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The effect of osmo-dehydration on antioxidant properties of dried black chokeberry

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Zásady pro vypracování

1. Use the databases available in the UPa library to find and process information on osmo-dehydration and its effect on the properties of final products (mainly fruits). Describe the chemical composition of the black chokeberry and the potential impact on consumer's health.

2. Prepare a powder from the black chokeberry samples (osmo-dehydrated) and determine its antioxidant properties.

3. Evaluate the results statistically, and find wether osmo-dehydration affects the antioxidant properties of prepared powder.

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"Keep your dreams alive. Understand that to achieve anything requires faith and belief in yourself, vision, hard work, determination, and dedication. Remember all things are possible for those who believe."

Gail Devers

Three years ago, I started one of the most amazing and enjoyable experiences of my short life; I decided to pursue my master's degree in a foreign country and I can firmly say that it was the best decision I have ever made.

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ANOTATION

Dried chokeberry fruits were pre-treated by ultrasound-assisted osmotic dehydrated using erythritol and xylitol at 0–45 min. A fine powder was obtained after subsequent forced-air drying and grinding of the samples. The effect of sugar and ultrasound treatment time on the colour, sorption properties and antioxidant characteristics was studied.

KEY WORD: Black chokeberry, moisture sorption, drying, antioxidant activity, flavonoid, anthocyanin.

TITLE

Vliv osmo-dehydratace na antioxidační vlastnosti prášku z plodů temnoplodce černoplodého

ANOTACE

Plody temnoplodce černoplodého (aronie) byly ošetřeny osmotickou dehydratací s využitím erythritolu a xylitolu a ultrazvukem po dobu 0–45 min. Prášek byl získán po vysušení v sušárně s nuceným oběhem vzduchu a následném rozemletí. V práci byl studován vliv použitých cukrů a doby aplikace ultrazvuku na barvu, sorpční vlastnosti a antioxidační charakteristiku.

KLÍČOVÁ SLOVA: Aronie, sorpce vlhkosti, sušení, antioxidančí aktivita, flavonoid, antokyanin

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LIST OF ABREVIATION

DPPH - Scavenging of the 1,1-diphenyl-2-picrylhydrazyl

UAE - Ultrasonically assisted extraction

FTIR- Fourier transform infrared

NIR – Near infrared

MIR – Mild infrared

FIR – Far infrared

UV-VIS -Ultraviolet visible

BET - Brunauer-Emmett-Teller

GAB - Guggenheim-Anderson-de Boer

CIELAB – A device-independent colour space

TEAC - Trolox equivalent antioxidant capacity

Introduction

Chokeberry (*Aronia melanocarpa*, Rosaceae) is native to eastern parts of North America, and in the 1900s, it was planted in Europe. Aronia fruits belong to the Aronia genus of the Rosaceae family, namely Maloideae subfamily. The Aronia ripen fruits contain different phenolic compound such as flavonoids, anthocyanins, phenolic acids etc. The composition and nutritive value of Aronia depends on a lot of factors such as environmental and climatic conditions, variety, maturity etc.

Nowadays, consumption of chokeberries is growing due to their antioxidant potential, that's why they are prevention of many diseases such as cancer, diabetes, heart disease etc. Chokeberry is widely used in functional food production, for example in fruit juice and wine for strengthening antioxidant properties. *Aronia melanocarpa* (Chokeberry) has received big attention in recent years as useful fruit for health. Its utilization as a functional food is growing day by day. In period of food processing and preservation may change the volatile components, antioxidants, phenolic compounds, and loose beneficial quality of food, that's why it important to study applying processing conditions and their influence of quality of food.

By reducing water, it is possible to prolong the shelf-life of the food product, due to the inhibition of some chemical reactions and preventing the multiplication of microorganisms. Water has important effect on food stability; It can change organoleptic character of food. There are several preservation processes, which are applied to low water in food product. One of them method is osmotic dehydration. The hypertonic solution is used during osmotic dehydration process and this time water is removed by a difference in osmotic pressure. Ultrasound-assisted osmotic dehydration influences on biogenic compounds.

The relationship between water activity and the equilibrium of the moisture content at constant temperature and pressure is called the moisture sorption isotherm. Each product has different interactions between the water and the solid components at different moisture contents. The knowledge of sorption isotherms is especially important in food production for the design and optimization of drying equipment, design of packages, stability of product, shelf-life and for observing and calculating moisture changes that may going on during storage. This research shows the effect of osmo-dehydration on antioxidant properties, colour change and moisture sorption isotherm of dried black chokeberry.

1. Literature review

1.1 Black chokeberry characteristics and use

Aronia, also called chokeberry, is a shrub. Aronia may grow more than three meters. Young shrubs are small, but mature bushes are like a sprawling tree. Leaves of chokeberry are oval. In summer and sprig period Aronia shrubs are green and autumn they become reddish brown. Fruits become ripe in late August and September. Mature chokeberry fruits are black and blue as it is shown on picture 1. Chokeberries are spherical. Due to different variety, their diameter ranges from 6.1 to 17.8 mm, whereas the weight of 100 fruits ranges from 32 to 111.7 g, sometimes is 280g. Chokeberries are harvested in autumn and have a strong, stable natural colour with a dry and sour flavour. Yields is up to 17 kilograms per bush. The chokeberry can be also mechanically harvested with equipment, which are available for blueberries. What's about smaller plants, the fruit is hand harvested by cutting the fruit clusters. Harvest is usually in late August to September when the fruit contains maximum level of sugar [1-4].

Aronia fruits belong to the Aronia genus of the Rosaceae family, namely Maloideae subfamily. Aronia fruits are represented by two species: *Aronia melanocarpa*, which is named black chokeberry and *Aronia arbutifolia* which is called red chokeberry. Black chokeberry contains a lot of natural colourants, that's why, nowadays, it is a widely utilized crop for the production of natural food colorants and due to its usage in processed products and nutraceutical utilization in the form of nutritional supplements [1-4].

The Aronia plant is native to eastern parts of North America, and in the 1900s, it was planted in Europe. Aronia has been commercially cultivated in the United States since early in the 20th century. Aronia berry are used in canning food industry. From Aronia juice extract are produced jelly, candies, pie and cookie fillings, yogurt, sorbet, flavoured milk, and other uses. Chokeberry has strongly colour and wonderful flavoured juice, that's why it is used in wine production In Russia, Denmark and Eastern Europe. Chokeberry became very popular fruits end of the 20^{th} century, that's why it is used in the food industry for the production of juices, nectars, syrups, jams, preserves, wines, tinctures, fruit desserts, jellies, fruit teas and dietary supplements. Due to Chokeberry includes a lot of anthocyanins, extract of chokeberry is added to foods as natural dyes [1-4].

The composition and nutritive value of Aronia depends on a lot of factors. Environmental and climatic conditions, variety, maturity, and other reason to meet different type and quantity

compounds in chokeberry fruit. Generally, in extract of chokeberry, levels of anthocyanins and flavonoids are five times greater than in cranberries extract. Aronia also contains antioxidants, polyphenols, minerals and vitamins. some of these substances significantly reduce the potential for cancer and heart disease. That's why hokeberry is widely used in functional food production! [1-8]

Picture 1. Aronia Melanocarpa [63]

1.2 The health benefits of chokeberry

Many beneficial health effects have been studied for Aronia fruit, its extract and for some isolated compounds. The Aronia berries have a wide range of potential medicinal and therapeutic effects. *Aronia Melanocarpa* is one of the richest fruits by antioxidant content. It is Basically consumed in Eastern Europe and Russia. In folk medicine Native Americans used Chokeberry as a treatment for the flu and same illness [1, 3-8].

There are several researches about the chemical composition of Aronia berries and their positive effect for human health, that's why that's why fruits and juice of chokeberry are using for prevention of different diseases. Health benefits of chemical compound chokeberry include hypotensive, lipid-lowering, gastroprotective, hepatoprotective, and anticarcinogenic effects. Also, according to various studies, Aronia products and their preparations have antiviral

activity, antiaging effect, protective effect against cadmium intoxication, and anti-inflammatory effect in patients, who has higher blood pressure. Consumption of Aronia fruit and its products improves human immunity [3, 5].

Furthermore, researchers studied positive role of chokeberry in the control of type 2 Diabetes. After regular consumption of Aronia sugar extract, diabetics showed a reduce in blood sugar levels and a decrease in total cholesterol levels too [3, 5].

Chokeberry contains important antioxidant such as procyanidins, anthocyanins, and phenolic acids etc. that's why its extract are used to reduce oxidative stress. People have oxidative stress when free radicals strengthen the body's defence mechanism against their harmful effects [1-8].

1.3 Chemical composition of chokeberry

The Aronia fruit pomace is a valuable source of nutritious components, such as proteins, fat dietary fibre, pectin, cell wall polysaccharides, vitamins, polyphenols, phospholipids, etc.

Black chokeberry is a source of wide range bioactive compounds, that has a wide spectrum of health-positive properties. Polyphenols are important compounds that determine the high bioactivity of chokeberries, which include anthocyanins, proanthocyanins, flavanols, flavanols, proanthocyanins, and phenolic acids. Aronia fruit and products have important antioxidant and health-promoting potential as they reduce the occurrence of free radicals. That's why chokeberry are extensively used in industry of functional and dietary food [1-8].

In our century, the Aronia melanocarpa fruit is characterized as an especially important source of bioactive components, such as polyphenols. They are considered to be very significant dietary antioxidants. Antioxidants are the substances that can scavenge free radicals, instable and reactive forms with an unpaired electron in the outer orbit. Free radicals could cause cell damage to the human body, when they strengthen the body's defence mechanism against their harmful effects. "Oxidative stress" that cause to a lot of chronic diseases, such as atherosclerosis, inflammation, cancer molecules and neurodegenerative diseases. Some dietary antioxidants may donate hydrogen radicals and replace the action of free radical scavengers. Therefore, polyphenols may prevent some important points in disorders' progress [3-6, 8].

To the most significant polyphenols of Aronia berries belong phenolic acids and flavonoids such as anthocyanins, flavanols, flavanols and proanthocyanins. AS the most important phenolic compounds were found and studied from the group of phenolic acids hydroxycinnamic

acids, namely, neochlorogenic acid, from anthocyanins, there were cyanidin-3-galactoside and cyanidin-3-arabinoside; from proanthocyanins, procyanidin B1. Flavanols (mainly quercetin glycosides and epicatechin) are insignificant components of Aronia fruits [1, 7].

Black Chokeberries are a rich of hydroxycinnamic acids, derivatives of cinnamic acid, which are slightly soluble in water. The most plentiful is chlorogenic acid, that is a complex of caffeic acid linked to quinin acid through an ester bond. The flavonoids present in the Aronia fruit are the major subgroups are anthocyanins, proanthocyanins, flavanols and catechins. 10% of total phenolics content are flavanol glycosides, from where prevail of quercetin 3-O-galactoside and quercetin 3-O-glucoside. In other berry extracts such as blueberries, black currant, blackberry, bilberry, red currant, red raspberry, and strawberry are significantly less amount of the content of total phenol [7-9].

Flavanols, namely quercetin glycosides and flavanols such as flavan-3-ols, epicatechin in proanthocyanins are also present in the Aronia berries, but only insignificant quantities. In the chokeberry fruit, a mixture of four different flavanols is present, mainly quercetin-3-galactoside, quercetin-3-glucoside, quercetin-3-rutinoside and quercetin-3-robinobioside. Furthermore, in Aronia fruits another flavanols, which are identified, are kaempferol and quercetin 3-vicianoside, but their quantity is extremely small amount [9,10].

Most of the interest in Black chokeberry fruit is focused on pigments, namely anthocyanins, glycosylated pigmented flavonoid compounds, and their content. In Aronia fruit, most important compounds are anthocyanins. The major anthocyanin of Aronia was identified as cyanidin-3-galactoside. More than 50% of anthocyanins of the present polyphenols. [14]

1.4 Chemistry of isolated compounds from chokeberry

Polyphenols

Polyphenols occur in different plant such Fruits, vegetables, whole grains and tea, chocolate, and wine. especially, Tea leaves, various berries, red wine are rich sources of polyphenols. Fruits like grapes, apple, pear, cherries, and berries contains up to 200–300 mg polyphenols per 100 grams fresh weight. Typically, a glass of red wine or a cup of tea or coffee contains about 100 mg of polyphenols [1,4,7].

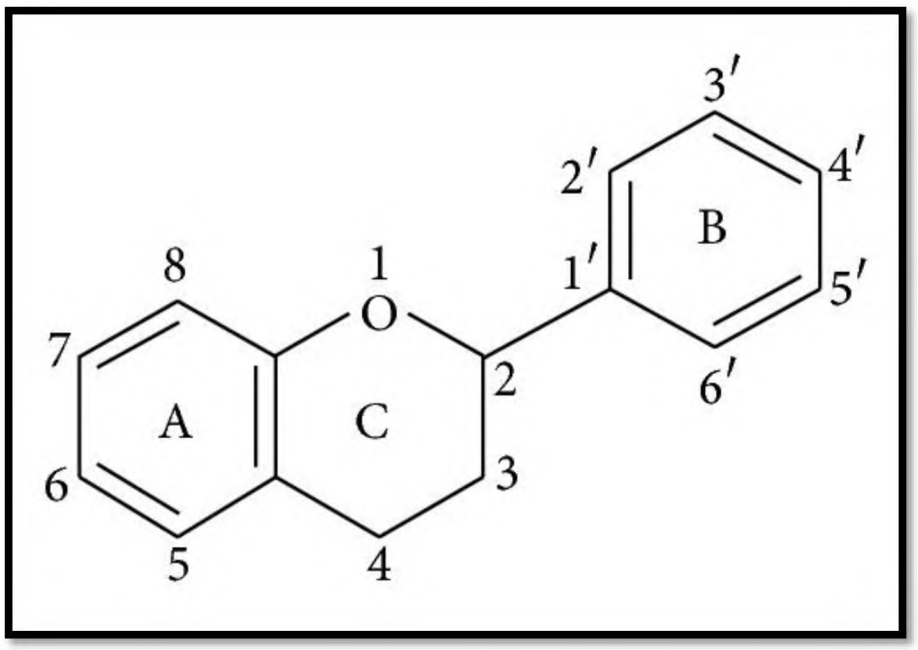
Approximately 8000 of polyphenolic compounds have been found and studied in Aronia berries. All plant phenolic compounds derivate from phenylalanine, or a shikimic acid. mainly, Polyphenols arise in conjugated forms, with one or more sugar residues linked to hydroxyl groups, although direct linkages of the sugar to an aromatic carbon also exist. The main group of Polyphenols include phenolic acids, flavonoids, anthocyanins, stilbenes and lignans [15-16].

Flavonoids

Flavonoids are widely spread group of plant phenolic compounds. They are occurring approximately in all plant parts, especially the photosynthesising plant cells. Major chemical compound of flowering plants are flavonoids. Flavonoids play an important role in the diet of humans and animals. Flavonoids have biological and pharmacological activities, which include antioxidant, cytotoxic, anticancer, antiviral, antibacterial, cardioprotective, hepatoprotective, neuroprotective, and antimalarial properties [4, 9-12].

Flavonoids was isolated from oranges in 1930. Flavonoids are natural compounds; these have variable phenolic structures and they are mainly obtained by extracting plants and are widely used in the food industry. At that time, it was considered to be a new type of vitamins and referred as vitamin P. Later, it was studied that this substance was a flavonoid. Nowadays, more than 4000 varieties of flavonoids have been identified [9].

Chemically flavonoids are a fifteen-carbon skeleton consisting of two benzene cores linked via a heterocyclic pyrene core. They can be classified into a variety of groups such as flavones, flavanols, flavanones, and others. Flavonoids are comprising 15 carbons, with two aromatic circles connected by a three-carbon bridge, henceforward C6–C3–C6. The basic flavonoid structure is aglycone, which structure is shown in picture 2. [9,12].



Picture 2. Basic flavonoid structures [64]

Flavonoids occur different types as aglycones, glycosides, and methylated derivatives. Six-member ring condensed with the benzene ring is either a α-pyrone or its dihydroderivative. the Flavonoids are divided into flavonoids (2-position) and isoflavonoids (3-position) according to the position of the benzenoid substituent [12].

Flavonoids have a lot of biochemical properties, but the most important property is their capacity to act as antioxidants. flavonoids functional groups and their arrangement of the nuclear structure are reason of the antioxidant activity. flavonoids, due to their configuration, substitution, and total number of hydroxyl groups substantially, influence several mechanisms of antioxidant activity such as radical scavenging and metal ion chelation ability [9-11].

