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OPTIMIZATION OF EXTRACTION CONDITIONS FOR THE EXTRACTION OF BIOACTIVE COMPOUNDS FROM ARONIA POMACE

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LIST OF ABBREVIATIONS

AAE – ascorbic acid equivalent

CGa– cyanidin-3- galactoside

CG – cyanidin-3- glucoside

CAr – cyanidin-3-arabinoside

CGE – cyanidin-3- glycoside equivalent

DM – dry mass

Et – ethanol

FM – fresh mass

FRAP – ferric reducing antioxidant capacity

GAE – gallic acid equivalent

GL – glycerol

HPLC – high performance liquid chromatography

MIN – minute

SFE – supercritical fluid extraction

SPM – secondary plant metabolites

TA – total anthocyanin concentration

TPC – total phenolic content

TPTZ – 2,4,6-Tri (2-pyridyl)-s-triazine

UAE – ultrasound-assisted extraction

USET – ultrasound exposure time

1. INTRODUCTION

Chokeberry (*Aronia melanocarpa*) is one of the berry types which found in North America, Eastern Europe and Asia with temperate rainforests and the best-known species within the genus Aronia (Lee et al., 2014). Nowadays, there is an increasing demand of using plant extracts for a reason of ingredients in food, beverages, pharmaceutical, nutraceutical and cosmetic industries. The by-product of such fruit and vegetable processing comprises of seed, skin, pomace which are not commonly consumed but possess valuable bioactive compounds particularly phytochemicals and secondary metabolites entrapped in tissue. In many case the concentration of bioactive compounds have been reported more in these by-products than the edible part of the fruit. The health benefit of these compounds includes anti-allergenic, anti-inflammatory, anti-microbial, anti-oxidant, antithrombotic, cardio protective, and vasodilatory effects. The valuable bioactive compounds, which should be utilized for development of functional or enriched food, are lost in want of economically viable extraction techniques. The by-product of chokeberry fruit is chokeberry pomace; which is presently known for a huge number of beneficial properties, is one of the fruits with the highest content in compounds as total phenolic compounds and total anthocyanins are some of special compounds, which gives superior antioxidant properties. The phenolic compounds in Aronia are much greater than other well-known fruits such as grapefruit, papaya, avocado, guava. Recently optimization is a highly concern on the extraction of bioactive compounds from several plants (Cheng & Hong, 2018; Ilghami et al., 2015). The efficiency of extraction is affected by various elements, such as solvent type and polarity, ratio of solid-liquid, time, pH and such must be optimized to minimize the cost of process The recovery of bioactive compounds involves extraction as a key step, which is achieved through conventional extraction methods such as, Soxhlet, supercritical extraction due to having their easy operation and simple equipment required have been applied. However, it offers low yield, use of extra solvent, the extraction time is high and high energy consumption. Currently there is an increasing demand to use alternative extraction methods like, ultrasound assisted extraction, microwave assisted extraction, supercritical fluids extraction methods. These methods are environmentally friendly and guarantee with both high quality and yield extract. UAE has advantages such as less time and energy requirement, extraction at low temperature and retention of the quality of the extract. Therefore, ultrasound assisted extraction method is the most crucial allows valuable bioactive compounds with high yield to be produced. With regard to this, A complete research focused on

all aspects of optimization of UAE of bioactive compounds from Aronia pomace is currently not available. Hence, the objective of this research is to optimize the extraction conditions assisted with ultrasound technique for maximum extraction of bioactive compounds from Aronia pomace.

1.1. Goal of the Study

The goal of this study is to optimize ultrasound-assisted extraction conditions through investigate the efficiency of different extraction conditions on Aronia bioactive compounds such as temperature, solvent extraction, and ultrasound with focusing on color parameters, anthocyanin content, polyphenol concentration, and antioxidant capacity. This topic is a part of a PhD research that I joined. By measuring color parameters and antioxidant capacity, the study aims to determine the quality and potential health benefits of the extracted compounds. The study also aims to understand the underlying mechanisms of extraction and how they are affected by different extraction conditions. This knowledge can be used to optimize the extraction process for different plant materials and bioactive compounds, leading to improved yields and quality of the extract. The efficiency of extraction solvents such as ethanol, glycerol, water, and citric acid can vary depending on several factors such as the type of bioactive compounds being extracted, the extraction method we apply, and the solvent properties. Ethanol is a widely used solvent for the extraction of a wide range of bioactive compounds. It has good solubility for a broad range of polar and nonpolar compounds, including polyphenols. It also relatively inexpensive and readily available, making it a popular choice for extraction. The other solvent glycerol which is sometimes considered a "green" solvent for the extraction of bioactive compounds from plant materials because of more environmentally friendly and sustainable approach to extractions, making it a "green" chemistry option. It is also non-toxic, viscous liquid, stable, and has a low volatility, which makes it a safer alternative to some other solvents. It can help to improve the solubility and stability of some bioactive compounds and may also have some antioxidant and antimicrobial properties. Water also a universal solvent that is able to dissolve a wide range of polar compounds such as phenolic acids. It is safe, inexpensive, and readily available. Besides, the organic solvent citric acid is a weak organic acid that act as a chelating agent, which improve the solubility and stability of bioactive compounds during extraction such as anthocyanins. Citric acid is considered also a “green” chemical against hydrochloric acid or formic acid for instance. Therefore, the efficiency of extraction solvents could vary depending on several factors and this study will help to develop more efficient and effective extraction conditions with optimal yield.

2. LITERATURE REVIEW

2.1 Aronia (Chokeberry)

Chokeberries (*Aronia melanocarpa* berries) are members of the Rosaceae family and were introduced to Europe at the beginning of the twentieth century. The most common aronia species are Aronia arbutifolia (red chokeberry), Aronia melanocarpa (black chokeberry), and their hybrid Aronia prunifolia (purple chokeberry). Differentiation allows through color and pubescence (Kulling & Rawel, 2008). Chokeberries are primarily composed of polyphenolic substances, including proanthocyanidins, anthocyanins, flavonoids, and phenolic acids (Kokotkiewicz et al., 2010). The content and qualities of aronia berries are also affected by cultivar. They differ in terms of juice extraction efficiency, total polyphenols, anthocyanins, and proanthocyanidin content, overall antioxidative capacity, and fruit weight and diameter (OCHMIAN et al., 2012). According to studies (Kokotkiewicz et al., 2010), A. melanocarpa is becoming more well-known as a result of its possible associations with health advantages like antioxidant, antibacterial, antiviral, antimutagenic, anticancer, cardioprotective, hepatoprotective, gastroprotective, antidiabetic, and anti-inflammatory effects. The following figure (1) shows a typical black chokeberry fruit.

Figure 1. Black chokeberry fruit (Internet 1)

2.1.1 Aronia pomace

Berry juice production yields roughly 70-80 percent target product and 20-30 percent by-product. Berry pomace, which remains after juice extraction, includes waste stems, wooden pieces, and leaf fragments in addition to the skins and seeds. Berry pomace typically has 5-6% (w/w) moisture after drying (Reque et al., 2014). It retains a significant amount of the phenolic compounds which found in the berry skins and seeds (White et al., 2010). In comparison to the raw material and other chokeberry fractions like juice, concentrate, jam, and syrup, chokeberry pomace has the

highest amounts of total phenols and anthocyanins, according to (CAPANOGLU, 2013); especially anthocyanins remain in the pomace to a great extent because they are less water soluble than other polyphenols and because they are linked to cell wall material in the fresh berry. Berry seeds are a component of berry pomace that is coated by a hard coat, making the compounds inside the seeds unavailable for extraction or consumption without further processing. Pressing residues of berry seeds have been demonstrated to have significant levels of antioxidant capacity (Helbig et al., 2008). Several research have looked at the antioxidant content of berries and pressing residues, with a focus on the distribution of different antioxidant components and their behavior throughout juice processing. Chokeberries contain 66% proanthocyanins (mainly tannins), 25% anthocyanins, 7.5% chlorogenic and neochlorogenic acid, and 1.3% flavonols. Epicatechin, o-diphenolics, cyanidin, and quercetin derivatives are the most potent antioxidants (Wawer et al., 2006). The antioxidant capacity of berries determines the scavenging potential of bioactive substances, which contributes to their ability to promote health (Wijngaard et al., 2009).

2.2 Bioactive Compounds

2.2.1 Polyphenols

Polyphenols are a type of secondary metabolite found in plant-based foods. There is a growing demand for the creation of green extraction methods that reduce extraction time, consumption of harmful solvents, and energy consumption. Ultrasound assisted extraction is one such method, with significant positive effects on phenolics extraction from different fruit and vegetal sources while using short time and energy (D'Alessandro et al., 2014). In this case, as shown from tables 17 to 19, the total phenolic content was extracted using ultrasound assisted. The extraction efficiency can be greatly affected by operating parameters such as temperature and solvent composition solid to solvent ratio (Cacace & Mazza, 2003), extraction time, and particle size (Bucić-Kojić et al., 2007). Polyphenols are flavonoids (including anthocyanins, flavanols, flavanones, flavones, flavonols, and isoflavonoids), tannins, stilbenes, and phenolic acids, as well as their derivatives (Zapolska-Downar et al., 2012). Although polyphenols are considered non-nutritive substances, their capacity to neutralize free radicals allows them to play a role in disease prevention and health improvement (Williamson, 2017). pH, temperature, interactions with other food components, availability to light and oxygen, and the presence and abundance of metal ions are all factors that can impact the stability of phenolic compounds (Cao et al., 2021). Polyphenols

are more stable in acidic than alkaline settings; also, keeping or processing foods at high temperatures reduces polyphenol concentration (Zeng et al., 2017). Some researchers reported raspberry pomace's phenolic content was characterized and TPC determined as 238.36 mg/100 g dry mass using HPLC. Anthocyanins were the most abundant phenolic components, accounting for about 83 percent of the total phenolic content (TPC), followed by ellagic acid and flavanols (Mildner-Szkudlarz et al., 2016). TPC concentrations in blackberry pomace extract from wild fruits varied from 48.28-50.16 mg GAE/g dm and from cultivated fruits ranged from 26.30-35.40 mg GAE/g dm, indicating that phenolic component concentrations are higher in wild blackberries (Jazić et al., 2019).

2.2.2 Antioxidants

Antioxidants protect food from oxidation processes such as browning. It has a significant positive impact on human physiology. Natural antioxidant extracts are used as food preservatives. Natural antioxidant extracts are derived primarily from berries such as black chokeberry, blueberry, and others. Biochemical processes alter food properties and can produce toxic compounds. Food compounds oxidize spontaneously when exposed to air, and antioxidants protect against this oxidation. Antioxidants are compounds that inhibit fermentation activity and prevent oxidative processes, which are responsible for reducing changes in the taste, color, and nutritional value of food. Natural antioxidants in food include ascorbic acid, carotenoids, phenolic compounds, and tocopherols (Santos-Sánchez et al., 2017). Chokeberry juice had a content of 1578.79 mg/100 g of DW, while pomace had a concentration of 8191.58 mg/100 g. Pomace had the highest antioxidant activity, as determined by TEAC, followed by fruit and juice (Oszmiański & Wojdylo, 2005)**.** (Jara-Palacios et al., 2019) stated that pomaces had a different qualitative and quantitative antioxidant activity and anthocyanin profile which depends on the type of berry. The antioxidant capacities of the extracts are strongly related to the solvent being used, owing to the various antioxidant potential of compounds with different polarities (Moure et al., 2001). The study showed black mulberry extracts obtained with ethanol/water/acetic acid (50/49.5/0.5, v/v/v) had the highest antioxidant capacity with values of 1490.61 mmol Fe2+/kg DW (Boeing et al., 2014). The method FRAP used to determine the antioxidant capacity based on the reaction of each method's specific reagent with electron donating or hydrogen radical (H) producing antioxidant compounds; the reducing capacity interpreted as the antioxidant capacity.

2.2.3 Anthocyanins

Anthocyanins come in a wide range of colors, from orange and red to purple and blue. Red berries, including blackberries, blueberries, raspberries, strawberries, elderberries, blackcurrants, red currants, and cranberries, are thought to be high in phenolic compounds, particularly anthocyanins (Bowen-Forbes et al., 2010). Cyanidin is a type of anthocyanin pigment that gives fruits their red, purple, and blue colors. The most well-known cyanidin compounds are Cyanidin-3-glucoside, Cyanidin-3-galactoside, and Cyanidin-3-arabinoside. These compounds chromatographic profile were studied by (Mayer-Miebach et al., 2012), (Veberic et al., 2015), (Wilkes et al., 2014), and (CAPANOGLU, 2013). Anthocyanin recovery from solid waste (pomace) is an appealing, sustainable, and cost-effective source of these chemicals, which might be included into foods to improve their biological value and also employed as natural colorants (Nile & Park, 2014). (Roda-Serrat et al., 2021) the total anthocyanin concentration in the pomace was 62.8 5.5 mg/g DW (Dry Weight), as determined by a thorough extraction with acidified methanol.In addition, anthocyanins have been linked to good health effects such as protection against oxidative stress and coronary heart disease, antibacterial, anti-inflammatory, and anticarcinogenic properties, obesity and diabetes control, and vision improvement (Santos-Buelga et al., 2014). (Jara-Palacios et al., 2019) showed blueberries had the anthocyanin content (1188 mg/100g.

2.3 Application of Bioactive Compounds

2.3.1 Bioactive compounds in food industry

Bioactive compounds have several roles in the food industry. They promote the quality characteristics of food for instance, anthocyanins, can impact the color of food. Anthocyanins are responsible for the red, purple, and blue colors in many fruits and vegetables, and are often used as natural colorants in food products. bioactive compounds in the food industry can improve the quality characteristics of food, making it more visually appealing, flavorful, and nutritious to consumers. Because consumers want functional foods and healthy products, the food industry has become increasingly mindful of the significance of using natural additives that bring a healthy added value to the final product. The study of pomaces such as byproducts from fruits is valuable for industrial reasons due to the increased need for nutraceutical, antioxidant chemicals, and natural colorants (Jara-Palacios et al., 2015). Chokeberry pomace extracts explored for use as an ingredient in chitosan-based packaging sheets. Due to anthocyanins' strong durability in acidic

environments, adding the extract to film formulation resulted in decreased solubility and a potential application as a pH-indicating film (Halász & Csóka, 2018). Berry pomace extracts can also be used to make natural antioxidant colorants (Jara-Palacios et al., 2019). The by-product, pomace could be reused in food industries as ingredient or natural additives due to bioactive compounds (Dueñas & García-Estévez, 2020).

2.3.2 Bioactive compounds in nutrition and health

Bioactive compounds play an important role in nutrition and health by providing a range of physiological benefits beyond basic nutrition. Incorporating foods rich in bioactive compounds into our diets can help promote overall health and well-being. They help to nutrient absorption that can improve nutrient absorption in the body, helping to maximize the nutritional value of the foods we eat. Polyphenols linked to a reduced risk of chronic diseases such as cancer, cardiovascular disease, and neurodegenerative diseases. Phenols and anthocyanins play important roles in nutrition and health by providing antioxidant and anti-inflammatory properties that can help protect against chronic diseases and promote overall well-being. Chokeberries contain several compounds, including carbohydrates, organic acids, amino acids, minerals, vitamins, fragrance compounds, and polyphenols (Kulling & Rawel, 2008). The chemical composition of chokeberry fruit is influenced by a variety of factors, including climate, soil composition, berry ripeness, harvest methods, and storage conditions, and it differs dramatically from other fruits with higher levels of polyphenols (Tolić et al., 2017). Polyphenols are carriers of flavor, smell, color, nutritional value, and antioxidative action (Robards et al., 1999). According to the data described by (Nawirska & Kwaśniewska, 2005), the dietary fiber of chokeberry pomace contains a high concentration of celluloses (35%), hemicelluloses (34%), lignin (24%), and pectin (24%). (8 percent). By-products of Aronia melanocarpa that are high in dietary fiber are regarded as valuable ingredients for food supplements and functional foods (Wawer et al., 2006). Chokeberries and their products have excellent antioxidant capabilities due to their high polyphenol content (Tolić et al., 2017). The anti-inflammatory characteristics of Aronia melanocarpa fruit have been linked to the prevention of chronic diseases such as diabetes, cardiovascular disease, and immune system disorders (Jurikova et al., 2017).

2.4. Conventional Extraction Methods

To take use of the beneficial qualities of bioactive compounds, efficient extraction systems that are inexpensive in cost, energy efficient, and work at low temperatures must be developed. Pretreatment, extraction, isolation/purification, and encapsulation are the four processes in the extraction of phenolic compounds (Routray & Orsat, 2012). Maceration, grinding, milling, homogenization, and drying are all ways of pre-treatment (Routray & Orsat, 2012). Homogenization disrupts the sample's cellular structure and increases the contact surface area between the solvent and the solute (Routray & Orsat, 2012).Drying can enhance the surface area of a product, as well as its bioavailability of phenolic compounds and shelf life (Brar, 2017). The extraction process employed is determined by the type of plant material used and the component being extracted.

2.4.1 Soxhlet extraction

Soxhlet extraction is a conventional method for recovering phenolic compounds. SE's main advantages are its simplicity, application at high temperatures, which improves process kinetics, inexpensive start-up costs, the absence of filtering, and the continual presence of solvent and sample during the extraction (Grigonis et al., 2005). Displacement of transfer equilibrium by repeatedly bringing fresh solvent to the solid matrix. Using heat from the distillation flask to maintain a relatively high extraction temperature. The extract does not need to be filtered. One of the key issues with the SE method is that it is limited due to its low extraction efficiency, lengthy procedure, and use of numerous solvents (Xiao et al., 2008). Figure 1 depicts a traditional Soxhlet extraction apparatus that utilize chemical solvents and heat. The solvent is boiled in the distillation column, and the vapors of the solvent flow to a condenser unit, where the solvent condenses into a liquid and falls onto the food source in the extraction vessel. Because the extracted solute is less volatile than the solvent, it is left in the extraction vessel while fresh solvent is recycled back into the distillation column (Singh & Orsat, 2014). This step is repeated until the extraction is finished. Soxhlet extraction has a low operational cost, is easy, and does not require subsequent filtration (Wang & Weller, 2006). However, the high temperatures used in Soxhlet extraction denature the nutraceutical components in food, decreasing their quality as well as the extraction yield (Singh & Orsat, 2014). Long operation times and a considerable amount of solvent are also required for

Soxhlet extraction (Wang & Weller, 2006). Other innovative methods may be employed for nutraceutical component extraction to address the limitations of the Soxhlet technique.

2.5 Novel Extraction Methods

One of the biggest issues with conventional extraction is that it takes much longer to finish the process, resulting in the destruction of thermosensitive compounds and the use of costly and pure solvents that evaporate quickly. Due to extraction constraints, various novel and one-of-a-kind extraction processes have been developed. Novel extraction methods are sometimes known as unconventional extraction techniques. Ultrasound, microwave, pulsed electric field, supercritical fluid, and pressurized liquid are some promising extraction techniques. In addition to these precautions, we may use less hazardous chemical synthesis strategies such as the development of safer compounds, the use of safe solvent auxiliaries, the use of renewable feedstock, and the reduction of derivatives (Handa, 2008b).

