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Sea Buckthorn as a Nutrient – Rich Resource for Food Ingredients

Comparison of different samples with sea buckthorn components

MASTER´S THESIS

to achieve the university degree of

Diplom – Ingenieur

Master´s degree programme: Biotechnology

submitted to

Graz University of Technology

Supervisor

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Institute of Biochemistry

Graz University of Technology

Graz, October 2018

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Danksagung

Meine Dankbarkeit ist bei weitem größer als es dieser Absatz mir erlaubt auszusagen. Es sind verschiedene Menschen mit verschiedensten Karaterren die einen Helfen, Türen öffnen und auch schließen, und am Ende auch Prägen, sodass man stets im Leben weiter kommt. Mit vielen höhen und noch mehr tiefen geht man seinen Schicksal entlang, stets bemüht dass Beste aus sich rauszuholen. Ohne diese Menschen und deren diversen Einflüße wäre ich nie an diesen Punkt angekommen im Leben.

Als erstes danke ich meiner Mentorin vom Bachelorstudium aus Sarajewo Frau Prof. Asima Akagic die den Grundstein legte für mein Masterstudium hier in Graz. Sie ermutigte und motivierte mich und letzendlich gab Sie mir Kraft, an mich zu glauben, um ein Studium im Ausland bewältigen zu können.

Von Herzen danke ich meinem jetzigen Betreuer und Mentor dieser Masterarbeit Herrn Assoc. Prof. Dipl. Ing. Dr. techn. Michael Murkovic für die Unterstützung und Bereitstellung seiner Resourcen, Geduld und Zeit. Mit hilfe seiner offenen Art und warmen Seele bekam ich den Mut die Herausforderungen in diesem Projekt zu bewältigen und so mein Wissen zu erweitern.

Bedanken möchte ich mich bei Frau Ciesarova Zuzana und Frau Kukurova Kristina vom "Food Research Institute" aus Bratislava für die Bereitstellung der Proben die ebenso an diesem Projekt mitwirkten wie auch Herrn Karel Cejpek vom "Department of Food Analysis and Nutrition" aus Prag. Die Ausflüge und die netten Gespräche halfen mir Wissen und Ideen zu dem Thema Sanddorn zu erlangen.

Ein großes Dankeschön geht an meine Laborkollgen und Freunde für das tolle Arbeitsklima. Ihr habt mir sehr geholfen und meine Arbeitstage bereichert. Bedanken möchte ich mich ebenso bei allen Freunden in Graz die mich aufbauten und ermutigten in Tagen die weniger erfolgreich waren. Insbesondere danke ich der bedingungslose Freundschaft von Nora Rezaeian die mich unterstützte, motivierte und stets zur Hilfe stand, wie kein Anderer am Institut.

Jedoch, mein größter Dank gebührt meinen Eltern, Vater Nezirevic Fehim und Mutter Nezirevic Sadeta für ihre Kraft, Motivation, Vertrauen und Liebe, die mir mein Studium ermöglicht und mich in all meinen Entscheidungen unterstützt haben. Vor allen meiner Mutter Sadeta, die meinen Abschluss leider nicht mehr miterleben kann, danke ich aus tiefster Seele für alles, was Sie für mich getan hat und fürs weitere Leben mitgab.

Diesen Abschluss erbrachte ich zu deinen Ehren!

Zusammenfassung

Das Ziel dieser Masterarbeit war, den Einfluss von Sanddorn als Bestandteil in verschiedensten Nahrungsmittelprodukten zu analysieren und die Konzentrationen der ausgewählten Nährstoffe zu bestimmen. Nach verschiedenen Literaturquellen gilt Sanddorn als eine außerordentlich interessante Pflanze deren nährstoffreiche Inhaltsstoffe eine sehr positive Auswirkung auf die Gesundheit des Menschen haben kann. Verschiedene Proben mit Sanddornbestandteilen wurden auf Konzentration von L - Ascorbinsäure, Gesamt Vitamin C-Gehalt, β - Carotin und Gesamt-Phenolgehalt analysiert.

Zusammenfassend lässt sich sagen, dass die Sanddornproben eine viel höhere Konzentration an L-Ascorbinsäure u somit auch Vitamin C aufwiesen als an β – Carotin und Gesamt – Penolgehalt. Dennoch überraschten einige Proben mit einem höheren β – Carotin sowie Gesamt – Phenolgehalt.

Demnach bestätigt die Pflanze ihre Vorzüge als nährstoffreiche Quelle, die einen positiven Einfluss auf die Ernährung des Menschen haben kann.

Die höchsten **Vitamin C-Konzentrationen** wurden in den drei Tinkturen im Bereich von 2410 – 3880 mg/ 100 g gefunden. Ebenso ist der höchste **Ascorbinsäure** Gehalt von 1293 – 3308 mg/ 100 g in den Tinkturen bestimmt worden. Es wurde ebenfalls eine sehr hohe Konzentration in Sanddornsaft und Sanddornsirup nachgewiesen, dessen Konzentrationen denen der Sanddornbeere ähnelten. Überraschend waren verschiedene Backwahren, Tee- und Honig-proben und Nebenprodukte die ebenfalls Gesamt Vitamin C und Ascorbinsäure enthielten.

Der Gehalt an **β – Carotin** war in der Tee-probe mit Sanddornblättern mit 8,08 mg/ 100 g am höchsten. Dicht gefolgt vom trocken gemahlenen Sanddorn Proben mit einem Gehalt von 7,08 mg/ 100 g. Interessanterweise wahr der Gehalt in den Sanddornbeeren sehr niedrig mit 1,99 mg/ 100 g für Erntejahr 2015 oder 1,66 mg/ 100 g Erntejahr 2016. Einen viel größeren Gehalt an β – Carotin hatte die Kuchenprobe mit Sanddorn in Konzentration von 4,48 mg/ 100 g.

Der **Gesamt-Phenolgehalt** in den Tinktur- und Tee – proben, sowie Nebenprodukt Proben und allen Backwaren war sehr hoch. Den höchsten Wert hatte die Tinktur bezeichnet als Tee mit 211 mg/ 100 g, gefolgt von den Nebenprodukt – Proben aus allen drei Chargen mit Konzentrationen von 137 – 164 mg/ 100 g. Erstaunlich wenig enthielten die Sanddornbeeren sowie Proben wie Sanddornsaft und Sirup, Honig oder Marmelade.

Abstract

The aim of this Master's thesis was to analyze the influence of sea buckthorn as a component in various food products and to determine the concentrations of selected nutrients. Various samples of sea buckthorn constituents were analyzed for concentration of L – ascorbic acid, total vitamin C content, β – carotene and total phenolic content.

In summary, it can be said that the sea buckthorn samples had a much higher concentration of L – ascorbic acid and therefore also vitamin C than β – carotene and total phenol content. Nevertheless, some samples surprised with a higher β – carotene and total phenolic content. Thus, the plant approves its advantages as a nutrient – rich source, which can have a positive effect on the human diet.

The highest **vitamin C** concentrations were found in the three tinctures in the range of 2410 to 3880 mg/ 100 g. As well, the highest ascorbic acid content of 1293 – 3308 mg/ 100 g was determined in the tinctures. Also, a very high concentration was found in sea buckthorn juice and sea buckthorn syrup, whose concentrations resembled those of sea buckthorn berry. Surprising were various bakery goods, tea and honey samples and by – products which also contained in total vitamin C and ascorbic acid.

The content of **β – carotene** was highest in the sea buckthorn tea leaves sample with 8,08 mg/ 100 g. Closely followed by milled dry sea buckthorn samples containing 7.08 mg/ 100 g. Interestingly, the content in the sea buckthorn berries was very low at 1.99 mg/ 100 g for crop year 2015 or 1.66 mg/ 100 g crop year 2016. In comparison, the cake with sea buckthorn had a much higher β – carotene content in a concentration of 4.48 mg/ 100 g.

The **total phenolic content** in the tincture and tea samples, as well as by – products or baked goods were very high. The tea tincture sample was highest at 211 mg/ 100 g, followed by by – product samples from all three batches at concentrations of 137 – 164 mg/ 100 g. Surprisingly small content contained the sea buckthorn berries and samples such as sea buckthorn juice and syrup, honey or jam.

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1. Introduction

The success of survival refers to the regular consumption of food and liquid. The last 10,000 years of human history has the development of agriculture permitted the population growth. The history of mankind is characterized by the success of culture production, gathering, and securing foods. In this sense, it is important to optimally use the diverse food supply and to tailor it to the individual needs of energy and nutrients.

Foods, and the nutrients derived from them, serve many ends. The body's needs are met by consuming the essential nutrients, both macronutrients and micronutrients, in amounts sufficient to maintain health. The nutrients together with water relate to metabolic requirements and the role these nutrients play in forming biological compounds.

Macronutrients - fats, carbohydrates, and proteins provide energy for the body. Micronutrients are required in small quantities for survival, they include vitamins and minerals. Other components that impact health are alcohol, dietary fiber (cellulose, lignin's...), and phytochemicals (alkaloids, anthocyanin's, carotenoids...).

The vitamins are classified as water soluble or fat soluble. The difference between these two types of vitamins lies in their solubility in water or in organic solvents, including fats and oils. Vitamins are essential for a large number of cellular and extracellular chemical reactions.

Phytochemicals or phytomolecules are non – nutrient molecules made by plants and found in diverse fruits, vegetables, grains, nuts, and seeds. Plants produce phytochemicals to provide a variety of diverse functions (for example fiber give rigidity to plant tissues and serves as a structural component of plant cell walls). They exist only in plant-based foods. Other phytochemicals act as antioxidants that protect cells against free radicals or highly reactive chemicals produced as a result of cellular metabolism.

Healthy dietary patterns bring all of the essential nutrients, energy, and phytochemicals. For good health, the diet should include mostly nutrient-rich foods because they have many micronutrients in inclusion to modest amounts of macronutrients for energy and protein. For good health, the daily intake of food should include mostly nutrient-rich foods because they have many micronutrients in inclusion to modest amounts of macronutrients for energy and protein.

In comparison, energy – dense or calorie – dense foods are generally not favored because they contain too much energy and too few micronutrients.

The three major reasons of food intake are availability of foods, purchasing power (money or barter), and social and cultural values placed on specific foods. The first two factors are more relevant in developing countries, whereas the third becomes more relevant in developed countries where purchasing power and a wide availability of foods allowance the selection of specific foods.

Food availability is highly determined by one's geographic location due to production. Distribution and cultivation being dependent on an area's climate, infrastructure and economy.

Functional foods are specially labeled as having health benefits or having advantages in reducing the risks of one or more chronic diseases. Functional foods are foods that have a potentially good effect on health beyond basic nutrition. A good example of a functional food is the sea-buckthorn berry, which is not only a good source of vitamins and minerals, but is also rich in phytochemicals. Sea buckthorn berries are good sources of phytochemicals such as vitamin C, polyphenols or β – carotene.

In the end, food supply the nutrient requirements and no nutrient phytochemicals that are crucial for both health promotion and disease prevention.

1.1. Vitamin C

Ascorbic acid is a six carbon compound similar to glucose. The term vitamin C characterized all compounds exhibiting the biological activity of ascorbic acid (2, 3-didehydro-l-threo-hexano-1, 4-lactone; also L-ascorbic acid). The vitamin also exist in the oxidized form, L-dehydroascorbic acid or dehydroascorbic acid. Biological activity lean on the 6-carbon lactone having a 2, 3-enediol structure. Ascorbic acid is a dibasic acid (pK_a values, 4.1 and 11.8) as both enolic hydroxyl groups can dissociate. It forms salts (sodium and calcium salts), the aqueous solutions of which are strongly acidic.

With a strong reducing agent, ascorbic acid is oxidized under mild conditions to dehydroascorbic acid via the radical intermediate semidehydroascorbic acid. (Combs & McClung, 2017)

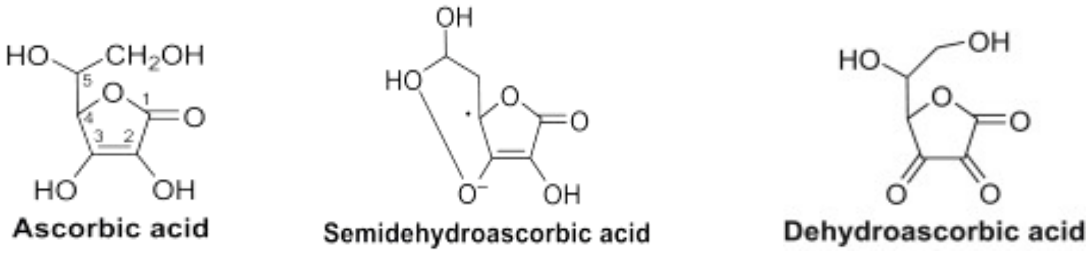

Figure 1. Chemical structure of ascorbic acid, semidehydroascorbic acid and dehydroascorbic acid (Combs & McClung, 2017)

Vitamin C can be synthesized from most of the higher animals (and probably all green plants). They generate it from glucose via the glucuronic acid pathway (Figure 2). (Combs & McClung, 2017)

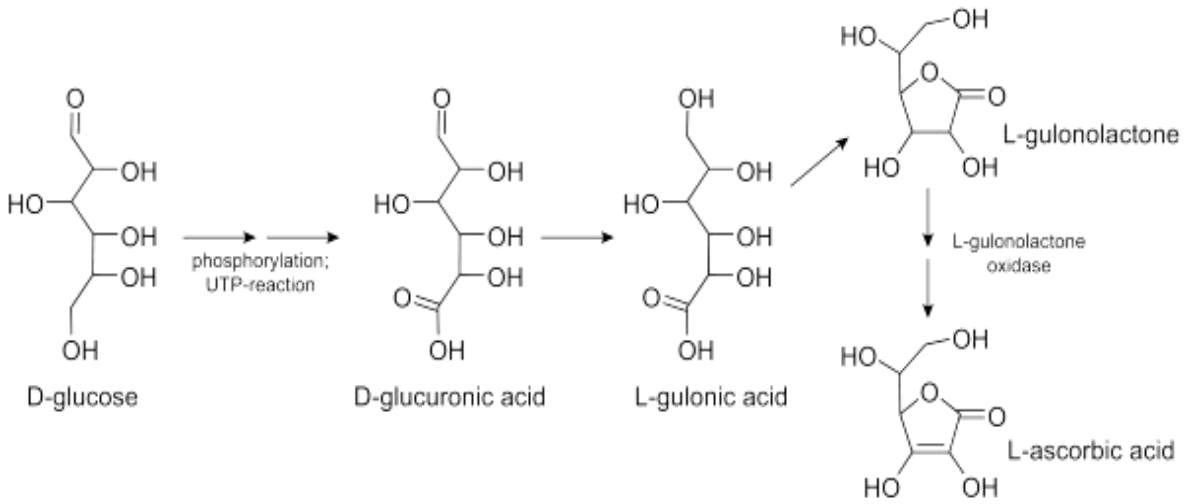

Figure 2. Glucuronic acid pathway from glucose to ascorbic acid (Combs & McClung, 2017)

The enzymes of this pathway are in the kidneys of amphibians, reptiles, egg-laying mammals, and the more primitive orders of birds; in both the kidneys and livers of many marsupials; but only in the livers of passerine birds and other mammals (Combs & McClung, 2017). Therefore, Vitamin C is an essential nutrient for a few species only, which, by virtue of a single enzyme deficiency, cannot synthesize it.

Vitamin C in most foods has biological activities comparable to that of purified L-ascorbic acid at doses in the nutritional range (15 – 200 mg). At higher doses, bioavailability falls due to declining absorption efficiency. Because dehydroascorbic acid can be reduced metabolically to yield ascorbic acid, both forms present in foods have vitamin activity. (Combs & McClung, 2017)

Vitamin C is widely appropriated in both plants and animals, occurring mostly (80–90%) as ascorbic acid but also as dehydroascorbic acid. Fruits, vegetables, and organ meats (e.g., liver and kidney) are generally the best origins. Plants synthesize L-ascorbic acid from carbohydrates. Most of the seeds do not contain ascorbic acid but start to synthesize it on sprouting. (Combs & McClung, 2017)

Some plants have high levels of the vitamin (e.g., fresh tea leaves, some berries, guava, and rose hips).

For practical sense, citrus and other fruits are good daily sources of vitamin C, as they are generally eaten raw and are, therefore, not expose to cooking procedures that can destroy vitamin C.

Table 1. Vitamin C contents of food (Combs & McClung, 2017)

Food	***Vitamin C, mg/ 100 g***
Orange	59
Guava	228
Apple	5
Banana	9
Rose hips	426
Strawberry	59
Potato	11
Carrot	3
Broccoli	89
Pepper	80 – 128
Cabbage	37
Cauliflower	48
Onion	7
Milk, cow	0 – 1
Milk, human	5
Beef	0

The role of vitamin C in a healthy diet is very important and well known. Therefore, in developed countries, food and drinks with a high content of vitamin C has become a popular component of a healthy diet.

Sea buckthorn is noticed as the next generation of new botanical because of its considerable medicinal value. Medicinal uses of sea buckthorn are documented in Asia and Europe. Historically, the Chinese used sea buckthorn medicinally for thousands of years.

The fruits, including seeds, have large amounts of essential oils and vitamin C. Nutritional fact of sea buckthorn fruit are based on its known composition (Beveridge et al., 1999) and the rapport of this composition to human nutritional requirements (Magherini, 1986). Generally, sea buckthorn fruits are very great in health promoting compounds (Jeppsson et al., 2000).

The vitamin C concentration in fruits alter depending on species, geographical location, and physiological maturity (Bernath and Foldesi, 1992; Zhou et al., 1991). Ascorbic acid in sea buckthorn species vary from 360 to 2500 mg/ 100 g (Beveridge et al., 1999) from general literature sources. These values are higher than in virtually any of the generally consumed fruits such as oranges (50 mg/ 100 g; Lu, 1992), strawberries (64 mg/ 100 g; Gontea and Barduta, 1974), or tomatoes (12 mg/ 100 g; Lu, 1992) and is comparable to kiwi fruit (100 – 400 mg/ g; Lu, 1992)

Ascorbic acid is sensitive to oxidation to dehydroascorbic acid, which itself can be rapidly and irreversibly degraded at neutral pH by irreversible hydrolytic opening of the lactone ring to yield 2, 3-diketogulonic acid. These reactions exist in the presence of O_2, even traces of metal ions, and are enhanced by heat and conditions of neutral to alkaline pH. The vitamin is also decreased by exposure to oxidases in plant tissues. Therefore, substantial loss of vitamin C can occur during storage, which is enhanced greatly during cooking. Alternatively, quick heating methods can protect vitamin C in food by inactivating oxidases, and acidic conditions stabilize dehydroascorbic acid (Combs & McClung, 2017).