Among flavonoids, antimicrobial compounds against a wide spectre of microorganisms have been found Many flavonoids including apigenin, galanin, flavone and flavanol glycosides, isoflavones, flavanones, and chalcones have been characterized strong antibacterial activity [9, 10].

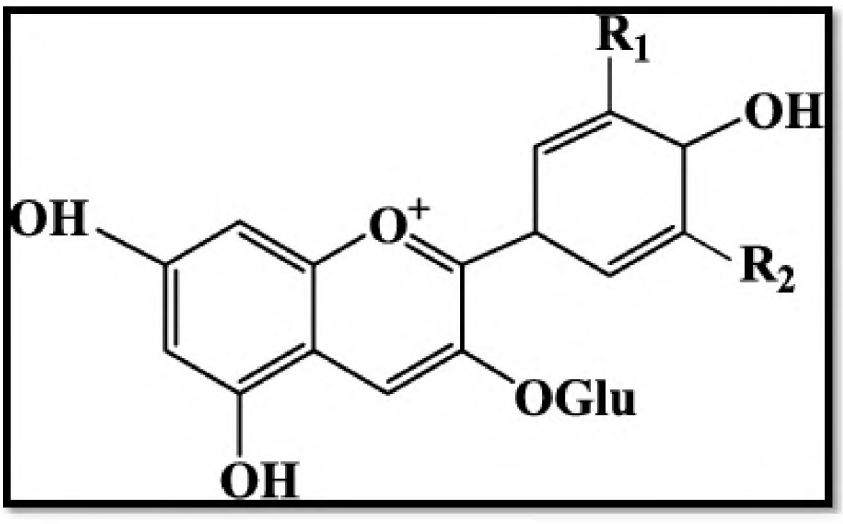
Catechins have been widely researched due to their antimicrobial activity. These compounds have antibacterial activity against *Vibrio cholerae, Streptococcus mutans, Shigella*, and other bacteria. The catechins can inactivate cholera toxin in *Vibrio cholera* and inhibit isolated bacterial glucosyltransferases in *S. mutans* because of their complexing activities. Also, another

research demonstrated inhibitory activity of quercetin, apigenin, and 3,6,7,3′,4′-pentahydroxyflavone against *Escherichia coli* DNA gyrase [9].

Anthocyanins

Anthocyanins belong to polyphenols. They were found in plants as colour pigments. Anthocyanins give different range of colour of many flowers, leaves, vegetables, and fruits. There is approximately one thousand anthocyanins, and more than 15 anthocyanidins belong of vegetal kingdom. Dietary intake of anthocyanins is allowed up to 200 mg/per day, which is higher than other flavonoids. From several researches, it was found that some plants containing anthocyanins have anticancer property. Anthocyanin isolates and anthocyanin-rich mixtures of bioflavonoids can protect from DNA cleavage, estrogenic activity, enzyme inhibition, boosting production of cytokines, anti-inflammatory activity, lipid peroxidation, decreasing capillary permeability and fragility, and membrane strengthening [14-16].

The anthocyanidins are the basic structures of the anthocyanins. The anthocyanidins consist of an aromatic ring bonded to an heterocyclic ring that contains oxygen, which is also bonded by a carbon–carbon bond to a third aromatic ring When the anthocyanidins are found in their glycoside form (bonded to a sugar moiety) they are known as anthocyanins. The basic anthocyanin structure is shown in picture 3. [16].



Picture3. The basic chemical structure anthocyanins [65]

1.5 Antioxidants

Antioxidant prevents oxidation process in food such as browning. it has a lot of positive effect on human physiology. For received maximum benefit, it is important to know concentration and absorption mechanism of antioxidant. Sometimes synthetic antioxidant has negative affect on health due to its toxic mixtures. antioxidants are classified two class: synthetic and natural. Synthetic antioxidants are polyphenolic compounds, for ex. BHA, BHT, EDTA etc. Natural antioxidants are divided enzymatic, which in turn is divided primary and secondary antioxidant and non-enzymatic. Non-enzymatic antioxidant is minerals (Se, Cu, Fe, Mg...), vitamins (vitamin A, C, B...), carotenoid (beta-carotene, lutein etc.), polyphenols (flavonoids, polyphenol acids etc.) and other antioxidant (protein and non-protein) [17,18].

Natural antioxidant extracts are used as food preservatives. Natural antioxidant extracts are basically received from berry, such as black chokeberry, blueberry etc. Biochemical processes change food properties and can produce toxic compounds. compounds of food spontaneously oxidize when exposed to air, and the antioxidants protect against this oxidation. Antioxidants are compounds that inhibit fermentation activity and prevent oxidative process, which are reasons for reducing changes in the taste, colour, and nutritive value of food. Ascorbic acid, carotenoids, phenolic compounds, and tocopherols are used in food as natural antioxidant [19].

According to mechanism antioxidants are classified into five types: radical scavenging antioxidants, chelators, which make complexes with metals and prevent generating of radicals; extinguishers, which deactivate high-energy oxidant species; oxygen scavengers, which remove oxygen and avoiding their destabilization; and regenerators of antioxidants, which restore other antioxidants in the food when they become radicals [19].

1.5.1 Scavenging of the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical

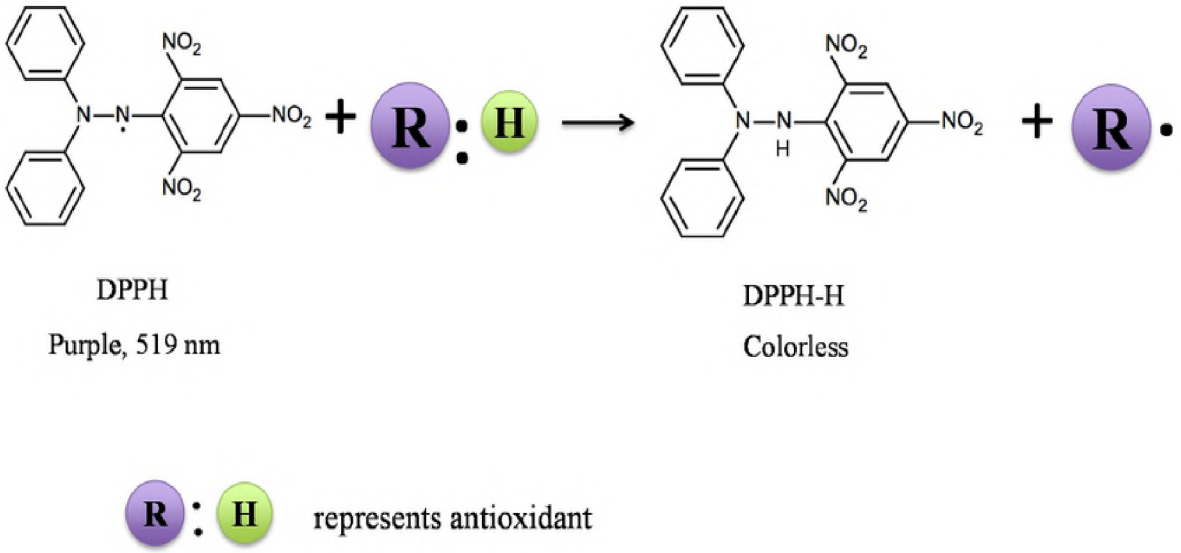
Free radicals are the products of normal cellular metabolism. A free radical can be defined as an atom or molecule containing one or more unpaired electrons in valency shell or outer orbit and is capable of independent existence. The odd number of electron(s) of a free radical makes it unstable, short lived and highly reactive. Because of free radical has high reactivity, they can abstract electrons from other compounds to reach stability. That's why, the attacked molecule loses its electron and becomes a free radical itself, beginning a chain reaction cascade which finally damages the living cell. Free radicals can negatively affect biological molecules such as

nucleic acids, lipids, and proteins, thereby changing the normal redox reaction and causing to increased oxidative stress. The free radicals induce oxidative stress, which is reason several diseases such as diabetes mellitus, neurodegenerative disorders, cardiovascular diseases, respiratory diseases, cataract development, rheumatoid arthritis and in various cancers. An antioxidant is substance that significantly delays or prevents oxidation of that substrate, which prevent us from developing various diseases [20,21].

Scavenging of the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical is best method for antioxidant activity testing. DPPH method is very rapid and easy. This method is based on of free electron-transfer that produces a violet solution in ethanol or in methanol. DPPH radical is stable at room temperature, but an antioxidant molecule is reduced in solution, giving rise to colourless ethanol solution. The DPPH assay can be evaluated antioxidants by UV-vis spectrophotometry that's why it can be useful to assess various products at a time [21-24].

The 2,2-diphenyl-1-picrylhydrazyl UV–Vis assay is the most popular used method for this goal. Although, it can give incorrect results if the spectroscopic properties of each substance are not considered. The 2,2-diphenyl-1-picrylhydrazyl assay is easy method to quantify the radical scavenging activity of pure substances, mixtures, or extracts [23].

DPPH mechanisms based on reaction of an antioxidant with the DPPH* radical, and the follow process depends on the antioxidant molecule. The reaction of antioxidant with 2,2-diphenyl-1-picrylhydrazyl causes disappearance of DPPH violet colour. Therefore, for measurement of changing colour are used UV-VIS spectrometry, which measure the absorbance of the remnant DPPH (λ max. around 517–520 nm). The different procedures to determine the antiradical activity measure the initial DPPH concentration and the remaining DPPH in the reaction medium after an incubation period (% DPPHR). Reaction mechanism of, 2-diphenyl-1-picrylhydrazyl with antioxidant are shown in picture 4. [23, 24].



picture 4. Reaction mechanism of 2,2-diphenyl-1-picrylhydrazyl with antioxidant. R : H = antioxidant radical scavenger; R = antioxidant radical. [66]

The percentage of remaining DPPH* can be calculated by the formula:

$$\%DPPHR = \frac{[DPPH*]t}{[DPPH*]0} . 100 = \frac{At/\varepsilon DPPH*b}{A0/\varepsilon DPPH*b/} . 100 = \frac{At}{A0} . 100, \qquad \text{Equation 1.}$$

Where At is the absorbance of the sample after incubation, A0 is the absorbance of the control with solvent instead of antioxidant at the same incubation time, ε is the molar absorptivity, and b is the optical path length.

1.5.2 Method of determination total phenolic content

Phenolic Quantification Assay is based on Folin-Ciocalteu method. The total phenolic content is determined spectrophotometrically according to the Folin - Ciocalteau (FC) method. The FC reagent contains phosphomolybdic/phosphotungstic acid complexes (composed of sodium tungstate, orthophosphoric acid, hydrochloric acid, lithium sulphate, sodium molybdate and bromine). The principle of the method is based on the reaction of an FC reagent with the hydroxyl group of phenolic substances. The method relies on the transfer of electrons in 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. The phosphotungsten-phosphomolybdenum complex is reduced to a blue color. The reduced Folin-Ciocalteu reagent is detectable with a spectrophotometer at 765 nm against the blank. Results are expressed as gallic acid equivalent [54].

1.5.3 Method of determination total anthocyanin content

The pH differential method has been applied widely by food technologies to assess the quality of fresh fruits and vegetables. The method can be used to determine total anthocyanins content. This method based on structural change of the anthocyanin chromophore between pH 1.0 and 4.5. The concept of determining number of anthocyanins present in food by measuring change in absorbance at two different pH values. The difference in absorbance at the (ca 520 nm) of the pigment is proportional to concentration of pigment. Absorbance should be measured at the vis-max of the pigment solution and the pigment content should be calculate by using the molecular weight an molar extinction coefficient of the major anthocyanin in the matrix. For example, the anthocyanin content of cyaning-3-glucoside by using a molar extinction coefficient of 26900l/mol.cm [55].

1.5.4 Method of determination total Flavonoid content

Method of determine total flavonoid content based on the formation of a complex between the aluminum ion, Al^{3+} and the carbonyl and hydroxyl groups of flavones and flavanols that produce a yellow color. Two widely used procedures can be differenced. In the first one, $AlCl_3$ solution in the concentration range of 2–10 % is added to a extract of Aronia fruits and can be applied in the presence of acid or acetate solution; Measurements were done after optimizing time of the addition of $AlCl_3$ at 425nm quercetin was used as the standard compound for the expression of results. In the second procedure, complexation reaction with NaNO2 in alkaline medium. The method is based on the nitration of any aromatic ring bearing a catechol group with its three or four positions unsubstituted or not sterically blocked. After addition of Al^{3+} a yellow solution of complex was formed, which then changed to red after addition of NaOH, and the value of absorbance is measured at 510 nm [56, 57].

1.5.5 Method of determination total antioxidant capacity

Three methods, TEAC, FRAP and DPPH were used for the estimation of antioxidant capacity based on the reaction of specific reagent of each method with electron donating or hydrogen radical (H) producing antioxidant compounds. The antioxidant capacity was interpreted as the reducing capacity. over 20 methods are applied for assessment of antioxidant capacities. For the methods based on similar principles of redox reactions (e.g. Trolox equivalent antioxidant capacity (TEAC) [58].

The DPPH method is based on the reaction of an antioxidant contained in a test sample with a stable violet-colored solution of the radical 2,2-diphenyl-1-picrylhydrazyl (DPPH). During the reaction, the reduction of DPPH by the antioxidants present to diphenylpicrylhydrazine (DPPH-H) is discussed. The results are determined spectrophotometrically at 517 nm as the loss of absorbance to a constant value against a blank. The DPPH method is based on the reaction of an antioxidant contained in a test sample with a stable violet-colored solution of the radical 2,2-diphenyl-1-picrylhydrazyl (DPPH). During the reaction, the reduction of DPPH by the antioxidants present to diphenylpicrylhydrazine (DPPH-H) is reduced.

$DPPH\bullet + AH \rightarrow DPPH^{-}H + A\bullet$

The results are determined spectrophotometrically at 517 nm as the loss of absorbance to a constant value against a blank [59] .

1.6 Osmotic dehydration

1.6.1 Osmotic dehydration and its influence on volatile

The ultrasound-assisted osmotic dehydration is basically used for food processing. The hypertonic solution is used during osmotic dehydration process and this time are removed water by a difference in osmotic pressure. It can prolong time of preservation of the food product. In the osmotic dehydration process can decrease moisture load and increase the nutrition and sensory quality [25-28].

Water has important affect food stability. It can change organoleptic character of food, such as juiciness, elasticity, tenderness, and texture. Quantity of water does not influence on food stability and texture, but its thermo-dynamic state that is responsible for its influence on ones. The thermodynamic state of water in food is represented by its activity, which is 0 for dry material and 1 for pure water. The lower the water activity the more stable is the food and its preservation can be long time [26-28].

Decrease of water activity is possibility in two way, either by addition of substance or by removal of solvent. In first case, it needs to add large amounts of sodium chloride, sugars, or polyols to food, that's why this way is acceptable by consumers. In second case, it needs large energy consumption, what make final product to be expensive [27].

Water interactions with solute characterize thermodynamic state of water in solution. Molecules have their own internal energy and interactions, also each substance need energy of the solution, which is in the defined energetic state. This state of the substance is named the chemical potential. Chemical potential is a character of concentration, temperature, and pressure. Under isothermal conditions, concentration and pressure determine chemical potential. Under conditions of higher soluble concentration, the chemical potential of the solvent is low [26, 27].

Osmotic dehydration has an influence on volatile compounds. During food processing and preservation, changes in volatile components content loose of beneficial quality of food may occur That's why it is important to study conditions of food processing and their influence of quality of food. Functional groups, methyl, aldehyde, ether, and acid compounds were studied in during all treatment and untreated. Fruit treated, namely plum, with ultrasound during osmotic dehydration preserve most of the volatile compounds, also in almond extract treated with ultrasound and wine manufactured were usefully preserve of the volatile compounds [27-31].

1.6.2 Influence of ultrasound-assisted osmotic dehydration on antioxidant and phenolics compounds and the advantages of osmotic dehydration

The research shows an increase of antioxidant activity in all ultrasound-assisted osmotic dehydrated plum, except one case, when plum was treated in glucose at 60 min. This loss of antioxidant activity was caused due to the higher migration of compounds during osmotic dehydration, what also cause insignificant structure deformation. When plum was treated in glucose at 30 min, its antioxidant activity was the highest, the increase also antioxidant capacity in sucrose at both 30 and 60 min of ultrasonic treatments. According to the given experiments, the retention of bioactive compounds depends upon osmotic solution and treatment time. According to this research, the ultrasound osmotic dehydration decreases the bioactive compounds during osmotic dehydration, but there is some of researches, where ultrasound application increase the retention of bioactive compounds with increase treatment time [29].