2.5.1 Ultrasound Assisted Extraction Method

Ultrasound-assisted extraction (UAE) is the extraction of bioactive substances using ultrasound-generating equipment and the appropriate solvent. According to Medina-Torres et al (Medina-Torres et al., 2017) ultrasound operates on mechanical waves that have length, amplitude, frequency, speed, power, and intensity. The frequency range of ultrasonic waves is 20 kHz to 100 MHz. It, like other waves, causes compression and expansion in a medium. Cavitation is the formation, expansion, and eventual collapse of bubbles. The kinetic energy of motion can be transferred into heating the contents of the bubble with a large quantity of energy. Cavitation can occur exclusively in liquids and liquids containing solids. Most importantly, the benefit of the UAE experiment could be noticed in the mobility of organic and inorganic components derived from the plant matrix aided by ultrasonic energy (Herrera & Luque de Castro, 2005). UAE's sustainability is attributed to lower solvent and energy usage due to shorter time and temperature requirements as compared to traditional extraction processes. (Kim et al., 2021) studied on phytochemicals and antioxidant activities of chokeberry. TPC, and TA contents of the fruit samples ranged from 17.05 to 135.55 mg of GAE/g DW, and 2.55 to 24.43 mg CGE/g DW respectively. UAE has been successfully employed to isolate bioactive components from fresh berry fruits. It resulted in a higher yield of polyphenols (and thus antioxidant activity) in extract derived from chokeberry fruits. Furthermore, increasing the temperature and adding ethanol to the

solvent improved the process's efficiency (d'Alessandro et al., 2012). A study found that the UAE permits the use of a lower temperature and lower solvent concentrations during the extraction of anthocyanins from blueberry fruits, producing extracts that are monomeric and rich in anthocyanins (Wang et al., 2016). (Woinaroschy et al., 2019) studied on bioactive compounds of blackberries with ethanol with 2% citric acid and the TPC found as 88.96±2.10 mg GAE/100ml; while the TPC was found as 60.67±0.91 and 37.66±1.72 mg GAE/100ml using the solvent water with 2% citric acid and 100% water respectively. Higher temperatures and longer treatment durations in the case of conventional extraction techniques reduce the antioxidant activity. According to a number of studies, ultrasound may have an effect on plant cells and tissues because the waves can increase temperature and then transfer that heat to the herb tissues (Jambrak et al., 2007).

2.5.2 Pulsed Electric Field

The application of short duration pulses (μs to ms) of moderate electric voltage (commonly 0.5-20 kV/cm) to a substrate of choice placed between two electrodes is known as pulsed electric field (PEF)-assisted extraction. PEF treatment intensities ranging from mild to moderate are frequently regarded as an effective pretreatment strategy for enhancing secondary metabolite extraction yields in cell cultures and plant systems (Heldman et al., 2010). Electroporation caused by dielectric breakdown of the cell membrane is the fundamental basis of PEF-assisted extraction (Pilwat et al., 1980). Due to the presence of free charges of opposite polarities across the membrane, it is hypothesized that cell membranes behave like a capacitor with a low dielectric constant and a natural trans-membrane potential. When an external electric field is applied, the transmembrane potential rises due to charge accumulation across the membrane. Subsequent exposure to an electric field raises the potential, resulting in electrostatic attraction between opposite charges across the membrane, producing membrane thinning. (Kumari et al., 2018) described a pulsed electric field (PEF) applied to a material positioned between two electrodes in short duration pulses of moderate voltage. PEF causes electroporation as a result of cell membrane damage. The intensity of the electric field, the duration of treatment, the waveform of the pulse, conductivity, porosity of the material, pH, and the ionic strength of the solvent are all factors in PEF-assisted extraction. PEF technology improves intracellular compound extraction by increasing intracellular substance diffusivity and mass transfer rates. PEF was used to increase the extraction of blueberry juice. (Pataro et al., 2017) examined anthocyanins from blueberry pomace. Before juice pressing,

blueberries were sliced in half and processed by PEF with varying input energy values 1 kJ/kg, 5 kJ/kg, or 10 kJ/kg. TAC and AA levels were increased in all pomace samples collected after pressing blueberries pretreated with PEF compared to the control (a sample of pomace remaining after pressing the juice from blueberries untreated with PEF). There was a relation between higher energy input and increased TAC and AA levels. Most studies suggest that a higher field strength resulted in better antioxidant compound yields. As the field strength is greater, the potential difference between the outside and inside of the cell membrane is greater than the critical membrane potential, which improves the disintegration rate of the cell membrane. However, using too strong a field may increase antioxidant degradation (Kumari et al., 2018). In addition, number of pulse and solvent type also influence the effectiveness of PEF (Zhou et al., 2015).

2.5.3 Supercritical fluid extraction

The use of solvents at temperatures and pressures above the critical values for temperature and pressure is referred to as supercritical fluid extraction (SFE). Solvents exhibit both gaseous and liquid characteristics under these conditions. Carbon dioxide (CO2) is often used in SFE because it has a low critical pressure and temperature, is non-toxic, non-flammable, and is inexpensive. It is a non-polar and hydrophobic solvent as well. SFE also delivers great extraction selectivity, which can be adjusted by modifying the extraction conditions (Wrona et al., 2017). (Milala et al., 2018) extracted oils from byproducts of chokeberries, raspberries, and strawberries. Oil yields ranged from 12% for raspberry pomaces to 18% for strawberry pomaces. The lipid fraction was collected at specific points during the extraction procedure. The oil's properties tocopherol concentration, fatty acid and pigment composition were affected by the time it was collected. In their study, (Basegmez et al., 2017) used SFE to generate polyphenol-rich extracts in blackcurrant pomace. The optimal process parameters were 45 MPa, 60 °C, and 120 minutes, yielding a TPC of 24.34 mg GAE/g extract.

2.5.4 Microwave-assisted extraction

Microwaves, which are non-ionizing electromagnetic waves, are used in microwave-assisted extraction to create changes in plant cell structure. This form of extraction involves the processes of heat and mass transfer that occur in only one direction. Because of molecular interactions with the electromagnetic field, microwave energy is delivered directly to material via the conversion of electromagnetic energy to thermal energy. Heat must then be dispersed volumetrically within the

sample. These effects promote cell penetration and compound internal and exterior diffusion, which leads to higher extraction yields (Veggi et al., 2012). Microwaves, which are non-ionizing electromagnetic waves, are used in microwave-assisted extraction to create changes in plant cell structure. This form of extraction involves the processes of heat and mass transfer that occur in only one direction. Because of molecular interactions with the electromagnetic field, microwave energy is delivered directly to material via the conversion of electromagnetic energy to thermal energy. Heat must then be dispersed volumetrically within the sample. These effects promote cell penetration and compound internal and exterior diffusion, which leads to higher extraction yields (Veggi et al., 2012). (Pap et al., 2013) investigated the best conditions for MAE of anthocyanins extracted from blackcurrant pomace. Variable values for power, time, solid-liquid ratio, and solvent pH were used. The maximum power (700 W) and solid-liquid ratio (1:20), but the shortest time (10 min) and lowest pH (2) values were found to be the most efficient conditions for anthocyanin extraction. The most prevalent anthocyanin in an HPLC study was delphinidin-3-rutoside. (Davis et al., 2021) were applied various types of solvents and power levels in MAE of pectin and polyphenolic-rich cranberry pomace extracts. An alkaline extraction procedure with a power value of 36 W/g yielded the highest polyphenol yields. When SLE and MAE were applied with different solvents of cranberry press residues, MAE resulted in a better extraction yield in every variant of the experiment. The values of quercetin equivalents of powdered cranberry residues were significantly higher for MAE with 100 percent acetone as a solvent than for water and ethanol extraction methods (Raghavan & Richards, 2007). Studies (Klavins et al., 2017) were used ethanol and trifluoroacetic acid as a solvent mixture to examine three methods of extracting phenolic chemicals from cranberry pomace: SLE, UAE, and MAE. The MAE extract had the lowest anthocyanin and polyphenol levels of any of the samples analyzed.

2.6 Selection of Solvents and Efficiency on Extraction of Aronia Pomace

Extraction solvent is one of the most important factors influencing the extraction efficiency of polyphenols and their associated health benefits (Ngo et al., 2017). Polar solvents often utilized for extracting polyphenols from plant materials (Do et al., 2014). The use of organic solvents such as ethanol, methanol, acetone or their aqueous mixtures generally preferred for extraction purpose (Wijekoon et al., 2011). Polyphenols are polar and are soluble in an aqueous mixture of polar solvents such as ethanol, methanol, or acetone and can be easily removed from the bio-active components. Researchers investigated the effect of different solvents on the final extraction

efficiency, 30%, 50%, 96% ethanol, and water. Previous research indicated that 50% ethanol was the best ethanol - water combination for Aronia polyphenol extraction (Galvan d'Alessandro et al., 2012). When 96% alcohol used in the extraction process, low amount total phenolic and anthocyanidin was extracted. The maximum amount of total phenolic extract obtained when 50% ethanol was used, and the maximum amount of anthocyanin was released when 70% alcohol was used (Galvan d'Alessandro et al., 2012). Different solvents like acidified water, ethanol, alcohols, glycerol, water used for the extraction of different compounds during UAE. The acidification of water has been done through citric acid. The efficiency of the UAE, expressed by the amount of antioxidants and antioxidative capacity, is mostly determined by the fruit species. There are also extraction conditions that influence the efficiency of UAE, which are time, temperature, and solvent type. Time: According to the researchers, the time of sonication had an effect on polyphenol or lipid fraction yields. Only in the case of one analyzed study, sonication period did not affect the extracts' polyphenol concentration (besides TAC). Yields increased with increasing sonication time; however, after reaching certain cutoff values, which may vary between studies and among the chemicals studied, yields decreased (Zafra-Rojas et al., 2016). Temperature: Studies (He et al., 2016) discussed the effect of temperature on extract yield and composition. In general, the effect of increasing temperature on polyphenol yield is similar to the effect of time on extraction yield. Polyphenol content increases with rising temperature, but at a certain point, it begins to decrease. When measuring the influence of temperature on specific polyphenol fractions, optimal temperature conditions may differ. Lower temperatures, for example, produce extracts with greater TAC and phenolic acids (Lončarić et al., 2020). Higher temperatures, on the other hand, result in enhanced quality and yield of oil recovered by UAE (Teng et al., 2016). When the final extraction yields obtained at 20 and 70 ℃ with 50% ethanol are compared, it is clear that increasing the extraction temperature had no positive impact on the extraction yields (Kechinski et al., 2010). (D'Alessandro et al., 2014) showed the highest TPC at 70 ℃ with 50% ethanol, 74.28 TPC in 240 minutes. Highest TA at 45 ℃ with 25% ethanol, 13.08 TA in 240 minutes. The extraction rate decreased with time in all conditions examined. Solvent type: When different solvents were examined, ethanol produced the highest polyphenol yields. When the final extraction yields obtained at 20 and 70 ℃ with 50% ethanol are compared, it is clear that increasing the extraction temperature had no positive impact on the extraction yields (Kechinski et al., 2010). (D'Alessandro et al., 2014) showed the highest TPC at 70 ℃ with 50% ethanol, 74.28 TPC in 240

minutes. Highest TA at 45 ℃ with 25% ethanol, 13.08 TA in 240 minutes. The extraction rate decreased with time in all conditions examined. Because of no toxicity, and low volatility glycerol used as an extraction solvent for biologically active compounds (Additives et al., 2017) too. Machado et al. (Machado et al., 2017) found that 70 percent ethanol was best for polyphenol extraction yields from blackberry and blueberry pomaces. The most widely used solvents for extracting phenolic compounds are water, ethanol, methanol, acetone, and their water mixtures, with acid or not (Michiels et al., 2012). The recovery of phenolic compounds is dependent on the solvent used in their extraction and its polarity (Alothman et al., 2009). (Boeing et al., 2014) reported that solvent combinations had a significant impact on the extraction of phenolic and anthocyanin components and their antioxidant capacity in Black mulberry. In comparison to their respective pure organic solvents, organic solvent-water combinations were more effective in extracting antioxidant chemicals.

2.7 Optimization of Ultrasound Assisted Extraction in Aronia Pomace

In optimizing an extraction our goal is to find a set of conditions that allow us to extract at maximum based on applied response surface methodology. (Zou et al., 2011) was used RSM to optimize UAE parameters including methanol concentration, extraction temperature, and liquid-to-solid ratio, to get the optimal conditions for extraction of anthocyanin from mulberry. The results revealed that methanol concentration, extraction temperature, and liquid-to-solid ratio all had a significant effect on anthocyanin extraction rate. 63.8 percent methanol (1 % TFA, v/v), 43.2 °C temperature, 23.8 liquid-to-solid ratio, and 40 minute extraction duration with ultrasonic irradiation were the optimal combination of response function. (Xu et al., 2017) optimal condition for the extraction of TPC: ethanol-water ratio of 0.69, ultrasonic duration of 52 minutes. The anthocyanin yield was 64.70 0.45 mg/g powder under optimal conditions; and According to the findings, the UAE can be an effective method for extracting some bioactive compounds from plant materials. (Aybasier et al., 2013) applied ultrasonic assisted extraction from blackberry leaves using response surface approach to optimize the phenolic compounds. The optimum methanol concentration was 61 and 64% (v/v), HCl concentration 0.41 and 0.45 M, extraction temperature 66 and 68 °C, and time 105 and 117 min. were determined for the highest possible extraction yield of phenolic compounds in relation to the total phenolic content and antioxidant activity. (Sady et al., 2019) studied on ultrasound assisted extraction of for bioactive compounds in chokeberry pomace using RSM to optimize extraction conditions. The results revealed that total phenolic content,

antioxidant activity, and total anthocyanin content were significantly influenced by ethanol concentration but not by sonication time. As the author mentioned the best extraction conditions for total phenolic content (188.5 mg GAE/g DM) and antioxidant capacity (49.2 mM Tr/100 g DM) were 60% ethanol and 20 minutes of sonication. The optimal condition for total anthocyanin content (89.3 mg C3GE/g) were a 65% ethanol concentration and a 13-minute sonication period. (Vázquez-Espinosa et al., 2019) optimized extraction conditions for extraction of TPC and TA using RSM method and the optimal conditions for simultaneous extraction in black Chokeberry were: 54% methanol as extraction solvent at pH 2.72 and 69.4 °C temperature, 70% amplitude, 0.7 s cycle, and 0.5:18.2 g:mL sample mass/solvent volume ratio. The developed methods showed a high precision level with coefficients of variation lower than 5%. (Zafra-Rojas et al., 2016) described RSM-based UAE optimization on blackberry. The experiment was conducted under continuous conditions of frequency 20 kHz, S-L ratio 1:24, and a temperature of 4 °C at the start and 25 °C at the finish. The ultrasonic amplitude was variable, ranging from 80 to 90 percent, and the extraction time was variable, ranging from 10-15 minutes. Mathematical analysis revealed that an amplitude of 91 percent and a time of 15 minutes, respectively, were the most beneficial parameters for TPC, TAC, and AA extracts on a dm basis.

2.8 Color Measurement

Color has a strong influence on a consumer's opinion about the food quality. Color and appearance draw a consumer's attention to a product and can help in impulse purchases (Pathare et al., 2013). In CIELAB Color measurement method, the location of color is determined by its color 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 (+) (Lunadei et al., 2011), as depicted below figure 2. (Kim et al., 2021) stated how the type of solvent used affects the color characteristics. (Ochmian et al., 2012) showed that the color of L*, a* and b* for cultivars of macerated chokeberry fruits were 14.13, 5.19, −12.98 respectively. (Samoticha et al., 2016) showed that the color values of L*, a* and b* for the whole freeze dried black chokeberry were 30, 10.9 and 3.7, respectively. (Horszwald et al., 2013) examined the powder of black chokeberry and found that the L*, a*, and b* color values were 24.35, 22.48, and 4.50, respectively. (Zielinska & Michalska, 2018) studied the color properties of blue berry pomace using hot air convective drying at 60 °C and found the values as L* 31.97 ± 0.14, a*1.50 ± 0.12, b* 1.56 ± 0.09, and the total color difference, ΔE* 2.27 ± 0.37.

Several studies have shown that different drying temperatures, drying processes, air flow rate, drying time, poor stability of pigmented compounds, oxidative reactions affected the color of dried product (Kerr & Varner, 2020). The type of solvent used also affects the color characteristics. Nero and Viking cultivars have greater quantity of fruit and pulp red- and blue-coloring substances. The color of L*, a* and b* for cultivars of macerated chokeberry fruits were 14.13, 5.19, −12.98 respectively (Ochmian et al., 2012). The study continued with other researcher (Samoticha et al., 2016) showed that the color values of L*, a* and b* for the whole freeze-dried black chokeberry were 30, 10.9 and 3.7, respectively; while (Horszwald et al., 2013) presented the powder of black chokeberry and found that the L*, a*, and b* color values were 24.35, 22.48, and 4.50, respectively. The variations between these studies, and our studies could be due to the different in genetic types of black chokeberry, drying process, and type of solvent used.

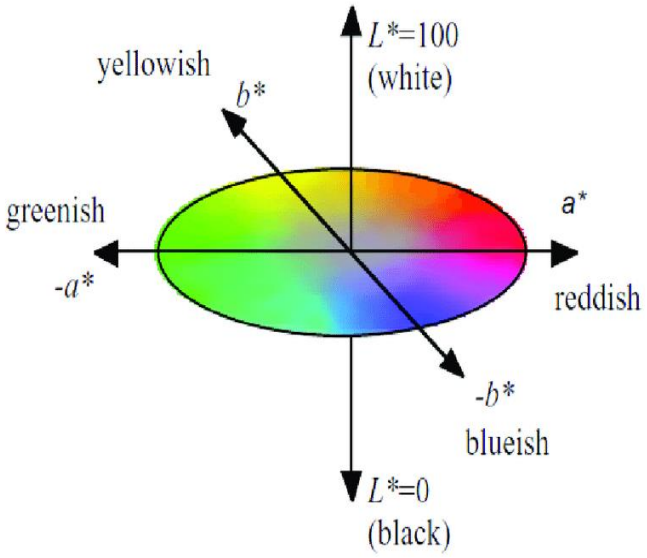


Figure 2. The CIELAB color space (Gabelaia, 2020)

3.MATERIAL AND METHODS

3.1 Biological Material

Chokeberry fruits (cultivar: 'Nero') were harvested from organic farming (Soroksár research site, Hungary) in 2022 have been used for these experiments. The washed fruits were then subjected to a pressing process to obtain the chokeberry pomace. This pomace was subsequently frozen and subjected to a freeze-drying process, which consisted of two stages: freezing (at –45°C) and drying (from 12 to 48 °C). The entire drying process was carried out for 12 hours. The freeze-dried chokeberry pomace was grounded to powder in Minichiller 300 (HUBER, Germany) for 15 seconds. The fraction obtained (with particle size of 8.89 μm) was then subjected to extraction. The following figure (3) shows the extraction of bioactive compounds from Aronia pomace. The method of this ultrasound-assisted extraction is a popular technique for the extraction of bioactive compounds from Aronia pomace. The Aronia pomace has been collected and grind it to a fine powder using a blender. The powder weighed with the desired amount and transferred it to a centrifuge tube and the solvent added to it in a ratio of 1:10 (w/v) of Aronia pomace: solvent. Placed to water bath with desired time and temperature and were put in an ultrasound apparatus and sonicate for a desired time (15 and 30) minutes and power (35kHz). After sonication, centrifuge the solution to separate the liquid extract from the solid residue and were transfer the liquid extract to a clean centrifuge tube and put to centrifuge until analysis made (Figure 3.).

