (n=5).

The high content of ALA (C18:3n-3) contributes to the favorable n-6/n-3 ratio in walnut- and kukui oil at 5.3 and 2.1 (Appendix III). These ratios are preferable since n-3 PUFAs are known to improve symptoms of depression (Husted & Bouzinova, 2016), and inflammatory conditions e.g. rheumatoid arthritis (James et al., 2000). Peanut oil had the third highest C18:3n-3 amount, 0.43 ± 0.02 g FA / 100 g oil respectively after walnut and kukui oil and had a n-6/n-3 ratio of 47.0. Though, tamanu oil has lower C18:3n-3 the C18:2n-6 amount was also lower hence lower n-6/n-3 ratio of 19.6. Argan oil, peanut oil, hazelnut oil, and almond oil all had n-3/n-6 ratios above 115 (Appendix III.). While in macadamia oil a n-6/n-3 ratio could not be obtained in this study. Additionally, n-6/n-3 ratios in hazelnut oil and peanut oil have been reported (Vingering et al., 2010). A low n-6/n-3 ratio is favorable since LA(C18:2n-6) could inhibit the uptake of ALA (C18:3n-3), therefore limit the ALA's potential availability as a metabolic precursors for EPA and DHA (Gerster, 1998).

Variation factors in nut oils FA compositions

Overall, the results in this study are in agreement with previously findings. However, the differences between the FA compositions in this study and earlier published FA compositions. These differences may be explained by several reasons. As said earlier the browning of the macadamia kernels had almost twice as high amounts of the FAs than non-browned kernels (Srichamnong & Srzednicki, 2015). Differences argan oils FA composition where found to come from geographical origins, and the extraction process/kernel type (Kharbach et al., 2019). In general differences in natural oil may come from different environmental conditions or genetics (Ako et al., 2005)

4.3 Free fatty acid and polar lipid fractions in nut oils

Some differences may come from the fact that this study quantified FAs from NL and PL. Because the utilized base-catalyzed transesterification in this study trans-esterify FAs in PL and NL. However, Li et al. (2006) reported that TAG is the predominant lipid ranging from 91.1 % to 98.4%. Meaning 91% to 98% of the lipid content in nut oils are TAG, hence the not quantified FFAs found in this study contribute only a few percent. The nut oils were fractionated into NL, PL and FFAs by SPE. NL fraction from SPE was not further analyzed in study only PL and FFAs. Since there were not found any FAs in the PL fraction for almond oil, macadamia oil, and kukui oil. It is therefore assumed that the quantified FA in these oils are from NL. Though in walnut oil, peanut oil, argan oil, hazelnut oil, and peanut oil FAs were found in the PL fraction (appendix IV). Therefore, it is uncertain if these FAs originates from PL or NL in these five nut oils.

Pistachio oil had seven FAs in the PL fraction which were identified and four could be integrated. The four FAs were C14:0, C16:0, C18:0 and C18:1n-9. In the FFAs fraction FAs were found in all the nut oils (appendix IV.). All nut oils had C14:0, C16:0, C18:0, C18:1n-9, and C18:2n-6. They contributed 0.8-2.0%, 15.6-31.9%, 6.8-27.3%, 9.4-58.1%, 1.9-26.1%, respectively of the total integrated FFAs areas. The FAs found in the FFA fraction are among the most abundant FAs in the total analysis, for all nut oils.

5. Conclusion

In conclusion nut oils are a rich source of unsaturated FAs ranging from 71% in tamanu oil to 93% in almond oil. All nut oils contained predominantly MUFAs, except for in walnut oil and kukui oil, where PUFAs were predominant. The MUFA C18:1n-9 and essential PUFA C18:2n-6 were the most abundant FAs in all nut oils. Kukui oil and walnut oil contained relative high amounts of the EFA, ALA (C18:3n-3) (13 and 9 g / 100g oil, respectively) and had therefore the lowest n-6/n-3 ratio at 2, and 5, respectively. However, kukui oil is not edible so further refining and detoxification are needed prior to consumption. Due to the high unsaturated FA content in nut oils, their consumption could contribute to several beneficial health effects such as CHD's and diabetes. Overall, walnut oil has a higher PUFA content than MUFA, and a favorable n-6/n-3 ratio. Therefore, walnut oil or walnut consumption can be argued to be the most health promoting nut oil.

6. Further work

Further studies on detoxification of the kukui oils are required so that the oil can be used to increase the n-3 PUFA content in the human diet. In addition, it would be interesting to investigate the total lipid content in the nut oils, including sterol esters, sterols, TAG and DAG.

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Appendices

Appendix I: Standards

Appendix II: RFF-values

Appendix III: Fatty acid profiles of nut oils

3.A Almond oil

3.B Hazelnut oil

3.C Peanut oil

3.D Walnut oil

3.E Argan oil

3.F Pistachio oil

3.G Tamanu oil

3.H Kukui oil

3.I Macadamia oil

3.J All oils

Appendix IV: FFAs and polar lipid FAs

Appendix V: Original bar charts

Appendix I: Standards

Internal standard

Table 1.A Internal standard used for quantification of the FA composition in all nut oils.