The phenolics have significant role in the food nutrition. They are the secondary metabolites. they are used in the dietary food. The bioactivity of phenolics is beneficial for human health, for ex. they can inhibition of lipoxygenase and free radical scavenging activity. In plump the highest of phenolics content was observed when it was treated in glucose at 30 min of ultrasound during osmotic dehydration. Like the Antioxidants, the phenolics content were reduced in glucose at 60 min and the phenolics activity was increased in sucrose at 30 and 60 min of ultrasound treatment during osmotic dehydration process [28,29].

The changing of Antioxidant activity and phenolic compound activity was similar in different sugar of ultrasound treatment during osmotic dehydration. The research showed that treatment of ultrasound during osmotic dehydration has similar influence on anthocyanins and phenolic compounds activity [30-32].

Osmotic dehydration is process, which reduce the water activity of food. However, unlike another drying process, the food does not reach very low water activity, for preservation must be making another complementary technology. Nowadays, osmotic dehydration is used mainly to reduce the weight and improve the drying process [26, 29].

Ultrasound improves the process, helps to increase in both water loss and solid gain. Because of osmotic dehydration is a liquid–solid operation, the energy loss due to ultrasound wave is very low or equal almost zero, unlike the drying process [25].

Most of time the ultrasonic bath and the ultrasonic probe are used to treat the food. Both systems are used to improve the osmotic dehydration process [25].

When we use ultrasound to help with osmotic dehydration, it causes a decrease in internal and external resistance. The external resistance is reduced by the acoustic streaming and the microjets, enhancing the mass transfer by convection. Also, the direct and indirect effects of ultrasound reduce the internal resistance. For example, conventional agitation could be less effective than ultrasound, because conventional agitation decreases only the external resistance [25-27].

On the other side, when ultrasound is used as a pre-treatment, it improves osmotic dehydration by reducing only the internal resistance, because structural changes and microchannel formation during the pre-treatment [25-27].

1.7 Ultrasound assisted extraction of Aronia

Extraction has been used since ancient times, probably after the discovery of fire. Old national people, such as Egyptians and Phoenicians, Jews and Arabs, Indians and Chinese, Greeks and Romans, and even Mayas and Aztecs, all of them knew to use innovative extraction and distillation processes. They are actively used in extraction and distillation processes for perfumes, cosmetics, or food. In modern life, Extraction processes are used in almost all fields such as food production, pharmaceuticals, cosmetic, nutraceutical, or bioenergy industries [33].

Ultrasound is important in achieving the objective of extraction. Ultrasound has a significant effect on the rate of different processes in the chemical and food industry. Using ultrasound is possibility to reduce the consumption of solvent, simplify working process, give more purity of the final product. one most important side of using ultrasound is ability to complete full extractions in minutes with high reproducibility. Nowadays, a lot of food components such as aromas, pigments, antioxidants, and other organic and mineral compounds have been extracted, analysed and formulated efficiently [33-35].

Various factors affect the extraction process, for example, time and temperature, solid–solvent ratio, type of solvent, particle size, ultrasound etc. Extraction time and temperature should be optimized to minimize process energy cost. In all the studies studied, obtained the number of polyphenols increases with time. The amount of extraction decreases progressively but the extraction was not finished during of experiment. The temperature has a positive effect on the extraction of different compounds from berries. According to various experiments, the effect of medium temperature on the amount of obtained polyphenols from Aronia is obvious. The positive effect of temperature could be explained by more solubility of polyphenols in the solvent, more diffusivities of the extracted molecules and the improved mass transfer at higher temperatures [33-35].

The effect of solid–solvent ratio on the extraction of polyphenols from Aronia berries was studied at 60°C with ground berries (fraction= 0.5–1.0 mm) and water as solvent. However, the yield of polyphenols at ratio 1:10 was sensibly lower than the yields at ratios 1:20 and 1:40 which were terribly similar. Consequently, the ratios 1:20 and 1:40 were considered as suitable to obtain highest yields and therefore the studies of the extraction of polyphenols from black chokeberry at different conditions were carried out using these ratios [34].

Today, alcohol and acetone are the most used solvents for extracting polyphenols from berries. Mono-component solvent systems is less efficient in extracting phenolic constituents than

water–alcohols mixtures. In food industry, namely, using of black chokeberry extracts, ethanol was preferred than methanol and acetone as solvent, but Commercially, ethanol is more expensive than methanol. 30%, 50% 96% ethanol and water were used to examine the effect of different solvents on the final extraction efficiency. When 96% alcohol was used in extraction process, total phenolic and anthocyanidin was extracted extremely low amount. The maximum amount of total phenolic extract was released when using 50% ethanol and the maximum amount of anthocyanin when using 70% alcohol [34,35].

Ultrasonically assisted extraction is cavitation which was first identified and characterized in 1895. Some type cavitation can be generated with power ultrasound. During the sonication of a vegetal material in a solvent the suspended solids promote asymmetric bubble collapse, which helps effective extraction. Asymmetric bubble collapse is major parameter of ultrasonically assisted extraction. The applied acoustic power must be large amount to carry cavitation. The main effect of cavitation in a heterogeneous extraction mixture is that the collapse of the bubbles is asymmetric resulting extremely high-speed jets of solvent targeting the vegetal material and this makes UAE (ultrasonically assisted extraction) so effective. This is the main phenomenon which must be considered when developing possible UAE extraction mechanisms [36].

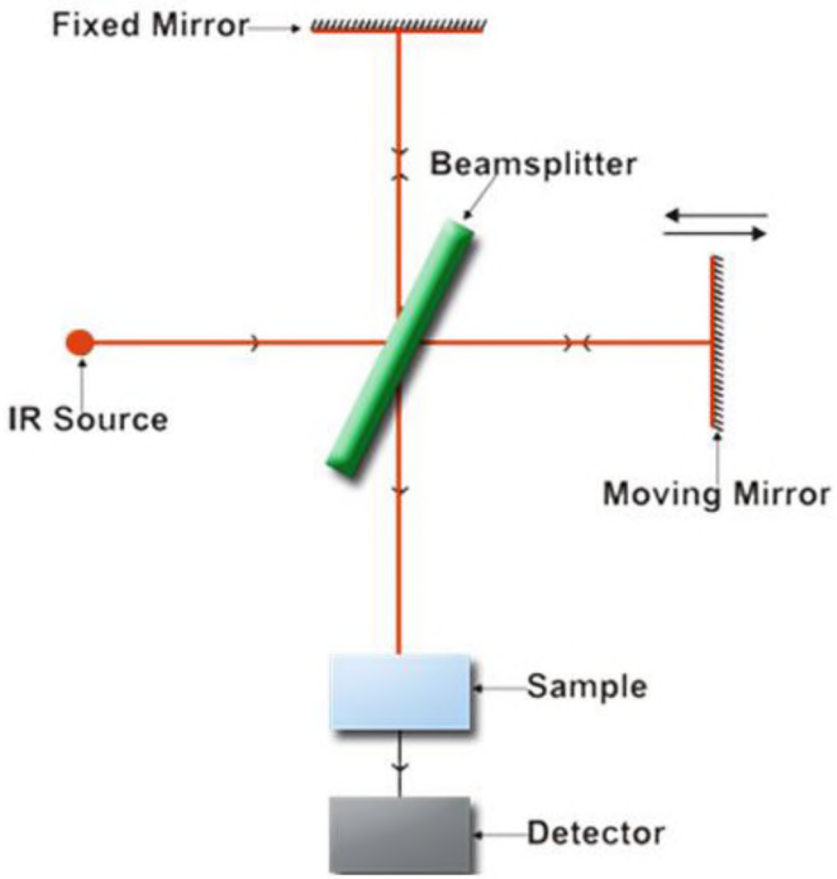
1.8 Fourier transform infrared spectroscopy

Infrared spectroscopy is used an excellent chemical analysis technique. Infrared spectrometry has changed for the last 40 years. In 1973 years, Herbert Laitinen used FT-IR spectrometers. Fourier transform infrared (FTIR) spectroscopy has become an attractive alternative for traditional analytical methods because little sample preparation is needed, analysis is rapid, and the use of hazardous solvents is minimized. Several researchers have attempted to utilize these advantages by applying FTIR to food science [37, 38].

IR spectra are produced by recording changes in absorption of IR radiation by molecules, which undergo mechanical motions (vibrational and rotational modes) due to the absorption of energy. Within any molecule, a given functional group gives characteristic IR absorption at specific, narrow frequency ranges regardless of their relationship with the rest of the molecule. There are three IR region of the electromagnetic spectrum spans: near IR is from14,000 to 4,000 cm−1, mid IR is 4,000 to 400 cm−1, and far IR is 400–50 cm−1. NIR spectra are weaker than another spectra. Its bands of fundamental vibrational transitions connected mainly with C-H, N-H, and O-H functional groups. NIR bands are complex vibrational motion of chemical bonds that deviate from harmonicity. These deviations create from transitions over two, three, or higher energy levels (12,500–4,000 cm−1, 800–2,500 nm) of the frequency of fundamental vibrations, causing to a decreasing NIR absorption intensity with growing rank of overtone. MIR spectra are used for structural identification of organic compounds because its absorption bands are received by fundamental vibrations of a specific functional group. MIR region between 1,200 and 700 cm−1, contains bands from lipids, proteins, carotenoids, and polysaccharides, and etc. MIR spectra are used for quantitative analysis because the intensities of the bands are proportional to the concentration of their functional group. FIR spectra are applied in astronomy and medicine [39].

1.8.1 Instrumentation of FTIR spectroscopy

FTIRs do not have a monochromator and use the Michelson interferometer principle to distinguish radiation of different wavelengths. Simplified block diagram of this device are shown on picture 5. It is arranged a fixed mirror and a moving mirror aligned at right angles to each other with the beam splitter positioned in the centre and equidistant from both. The beam splitter splits the incident beam into two parts and recombines it after introducing the path difference due to reflection from the moving mirror. On recombination an interference pattern is created due to the continuous variation of the path difference of the two beams [40].



Picture 5. FTIR spectroscopy [67]

FTIR spectroscopy is used as detector for chromatography. FTIR spectrometer is widely applied in food system for identify organic compounds.

1.9 UV-VIS spectrometer

Nowadays, ultraviolet visible (UV-VIS) spectroscopy is widely used in analytical chemistry, food scientists and industry.

UV/VIS spectroscopy is based on the absorption of light by a sample. Ultraviolet and visible lights range are from 10 nm to 400nm and 400nm to 780 nm, for UV spectrum is from 10 nm to 400nm and for UV-VIS is from 400nm to 780nm.

The principle of ultraviolet-visible absorption lies in the fact that molecule has a bond, the atoms in a bond have their atomic orbitals merged to form molecular orbitals which can be occupied by electrons of different energy levels. The electrons in a molecule can be of one of three types: namely σ (single bond), π (multiple-bond), or non-bonding (n- caused by lone pairs). σ-bond electrons have the lowest energy level and are the most stable electrons. These would require a lot of energy to be displaced to higher energy levels. As a result, these electrons generally absorb light in the lower wavelengths of the ultraviolet light and these transitions are rare. n-electrons or non-bonding electrons are generally electrons belonging to lone pairs of atoms. These are of higher energy levels than π-electrons and can be excited by ultraviolet and visible light as well. Most of the absorption in the ultraviolet-visible spectroscopy occurs due to π-electron transitions or n-electron transitions [41].

UV/Vis spectroscopy is widely applied in analytical chemistry for the quantitative determination of different samples. UV / Vis spectroscopy determines the concentration of the absorber in the solution, according to the Bouguer-lambert-Beer low or applying calibration methods.

The Bouguer-Lambert-Beer low forms the Mathematical basis of light absorption measurement on gases and solutions in the UV-VIS and IR region.

$$A = lg\left(\frac{I_0}{I}\right) = \lg\left(\frac{100}{T\%}\right) = \varepsilon * c * d\ , \qquad \text{Equation 2}$$

Where I_0 is intensity of the monochromatic light entering the sample and I is the intensity of this light of this emerged from the sample, c is concentration of light absorption substance and d is the pathlength of the sample in cm [42].

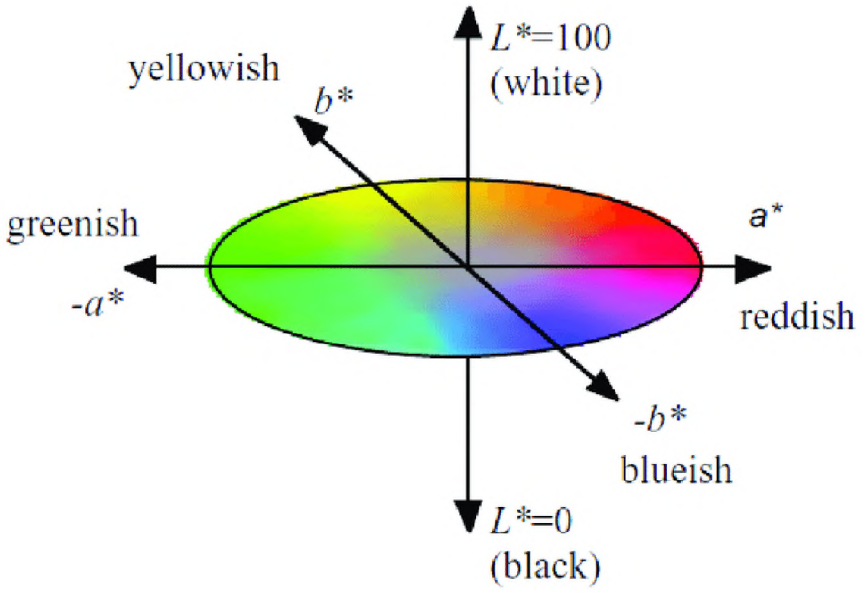
1.10 Colour measurement

Visual appearance of the food, especially colour has a strong influence on a consumer's opinion about the food quality. Colour is a phenomenon that depends on the observer and the conditions in which the colour is observed. Quality is not a single well-defined attribute but comprises many properties or characteristics. Appearance is one of the important factors the consumer uses to evaluate the quality of food products. Colour and appearance attract a consumer to a product and can help in impulse purchases [43,44].

The colour of an object can be described by several colour coordinate systems. Some of the most popular systems are RGB - red, green and blue, which is used in colour video monitors; Hunter Lab (CIE) L*a*b*, CIE XYZ, CIE L*u*v*, CIE Yxy, and CIE LCH.

a) The CIE L*a*b* (CIELAB) colour spaces.

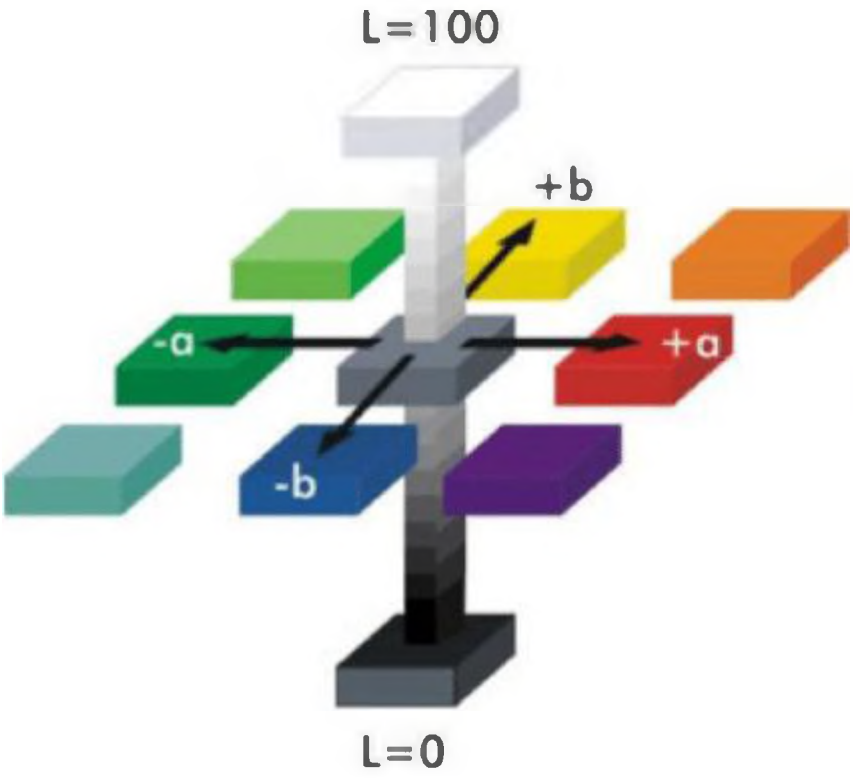
The CIELAB Colour measurement method was developed in 1976. The location of colour in the CIELAB colour spaces is determined by its colour coordinates: L* a* and b*. L* represents the difference between light (L*=100) and dark (L*=0). The component a* is difference between green (-) and red (+) and coordinate b* represent the between blue (-) and yellow (+), as it is shown picture 6. C* is chroma coordinate. Chroma (C*) is considered the quantitative attribute of colourfulness, which is used to study the degree of difference of a hue in comparison to a grey colour with the same lightness. The more the chroma values, the more is the colour intensity of samples perceived by humans [44,45].