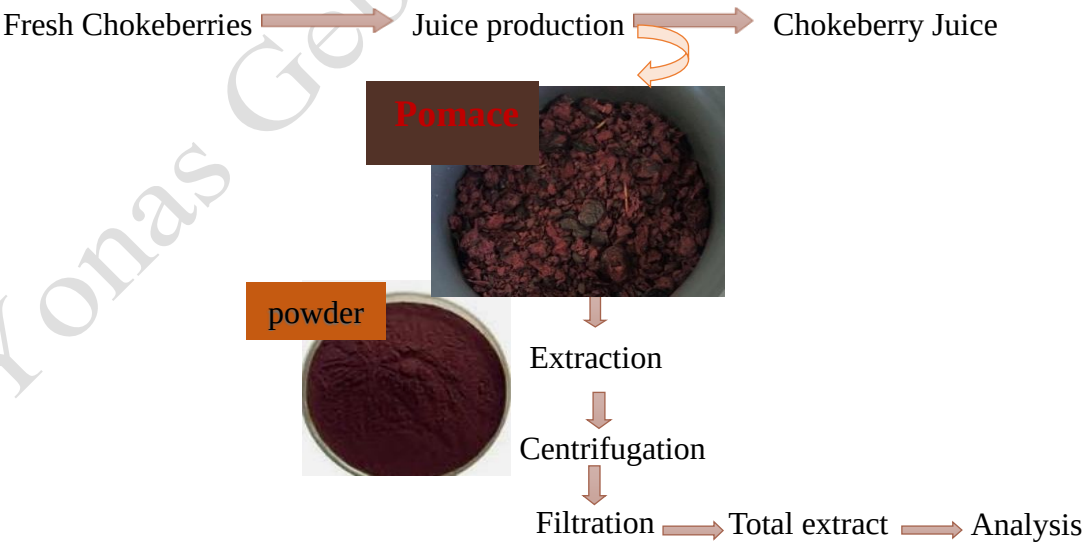


Figure 3. Procedure for ultrasound-assisted extraction of bioactive compounds from Aronia pomace

3.2 Solvents and Reagents

Folin-Ciocalteu reagent, 3,4,5-trihydroxybenzoic acid,Gallic acid, sodium carbonate, potassium chloride, acetic acid, sodium acetate, ethanol, glycerol, citric acid, distilled water, ascorbic acid, hydrochloric acid, ferric chloride hexahydrate (FeCl3.6H2O), methanol and 2,4,6-Tripyridyl-S-triazine (TPTZ) were used for this experiment. All chemicals and reagents were of analytical grade. The freeze-drying process were carried out using a lyophilizer in the laboratory from department of Livestock Products and Food Preservation Technology. The entire extraction process used a BANDELIN SONOREX-RK52 ultrasonic bath (35 kHz). Spectrophotometric assays were performed using a (HITACHI U-2900) spectrophotometer.

3.3 Ultrasound-Assisted Extraction

The following picture shows a typical SONOREX-RK52 ultrasonic bath (Figure 4.) used in the laboratory for sonication of Aronia pomace. Ultrasound assisted extraction were carried out with triplicate for each experiment under controlled temperature.

Figure 4. Ultrasonic bath (Internet 2)

The variables for this experiment were temperature, water bath time, sonication time, and concentration of solvents as shown in the following Table.

Table 1. 50% ethanol with 1% citric acid (A),50% glycerol with 1% citric acid (B), 100% water with 1% citric acid (C)

Solvents:		Extraction conditions						
Solvent A, B, C	Operation	Temperature(°C)			Time(minute)		US (minute)	
		40	50	60	60	120	15	30
	1	x			x		x	
	2	x			x			x
	3	x				x	x	
	4	x				x		x
	5		x		x		x	
	6		x			x	x	
	7		x			x		x
	8		x		x			x
	9			x	x		x	
	10			x		x	x	
	11			x		x		x
	12			x	x			x

A carefully measured of 300 mg of the lyophilizate was added to 9 mL of the following solvent mixtures; 50% ethanol with 1% citric acid, 50% glycerol with 1% citric acid, and 100% water with 1% citric acid. The sample were subjected to water bath with different temperature 40,50,60 ℃ for 60 and 120 minutes to assure uniform heat through the sample mixture. After this operation samples were added to the ultrasonic to assist the extraction. During extraction, the amount of ultrasonic energy was injected into the sample which allows it to establish whether the phenomenon of cavitation occur which cavitation effect increases the molecular motion and solvent penetration. The sonication time were 15 minutes for each batch of sonication after this the samples were subjected to centrifuge (HERAEUS MEGAFUGE 8 Centrifuge) for 5 minutes spinning time and finally the filtrate transferred to centrifuge tube. The extracted samples were kept at -20°C until analysis.

3.4 Total Polyphenol Content

Folin-Ciocalteu procedure were used to determine the TPC content. It consists of 1.25 mL of Folin-Ciocalteu reagent, 0.05 mL of sample, 0.2 mL MeOH: Distilled water, and 1 mL sodium carbonate solution were added to each test tube for analysis. The method was applied with a modified from (Singleton & Rossi, 1965). The absorbance was measured at 760 nm and TPC calculated as mg GAE/g FM).

$$TPC = \frac{A}{tga} * \frac{Vall}{Vsample} * D$$

TPC −Total Polyphenol Content

A −Absorbance

tga – Slope of the calibration line (0.???)

$Vall$ − Final volume (2.5 mL)

$Vsample$ − Volume of the sample

$D - Dilution$

3.5 Antioxidant Capacity

The antioxidant activity was determined modified from (Benzie & Strain, 1996) by ferric reducing ability of plasma (FRAP) assay. A pH 3.6 of 300mM of Anhydrous sodium acetate were prepared with a 500 ml acetic acid. Ferric chloride hexahydrate ($FeCl_3$.6H2O) with 0.27 g were measured using electronic balance and diluted with 50 ml of distilled water. In addition, 0.161 g of 2,4,6-Tripyridyl-S-triazine (TPTZ), and a 168 µl of hydrochloric acid were measured and subjected to mix altogether with 50 mL of distilled water. These reagents of sodium acetate buffer, ferric

chloride hexahydrate and TPTZ were transferred to a 500 mL of beaker to make the FRAP ready which the beaker was covered to protect from light. The calibration curve made, a 1.5 mL of FRAP added to different concentration10, 20,30,40, and 50 µL of ascorbic acid solution using spectrophotometry. Ascorbic Acid Equivalent (AAE) was used as a standard and the absorbance of the samples measured at 593 nm with 5 minutes of reaction time. The FRAP content were calculated milligram of ascorbic acid equivalent per gram of dry mass of the sample, mg AAE/g FM.

3.6 Total Anthocyanin Content

The determination of total anthocyanin content was based on pH differential spectrophotometric method with the absorbance at two different wavelengths 520 and 700 nm following the method (Lee et al., 2005) TAC was expressed as mg cyanidin 3-glucoside (C3GE) equivalent per g of FM of the extract. Spectrophotometric assay was performed in triplicate for each batch. The absorbance (Ad) was calculated using Equation 2:

$A = (A520 - A700)pH1.0 - (A520 - A700)pH4.5$

Anthocyanin concentration in the extract was calculated and expressed as cyanidin-3-glycoside equivalent (C3G): Where A is Absorbance difference, MW is molecular weight for cyanidin-3-glucoside (449.2 g/mol), DF is the dilution factor of the samples, and ε is the molar absorptivity of cyanidin-3-glucoside (26.900 M/cm). Results were expressed as mg of cyanidin-3-glucoside equivalents per ml of liquid extract (mg C3G/g FM).

3.7 HPLC Analysis

The most abundance anthocyanin compounds in Aronia namely cyanidin-3-glucoside, cyanidin3-galactoside, and cyanidin-3 arabinoside were quantified in the extracts using HPLC standards from (Sigma Aldrich) for identification of the peaks. The analysis of anthocyanins was done using an Shimadzu HPLC system (s). A LC column (150 x 4.6 mm) (Phenomenex Technologies co; LTD) was used; The mobile phases were water with 0.5% formic acid (A) and acetonitrile with 0.5% formic acid (B). The flow rate was maintained at 0.5 mL/min. The absorbance was obtained at 520 nm. The following gradient (Table 2) was used:

Table 2. Gradient program

Time(min.)	B (%)
0.01	5.0
5.0	25.0
10.0	100.0
15.0	100.0
25.0	5.0
35.0	5.0

3.8. Color Measurement

The color was determined using a digital colorimeter (Konica Minolta, Chroma-400) was used to determine the color difference of pomace extract according to (Mokrzycki and Tatol, 2011). It is a handheld, portable measurement equipment used to assess the color of things, particularly those with smoother surfaces or less color change. To evaluate the color of the pomace sample the color parameters L*, a*, and b* were measured. The values L* represents the lightness or brightness(+L*) or darkness(−L*). a* indicates redness (+a*) or greenness(−a*), and b* refers yellowness (+b*) or blueness (−b*). The total color difference (ΔE*) was calculated based on the following Equation 3:

$$\Delta E^* = \sqrt{(L^* - L_0^*)^2 + (a^* - a_0^*)^2 + (b^* - b_0^*)^2}$$

E1: 0 < ΔE < 1, observer does not notice the difference; E2: 1 < ΔE < 2, only experienced observer can notice the difference; E3: 2 < ΔE < 3.5, unexperienced observer also notices the difference; E4: 3.5 < ΔE < 5, clear difference in color is noticed; E5: 5 < ΔE, observer notices two different colors.

3.9. Statistical Analysis

All analyses were done with triplicate, and these values were then presented as means ± SD (standard deviation). IBM SPSS statistics software version 27.0.1 were used to analyze the multivariate test. Minitab software version 21.3.1 were used to study both between and interaction effect of the extraction conditions and to develop the fitting model for optimal extraction conditions to this particular study. A value of $P < 0.05$ was considered statistically significant. The skewness and kurtosis also considered based on (Demir, 2022).

4. RESULT AND DISCUSSION

4.1 Ultrasound Assisted Extraction

In this study, a total of 36 Aronia pomace samples were made from the *Aronia/Chokeberry fruit*, "Nero" cultivar, were selected to determine the total phenolic contents, total and individual anthocyanin content, the total antioxidant activity and color parameters. UAE is a green technology for extracting bioactive components in a short period of time along with less energy consumption. It is also considered as a boosting technique because it uses much less energy than conventional extraction methods and increases the yield of bioactive components through the cavitation process. USET, temperature, and water bath time play a major role in the extraction. Because of bubble explosions, ultrasound extraction takes less time. The TPC, FRAP, and TA for ultrasound-assisted extraction with three solvents for solvent A (50% ethanol with 1% citric acid, solvent B (50% glycerol with 1% citric acid), and solvent C (100% water with 1% citric acid) were determined. The details based on the three solvents are discussed as follows: Table 6 depicted the statistical data of TPC, TA, and FRAP for ultrasound assisted extraction with solvent A at different temperature, ultrasound exposure time and water bath time. The mean value for TA were 639.03 mg CGE/100g FM; while 2744.5 mg AA/100g FM, and 3449.4 mg GAE/100g FM found for FRAP and TPC respectively. The minimum and maximum value found as 425.84 & 881.28 CGE/100g FM, 1540.4 & 4393.9 AA/100g FM, and 2622.4 & 4527.8 GAE/100g FM for TA, FRAP, and TPC respectively. skewness and kurtosis are important measures of the distribution of the data set. As shown in table 3 the skewness -0.26, 0.48, and 0.26 and the kurtosis 0.07, -0.16, and -0.53 were found for TA, FRAP and TPC respectively and data is considered as normal. (Demir, 2022) described the data considered to be normal if skewness is between -2 to +2 and kurtosis is between -7 to +7. Multivariate test was made to see the effects between and it can be observed that in table 8, temperature is highly significant on the response variables TPC, FRAP, and TA. The interaction effect of temperature and ultrasound exposure time was highly significant ($p<0.05$). The interaction effect of temperature and ultrasound exposure time also have high effect on TA and TPC responses. In this study under solvent A, time didn`t show a significant effect however a little effect observed on TA along with interaction effects of temperature and ultrasound exposure time. The F-value found that temperature has greater F value on TA, FRAP, and TPC; similarly, ultrasound exposure time showed high F value on TPC and TA as well.

Table 3. Statistical data for 50% ethanol with 1% citric acid

Variable	Total Count	Mean	SE Mean	StDev	CoefVar	Minimum	Maximum	Skewness	Kurtosis
TA	108	639.03	9.26	96.23	15.06	425.84	881.28	-0.26	0.07
FRAP	108	2744.5	59.2	615.7	22.44	1540.4	4393.9	0.48	-0.16
TPC	108	3449.4	42.1	438.0	12.70	2622.4	4527.8	0.26	-0.53

50% glycerol with 1% citric acid solvent: It was the other extraction solvent for the extraction of TPC, TA, and FRAP with ultrasound assisted. In this type of extraction, the total polyphenol content, total anthocyanin content and ferric reduction antioxidant power found as a mean of 3255.2 mg GAE/100g FM, 564.2 mg CGE/100g FM, and 3134.0 mg AA/100g FM respectively as shown in Table (4). The coefficient of variation for TA found that 24.93, FRAP showed 18.76, and 9.18 obtained on TPC. Skewness and kurtosis were taken which shows the distribution of data set. As a result, the skewness as -0.5, -2.15, and 1.75 were found on TPC, TA, and FRAP respectively and kurtosis 1.88, 20.18 and 6.59 obtained for TPC, TA, and FRAP as well. A pairwise Pearson correlation coefficient used to see the association between responses. A multivariate test was used to show which factor(s) is/are significant or not for the extraction of response variables, TPC, TA, and FRAP. Temperature was highly significant on the extraction of those responses at ($p < 0.05$). In addition, the interaction effect of temperature and ultrasound assisted exposure time were significant in all the three responses. The interaction effect of Ultrasound exposure time, temperature and time obtained as significant on FRAP.

Table 4. Statistical data of 50% glycerol with 1% citric acid solvent

Variable	Total Count	Mean	SE Mean	StDev	CoefVar	Skewness	Kurtosis
TPC	108	3255.2	28.7	298.7	9.18	-0.50	1.88
TA	108	564.2	13.5	140.7	24.93	-2.15	20.18
FRAP	108	3134.0	56.6	588.0	18.76	1.75	6.59

100% water with 1% citric acid: For the extraction of bioactive compounds from Aronia pomace the solvent 100% water with 1% citric acid was applied. In this solvent extraction the total

anthocyanin content was found as 341.46 mg CGE/100g FM as a mean value described in the table 12. As compared to this TA value with other total anthocyanin content extracted with other solvents 50% ethanol with 1% citric acid and 50% glycerol with 1% citric acid, it is found that the TA value as a mean extracted with 100% water with 1% citric acid solvent is found lower than total anthocyanin content extracted with other both solvents. The highest content of TA obtained as 639.03 mg CGE/100g FM extracted from solvent A, 50% ethanol with 1% citric acid. On the other hand, the TA extracted with solvent B, 50% glycerol with 1% citric acid was found as 564.2 mg CGE/100g FM which is lower than the one extracted with solvent B but higher extracted than solvent C, 100% water with 1% citric acid. The total polyphenol content in this solvent C extraction presented as 1021.0 mg GAE/100g FM as a mean value. However, the TPC was determined with other solvents, solvent A and solvent B and showed higher content as compared to solvent C depicted in the table. The TPC extracted with 50% ethanol with 1% citric acid showed higher mean value as 3449.4 mg GAE/100g FM; conversely TPC extracted with 100% water with 1% citric acid determined as three times lower than the TPC extracted with solvent A. This showed that how different solvents have different extraction efficiency and the necessities for the selection of solvents on the extraction of bioactive compounds from Aronia pomace. The TPC extracted with 50% glycerol with 1% citric acid could be taken as a middle value between the two solvents which is found as 3255.2 mg GAE/100g FM. This content is giving higher value as compared to the solvent 100% water with 1% citric and lower than 50% ethanol with 1% citric acid solvent. The ferric reducing antioxidant potential as depicted in table 13 it is 1262.8 mg AA/100g FM. However, as compared to other solvents, the FRAP extracted with 100% water with 1% citric acid found as lower and the green chemistry, 50% glycerol with 1% citric acid showed higher FRAP value as 3134.0 mg AA/100g FM. The solvent 50% ethanol with 1% citric acid produced 2744.5 mg AA/100g FM is higher than the other extract solvent, solvent C. As compared to the three solvents, the solvent 100% water with 1% citric acid showed lower yield on TPC, FRAP, and TA responses as a mean value. The total anthocyanin content could be described as a range minimum and maximum value. TA extracted with 100% water with 1% citric acid fond as 242.07 and 459.92 mg CGE/100g FM as minimum and maximum content respectively. On the other hand, FRAP resulted 742.2 and 2836.3 mg AA/100g FM as minimum and maximum potential respectively; and for TPC it is found as 647.2 and 1470.3 mg GAE/100g FM content as well. The shape of distribution of the dataset could be described as skewness and kurtosis**.** Skewness measures the asymmetry of a

distribution of the data and value could be positive or negative. Negative skew shows that the tail of the distribution is on the left side and extends towards negative values and where as positive skew shows the tail is on the right side of the distribution, extending towards more positive values. Therefore, the skewness of the total anthocyanin content found to be -0.01 indicating that the distribution is left-skewed. Conversely, the skewness for the ferric reducing antioxidant power and total phenolic content found as 1.75 and 0.42 respectively showed that the distribution is right-skewed. Kurtosis determines whether a distribution is heavy-tailed or light-tailed relative to a normal distribution. The kurtosis of TA, FRAP, and TPC found to be 0.66, 3.42, and 0.07 respectively, indicating that the data distribution was less-heavy tailed, implies produce fewer or less extreme outliers. The pairwise Pearson correlation was found and the highest correlation was obtained on total polyphenol content with total anthocyanin content which has a correlation of 0.598 at 95% confidence interval with a P-value of (p = 0.000); conversely the correlation FRAP with TA, and TPC with FRAP showed lower correlation which implies their association is weaker in this type of solvent extraction. Alike other solvents, 50% ethanol with 1% citric acid and 50% glycerol with 1% citric acid, a multivariate test was made. The F-value 27.744 and 18.974 found on the temperature for TPC and TA respectively with p-value (P = 0.000). Time showed an F-value of 6.123 for TA with p-value of (p = 0.015). Besides, ultrasound exposure time with F-value of 3.794 on were found on FRAP at p-value of (p=0.054); and the interaction effect of temperature and ultrasound exposure time on FRAP obtained F-value of 8.620 at p-value (p = 0.000). Ultrasound exposure time with time effect also showed F-value 8.029 on FRAP at p-value (p = 0.006); and interaction of temperature, time and ultrasound exposure time found F-value 4.991 for TPC at p-value (p = 0.009).

T**able 5.** Statistical data of 100% water with 1% citric acid solvent

Variable	Total Count	Mean	SE Mean	StDev	CoefVar	Minimum	Maximum	Skewness	Kurtosis
TA	108	341.46	3.95	41.03	12.02	242.07	459.92	-0.01	0.66
FRAP	108	1262.8	38.9	404.7	32.05	742.2	2836.3	1.75	3.42
TPC	108	1021.0	16.4	170.9	16.74	647.2	1470.3	0.42	0.07

4.2. Correlation Between Spectrophotometric Assays

The correlation coefficients (R^2) for spectrophotometric assays of the three solvents namely,50% ethanol with 1% citric acid, 50% glycerol with 1% citric acid, and 100% water with 1% citric acid ranged from 0.7171 to 0.824 in Table A7 (Annex A) and figure 5. TPC and FRAP methods showed a high correlation coefficient of R^2= 0.824. The highest correlation was demonstrated between TPC and TA (R^2 = 0.804). Correlation coefficient was found to be comparatively low (R^2 = 0.717) between TA and antioxidant activity assay, FRAP. These results imply that TPC were the major contributor to the antioxidant capacity of the studied Aronia pomace extract. A boxplot is a standardized method of representing the distribution of a data-based set. It can provide information about your outliers and their values. Boxplots can also tell us how closely the data is clustered, and whether or not the data is skewed. Figure 5, shows how the data is normally distributed for TPC, FRAP and TA. As we see the figure, there are some outliers; this cause may happen due to reasons such as from instrument, calibration, measurement, and other factors. However, as a judgement the figure shows the data distributed in a good manner.