Internal standard	Molecular weight (g/mol)	Concentration (mg/mL)	IS volume (µL)	Moles Fatty Acid
C19:0 TG	933.6	10	50	$1.60675 * 10^{-6}$

Reference standards

Table 1.B 37 FAMEs in the Restek Food Industry FAME MIX used for quantification of FAs in all nut oils. All listed in elution order with systematic name. retention time and peak area.

FAME	Systematic name	Retention time (RT)	AREA
C4:0	Butanoic acid	4.65	34224
C6:0	Hexanoic acid	6.329	1588.299
C8:0	Octanoic acid	7.611	3099.511
C10:0	Decanoic acid	8.959	3703.035
C11:0	Undecanoic acid	9.795	1745.178
C12:0	Dodecanoic acid	10.845	3857.027
C13:0	Tridecanoic acid	12.248	1790.024
C14:0	Tetradecanoic acid	14.135	3836.162
C14:1n-7	*cis-9*-tetradecanoic acid	15.891	1607.751
C15:0	Pentadecanoic acid	16.765	1720.355
C15:1n-7	*cis-9*-pentadecanoic	19.144	1537.408
C16:0	Hexadecanoic acid	20.352	6043.247
C16:1n-7	*cis-9*-hexadecanoic acid	22.861	1544.667
C17:0	Heptadecanoic acid	25.278	1616.868
C17:1n-7	*cis-10*-heptadecanoic acid	28.66	1438.296
C18:0	Octadecanoic acid	32.183	3451.612
C18:1n-9t	trans-9-octadecanoic acid	34.934	1719.794
C18:1n-6c	*cis-9*-octadecanoic acid	35.928	3256.665
C18:2n-6t	trans-9.12-octadecadienoic acid	40.918	1165.047
C18:2n-6c	*all cis-9.12*-octadecadienoic acid	42.879	1231.495
C18:3n-6c	*all cis-6.9.12*- octadecatrienoic	46.745	1115.172
C20:0	Eicosanoic acid	48.818	3090.385
C18:3n-3c	*all cis-9.12.15*- octadecatrienoic	49.152	1193.297
C20:1n-9	*cis-11*-eicosenoic acid	51.132	1304.74
C21:0	Heneicosanoic acid	54.319	1287.212
C20:2n-6c	*cis-11.14*-eicosenoic acid	55.054	1213.284
C20:3n-3t	all trans-8.11.14- eicosatrienoic acid	57.405	1088.318

C22:0	Docosanoic acid	58.873	2715.916
C20:4n-6c	*all cis-5.8.11.14*-eicosatetraenoic acid	59.105	2686.783
C20:3n-3c	*all cis-11.14.17*- eicosatrienoic acid	59.105	2686.783
C22:1n-9c	*cis-13*-docosenoic acid	60.5	1146.513
C23:0	Tricosanoic acid	62.823	2168.524
C20:5n-3c	*all cis-5.8.11.14.17*-eicosapentaenoic acid	62.823	2168.524
C22:2n-6c	*cis-13.16*-docosenoic acid	63.39	1128.504
C24:0	Tetracosanoic acid	66.289	2011.393
C24:1n-9t	*cis-15*-tetracosenoic acid	67.637	995.539
C22:6n-3c	*all cis-4.7.10.13.16.19*-docosahexaenoic acid	71.205	706.191

Table 1.C Other reference standards used in addition to Restek Food Industry FAME MIX for C18:1n-7c for all nut oils and C26 for … nut oils

FAME	Systematic name	Retention time (RT) [min]	Concentration	Diluted in
C18:1n-8c	*cis-12*-octadecanoic acid. ME	41.92	1 mg/mL	Heptane
C18:1n-7c	*cis-11*-octadecanoic acid. ME	40.91	1 mg/mL	Hexane
C26:0	Hexacosanoic acid. ME	73.33	1 mg/mL	Hexane

Appendix II: Relative response factors

For the FAs found in the nut oils not represented the previously determined RRF in table 2.A were assigned the RRF they were most alike. The C16:1n-? isomer was assigned the same RRF as C16:1n-7. Then C17:1n-7 was assigned the same RRF as C17:0, C18:1n-7 FA as C18:1n-9 FA and C26:0 as C24:0, respectively.

Table 2.A RRF-values used for quantification of FAs previously determined by (Devle et al., 2009)

Short name	**Systematic name**	**Molecular weight (g/mol)**	**RRF**
C6:0	Hexanoic acid	116.16	0.67
C8:0	Octanoic acid	144.22	0.81
C9:0	Nonanoic acid	158.24	0.74
C10:0	Decanoic acid	172.27	0.85
C11:0	Undecanoic acid	186.3	1.03
C12:0	Dodecanoic acid	200.33	0.94
C13:0	Tridecanoic acid	214.35	1.11
C14:0	Tetradecanoic acid	228.38	1.01
C14:1	*cis-9*-tetradecanoic acid	226.38	1.24
C15:0	Pentadecanoic acid	242.41	1.22
C16:0	Hexadecanoic acid	256.43	1.09
C16:1n-7c	*cis-9*-hexadecanoic acid	254.43	1.18
C17:0	Heptadecanoic acid	270.46	1.22
C18:0	Octadecanoic acid	284.48	1.19
C18:1n-9c	*cis-9*-octadecanoic acid	282.48	1.16
C18:2n-6c	*all cis-9.12*-octadecadienoic acid	280.48	1.01
C18:2n-6t	trans-9.12- octadecadienoic	280.48	1.04
C18:3n-6c	*all cis-6.9.12*- octadecatrienoic	278.48	0.99
C19:0	Nonadecanoic acid	298.52	1.00
C18:3n-3c	*all cis-9.12.15*- octadecatrienoic	278.48	0.98
C20:0	Eicosanoic acid	312.54	1.17
C20:1n-9c	*cis-11*-eicosenoic acid	310.54	1.13
C20:2n-6c	*cis-11.14*-eicosenoic acid	308.54	1.06
C20:3n-6c	*all cis-8.11.14*- eicosatrienoic acid	306.53	1.17
C21:0	Heneicosanoic acid	326.57	1.18
C20:4n-6c	*all cis-5.8.11.14*-eicosatetraenoic acid	304.52	1.00
C20:3n-3c	*all cis-11.14.17*- eicosatrienoic acid	306.53	0.96
C20:5n-3c	*all cis-5.8.11.14.17*-eicosapentaenoic acid	302.52	0.96
C22:0	Docosanoic acid	340.59	1.18