However, due to the reversible nature of this reaction, the DHA is then converted into AA by dehydroascorbate reductase (DHAR) in different cells such as erythrocytes, smooth muscle cells and hepatocytes (Wilson, 2002). Because fruits and vegetables are leading source of vitamin C, accurate quantification of AA and DHA is warranted. Fruits and vegetables have complex matrices with enough nonspecific compounds that interfere with quantification of AA and DHA (Fontannaz, Kilinç, & Heudi, 2006; Patil, Jayaprakasha, Chidambara Murthy, & Vikram, 2009). Therefore, quantification of these compounds in fruits and vegetables without degradation of vitamin C is challenging. BME (β – mercapto ethanol) and DTT (dithiothreitol) are the two generally used reducing agents for DHA analysis by derivatization (Deutsch & Santhosh-Kumar, 1996; Schorah et al., 1996). TCEP (tris (2- carboxy ethyl) phosphine hydrochloride) is a non-volatile, odorless and comparatively inexpensive reducing agent (Han & Han, 1994). Unlike DTT, the utilization of TCEP in the field of biochemistry was not popular until recently (Getz et al., 1999).

In a study in the USA it was shown that the presence of the TCEP in grapefruit samples, stabilized the total ascorbic acid (TA) at all concentrations of MPA (metaphosphoric acid) and TCA extracts (trichloroacetic acid). As TCEP presents a higher reducing efficiency compared to other reducing agents tested, it was selected to reduce the grapefruit samples extracted with MPA (metaphosphoric acid) and TCA (trichloracetic acid). (Chebrolu et al., 2012)

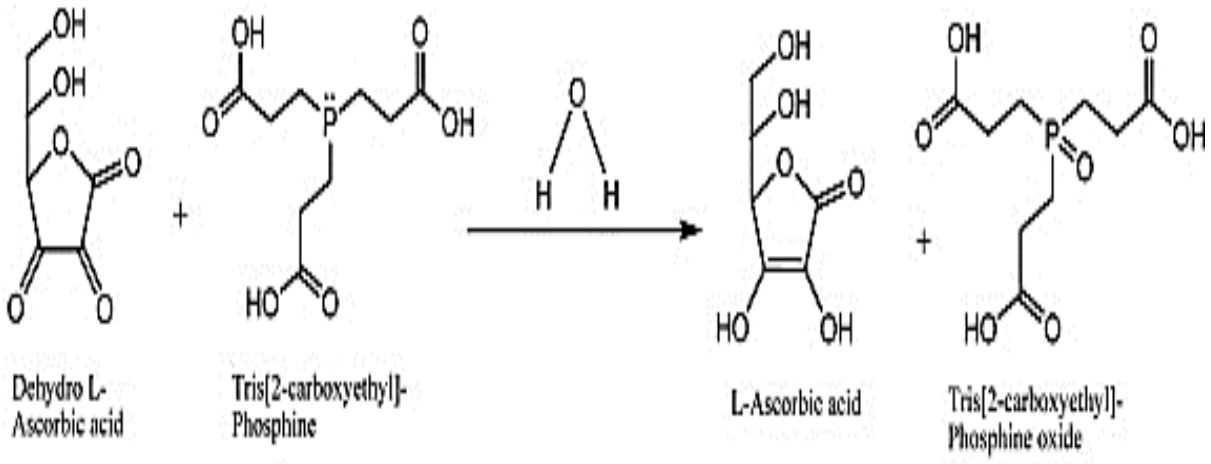

Figure 3. The schematic representation of reduction of dehydroascorbic acid to ascorbic acid facilitated by tris-(2-carboxy ethyl)-phosphine-hydrochloride (Chebrolu et al., 2012)

1.2. Carotenoids

Biological materials are generally classified as fats, proteins, carbohydrates, and minerals. 'Fat extracts' may be collected of a complex mixture of substances with widely different chemical structures, but with similar solubility in non-polar solvents. The term presently used to contain this diverse group of substances is Lipid. Lipids are still most often characterized according to their solubility properties. (Weber, 1980)

Most lipids are composed of carbon, hydrogen, and oxygen but some may contain only the first two elements. Lipids may also have phosphorus, nitrogen, and/or sulfur. General systems of lipid classification are based on the chemical structure. Classification of lipids are based on the presence of functional groups, types of linkages between molecules, and the presence of polar substances and elements other than carbon, hydrogen, and oxygen. Lipids area in structure from simple short hydrocarbon chains to more complex molecules, including triacylglycerol'-s, phospholipids and sterols as well as their esters. Lipids within each class may vary structurally. (Burdge & Calder, 2015)

Lipids have been divided into eight categories: Fatty acyls, glycerolipids, glycerol-phospholipids, sphingolipids, saccharolipids and polyketides (derived from condensation of ketoacyl subunits); and sterol lipids and prenol lipids (derived from condensation of isoprene subunits). (Fahy et al., 2011)

Most of the various yellow to red pigments (as carotenes) found widely in plants and animals and characterized chemically by a long aliphatic polyene chain composed of eight isoprene units are termed carotenoids.

Carotenoids belong to the class of tetraterpenoids (i.e. they contain 40 carbon atoms, being built from four terpene units each containing 10 carbon atoms). Structurally, carotenoids take the form of a polyene hydrocarbon chain which is sometimes completed by rings, and may or may not have additional oxygen atoms attached. Terpenes are molecules formed from repeating isoprene (2-methyl-1,3-butadiene) units.

Terpene is formed from two isoprene molecules. Examples of terpenes are the diterpene retinol (vitamin A) and the plant compound *β*-carotene, a tetraterpene that can act as a precursor of retinol.

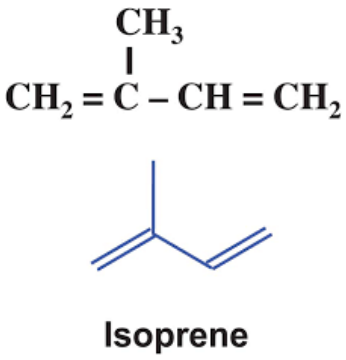

Figure 4. Chemical structure and molecular configuration of the isoprene (Zimmerman et al., 2018)

Carotenoids are a terpene subclass that is usually associated with such foods as tomatoes, parsley, oranges, and pink grapefruits. Carotenoids are a class of natural pigments that are extensively distributed in vegetables and fruits and also used as food additives. Presently, more than 600 different types of carotenoids have been found in nature. Like terpenes, carotenoids are also divided into smaller classes. Possible the two best known, and most studied, carotenoids are β-carotene and xanthophylls (Zimmerman et al., 2018).

As mentioned, carotenoids are lipid-soluble pigments responsible for the color of a wide variety of foods. They can be divided into these two groups (Shen et al., 2009):

1. Xanthophyll'-s, molecules containing oxygen, such as lutein and zeaxanthin; and,
2. Carotenes, non-oxygenated molecules such as β – carotene and lycopene.

Some of them are pro-vitamin A carotenoids, subsequently transformed into vitamin A, which can prevent eye diseases, such as night blindness, susceptibility to infection, rough, scaly skin, and limited tooth and bone development. There are about 700 carotenoids in nature, but only about 50 are with pro-vitamin A activity. Of those 50 compounds, we found the three most important forerunner of vitamin A in humans to be α – carotene, β – cryptoxanthin and β – carotene, which are the major pro-vitamin A components of most carotenoid-containing foods. (Lozano-Alejo et al., 2007; Jaswir et al., 2011)

Although carotenoids are present in many common human foods, deeply pigmented fruits, juices and vegetables create the major dietary sources with:

1. Yellow-orange vegetables and fruits give most of the β-carotene and the α-carotene;
2. Orange fruits contains α-cryptoxanthin;
3. Dark green vegetables and egg yolk have lutein and zeaxanthin and tomatoes lycopene (Rao & Rao, 2007)

Carotenoids can also be found in the animal kingdom (bird plumage, fish, crustaceans, and insects). However, animals and humans cannot synthesize carotenoids, so food is their only source of these compounds. (Tanaka et al., 2012; Burri et al., 2011)

The carotenoids may be a component of a larger cellular structure, such as chloroplast in green tissue, dissolved in fat as in margarine, or in a crystalline state as lycopene in the tomato (Simpson & Chichester, 1981).

But, the level of phytochemicals, such as carotenoids, varies in different fruits and vegetables, according to the variety cultivated.

The content of phytochemical substances is also influenced by numerous factors, such as ripening time, genotype, cultivation techniques, and climatic conditions that occur during the pre-harvest period. (Tavarini et al., 2008; Kamffer et al., 2010)

Other than pre-harvest factors, numerous post-harvest steps, including food-processing operations, also have a great influence on the stability of phytochemicals in fruit and vegetables and in their products.

1.2.1. β – Carotene

Over the past years, a large number of compounds which can inhibit the development of cancer has been identified.

Many of these compounds are found to exist naturally in food or elsewhere in nature.

β – Carotene is a carotenoid that has become an attractive subject in research today.

A large body of observational epidemiologic studies has consistently demonstrated that people eating more fruits and vegetables (which are rich in carotenoids) and people having bigger β-carotene levels have a lower risk of cancer. (Mayne, 1996; Krinsky et al., 1992)

Moreover, a number of animal and laboratory studies have shown that β – carotene can block certain carcinogenic processes and suppress tumor cell growth. (Krinsky et al., 1992; DeFlora et al., 1999)

Some mechanisms for these actions are that β – carotene may:

- function as an antioxidant, (Burton & Ingold, 1984; Krinsky, 1992)
- be a precursor for retinoic acid, (Napoli, 1988; Wang et al., 1996)
- raise immunologic function, (Bendich, 1986; Santos et al., 1996)
- activate carcinogen-metabolizing enzymes (Edes et al., 1989)

Table 2. β – Carotene in raw fruits and vegetables (Bushway, 1986)

Product	***β - carotene μg/ 100 g***
Spinach	4328 – 4737
Broccoli	576 – 707
Asparagus	289 – 353
Squash	3911 – 4925
Carrots	7876 – 9344
Pink grapefruit	231 – 346
Green Pepper	75 – 88
Sweet potato	7296 – 11158

β - Carotene (important provitamin A), generally distributed in dark green leaf, vegetables, carrot, and certain red and yellow fruits (Table 2), has been used as a protective agent against mutagenesis (Salvadori et al. 1992). Some studies have advised that β – carotene has an inhibitory effect on the genotoxic activity of several compounds.

β – Carotene is the primary plant – based source of vitamin A; thus, a clear understanding of factors influencing its potential for utilization as vitamin A has relevant public health implications. Vitamin A deficiency continues to appoint a major health impact throughout much of the developing world. The negative consequences of vitamin A deficiency include impaired resistance to infection, blindness, and increased risk of mortality. (Novotny et al., 2010)

Based on chemical data, the main function of β – carotene is as an optimal, naturally occurring, provitamin A. Structurally and functionally is β – carotene different from other carotenoids. No difference is between naturally occurring and chemically synthesized β-carotene (Grune et al., 2010).

As a tetraterpenoid β – carotene has 40 carbon atoms in a core structure of conjugated double bonds substituted with 2 β – ionone rings.

Due to its extended system of 9 fully conjugated double bonds, β – carotene has a major absorption peak in the visible spectrum with a maximum at ~450 nm, responsible for the orange to red color of the compound. In biological systems, the dominant isomer is the all-*E*-β – carotene. *Z*-isomers have been detected in living organisms and food samples, among them are 9-*Z*-, 13-*Z*-, and 15-*Z*-β – carotene, in addition to several di - and poly - *Z* analogs. (Grune et al., 2010)

β – Carotene is an important carotenoid in the skin and is enriched in this tissue upon supplementation. Human intervention studies demonstrate moderate UV protective effects of β – carotene in the skin. Due to its unique structure and cleavage efficacy, β – carotene is the most active provitamin A carotenoid. As an antioxidant, the compound destroys molecular singlet oxygen and scavenges reactive oxygen species, especially peroxyl radicals. Singlet oxygen destroying is probably to be restricted to the skin as the only light exposed tissue that contains higher levels of β – carotene; other carotenoids demonstrate comparable activity. Upon radical scavenging, β – carotene decays and cannot be regenerated. Thus, it is proposed that the major function of β – carotene in human nutrition is that of a provitamin A. (Grune et al., 2010)

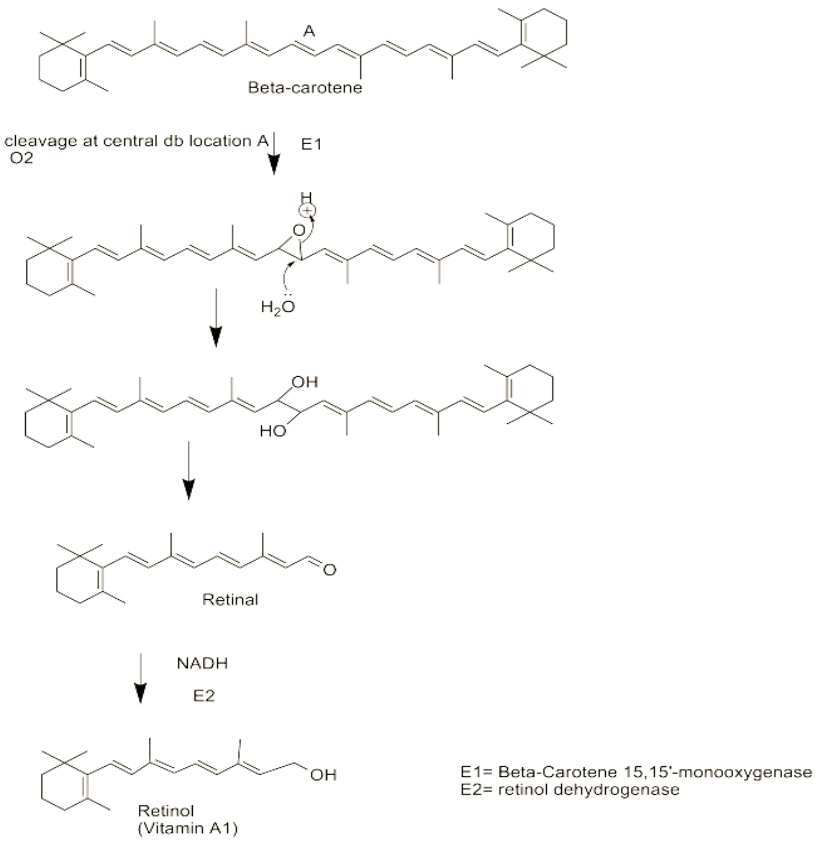

Figure 5. Conversion of β – carotene to retinol (Vitamin A) (Dewick & Paul, 2009)

Retinol is synthesized from the dismantling of β – carotene. First the β – carotene 15-15'-monooxygenase divide β – carotene at the central double bond, creating an epoxide. This epoxide is then attacked by water resulting two hydroxyl groups in the center of the structure. The cleavage arises when these alcohols are reduced to the aldehydes using NADH. This resulting compound is called retinal and is then reduced to retinol by the enzyme retinol dehydrogenase (Figure 5). (Dewick & Paul, 2009)

The type of food matrix in which carotenoids are located regulate their bioavailability to a great extent. Processing, such as mechanical homogenization or heat treatment, has the potential to increase the bioavailability of carotenoids from vegetables.

However, for β – carotene, this will not result in bioavailability similar to that observed for the pure compound. The amount of dietary fat required to provide carotenoid absorption seems to be low (3–5 g per meal), although it depends on the physicochemical characteristics of the carotenoids ingested. Unabsorbable, fat-soluble compounds decrease carotenoid absorption and interaction among carotenoids may also result in a weaker carotenoid bioavailability (Hof et al., 2000).

Interesting is also that in one study from the university in London they did not find convincing evidence that antioxidant supplements have beneficial effects on mortality. Even more, β – carotene, vitamin A, and vitamin E as a supplements seem to increase the risk of death (Bjelakovic et al., 2007). Therefore, the intake of β – carotene by food should be advocated more.

1.3. Polyphenols

Polyphenols are a huge family of phytochemicals with great chemical diversity, known to be bioactive compounds of foods, nutraceuticals and medicinal plants.

Polyphenols are a structural class of mainly natural, but also synthetic or semi-synthetic, organic chemicals that are defined by the presence of one or more aromatic ring manner one or more hydroxyl moieties. (Dewick, 2009; Ververidis et al., 2007)

With over 8000 structural variations, polyphenols are the largest family of secondary metabolites created by the shikimate/phenyl propanoic or polyketide pathways of plants.

They are involved in protection against ultraviolet radiation, cold temperatures and droughts, help plants defend themselves against herbivores, parasites and pathogens, and contributing to the organoleptic properties of leaves, berries and fruits, and their products, such as wine or olive oil.

The structure of polyphenols ranges from simple molecules, such as phenolic acids, to highly polymerized molecules, such as condensed tannins (Figure 6). (Haborne, 1980)

The main polyphenol subgroups are characterized according to the number of phenol rings and structural elements that bind these rings to one another: phenolic acids, flavonoids, stilbenes and lignans (Quideau et al., 2011).

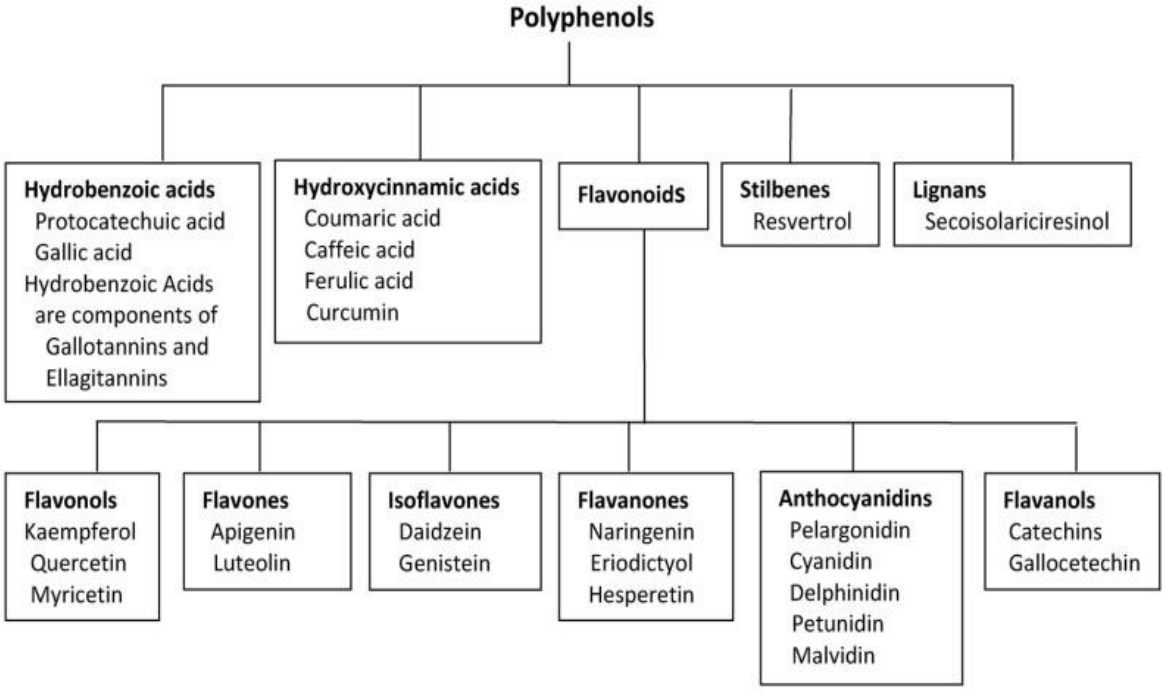

Figure 6. Polyphenol classifications and their relationships to each other (Elaine Hardman 2014).