Table 6. Statistical result of the three solvents

Variable	Total Count	Mean	SE Mean	StDev	CoefVar	Skewness	Kurtosis
TA	324	514.90	8.99	161.88	31.44	-0.30	1.99
FRAP	324	2380.4	54.0	972.7	40.86	0.11	-0.39
TPC	324	2575.2	63.8	1149.1	44.62	-0.49	-1.40

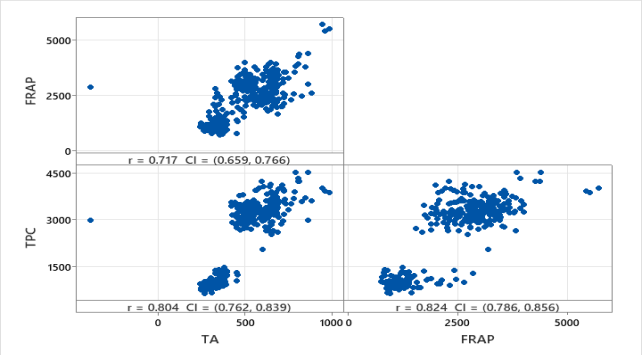


Figure 5. A 95% CI Pearson correlation Matrix Plot of TA, FRAP, and TPC for all three solvents

4.3. Determination of Total Polyphenol Content

There is a growing demand for the creation of green extraction methods that reduce extraction time, consumption of harmful solvents, and energy consumption. Ultrasound assisted extraction is one such method, with significant positive effects on phenolics extraction from different fruit and vegetal sources while using short time and energy (D'Alessandro et al., 2014). In this case, as shown from Tables A8 to A10 (Annex A), the total phenolic content was extracted using ultrasound assisted. The extraction efficiency can be greatly affected by operating parameters such as temperature and solvent composition solid to solvent ratio (Cacace & Mazza, 2003), extraction time, and particle size (Bucić-Kojić et al., 2007). The total polyphenol content for solvent A is presented in Table A8. The TPC extracted was calculated using the Folin-Ciocalteu method in terms of Gallic acid equivalent (GAE) in mg/100g of FM extract. The TPC values were obtained from the calibration curve $y = 0.0114x$ with $R^2 = 0.9861$, where x is the absorbance and 'y' is the concentration of gallic acid solution (µg/mL) expressed as mg GAE/ml. The total polyphenol content ranges from 3017.68 to 3904.02 mg GAE/100g FM as shown in Table A8. The total polyphenol content found from 2925.75 to 3496.96 mg GAE/100g FM as presented in Table A9 using the solvent 50% glycerol with 1% citric acid. As we see across the table there is a reasonable quantitative change on the content of polyphenols which is due to the nature of the solvent and varying extraction conditions as temperature, time and ultrasound exposure time. The total polyphenol content of solvent C, 100% water with 1% citric acid depicted in Table A10, which accounts from 816.1 to 1236.88 mg GAE/100g FM. (Woinaroschy et al., 2019) studied on bioactive compounds of blackberries with ethanol with 2% citric acid and the TPC found as 88.96 ± 2.10 mg GAE/100ml; while the TPC was found as 60.67 ± 0.91 and 37.66 ± 1.72 mg GAE/100ml using the solvent water with 2% citric acid and 100% water respectively. The effect of temperature, extraction time, and USET on total phenolic content has been shown in Figure 7. It was found that the TPC extracted with 50% ethanol with 1% citric acid increases as temperature and ultrasound exposure time, USET increases until a certain level but the lowest extraction yield found as minimal temperature and ultrasound extraction time applied; in this case as the temperature of water bath at 40 ℃ and sonication time 15 minutes it is found that the lowest yield recorded. In details, the temperature at 40 ℃, extract exposed for 60 minutes in water bath, and a 15 minutes of sonication time, the total polyphenol content found that 3112.5 mg GAE/100g FM, with this, as the set point increases for each factor to, 50 ℃, 120 minutes, and a 30-minutes of sonication time,

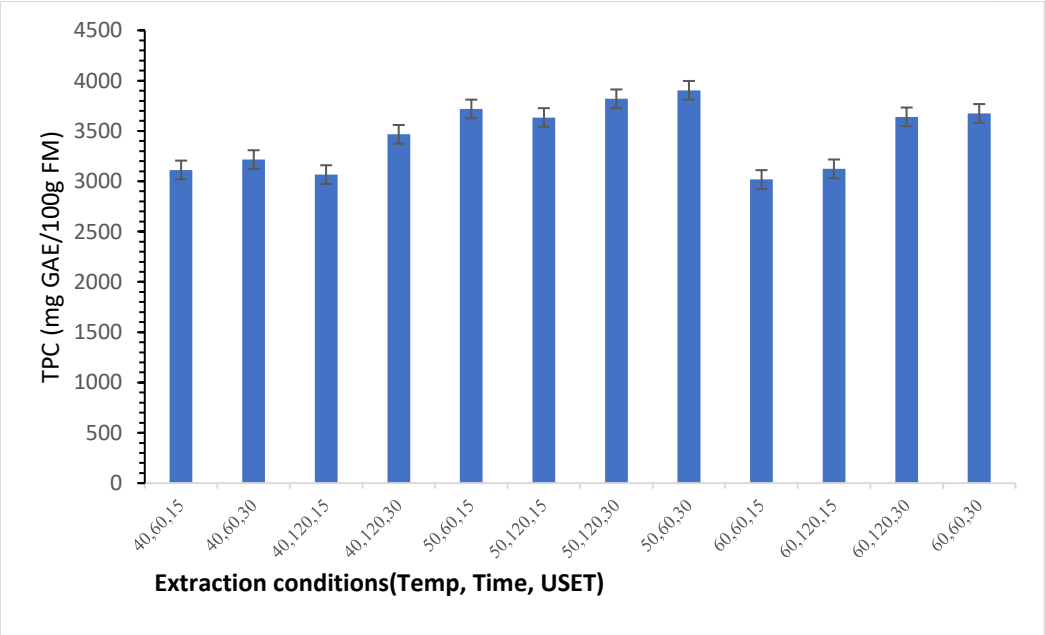
we found the TPC content as 3819.82 mg GAE/100g FM. As shown in in the multivariate test on Table A2 (Annex A) temperature and USET has a significant effect on extraction of TPC with 50% ethanol with 1% citric acid solvent. As the time of extract treated USET increases from 15 minutes to 30 minutes by keeping the temperature 40 ℃, and water bath time 60 minutes, the TPC was found that 3215.37 mg GAE/100g FM, while with no changing the temperature at 40℃, and the water bath time 120 minutes, the extract was treated with ultrasound assisted in 15 and 30 sonication time. The total polyphenol content treated with 40 ℃, in 120 water bath time and a-15 minutes of ultrasound exposure time found as 3066.39 mg GAE/100g FM; and at 40 ℃, in 120 minutes of water bath time, and a-30 minutes sonication time the TPC was 3466.46 mg GAE/100g FM. The temperature of the extract was raised intentionally to 50 ℃ to study its effect along with other extraction conditions. In this case, the temperature kept at 50 ℃, but the water bath time and ultrasound exposure time changed and switched each other, meaning 60 minutes with 15 minutes of USET, 60 minutes with 30 minutes of USET, and the same principle applied for 120 minutes too. The total polyphenolic content determined 3718.46 mg GAE/100g FM for 50 ℃, 60 minutes of water bath, and 15 minutes of USET. And the highest TPC presented as 3904.02 mg GAE/100G FM for a temperature of 50 ℃, 60 minutes, and 30 minutes of sonication time for the entire extraction with 50% ethanol with 1% citric acid. Conversely, the pomace extract treated with 50 ℃, exposed for 120 minutes in water bath, and 15 sonication time, the TPC found as 3633.74 mg GAE/100g FM. In addition, as the sonication time set to 30 minutes, with 120 minutes of water bath and 50 ℃ was 3819.82 mg GAE/100g FM. The pomace was also treated by increasing the temperature and varying the water bath time and the ultrasound exposure time. In this way, the temperature was set to 60 ℃, the time varying 60, and 120 minutes and the sonication time was 15, and 60 minutes which the extract exposed with different sonication and water bath time. Thus, the total polyphenolic content was presented as 3017.68 mg GAE/100g FM at a temperature 60 ℃, 60 minutes, and a 15-minutes of USET. From there, only the sonication time was changed to 30 minutes (60 ℃, 60 minutes of water bath, and 30 minutes for sonication) the TPC found as 3674.55 mg GAE/100g FM, while the TPC yield was 3640.09 mg GAE/100g FM as the extract exposed to 60 ℃, 120 minutes, and 30 minutes of ultrasound exposure time in 50% ethanol with 1% citric acid solvent. On the other hand, the sample extracted with 50% glycerol with 1% citric acid showed the total polyphenol is highly influenced by the extraction conditions. Temperature and their interaction: temp.*time*USET had a significant impact on TPC extraction with the

solvent, 50% glycerol with 1% citric acid, as shown in the multivariate test in Table A4 (Annex A). The highest TPC yield with this solvent found as 3508.5 mg GAE/100g FM. At a temperature of 50 ℃, 120 minutes, and a 30-minutes of sonication time, the TPC content found as 3034.05 mg GAE/100g FM. At a 30 minutes of sonication time, a temperature of 40 ℃, and water bath time 60 minutes, the TPC was found that 3158.4 mg GAE/100g FM, while with the same temperature 40℃, and the water bath time 120 minutes, the extract was treated with ultrasound assisted in 15 and 30 sonication time. The total polyphenol content treated with 40 ℃, in 120 water bath time and a-15 minutes of ultrasound exposure time found as 3461.26 mg GAE/100g FM; and at 40 ℃, in 120 minutes of water bath time, and a-30 minutes sonication time the TPC was 3351.48 mg GAE/100g FM. The temperature of the extract was set to 50 ℃ and the total polyphenolic content determined 3043.03 mg GAE/100g FM for 50 ℃, 60 minutes of water bath, and 15 minutes of USET; with same time and temperature but the sonication time set to 30 minutes 2966.14 mg GAE/100G FM total polyphenol content was found. On the contrary, the extract treated with 50 ℃, exposed for 120 minutes in water bath, and 15 minutes of sonication time, the TPC presented as 2925.75 mg GAE/100g FM. Likewise, at a temperature of 50 ℃, the sonication time 30 minutes, and a120 minutes of water found that 3034.05 mg GAE/100g FM. The pomace was also extracted by increasing the temperature and varying the water bath time and the ultrasound exposure time. In this way, the temperature was set to 60 ℃, the time varying 60, and 120 minutes and the sonication time was 15, and 60 minutes which the extract exposed with different sonication and water bath time. Thus, the total polyphenolic content was presented as 3323.39 mg GAE/100g FM at a temperature 60 ℃, 60 minutes, and a 15-minutes of USET. In the meantime, sonication time was set to 30 minutes (60 ℃, 60 minutes of water bath, and 30 minutes for sonication) the TPC found as 3397.02 mg GAE/100g FM, while the TPC yield was found as 3496.96 mg GAE/100g FM as the extract exposed to 60 ℃, 120 minutes, and 30 minutes of ultrasound exposure time. (Kim et al., 2021) studied on phytochemicals and antioxidant activities of chokeberry. TPC, and TA contents of the fruit samples ranged from 17.05 to 135.55 mg of GAE/g DW, and 2.55 to 24.43 mg CGE/g DW respectively. As shown in figure 6, Temperature, and interaction effect with time and ultrasound exposure time had an effect on the TPC yield. For instance, the temperature at 40 ℃, extract exposed for 60 minutes in water bath, and a 15 minutes of sonication time, the total polyphenol content found that 816.1 mg GAE/100g FM, with this, as we increase their set point for each factor as, 50 ℃, 120 minutes, and a 30-minutes of sonication time, we found the TPC

content as 1037.18 mg GAE/100g FM. A shown in in the multivariate test on Table A6 (Annex A) temperature has a significant effect, also interaction effect Temp * Time * USET has significant effect too on extraction of TPC with 100% water with 1% citric acid solvent. As the time of extract treated USET increases from 15 minutes to 30 minutes without changing the temperature 40 ℃, and water bath time 60 minutes, the TPC was found that 966.71mg GAE/100g FM. By keeping the temperature at 40℃, and the water bath time 120 minutes, the extract was treated with ultrasound assisted in 15 and 30 sonication time. The total polyphenol content treated with 40 ℃, in 120 water bath time and a-15 minutes of ultrasound exposure time found as 919.72 mg GAE/100g FM; and at 40 ℃, in 120 minutes of water bath time, and a-30 minutes sonication time the TPC was 937.03 mg GAE/100g FM. The temperature of the extract was raised intentionally to 50 ℃ to study its effect along with other extraction conditions. In this case, the temperature kept at 50 ℃, but the water bath time and ultrasound exposure time changed and switched each other, meaning 60 minutes with 15 minutes of USET, 60 minutes with 30 minutes of USET, and the same principle applied for 120 minutes. The total polyphenolic content determined 1065.05 mg GAE/100g FM for 50 ℃, 60 minutes of water bath, and 15 minutes of USET. And 946.34 mg GAE/100G FM for a temperature of 50 ℃, 60 minutes, and 30 minutes of sonication time. Conversely, the pomace extract treated with 50 ℃, exposed for 120 minutes in water bath, and 15 sonication time, the TPC found as 976.79 mg GAE/100g FM. In addition, as the sonication time gets to 30 minutes, with 120 minutes of water bath and 50 ℃ was 1037.18 mg GAE/100g FM. The pomace was also treated by increasing the temperature and varying the water bath time and the ultrasound exposure time. In this way, the temperature was set to 60 ℃, the time varying 60, and 120 minutes and the sonication time was 15, and 60 minutes which the extract exposed with different sonication and water bath time. Thus, the total polyphenolic content was presented as 1062.98 mg GAE/100g FM at a temperature 60 ℃, 60 minutes, and a 15-minutes of USET. From here, only the sonication time was changed to 30 minutes (60 ℃, 60 minutes of water bath, and 30 minutes for sonication) the TPC found as 1156.49 mg GAE/100g FM, while the TPC yield was 1131.01 mg GAE/100g FM as the extract exposed to 60 ℃, 120 minutes, and 30 minutes of ultrasound exposure time. The total phenolic values range from 8.6 g/kg to 10.8 g/kg fresh weight chokeberry was reported by (Jakobek et al., 2012). The highest yield found on solvent A at a temperature of 50℃,60 minutes, and 30 minutes of ultrasound exposure time which is about 3904.02 mg GAE/100g FM. Different chokeberry varieties were studied (Jakobek et al., 2012),

total phenolic content ranged from 8563.8 to 12055.7 mg of GAE/KG FM. Our statement may show a different result because the TPC, FRAP, and TA value depends on the many factors such as the type of extraction method, drying method, type of solvent used, storage conditions. These reasons agree with (Denev et al., 2012) lower or higher values reported in the literature could be due to different extraction methods used for analysis, differences in analytical procedures used, different processing technologies and storage conditions, or differences in chokeberry cultivars.

A.



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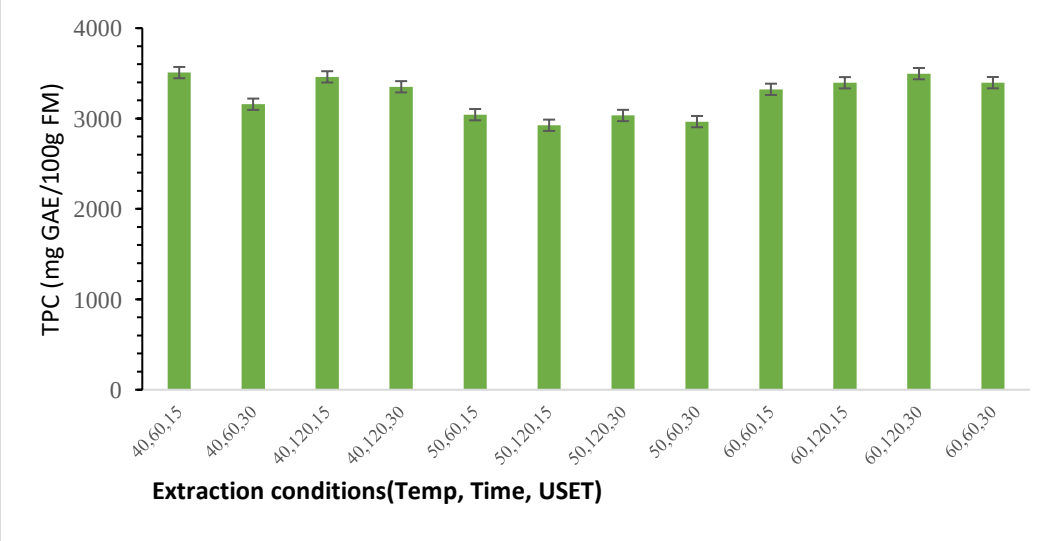


C.