C22:1n-9c	*cis-13*-docosenoic acid	338.59	1.10
C22:2n-6c	*cis-13.16*-docosenoic acid	336.57	1.03
C23:0	Tricosanoic acid	354.62	1.17
C24:0	Tetracosanoic acid	368.65	1.19
C22:6n-3c	*all cis-4.7.10.13.16.19*-docosahexaenoic acid	328.57	1.01
C24:1n-9c	*cis*-15-tetracosenoic acid	366.65	1.01

Appendix III: Fatty acid profiles of nut oils

Table 3.A Summary table for almond *(Prunus dulcis)* oil with 4 mg/mL concentration. Match factor and probability obtained through NIST 08 library searches from spectral information. Average retention times. RFF-values and average amount of each FA in g/100g (n=5).

FA	Retention time [min]	A. Areal	Match factor	Probability [%]	Molecular weight [g/mol]	RFF	A. Amount FA [g/100g]	S.D.
C14:0	14.16	156.80	883	78.3	228.38	1.01	0.02	<0.01
C15:0	16.77	34.17	815	41.3	242.41	1.22	<0.01	<0.01
C16:0	20.38	27791.60	944	78.2	256.43	1.09	3.88	0.27
C16:1 others*	22.41	64.93	746	22.9	254.43	1.18	0.01	<0.01
C16:1n-7	22.84	1445.65	922	28.6	254.43	1.18	0.19	0.01
C17:0	25.30	87.60	807	67.5	270.46	1.22	0.01	<0.01
C17:1n-7	28.35	199.80	808	28.8	268.43	1.22	0.03	<0.01
C18:0	32.68	5673.31	935	72.5	284.48	1.19	0.81	0.07
C18:1n-9	36.73	333258.76	952	10.4	282.48	1.16	48.42	1.09
C18:1n-7*	37.17	4180.00	907	6.04	282.46	1.16	0.61	0.10
C18:2n-6c	43.18	107506.18	956	37.1	280.48	1.01	17.82	0.30
ΣC18:2 others*	53.631 + 69.62	135.38	780	17.4	280.48	1.01	0.02	<0.01
C18:3n-3c	49.27	381.00	854	41.4	278.48	0.98	0.06	<0.01
Σ18:3 others*	64.216 & 64.96	149.02	707	58.2	278.48	0.98	0.03	0.01
C20:0	48.92	195.25	856	73.9	312.54	1.17	0.03	<0.01
C20:1n-9	51.25	133.40	858	25.5	310.54	1.13	0.02	<0.01
C21:0	54.337	32.40	748	79.5	326.57	1.18	0.01	<0.01
C22:0	58.854	36.13	719	49	340.59	1.18	0.01	<0.01

* Not represented in the Restek Food Industry FAME-mix reference standard

Table 3.B Summary table for hazelnut *(Corylus avellana)* oil with 2 mg/mL concentration. Match factor and probability obtained through NIST 08 library searches from spectral information. Average retention times. RFF-values and average amount of each FA in g/100g (n=5).

FA	Retention time [min]	A. Areal	Match factor	Probability [%]	Molecular weight [g/mol]	RFF	A. Amount FA [g/100g]	S.D.
C14:0	14.153	148.25	886	67.3	228.38	1.01	0.02	<0.01
C15:0	16.774	12.93	809	47.5	242.41	1.22	<0.01	<0.01
C16:0	20.352	5369.72	946	80.5	256.43	1.09	3.32	0.06
C16:1 others*	22.387	53.58	806	20.4	254.43	1.18	0.08	<0.01
C16:1n-7	22.833	20905.82	916	27.3	254.43	1.18	0.01	<0.01
C17:0	25.268	93.35	827	79.7	270.46	1.22	0.01	<0.01
C17:1n-7	28.344	590.55	712	7.65	268.43	1.22	0.01	<0.01
C18:0	32.675	2630.31	936	73.2	284.48	1.19	1.60	0.05
C18:1n-9	36.755	85545.88	949	9.88	282.48	1.16	56.24	1.09
C18:1n-7*	37.136	10894.60	914	6.75	282.46	1.16	0.76	0.03
C18:1n-6c	43.083	349123.06	952	34.6	280.48	1.01	8.83	0.07
C18:1 other*	67.98	61.71	668	6.93	278.48	0.98	0.02	<0.01
C18:3n-3c	49.263	46801.33	855	39.5	278.48	0.98	0.03	<0.01
C20:0	48.901	4788.31	915	69.7	312.54	1.17	0.11	<0.01
C20:1n-9	51.224	736.28	905	27.7	310.54	1.13	0.10	0.01
C21:0	54.347	189.16	796	70.4	326.57	1.18	0.01	<0.01
C22:0	58.854	1023.99	923	55	340.59	1.18	0.37	0.02
C23:0	62.757	64.44	712	76.3	354.62	1.17	0.01	<0.01
C24:0	66.26	1980.33	867	93.1	368.65	1.19	0.11	0.01