Picture 6. Illustration – CIELAB colour space [66].

b) Hunter Lab colour space

The Hunter lab is widely applied in food industry. This is based on L, a and b measurements. The L value represents lightness and changes from 0 (black) to 100 (white). The value changes from (-) greenness to (+) redness. The b value represents to change from (-) blueness to (+) yellowness. The Hunter scale is derived from X,Y,Z values. The illustration of Hunter lab is shown on picture 7.



Picture 7. Hunter lab colour space [69].

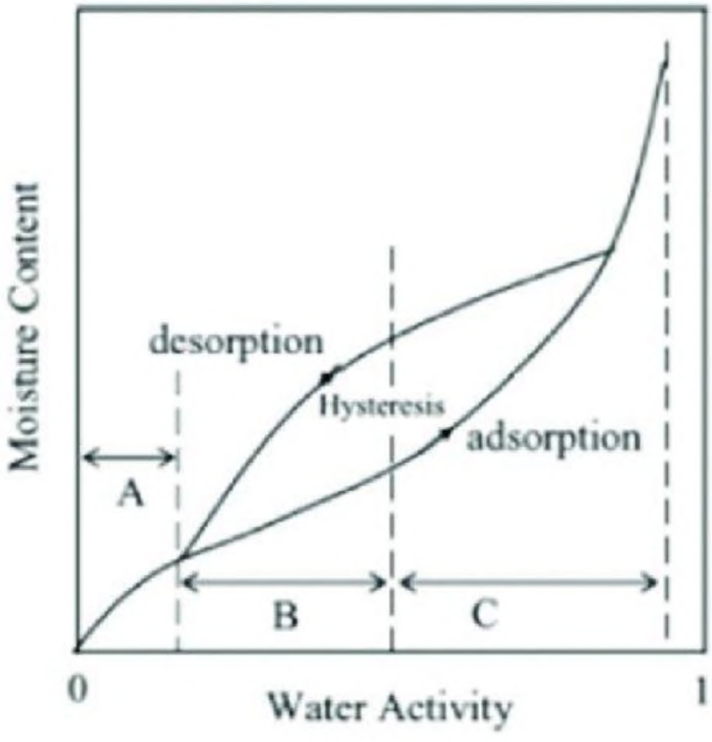
For colour measurement instrumental method or visual illustration are used. Applied instrumental methods are basically colorimeter or spectrophotometer. Colorimeters measure the colour of emit light and reflect or transmit external light. Spectrophotometers measure the spectral distribution of transmittance or reflectance of the sample [44,45].

1.11 Sorption Isotherm

The relationship between water activity and the equilibrium of the moisture content at constant temperature and pressure is called the moisture sorption isotherm. Each product has different interactions between the water and the solid components at different moisture contents, that's why the thermodynamic relationship is complex and different in all of them. The knowledge of sorption isotherms is especially important in food production for the design and optimization of drying equipment, design of packages, stability of product, shelf-life and for observing and calculating moisture changes that may going on during storage [48-50].

Sorption isotherms can be created from an adsorption process or a desorption process; the different moisture contents for the same aw is called moisture sorption hysteresis, which differences in the filling and emptying of pores and capillaries, swelling of polymeric materials and supersaturation of some solutes during desorption. Hysteresis defines the difference between these curves, which reflect desorption and adsorption process, as it is shown in picture 8. Three regions are show in giving picture [49-51].

Region A represents that enthalpy of vaporization is significantly more than the pure water. The bound water is monolayer water and H-bonded water. Monolayer water is absorbed by the hydrophilic and polar groups of food components, namely polysaccharides, proteins, etc.

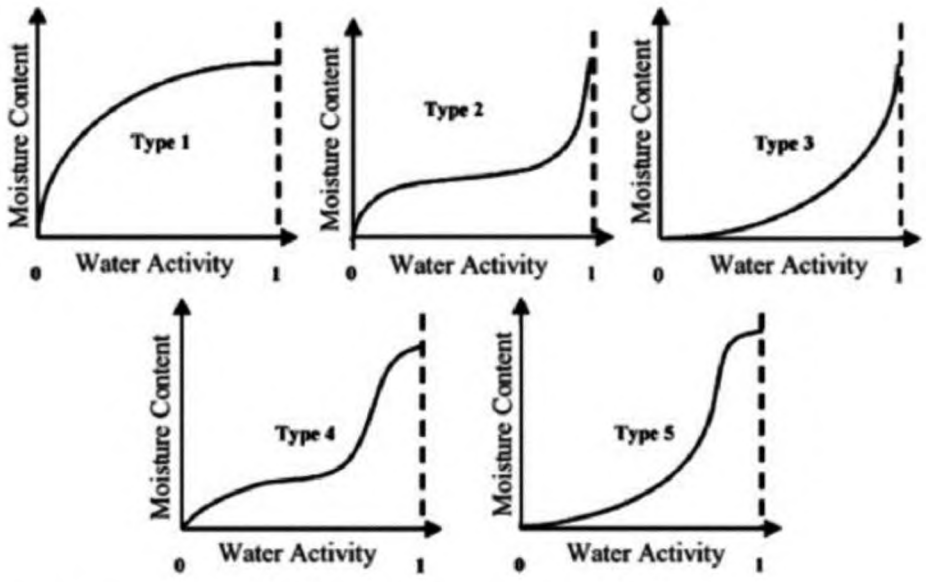


Picture 8. Sorption isotherm, showing the hysteresis.[70]

In region B, water molecules bind present in small capillaries. The vaporization enthalpy is a little more than the one of pure water. In region C, Water is roughly bounded with nutrients. the phenomenon of hysteresis is exhibited in foods nutrients, which have insignificantly capillaries or pores [49,53].

1.11.1 Classification and measurement of sorption isotherms

Sorption isotherm is classified five different types by Brunauer-Emmett-Teller, as it is shown in picture 8. first type represents a characteristic rise in water activity related to the increasing moisture content. Second type is sigmoidal sorption isotherm, that show the existence of multilayers at the internal surface of a material. Third type isotherm is known as the Flory-Huggins isotherm. it accounts for a solvent above the glass transition temperature. Forth type isotherm describes the adsorption of a hydrophilic solid until a site of hydration is reached maximum. Five type isotherm is known as the Brunauer-Emmett-Teller (BET) isotherm. It is related to the isotherms type 1 and 2 and it presents multilayer adsorption isotherm [49].



Picture 9. Types of isotherms described by Brunauer. [71]

Three different sorption isotherm measuring technique are used in food products. They are gravimetric, manometric or hygrometric. The gravimetric methods represent to measure of sample weight with balance. The manometric methods represent to measure of water vapour pressure, when it is equilibrium with a sample at given moisture content. The hygrometric

methods represent to measure the equilibrium relative humidity with a sample at a given moisture content. [49, 50]

1.11.2 Mathematical models of sorption isotherms

Mathematical models representative the interaction between the water activity of product and its moisture content. There are a lot of mathematical model, which describe sorption isotherm. In food science are basically used the Langmuir equation, the BET equation, the Oswin model, the Smith model, the Halsey model, the Henderson model, the Iglesias-Chirife equation, the GAB model, and the Peleg model etc. In Food product the most common used mathematical models are Brunauer-Emmett-Teller (BET) equation and Guggenheim-Anderson-de Boer (GAB) model [49,51].

Brunauer-Emmett-Teller (BET) equation

The BET equation was first developed by Brunauer, Emmett and Teller. It is most frequently used model in food system, which representatives the multilayer sorption isotherm. The BET is combination of the type II and III. It estimates the number of bound waters in specific polar sites of dehydrated food systems.

This is the BET equation formulation:

$$M_w = \frac{M_0 C a_w}{(1-a_w)(1+(C-1)a_w)}, \qquad \text{Equation 3.}$$

where M_w is equilibrium moisture content (kg water/kg dry matter), M_0 is the monolayer moisture content and C is energy constant related to the net heat of sorption.

The BET equation representatives of isothermal sorption multilayers and it has been used in gas adsorption and porous steam in surfaces and solids. For food preservation and good storage stability, the moisture conditions must be determine. The BET equation is considered one of the best model for measuring the optimum moisture conditions [49, 51].

Guggenheim-Anderson-de Boer (GAB) model

Guggenheim, Anderson, de Boer are scientist, who independently developed the equation, Accordingly the term GAB models comes to their names. The GAB model has more advantages than other mathematical models because it presents sophisticated version of BET and Langmuir.

Langmuir developed physical adsorption model based on unimolecular layers with identical and independent sorption sites and formulated as given in equation:

$$a_w\left(\frac{1}{M_w}-\frac{1}{M_0}\right)=\frac{1}{CMM_0}, \qquad \text{Equation 4}$$

where M_w is equilibrium moisture content (kg water/kg dry matter), M_0 is the monolayer sorbent (kg water/kg dry matter), and C is a constant.

The GAB model describes both the classical mono-molecular layer and the multidimensional adsorption, because the theoretical background of the model is the BET and Langmuir theories of physical adsorption. The GAB model is formulated as given in equation:

$$M_w=\frac{M_0CKa_w}{(1-Ka_w)((1-Ka_w+CKa_w}, \qquad \text{Equation 5}$$

Where M_0 is the monolayer moisture content, K and C are adsorption constant.

The GAB model is very suitable for analysing of fruit and vegetable, because it describes the sorption behaviour in a wide range of a_w (0-0.9) [49, 51].

2. Materials and methods.

2.1 List of used instruments and material

1) UV-VIS spectrometer (UV-2600 Shimadzu, Japan)
2) FTIR spectroscope Nicolet iS50 FT-IR (Fisher Scientific s. r. o., Czechia)
3) Ultra Scan VIS (HunterLab, Virginia, USA)
4) Ultrasound bath Sonorex RK31 (Bandelin Electronic, Germany)
5) Automatic pipet (Sartorius, Göttingen, Germany)
6) Laboratory scale KERN 440 – 35N (Kern & Sohn, German)
7) Centrifuge Universal 320 (Andreas Hettich GmbH & Co. KG, Germany)
8) Vibratory grinder BVM-2 (Brio Hranice, Czechia)
9) pH-meter CG 842 (Schott, Mainz, Germany)
10) Thermostat BSK ET618 (Lovibond®, UK)
11) Forced-air oven HS 62-A (Chirana, Czechia)
12) Plastic desiccator
13) Desiccator
14) Laboratory utensils

2.2 List of used chemical reagents:

chemicals for analytical analysis

1) Potassium chloride (KCl) – (analytical grade, Penta s. r. o., Czechia)
2) Sodium acetate trihydrate (CH_3CH_2Na . $3H_2O$) – (analytical grade, Penta s. r. o., Czechia)
3) Aluminum chloride trihydrate ($AlCl_3$. $3H_2O$) (99 %, purity Sigma Aldrich, Germany)
4) Hydrogen chloride (HCl) – (35 %, analytical grade, Penta s. r. o., Czechia)
5) Quercetin hydrate - 2-(3,4-Dihydroxyphenyl)-3,5,7-trihydroxy-4H-chromen-4-one hydrate – (≥ 95 % Sigma Aldrich, Germany)
6) Sodium hydroxide (NaOH) – (analytical grade, Penta s. r. o., Czechia)
7) Sodium Nitrite ($NaNO_2$) – (analytical grade, Penta s. r. o., Czechia)
8) Catechin hydrate - (2*R*,3*S*)-2-(3,4-Dihydroxyphenyl)-3,4-dihydro-2*H*-chromene-3,5,7-triol – (99.9 %, Sigma Aldrich, Germany)
9) MeOH – (Chromasolve grade, 99.9%, Honeywell, NC, USA)
10) Folin-Ciocalteau solution - (Sigma Aldrich, Germany)
11) Gallic acid – 3,4,5-Trihydroxybenzoic acid – (99.0 %, Sigma Aldrich)
12) DPPH- 2,2-diphenyl-1-picrylhydrazyl – (Sigma Aldrich, Germany)
13) Trolox- 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid – (>98% Sigma Aldrich, Germany)
14) Formic acid (HOOOH) – (99.9 % Sigma Aldrich, Germany)

Chemicals for determination moisture sorption

1) Potassium acetate (CH_3COOK) – (analytical grade, Penta s.r.o., Czechia)
2) Magnesium chloride ($MgCl_2$) – (analytical grade, Penta s.r.o., Czechia)
3) Potassium carbonate (K_2CO_3) – (analytical grade, Penta s.r.o., Czechia)
4) Magnesium nitrate ($Mg(NO_3)_3$ – (analytical grade, Penta s.r.o., Czechia)
5) Cobalt chloride ($CoCl_2$) – (analytical grade, Penta s.r.o., Czechia)
6) Sodium chloride (NaCl) – (analytical grade, Penta s.r.o., Czechia)
7) Potassium chloride (KCl) – (analytical grade, Penta s.r.o., Czechia)

2.3 Sample preparation

Sample, namely black chokeberry, was sent from University of Krakow (Poland) and it was already treated by ultrasound-assisted osmotic dehydration at different time. Erythritol and xylitol were used for osmo-dehydration with ultrasound applied at 0, 5, 15, 30 and 45 min. Osmo-dehydration with saccharose but without ultrasound assistance was used as a control. Each berry was cut in four parts and dried in a forced-air oven for 22-23 hours at 45°C. Dry samples were stored in desiccator above freshly-dried silica gel.

2.3.1 Method of extraction

In a glass tube, 0.5 g powder of chokeberry was added, which was treated by ultrasound-assisted osmotic dehydration, 10ml 90% methanol solution and 30 µl pure formic acid. The Extraction was provided in ultrasound bath for 30 minutes. After this process, given mixture was removed to plastic tube and centrifugation was made at 3000 rpm for 5 minutes. After centrifugation, the supernatant was removed and stored at -20^0C until analyzed transparent solution without precipitate.

2.4 Spectrophotometric analysis

The total content of phenolic compounds, the total content of Anthocyanins, the total content flavonoids by two different methods, and the total antioxidant activity (DPPH assay) were determined. Each sample was extracted in duplicate and each extract was applied in duplicate in spectrophotometric assays (N = 4).

2.4.1 Preparation of standard calibration solution and optimization time

Time of optimization

For DPPH and Folin-Ciocalteu methods were optimized time if what time it needs to complete the reaction and achieve stable absorption. For both assays, the optimal time was set to 30 min as it is shown on appendix 1. and 2.

Calibration solutions of quercetin

A stock solution of quercetin at a concentration of 500 µg/ml was prepared by weighing 0.05 g of quercetin into a 100 ml volumetric flask, which was made up to the mark with methanol. Calibration solutions in the concentration range from 50 to 500 mM were prepared from the stock solution by dilution.

Calibration solutions of catechin

A stock solution of catechin at a concentration of 500 µg/ml was prepared by weighing 0.05 g of catechin into a 100 ml volumetric flask, which was made up to the mark with methanol. Calibration solutions in the concentration range from 50 to 500 mM were prepared from the stock solution by dilution.

Calibration solutions of gallic acid

A stock solution of gallic acid at a concentration of 500 µg/ml was prepared by weighing 0.05 g of gallic acid into a 100 ml volumetric flask, which was made up to the mark with methanol. Calibration solutions in the concentration range from 25 to 250 mg/l were prepared from the stock solution by dilution.

Calibration solutions of Trolox

A stock solution of Trolox at a concentration of 500 µg/ml was prepared by weighing 0.05 g of Trolox into a 100 ml volumetric flask, which was made up to the mark with methanol. Calibration solutions in the concentration range from 5 to 75 mg/l were prepared from the stock solution by dilution.