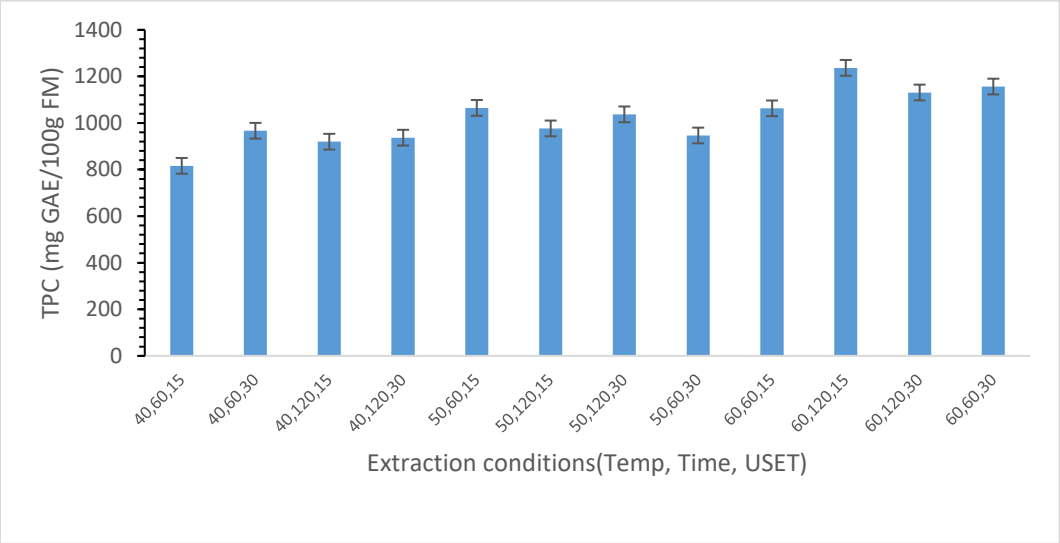


Figure 6. Effect of temperature, time and USET (n=3) on TPC yield: 50% ethanol with 1% citric acid (A), 50% glycerol with 1% citric acid (B), 100% water with 1% citric acid (C)

4.3.1 Optimization of total polyphenol content

For the determination of total polyphenolics, total anthocyanin content and antioxidant capacity from Aronia pomace extracts, different extraction conditions such as 50% ethanol with 1% citric acid (solvent A), 50% glycerol with 1% citric acid (solvent B), 100% water with 1% citric acid (solvent C), ultrasound assisted, temperature, and ultrasound exposure time used. With this, the following discussion for optimization consider the three solvents which are explained separately, as solvent A, solvent B, and solvent C along with their fitting model. Optimization is an effective approach to achieve the best solution, where the objective is maximizing the yield of TPC, FRAP, and TA through optimizing the extraction parameters temperature, time, and ultrasound exposure time. However, to optimize the extraction conditions, the factors that have significant effect on the TPC were identified and optimization performed. In this study temperature and USET had significant effect on solvent A, and solvent B, while 'time' were identified as insignificant with p-value ($p>0.05$). On the other hand, temperature was identified as the significant factor for solvent C as shown on the standard pareto chart, (figure B1 for solvent A, B, and C respectively), which graphically represented both the significant and insignificant factors. Standard Pareto chart is a graphical tool provides a visual representation of the extraction conditions making it easier to communicate, identify and prioritize the most significant factors contributing for extraction of

bioactive compounds from Aronia pomace. The standard Pareto chart is a powerful tool often used in optimization of the extraction conditions allowing researchers and scientists to identify the most significant factors, prioritize optimization efforts, and achieving the optimal extraction yield more efficiently. In pareto chart, the length of each bar is proportional to the value of the standardized effect it represents. The vertical line indicates the 95% confidence level statistically significant bound. Significant factors are those that surpass this reference line. Taking all of this into account, the same information mentioned above in the multivariate test was observed. The extraction of bioactive compounds from Aronia pomace involves the transfer of these compounds from the sample matrix into the solvent. The process is influenced by various factors, including temperature, water bath time, and sonication time. Therefore, In the context of the extraction of bioactive compounds, the Pareto chart could be used to identify and prioritize the factors that have the greatest impact on the yield of TPC, FRAP, and TA compounds. Temperature, time, and USET are important factors that can affect the extraction of bioactive compounds from the sample. Temperature had a high significant effect on the extraction yield shown in figure B1 (Annex B) on the Pareto chart; it influences the solubility of the bioactive compounds in the solvent, the diffusion rate, and the kinetic energy of the molecules. This is because the increasing the temperature can increase the amount of compounds that can be dissolved in the solvent as the solvent penetrate more easily, and faster mass transfer rate, resulting in higher extraction of bioactive compounds. while USET influence the extraction yield and the stability of the bioactive compounds by inducing cavitation, which generates microjets and shockwaves that can break down cell walls and increase the mass transfer of the bioactive compounds. However, the effect of ultrasound exposure on the extraction yield is highly dependent on various factors such as the duration of sonication, the nature of the solvent, and the property of pomace material. Hence, ultrasound exposure time had small significant effect compared to temperature on the extraction of TPC, FRAP, and TA shown in figure B1 on the Pareto chart. Water bath time is another factor that might not significantly affected the extraction yield as shown on the pareto chart. This is water bath often used to maintain a constant temperature during the extraction. Moreover, the Pareto chart suggest that temperature followed by ultrasound exposure time is the most significant factor affecting the extraction yield. Therefore, optimizing the temperature range should be a primary focus for improving the extraction yield. However, ultrasound exposure time is also a significant factor, and finding the optimal balance between temperature and sonication time is crucial for achieving the

highest yield of TPC, TA, and FRAP. Among the pomace extracts analyzed, the extract samples with solvent A the highest total phenolic content at 50 ℃ water bath temperature, for 60 minutes, and a 30-minute USET. Samples extracted with solvent B, the highest total polyphenolic content found as 40 ℃ water bath temperature for 60 minutes and a 15-minute USET. Besides for solvent C, it was that 60 ℃, 60 minutes, and 15 minutes of temperature of the water bath, duration of samples exposed to water bath and sonication time respectively. The lowest values of TPC extracted with solvent A was 60 ℃, 60 minutes in water bath, and ultrasound exposure time for 15 minutes, while TPC with temperature of 50 ℃, the extracts in water bath for 120 minutes, and 15 minutes of sonication time were the lowest for solvent B. And extracts using solvent C a temperature of water bath at 40 ℃, in 60 minutes, and for ultrasound exposure time of 15 minutes were the lowest content of total polyphenol. Total polyphenolic content in the extract samples ranges from 3017.68 to 3904.02 mg GAE/100 G FM using solvent A; and it is from 2925.75 to 3508.5 mg GAE/ 100g FM for solvent B, while solvent C was found from 816.1 to 1236.88 mg GAE/100 g FM. Simi et al. (2016) found the optimal extraction time and ethanol concentration for extracting the optimal TPC from whole chokeberries were 15 minutes and 53.8%, respectively. Cvetkovi et al. (2018) demonstrated that extraction conditions such as solvent type and concentration, reaction time, and temperature had a significant effect on total phenolic content and antioxidant activity in chokeberry leaves. The optimal extraction parameters for commercially available frozen chokeberries yield were 15 min of extraction time and 62.5% ethanol concentration (Simi et al., 2018). Xu et al. (2017) studied the optimal extract phenolic compounds from chokeberry pomace using ultrasound assisted. The authors used RSM to determine the optimal parameters, which were 69% ethanol concentration (from a range of 60-80%) and 50 minutes of sonication time (from a range of 40-60 minutes), allowing them to extract 68.15 mg GAE/g of TPC. Temperature and ultrasound exposure time were optimized and the fitting model developed. (Galvan d'Alessandro et al., 2012) showed the effect of various parameters extraction time, temperature, and solid-solvent ratio, with a clear effect of ultrasound up to 85% increase in extracted polyphenol yield. The presence of ethanol in the solvent, as well as the high temperature significantly improved the extraction process. The ultrasound helps to shorten the extraction time. In all water extraction experiments, 15 minutes of sonication time resulted in higher extraction yields than 60 minutes of extraction without ultrasound. The effect of solid–solvent ratio, water as a solvent 1:20 and 1:40 were found as suitable to obtain optimal yields

for the extraction of polyphenols from black chokeberry. The extraction of polyphenols from Aronia berries was studied at 60 ℃ from ground berries water as solvent. (Xu et al., 2017) optimal condition for the extraction of TPC: ethanol-water ratio of 0.69, ultrasonic duration of 52 minutes. Thus, different results discussed form different literatures, this study result agrees with the literatures discussed because of the extraction of bioactive compounds is greatly affected with the type and ratio of solvent used, the extraction method, and the genetic variability as well. Therefore, the optimization model for this study presented as follows: A second order polynomial equation in terms of factors with only significant terms was developed to study the relationship between total phenolic content and independent variables. Thus, the model form regression equation on total phenolic content (mg GAE/100g FM of different solvents as follows: TPC for solvent A = −8003 + 466.8 Temp. + 1.77 Time − 38.4 USET − 4.795 $Temp^2$. − 0.056 Temp.*Time + 1.118 Temp.*USET + 0.058 Time*USET

TPC solvent B= 14344 − 417.5 Temp. − 3.58 Time − 70.8 USET + 3.944 $Temp^2$. + 0.0111 Temp.*Time + 1.058 Temp.*USET + 0.1678 Time*USET

TPC solvent C= 570 − 6.2 Temp. + 0.35 Time + 21.2 USET + 0.220 $Temp^2$. + 0.0310 Temp.*Time − 0.300 Temp.*USET − 0.0569 Time*USET

The sign and magnitude of the coefficients in the above equation show the effect of independent variables on the total phenolic content of the extract.

4.4. Determination of Total Antioxidant Capacity

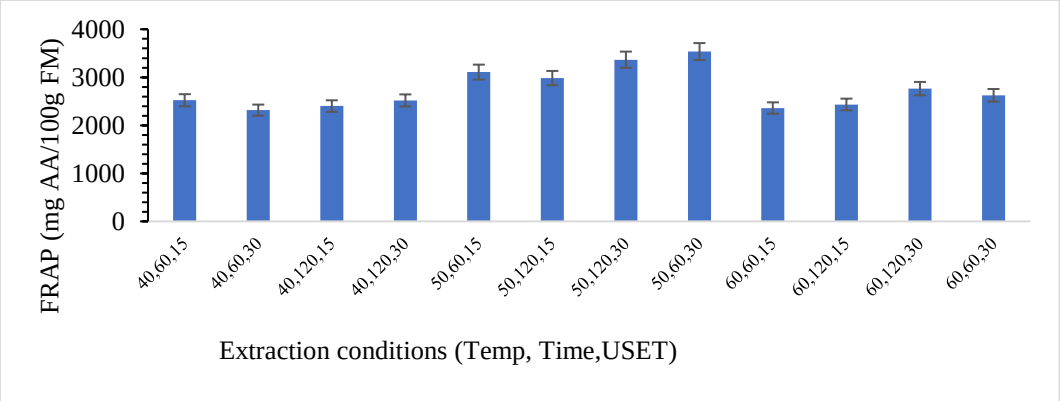
In comparison to juice and fruits, pomace has a much greater phenolic content. Chokeberry juice had a content of 1578.79 mg/100 g of DW, while pomace had a concentration of 8191.58 mg/100 g. Pomace had the highest antioxidant activity, as determined by TEAC, followed by fruit and juice (Oszmiański & Wojdylo, 2005)**.** The antioxidant capacities of the extracts are strongly related to the solvent being used, owing to the various antioxidant potential of compounds with different polarities (Moure et al., 2001). The study showed black mulberry extracts obtained with ethanol/water/acetic acid (50/49.5/0.5, v/v/v) had the highest antioxidant capacity with values of 1490.61 mmol $Fe2^+$/kg DW (Boeing et al., 2014). An assay called FRAP, or ferric reducing antioxidant power, is used to measure the antioxidant power. The assay is based on the rapid reduction in ferric-tripyridyltriazine ($Fe3^+$ -TPTZ) by antioxidants present in the samples forming

ferrous-tripyridyltriazine ($Fe2^{+}$ -TPTZ), a blue-colored product (Benzie & Strain, 1996). therefore, the antioxidant activity of pomaces was measured by the FRAP assay with different solvents (Table A8, A9, and A10). In this study, the ability of phenolic extracts from pomaces to scavenge the radical is measured using ascorbic acid as reference antioxidant compound. The total antioxidant capacity, FRAP for solvent A is presented in Table A8. The antioxidant capacity of the extract was studied as the ferric reducing antioxidant power, FRAP assay. The FRAP values were obtained from the calibration curve y = 0.2523x with $R^2 = 0.9992$, where x is the absorbance at 593 nm and y is the concentration of ascorbic acid (µg/mL). The FRAP content ranges from 2315.93 to 3535.27 mg AA/100g FM as shown in the Table A8. The FRAP content found from 2617.88 to 4039.01 mg AA/100g FM as shown in the Table A9 for the solvent 50% glycerol with 1% citric acid; and the FRAP content on 100% water with 1% citric acid solvent was found from 966.81 to 1693.84 mg AA/100g FM depicted in Table A10. As we see across the table there is a reasonable quantitative change on the content of FRAP which is due to the nature of the solvent and varying extraction conditions as temperature, time and ultrasound exposure time. The effect of temperature, extraction time, and USET on the antioxidant capacity shown in Figure 7. It was presented that the FRAP extracted in 50% ethanol with 1% citric acid increases as temperature and ultrasound exposure time, USET increases until a certain level but the highest extraction yield found at a temperature of 50 ℃, 60 minutes for water bath, and 30 minutes of ultrasound exposure time was 3535.27 mg AA/100g FM. The temperature at 40 ℃, extracts exposed for 60 minutes in water bath, and a 15 minutes of sonication time, the FRAP found as 2522.06 mg AA/100g FM; as the condition increases to, 50 ℃, 120 minutes, and a 30-minutes of sonication time, it's found that the FRAP content as 3366.53 mg AA/100g FM. As shown in in the multivariate test on Table A2 temperature and USET influences the extraction of FRAP with the solvent, 50% ethanol with 1% citric acid. As the USET set from 15 minutes to 30 minutes with keeping the temperature 40 ℃, and water bath time 60 minutes, the FRAP was found that 2315.93 mg AA/100g FM, while, the FRAP treated with 40 ℃, in 120 minutes of water bath time and a-15 minutes of ultrasound exposure time found as 2401.15 mg AA/100g FM; and at 40 ℃, in 120 minutes of water bath time, and a-30 minutes sonication time the FRAP was 2517.94 mg AA/100g FM. Deliberately, the temperature of the extract was set to 50 ℃ to study its effect along with other extraction conditions. In this case, the temperature kept at 50 ℃, but the water bath time and ultrasound exposure time switched as 60 minutes with 15 minutes of USET, 60 minutes with 30 minutes of USET, and the

same principle applied for 120 minutes too. The FRAP determined 3107.96 mg AA/100g FM for 50 ℃, 60 minutes of water bath, and 15 minutes of USET. On the other hand, the pomace extract treated with 50 ℃, exposed for 120 minutes in water bath, and 15 sonication time, the FRAP found as 2983.35 mg AA/100g FM. In addition, as the sonication time set to 30 minutes, with 120 minutes of water bath and 50 ℃ was 3366.53 mg AA/100g FM. The pomace was also treated by increasing the temperature and varying the water bath time and the ultrasound exposure time. Thus, the temperature set to 60 ℃, the time varying 60, and 120 minutes and the sonication time was 15, and 60 minutes which the extract exposed with different sonication and water bath time. Thus, the FRAP was presented as 2360.53 mg AA/100g FM at a temperature 60 ℃, 60 minutes, and a 15-minutes of USET. In addition, the FRAP yield found as 2764.77 mg AA/100g FM as the extract exposed to 60 ℃, 120 minutes, and 30 minutes of ultrasound exposure, while 2624.71 mg AA/100g FM were found at a temperature of 60 ℃, with 60 minutes of in water bath, and a 30 minutes of sonication time with the solvent, 50% ethanol with 1% citric acid solvent. On the contrary, the sample extracted with 50% glycerol with 1% citric acid showed that the FRAP is highly influenced by temperature and USET which had a significant impact as shown in the multivariate test in Table A4. The highest FRAP yield with this solvent found as 4039.01 mg AA/100g FM as shown in figure 9. At a temperature of 50 ℃, 120 minutes, and a 30-minutes of sonication time, the FRAP found as 2992.11 mg AA/100g FM. Multivariate test was made, shown on Table A6, temperature and USET had influence on extraction of FRAP in a 50% glycerol with 1% citric acid solvent. At a 30 minutes of sonication time, a temperature of 40 ℃, and water bath time 60 minutes, the FRAP was found that 3286.13 mg AA/100g FM, while with the same temperature 40℃, and the water bath time 120 minutes, the extract was treated with ultrasound assisted in 15 and 30 sonication time. The FRAP treated with 40 ℃, in 120 minutes of water bath time, and a-30 minutes sonication time the FRAP was 3055.08 mg AA/100g FM. The temperature of the extract was set to 50 ℃ and the FRAP determined 3139.43 mg AA/100g FM for 50 ℃, 60 minutes of water bath, and 15 minutes of USET; with same time and temperature but the sonication time set to 30 minutes 2883.34 mg AA/100G FM was found. On the other hand, the extract treated with 50 ℃, exposed for 120 minutes in water bath, and 15 minutes of sonication time, the FRAP presented as 3148.15 mg AA/100g FM. Similarly, at a temperature of 50 ℃, the sonication time 30 minutes, and a120 minutes of water it's found that 2992.11 mg AA/100g FM. The pomace was also extracted by increasing the temperature and changing the water bath time and the ultrasound

exposure time. In this case, the temperature was set to 60 ℃, the time varying 60, and 120 minutes and the sonication time was 15, and 60 minutes which the extract exposed with different sonication and water bath time. Thus, the FRAP was found as 2931.16 mg AA/100g FM at a temperature 60 ℃, 60 minutes, and a 15-minutes of USET. Meanwhile, sonication time was set to 30 minutes (60 ℃, 60 minutes of water bath, and 30 minutes for sonication) the FRAP found as 3166.09 mg AA/100g FM, though the FARP yield was found as 3121.87 mg AA/100g FM as the extract exposed to 60 ℃, 120 minutes, and 30 minutes of ultrasound exposure time. Besides, the FRAP extracted with100% water with 1% citric acid also shown in Figure 7. The temperature at 40 ℃, 60 minutes in water bath, and a 15 minutes of sonication time, the FRAP was found as 1458.71 mg AA/100g FM, with this, as the temperature set at 50 ℃, 120 minutes, and a 30-minutes of sonication time, we found the FRAP as 1063.73 mg AA/100g FM. As shown in the multivariate test on Table A6 USET and time had a significant effect on the extraction of FRAP with 100% water with 1% citric acid solvent. With sonication time of 30 minutes, temperature of 40 ℃, and water bath time 60 minutes, the FRAP was found that 1261.63 mg AA/100g FM. By keeping the temperature at 40 ℃, and the water bath time 120 minutes, the extract was treated with ultrasound assisted in 15 and 30 sonication time. The FRAP treated with 40 ℃, in 120 water bath time and a-15 minutes of ultrasound exposure time found as 1266.61 mg AA/100g FM; and at 40 ℃, in 120 minutes of water bath time, and a-30 minutes sonication time the FRAP was 966.81 mg AA/100g FM. The FRAP determined 1023.37 mg AA/100g FM for 50 ℃, 60 minutes of water bath, and 15 minutes of USET. And 1609.52 mg AA/100g FM for a temperature of 50 ℃, 60 minutes, and 30 minutes of sonication time. In contrast, the pomace extract treated with 50 ℃, exposed for 120 minutes in water bath, and 15 sonication time, the FRAP found as 1134.85 mg AA/100g FM. In addition, as the sonication time changed to 30 minutes, with 120 minutes of water bath and 50 ℃ was 1063.73 mg AA/100g FM. The pomace was also treated by increasing the temperature and varying the water bath time and the ultrasound exposure time. In this way, the temperature was set to 60 ℃, the time varying 60, and 120 minutes and the sonication time was 15, and 60 minutes which the extract exposed with different sonication and water bath time. Thus, the FRAP was presented as 1399.19 mg AA/100g FM at a temperature 60 ℃, 60 minutes, and a 15-minutes of USET. The FRAP yield was 1084.04 mg AA/100g FM as the extract exposed to 60 ℃, 120 minutes, and 30 minutes of ultrasound exposure time.

A.