* Not represented in the Restek Food Industry FAME-mix reference standard

Table 3.C Summary table for peanut *(Arachis hypogaea)* oil with 2 mg/mL concentration. Match factor and probability obtained through NIST 08 library searches from spectral information. Average retention times. RFF-values and average amount of each FA in g/100g (n=5).

FA	Retention time [min]	A. Areal	Match factor	Probability [%]	Molecular weight [g/mol]	RFF	A. Amount FA [g/100g]	S.D.
C14:0	14.16	113.72	860	63	228.38	1.01	0.02	<0.01
C15:0	16.77	36.11	738	34.5	242.41	1.22	0.01	<0.01
C16:0	20.39	28000.86	948	79.8	256.43	1.09	4.59	0.91
C16:1 other	22.39	83.85	786	28.7	254.43	1.18	0.01	<0.01
C16:1n-7	22.84	201.31	865	31.9	254.43	1.18	0.03	0.01
C17:0	25.27	144.03	872	68	270.46	1.22	0.02	<0.01
C17:1n-7	28.35	103.53	826	25	268.43	1.22	0.02	<0.01
C18:0	32.63	8779.76	910	70.6	284.48	1.19	1.46	0.24
C18:1n-9c	36.65	208758.80	952	9.79	282.48	1.16	35.62	4.87
C18:1n-7c*	37.08	2119.39	864	6.56	282.46	1.16	0.36	0.10
C18:1 iso*	67.97	141.73	635	8.3	278.48	0.98	0.03	0.01
C18:2n-6c	43.16	5714.00	956	37.1	280.48	1.01	11.14	1.78
C18:3n-3c	49.24	475.72	885	37.2	278.48	0.98	0.09	0.02
C20:0	48.92	3104.37	930	56.3	312.54	1.17	0.58	0.10
C20:1n-9	51.24	4439.98	930	25.3	310.54	1.13	0.86	0.17
C21:0	54.34	61.77	787	70.1	326.57	1.18	0.01	<0.01
C22:0	58.91	7422.97	934	73.5	340.59	1.18	1.50	0.26
C22:1n9	60.48	235.72	843	56.6	354.62	1.10	0.05	0.01
C23:0	62.77	43.624	797	76.2	354.62	1.17	0.01	<0.01
C24:0	66.30	3669.56	926	89.4	368.65	1.19	0.79	0.12
C26:0*	72.41	352.47	847	93.3	396.40	1.19	0.08	0.03

* Not represented in the Restek Food Industry FAME-mix reference standard

Table 3.D Summary table for walnut *(Juglans regia)* oil with 2 mg/mL concentration. Match factor and probability obtained through NIST 08 library searches from spectral information. Average retention times. RFF-values and average amount of each FA in g/100g (n=5).

FA	Retention time [min]	A. Areal	Match factor	Probability [%]	Molecular weight [g/mol]	RFF	A. Amount FA [g/100g]	S.D.
C12:0	10.93	83.11	854	61.7	200.33	0.94	0.01	<0.01
C14:0	14.172	178.00	866	56.5	228.38	1.01	0.03	0.01
C15:0	16.784	47.62	824	44.8	242.41	1.22	0.01	<0.01
C16:0	20.371	35132.11	944	79	256.43	1.09	5.55	0.96
C16:1 other*	22.396	166.17	815	29.5	254.43	1.18	0.02	<0.01
C16:1n-7	22.861	217.97	823	20.1	254.43	1.18	0.03	0.01
C17:0	25.296	147.01	830	77	270.46	1.22	0.02	<0.01
C17:1n-7	28.391	46.02	758	11.6	268.43	1.22	0.01	<0.01
C18:0	32.489	11492.18	943	76	284.48	1.19	1.84	0.30
C18:1n-9	36.188	63920.52	929	4.51	282.48	1.16	10.50	1.73
C18:1n-7*	36.978	2671.76	868	5.87	282.46	1.16	0.44	0.09
C18:2n-6c	43.297	267720.60	956	37.2	280.48	1.01	50.08	8.64
C18:2 other*	53.612	139.07	618	10.2	280.48	1.01	0.03	<0.01
C18:3n-3c	49.272	49967.12	954	69.5	278.48	0.98	9.60	1.88
ΣC18:3 others*	56.94. 57.36. 57.73. 47.83	2719.64	889	44.1	278.48	0.98	0.52	0.10
C20:0	48.882	541.83	750	35.6	312.54	1.17	0.10	0.02
C20:1n-9	51.206	696.55	873	17.8	310.54	1.13	0.13	0.02
C20:2n-6c	55.099	69.11	735	2.75	308.54	1.06	0.01	<0.01
C21:0	54.337	64.80	779	74.2	326.57	1.18	0.01	<0.01
C22:0	58.872	59.76	785	74.8	340.59	1.18	0.01	<0.01

* Not represented in the Restek Food Industry FAME-mix reference standard

Table 3.E Summary table for argan *(Argania spinosa)* oil with 2 mg/mL concentration. Match factor and probability obtained through NIST 08 library searches from spectral information. Average retention times. RFF-values and average amount of each FA in g/100g (n=5).