Preparation of buffer solution

a) pH 1.0 buffer (potassium chloride, 0.025 M.)
   0.186 g KCl was weighted into a beaker and was added distilled water to ca 98 ml. the solution was removed to volumetric flask and was measured the pH, and adjusted pH to 1.0 with HCl.
b) pH 4.5 buffer (sodium acetate 0.4 M).
   5.443 g $CH_3CO_2Na.3H_2O$ was weighted into a beaker and was added distilled water to ca 98 ml. The solution was removed to volumetric flask and was measured the pH, and adjust pH to 4.5 with HCl [54].

2.4.2 Determination of total anthocyanins content (pH differ methods)

Tube 1. 0.5ml of extracts were added to 5.0 ml of buffer (HCl/KCl, pH 1.0) and the mixture was shaken.

Tube 2. 0.5ml of extracts were added to 5.0 ml of buffer (acetate, pH 4.5) and the mixture was shaken.

After 15 minutes of incubation (dark), absorbance at 510 and 700 nm was measure. Anthocyanin content was calculated with molar absorption coefficient of cyaning-3-glucoside (ε=26900l/mol.cm).

Calculate :

1. $A=(A_{510nm}-A_{700nm})_{pH1.0}-(A_{510nm}-A_{700nm})_{pH4.5}$ Equation 6.
2. $C=A\ \varepsilon\ M$ Equation 7.

Where C - Concentration

A- Absorbance

ε- Molar absorption coefficient

M- Molar weight

Each sample was measured 4 times. [54]

2.4.3 Total flavonoid content

Procedure 1:

An aliquant of $AlCl_3$ solution (1 ml, 2%, w/v) was added to 2 ml of the test solution (standard or sample) and subsequently 1 ml of water, HCl, CH_3COONa (each at concentration of 1 M) was added. The concentrations of standard solutions of flavonoids were 100 μM. The mixture was vigorously shaken and then after 10 min of incubation at room temperature, subjected to spectral analysis in the range of 300-600 nm. The amount of $AlCl_3$ solution was substituted by the same amount of water in blank. For quantitative analysis, quercetin was chosen as the reference compound as it is widely found in plants and the measurements were done at 425 nm. Calibration curve for quercetin using total flavonoid content assay is shown on appendix 3 [56].

Procedure 2:

Of the test solution (standard of sample), 1ml was mixed with 0.6 ml of $NaNO_2$ (5%, w/v) and after 5 min, 1 ml of $AlCl_3$ (2%, w/v) was added. Flavonoid standard solutions of 100 μM were used. A sample was mixed, and 6 min later was neutralized with 1 ml of 1 M NaOH solution.

The mixture was left for 10 min at room temperature and then subjected to spectral analysis in the range of 300-600 nm against the blank, where $AlCl_3$ solution was substituted by water. Catechin (in the 50-500μM concentration range) was the standard of choice for the expression of results at 510 nm. Calibration curve for catechin using total flavonoid content assay is shown on appendix 4 [56].

2.4.4 Determination of total phenolic content (Folin-Ciocalteau method)

Mix. 1.0 ml of extract + 1.0 ml of methanol + 5ml distilled water + 0.5 ml of folin-Ciocalteau solution (already prepared). After 5 min. of standing, add 1.0 ml of 5% (w/w) of Na_2CO_3. Let keep it in dark for XXX min. (30 min.) and measured at 765 nm. Blank contains 1.0 ml of extraction solution (i.e. 90% methanol with formic acid) instead of extract. Express as Gallic acid equivalent. Calibration curve for gallic acid using total phenolic content assay is shown on appendix 5 [56].

2.4.5 Determination of antioxidant capacity

5.0 g DPPH was added 200 ml pure methanol. 0.5 ml extract was mixed 5.0 ml of DPPH solution and was kept in dark for 30 min. and it was measured absorbance at 517 nm. Methanol was used instead of sample extract, results expressed as Trolox equivalent. Calibration curve for Trolox using total antioxidant capacity assay is shown on appendix 6. Blank, DPPH solution, was measured before each day of measurement.

$$Inhibition(\%)=(A_{DPPH}-A_{sample})/A_{DPPH}*100 \qquad \text{Equation 8.}$$

2.4.6 Statistical analysis

The effects of sugar and ultrasound time treatment on antioxidant properties where studied using two-factor analysis of variance (Anova). Duncan's test was used for multiply comparison. All the statistical analysis was performed on the probability level of $p=0.05$ using Statistica (version 12, StatSoft Inc., USA)

2.5 Static gravimetric determination of sorption isotherm

Prior to experiment, all the Aronia berries samples were kept in desiccator above dried silica gel at laboratory temperature in order to obtain dry samples. Desiccator was covered to avoid the sunlight.

Each sample was placed on aluminium pan (approx. 1.0 g). The pans with samples were placed in desiccators with the slurries of inorganic salts (potassium acetate, magnesium chloride, potassium carbonate, magnesium nitrate, cobalt chloride, sodium chloride and potassium chloride), which corresponded to various water activity levels (0.227; 0,328; 0,432; 0,529; 0,649; 0,753; 0,843). A few crystals of thymol was placed in desiccators with water activity higher than 0.60 to avoid fungi deterioration of samples. All the desiccators were kept at 25 °C until equilibrium was reached (at least 3 weeks), then moisture content was determined at 103 °C (3 h). The results were expressed as g of water per g of dry solid according to equation:

$$m = \frac{[w_2 - w_2]}{w_1} = \frac{g\ water}{g\ dry\ solids}, \qquad \text{Equation 9}$$

Where W_1 = initial weight

W_2 = final weight

Exponential and polynomial models were drawn on excel shift. F1(differences factor) method was used to measure the percent of error between two curves.

F1=(Mexp-Mprep)/Mexp. Equation 10.

Error of exponential and polynomial models were compared each other, then was decided which model should be used for obtaining moisture isotherm, plot moisture.

3. Results and discussion

3.1 The effect of osmo-dehydration on total anthocyanin content

The figures 1 and 2 show how many milligrams of total anthocyanins are contained in 1 gram of dry sample. The increased amount of anthocyanins were observed in all ultrasound-assisted osmotic dehydrated chokeberry in compared to control (saccharose) except the sample treated by xylitol at 5 min as shown Figures 1 and 2. The highest amount of anthocyanins were observed in xylitol and erythritol at 30 min. The decrease number of anthocyanins were observed in ultrasound-assisted osmotic dehydrated chokeberry in both of sugar at 45 min in compared to the treated in xylitol and erythritol at 30. This decrease of number anthocyanins happened may be due to the higher migration of compounds during osmotic dehydration. [29]

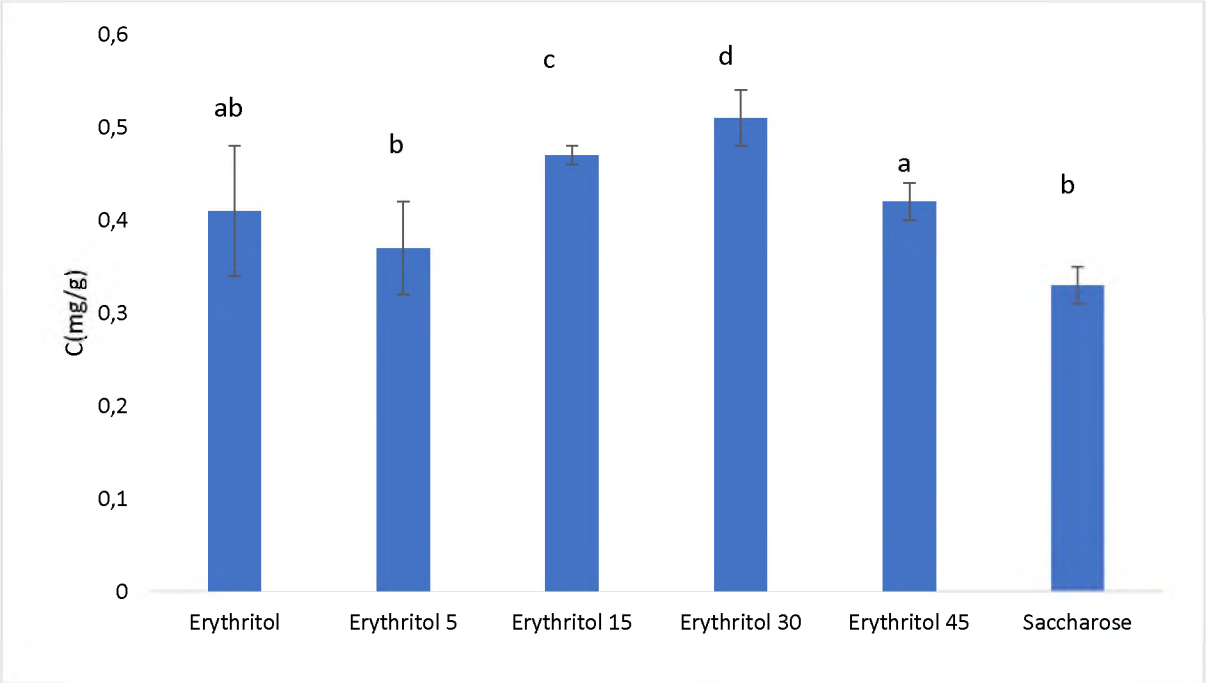


Figure 1. Impact of ultrasound assisted osmotic dehydration using erythritol on total anthocyanin compound in Aronia powder dried for 22-23 hours at 45°C. *The numbers expressed the ultrasound treatment time, i.e. erythrito5, erythritol15 mean 5 and 15 min, etc. An average mean with standard deviation (N=4). Different letters indicate statistical differences {p<0.05}*

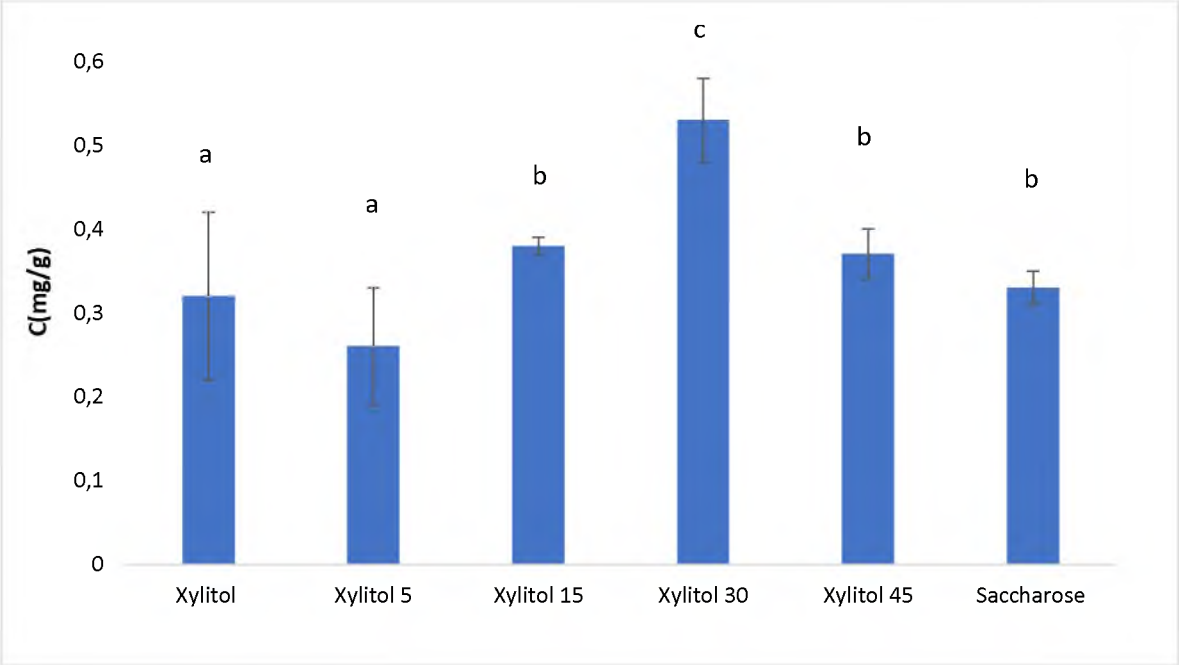


Figure 2. Impact of ultrasound assisted osmotic dehydration using xylitol on total anthocyanin compound in Aronia powder dried for 22-23 hours at 45°C. The numbers expressed the ultrasound treatment time, i.e. xylitol 5, xylitol 15 mean 5 and 15 min, etc. An average mean with standard deviation (N=4). Different letters indicate statistical differences {p<0.05}

According to ANOVA procedure, osmo-dehydration with erythritol gave the higher anthocyanin contents in comparison with xylitol ($p < 0.001$).The effect of ultra-sound treatment time was found to be significant ($p < 0.001$) showing that 30 min resulted in higher anthocyanin values.

3.2 The effect of osmo-dehydration on total flavonoid content

Procedure 1.

The below figures 3. and 4. show how many milligrams of total flavonoid compounds are contained in 1 gram of dry sample. The increased number of flavonoids were observed in all ultrasound-assisted osmotic dehydrated chokeberry in compared to control except the treated in xylitol at 5 and in erythritol at 5 min as shown Figures 3 and 4. The highest amount of flavonoids was observed in xylitol and erythritol at 30 min. The decrease number of flavonoids were observed in ultrasound-assisted osmotic dehydrated chokeberry in both of sugar at 45 min in compared to the treated in xylitol and erythritol at 30.

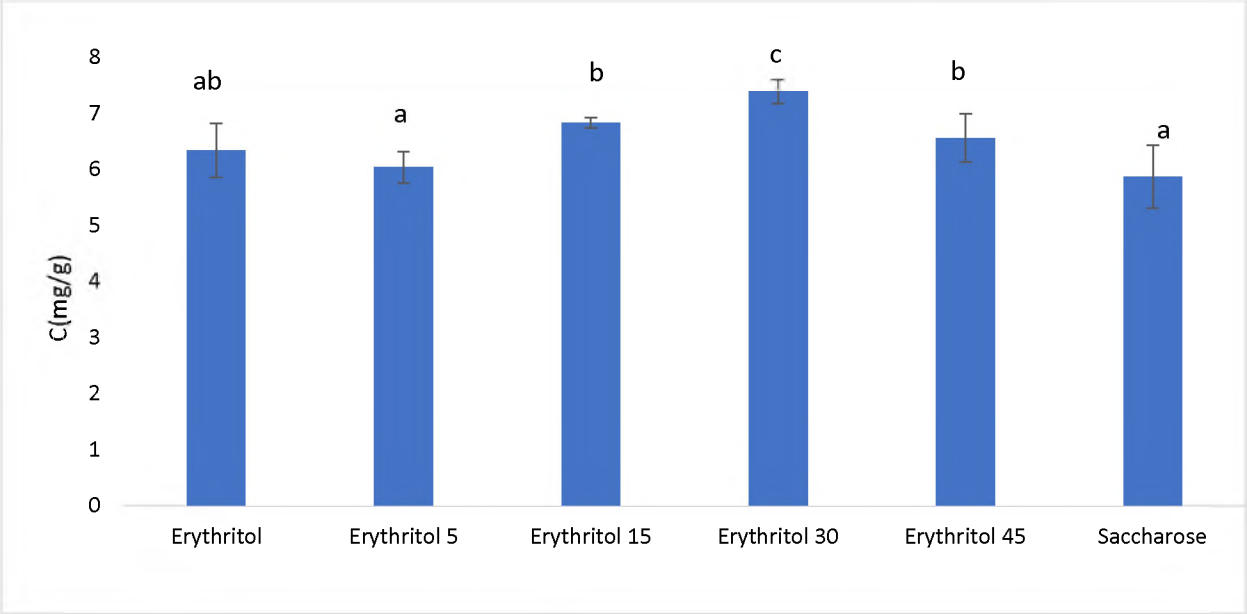


Figure 3. Impact of ultrasound assisted osmotic dehydration using erythritol on total flavonoid compound in Aronia powder dried for 22-23 hours at 45°C. The numbers expressed the ultrasound treatment time, i.e. erythrito5, erythritol15 mean 5 and 15 min, etc. An average mean with standard deviation (N=4). Different letters indicate statistical differences {p<0.05}. (procedure 1).