The broadest group of phenolics are flavonoids (Figure 6). These have the ordinary structure of diphenylpropanes (C6-C3-C6), which is made up of two aromatic rings linked via three carbons that usually form an oxygenated heterocycle. (Haborne, 1980)
The flavonoid can be subdivided into 14 subclasses established on the degree of oxidation of the heterocyclic ring: chalcones, aurones, dihydrochalcones, flavones, flavonols, dihydroflavonols, flavanones, flavanols, flavandiols, isoflavones, flavonones, anthocyanidins and proantocyanidins. (Bravo, 1998)
The immense complexity of polyphenol universe also lies in the potential for multiple interactions with different groups such as sugars, alcohols and acids. Most common flavonoids are often found in foods attached to sugars, acids or alcohols and these compounds can be also biological active (Storniolo et al., 2014).
Fruits and vegetables consumption is correlated to the reduction of the risk of developing high-prevalence diseases such as cardiovascular diseases and cancer. (Birt et al., 2001)
Interestingly, the beneficial effects of consuming grains, legumes, fruits, vegetables, tea and wine have been associated, at least in part, to the biological effects of their polyphenol content inflecting the mechanisms involved in low-grade inflammation (Bravo, 1998; Med, 1999; Harborne, 1992; Scalbert, 2005). Polyphenols have been determined in a wide range of fruit and vegetables, including berries, whole-grain cereals and cacao, beverages such as coffee, tea and wine. (USDA database, Neveu et al., 2010)
The Prevención con Dieta Mediterránea (PREDIMED) study found that the mortality, the risk of cardiovascular diseases and diabetes were decreased in individuals with a diet rich in polyphenols. (Tressera-Rimbau et al., 2014)
Most of the publications in recent decades have concluded, that, the putative beneficial effects of polyphenols are frequently related to their antioxidant activity. The antioxidant properties of polyphenols, which are higher than or similar to those of vitamin E are deliberated by the presence of hydroxyl groups that are readily oxidized to produce the corresponding o-quinones. Thus, these compounds are active scavengers of reactive oxygen species (ROS) (Tresserra-Rimbau, et al., 2018).
Interestingly, the polyphenol content is determined widely by the plant species. Some food and beverages may be especially rich in a specific polyphenol class, e.g. phenolic acids in coffee, anthocyanidins in red wine, flavanones in citrus fruits, and isoflavones in soy products. (Perez-Jimenez et al., 2010)

Main sources of polyphenols depend on few foods and beverages depending on country. As example, tea intake has a bigger influence in Asian countries and UK, whereas wine, fruits, olives and olive oil intake influences polyphenolic intake in Mediterranean diets. It is important to define the polyphenol pattern and consider that two diets with the same total polyphenol content may have completely different pattern of polyphenol intake.

The consumption of specific polyphenol groups has recently been associated with a reduced risk of certain diseases. (Tressera-Rimbau et al., 2016; Creus-Cuadros et al., 2017)

It is important to recognize that the polyphenol content and profile are markedly influenced by plant variety, growth conditions, crop management, stage of maturity at harvest, postharvest handling, storage and food processing methods, and these factors account for the great high variability in the polyphenol content of processed products such as wine and virgin olive oil. (Hernandez-Jimenez et al., 2013; Franco et al., 2014)

Other foods also present a great variability of the polyphenol content (Table 3) and profile such as coffee and tea, and there is substantial variation in polyphenol content in different species of berries (Table 3). Food processing can modify their polyphenol composition of the original food. Thus, coffee chlorogenic acid content varies lean on different aspects, being the most important the specie and the roasting degree that decrease polyphenol content. (Moreira et al., 2017)

Table 3. Selected foods and their total phenolic content as TPC mg/ 100 g

Fruit and vegetables	***TPC mg/ 100 g***	***References***
Cashew nut	381	(Abe, et al. 2010)
Walnut	2500	(Abe, et al. 2010)
Cranberry	387	(Tarko et al., 2017)
Goji berry	374	(Tarko et al., 2017)
Sea buckthorn berry	190 – 480	(Sharma et al. 2008)
Banana	99.9	(Murillo et al., 2012)
Orange	86.7	(Murillo et al., 2012)
Melon	88.2	(Murillo et al., 2012)
Mango	138	(Murillo et al., 2012)
Coffee plum	506	(Murillo et al., 2012)
Carrot	19 – 342	(Leja et al., 2013)
Onion	90	(Vinson et al., 1998)

Fermentation can massively influence polyphenol content, thus, each tea variety also had a unique polyphenol profile dependent in great form of the fermented processes. (Wu et al., 2012)

Another method to rise the polyphenol content is to use berry purees in products rather than clarified juices. The purees have the added advantage of the non-extractable polyphenols, which are either covalently attached to cell walls or embedded in the tissue matrix. Thus, agricultural practice, cultural habits and food preferences are main factors in polyphenol intake, and explain the extremely high inter-individual variability in polyphenol intake. (Scalbert & Williamson, 2000)

However, careful uptake of dietary supplements that contain specific polyphenols is important. The consumption on these supplements increases. In addition, the effect of novel extraction and processing methods (e.g. supercritical CO2, pressurized water, microwave-enabled systems, etc.) on polyphenol content and composition (Tresserra-Rimbau, et al. 2018) has to be considered.

As mentioned previously, it is currently recognized that polyphenols are synthesized mainly by plants in response to stress, and fruits and vegetables are cultivated and eaten in Western societies where, stress and pathogens are kept to a minimum to increase production, but low amounts of polyphenols are present as a result.

In human nutrition, food preferences depend on a wide range of factors, including culture, tradition, ethics, the environment, consumption patterns and personal decisions. Currently, there has been an increasing focus on the nutritional properties of traditional dishes and recipes. It exists a significant knowledge gap regarding their composition currently. (Vallverdu-Queralt et al., 2014)

1.4. Sea buckthorn *(Hippophae rhamnoides L.)* (Thomas et al., 2003)

Hippophae rhamnoides L. (Sea buckthorn) is a hardy, deciduous shrub belonging to the family Elaeagnaceae. It bears yellow or orange fruits that have been used for centuries in both Europe and Asia for food, therapeutic, and pharmaceutical reasons.

Sea buckthorn is widely distributed as a native plant in China, Mongolia, Russia, United Kingdom, France, Denmark, Netherlands, Germany, Poland, Finland, Sweden, and Norway. A sea buckthorn industry has been booming in Russia since the 1940's when scientists began to investigate the biologically active substances found in the fruit, leaves, and bark. The Chinese practice with sea buckthorn products is more recent, although traditional uses of this crop date back many centuries.

Research and plantation establishment in China were introduced in the 1980's. Since 1982 over 500 000 ha of sea buckthorn have been planted and 150 processing factories have been well-established, producing over 200 products.

During the last time, sea buckthorn has attracted big attention from researchers around the world, most recently in North America, mainly for its nutritional and medicinal value. Sea buckthorn can be cultivated, by temperatures from – 43 °C to + 40 ˚C. Sea buckthorn can be used for many aspirations and, thus, has considerable economic potential (Table 4). A sea buckthorn habitat can yield 0.75–1.5 t/ ha of fruits (Figure 7).

Figure 7. Sea buckthorn plant (John K., 2012)

Table 4. Various sea buckthorn plant parts and their possible uses

Plant part	***Possible uses***
Bark	Pharmaceuticals, cosmetics
Leaves	Pharmaceuticals, cosmetics, tea, Animal feeds
Fruits	Volatile oil (Pharmaceuticals, Drinks, Food), Juice (Sports drinks, health drinks, Beverages, Brewery), Pulp (Cosmetics, Pigments, Animal feeds)
Seeds	Oil (Pharmaceuticals, cosmetics), Residues (Animal feeds)
Roots	Soil conservation, soil erosion

The fruit of the sea buckthorn plant weighs between 270 and 480 mg and averages 350 mg. It depends on the variety and maturity. Pressing these fruits yields 60 – 85 % juice. The composition of fruits or juice is detailed in Table 5.

Table 5. Composition and characteristics of sea buckthorn fruits/juice

Attribute (Units)	***Average***
Vitamin C (mg/ 100 g)	709
Vitamin C (mg %) raw juice	105.3
Total carotenoid (mg/ 100 g)	7.6
Carotenoid content (oil mg/ 100 g)	1167
β - Carotene (mg %) raw juice	2.1
β - Carotene (mg %) retentate[a]	21.62
Total phenolic content (mg/ g)	1.9 – 4.8
Vitamin E (mg/ 100 g)	64.4
Seed oil (%,w/ w)	14.2
Juice oil Content (%)	0.903
Glucose (% of total)	54.2
Fructose (% of total)	45.4
Xylitol (µg/ g)	39.2
Xylose (% of total)	0.42
Organic acid (% malic)	4
Lipid (%) raw juice	0.83
Lipid (%) retentate[a]	7.9
Protein (%) raw juice	0.8
Protein (%) retentate[a]	4.18
Potassium (µg/ mL)	497
Calcium (µg/ mL)	143
Aspartic acid (mg/ 100 g)	426.6

[a]Retentate: separation from sea buckthorn fruit raw juice using cellulose acetate membranes

Vitamin C (ascorbic acid) is a nutrient of major importance in sea buckthorn juice because it is present in large quantities, ranging from about 105 – 2500 mg/ 100 mL. Considering that orange juice provides 35 – 56 mg/ 100 mL (Araujo, 1977), the value of sea buckthorn as a new and important source of vitamin C is very interesting. Carotenoid pigments are primarily associated with the suspended solids and pulp oil obtained by an ultra-filtration membrane. The concentration ranges from 2 to 34.5 mg/ 100 mL juice. The variation associated with this wide range of values is probably dependent on the method of juice production. Nevertheless the high levels of vitamin C and carotenoid negotiate the unique nutritional benefit provided by sea buckthorn juice. If pulp oil can be incorporated into the juice or drinks prepared from juice, carotenoid levels can be enhanced. The same applies to vitamin C. The findings of some authors reported that sea buckthorn pomace without seeds is one of the important resources as an antioxidant in food, pharmaceuticals, cosmetics, or nutraceutical industries.

2. Materials and Methods

2.1. Materials

2.1.1. Instruments and Chemicals

The next Table 6 lists all instruments used for sample preparation and analysis

Table 6. Instruments and their production companies used in these analyzes

Instrument	***Producer***
Analysis Level AG135 & AG245	Mettler Toledo GmbH, Vienna, Austria
Centrifuge 5804 R	Eppendorf AG, Hamburg, Germany
Centrifuge Hermle Z323	Hermle Labortechnik GmbH, Wehingen
High Performance Liquid Chromatograph, Series 1100	Hewlett Packard, California, USA
Vortex	Scientific Industries, New York, USA
DU® Photometer	Beckman Coulter ™ Inc, Vienna, Austria
ThermoStat plus	Eppendorf AG, Hamburg, Germany
Lyophiliser	Savant Instrument Inc., USA

In the next Table 7 is a list of used chemicals, standard reagents and solutions for the HPLC.

Table 7. Chemicals and standard reagents with their production companies used in these analyzes

Standard reagents	***Producer***
L – Ascorbic acid	Sigma Aldrich Chemie GmbH, Vienna, Austria
β – Carotene	Sigma Aldrich, St. Louis, USA
Gallic acid	Sigma Aldrich Chemie GmbH, Vienna, Austria
Chemicals	***Producer***
Acetic acid	ChemLab analytical, Zedelgem, Belgium
Acetonitrile	ChemLab analytical, Zedelgem, Belgium
Ethyl acetate	ChemLab analytical, Zedelgem, Belgium
Di – Sodiumhydrogenphosphate	Merck, Darmstadt, Germany
Folin – Ciocalteu`s phenol reagent	Sigma Aldrich Chemie GmbH, Vienna, Austria
Methanol	ChemLab analytical, Zedelgem, Belgium
metha-Phosphoric acid 2 %	Carl Roth GmbH, Karlsruhe, Germany
ortho-Phosphoric acid 75 %	Fluka, Vienna, Austria
Sodiumcarbonat	Merck, Darmstadt, Germany
Tris (2 – carboxyethyl) phosphin	Sigma Aldrich Chemie GmbH, Vienna, Austria
Water	Ultra – Clear water system – Siemens

2.1.2. Raw materials and different samples

Table 8. Raw materials and different samples from Slovakia

Raw materials	***Country of origin + harvest***
Sea buckthorn berries	Austria, 2017
Sea buckthorn berries	Slovakia, 2016
Sea buckthorn berries	Slovakia, 2015
Samples	***Samples from Slovakia + explanations***
Buckwheat flour	Biomila, Slovakia
Milled dry sea buckthorn	Tvrdošovce, Slovakia
Control cookies (Buckwheat)	Danube strategy, Slovakia, April 2017
10% sea buckthorn cookies	Danube strategy, Slovakia, April 2017
25% sea buckthorn cookies	Danube strategy, Slovakia, April 2017
50% sea buckthorn cookies	Danube strategy, Slovakia, April 2017
100% sea buckthorn cookies	Danube strategy, Slovakia, April 2017
Control cake (Buckwheat)	Danube strategy, Slovakia, April 2017
Cake with sea buckthorn	Danube strategy, Slovakia, April 2017
Organic juice of sea buckthorn inlayed with oil	Tvrdošovské Zlato, Slovakia
Syrup of sea buckthorn	Tvrdošovské Zlato, Slovakia
Organic honey of sea buckthorn	Tvrdošovské Zlato, Slovakia
Apricot plum jam with sea buckthorn	Tvrdošovské Zlato, Slovakia
Tincture 1 - from “skin” selection	(light mass accumulated in specific part of juice pressing apparature, 250g/ 2L, 80% ethanol)
Tincture 2 - from “tea”	(skin+seeds=rest after juice processing that is using as a tea, 250g/ 1.5L, 80% ethanol)
Tincture 3 - from “leaves”	(with small branches, 250g/ 1.5L, 80% ethanol)
Special dry sea buckthorn	Tvrdošovské Zlato, byproduct, April 2017
Special dry sea buckthorn	Tvrdošovské Zlato, byproduct, April 2016
Special dry sea buckthorn	Tvrdošovské Zlato, byproduct, May 2016
0% Special dry sea buckthorn	+ 100% Buckwheat flour, April 2017
10% Special dry sea buckthorn	+ 90% Buckwheat flour, April 2017
25% Special dry sea buckthorn	+ 75% Buckwheat flour, April 2017
50% Special dry sea buckthorn	+ 50% Buckwheat flour, April 2017
100% Special dry sea buckthorn	+ 0% Buckwheat flour, April 2017
Cookies	VUP Food Research Institute, Bratislava, Slovakia, 2017
Muffins	VUP Food Research Institute, Bratislava, Slovakia, 2017
Cookies with 10% special dry sea buckthorn (april 2017)	VUP Food Research Institute, Bratislava, Slovakia, 2017
Tea 1 - sea buckthorn leaves	Tvrdošovské Zlato, 2017
Tea 2 - sea buckthorn commercial tea with rosehips	Bratislava, Slovakia, 2017

2.2. Methods

High Performance Liquid Chromatography (HPLC)

In this study HPLC was used for the determination of vitamin C and β - carotene in all samples from Table 8.

In Reversed-phase high-performance liquid chromatography (RP-HPLC) compounds are separated based on their hydrophobic character.

The separation depends on the hydrophobic binding of the solute molecule from the mobile phase to the immobilized hydrophobic ligands sticks to the stationary phase, i.e., the sorbent. The solute mixture is initially applied to the sorbent in the presence of aqueous buffers, and the solutes are eluted by the adding of organic solvent to the mobile phase. Elution can go on either by isocratic conditions where the concentration of organic solvent is constant, or by gradient elution whereby the amount of organic solvent is increased over a period of time.

For vitamin C and β – carotene measurements, the C18 column was used. The HPLC-instrument was used (consisting of a vacuum-degasser, a quaternary pump, an auto sampler, a UV / VIS and a fluorescence detector) by Hewlett Packard, Series 1100. For data evaluation was the software ChemStation for LC 3D© 1990 – 2003 by Agilent Technologies used.

Foli – Ciocalteu assay

Phenolic Quantification Assay is established on Folin – Ciocalteu method. The FC reagent consist of phosphomolybdic / phosphotungstic acid complexes. The method based on the transfer of electrons in an alkaline medium from phenolic compounds to form a blue chromophore constituted by a phosphotungstic / phosphomolybdenum complex where the maximum absorption depends on the concentration of phenolic compounds. Sodium carbonate is added to the samples to achieve a pH value around 10. The reduced Folin – Ciocalteu reagent is measurable with a spectrophotometer in the range from 690 to 765 nm. The product of this redox-reaction is a blue colored complex. Commonly, gallic acid is used as the reference standard compound and results are showed as gallic acid equivalents (mg/ ml). The absorption can be measured by using a visible-light spectrophotometer (Jadhav et al., 2012).

Some restrictions due to interfering compounds (sugars, organic acids, enediols such as ascorbic acid, reductones and aromatic amines) can occur (Prior et al., 2005).

Sample preparation

Before sampling, the raw material and the various samples from Slovakia were stored in the freezer. The sea buckthorn berries were lyophilized overnight (24h) until the day of the analysis to achieve a better extraction.

Lyophilization, also referred to as freeze-drying or sublimation drying, is a process for the gentle drying of products. Lyophilisation is based on the physical process of sublimation: The ice crystals sublimate directly into the gaseous state without the intervening appearance of a liquid phase (Davies, 1968).