FA	Retention time [min]	A. Areal	Match factor	Probability [%]	Molecular weight [g/mol]	RFF	A. Amount FA [g/100g]	S.D.
C12:0	11.04	96.19	890	69.5	200.33	0.94	< LOQ	
C14:0	14.26	424.80	918	67.6	228.38	1.01	0.09	0.04
C15:0	16.90	170.80	884	58.2	242.41	1.22	0.03	0.01
C16:0	20.59	46163.60	937	74.2	256.43	1.09	8.70	0.61
C16:1n-7	23.02	198.60	838	52.5	254.43	1.18	0.03	0.01
C17:0	25.51	177.40	852	63.5	270.46	1.22	0.03	<0.01
C17:1n-7	28.53	49.80	603	18.2	268.43	1.22	0.01	<0.01
C18:0	33.01	21772.43	906	73.2	284.48	1.19	4.22	0.16
C18:1n-9c	36.90	176933.00	946	9.07	282.48	1.16	35.43	1.42
C18:1n-7c*	37.42	653.20	739	5.54	282.46	1.16	0.14	0.03
C18:2n-6c	43.49	110068.40	954	36.7	280.48	1.01	24.63	1.85
C18:3n-3c	49.46	152.20	669	6.65	278.48	0.98	0.04	0.01
C20:0	49.19	877.40	912	60.3	312.54	1.17	0.19	0.01
C20:1n-9	51.46	785.00	844	28.2	310.54	1.13	0.17	0.02
C21:0	54.60	36.20	766	78.5	326.57	1.18	0.01	<0.01
C22:0	59.12	278.65	883	69.8	340.59	1.18	0.07	0.01
C24:0	66.53	125.60	801	81.2	368.65	1.19	0.04	0.01

* Not represented in the Restek Food Industry FAME-mix reference standard

Table 3.F Summary table for pistachio (*Pistacia vera*) oil with 1 mg/mL concentration. Match factor and probability obtained through NIST 08 library searches from spectral information. Average retention times. RFF-values and average amount of each FA in g/100g (n=5).

FA	Retention time [min]	A. Areal	Match factor	Probability [%]	Molecular weight [g/mol]	RFF	A. Amount FA [g/100g]	S.D.
C14:0	14.27	108.45	842	51	228.38	1.01	0.03	<0.01
C16:0	20.62	25343.40	949	79.4	256.43	1.09	6.98	0.42
C16:1 other*	22.58	127.11	762	22.7	254.43	1.18	0.03	0.01
C16:1n-7	23.03	1666.20	932	34.8	254.43	1.18	0.42	0.03
C17:0	25.54	139.37	724	63.5	270.46	1.22	0.04	0.01
C17:1n-7	28.61	109.98	541	2.25	268.43	1.22	0.03	0.01
C18:0	33.05	2942.99	890	74.7	284.48	1.19	0.82	0.03
C18:1n-9c	37.09	116911.20	936	8.71	282.48	1.16	33.49	1.64
C18:1n-7c*	37.56	3860.40	870	6.59	282.46	1.16	1.10	0.07
C18:2n-6c	43.54	61704.20	946	36.1	280.48	1.01	20.18	1.34
ΣC18:2 others*	53.81	73.54	599	7	280.48	1.01	0.02	0.01
C18:3n-3c	49.47	1281.10	870	42.6	278.48	0.98	0.43	0.02
C20:0	49.23	262.33	842	53.5	312.54	1.17	0.08	0.01
C20:1n-9	51.48	537.57	785	25.1	310.54	1.13	0.17	0.01
ΣC20:1 others*	50.564 + 51.168	262.79	675	12.9	310.54	1.13	0.08	0.01
C20:2n-6	55.33		727	22.3	308.54	1.06	<LOQ	
C21:0	54.64	43.86	557	59.7	326.57	1.18	0.01	0.01
C22:0	59.12	61.00	659	74.6	340.59	1.18	0.02	<0.01
C22:1n-9	60.71	25.00	654	15.6	338.59	1.10	0.01	<0.01

* Not represented in the Restek Food Industry FAME-mix reference standard

Table 3.G Summary table for tamanu *(Calophyllum inophyllum)* oil with 2mg/mL concentration. Match factor and probability obtained through NIST 08 library searches from spectral information. Average retention times. RFF-values and average amount of each FA in g/100g (n=5).