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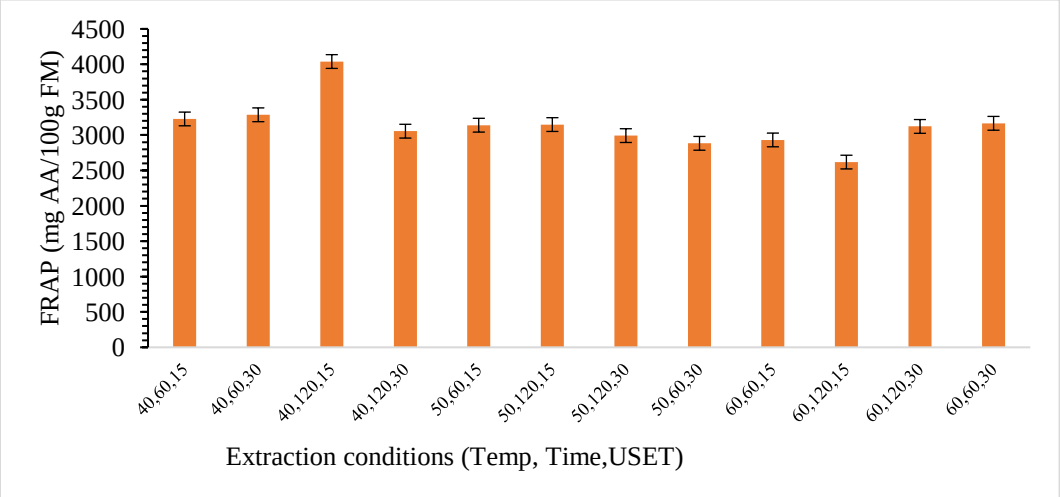

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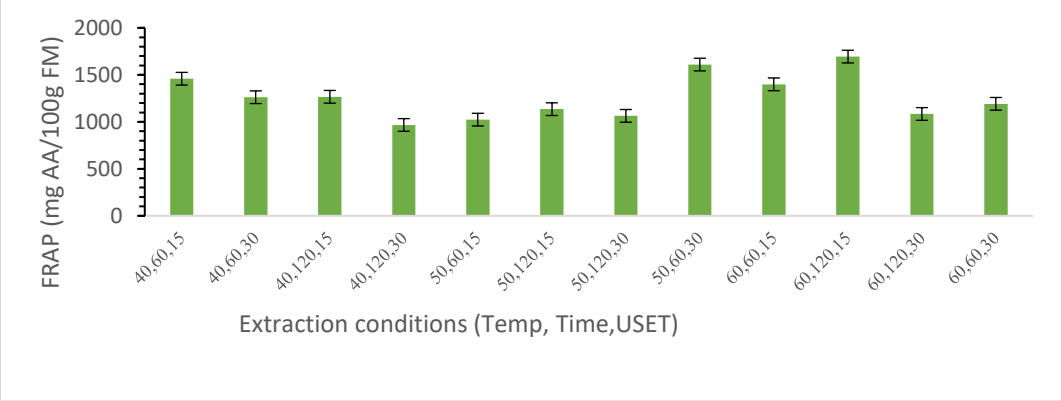


Figure 7. Effect of temperature, time, and USET (n=3) on FRAP yield: 50% ethanol with 1% citric acid (A), 50% glycerol with 1% citric acid (B), 100% water with 1% citric acid (C)

4.4.1. Optimization of total antioxidant capacity

Previous research indicated that 50% ethanol was the best ethanol - water combination for Aronia polyphenol extraction (Galvan d'Alessandro et al., 2012). (Clinical hospital Dubrava, Avenija Gojka Šuška 6, HR-10000 Zagreb, Croatia et al., 2015) analyzed chokeberry products and found that they contain a significant level of phenols (1494 to 5292 mg per 100 g of dry matter) but a small amount of total anthocyanins. (141 to 2468 mg per 100 g of dry matter). The standard pareto chart, (from figure 10 for solvent A, B, and C respectively) graphically showed the extraction parameters whether they are significant or insignificant. In this study temperature and USET had significant effect for antioxidant activity on solvent A, while temperature, and interaction of temperature and USET had significant effect on solvent B; also, the interaction of water bath time and USET were identified as significant factor for antioxidant activity in solvent C as shown in figure B2 (Annex B) on the pareto chart shown in figure 10 for solvent A, B, and C respectively. The chart graphically represented both the significant and insignificant factors. It is a graphical tool provides a visual representation of the extraction conditions making it easier to communicate, identify and prioritize the most significant factors contributing for extraction of bioactive compounds from Aronia pomace. The standard Pareto chart is a powerful tool often used in optimization of the extraction conditions allowing researchers and scientists to identify the most significant factors, prioritize optimization efforts, and achieving the optimal extraction yield more efficiently. In pareto chart, the length of each bar is proportional to the value of the standardized effect it represents. The vertical line indicates the 95% confidence level statistically significant bound. Significant factors are those that surpass this reference line. Taking all of this into account, the same information mentioned above in the multivariate test was observed. The extraction of bioactive compounds from Aronia pomace involves the transfer of these compounds from the sample matrix into the solvent. The process is influenced by various factors, including temperature, water bath time, and sonication time. Therefore, In the context of the extraction of bioactive compounds, the Pareto chart could be used to identify and prioritize the factors that have the greatest impact on the yield of TPC, FRAP, and TA compounds. Temperature, time, and USET are important factors that can affect the extraction of bioactive compounds from the sample. Temperature had a high significant effect on the extraction yield shown in figure B2 (Annex B) A, and B on the Pareto chart; it influences the solubility of the bioactive compounds in the solvent, the diffusion rate, and the kinetic energy of the molecules. This is because the increasing the

temperature can increase the amount of compounds that can be dissolved in the solvent as the solvent penetrate more easily, and faster mass transfer rate, resulting in higher extraction of bioactive compounds. while USET influence the extraction yield and the stability of the bioactive compounds by inducing cavitation, which generates microjets and shockwaves that can break down cell walls and increase the mass transfer of the bioactive compounds. However, the effect of ultrasound exposure on the extraction yield is highly dependent on various factors such as the duration of sonication, the nature of the solvent, and the property of pomace material. Hence, ultrasound exposure time had small significant effect compared to temperature on the extraction of TPC, FRAP, and TA shown in figure B2 on the Pareto chart. The water bath time on solvent C had significantly affected the antioxidant activity as shown on the pareto chart. This is might be the reason that water bath time could influence the rate of solvent penetration into the sample, and this can affect the amount of polyphenolic compounds extracted. Longer water bath times could lead to increased solvent penetration and subsequent extraction of more phenolic compounds, leading increase the antioxidant activity. Therefore, the Pareto chart can be a useful tool for analyzing the significancy of temperature, time and USET on the extraction of bioactive compounds. By prioritizing the factors that have the greatest impact on the yield, the chart can help optimize the extraction process and improve the yield. The highest ferric reducing antioxidant power, FRAP were found as the sample treated in a 50 ℃, 60 minutes in water bath, and a 30-minute of sonication time for solvent A, while a temperature of 40 ℃, for water bath 120 minutes and, a 15 minutes of ultrasound exposure time was for solvent B, and the temperature of the water bath was 60 ℃ for about 120 minutes and 15 minutes of sonication time for solvent C. Experimental values of FRAP yield for solvent A, B, and C obtained under different extraction conditions are shown in Table A8, A9, and A10 for solvent A, B, and C respectively. The highest value of the investigated response was 3535.27 mg AA/100g FM, 4039.01 mg AA/100g FM, and 1693.84 mg AA/100g FM for solvent A, B, and Solvent C respectively. (Jara-Palacios et al., 2019) stated that pomaces had a different qualitative and quantitative antioxidant activity and anthocyanin profile which depends on the type of berry. Experimental results were fitted to regression equation model and the equation could be expressed as:

FRAP (for solvent A) = −14449 + 730 Temp. − 5.6 Time − 53.9 USET − 7.56 $Temp^2$. + 0.055 Temp.*Time + 1.141 Temp.*USET + 0.128 Time*USET

FRAP (solvent B) = 8565 − 189 Temp. + 26.1 Time − 122.9 USET + 1.40 $Temp^2$. − 0.391 Temp.*Time + 2.774 Temp.*USET − 0.249 Time*USET

FRAP (solvent C) = 3184 − 90.5 Temp. − 6.39 Time + 56.6 USET + 0.825 $Temp^2$. + 0.281 Temp.*Time - 0.534 Temp.*USET − 0.431 Time*USET

The sign and magnitude of the coefficients in the above equation show the effect of independent variables on the ferric reducing antioxidant power of the pomace extract.

4.5. Determination of Total Anthocyanin Content

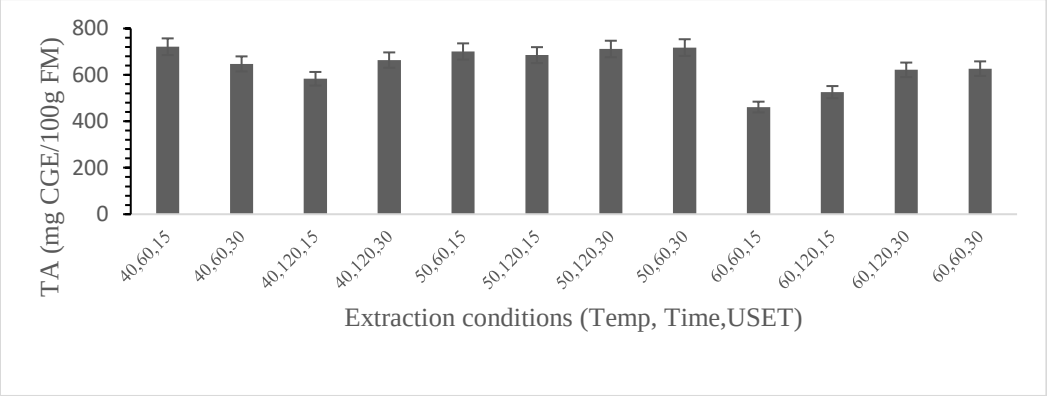
The polarity of the extracting solvents used had a significant impact on the extraction of Aronia anthocyanins. The extraction yields obtained with 50% ethanol were approximately three times greater than those obtained with aqueous extractions. Aronia anthocyanins are not stable at high temperatures; blueberry anthocyanins have been found to be thermally unstable. When the final extraction yields obtained at 20 and 70 ℃ with 50% ethanol are compared, it is clear that increasing the extraction temperature had no positive impact on the extraction yields (Kechinski et al., 2010). (D'Alessandro et al., 2014) showed the highest TPC at 70 ℃ with 50% ethanol, 74.28 TPC in 240 minutes. Highest TA at 45 ℃ with 25% ethanol, 13.08 TA in 240 minutes. The extraction rate decreased with time in all conditions examined. Ultrasound assisted and rise temperature improved polyphenol extraction in all investigated conditions (Jara-Palacios et al., 2019) showed blueberries had the anthocyanin content (1188 mg/100g. The total anthocyanin content of pomace extract determined using pH differential method with the absorbance of two different wavelengths 520 and 700 nm following (Lee et al., 2005) with some modification and the most abundant anthocyanins found in the extract cyanidin-3-glucoside, cyanide-galactoside, and cyanide-arabinose content were determined using High performance Liquid chromatography. The total anthocyanin content, TA for solvent A, 50% ethanol with 1% citric acid is shown in table A8. The total anthocyanin content of the pomace extract ranges from 626.98 to 721.36 mg CGE/100g FM as shown in the table A8. The TA content found from 456.18 to 660.45 mg CGE/100g FM as shown in the table A9 for the solvent B, 50% glycerol with 1% citric acid. Similarly, the total anthocyanin content with the solvent C, 100% water with 1% citric acid was found from 302.88 to 389.67 mg CGE/100g FM shown in table A10. As we see across the table a significant change observed on the content of total anthocyanin which is due to the nature of the solvent and varying extraction conditions as temperature, time and ultrasound exposure time. The total anthocyanin

content on extraction conditions of temperature, time and ultrasound exposure time for the three solvents are described in figure 8. The total anthocyanin, extracted in 100% ethanol with 1% citric acid which the highest TA yield found at a temperature of 40 ℃, 60 minutes of water bath, and 15 minutes of ultrasound exposure time was 721.36 mg CGE/100g FM; and as the condition increases to, 50 ℃, 120 minutes, and a 30-minutes of sonication time, it's found that the TA content as 647.43 mg CGE/100g FM. As shown in the multivariate test on Table 8 temperature and USET influences the extraction of TA with the solvent, 50% ethanol with 1% citric acid. As the USET set from 15 minutes to 30 minutes with keeping the temperature 40 ℃, and water bath time 60 minutes, the TA was found that 461.69 mg CGE/100g FM, while, the TA treated with 40 ℃, in 120 minutes of water bath time and a-15 minutes of ultrasound exposure time found as 525.77 mg CGE/100g FM; and at 40 ℃, in 120 minutes of water bath time, and a-30 minutes sonication time the TA was 583.63 mg CGE/100g FM. Purposely, the temperature of the extract was set to 50 ℃ to study its effect along with other extraction conditions. In this case, the temperature kept at 50 ℃, but the water bath time and ultrasound exposure time switched as 60 minutes with 15 minutes of USET, 60 minutes with 30 minutes of USET, and the same principle applied for 120 minutes too. The TA determined 622.05 mg CGE/100g FM for 50 ℃, 60 minutes of water bath, and 15 minutes of USET. On the other hand, the pomace extract treated with 50 ℃, exposed for 120 minutes in water bath, and 15 sonication time, the TA found as 626.98 mg CGE/100g FM. In addition, as the sonication time set to 30 minutes, with 120 minutes of water bath and 50 ℃ was 647.43 mg CGE/100g FM. The pomace was also treated by increasing the temperature and varying the water bath time and the ultrasound exposure time. Thus, the temperature set to 60 ℃, the time varying 60, and 120 minutes and the sonication time was 15, and 60 minutes which the extract exposed with different sonication and water bath time. Thus, the TA was presented as 685.28 mg CGE/100g FM at a temperature 60 ℃, 60 minutes, and a 15-minutes of USET. In addition, the TA yield found as 711.73 mg CGE/100g FM as the extract exposed to 60 ℃, 120 minutes, and 30 minutes of ultrasound exposure, while 717.84 mg CGE/100g FM were found at a temperature of 60 ℃, with 60 minutes of in water bath, and a 30 minutes of sonication time with the solvent, 50% ethanol with 1% citric acid solvent. On the contrary, the sample extracted with 50% glycerol with 1% citric acid showed that the TA was highly influenced by temperature and USET which had a significant impact as shown in the multivariate test in Table A9. Multivariate test was made as shown on Table A4, temperature and USET had an influence on extraction of TA in a 50% glycerol with 1%

citric acid solvent. Because of no toxicity, and low volatility glycerol used as an extraction solvent for biologically active compounds (Additives et al., 2017). (Kowalska et al., 2021) presented the highest anthocyanin concentration was found for a water-glycerol system with 50% glycerol concentration, at extraction temperatures of 20 ℃ and 50 ℃. At a temperature of 50 ℃, 120 minutes, and a 30-minutes of sonication time, the TA was found as 509.96 mg CGE/100g FM. At a 30 minutes of sonication time, a temperature of 40 ℃, and water bath time 60 minutes, the TA was found that 583.87 mg CGE/100g FM, while with the same temperature 40℃, and the water bath time 120 minutes, the extract was treated with ultrasound assisted in 15 and 30 sonication time. The TA treated with 40 ℃, in 120 minutes of water bath time, and a-30 minutes sonication time the TA was 586.51 mg CGE/100g FM. The temperature of the extract was set to 50 ℃ and the TA determined 529.59 mg CGE/100g FM for 50 ℃, 60 minutes of water bath, and 15 minutes of USET; with same time and temperature but the sonication time set to 30 minutes 457.99 mg CGE/100g FM was found. In contrast, the extract treated with 50 ℃, exposed for 120 minutes in water bath, and 15 minutes of sonication time, the TA presented as 577.97 mg CGE/100g FM. In the same way, at a temperature of 50 ℃, the sonication time 30 minutes, and a120 minutes of water it's found that 509.96 mg CGE/100g FM. The pomace was also extracted by increasing the temperature and changing the water bath time and the ultrasound exposure time. In this case, the temperature was set to 60 ℃, the time varying 60, and 120 minutes and the sonication time was 15, and 60 minutes which the extract exposed with different sonication and water bath time. Thus, the TA was found as 518.39 mg CGE/100g FM at a temperature 60 ℃, 60 minutes, and a 15-minutes of USET. For the meantime, sonication time was set to 30 minutes (60 ℃, 60 minutes of water bath, and 30 minutes for sonication) the TA found as 577.69 mg CGE/100g FM, though the TA yield was found as 456.18 mg CGE/100g FM as the extract exposed to 60 ℃, 120 minutes, and 30 minutes of ultrasound exposure time. In addition to other factors, the yield is affected by type of extraction solvents. (Roda-Serrat et al., 2021) the total anthocyanin concentration in the pomace was 62.8 5.5 mg/g DW (Dry Weight), as determined by a thorough extraction with acidified methanol. On the other case, the TA extracted with100% water with 1% citric acid shown in Figure 8 The temperature at 40 ℃, 60 minutes in water bath, and a 15 minutes of sonication time, the TA was found as 302.88 mg CGE/100g FM, with this, as the temperature set at 50 ℃, 120 minutes, and a 30-minutes of sonication time, we found the TA as 332.44 mg CGE/100g FM. As shown in the multivariate test on Table A6 temperature and time had a significant effect on the extraction of TA

in 100% water with 1% citric acid. With sonication time of 30 minutes, temperature of 40 ℃, and water bath time 60 minutes, the TA was found that 310.04 mg CGE/100g FM. By keeping the temperature at 40℃, and the water bath time 120 minutes, the extract was treated with ultrasound assisted in 15 and 30 sonication time. The TA treated with 40 ℃, in 120 water bath time and a-15 minutes of ultrasound exposure time found as 317.82 mg CGE/100g FM; and at 40 ℃, in 120 minutes of water bath time, and a-30 minutes sonication time the TA was 324.27 mg CGE/100g FM. The temperature of the extract was raised intentionally to 50 ℃ to study its effect along with other extraction conditions. In this case, the temperature kept at 50 ℃, but the water bath time and ultrasound exposure time changed and switched each other, meaning 60 minutes with 15 minutes of USET, 60 minutes with 30 minutes of USET, and the same principle applied for 120 minutes. The TA determined 389.67 mg CGE/100g FM for 50 ℃, 60 minutes of water bath, and 15 minutes of USET. And 350.28 mg CGE/100g FM for a temperature of 50 ℃, 60 minutes, and 30 minutes of sonication time. In contrast, the pomace extract treated with 50 ℃, exposed for 120 minutes in water bath, and 15 sonication time, the TA found as 334.56 mg CGE/100g FM. In addition, as the sonication time changed to 30 minutes, with 120 minutes of water bath and 50 ℃ was 332.44 mg CGE/100g FM. The pomace was also treated by increasing the temperature and varying the water bath time and the ultrasound exposure time. In this way, the temperature was set to 60 ℃, the time varying 60, and 120 minutes and the sonication time was 15, and 60 minutes which the extract exposed with different sonication and water bath time. Thus, the TA was presented as 374.25 mg CGE/100g FM at a temperature 60 ℃, 60 minutes, and a 15-minutes of USET. The TA yield was 332.72 mg CGE/100g FM as the extract exposed to 60 ℃, 120 minutes, and 30 minutes of ultrasound exposure time.

A.