Lyophilisation is often used at the laboratory level to dehydrate fresh biological material for storage because no enzymatic reactions can arise in the dry state. Freeze-dried botanical samples can also be found as commercial products in the general. It is commonly assumed that the lyophilization process itself does not alter the composition of the plant material. However, few analytical data are available to ratify this assumption (Abascal et al., 2005). The other samples from Slovakia were not freeze-dried.

2.2.1. Analysis of L – ascorbic acid and total vitamin C content

Sample preparation

The sea buckthorn berries were weighed in before and after freeze-drying.
After freeze-drying crushed with a mortar and diluted with 10 mL 2% m - phosphoric acid the samples were vortexed for 5 minutes.
The same procedure was for other samples without freeze-drying and direct dilution in 1mL Eppendorf tube of 2% m - phosphoric acid after weighing.
Most of the samples were centrifuged for 10 minutes at 14,000 rpm. Only samples such as the sea buckthorn berries, honey, jam, syrup and oil were centrifuged for 5 minutes at 5,000 rpm twice. Then the supernatant was transferred to vials.
In some samples, the dehydroascorbic acid was reduced to ascorbic acid. For this purpose, a tris(2-carboxyethyl) phosphine (TCEP) solution (20 mM) was added half an hour before the measurement in the HPLC. Samples were analyzed using isocratic RP-HPLC. The standard of L-ascorbic acid was solved in 2 % m - phosphoric acid and concentrations of 40 μg – 640 μg/ mL were prepared.

HPLC measurement

The method of the parameters of the HPLC measurement is according to the method from De Zuani (Stability of selected nutrients in home-made fruit juices under oxygen-free storage conditions) with small modifications. The parameters for the HPLC follow in Table 9, and the standard chromatogram can be seen in Figure 8. For the L – ascorbic acid standard, the retention time was 1.6 minutes.

Table 9. HPLC parameters for the analysis of L – ascorbic acid

Pre-Column	Phenomenex® AJ0 – 9297, EVO C18,
Column	Phenomenex Kinetex, 5µm EVO C18 100Å
Column temperature	25 °C
Detection	UV/VIS Detector, 245 nm
Flow rate	0.5 mL/ min
Mobile phase	100%, 50 mM phosphate buffer, pH 2.7
Injection Volume	5 µL
Stop time	10 min.

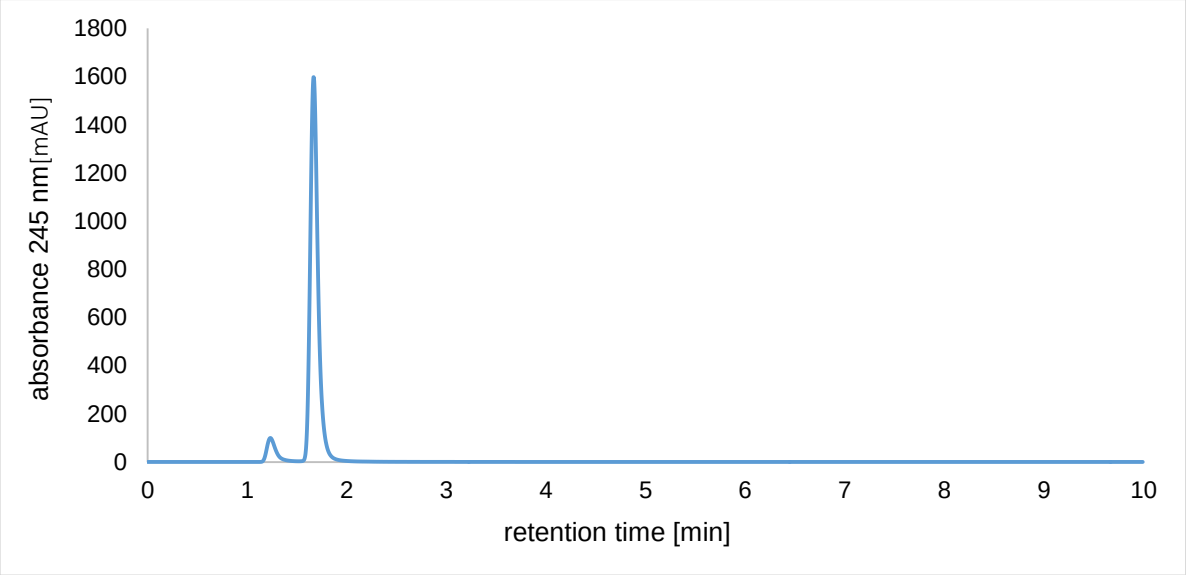

Figure 8. Concentration of L – ascorbic acid standard with 160 µg/ ml shown as a chromatogram measured at 245 nm.

2.2.2. Analysis of β – carotene

Sample preparation

The sample preparation was carried out with freeze drying as for vitamin C only for the sea buckthorn berries. Weighed and mortared according to the same principle as for vitamin C. Also for the same samples.

Samples such as the sea buckthorn berries, honey, jam, syrup and oil were diluted in 10 mL of 100 % ethyl acetate. The other samples were diluted in 1 ml of 100 % ethyl acetate. Extraction took 15 minutes for all samples by vortexing. The centrifugation took 15 minutes at 5,000 rpm for the above mentioned samples. For the remaining samples, it also took 15 minutes, but at 14,000 rpm. After that, the supernatant was transferred into vials.

Using isocratic RP-HPLC samples were analyzed. The β – carotene standard was dissolved in 100 % ethyl acetate, and concentrations of 5 μg – 160 μg/ mL were prepared.

HPLC measurement

The method of the parameters of the HPLC measurement is according to the method from Nora Rezaeian (Development of HPLC methods for the analysis of β – carotene and β-Apo-8´-carotenal degradation kinetics during thermal oxidation in triacylglycerol model systems) with small modifications. The parameters for the HPLC measurements are summarized in Table 10. One example of the standard chromatogram is shown in Figure 9. The retention time of the β – carotene standard was 20 minutes.

Table 10. HPLC parameters for the analysis of β – carotene

Pre-column	Security Guard Catridges Phenomenex® AJ0 - 7596, Gemini®C18, 4 × 0.2 mm ID
Column	Phenomenex®, Gemini®, 3 μm C18, 110Å, LC Column 150 × 3 mm
Column temperature	25°C
Detection	DAD array 450 nm
Flow rate	0.6 mL/ min
Mobile phase	100% Methanol
Injection volume	5 μL
Stop time	26 min.

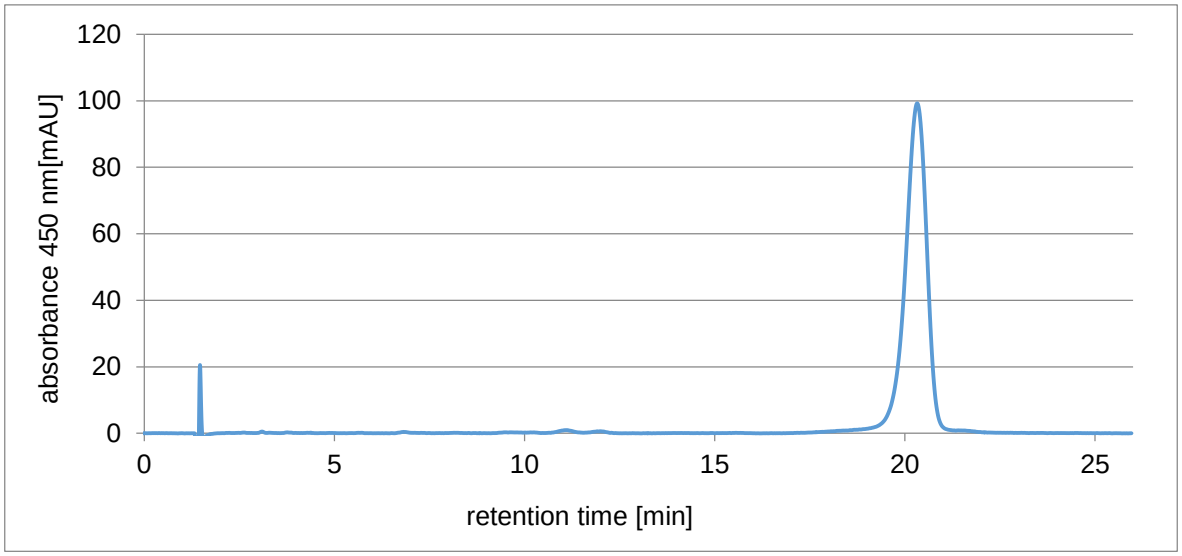

Figure 9. Chromatogram of the β – carotene standard with the concentration of 100 μg/ ml. The fluorescence was measured at 450 nm.

2.2.3. Analysis of the total phenolic content

Sample Preparation

About 1g of the extract was dissolved in 10 mL methanol – water (9:1) and shacked for 24 h at 25 °C in the ThermoStat plus shaker followed by centrifugation at 4.000 rpm for 10 min. The clear supernatant was collected and ready for analysis. The sea buckthorn berries were crushed with a mortar after freeze-drying and diluted as described above.

Total Phenolic Content

The total phenolic of the extracts were determined using the Folin and Ciocalteu reagent, following the method described by Singleton et al. (1999) with slight modifications. Sample and standard readings were made using a spectrophotometer (Beckman Coulter DU® 800 photometer, and the associated software DU® 800 Spectrophotometer) at 765 nm against the reagent blank.

The test sample (10 µL) was mixed with 0.6 µL of deionized water and 50 µL of Folin-Ciocalteu's phenol reagent in 1cm plastic cuvettes. After 1 min, 150 µL of saturated sodium carbonate solution (7.5 % w/ v in water) were added to the mixture and after eight more minutes another 150 µL sodium carbonate solution were added. The reaction was kept in the dark for 2 hours and the absorption was measured at 765 nm by using a photometer. Instead of a sample 10 µL deionized water were used for the blank. The phenolic content was calculated as gallic acid equivalents GAE/ g of dry material on the basis of a standard curve of gallic acid (200 – 3200 µg/ mL). All determinations were carried out in triplicate.

3. Results and Discussion

3.1 L – Ascorbic acid and total vitamin C

As described in the chapter “Materials and Methods” the sea buckthorn fruits were freeze-dried, extracted and analyzed by using HPLC. All other samples were directly extracted without freeze-drying. Averages and standard deviations were calculated for all obtained data. The results from L-ascorbic acid plus total vitamin C are presented in tabular and graphical form. In the appendix are listed the standard calibration, measured peak areas and sample chromatogram.

3.1.1 L – Ascorbic acid in sea buckthorn berries

The L-ascorbic acid content of sea buckthorn berries from Austria, harvest year 2017, is significantly higher than that of the other two samples from Slovakia (Figure 10). In comparison, in the sample from Slovakia, where the berries were harvested in 2016, about 45% of the ascorbic acid content was lower. For the sample harvested in 2015, the ascorbic acid content was only 20% lower than for the sample from Austria. When comparing samples of sea buckthorn from the same country of origin (2015 and 2016), the harvest year 2016 shows a 30% lower amount of ascorbic acid.

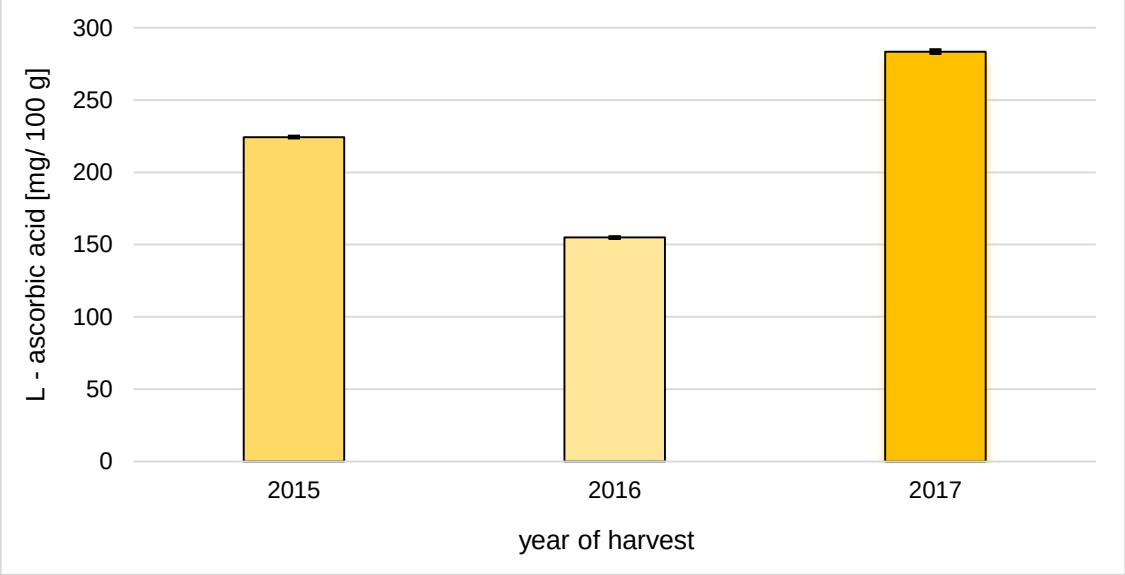

Figure 10. Comparison of L-ascorbic acid of sea buckthorn berries from different harvest years.

Referring to the literature where the content of vitamin C in sea-buckthorn is 190 - 480 mg/ 100 g (Sharma et al., 2008), two of the samples are in the range (Table 11). Only the sample from the harvest year 2016 is below the reference, but not too far away. One of the reasons why there are differences between the samples can be explained by the differences between the harvest years. Through various literature data, the nutrient enrichment in the plant is influenced by the growth conditions (soil nutrients, climate factors, health of the plant itself). Because all three samples have different content of ascorbic acid and have been harvested in different years.

It is also important to consider the difference between the sea buckthorn species. The sea buckthorn sample with the highest content of ascorbic acid grew in Austria and is a different variety than the samples of sea buckthorn from Slovakia.

Another reason for the differences between the samples with different countries of origin are the storage conditions. The sea buckthorn sample from Austria was frozen immediately after harvesting and after a few months processed directly with the freeze dryer. Sea buckthorn samples from Slovakia had another way with intermittent thawing to processing. Under such conditions, degradation of ascorbic acid occurs.

Table 11. Comparison of L – ascorbic acid of sea buckthorn berries from different harvest years. Averages and standard deviations were obtained from three separate measurements

L – Ascorbic acid [mg/ 100 g]	
Sea buckthorn berries, harvest year 2015 from Slovakia	**224** ± 0.3
Sea buckthorn berries, harvest year 2016 from Slovakia	**155** ± 0.3
Sea buckthorn berries, harvest year 2017 from Austria	**284** ± 1

3.1.2 L – Ascorbic acid from various sea buckthorn by – products

The highest amount of ascorbic acid have the by-product of charge 2017. This by-products (three samples are shown in Figure 11) are produced during juice production of sea buckthorn. In comparison a much lower level of ascorbic acid was detected by samples that comes from production of by-products from the batches of two months (April, May) 2016. The batch by-product 2017 was mixed in different proportions with buckwheat flour, for example as an ingredient in food. As you can see in the middle of Figure 11, sample proportions are shown from 0% to 100%. It can also be seen that the ascorbic acid content increases continuously, considering the standard deviation. Cookies were made with 10% by-product of 2017, and the content of ascorbic acid is very close to the sample of 100% by-product. The last two samples shown on the right in Figure 11 are tea examples. Comparing these two samples there is a big difference between the leaves of sea buckthorn and a commercial sea buckthorn tea mixed with rosehips. The sample with leaves has much lower content of ascorbic acid than the commercial tea.

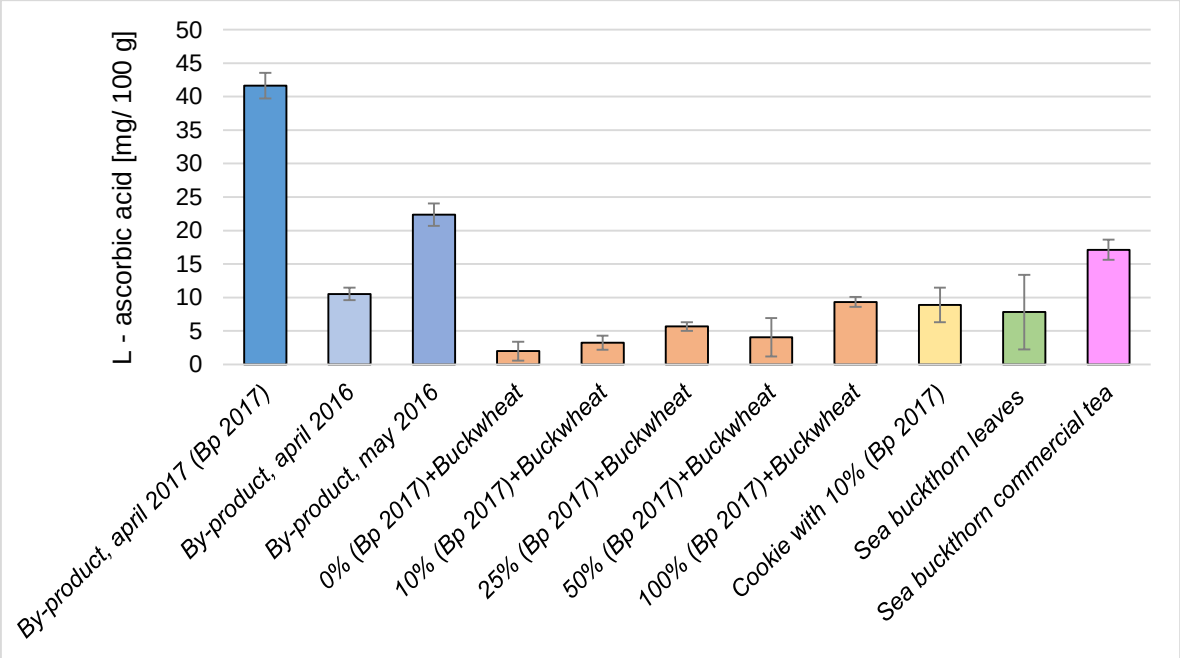

Figure 11. Comparison of L – ascorbic acid from various sea buckthorn by – products themselves as well as ingredient and tea samples.

When comparing the ascorbic acid concentrations of fruits and vegetables like orange and strawberry 59 mg/ 100 g, potatoes 11 mg/ 100 g, banana 9 mg/ 100 g or milk from cow with only 0 - 1 mg/ 100 g (Combs and McClung, 2017) the content of the respective samples (Table 12) is very good.