FA	Retention time [min]	A. Areal	Match factor	Probability [%]	Molecular weight [g/mol]	RFF	A. Amount FA [g/100g]	S.D.
C14:0	14.27	20.40	824	54.6	228.38	1.01	<0.01	<0.01
C16:0	20.56	31354.25	946	74.6	256.43	1.09	5.50	0.70
C16:1n-7	23.01	314.73	847	44.7	254.43	1.18	0.05	0.01
C17:0	25.49	109.40	804	64.8	270.46	1.22	0.02	0.01
C17:1n-7	28.59	49.99	528	5.12	268.43	1.22	0.01	<0.01
C18:0	32.90	35101.27	942	73.6	284.48	1.19	6.29	0.66
C18:1n-9	36.63	87364.12	938	7.11	282.48	1.16	16.11	1.10
C18:1n-7*	37.26	1138.17	862	6.69	282.48	1.16	0.21	0.03
C18:2n-6c	43.36	53124.10	950	35.6	280.48	1.01	13.20	1.10
C18:3n-3c	49.37	1342.30	800	41.1	278.48	0.98	0.07	0.01
C20:0	49.14	63129.20	892	53.3	312.54	1.17	0.27	0.01
C20:1n-9	51.41	330.09	824	22.8	310.54	1.13	0.04	<0.01
C21:0	54.58	211.41	698	49.1	326.57	1.18	0.02	<0.01
C22:0	59.11	77.11	859	72.2	340.59	1.18	0.06	<0.01
C23:0	63.02	?	590	51	354.62	1.06	< LOQ	
C24:0	66.52	276.21	674	77.9	368.65	1.19	0.02	0.01

* Not represented in the Restek Food Industry FAME-mix reference standard

** Not able to separate the peaks

Table 3.H Summary table for Kukuinut *(Aleurites moluccans)* oil with 2mg/mL concentration. Match factor and probability obtained through NIST 08 library searches from spectral information. Average retention times. RFF-values and average amount of each FA in g/100g (n=5).

FA	Retention time [min]	A. Areal	Match factor	Probability [%]	Molecular weight [g/mol]	RFF	A. Amount FA [g/100g]	S.D.
C14:0	14.23	424.80	874	60.7	228.38	1.01	0.02	<0.01
C15:0	16.86	170.80	769	36.2	242.41	1.22	<0.01	<0.01
C16:0	20.51	46163.60	947	80.3	256.43	1.09	3.77	0.56
C16:1n-7	22.97	198.60	852	20.7	254.43	1.18	0.03	<0.01
C17:0	25.46	177.40	673	33.3	270.46	1.22	0.01	<0.01
C17:1n-7	28.52	49.80	547	2.02	268.43	1.22	<LOQ	-
C18:0	32.92	21772.43	931	73.4	284.48	1.19	2.25	0.26
C18:1n-9	36.70	176933.00	932	5.83	282.48	1.16	16.67	1.69
C18:1n-7*	37.34	653.20	795	7.31	282.48	1.16	0.34	0.08
C18:2n-6c	43.46	110068.40	956	37.5	280.48	1.01	26.96	3.09
C18:3n-3c**	49.49	152.20	943	66.3	278.48		13.04	1.85
ΣC18:3 others*	163.24	642.38	785	45	278.48	0.98	0.133	0.035
C20:0**	49.49	877.40	-	-	312.54	1.17	<LOQ	-
C20:1n-9	51.41	785.00	876	20.2	310.54	1.13	0.06	0.02
C21:0	54.54	36.20	725	69.9	326.57	1.18	0.01	<0.01
C22:0	59.08	278.65	907	72.5	340.59	1.18	0.20	0.02
C24:0	66.48	125.60	864	92.6	368.65	1.19	0.05	0.01

* Not represented in the Restek Food Industry FAME-mix reference standard

** Not able to separate the peaks

Table 3.I Summary table for macadamia *(Macadamia tetraphylla)* oil with 2mg/mL concentration. Match factor and probability obtained through NIST 08 library searches from spectral information. Average retention times. RFF-values and average amount of each FA in g/100g (n=5).

FA	Retention time [min]	A. Areal	Match factor	Probability [%]	Molecular weight [g/mol]	RFF	A. Amount FA [g/100g]	S.D.
C12:0	10.98	94.73	881	64.8	200.33	0.94	0.019	0.003
C14:0	14.25	1309.18	936	69.7	228.38	1.01	0.269	0.019
C16:0	20.49	16533.01	945	75	256.43	1.09	3.472	1.965
C16:1n-7	23.01	34219.36	951	39.8	254.43	1.18	6.475	3.684
C16:1 other*	23.67	8809.48	859	39	254.43	1.18	1.892	4.026
C17:0	25.47	93.74	668	59.1	270.46	1.22	0.019	0.014
C17:1n-7	28.60	137.19	599	3.32	268.43	1.22	0.030	0.018
C18:0	32.51	6414.87	943	78.2	284.48	1.19	1.360	0.766
C18:1n-9	36.37	143124.89	946	9.22	282.48	1.16	32.724	4.480
C18:1n-7*	37.06	8501.99	939	8.64	282.48	1.16	1.877	0.228
C18:2n-6c	43.11	4058.82	908	18.3	280.48	1.01	1.005	0.128
C20:0**	49.08	7157.99	934	54.1	312.54	1.17	1.719	0.301
C18:3n-3c**	49.29	5.38	0	0	278.48	0.98	0.005	0.011
C20:1n-9	51.35	5828.60	946	27	310.54	1.13	1.455	0.252
C20:1 other*	51.94	235.92	822	15.9	310.54	1.13	0.057	0.013
C21:0	54.55	69.43	651	77.4	326.57	1.18	0.019	0.006
C22:0	59.11	1723.47	902	72.6	340.59	1.18	0.442	0.068
C22:1n-9	60.69	409.72	896	40.4	338.59	1.10	0.111	0.022
C24:0	66.51	512.57	892	92.7	368.65	1.19	0.143	0.032