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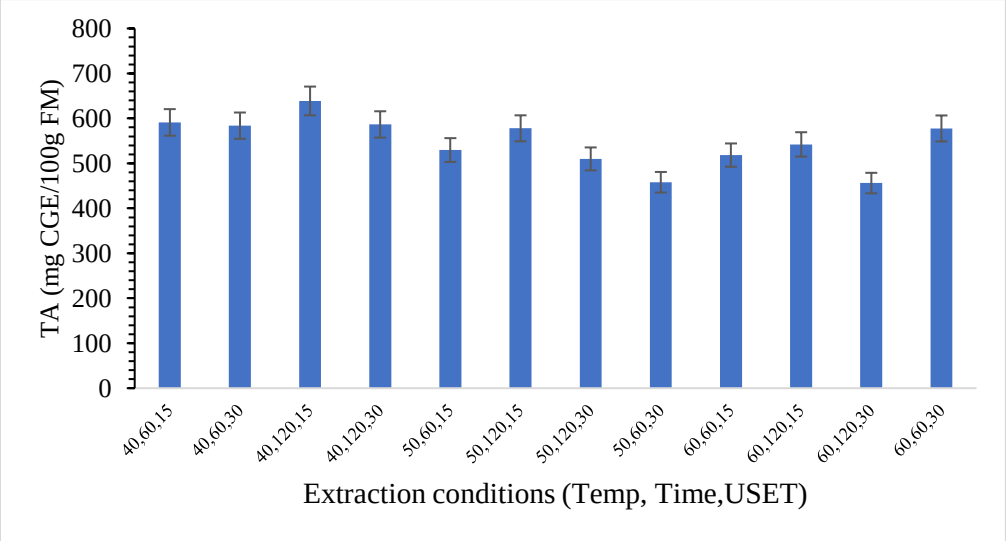

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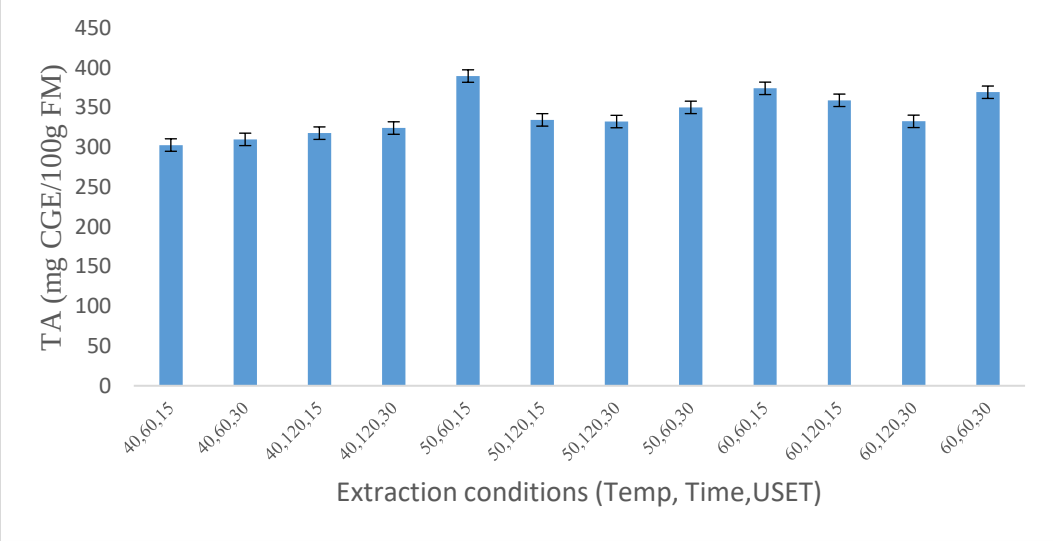

Figure 8. Effect of temperature, time, and USET (n=3) on TA yield: 50% ethanol with 1% citric acid (A), 50% glycerol with 1% citric acid (B), 100% water with 1% citric acid (C)

4.5.1 Optimization of total anthocyanin content

In optimization of extraction conditions to maximize the total anthocyanin content with different solvents from pomace extract the first and foremost was identifying the key parameters that are significant and insignificant on the extraction of compounds. The parameters having significance on extraction of total anthocyanin content with 50% ethanol with 1% citric acid was temperature, ultrasound exposure time, USET and their interaction effects as shown on figure B3 A; however, the parameter, time is insignificant, meaning it doesn`t have an effect on the extraction of total

anthocyanin with this solvent. Therefore, the optimization made by optimizing temperature and USET. On the other hand, the chart shown on B (in figure B3) the significant parameter on the extraction of TA with 50% glycerol and 1% citric acid is temperature. This was compared with the above multivariate test and it might have bit difference as the multivariate test showed temperature and USET could take as significant; thus, temperature and USET would optimize to maximize the TA content. In a similar way, temperature and time were optimized to maximize the total anthocyanin content using 100% water with 1% citric acid solvent. Moreover, the standard pareto chart from figure B3 with solvent A, B, and C respectively, graphically shows the significant and insignificant extraction conditions with their respective solvent. In this study temperature, time and USET along with their interaction had significant effect for TA on solvent A, while temperature had a significant effect on solvent B; also, with a great effect on temperature, and a minor effect on time observed positive effect for TA in solvent C as shown on pareto chart in figure B3. The chart graphically represented both the significant and insignificant factors. It is a graphical tool provides a visual representation of the extraction conditions making it easier to communicate, identify and prioritize the most significant factors contributing for extraction of bioactive compounds from Aronia pomace. The standard Pareto chart is a powerful tool often used in optimization of the extraction conditions allowing researchers and scientists to identify the most significant factors, prioritize optimization efforts, and achieving the optimal extraction yield more efficiently. In pareto chart, the length of each bar is proportional to the value of the standardized effect it represents. The vertical line indicates the 95% confidence level statistically significant bound. Significant factors are those that surpass this reference line. Taking all of this into account, the same information mentioned above in the multivariate test was observed. The extraction of bioactive compounds from Aronia pomace involves the transfer of these compounds from the sample matrix into the solvent. The process is influenced by various factors, including temperature, water bath time, and sonication time. Therefore, In the context of the extraction of bioactive compounds, the Pareto chart could be used to identify and prioritize the factors that have the greatest impact on the yield of TPC, FRAP, and TA compounds. Temperature, time, and USET are important factors that can affect the extraction of bioactive compounds from the sample. Temperature had a high significant effect on the extraction yield shown in figure B3 (Annex B) A, and B on the Pareto chart; it influences the solubility of the bioactive compounds in the solvent, the diffusion rate, and the kinetic energy of the molecules. This is because the increasing the

temperature can increase the amount of compounds that can be dissolved in the solvent as the solvent penetrate more easily, and faster mass transfer rate, resulting in higher extraction of bioactive compounds. while USET influence the extraction yield and the stability of the bioactive compounds by inducing cavitation, which generates microjets and shockwaves that can break down cell walls and increase the mass transfer of the bioactive compounds. However, the effect of ultrasound exposure on the extraction yield is highly dependent on various factors such as the duration of sonication, the nature of the solvent, and the property of pomace material. Hence, ultrasound exposure time had small significant effect compared to temperature on the extraction of TPC, FRAP, and TA shown in figure B3 on the Pareto chart. The water bath time on solvent A and C had a minor effect on TA as shown on the pareto chart. This is might be the reason that the water bath time can also affect the pH of the extraction solvent, and this can influence the stability and solubility of the anthocyanin compounds. Anthocyanins are most stable at acidic pH, and an increase in pH can lead to degradation or loss of anthocyanins.

Therefore, the water bath time could have affected the extraction of anthocyanin compounds by altering the pH of the extraction solvent. In addition, this might also be the reason that because anthocyanins are sensitive to temperature changes, and they can degrade or break down if exposed to high temperatures for prolonged periods. Thus, the water bath time could have affected the extraction of anthocyanin compounds by influencing the temperature of the extraction solvent. Therefore, by prioritizing the factors that have the effect on TA yield, the pareto chart can help to optimize the extraction process and maximize the yield. And the fitting model finally developed. The total anthocyanin content ranged from 461.69 to 721.36 mg CGE /100g FM for solvent A; 456.18 to 638.86 mg CGE/100g FM found extracted with solvent B; and 302.88 to 389.67 mg CGE/100g FM was found using solvent C. The extract samples with the highest total anthocyanin content had a 40 ℃ for 60 minutes, and 15 minutes of ultrasound exposure time. The highest TAC was found for solvent B as temperature of 40 ℃ for 120 minutes in water bath and 15 minutes of ultrasound exposure time. On the other hand, the total anthocyanin content with solvent C was found as 50 ℃, for 60 minutes in water bath and 15 minutes of sonication time; and the lowest total anthocyanin content for solvent A were a temperature of 60 ℃, for 60 minutes in water bath, and 15 minutes of ultrasound exposure time. The sample extracted with solvent B the lowest total anthocyanin content were 60 ℃, 120 minutes in water bath, and 30 minutes of sonication time, while extracts extract with solvent C the lowest total anthocyanin content were a temperature of

40 ℃, water bath time of 60 minutes and, ultrasound exposure duration of 15 minutes. Gao et al. (2016) studied that the ethanol concentration 56% was optimum for ultrasound extraction of phenolic compounds from chokeberry pulp. The best ultrasound-assisted extraction conditions for determining TAC in chokeberries, according to Chen et al. (2018), were 62% ethanol concentration and 44 min ultrasonic time. The authors obtained 4.319 mg C3GE/g of anthocyanins under these conditions (ultrasonic power 198 W and liquid-solid ratio 19 mL/g). (Roda-Serrat et al., 2021) studied the optimal conditions for maximizing both anthocyanin concentration and total anthocyanin content extracted from chokeberry juice pomace were 1.5 wt% citric acid, 45 °C, and 34 g solvent/g fresh pomace; while examined the operative parameters employed for batch extraction of chokeberry pomace that the optimal extraction temperature was about 70 ℃. Thus, different optimal extraction conditions set from different literatures this study agrees with the mentioned literatures as the method of extraction, and solvents governs for setting up the optimal extraction conditions. For instance, (D'Alessandro et al., 2014) found that extraction with pure water or a water-ethanol 50% vol solution, with or without sonication, resulted in a decrease in TA at 70 ℃. Table A8, A9, and A10 (Annex A) shows the experimental values of total anthocyanin content with solvent A, B, and C under different extraction conditions. The highest value of the investigated response was 721.36 mg CGE/100g FM, 638.86 mg CGE/100g FM, and 389.67 mg CGE/100g FM for solvent A, B, and Solvent C respectively. Experimental results were fitted to regression equation model and the equation could be expressed as:

TA (solvent A) = −661 + 76.2 Temp. − 4.78 Time − 20.97 USET − 0.973 $Temp^2$. + 0.0752 Temp.*Time + 0.426 Temp.*USET + 0.0350 Time*USET

TA (solvent B) = 2379 − 73.8 Temp. + 3.88 Time − 14.6 USET + 0.680 $Temp^2$. − 0.0473 Temp.*Time + 0.388 Temp.*USET − 0.0564 Time*USET

TA (solvent C) = −333 + 22.40 Temp. + 1.294 Time + 2.59 USET − 0.1542 $Temp^2$. − 0.0337 Temp.*Time − 0.0750 Temp.*USET + 0.0055 Time*USET

The sign and magnitude of the coefficients in the above equation show the effect of independent variables on the total anthocyanin content of the pomace extract.

4.6. HPLC Analysis

Anthocyanins in Aronia pomace samples were identified by using reversed phase-HPLC (Table. 7). For quantification of anthocyanins, standards were used and the content of individual anthocyanins were calculated using the standards. The main anthocyanins cyanidin- 3-galactoside, cyanidin-3-glucoside, and cyanidin-3-arabinoside were detected in Aronia pomace sample shown in (Figure 9); The RP-HPLC analysis showed three distinct peaks corresponding to those main three anthocyanins under investigation. The peak corresponding to cyanidin-3-galactoside eluted first, followed by the peak corresponding to cyanidin-3-glucoside, and finally, the peak corresponding to cyanidin-3-arabinoside. The order of the anthocyanin peaks observed in this study is consistent with previous reports of the elution order of anthocyanins on reversed phase-HPLC (Meng et al., 2019), (CAPANOGLU, 2013). The elution order is determined by the hydrophobicity and degree of glycosylation of the anthocyanins. Cyanidin-3-galactoside is the most hydrophobic and least glycosylated of the three anthocyanins studied, which explains why it eluted first. Cyanidin-3-arabinoside, on the other hand, is the least hydrophobic and most glycosylated, which explains why it eluted last. Cyanidin-3-glucoside is intermediate in terms of hydrophobicity and glycosylation, which explains why it eluted between the other two peaks. Aronia pomace is a rich source of anthocyanins, and the order of the anthocyanin peaks observed in this study provides important information for the quantification and characterization of these compounds in Aronia pomace. The chromatographic pattern of anthocyanins was compared to others described in the literature, and it showed highly similar characteristics to the respective chromatograms published by (Mayer-Miebach et al., 2012), (Veberic et al., 2015), (Wilkes et al., 2014), and (CAPANOGLU, 2013). Therefore, the anthocyanin peaks using reversed phase-HPLC was cyanidin-3-galactoside, cyanidin-3-glucoside, and cyanidin-3-arabinoside in order, which is consistent with previous reports. The following figure (9) shows the HPLC chromatogram of Aronia pomace recorded at 520 nm.

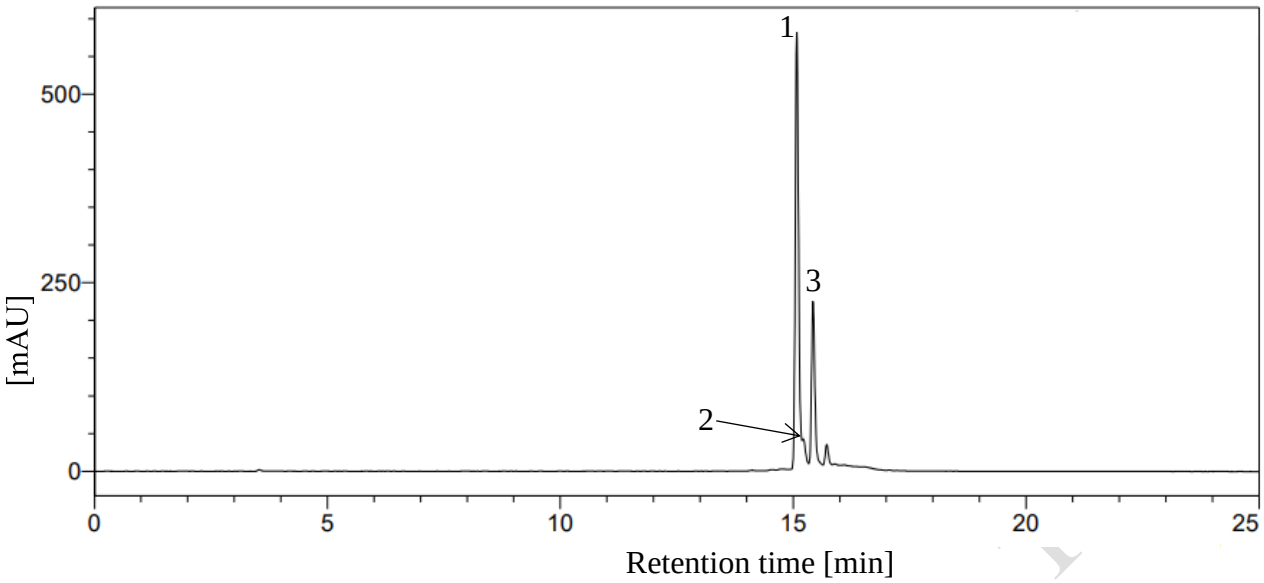

Figure 9. HPLC chromatogram of Aronia pomace recorded at 520 nm. 1 – cyanidin-3-galactoside, 2 – cyanidin-3-glucoside, 3 – cyanidin-3-arabinoside.

Table 7. Individual Anthocyanin contents in Aronia pomace samples

Samples	Cyanidin-3-galactoside	Cyanidin-3-glucoside	Cyanidin-3-arabinoside	Sum of identified anthocyanin components
	(mg/100g FW)	(mg/100g FW)	(mg/100g FW)	(mg/100g FW)
Pomace 111	210.5 ± 256.5	14.1 ± 16.5	93.4 ± 115.6	318.0
Pomace 212	216.5 ± 272.5	14.5 ± 18.6	98.9 ± 124.1	330.0
Pomace 221	250.1 ± 340.8	16.7 ± 22.9	103.6 ± 138.5	370.4
Pomace 222	207.3 ± 267.9	13.8 ± 17.7	94.1 ± 121.0	315.1
Pomace 211	229.4 ± 291.2	15.3 ± 19.5	102.2 ± 131.5	346.9

4.7 Color Measurement

Food colorants derived from natural sources that can replace the usage of synthetic dyes are in high demand. Because anthocyanin-rich byproducts are recognized as natural pigment sources, they have the potential to be used as natural colorants.

In this regard, measuring the color of Aronia pomace extracts is very useful for determining the optimal chromatic characteristics that define the extract color. As a result, the extract color could be used as important parameter concerning the preferences and quality of final product in areas of food industries and pharmaceuticals. The sample was extracted with different solvents and the total color difference, ΔE examined. The overall experimental L*, a*, and b* values of the pomace extract varied from –0.6 -2.8, –1.9-1.1, –0.5-0.5 respectively. The sample extracted with 50% ethanol with 1% citric acid solvent was compared with the sample extracted with 50% glycerol with 1% citric acid solvent because of each sample was treated with different extraction conditions. Their ΔL*, Δa*, Δb*, and their total color difference, ΔE* have shown in tables (Annex A11 to A13). In this scenario the total color difference was found as ΔE* = 1.3, which is only experienced observer can notice the color difference. On the other hand, the extract treated with 50%ethanol with 1% citric acid evaluated with the solvent 100% water with 1% citric acid. The total color difference found as ΔE* = 1.0; meaning, not perceptible by human eyes or observer does not notice the difference. However, the total color difference of the extract extracted with 50% glycerol with 1% citric acid and 100% water with 1% citric acid the total color difference was presented as ΔE* = 1.4 which is only experienced observer can notice the difference. Several studies have shown that different drying temperatures, drying processes, air flow rate, drying time, poor stability of pigmented compounds, oxidative reactions affected the color of dried product. (Kim et al., 2021) stated how the type of solvent used affects the color characteristics. (Ochmian et al., 2012) showed that the color of L*, a* and b* for cultivars of macerated chokeberry fruits were 14.13, 5.19, −12.98 respectively. (Samoticha et al., 2016) showed that the color values of L*, a* and b* for the whole freeze dried black chokeberry were 30, 10.9 and 3.7, respectively. (Zielinska & Michalska, 2018) studied the color properties of blue berry pomace using hot air convective drying at 60 °C and found the values as L* 31.97 ± 0.14, a*1.50 ± 0.12, b* 1.56 ± 0.09, and the total color difference, ΔE* 2.27 ± 0.37. The variations between these studies, and our studies could be due to the different in genetic types of black chokeberry, drying process, and type of solvent used.

5. SUMMARY

Aronia pomace is a byproduct of the juice-making process that contains a wide range of bioactive compounds such as polyphenols, anthocyanins, and antioxidants that contribute to its color, flavor, and health benefits. Optimization of ultrasound-assisted extraction (UAE) involves determining the optimal extraction conditions for extracting bioactive compounds from Aronia pomace using UAE technology. The extraction conditions involved are type and concentration of solvent, sonication time, water bath time, temperature, and the ratio of Aronia pomace sample to solvent. These conditions could significantly affect the yield, and the optimal conditions vary depending on the target bioactive compounds. In UAE, Aronia pomace sample is mixed with the solvent and subjected to ultrasonic waves, which create high-pressure waves that cause the formation of microscopic bubbles. It is a green and sustainable technique that can be applied to a variety of plant materials, including Aronia pomace. UAE improves extraction efficiency, allowing extraction of bioactive compounds from Aronia pomace with improved yield. The extracted bio active compounds could have applications in food, pharmaceutical, and cosmetic industries, and the research could help to the development of ways of utilizing fruit by-products efficiently. Ultrasound-assisted extraction of bioactive compounds from Aronia pomace aimed to optimize extraction conditions through investigate the efficiency of extraction conditions with focusing on color parameters, anthocyanin content, polyphenol concentration, and, antioxidant capacity. The thesis investigated the effects of ultrasound parameters such as sonication time on the extraction efficiency of bioactive compounds from Aronia pomace. Various extraction solvents ethanol, glycerol, citric acid, and water with their respective proportion were used to evaluate their effectiveness in extracting the target bioactive compounds. The extracted compounds total polyphenol content, total anthocyanin content, and the antioxidant activity were characterized and quantified using UV/Vis. spectrophotometry and the main anthocyanin content were quantified using the analytical technique high-performance liquid chromatography (HPLC). The obtained results allowed optimizing the conditions for the extraction of from Aronia pomace by using a technique considered as environmentally friendly. The optimal extraction conditions treated with 50% ethanol with 1% citric acid, total phenolic content (3904.02 mg GAE/100 g FM) and antioxidant capacity (3535.27 mg AA/100 g FM) were 50 ℃, 60 minutes water bath time, and 30 minutes of sonication time; while total anthocyanin content (721.36 mg CGE/100g FM) were 40

℃, 60 minutes water bath time, and 15 minutes of sonication time. The optimal extraction conditions treated with 50% glycerol with 1% citric acid, total phenolic content (3508.5 mg GAE/100 g FM) were 40 ℃, a 60-minute water bath time, and 15 minutes of sonication time; while antioxidant capacity (4039.01 mg AA/100 g FM) and total anthocyanin content (638.86 mg CGE/100g FM) were 40 ℃, 120 minutes water bath time, and 15 minutes of sonication time. Besides, the optimal extraction conditions treated with 100% water with 1% citric acid, total phenolic content (1236.88 mg GAE/100 g FM) and antioxidant capacity (1693.84 mg AA/100 g FM) were 60 ℃, 120 minutes water bath time, and 15 minutes of sonication time; while total anthocyanin content (389.67mg CGE/100g FM) were 50 ℃, 60 minutes water bath time, and 15 minutes of sonication time. The results of this study could have important implications for the food and beverage industries, as the use of ultrasound-assisted extraction could significantly boost the yield and quality of bioactive compounds from Aronia pomace. The main anthocyanins cyanidin-3-galactoside, cyanidin-3-glucoside, and cyanidin-3-arabinoside were detected in Aronia pomace sample using reversed phase-High Performance Liquid Chromatography (HPLC). Color analysis were done and the color difference evaluates the degree of color change between two samples (reference sample and test sample) in the color analysis of bioactive compounds in Aronia pomace. from Aronia pomace extract under optimal conditions. The extraction conditions that had maximum influence were temperature, and ultrasound exposure time. It can be concluded from the result that the extraction conditions assisted with ultrasound exposure can effectively, easily, and quickly extract bioactive compounds with optimal yield.