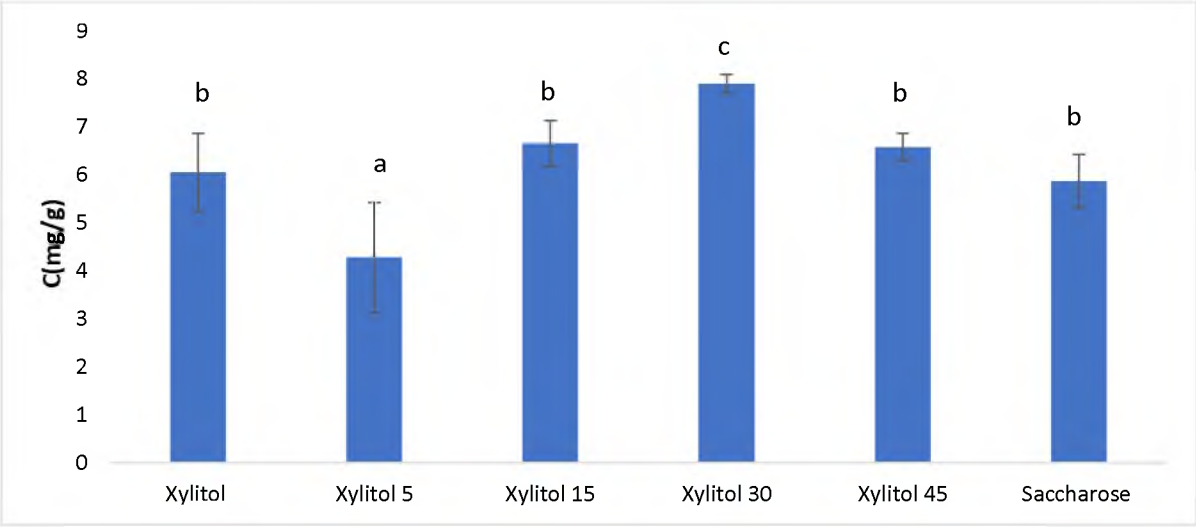


Figure 4. Impact of ultrasound assisted osmotic dehydration using xylitol on total flavonoid compound in Aronia powder dried for 22-23 hours at 45°C. The numbers expressed the ultrasound treatment time, i.e. xylitol 5, xylitol 15 mean 5 and 15 min, etc. An average mean with standard deviation (N=4). Different letters indicate statistical differences {p<0.05}. (procedure 1)

Anova procedure showed that there is no effect of sugar used for osmo-dehydration on the flavonoid content (procedure 1), however the effect of ultra-sound treatment time has significant effect on total flavonoid content (procedure 1); i.e. $p < 0.001$. Osmo-dehydration coupled with 30 min ultra-sound treatment resulted in the highest total flavonoid content.

Procedure 2.

The below figures 5. and 6. show if how many milligrams of total flavonoid compounds are contained in 1 gram of dry sample and effects of ultrasonic assisted osmo-dehydration on flavonoids according to procedure 2. The increased amount of flavonoids were observed in all ultrasound-assisted osmotic dehydrated chokeberry in compared to control except the treated erythritol at 5 min as shown Figure 5. The highest amount of flavonoids was observed in erythritol at 30 min. The decrease number of anthocyanins were observed in ultrasound-assisted osmotic dehydrated chokeberry in both of sugar at 45 min in compared to the treated in erythritol at 30.

The decrease amount of flavonoids were observed in all ultrasound-assisted osmotic dehydrated the treated xylitol of chokeberry in compared to control as shown figure 6. The highest amount of flavonoids was observed in xylitol at 0 min. The lowest number of flavonoids were observed in xylitol at 45 min. The data in the figure 5. are radically different from experiments of other flavonoids, which may have been caused by an error during the test.

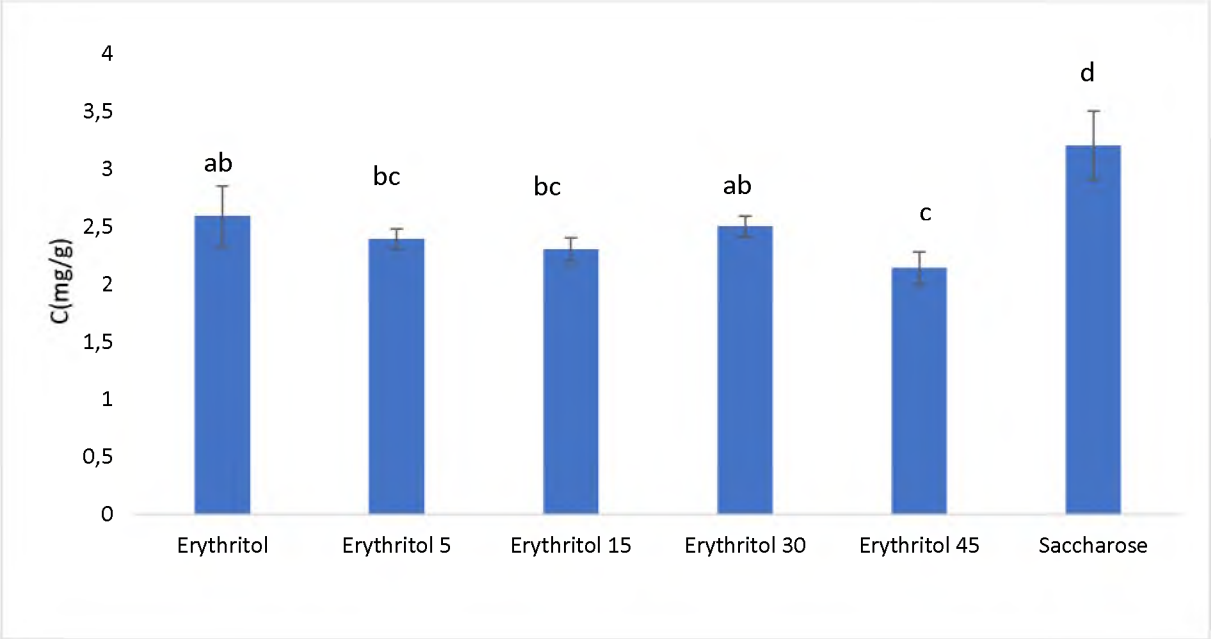


Figure 5. Impact of ultrasound assisted osmotic dehydration using erythritol on total flavonoid compound in Aronia powder dried for 22-23 hours at 45°C. The numbers expressed the ultrasound treatment time, i.e. erythrito5, erythritol15 mean 5 and 15 min, etc. An average mean with standard deviation (N=4). Different letters indicate statistical differences {p<0.05} (procedure 2.)

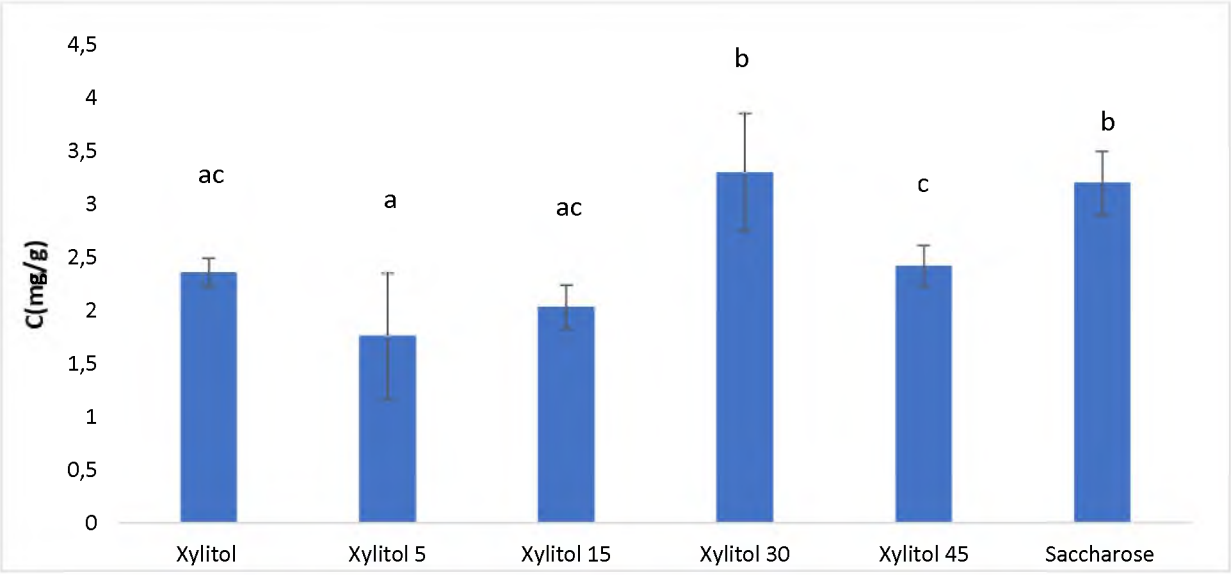


Figure 6. Impact of ultrasound assisted osmotic dehydration using xylitol on total flavonoid compound in Aronia powder dried for 22-23 hours at 45°C. The numbers expressed the ultrasound treatment time, i.e. xylitol 5, xylitol 15 mean 5 and 15 min, etc. An average mean with standard deviation (N=4). Different letters indicate statistical differences {p<0.05} (procedure 2)

Regarding total flavonoids content (procedure 2), the of sugar was not confirmed ($p>0.05$) but ultra-sound treatment time played a key role in releasing flavonoids in this assay ($p < 0.01$). Similarly, the highest content was found for osmo-dehydrated samples assisted with 30 min of ultra-sound treatment.

Procedure 1 can only be used to determine flavanol content and from flavone group subgroup - luteolin. Flavanols exhibited the maximum absorbance at 415-425 nm and flavone at 405-420. Procedure 2. is much less selectivity for determination flavonoids in comparison with the procedure 1. In case of procedure 2, non-flavonoid compound can be measured [56]. That's why, number of total flavonoids was significant difference between the two experiments.

3.3 The effect of osmo-dehydration on total phenolic content

The below figures 7. and 8. show if how many milligrams of total phenolic compounds are contained in 1 gram of dry sample and effects of ultrasonic assisted osmo-dehydration on flavonoids according to procedure 2. The increased number of total phenolic were observed in all ultrasound-assisted osmotic dehydrated chokeberry in compared to control except the treated in xylitol and erythritol at 5 min as shown Figures 7 and 8. The highest amount of Phenolics was observed in xylitol and erythritol at 30 min. The decrease number of total phenolic were observed in ultrasound-assisted osmotic dehydrated chokeberry in both of sugar at 45 min in compared to the treated in xylitol and erythritol at 30.

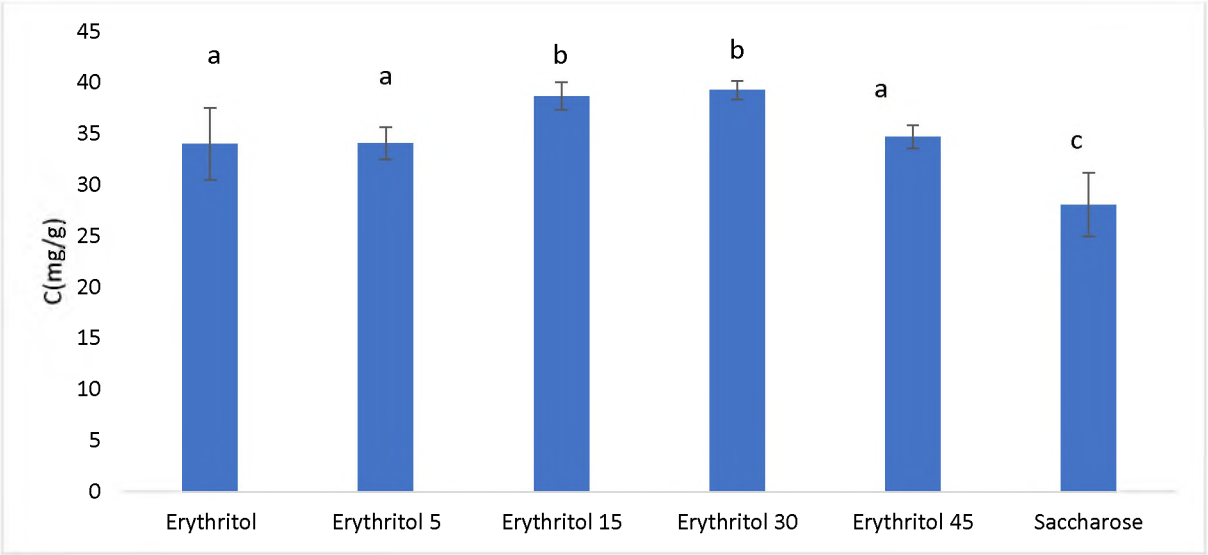


Figure 7. Impact of ultrasound assisted osmotic dehydration using erythritol on total phenolic compound in Aronia powder dried for 22-23 hours at 45°C. The numbers expressed the

ultrasound treatment time, i.e. erythrito5, erythritol15 mean 5 and 15 min, etc. An average mean with standard deviation (N=4). Different letters indicate statistical differences {p<0.05}

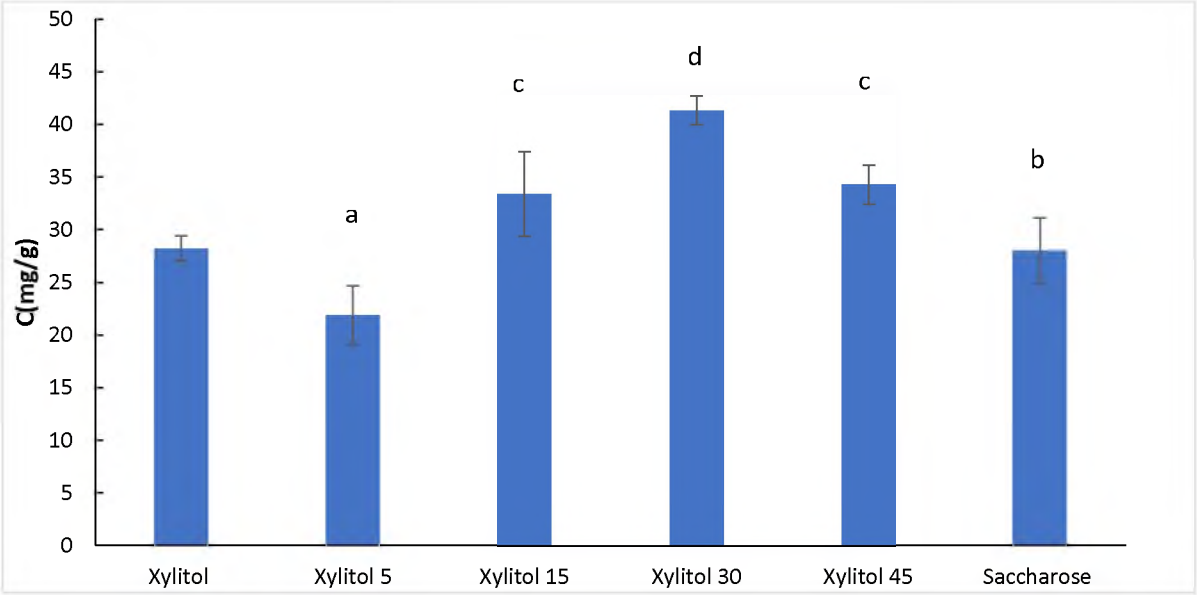


Figure 8. Impact of ultrasound assisted osmotic dehydration using xylitol on total phenolic compound in Aronia powder dried for 22-23 hours at 45°C. The numbers expressed the ultrasound treatment time, i.e. xylitol 5, xylitol 15 mean 5 and 15 min, etc. An average mean with standard deviation (N=4). Different letters indicate statistical differences {p<0.05}

The both effects of sugar and ultra-sound treatment time used for osmo-dehydration were found to be significant ($p<0.001$). The highest total phenolic content was for samples treated by erythritol and for those treated by 30 min of ultra-sound during osmo-dehydration.

3.4 The effect of osmo-dehydration on total antioxidant capacity

The below figures 9. and 10. show if how many milligrams of antioxidants are contained in 1 gram of dry sample and effects of ultrasonic assisted osmo-dehydration on flavonoids according to procedure 1The antioxidant capacity of ultrasound-assisted osmotic dehydrated chokeberry was determined by DPPH assay method. The increased antioxidant activity was observed in all ultrasound-assisted osmotic dehydrated chokeberry in compared to control except the treated in xylitol and erythritol at 15 min. and xylitol 5 min. as shown Figure 9 and 10. The highest antioxidant activity was observed in erythritol at 30 min and in each case of xylitol, antioxidant activity is approximately equal. The decrease antioxidant activity was observed in ultrasound-

assisted osmotic dehydrated chokeberry in erythritol at 45 min in compared to the treated in erythritol at 30 and 15 min.