Especially in the byproduct of 2017, the biggest concentration is probably due to the harvest year. Also, a much lower level of ascorbic acid was detected by a sample derived from batch by-product production in April 2016 compared to May 2016. This could be based on the technology of processing in the respective batches. Influenced by the heat treatment during manufacturing in the industry and therefore the ascorbic acid content was degraded. In samples with different proportions from 0% to 100% (with by-product 2017), ascorbic acid increases continuously, and taking into account the standard deviation. This was also to be expected by the higher amount of by-product 2017. It was also interesting that the content of ascorbic acid in the cookie sample with only 10% by-product 2017 was similar to that of 100% by-product 2017 + Buckwheat. One explanation would be that the content of fruit components of sea buckthorn berries in the by-product itself was higher. Because different parts of the fruit have a different content of nutrients. The sea buckthorn leaves also showed a considerable amount of ascorbic acid. Compared to commercial tea (Table 12), where the content is higher by half, but the ascorbic acid content is additionally increased by the rosehip and sea buckthorn berries. Consider that there are dried leaves because during the heat treatment the concentration decreases.

Table 12. Comparison of L – ascorbic acid from various sea buckthorn by-products themselves as well as ingredient and tea samples. Averages and standard deviations were obtained from three separate measurements

L – Ascorbic acid [mg/ 100 g]	
Special dry sea buckthorn, by-product, april 2017	**41.6** ±1.92
Special dry sea buckthorn, by-product, april 2016	**10.5** ± 0.94
Special dry sea buckthorn, by-product, may 2016	**22.4** ± 1.67
0% Special dry sea buckthorn, by-product, april 2017 + Buckwheat	**2.01** ± 1.41
10% Special dry sea buckthorn, by-product, april 2017 + Buckwheat	**3.25** ± 1.06
25% Special dry sea buckthorn, by-product, april 2017 + Buckwheat	**5.67** ± 0.63
50% Special dry sea buckthorn, by-product, april 2017 + Buckwheat	**4.06** ± 2.87
100% Special dry sea buckthorn, by-product, april 2017 + Buckwheat	**9.34** ± 0.74
Cookies with 10% special dry sea buckthorn, by-product, april 2017	**8.89** ± 2.56
Sea buckthorn leaves	**7.82** ± 5.57
Sea buckthorn commercial tea with rosehips	**17.1** ± 1.52

3.1.3 L – Ascorbic acid and total vitamin C content from various sea buckthorn by – products

The vitamin C content was compared with the ascorbic acid in various sea buckthorn by – products. A significantly highest content was in the organic juice + oil samples. In both cases in the sample for ascorbic acid as well as in the sample for the total vitamin C. In this case, it is clear that the largest concentration compared to all other samples is the one with the total vitamin C in organic juice + oil. In the cases with the syrup samples there are no big differences between the ascorbic acid content and the total vitamin C (5% more). But still a good concentration compared to the other samples on the left (Figure 12), but by a half lower compared to the organic juices + oils. Samples such as honey, milled dry sea buckthorn, cookies and muffins have no differences in concentration between ascorbic acid and total vitamin C. Honey has the lowest levels of all, followed by muffins with slightly higher levels, and cookies and milled dry sea buckthorn samples still have twice as much.

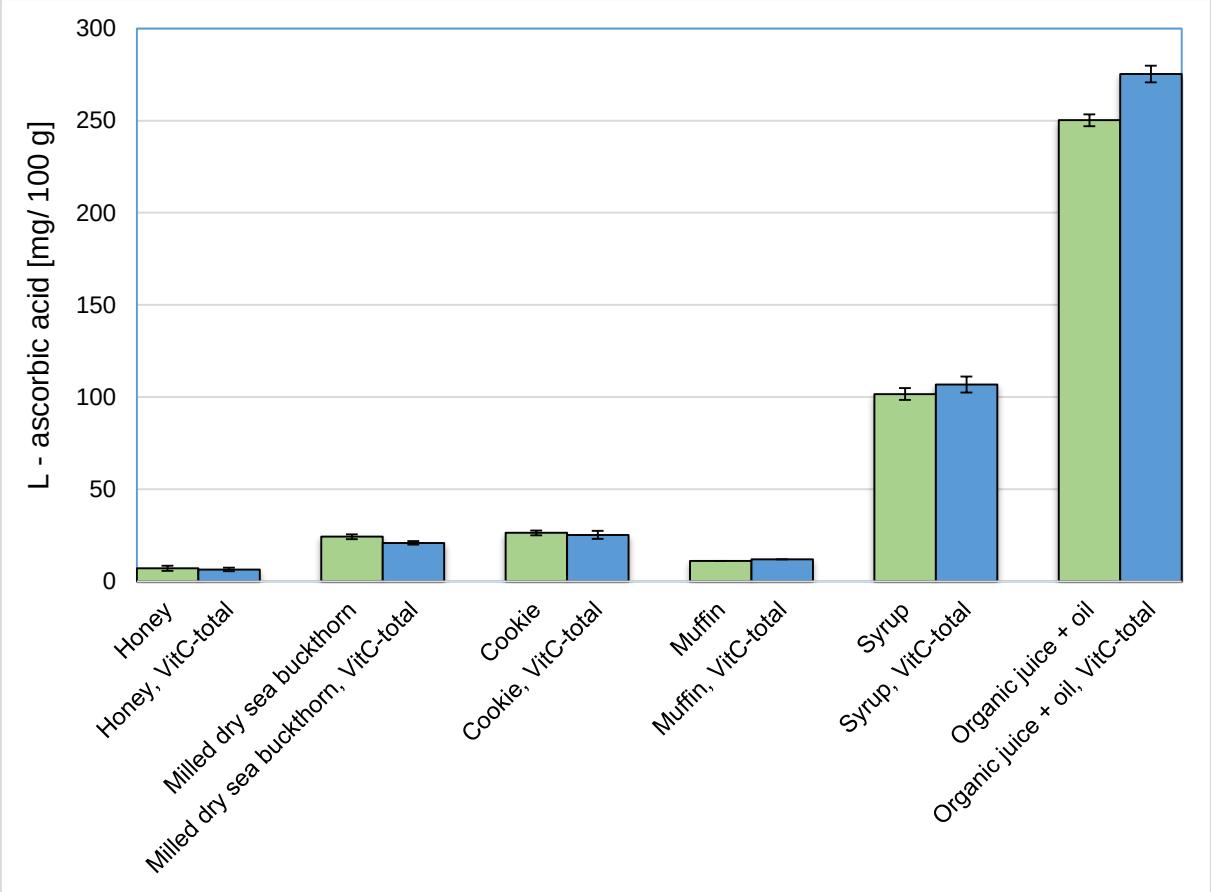

Figure 12. Comparison of L – ascorbic acid with total vitamin C content from samples of sea buckthorn as an ingredient in various food samples.

In literature references, the sea buckthorn juice contains 168 to 184 mg/ 100 g (Arimboor et al., 2006), in another study the concentration was found to be in the range of 114 - 1550 mg/ 100 g (Dharmananda, 2004).The concentrations mainly depends on the species and regions of the plant. In both cases, it is in the organic juice + oil ranges as in the Dharmanda range, 2004, and much higher than Arimboor et. al., 2006. Especially is the ascorbic acid content in the organic juice + oil the same like as it described on the package (250 mg/ 100 g). Interestingly, the total content of vitamin C (275 mg/ 100 g) is higher than described on the packaging (Table 13). This means that ascorbic acid is susceptible to oxidation to dehydroascorbic acid, which itself can be rapidly degraded.

TCEP was needed to reduce the samples, so dehydroascorbic acid is then converted to ascorbic acid. For this reason, the total content of vitamin C in the case of organic juice + oil is higher than the other one. The reason why the reduction method is not so clear with the other samples is probably due to the fact that the dehydroroascorbic acid was rapidly degraded further and thus a reduction was not possible. According to Thomas and Thomas (2003), the content of ascorbic acid in juices is in a range of about 105 to 2500 mg/ 100 ml. Based on this value, it is obtained from the syrup samples, but not in the case from the references above. With honey it is the non-acidic condition which testifies to a low vitamin C content. In the cases of milled dry sea buckthorn, cookies and muffins they are also a respectable part of vitamin C as well as ascorbic acid. All three variants were heated by their production, which brought the acidic conditions into the foreground and the heating itself was not considered as so important for the degradation of vitamin C. It is always important to consider the difference in the possible variable fruit parts in the samples themselves as a possible explanation

Table 13. Comparison of L – ascorbic acid with total vitamin C content from samples of sea buckthorn as an ingredient in various food samples. Averages and standard deviations were obtained from three separate measurements

L – Ascorbic acid and total vitamin C content [mg/ 100 g]	
Honey with sea buckthorn	**7.13** ± 1.46
Honey with sea buckthorn, Vitamin C-total	**6.47** ± 0.97
Milled dry sea buckthorn	**24.3** ± 1.36
Milled dry sea buckthorn, Vitamin C-total	**20.9** ± 1.02
Cookie with sea buckthorn	**26.3** ± 1.27
Cookie with sea buckthorn, Vitamin C-total	**25.3** ± 2.18
Muffin with sea buckthorn	**11.2** ± 0.92
Muffin with sea buckthorn, Vitamin C-total	**12.0** ± 0.89
Syrup of sea buckthorn	**102** ± 3.23
Syrup of sea buckthorn, Vitamin C-total	**107** ± 4.29
Organic juice of sea buckthorn inlayed with oil	**250** ± 3.19
Organic juice of sea buckthorn inlayed with oil, Vitamin C-total	**275** ± 4.5

3.1.4 L – Ascorbic acid and total vitamin C content of sea buckthorn as an ingredient in tinctures.

The vitamin C content was compared with the ascorbic acid in various sea buckthorn tinctures. The highest content was in the tinctures with leaves (Figure 13), then in the tea tincture and the smallest in skin tincture from sea buckthorn. All three samples show a higher content of vitamin C than ascorbic acid. The difference is very small only in the tea tincture, in the other tinctures the difference is much greater. It is important to mention that the measurement of the tinctures was done once because there were not enough samples.

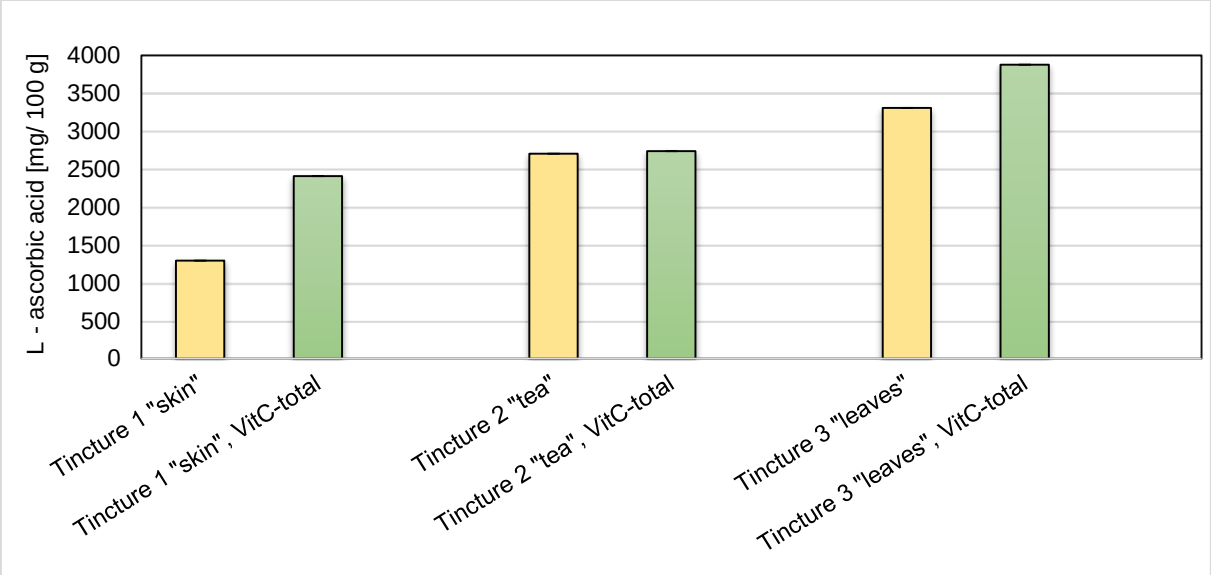

Figure 13. Comparison of L – ascorbic acid with the total content of vitamin C from samples of sea buckthorn as an ingredient in tinctures.

The large amount of vitamin C in leaf tincture is a peculiarity from all other samples previously. Because they have a vitamin C concentration of 3880 mg/ 100 ml and a reference in the literature is 250 - 1660 mg/ 100 ml (Zheng et al., 2011) for sea buckthorn plant. One explanation could be how the mixed leaves and twigs were stored in 250 g/ 2 L of 80% ethanol. And in this way could be the best to storage the most of the nutrients. Especially when it was done very fast directly from the plant. This means for the samples of all tinctures. All tinctures are above the literature range, only a skin tincture for ascorbic acid is within the limits. But in the samples of skin tincture is the biggest difference between the content of ascorbic acid and total vitamin C.

In particular, the content of vitamin C is greater by half (Table 14) than that of ascorbic acid. This means that most of the dehydroascorbic acid with the TCEP was directly reduced to ascorbic acid. Also to recognize that the dehydroascorbic acid in the ethanol solution was also very well stabilized. This finally results to an accumulation of vitamin C in this large amount. In the case of tea tincture 2, there was no difference in the concentration of the two samples. Perhaps the handling by sample preparation was wrong or the reducing agent did not work, because the concentrations of both samples of tea texture 2 are very close to each other. Differences between the samples of leaf tincture 3 are not so great. Vitamin C content is for 15% higher than in the sample of leaves tincture for ascorbic acid. It is difficult to give an explanation for samples that were measured only once. For this reason, the results are interesting, but they require many more samples to repeat this measurements, and finally, to give a good scientific assessment.

Table 14. Comparison of L – ascorbic acid with the total content of vitamin C from samples of sea buckthorn as an ingredient in tinctures. Measurements were performed only once for all samples of the tinctures

L – Ascorbic acid and total vitamin C content [mg/ 100 g]	
Tincture 1 - from "skin" selection *(light mass accumulated in specific part of juice pressing apparature)*	**1293**
Tincture 1 - from "skin" selection *(light mass accumulated in specific part of juice pressing apparature), Vitamin C-total*	**2410**
Tincture 2 - from "tea" *(skin + seeds=rest after juice processing that is using as a tea)*	**2703**
Tincture 2 - from "tea" *(skin + seeds=rest after juice processing that is using as a tea), Vitamin C-total*	**2740**
Tincture 3 - from "leaves" with small branches	**3308**
Tincture 3 - from "leaves" with small branches, Vitamin C-total	**3880**

In Table 15 are samples without any vitamin C concentration. In case of buckwheat it was expected. The same for control cookies and control cake. It is more likely that any amount of ascorbic acid was expected for 100% sea buckthorn cookies or apricot jam with sea buckthorn. The probability could be the handling of the sample material or the storage of the samples and the transport from abroad.

Table 15. Samples without L – ascorbic acid and vitamin C content. Measurements were performed in triplicate

L – Ascorbic acid and total vitamin C content [mg/ 100 g]	
Buckwheat flour	ND
Control cookies (Buckwheat)	ND
10% sea buckthorn cookies	ND
25% sea buckthorn cookies	ND
50% sea buckthorn cookies	ND
100% sea buckthorn cookies	ND
Control cake (Buckwheat)	ND
Cake with sea buckthorn	ND
Apricot plum jam with sea buckthorn	ND

ND = not detected

3.1.5 L – Ascorbic acid and total vitamin C content of sea buckthorn and muffins

This experiment was carried out at the "Food Research Institute, Department of Chemistry and Food Analysis, National Agricultural and Food Center in Bratislava, Slovakia" with the great support of colleagues Ciesarova Zuzana, Kukurova Kristina and Belajova Elena. The complete execution was based on the method of the institute in Bratislava. The aim of this project was to compare the results of vitamin C measurements.

The vitamin C content was compared with the ascorbic acid of sea buckthorn berries and muffin samples. A significantly highest content was in the fresh weighted sea buckthorn berries. In both cases in the sample for ascorbic acid as well as in the sample for the total vitamin C (Figure 14). In this case, it is clear that the largest concentration compared to all other samples is the one with the total vitamin C in sea buckthorn berries. In the cases with the muffin samples over time there are also differences between the ascorbic acid content and the total vitamin C. The biggest difference was at the sample Muffin 3 with 40%, with the other three muffins 30%. L-ascorbic acid and vitamin C were degraded for 24 hours under normal room conditions.

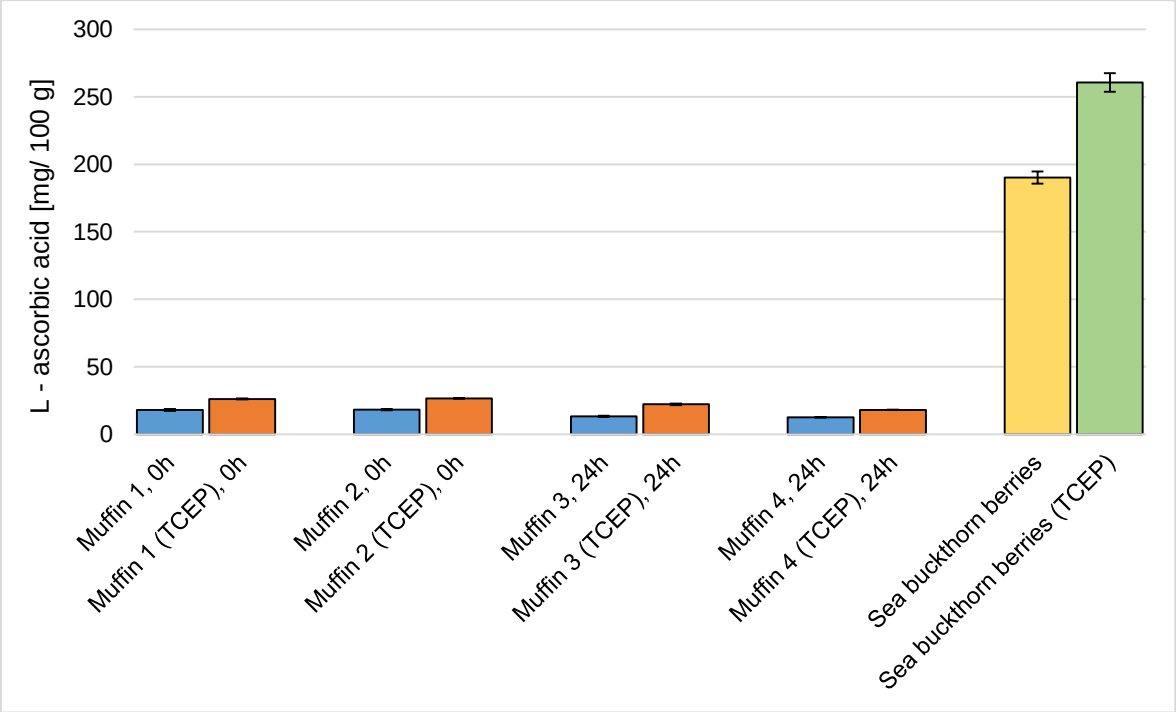

Figure 14. Comparison of L – ascorbic acid with the total content of vitamin C from samples of muffins over time and sea buckthorn berries.