* Not represented in the Restek Food Industry FAME-mix reference standard

** Hard to detect the two different peaks. sometimes one broad peak. and other times two double peaks

Table 3.J Summarizing all nut oils and their fatty acid composition given in g/100g. Including total FAs, SFA, PUFA, SFA, n-3, n-6, n-6/n-3 ratio and MUFA/SFA (n = 5)

FA	Almond oil	Hazelnut oil	Peanut oil	Walnut oil	Argan oil	Tamanu oil	Kukui oil	Macadamia oil	Pistachio oil
C12:0	N.D.	N.D.	N.D.	0.01 ± <0.01	<LOQ	N.D.	N.D.	0.02 ± <0.01	N.D.
C14:0	0.02 ± <0.01	0.02 ± <0.01	0.02 ± <0.01	0.03 ± 0.01	0.09 ± 0.04	<0.01 ± <0.01	0.02 ± <0.01	0.27 ± 0.02	0.03 ± <0.01
C15:0	<0.01 ± <0.01	<0.01 ± <0.01	0.01 ± <0.01	0.01 ± <0.01	0.03 ± 0.01	N.D.	<0.01 ± <0.01	N.D.	N.D.
C16:0	3.88 ± 0.27	3.32 ± 0.06	4.59 ± 0.91	5.55 ± 0.96	8.70 ± 0.61	5.50 ± 0.70	3.77 ± 0.56	3.97 ± 0.42	6.98 ± 0.42
C16:1 iso	0.01 ± <0.01	0.01 ± <0.01	0.01 ± <0.01	0.02 ± <0.01	N.D.	N.D.	N.D.	0.09 ± 0.01	0.03 ± 0.01
C16:1n-7	0.19 ± 0.01	0.08 ±<0.01	0.03 ± 0.01	0.03 ± 0.01	0.03 ± 0.01	0.05 ± 0.01	0.03 ± <0.01	7.65 ± 1.07	0.42± 0.03
C17:0	0.01 ± <0.01	0.01 ± <0.01	0.02 ± <0.01	0.02 ± <0.01	0.03 ± <0.01	0.02 ± 0.01	0.01 ± <0.01	<0.01± <0.01	0.04 ± 0.01
C17:1n-7	0.03 ± <0.01	0.01 ± <0.01	0.02 ± <0.01	0.01 ± <0.01	0.01 ± <0.01	0.01 ± <0.01	N.D.	0.01 ± <0.01	0.03 ± 0.01
C18:0	0.81 ± 0.07	1.60 ± 0.05	1.46 ± 0.24	1.84 ± 0.30	4.22 ± 0.16	6.29 ± 0.66	2.25 ± 0.26	1.49 ± 0.19	0.82 ± 0.03
C18:1n-9	48.42 ± 1.09	56.24 ± 1.09	35.62 ± 4.87	10.50 ± 1.73	35.43 ± 1.42	16.11 ± 1.10	16.67 ± 1.69	32.72 ± 4.48	33.49 ±1.64
C18:1n-7	0.61 ± 0.10	0.76 ± 0.03	0.36 ± 0.10	0.44 ± 0.09	0.14 ± 0.03	0.21 ± 0.03	0.34 ± 0.08	1.88 ± 0.23	1.10 ± 0.07
C18:1 ISO	N.D.	0.02 ± <0.01	0.03 ± 0.01	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
C18:2n-6c	17.82 ± 0.30	8.83 ± 0.07	11.14 ± 1.78	50.08 ± 8.64	24.63 ± 1.85	13.20 ± 1.10	26.96 ± 3.09	1.01 ± 0.13	20.18 ± 1.34
C18:3n-3c	0.06 ± <0.01	0.03 ± <0.01	0.09 ± 0.02	9.60 ± 1.88	0.04 ± 0.01	0.07 ± 0.01	13.04 ± 1.85	<LOQ	0.43 ± 0.02
ΣC18:3 others	0.03 ± 0.01	N.D.	N.D.	0.52 ± 0.10	N.D.	N.D.	0.13 ± 0.03	N.D.	N.D.
C18:3 ISO	N.D.	N.D.	N.D.	0.03 ± <0.01	N.D.	N.D.	N.D.	N.D.	N.D.
C18:2 ISO	0.02 ± <0.01	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	0.02 ± 0.01
C20:0	0.03 ± <0.01	0.11 ± <0.01	0.58 ± 0.10	0.10 ± 0.02	0.19 ± 0.01	0.27 ± 0.01	N.D.	1.42 ± 0.22	0.08 ± 0.01
C20:1 n-9	0.02 ± <0.01	0.10 ± 0.01	0.86 ± 0.17	0.13 ± 0.02	0.17 ± 0.02	0.04 ± <0.01	0.06 ± 0.02	1.45 ± 0.25	0.17 ± 0.01
C20:1 ISO	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	0.06 ± 0.01	0.08 ± 0.01
C20:2n-6c	N.D.	N.D.	N.D.	0.01 ± <0.01	N.D.	N.D.	N.D.	N.D.	<LOQ
C21:0	0.01 ± <0.01	0.01 ± <0.01	0.01 ± <0.01	0.01± <0.01	0.01 ± <0.01	0.02 ± <0.01	0.01 ± <0.01	0.02 ± 0.01	0.01 ± 0.01
C22:0	0.01 ± <0.01	0.37 ± 0.02	1.50 ± 0.26	0.01 ± <0.01	0.07 ± 0.01	0.06 ± <0.01	0.20 ± 0.02	0.44 ± 0.07	0.02 ± <0.01
C22:1n-9	N.D.	N.D.	0.05 ± 0.01	N.D.	N.D.	N.D.	N.D.	0.11 ± 0.02	0.01 ± <0.01
C23:0	N.D.	0.01 ± <0.01	0.01 ± <0.01	N.D.	N.D.	D.	N.D.	N.D.	N.D.
C24:0	N.D.	0.11 ± 0.01	0.79 ± 0.12	N.D.	0.04 ± 0.01	0.02 ± 0.01	0.05 ± 0.01	0.14 ± 0.03	N.D.
C26:0	N.D.	N.D.	0.08 ± 0.03	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.