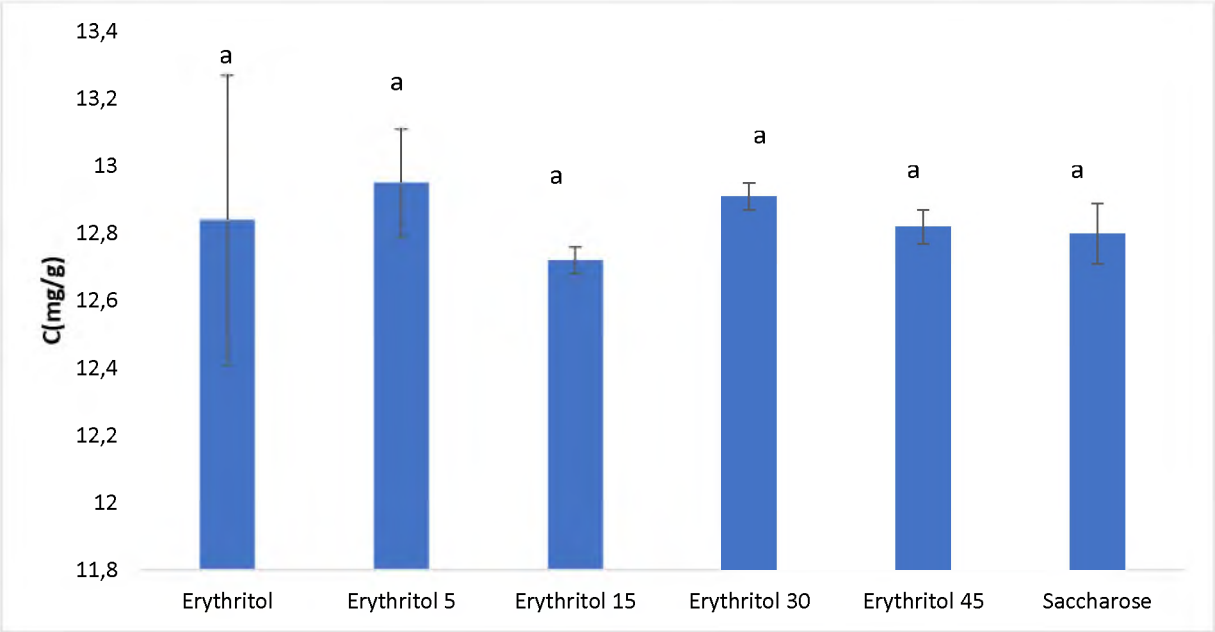


Figure 9. Impact of ultrasound assisted osmotic dehydration using erythritol on total antioxidant capacity in Aronia powder dried for 22-23 hours at 45°C. The numbers expressed the ultrasound treatment time, i.e. erythrito5, erythritol15 mean 5 and 15 min, etc. An average mean with standard deviation (N=4). Different letters indicate statistical differences {p<0.05}

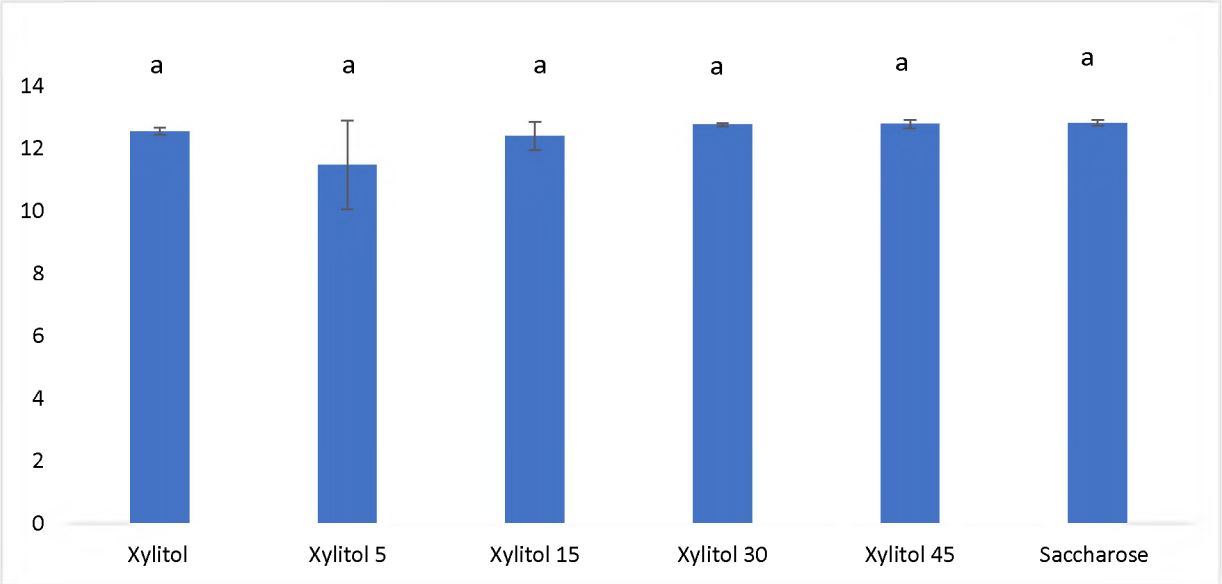


Figure 10. Impact of ultrasound assisted osmotic dehydration using xylitol on total antioxidant capacity in Aronia powder dried for 22-23 hours at 45°C. The numbers expressed the ultrasound treatment time, i.e. xylitol 5, xylitol 15 mean 5 and 15 min, etc. An average mean with standard deviation (N=4). Different letters indicate statistical differences {p<0.05}

Regarding DPPH assay, the effect of sugar used for osmo-dehydration process was significant ($p<0.01$), i.e. higher values were determined for samples treated by erythritol in comparison with xylitol. Surprisingly, the effect of ultra-sound treatment time was found to be negligible ($p > 0.05$).

3.5 Fourier transform infrared analyses method

The used conditions for infrared spectroscopy were as follows. The background was scanned automatically after 30 minutes. If impurities appeared in the spectrum, the background was scanned manually. A total of 64 scans were performed for both the background scan and the sample scan. The resulting spectra were stored as the dependence of transmittance [%] on wavelength [cm-1].

Fourier transform infrared analyses method are used to determine functional group of sample. The infrared (IR) spectroscopy absorbs band of the chemical compounds, which are in powder of ultrasound-assisted osmotic dehydrated chokeberry. The wave numbers were showed insignificantly variations on signals of compounds in treated of ultrasound-assisted osmotic dehydrated chokeberry. This means that in each sample is same compounds. This means that in each sample is same compounds. This is shown in each treatment Fig. 10 and fig. 11. Thus, on figure 10 and 11 show that structure of biological compound is not changed, but percentage of transmittance of identical carbonyl are different in ultrasound assisted osmotic dehydrated chokeberry powder at different time intervals.

FTIR spectra of our samples determine several substantial peaks. Wavelength 1475 and 1350 cm^{-1} corresponds the C-H deformation vibrations and absorption bands 3400 and 2800 cm^{-1} show peak of C-H. Absorption bands at 2950–2800 cm^{-1} show to vibrations of methyl (CH3) groups. The chokeberry powder is shown by the broad absorbance peak of O-H valance vibration between 3400 and 3200 cm^{-1}. Wavelength 1050+1350-1410 cm^{-1} corresponds the primary alcohol. Absorption bands 1200+1310-1410 cm^{-1} show phenolic group. Wavelength 810-890 + 680-730 corresponds trisubstituted aromatic ring. Also, aliphatic secondary amines are subsisting near 1500 cm^{-1} and weak, disulphides are represented 400-500 cm^{-1}. Wavelength ˜1100 cm-1 corresponds C-O banding in ether. This investigation shows presence of phenolic compound, ether and amines.

Variation of transmittance especially relieved on wavelength 1050 cm-1 and 3290 cm-1, which corresponds C-O and O-H bands. The biggest transmittance change has been revealed on carbonyl compound (C-O). Compared to the standard (saccharose 0 min.), the absorbance of all samples was reduced and increase transmittance, except powder, which was treated in erythritol at 15 minute and in xylitol at 45 minute. The increase in transmittance percentage, especially, in ester may cause the formation of colour and release of volatiles during osmotic dehydration.

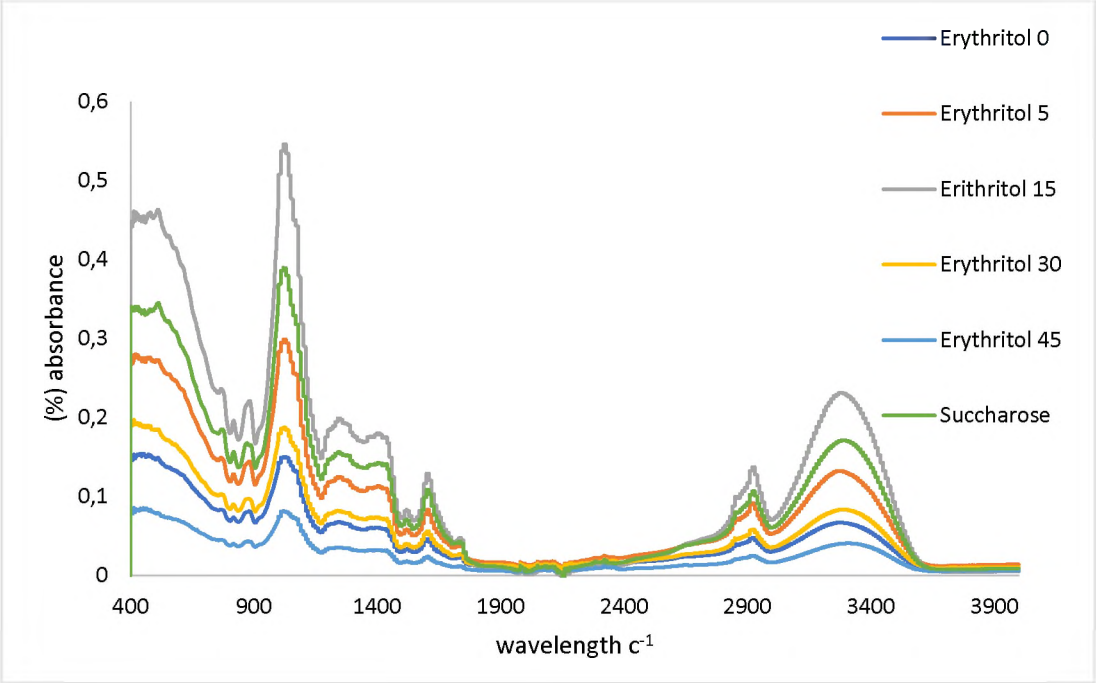


Figure 11. Influence of ultrasound assisted osmotic dehydration on functional group of bioactive compounds in Aronia by FTIR.

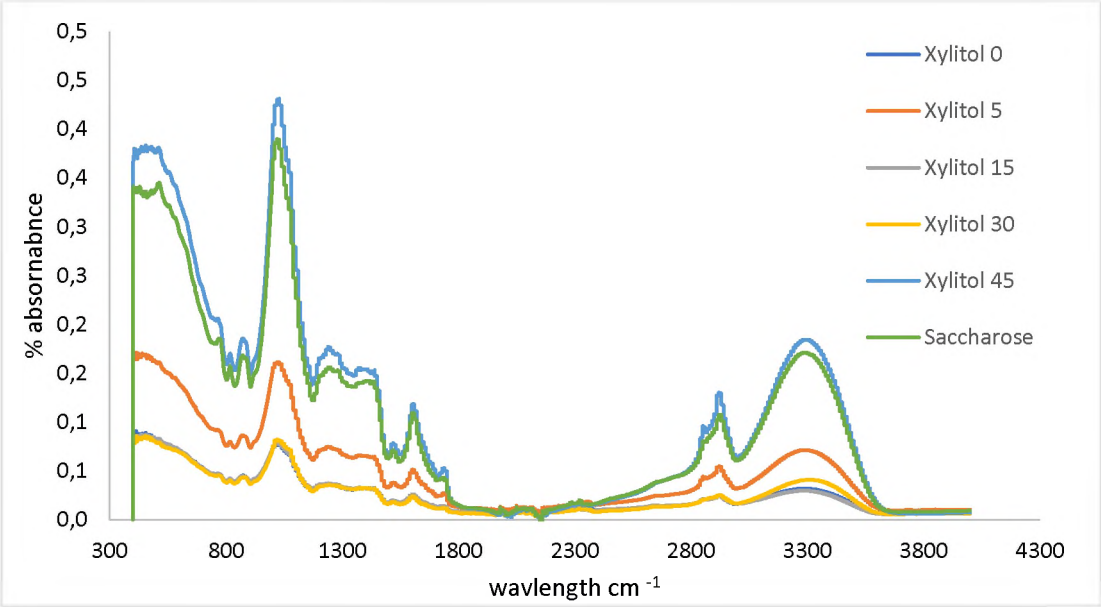


Figure 12. Influence of ultrasound assisted osmotic dehydration on functional group of bioactive compounds in Aronia by FTIR.

3.6 Colour measurement

The colour of the food product is one of the important properties in business, because colour create effectiveness and attractiveness of food, which affect the acceptability of consumers. The change in colour of ultrasound-assisted osmotic dehydrated chokeberry after drying are shown in Table 1.

Hunter L,a,b colour space is a 3-dimensional rectangular colour space base on Opponent-Colours Theory.

- L (lightness) axis-0 is black, 100 is white, and 50 is middle grey
- a (red-green) axis – positive values are red, negative values are green and 0 is neutral
- (blue-yellow) axis – positive values are yellow, negative values are blue and 0 is neutral

Table 1. Influence of ultrasound assisted osmotic dehydration on colour attributes of dried chokeberry

Sample	L	A	B
Saccharose 0	18.63	9.93	7.17
Xylitol 0	17	6.7	3.36
ylitol 5	17.61	4.94	2.36
xylitol 15	16.53	7.07	4.01
xylitol 30	15.56	8.15	5.53
xylitol 45	16.3	6.53	4.42
erythritol 0	16.37	8.92	6.25
erythritol 5	16.92	7.06	4.08
erythritol 15	15.99	7.98	4.75
erythritol 30	14.75	7.11	4.42
erythritol 45	16.24	8.07	5.58

According to table 1, the lightness of the dried chokeberry without ultrasonic osmotic process and with ultrasound osmo-dehydrated chokeberry are approximately same in each sample. However, compared to the standard, namely saccharose, all compounds have a slight colour change where the sample become more black, but compared to xylitol 0 and erythritol 0 are not trend.

The redness of the dried chokeberry without ultrasonic osmotic process was increased as compare to ultrasound osmo-dehydrated chokeberry. This may happen just because inhibition of free radicals by antioxidant. It is also worth noting that redness is more with ultrasonic

osmotic dehydrated for 30 than for 45 minutes. The change of the redness and antioxidant activity is consistent each other in chokeberry powder.

The decrease in yellowness was also observed in all osmo-dehydrated chokeberry and in my view, turn in to the browning. Browning may be caused the reduction of red colour and its conversion in dark colour by Millard reaction.

It should be noted that the change in the colour of each sample was insignificant and in giving every case was not trendline, but every case was difference from standard. It means that ultrasound assist osmatic dehydration has effect on changing of colour in food product.

3.7 Moisture Isotherm plot.

Most food isotherms have a sigmoidal shaped curve, in which the curves concave upwards. The moisture sorption isotherms of osmotically dehydrated chokeberries, which were treated by ultrasonically in erythritol, xylitol and saccharose at different time, are found to be sigmoid type it is shown in figure 13. and 14. Exponential model is used for assessment of isotherm curve. In table 2. is shown percentage of errors according to Exponential and polynomial model. Their errors were compared each other, then it was decided that we would use exponential model for assessment moisture sorption isotherm of our samples.

Table 2. percentage errors of exponential and polynomial trendline.