Referring to the literature where the content of vitamin C in sea-buckthorn is 190 - 480 mg/ 100 g (Sharma et al., 2008), both of the samples are in the range.

The sea buckthorn juice contains 168 to 184 mg/ 100 g (Arimboor et al., 2006), in another study the concentration was found to be in the range of 114 - 1550 mg/ 100 g (Dharmananda, 2004).The concentrations mainly depends on the species and regions of the plant. In both cases, it is in the sea buckthorn berries range as in the Dharmanda range, 2004, and much higher than Arimboor et. al., 2006.

L-Ascorbic acid is susceptible to oxidation to dehydroascorbic acid, which itself can be rapidly degraded. TCEP was needed to reduce the samples, so dehydroascorbic acid is then converted to ascorbic acid. For this reason, the total content of vitamin C in the case of all samples presented in Figure 14 is higher than the other one. All samples had a difference between L-ascorbic acid and vitamin C of ≈ 30%.The biggest difference was in muffin 3 with 40% between the ascorbic acid and total vitamin C content. . It is always important to consider the difference in the possible variable fruit parts in the samples themselves as a possible explanation

When comparing the ascorbic acid concentrations of fruit and vegetable like orange 59 mg/ 100 g and potato (Combs and McClung, 2017) the content of the muffin samples (Table 16) is very good. What we can consider is the lower amount of ascorbic acid and vitamin C after 24 hours in the muffin samples. This means that the degradation of both takes place for 30% in the room condition. It is also important to note that all concentrations in this experiment are similar to those discussed in this Master's thesis for Vitamin C.

Table 16. Comparison of L – ascorbic acid with the total content of vitamin C from samples of muffins over time and sea buckthorn berries. Measurements were performed in triplicate

L – Ascorbic acid and total vitamin C content [mg/ 100 g]	
Muffin 1, 0h	**17.9** ± 0.74
Muffin 1, 0h, (TCEP) with reduction	**26.1** ± 0.32
Muffin 2, 0h	**18.1** ± 0.53
Muffin 2, 0h, (TCEP) with reduction	**26.5** ± 0.16
Muffin 3, 24h	**13.3** ± 0.41
Muffin 3, 24h, (TCEP) with reduction	**22.2** ± 0.63
Muffin 4, 24h	**12.5** ± 0.29
Muffin 4, 24h, (TCEP) with reduction	**18** ± 0.14
Sea buckthorn berries, fresh measuring	**190** ± 4.46
Sea buckthorn berries, fresh measuring	**261** ± 6.89

3.2 β – Carotene

As described in the chapter “Materials and Methods” the sea buckthorn fruits were freeze-dried, extracted and analyzed by using HPLC. All other samples were directly extracted without freeze-drying. Averages and standard deviations were calculated for all obtained data. The results from β – carotene are presented in tabular and graphical form. In the appendix are listed the standard calibration, measured peak areas and sample chromatogram.

3.2.1 β – carotene in sea buckthorn berries

β – carotene content in the sea buckthorn berries from Slovakia, harvest year 2015 and 2016 was present, the sample from Austria had no content of β – carotene (Figure 15). In comparison, in the samples from Slovakia, where the berries were harvested in 2016, about 27% of the β – carotene content was lower than for the sample harvested in 2015.

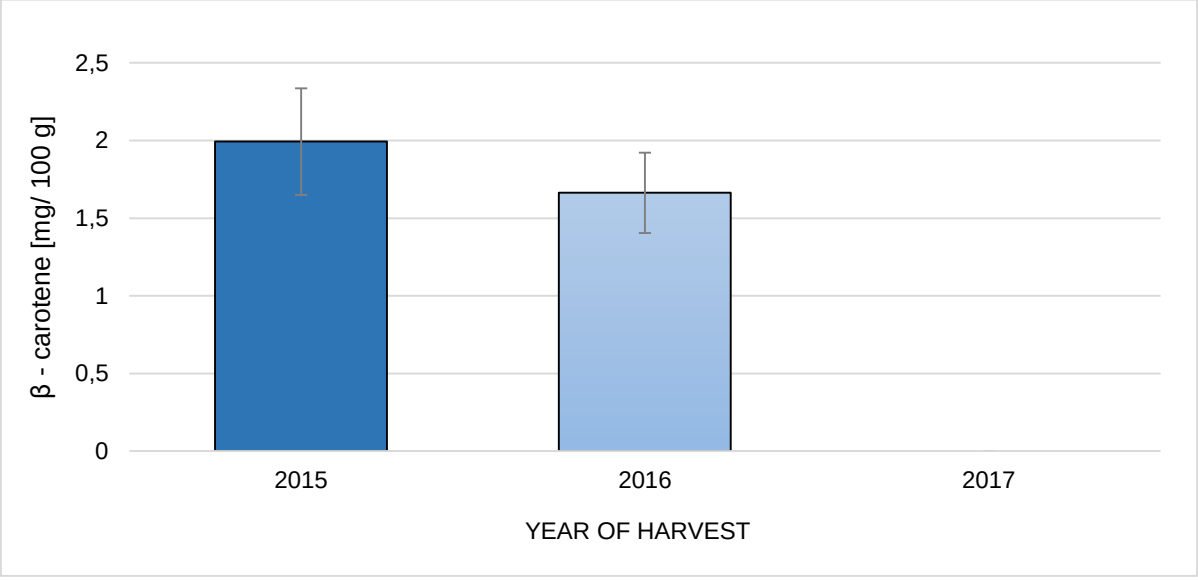

Figure 15. Comparison of β – carotene content of sea buckthorn berries from different harvest years.

Referring to the literature where the content of β – carotene in sea buckthorn is 1 - 120 mg/ 100 g (Yang, 2001), both samples from Slovakia are in the range (Table 17). One of the reasons why there are differences between the samples can be explained by the differences between the harvest years. Through various literature data, the nutrient enrichment in the plant is influenced by the growth conditions (soil nutrients, climate factors, health of the plant itself). Because both samples have different content and have been harvested in different years. It is also important to consider the difference between the sea buckthorn species. Another reason could be that samples only after a few months with the freeze dryer were processed. One year had passed from the harvest to the first measurement. Under such conditions, the degradation of β – carotene occurs. This may also be one reason why the concentration of β – carotene was generally so low in both samples. The bizarre was that with the sea buckthorn berries 2017 from Austria no concentration at all was detected. The appearance of the sea buckthorn berries in 2017 was very specific, as the berries were much smaller than the samples from Slovakia. In addition, the color of the Austrian berries was much paler and less intense than the others. Also to mention that the extract solution itself was much more colorless. All of this factors could be the reason why in sample 2017 nothing of β – carotene was detectable.

Table 17. Comparison of β – carotene content of sea buckthorn berries from different harvest years. Averages and standard deviations were obtained from three separate measurements

β – carotene [mg/ 100 g]	
Sea buckthorn berries, harvest year 2015 from Slovakia	**1.99** ± 0.34
Sea buckthorn berries, harvest year 2016 from Slovakia	**1.66** ± 0.26
Sea buckthorn berries, harvest year 2017 from Austria	**-**

3.2.2 β – Carotene from various sea buckthorn by – products

The highest amount of β – carotene have the sea – buckthorn leaves. Followed by the milled dry sea buckthorn and the sea buckthorn commercial tee with rosehips.

The by – products had similar concentrations for May 2016 and April 2017, with the by – product 2017 having 10% more β – carotene. This by – products (two samples of three are shown in Figure 16) are produced during juice production of sea buckthorn. In comparison no level of β – carotene was detected by sample that comes from production of by – product from the batch of April 2016. It is also interesting that the control cake sample contained only 30% less β – carotene than the cake with sea buckthorn. Syrup and organic juice + oil are very similar in concentration and the jam sample contained the lowest concentration of β – carotene.

Comparing the two tea samples there is a big difference between the leaves of sea buckthorn and a commercial sea buckthorn tea mixed with rosehips. The sample with leaves has much bigger content of β – carotene than the commercial tea (27 % lower).

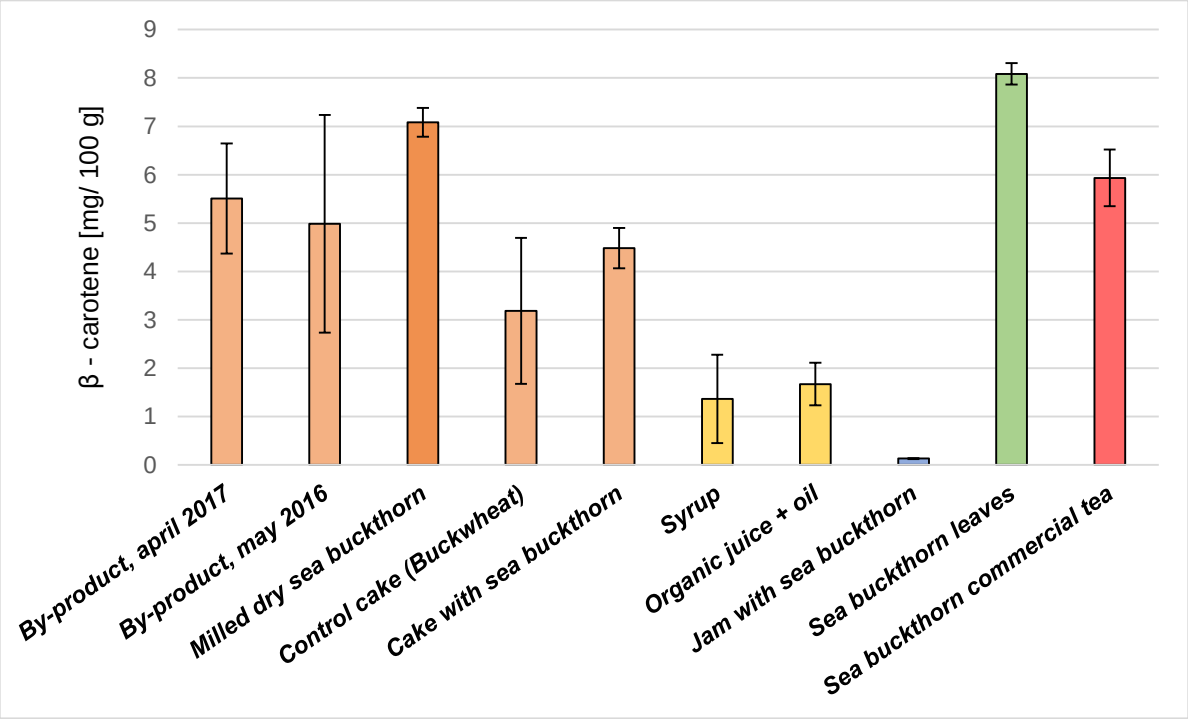

Figure 16. Comparison of β – carotene content from samples of sea buckthorn as an ingredient in various food samples

When comparing the β – carotene concentrations of fruits and vegetables like sweet potato 7.3 – 11.16 mg/ 100 g, carrots 7.8 – 9.34 mg/ 100 g, and pink grapefruit 0.23 – 0.35 mg/ 100 g (Bushway, 1986) the content of the respective samples (Table 18) is very good. In the case of sea buckthorn leaves, the greatest concentration may be due to chance.

According to Salvadori et al. (1992), β – carotene is generally distributed in dark green leaves. The amount of total phenolics, carotenoids, and chlorophylls of fresh sea buckthorn leaves decrease with drying (Guan et al., 2005). A higher reduction was observed in the leaves dried at higher temperatures (80 °C or 100 °C) for longer times compared with those dried at lower temperatures (50 °C or 60 °C). The authors suggest that dried leaves from sea buckthorn were of a high nutraceutical quality comparable to those of frequently consumed vegetables (Guan et al., 2005). The temperature may have been low during production at the factory, which would explain the high concentration of β – carotene in leaves. Also interesting is the second highest sample milled dry sea buckthorn, which was also dried and still has high value. The reason could be the same as by the leaves samples.

A lower level of β – carotene was detected by a sample derived from batch by – product production in May 2016 compared to April 2017. This could be based on the technology of processing in the respective batches. Influenced by the heat treatment during manufacturing in the industry and therefore the β – carotene content was degraded. Another reason why there are various between the samples can be explained by the differences between the harvest years.

Logically, samples with and without sea buckthorn in buckwheat flour differ, β – carotene is lower in the control cake. But both cake samples are very high compared to all other samples, especially in the case for samples without β – carotene (Table 19). Very good results are in the case of syrup and organic juice + oil samples compared to the literature for carotenoids in juices, where according to Zhang et. al (1989), the concentration is between 2.0 - 16.1 mg/ 100 ml or, in the case of Ma et. al (1989) they are between 4.6 - 12.0 mg/ 100 ml. And in this experiment, the concentration was determined only with β – carotene. The sample organic juice + oil also describes on the packaging the concentration of vitamin A in an amount of 0.24 mg/ 100 g, which additionally confirms the β – carotene content.

Compared to all other samples in Figure 16 commercial tea have also very high content of β – carotene, where the content is lower than by the leaves for 27 %. Maybe additionally increased by the rosehips in this mixture.

One explanation would be that the content of fruit components of sea buckthorn berries in this samples was higher. Because different parts of the fruit have a different content of nutrients.

Table 18. Comparison of β – carotene content from samples of sea buckthorn as an ingredient in various food samples. Averages and standard deviations were obtained from three separate measurements

β – carotene [mg/ 100 g]	
Special dry sea buckthorn, by-product, April 2017	**5.51** ±1.14
Special dry sea buckthorn, by-product, may 2016	**4.99** ± 2.25
Milled dry sea buckthorn	**7.08** ± 0.30
Control cake (Buckwheat)	**3.19** ± 1.51
Cake with sea buckthorn	**4.48** ± 0.42
Syrup of sea buckthorn	**1.36** ± 0.92
Organic juice of sea buckthorn inlayed with oil	**1.67** ± 0.44
Apricot plum jam with sea buckthorn	**0.13** ± 0.01
Sea buckthorn leaves	**8.08** ± 0.22
Sea buckthorn commercial tea with rosehips	**5.93** ± 0.59

There is extreme variation in the carotenoid concentration in sea buckthorn. About 10 fold variation was observed in the same species (1 to 120 mg/ 100 g) belongs to the same population (Yang, 2001), this means the same for β – carotene. The storage of the samples, the distribution and the long waiting time to the measurement may also have affected the poor results described in Table 19. Also to consider that many scientific articles testify that β – carotene is very sensitive to heat, light and oxygen, which greatly increases the error rate in measurements.

Table 19. Samples without β – carotene content. Measurements were performed in triplicate, only once for three samples of the tinctures

β – carotene [mg/ 100 g]	
Special dry sea buckthorn, by-product, April 2016	ND
0% Special dry sea buckthorn, by-product, April 2017 + Buckwheat	ND
10% Special dry sea buckthorn, by-product, April 2017 + Buckwheat	ND
25% Special dry sea buckthorn, by-product, April 2017 + Buckwheat	ND
50% Special dry sea buckthorn, by-product, April 2017 + Buckwheat	ND
100% Special dry sea buckthorn, by-product, April 2017 + Buckwheat	ND
Cookies with 10% special dry sea buckthorn, by-product, April 2017	ND
Honey with sea buckthorn	ND
Cookie with sea buckthorn	ND
Muffin with sea buckthorn	ND
Tincture 1 - from "skin" selection *(light mass accumulated in specific part of juice pressing apparature)*	ND
Tincture 2 - from "tea" *(skin + seeds=rest after juice processing that is using as a tea)*	ND
Tincture 3 - from "leaves" with small branches	ND
Buckwheat flour	ND
Control cookies (Buckwheat)	ND
10% sea buckthorn cookies	ND
25% sea buckthorn cookies	ND
50% sea buckthorn cookies	ND
100% sea buckthorn cookies	ND

ND = not detected

3.3 Total phenolic content

As described in the chapter “Materials and Methods” the sea buckthorn fruits were freeze – dried, extracted and analyzed by using HPLC. All other samples were directly extracted without freeze – drying. Averages and standard deviations were calculated for all obtained data. The results from total phenolic content are presented in tabular and graphical form. In the appendix are listed the standard calibration and measured peak areas.

3.3.1 Total phenolic content in sea buckthorn berries

The total phenolic content of sea buckthorn berries from Austria, harvest year 2017, is significantly higher than that of the other two samples from Slovakia (Figure 17). In comparison, in the sample from Slovakia, where the berries were harvested in 2016, about 50 % of the total phenolic content was lower. For the sample harvested in 2015, the ascorbic acid content was also 40 % lower than for the sample from Austria. When comparing samples of sea buckthorn from the same country of origin (2015 and 2016), the harvest year 2016 shows a 16 % lower amount of ascorbic acid.