N.D. – Not detected
<LOQ – under quantification limit

Table 3.J *Continues*

FA	Almond oil	Hazelnut oil	Peanut oil	Walnut oil	Argan oil	Tamanu oil	Kukui oil	Macadamia oil	Pistachio oil
Tot. FAs	71.97 ± 1.44	71.63 ± 1.39	57.27 ± 8.40	78.94 ± 13.7	73.83 ± 3.68	41.87 ± 3.47	63.43 ±7.60	52.62 ± 6.67	63.96 ± 3.45
SFA	4.77 ± 0.34	5.55 ± 0.13	9.07 ± 1.48	7.58 ± 1.28	13.38 ± 0.74	12.18 ± 1.34	6.32 ± 0.85	7.78 ± 0.89	7.98 ± 0.45
MUFA	49.27 ± 1.17	57.21 ± 0.99	36.97 ± 4.60	11.14 ± 1.84	35.78 ± 1.43	16.42 ± 1.11	17.11 ± 1.76	43.98 ± 5.77	35.35 ± 1.72
PUFA	17.93 ± 0.31	8.86 ± 0.06	11.24 ± 1.61	60.24 ± 10.63	24.67 ± 1.85	13.27 ± 1.10	40.00 ± 4.93	1.01 ± 0.13	20.63 ± 1.36
n-6	17.82 ± 0.30	8.83 ± 0.06	11.14 ± 1.59	50.09 ± 8.65	24.63 ± 1.85	13.20 ± 1.10	26.96 ± 3.09	1.01 ± 0.13	20.18 ± 1.34
n-3	0.06 ± <0.01	0.03 ± <0.01	0.09 ± 0.02	9.60 ± 1.88	0.04 ± 0.01	0.07 ± 0.01	13.04 ± 1.85	-	0.43 ± 0.02
n-6/n-3 ratio	275.51 ± 5.75	253.12 ± 4.84	119.11 ± 9.68	5.24 ± 0.17	668.32 ± 145.80	185.65 ± 19.63	2.07 ± 0.08	-	47.03 ± 1.78
MUFA/SFA	10.36 ± 0.67	10.31 ± 0.06	4.08 ± 0.28	1.47 ± 0.04	2.67 ± 0.10	1.35 ± 0.07	2.71 ± 0.11	5.65 ± 0.31	4.43 ± 0.12

Appendix IV: FFAs and polar lipid FAs

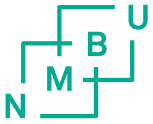
Table 4.A Identified free fatty acids in all nut oils given in integrated peak area (n=1).

FA	Hazelnut	Argan	Walnut	Peanut	Pistachio	Almond	Macadamia	Kukui	Tamanu
C14:0	94	94	89	79	103	149	244	97	-
C16:0	1774	2752	1357	1386	2015	1505	4668	1381	75504
C16:1n-9	-	-	-	-	-	101	4772	-	-
C17:0	-	-	-	-	-	472	106	-	454
C18:0	1812	2485	1509	1577	1723	1510	2129	-	60298
C18:1	6326	5401	572	1876	2477	4105	16933	1516	196272
C18:1 other	-	-	-	-	-	-	833	-	2381
C18:2n-6	886	3792	2232	423	-	1123	582	1289	145286
C18:3n-3	-	-	291	-	-	-	-	652	-
C19:0	-	-	-	-	-	108	-	-	-
C20:0	-	-	-	-	-	-	524	-	2584
C20:1n-9	-	-	-	-	-	-	409	-	942
C22:0	-	-	-	-	-	-	123	-	-
Tot. Area	10892	14524	6050	5341	6318	9073	31323	4935	483267

Table 4.B Identified fatty acids from polar lipid (PL) fraction in all nut oils given in integrated peak area (n=1).

FA	Hazelnut	Argan	Walnut	Peanut	Pistachio
C14:0	I.	-	-	-	463
C16:0	I.	I.	I.	I.	757
C16:1n-9	-	-	-	-	I.
C18:0	I.	I.	I.		337
C18:1n-9	I.	-	-	-	551
C18:2n-6	-	I.	I.	-	-
C20:1	-	-	-	-	I.
C22:6	-	-	-	-	I.
Tot. Area	-	-	-	-	2108

I. – Identified peaks, but too small for integration.



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