	Exponential model		Polynomial model	
		Errors %		Errors %
Saccharose	$y = 0.0222e^{3.0922x}$	8.6696	$y = 0.6603x^2 - 0.2946x + 0.0829$	5.5128
Erythritol	$y = 0.0191e^{3.3814x}$	6.0430	$y = 0.6459x^2 - 0.2464x + 0.0661$	6.9366
Erythritol 5	$y = 0.018e^{3.397x}$	6.0134	$y = 0.8264x^2 - 0.4353x + 0.103$	6.0817
Erythritol15	$y = 0.017e^{3.59x}$	4.2051	$y = 0.7932x^2 - 0.3641x + 0.0844$	5.1489
Erythritol 30	$y = 0.0235e^{3.0183x}$	9.0249	$y = 0.8464x^2 - 0.4847x + 0.125$	9.7119
Erythritol 45	$y = 0.0301e^{2.6281x}$	11.6914	$y = 0.9859x^2 - 0.6629x + 0.1749$	15.2352
Xylitol	$y = 0.0251e^{2.9577x}$	8.2009	$y = 0.7841x^2 - 0.4173x + 0.1134$	4.8383
Xylitol 5	$y = 0.0226e^{3.0579x}$	9.3969	$y = 0.5544x^2 - 0.1938x + 0.0633$	9.5097
Xylitol 15	$y = 0.0282e^{2.6795x}$	10.4910	$y = 0.678x^2 - 0.3613x + 0.1093$	6.1799
Xylitol 30	$y = 0.0253e^{2.8925x}$	8.1346	$y = 0.7043x^2 - 0.3576x + 0.1021$	7.0417
Xylitol 45	$y = 0.0303e^{2.5477x}$	7.0015	$y = 0.5059x^2 - 0.2081x + 0.0811$	7.7806

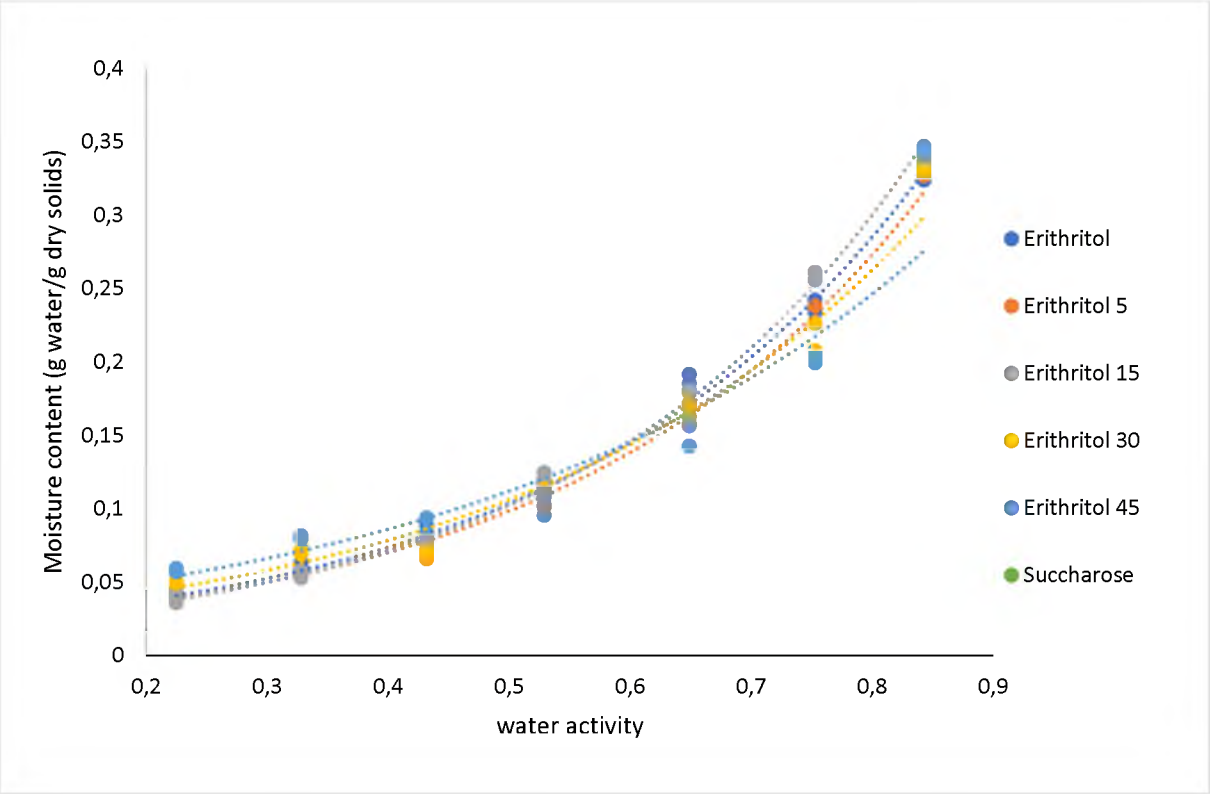


Figure 13. The moisture sorption isotherms of osmotically dehydrated chokeberries.

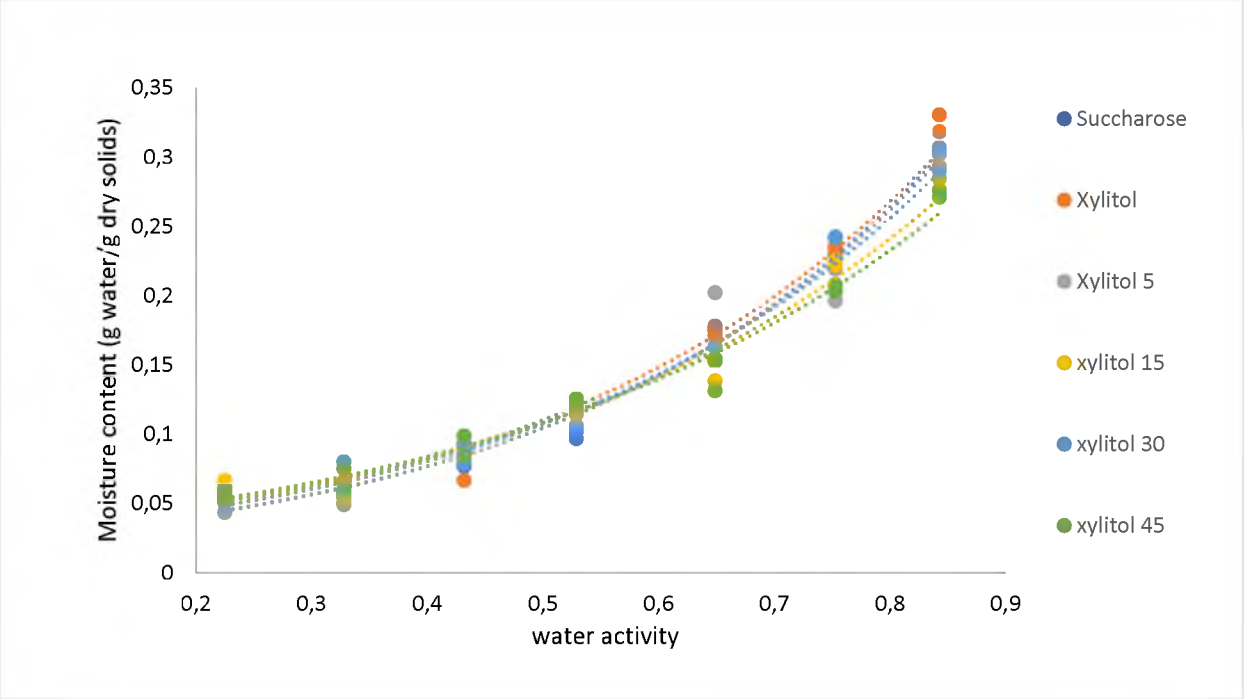


Figure:14. The moisture sorption isotherms of osmotically dehydrated chokeberries.

IN CONCLUSION:

The investigate of ultrasound-assisted osmotic dehydration on black chokeberry in erythritol, xylitol and saccharose at 0. 5, 15, 30 and 45 min shows that the application of ultrasound during osmotic dehydration increases the water loss and solid gain in the fruit. The experiments revealed that the maximum amount of biogenic compound was maintained, when sample, namely black chokeberry, was treated of ultrasonic assisted osmotic dehydration at 30 minute in both of sugar (erythritol and xylitol). the capacity of antioxidants were investigated by DPPH method, but Duncan's test shows that probability level was more than 0.05, what means that the test result is not accurate.

FTIR method shows that ultrasound assisted osmotic dehydration influence on functional group is very low, but In every sample their absorbance reduced or transmittance increased to compare standard (saccharose 0) except one case, when Aronia fruit was treated in erythritol at 15 minute. The colour change was measured through Hunter Lab. In all samples were different colour, but there was not trendline, which determined if which drying conditions were most useful. By moisture sorption isotherm method investigated to have sigmoidal curve, such as it is most food isotherms.

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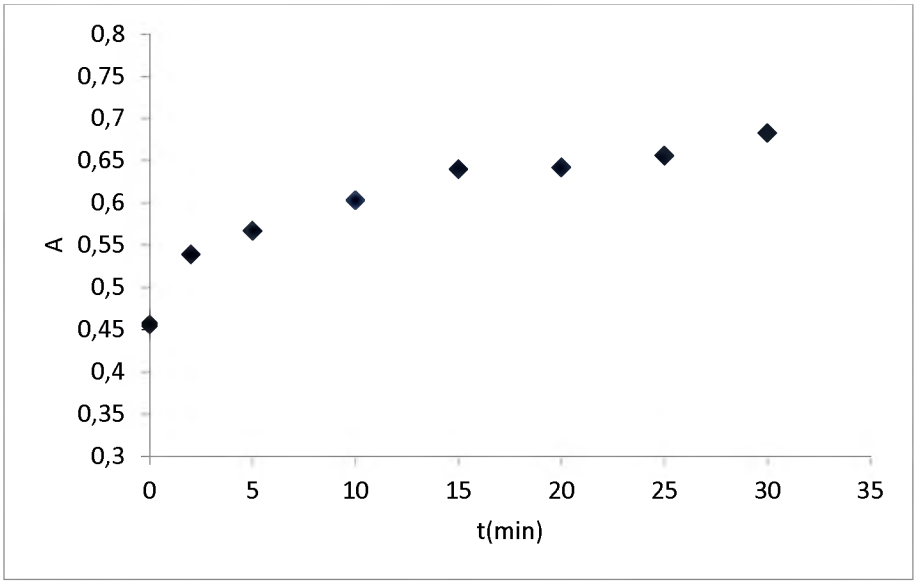
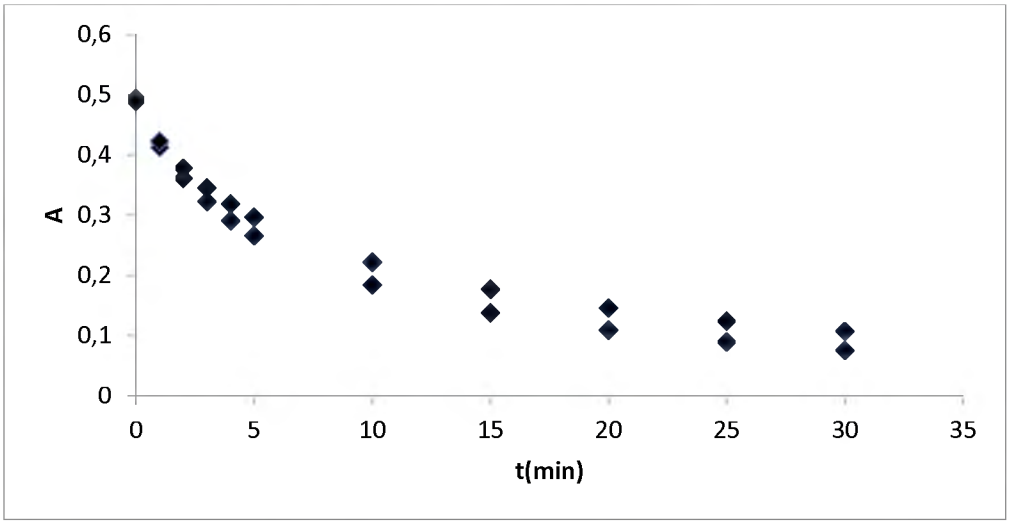
List of appendices

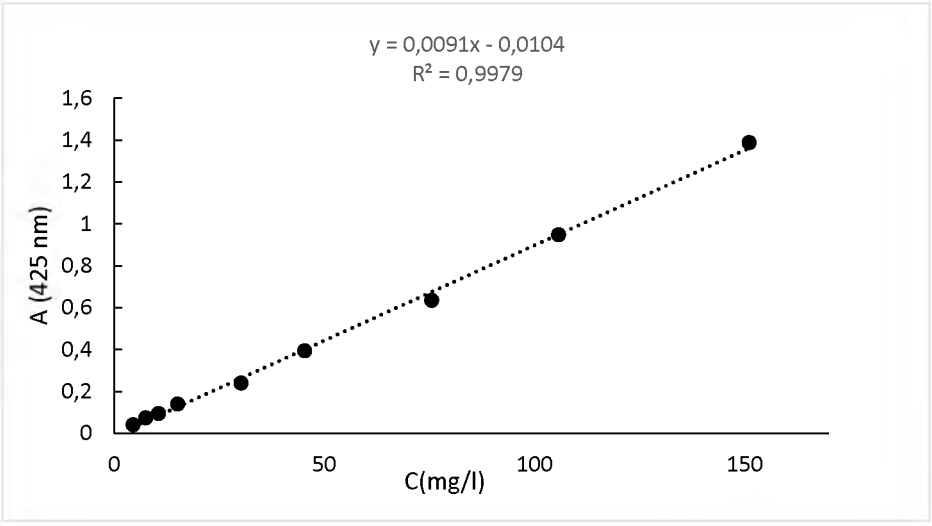
Appendix 1. Optimization time for DPPH



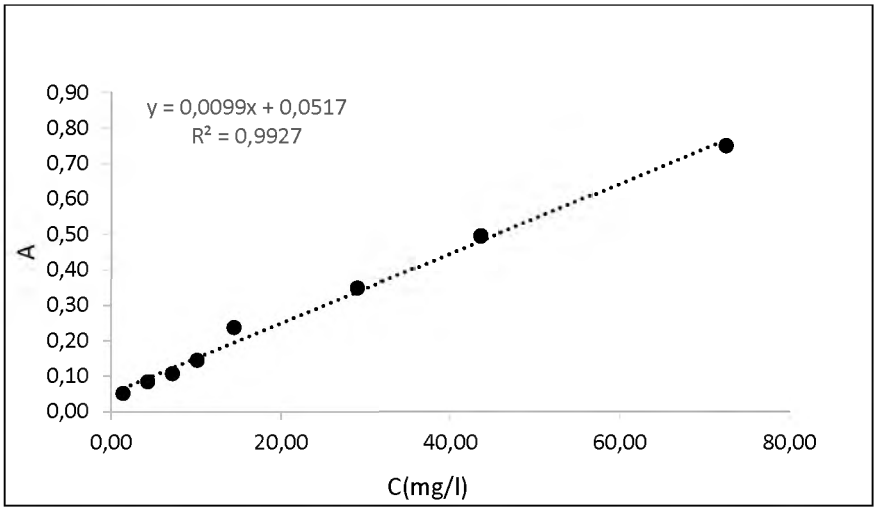
Appendix 2. Optimization time for folin-ciolacuteau



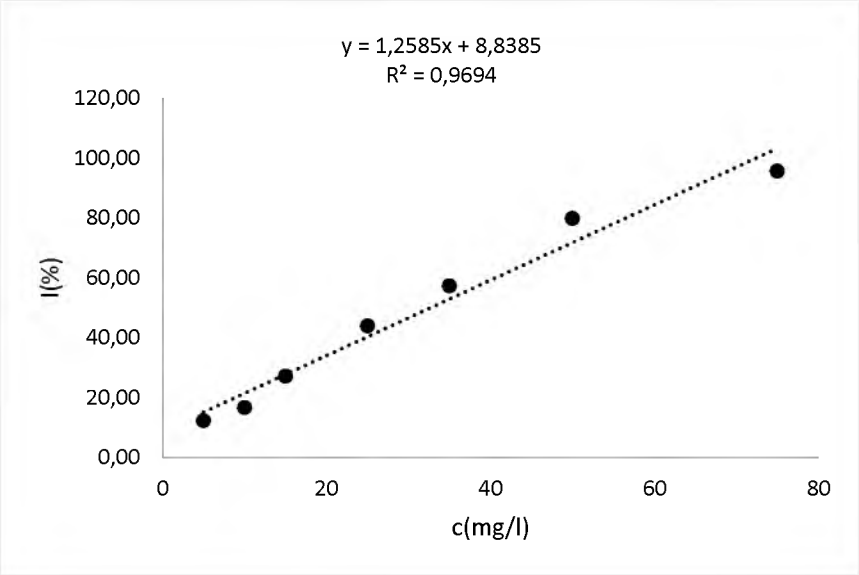
Appendix 3. Calibration curve for quercetin using total flavonoid content assay (procedure 1).



Appendix 4. Calibration curve for catechin using total flavonoid content assay (procedure 2).



Appendix5 . Calibration curve for Trolox using total antioxidant capacity assay.



Appendix 6. Calibration curve for gallic acid using total phenolic content assay.