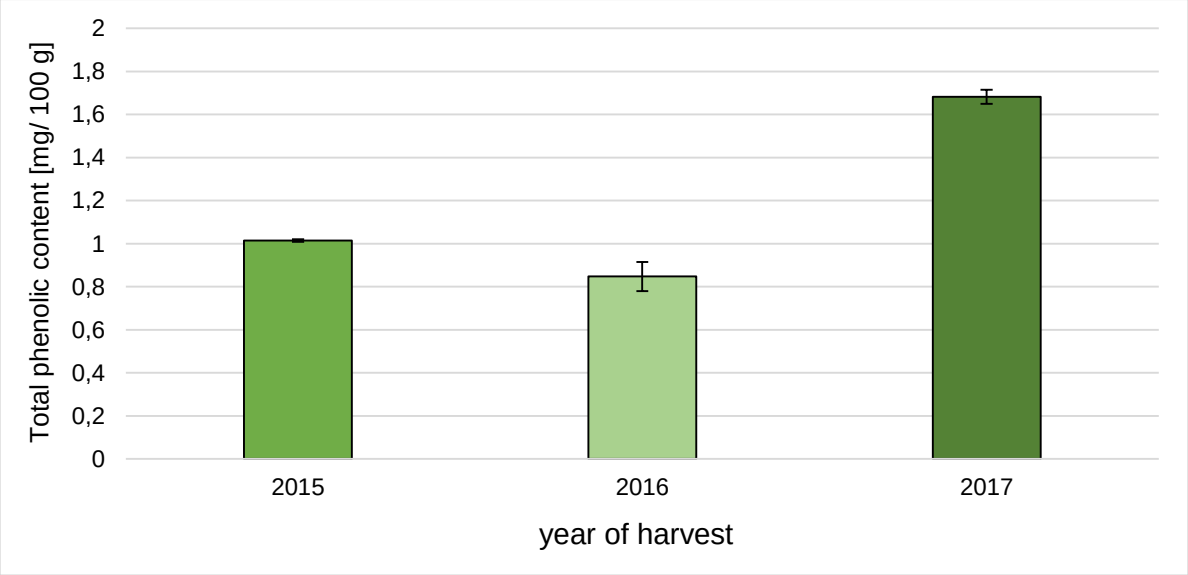

Figure 17. Comparison of total phenolic content of sea buckthorn berries from different harvest years.

Referring to the literature where the total phenolic content in sea-buckthorn berries is 190 - 480 mg/ 100 g (Sharma et al., 2008), all of the three samples are not in the range. Only the sample from the harvest year 2017 is the highest to reference, but also too less (Table 20). One of the reasons why there are differences between the samples itself can be explained by the differences between the harvest years. Through various literature data, the nutrient enrichment in the plant is influenced by the growth conditions (soil nutrients, climate factors, health of the plant itself). Because all three samples have different content of total phenolic content and have been harvested in different years.

It is also important to consider the difference between the sea buckthorn species. The sea buckthorn sample with the highest content of total phenolic content grew in Austria and is a different variety than the samples of sea buckthorn from Slovakia.

Another reason for the differences between the samples with different countries of origin are the storage conditions. The sea buckthorn sample from Austria was frozen immediately after harvesting and after a few months processed directly with the freeze dryer. Sea buckthorn samples from Slovakia had another way with intermittent thawing to processing. Under such conditions, degradation of phenolic compound occurs. Arimboor et al. (2008) determined the free and bound phenolic acid in sea buckthorn plant. The authors found seed kernels contain high amount (5741 mg/ kg) of phenolic acid than seed coat. Polyphenols however vary from species to species and from parts to the part. Also to consider, potentials also differ when the site of presence is different.

Table 20. Comparison of total phenolic content of sea buckthorn berries from different harvest years. Averages and standard deviations were obtained from three separate measurements

Total phenolic content [mg/ 100 g]	
Sea buckthorn berries, harvest year 2015 from Slovakia	**1.01** ± 0.01
Sea buckthorn berries, harvest year 2016 from Slovakia	**0.85** ± 0.07
Sea buckthorn berries, harvest year 2017 from Austria	**1.68** ± 0.03

3.3.2 Total phenolic content in by-product samples as a component in buckwheat, tea and tincture

The highest amount of polyphenols contains the tea tincture 2. The amount of the other tinctures (leaves and skin) was 40 % lower. The highest amount of polyphenols from the by-products was by the sample charge 2017.

This by-products (three samples are shown in Figure 18 at the top) are produced during juice production of sea buckthorn. In comparison a much lower level of polyphenols was detected by samples that comes from production of by – products from the batches of two months (April, May) 2016. The batch by – product 2017 was mixed in different proportions with buckwheat flour, for example as an ingredient in food. As you can see in the middle of Figure 18, sample proportions are shown from 0 % to 100 %. It can also be seen that the total phenolic content increases continuously. Cookies were made with 10 % by – product of 2017, and the content of polyphenols is very close to the sample of 0 % by – product. The two samples between cookies and tinctures shown in Figure 18 are tea examples. Comparing these two samples there is a big difference between the leaves of sea buckthorn and a commercial sea buckthorn tea mixed with rosehips. The sample with leaves has much higher content of polyphenols than the commercial tea. It is important to mention that the measurement of the tinctures was done once because there were not enough samples.

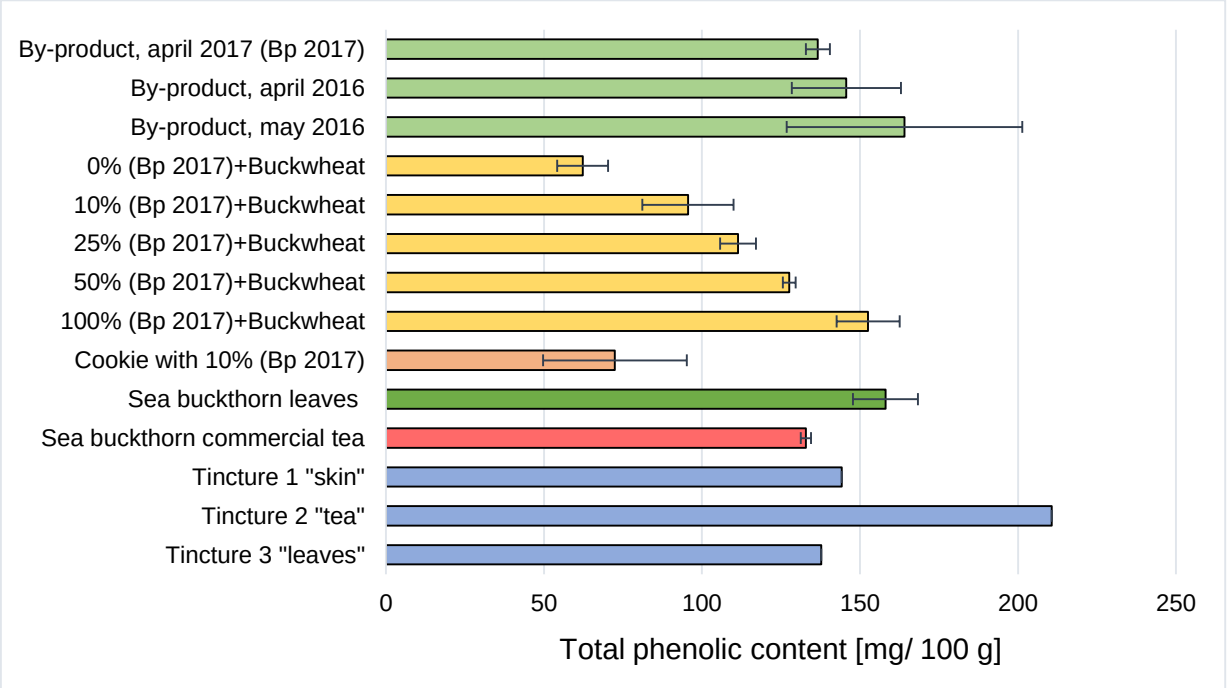

Figure 18. Comparison of total phenolic content from samples of sea buckthorn by – product as an ingredient in various food samples

When comparing the polyphenol concentrations of fruits and vegetables like orange with 86.67 mg/ 100 g, banana 99.90 mg/ 100 g (Murillo, et al., 2012), carrot 19 – 342 mg/ 100 g (Leja, et al., 2013), or onion with only 90 mg/ 100 g (Vinson, et al., 1998) the content of the respective samples (Table 21) is very good.

Especially in the by – product of May 2016, the biggest concentration is probably due to the harvest year. Also, a nice level of polyphenols was detected by both samples derived from batch by-product production in April 2016 and April 2017 compared to May 2016. This could be based on the technology of processing in the respective batches. Influenced by the heat treatment during manufacturing in the industry and therefore the total phenolic content was degraded. In samples with different proportions from 0 % to 100 % (with by – product 2017), polyphenols increases continuously, and taking into account the standard deviation. This was also to be expected by the higher amount of by – product 2017 in the buckwheat. It was also interesting that the content of polyphenols in the cookie sample with only 10 % by - product 2017 was similar to that of 0 % by – product 2017 + buckwheat. Compared with the sample of 10 % by – product, this is a 25 % difference, meaning 25 % lower concentration of polyphenols in the cookies. One explanation would be that the content of buckwheat itself was lower. Therefore, when we look at the sample of buckwheat with a phenol content of 108.6 mg/ 100 g (Table 22) and sample 0 % by – product 2017 with 62.3 mg/ 100 g (Table 21), we can say that polyphenols are representative and variable ingredients and that could alter the content of the cookie sample, regardless from by - product 2017. The sea buckthorn leaves also showed a considerable amount of polyphenols. Compared to commercial tea (Table 21), where the content is 26 % lower, it is interesting that the additional component of the rose hips does not increase the concentration of the polyphenols too much. To consider, according to literature, the polyphenol concentration in sea buckthorn leaves is 270 - 1080 mg/ 100 g (Sharma, et al., 2008). In this case, it is not within this range of this reference, but reason could be a variety characteristic.

The large amount of polyphenol in tea tincture is a peculiarity from all other samples previously. Because they have a polyphenol concentration of 211 mg/ 100 ml and a reference in the literature is 270 - 1080 mg/ 100 ml (Sharma et al., 2008) for sea buckthorn leaves.

All tinctures are under the literature range, only a tea tincture is near the limit. But comparing the skin sample with the leaf sample of the tinctures shows almost no difference. But the comparison of the skin sample with the leaf sample of the tinctures shows almost no difference. Definitely all tincture samples are interesting for a deeper research in the future. Because these results of this experiment are limited by their one-time measurement.

Table 21. Comparison of total phenolic content from samples of sea buckthorn by – product as an ingredient in various food samples. Averages and standard deviations were obtained from three separate measurements, only once for three samples of the tinctures

Total phenolic content [mg/ 100 g]	
Special dry sea buckthorn, by-product, April 2017	**137** ± 3.81
Special dry sea buckthorn, by-product, April 2016	**146** ± 17.3
Special dry sea buckthorn, by-product, May 2016	**164** ± 37.3
0% Special dry sea buckthorn, by-product, April 2017 + Buckwheat	**62.3** ± 8.0
10% Special dry sea buckthorn, by-product, April 2017 + Buckwheat	**95.6** ± 14.5
25% Special dry sea buckthorn, by-product, April 2017 + Buckwheat	**111** ± 5.7
50% Special dry sea buckthorn, by-product, April 2017 + Buckwheat	**128** ± 2.0
100% Special dry sea buckthorn, by-product, April 2017 + Buckwheat	**153** ± 10
Cookies with 10% special dry sea buckthorn, by-product, April 2017	**72.5** ± 22.7
Sea buckthorn leaves	**158** ± 10.3
Sea buckthorn commercial tea with rosehips	**133** ± 1.6
Tincture 1 - from "skin" selection *(light mass accumulated in specific part of juice pressing apparature)*	**144**
Tincture 2 - from "tea" *(skin + seeds=rest after juice processing that is using as a tea)*	**211**
Tincture 3 - from "leaves" with small branches	**138**

3.3.3 Total phenolic content from various products with sea buckthorn

The highest amount of polyphenols have the sample milled dry sea buckthorn. Followed by the buckwheat flour and in almost the same concentration as the cake with sea buckthorn. Then, as expected, the control cake was the next one with the lower concentration. As you can see in the middle of Figure 19, sample proportions are shown from 0 % to 100 %. It can also be seen that the total phenolic content increases but variably with the percentage, considering the standard deviation.

In comparison of samples below in the Figure 19 had a much lower level of polyphenols that the other on the top. All of them are under 10 mg/ 100 g. Only the samples of cookies and muffins with a slightly higher concentration were better than the samples with the very similar amount of polyphenols in a concentration of ≈ 1.4 mg/ 100 g. When comparing only these samples, there are almost no differences between the concentrations of total phenol content.

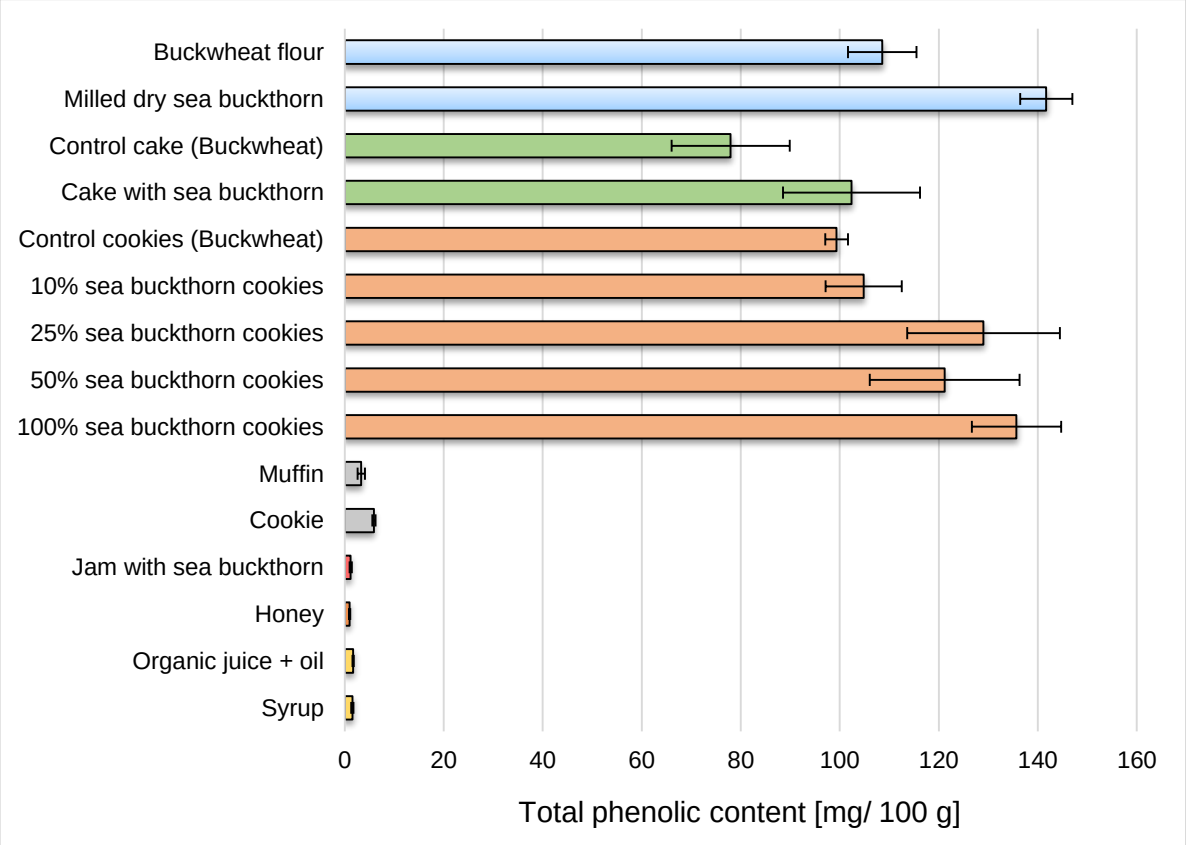

Figure 19. Comparison of total phenolic content from samples of sea buckthorn as an ingredient in various food samples.

All samples with buckwheat, including the milled dry sea buckthorn sample as the highest, have a very high concentration of polyphenols. It is very satisfactory compared to the literature of sea buckthorn berries with 190 - 480 mg/ 100 g. Especially when we compare it like in the previous section with orange (86.67 mg/ 100 g) or banana (99.90 mg/ 100 g) (Murillo, et al., 2012). Interestingly, the buckwheat sample and the control cake sample (such as the buckwheat sample) make a big difference. The reason could be a buckwheat content difference or a measurement error. But as expected, a larger amount of polyphenol shows the cake with sea buckthorn than the control cake (Table 22). Also a nice total phenol content shows the samples with more and more sea buckthorn in buckwheat for cookies samples. But it still varies in high concentration at all percentages. Even in these cases, the reason might be that the polyphenols of buckwheat content changes the results. To note that the control cookie is similar in concentration of buckwheat sample. All of the aforementioned samples were in a crumbled structure. The cookies and muffin samples were in a normal baked form as a finished product to be consumed. This may be one reason why they had such low concentrations of polyphenols. Because the cookie and muffin samples are older and were measured after one year. For the other samples, which are in Figure 19 above, the measurement was made after 5 months. The same reason could be for the samples jam, honey, syrup and organic juice + oil. Means that the samples were either too old or had been measured with errors. In particular, a higher concentration was to be expected for syrup and organic juice + oil. According to the literature, in the pulp a polyphenol concentration of 190 – 480 mg/ 100 g can be expected. Also note that the concentration of polyphenols, especially in oil, is 338 mg/ 100 g (Zeb and Ullah, 2014). And for these cases of samples, it is very unacceptable to be satisfied with the polyphenol concentrations.

Table 22. Comparison of total phenolic content from samples of sea buckthorn as an ingredient in various food samples. Averages and standard deviations were obtained from three separate measurements, only once for three samples of the tinctures

Total phenolic content [mg/ 100 g]	
Buckwheat flour	**109** ± 6.92
Milled dry sea buckthorn	**142** ± 5.27
Control cake (Buckwheat)	**77.9** ± 11.9
Cake with sea buckthorn	**102** ± 13.8
Control cookies (Buckwheat)	**99.4** ± 2.28
10% sea buckthorn cookies	**105** ± 7.72
25% sea buckthorn cookies	**129** ± 15.4
50% sea buckthorn cookies	**121** ± 15.1
100% sea buckthorn cookies	**136** ± 9.03
Muffin with sea buckthorn	**3.33** ± 0.75
Cookie with sea buckthorn	**5.92** ± 0.33
Apricot plum jam with sea buckthorn	**1.22** ± 0.21
Honey with sea buckthorn	**1.01** ± 0.12
Organic juice of sea buckthorn inlayed with oil	**1.71** ± 0.14
Syrup of sea buckthorn	**1.55** ± 0.22

IV. Appendix

Data of the L – ascorbic acid and total vitamin C measurements

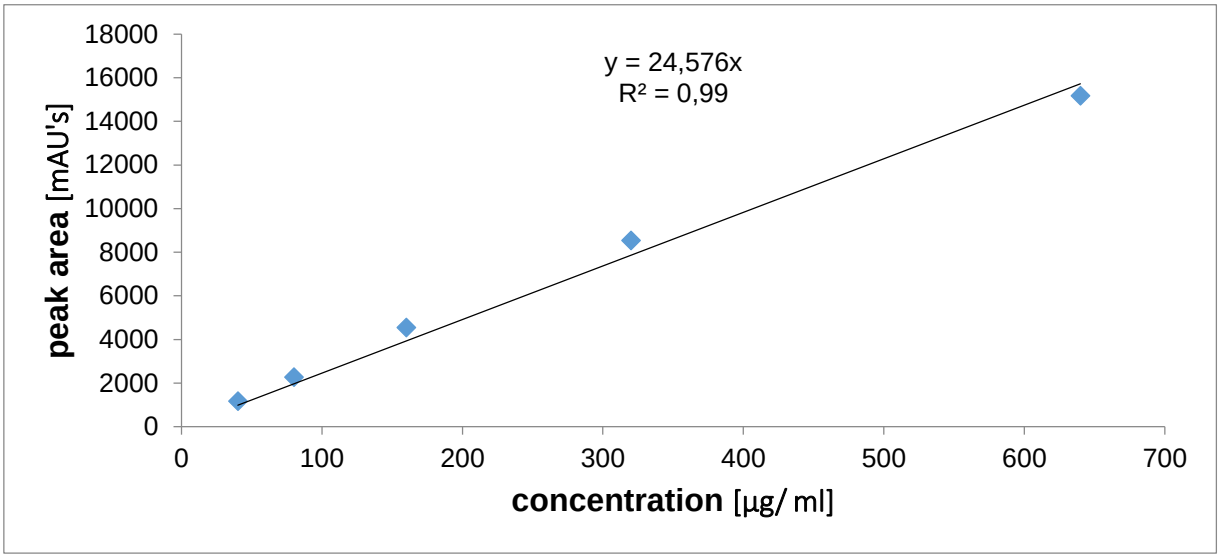

Figure 20. Standard calibration curve of L – ascorbic acid. Calibration equation was used to calculate the total vitamin C in all samples. Calibration equation: y = 24,576x

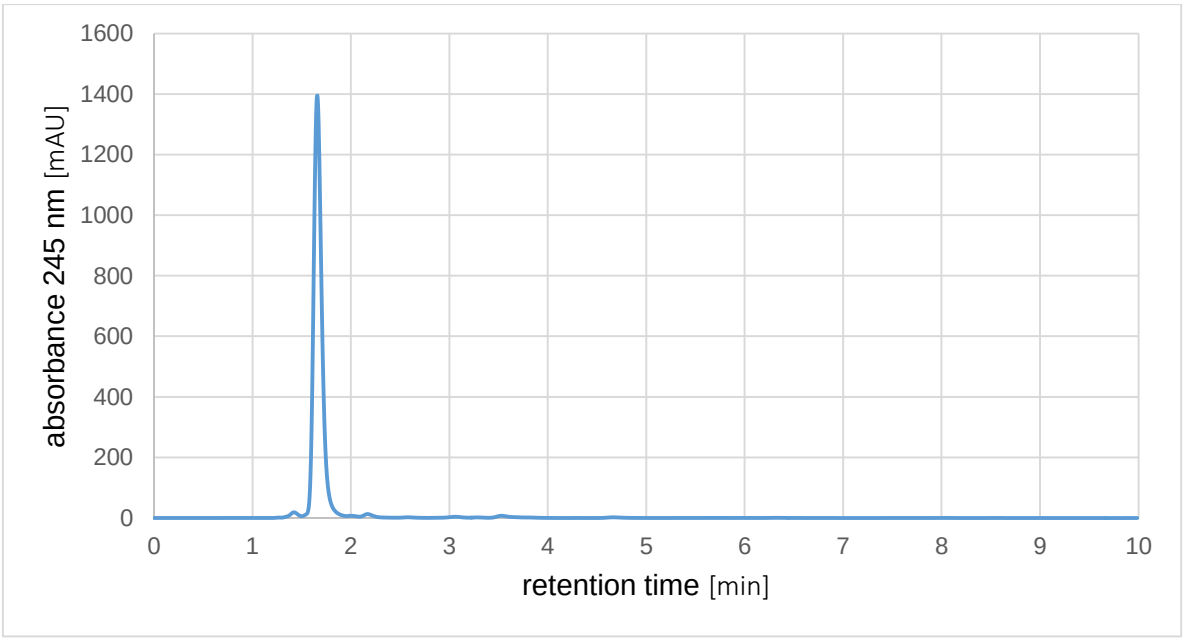

Figure 21. Sample chromatogram of the L – ascorbic acid measurement in sea buckthorn berry harvest year 2017

Table 23. Peak areas from the analysis of vitamin C in all samples

Samples	***Sea buckthorn berries 2017***	***Sea buckthorn berries 2016***	***Sea buckthorn berries 2015***
Peak areas [mAU's]	7973.5	10646.6	15354.6
	8042.1	10689.8	15304
	8026.7	10642.3	15343.8
Samples	***Buckwheat flour***	***Milled dry sea buckthorn***	***Control cookies (Buckwheat)***
Peak areas [mAU's]	0	140.8	0
	0	163.8	0
	0	194.8	0
Samples	***10% sea buckthorn cookies***	***25% sea buckthorn cookies***	***50% sea buckthorn cookies***
Peak areas [mAU's]	0	0	0
	0	0	0
	0	0	0
Samples	***100% sea buckthorn cookies***	***Control cake (Buckwheat)***	***Cake with sea buckthorn***
Peak areas [mAU's]	0	0	0
	0	0	0
	0	0	0
Samples	***Organic juice + oil***	***Syrup of sea buckthorn***	***Organic honey of sea buckthorn***
Peak areas [mAU's]	12077.4	4808.3	317.9
	12395.2	4983.3	268.1
	12418.5	5194.9	305.9
Samples	***Jam with sea buckthorn***	***Tincture 1 - from "skin" selection***	***Tincture 2 - from "tea"***
Peak areas [mAU's]	0	227	474.5
	0	227	474.5
	0	227	474.5
Samples	***Tincture 3 - from "leaves"***	***Special dry sea buckthorn, April 2017***	***Special dry sea buckthorn, April 2016***
Peak areas [mAU's]	580.7	196.6	55.7
	580.7	224.5	60
	580.7	190.5	46.2
Samples	***Special dry sea buckthorn, May 2016***	***0% Special dry sea buckthorn***	***10% Special dry sea buckthorn***
Peak areas [mAU's]	114.3	0	30.4
	132.1	29.4	23.2
	96.5	35.7	37.6
Samples	***25% Special dry sea buckthorn***	***50% Special dry sea buckthorn***	***100% Special dry sea buckthorn***
Peak areas [mAU's]	48.8	45.6	85.5
	45.9	0	74.5
	49.7	60	89.1

Samples	***Cookies***	***Muffins***	***Cookies with 10% by-product (April 2017)***
Peak areas [mAU's]	699	0	103.2
	773.9	444.8	123.1
	800.3	0	56.1
Samples	**Tea 1 - sea buckthorn leaves**		**Tea 2 - commercial tea**
Peak areas [mAU's]	49.2		121.1
	41.9		131.5
	0		120.8

Data of the β – carotene measurements

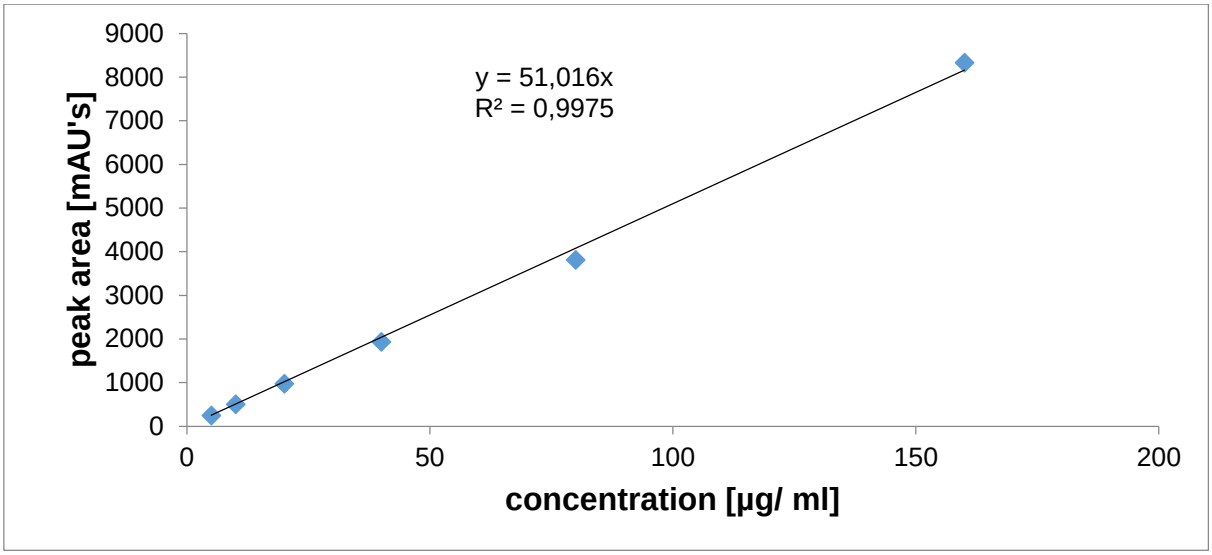

Figure 22. Standard calibration curve of β – carotene. Calibration equation was used to calculate the β – carotene concentration in all samples. Calibration equation: y = 51,016x

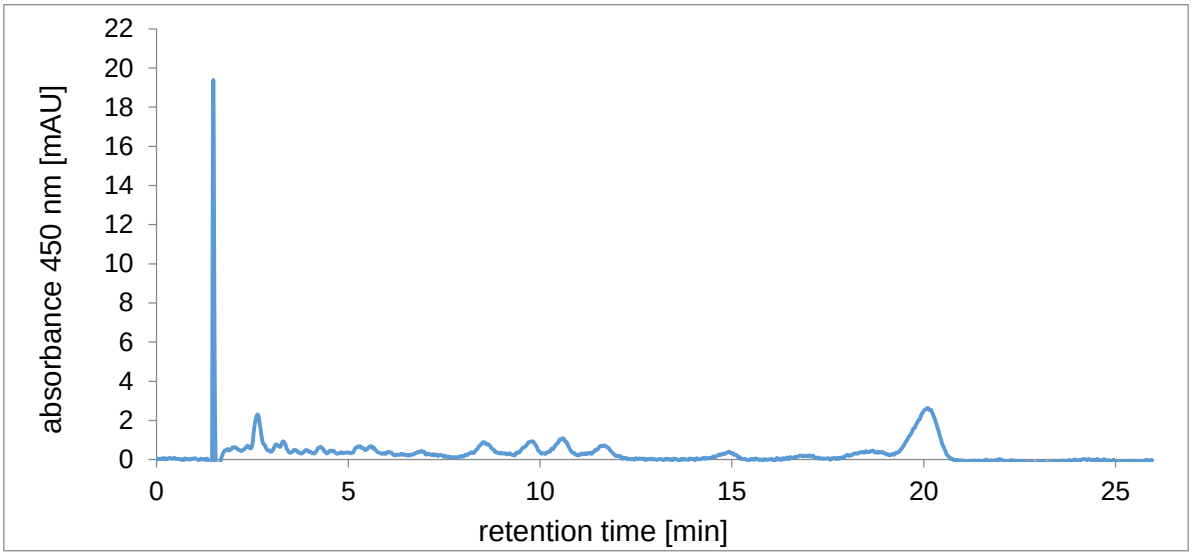

Figure 23. Sample chromatogram of the β – carotene measurement in sample milled dry sea buckthorn

Table 24. Peak areas from the analysis of β – carotene in all samples

Samples	***Sea buckthorn berries 2017***	***Sea buckthorn berries 2016***	***Sea buckthorn berries 2015***
Peak areas [mAU's]	0	333.1	334.1
	0	362.2	346.1
	0	284	258.1
Samples	***Buckwheat flour***	***Milled dry sea buckthorn***	***Control cookies (Buckwheat)***
Peak areas [mAU's]	0	143.3	0
	0	105.4	0
	0	119.1	0
Samples	***10% sea buckthorn cookies***	***25% sea buckthorn cookies***	***50% sea buckthorn cookies***
Peak areas [mAU's]	0	0	0
	0	0	0
	0	0	0
Samples	***100% sea buckthorn cookies***	***Control cake (Buckwheat)***	***Cake with sea buckthorn***
Peak areas [mAU's]	0	77.9	148.5
	0	91	136.2
	0	0	167.8
Samples	***Organic juice + oil***	***Syrup of sea buckthorn***	***Organic honey of sea buckthorn***
Peak areas [mAU's]	116.1	57.2	0
	63.5	19.7	0
	76.5	132.1	0
Samples	***Jam with sea buckthorn***	***Tincture 1 - from "skin" selection***	***Tincture 2 - from "tea"***
Peak areas [mAU's]	15.6	0	0
	13.6	0	0
	16	0	0
Samples	***Tincture 3 - from "leaves"***	***Special dry sea buckthorn, April 2017***	***Special dry sea buckthorn, April 2016***
Peak areas [mAU's]	0	119.5	0
	0	71.2	0
	0	57.4	0
Samples	***Special dry sea buckthorn, may 2016***	***0% Special dry sea buckthorn***	***10% Special dry sea buckthorn***
Peak areas [mAU's]	95.2	0	0
	46.6	0	0
	33.2	0	0
Samples	***25% Special dry sea buckthorn***	***50% Special dry sea buckthorn***	***100% Special dry sea buckthorn***
Peak areas [mAU's]	0	0	0
	0	0	0
	0	0	0

Sample	***Cookies***	***Muffins***	***Cookies with 10% by-product (April 2017)***
Peak areas [mAU's]	0	0	0
	0	0	0
	0	0	0
Sample	***Tea 1 - sea buckthorn leaves***		***Tea 2 - commercial tea***
Peak areas [mAU's]	71.6		132.4
	60.6		115.8
	65.6		157.6

<u>Data of the total phenolic content measurements</u>

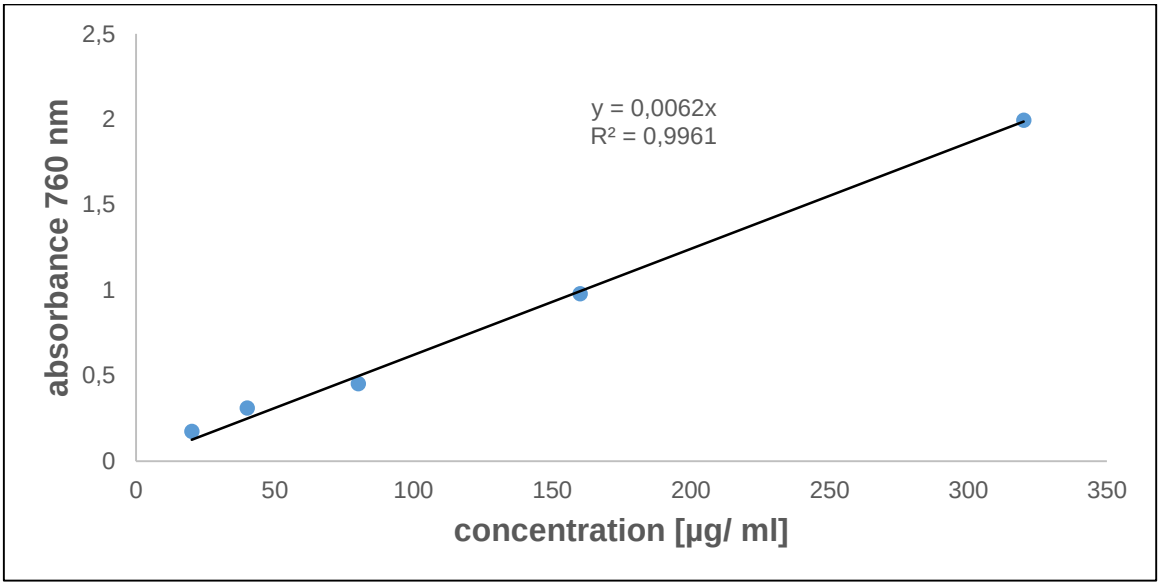

Figure 24. Standard calibration curve of gallic acid. Calibration equation was used to calculate the total phenolic content in all samples. Calibration equation: y = 0,0062x

Table 25. Absorption obtained from the total phenolic content measurements in all samples

Samples	***Sea buckthorn berries 2017***	***Sea buckthorn berries 2016***	***Sea buckthorn berries 2015***
Peak areas [mAU's]	0.101	0.140	0.150
	0.108	0.153	0.142
	0.106	0.178	0.190
Samples	***Buckwheat flour***	***Milled dry sea buckthorn***	***Control cookies (Buckwheat)***
Peak areas [mAU's]	0.346	0.438	0.313
	0.307	0.420	0.313
	0.357	0.460	0.298
Samples	***10% sea buckthorn cookies***	***25% sea buckthorn cookies***	***50% sea buckthorn cookies***
Peak areas [mAU's]	0.292	0.403	0.333
	0.335	0.457	0.353
	0.348	0.340	0.441
Samples	***100% sea buckthorn cookies***	***Control cake (Buckwheat)***	***Cake with sea buckthorn***
Peak areas [mAU's]	0.397	0.275	0.287
	0.460	0.260	0.378
	0.405	0.190	0.287
Samples	***Organic juice + oil***	***Syrup of sea buckthorn***	***Organic honey of sea buckthorn***
Peak areas [mAU's]	0.118	0.078	0.056
	0.100	0.110	0.073
	0.100	0.100	0.058
Samples	***Jam with sea buckthorn***	***Tincture 1 - from "skin" selection***	***Tincture 2 - from "tea"***
Peak areas [mAU's]	0.093	0.447	0.653
	0.072	0.447	0.653
	0.061	0.447	0.653
Samples	***Tincture 3 - from "leaves"***	***Special dry sea buckthorn, april 2017***	***Special dry sea buckthorn, april 2016***
Peak areas [mAU's]	0.427	0.407	0.527
	0.427	0.433	0.420
	0.427	0.431	0.408
Samples	***Special dry sea buckthorn, may 2016***	***0% Special dry sea buckthorn***	***10% Special dry sea buckthorn***
Peak areas [mAU's]	0.426	0.220	0.233
	0.428	0.160	0.330
	0.672	0.199	0.326
Samples	***25% Special dry sea buckthorn***	***50% Special dry sea buckthorn***	***100% Special dry sea buckthorn***
Peak areas [mAU's]	0.340	0.389	0.487
	0.327	0.404	0.502
	0.369	0.394	0.430

Sample	***Cookies***	***Muffins***	***Cookies with 10% by-product (April 2017)***
Peak areas [mAU's]	0.393	0.230	0.320
	0.365	0.142	0.152
	0.343	0.248	0.202
Sample	***Tea 1 - sea buckthorn leaves***		***Tea 2 - commercial tea***
Peak areas [mAU's]	0.497		0.416
	0.525		0.405
	0.448		0.415

V. Literature

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