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ANNEXES: TABLES AND FIGURES

ANNEX IA TABLES:

Table A1 Pairwise Pearson Correlations for solvent 50% ethanol with 1% citric acid

1	2	N	Correlation	95% CI for ρ	P-Value
FRAP	TA	108	0.469	(0.307, 0.604)	0.000
TPC	TA	108	0.506	(0.351, 0.634)	0.000
TPC	FRAP	108	0.467	(0.305, 0.603)	0.000

Table A2. Multivariate tests of between effects for solvent 50% ethanol with 1% citric acid

Factor	Variables	DF	F-value	P-value
Temp	TA	2	55.541	0.000
	FRAP	2	28.047	0.000
	TPC	2	27.789	0.000
Time	TA	1	1.504	0.223
	FRAP	1	0.000	0.998
	TPC	1	0.081	0.776
USET	TA	1	20.722	0.000
	FRAP	1	5.240	0.024
	TPC	1	29.558	0.000
Temp * Time	TA	2	5.245	0.007
	FRAP	2	0.627	0.536
	TPC	2	0.759	0.471
Temp * USET	TA	2	12.204	0.000
	FRAP	2	2.003	0.141
	TPC	2	3.911	0.023
	TA	1	1.911	0.170
Time * USET	FRAP	1	0.361	0.549
	TPC	1	0.175	0.677
Temp * Time *	TA	2	8.219	0.001
USET	FRAP	2	0.322	0.726
	TPC	2	1.054	0.352

Table A3. Pairwise Pearson Correlations for solvent 50% glycerol with 1% citric acid

Sample 1	Sample 2	N	Correlation	95% CI for ρ	P-Value
FRAP	TA	108	0.403	(0.232, 0.550)	0.000
TPC	TA	108	0.248	(0.062, 0.417)	0.010
TPC	FRAP	108	0.308	(0.127, 0.470)	0.001

Table A4. Multivariate tests of between effects for solvent 50% glycerol with 1% citric acid

Factors	Variables	DF	F-value	P-value
Temperature	TA	2	3.284	0.042
	FRAP	2	7.450	0.001
	TPC	2	36.632	0.001
Time	TA	1	0.307	0.581
	FRAP	1	0.324	0.571
	TPC	1	1.059	0.306
USET	TA	1	0.026	0.872
	FRAP	1	1.002	0.319
	TPC	1	0.943	0.334
Temp*Time	TA	2	0.839	0.435
	FRAP	2	1.844	0.164
	TPC	2	0.644	0.527
Temp*USET	TA	2	3.167	0.047
	FRAP	2	6.092	0.003
	TPC	2	4.865	0.010
Time*USET	TA	1	0.924	0.339
	FRAP	1	1.268	0.263
	TPC	1	3.004	0.086
Temp*Time*USET	TA	2	0.393	0.676
	FRAP	2	4.269	0.017
	USET	2	0.536	0.587

Table A5. Pairwise Pearson Correlations for solvent 100% water with 1% citric acid

1	2	N	Correlation	95% CI for ρ	P-Value
FRAP	TA	108	0.053	(-0.137, 0.240)	0.583
TPC	TA	108	0.598	(0.462, 0.707)	0.000
TPC	FRAP	108	0.149	(-0.041, 0.329)	0.123

Table A6. Multivariate tests of between effects for solvent 100% water with 1% citric acid

Factor	Variable	DF	F-value	P-value
Temp	TA	2	18.974	0.000
	FRAP	2	1.415	0.248
	TPC	2	27.744	0.000
Time	TA	1	6.123	0.015
	FRAP	1	3.205	0.077
	TPC	1	2.060	0.154
USET	TA	1	2.362	0.128
	FRAP	1	3.794	0.054
	TPC	1	0.385	0.537
Temp * Time	TA	2	5.852	0.004
	FRAP	2	2.504	0.087
	TPC	2	0.649	0.525
Temp * USET	TA	2	1.735	0.182
	FRAP	2	8.620	0.000
	TPC	2	1.747	0.180
Time * USET	TA	1	0.151	0.699
	FRAP	1	8.029	0.006
	TPC	1	0.960	0.330
Temp * Time * USET	TA	2	1.795	0.172
	FRAP	2	1.374	0.258
	TPC	2	4.991	0.009

Table A7. Pairwise Pearson Correlations; (three Solvents)

1	2	N	Correlation (R^2)	95% CI for ρ	P-Value
FRAP	TA	324	0.717	(0.659, 0.766)	0.000
TPC	TA	324	0.804	(0.762, 0.839)	0.000
TPC	FRAP	324	0.824	(0.786, 0.856)	0.000

Table A8. UAE of bioactive compounds with solvent 50% ethanol with 1% citric acid

	Extraction conditions			Analytical results		
Sample ID	Temp. ℃	Time minute	USET minute	TA mg CGE/100g FM	FRAP mg AA/100g FM	TPC mg GAE/100g FM
A2111	40	60	15	721.36 ± 85.4	2522.06 ± 45.1	3112.5 ± 38.28
A2112	40	60	30	647.43 ± 44.4	2315.93 ± 30.64	3215.37 ± 15.96
A2113	40	120	15	583.63 ± 75.9	2401.15± 48.87	3066.39 ± 24.73
A2114	40	120	30	663.81 ± 67.1	2517.94± 41.31	3466.46 ± 27.52
A2115	50	60	15	700.82 ± 32.4	3107.96 ± 42.78	3718.46 ± 23.75
A2116	50	120	15	685.28 ± 25.4	2983.35 ± 47.12	3633.74 ± 31.28
A2117	50	120	30	711.73 ± 62.2	3366.53 ± 47.83	3819.82 ± 39.78
A2118	50	60	30	717.84 ± 82.4	3535.27 ± 72.62	3904.02 ± 37.88
A2119	60	60	15	461.69 ± 24.6	2360.53 ± 66.86	3017.68 ± 27.61
A2120	60	120	15	525.77 ± 79.4	2433.91 ± 41.96	3123.46 ± 15.98
A2121	60	120	30	622.05 ± 27.9	2764.77 ± 48.88	3640.09 ± 55.18
A2122	60	60	30	626.98 ± 50.1	2624.71 ± 51.42	3674.55 ± 34.1

Table A9. UAE of bioactive compounds with solvent 50% glycerol with 1% citric acid

	Extraction conditions			Analytical results		
	Temp.	Time	USET	TA	FRAP	TPC
Sample ID	℃	minute	minute	mg CGE/100g FM	mg AA/100g FM	mg GAE/100g FM
B4111	40	60	15	591.07 ± 12.4	3227.6 ± 37.16	3508.5 ± 29.26
B4112	40	60	30	583.87 ± 81	3286.13 ± 42.81	3158.4 ± 83.88
B4113	40	120	15	638.86 ± 24.2	4039.01 ± 58.15	3461.26 ± 39.15
B4114	40	120	30	586.51 ± 66.6	3055.08 ± 35.03	3351.48 ± 28.94
B4115	50	60	15	529.59 ± 18.7	3139.43 ± 73.32	3043.03 ± 20.96
B4116	50	120	15	577.97 ± 35.6	3148.15 ± 54.42	2925.75 ± 41.42
B4117	50	120	30	509.96 ±13.6	2992.11 ± 54.19	3034.05 ± 20.96
B4118	50	60	30	457.99 ± 32.3	2883.34 ± 63.08	2966.14 ± 21.19
B4119	60	60	15	518.39 ± 29.6	2931.16 ± 39.82	3323.39 ± 77.89
B4120	60	120	15	542.16 ± 67.7	2617.88 ± 58.25	3395.91 ± 57.04
B4121	60	120	30	573.63 ± 89.8	3121.87 ± 58.19	3496.96 ± 96.51
B4122	60	60	30	660.45 ± 18.7	3166.09 ± 31.5	3397.02 ± 45.71

Table A10. UAE of bioactive compounds with solvent 100% water with 1% citric acid

	Extraction conditions			Analytical results		
Sample ID	Temp. ℃	Time minute	USET minute	TA mg CGE/100g FM	FRAP mg AA/100g FM	TPC mg GAE/100g FM
C6111	40	60	15	302.88 ± 28.4	1458.71 ± 52.37	816.1 ± 48.81
C6112	40	60	30	310.04 ± 28.4	1261.63 ± 32.53	966.71 ± 19.25
C6113	40	120	15	317.82 ± 38.9	1266.61 ± 25.55	919.72 ± 99.85
C6114	40	120	30	324.27 ± 26.8	966.81 ± 25.39	937.03 ± 85.13
C6115	50	60	15	389.67 ± 51.5	1023.37 ± 23.15	1065.05 ± 35.78
C6116	50	120	15	334.56 ± 13.7	1134.85 ± 22.48	976.79 ± 31.34
C6117	50	120	30	332.44 ± 30.5	1063.73 ± 13.79	1037.18 ± 10.6
C6118	50	60	30	350.28 ± 16.7	1609.52 ± 55.7	946.34 ± 95.64
C6119	60	60	15	374.25 ± 11.7	1399.19 ± 20.77	1062.98 ± 10.42
C6120	60	120	15	359.2 ± 29.5	1693.84 ± 58.77	1236.88 ± 13.39
C6121	60	120	30	332.72 ± 64.7	1084.04 ± 23.19	1131.01 ± 24.72
C6122	60	60	30	369.35 ± 17.6	1191.81 ± 17.79	1156.49 ± 15.32

Table A11. summary of results obtained from color evaluation of Aronia pomace extracts extracted with 50% ethanol with 1% citric acid, and 50% glycerol with 1% citric acid.

	GL111				GL112				GL121				GL122				GL211				GL221			
	ΔL*	Δa*	Δb*	ΔE*	ΔL*	Δa*	Δb*	ΔE*	ΔL*	Δa*	Δb*	ΔE*	ΔL*	Δa*	Δb*	ΔE*	ΔL*	Δa*	Δb*	ΔE*	ΔL*	Δa*	Δb*	ΔE*
Et111	1.5	-1.6	-0.6	2.3	2.6	-0.5	-0.3	2.6	2.4	-0.1	0.1	2.4	1.2	-0.5	-0.1	1.3	0.5	-0.7	-0.3	0.9	0.4	-0.2	0.0	0.4
Et112	1.6	-1.5	-0.7	2.3	2.6	-0.4	-0.4	2.6	2.4	0.0	0.0	2.4	1.2	-0.4	-0.2	1.3	0.5	-0.6	-0.3	0.8	0.4	-0.1	-0.1	0.4
Et121	1.6	-1.5	-0.6	2.3	2.7	-0.4	-0.3	2.7	2.5	0.0	0.1	2.5	1.3	-0.4	-0.1	1.3	0.6	-0.6	-0.3	0.9	0.4	-0.2	0.0	0.5
Et122	1.7	-1.7	-0.7	2.5	2.8	-0.6	-0.4	2.8	2.6	-0.1	0.0	2.6	1.4	-0.6	-0.2	1.5	0.7	-0.7	-0.3	1.1	0.5	-0.3	-0.1	0.6
Et211	1.6	-1.7	-0.7	2.4	2.6	-0.6	-0.4	2.7	2.5	-0.1	0.0	2.5	1.3	-0.5	-0.2	1.4	0.6	-0.7	-0.4	1.0	0.4	-0.3	-0.1	0.5
Et221	1.5	-1.8	-0.7	2.4	2.5	-0.7	-0.4	2.6	2.3	-0.2	0.0	2.3	1.1	-0.7	-0.1	1.3	0.4	-0.8	-0.3	1.0	0.3	-0.4	-0.1	0.5
Et222	1.3	-1.7	-0.7	2.2	2.4	-0.6	-0.4	2.5	2.2	-0.1	0.0	2.2	1.0	-0.6	-0.2	1.1	0.3	-0.7	-0.3	0.9	0.2	-0.3	-0.1	0.4
Et212	1.5	-1.9	-0.7	2.5	2.5	-0.8	-0.4	2.7	2.3	-0.4	0.0	2.4	1.1	-0.8	-0.2	1.4	0.5	-1.0	-0.4	1.2	0.3	-0.6	-0.1	0.7
Et311	1.6	-1.4	-0.6	2.2	2.6	-0.3	-0.3	2.6	2.4	0.2	0.1	2.5	1.2	-0.2	-0.1	1.3	0.6	-0.4	-0.2	0.7	0.4	0.0	0.0	0.4
Et321	1.7	-1.3	-0.6	2.2	2.7	-0.2	-0.3	2.7	2.5	0.3	0.1	2.6	1.3	-0.1	-0.1	1.3	0.7	-0.3	-0.2	0.8	0.5	0.1	0.0	0.5
Et322	1.7	-1.7	-0.7	2.5	2.7	-0.6	-0.5	2.8	2.5	-0.2	-0.1	2.6	1.3	-0.6	-0.2	1.5	0.7	-0.8	-0.4	1.1	0.5	-0.4	-0.2	0.7
Et312	1.7	-1.5	-0.6	2.4	2.7	-0.4	-0.4	2.8	2.6	0.0	0.0	2.6	1.4	-0.4	-0.1	1.4	0.7	-0.6	-0.3	1.0	0.5	-0.2	-0.1	0.6

	GL222				GL212				GL311				GL321				GL322				GL312				
	ΔL*	Δa*	Δb*	ΔE*	ΔL*	Δa*	Δb*	ΔE*	ΔL*	Δa*	Δb*	ΔE*	ΔL*	Δa*	Δb*	ΔE*	ΔL*	Δa*	Δb*	ΔE*	ΔL*	Δa*	Δb*	ΔE*	
Et111	0.4	-0.2	0.0	0.5	0.3	-0.3	0.0	0.5	0.5	-0.3	-0.1	0.6	0.6	-0.3	0.0	0.71	0.3	-0.3	-0.1	0.5	0.6	-0.4	-0.1	0.72	
Et112	0.4	-0.1	-0.1	0.4	0.4	-0.2	-0.1	0.4	0.5	-0.2	-0.1	0.6	0.7	-0.2	-0.1	0.69	0.3	-0.2	-0.1	0.4	0.6	-0.3	-0.1	0.69	
Et121	0.5	-0.2	-0.1	0.5	0.4	-0.2	0.0	0.5	0.6	-0.3	-0.1	0.6	0.7	-0.2	0.0	0.76	0.4	-0.3	-0.1	0.5	0.7	-0.3	-0.1	0.76	
Et122	0.6	-0.3	-0.1	0.7	0.5	-0.4	-0.1	0.7	0.7	-0.4	-0.1	0.8	0.8	-0.4	-0.1	0.91	0.5	-0.4	-0.1	0.7	0.8	-0.5	-0.1	0.92	**ΔE* 1.3**
Et211	0.5	-0.3	-0.1	0.6	0.4	-0.4	-0.1	0.6	0.5	-0.4	-0.1	0.7	0.7	-0.4	-0.1	0.79	0.4	-0.4	-0.1	0.6	0.7	-0.5	-0.1	0.81	
Et221	0.3	-0.4	-0.1	0.5	0.3	-0.5	-0.1	0.6	0.4	-0.5	-0.1	0.7	0.6	-0.5	0.0	0.74	0.3	-0.5	-0.1	0.6	0.5	-0.6	-0.1	0.78	
Et222	0.2	-0.3	-0.1	0.4	0.1	-0.4	-0.1	0.4	0.3	-0.4	-0.1	0.5	0.4	-0.4	-0.1	0.58	0.1	-0.4	-0.1	0.4	0.4	-0.5	-0.1	0.62	
Et212	0.3	-0.6	-0.2	0.7	0.3	-0.7	-0.1	0.7	0.4	-0.7	-0.2	0.8	0.6	-0.6	-0.1	0.88	0.3	-0.7	-0.2	0.7	0.5	-0.7	-0.2	0.93	
Et311	0.4	0.0	0.0	0.4	0.4	-0.1	0.0	0.4	0.5	-0.1	0.0	0.5	0.7	-0.1	0.0	0.69	0.4	-0.1	0.0	0.4	0.6	-0.1	0.0	0.66	
Et321	0.5	0.1	0.0	0.6	0.5	0.0	0.0	0.5	0.6	0.0	0.0	0.6	0.8	0.0	0.0	0.79	0.5	0.0	0.0	0.5	0.7	0.0	0.0	0.74	
Et322	0.6	-0.4	-0.2	0.7	0.5	-0.5	-0.2	0.7	0.6	-0.5	-0.2	0.8	0.8	-0.5	-0.1	0.92	0.5	-0.5	-0.2	0.7	0.8	-0.5	-0.2	0.95	
Et312	0.6	-0.2	-0.1	0.6	0.5	-0.3	-0.1	0.6	0.7	-0.3	-0.1	0.7	0.8	-0.3	0.0	0.85	0.5	-0.3	-0.1	0.6	0.8	-0.3	-0.1	0.84	

Table A12. summary of results obtained from color evaluation of Aronia pomace extracts extracted with 50% ethanol with 1% citric acid, and 100% water with 1% citric acid.

	W111				W112				W121				W122				W211				W221			
	ΔL*	Δa*	Δb*	ΔE*	ΔL*	Δa*	Δb*	ΔE*	ΔL*	Δa*	Δb*	ΔE*	ΔL*	Δa*	Δb*	ΔE*	ΔL*	Δa*	Δb*	ΔE*	ΔL*	Δa*	Δb*	ΔE*
Et111	0.1	-0.9	-0.3	1.0	-0.3	-0.8	-0.2	0.9	-0.4	-0.9	-0.3	1.0	-0.2	-0.8	-0.2	0.8	0.5	-0.8	-0.3	1.0	-0.6	-0.7	-0.2	0.9
Et112	0.2	-0.8	-0.4	0.9	-0.2	-0.7	-0.3	0.8	-0.4	-0.8	-0.3	1.0	-0.2	-0.7	-0.3	0.8	0.5	-0.7	-0.3	1.0	-0.5	-0.6	-0.2	0.8
Et121	0.2	-0.9	-0.3	0.9	-0.2	-0.7	-0.2	0.8	-0.3	-0.8	-0.3	0.9	-0.2	-0.7	-0.2	0.8	0.6	-0.8	-0.3	1.0	-0.5	-0.6	-0.2	0.8
Et122	0.3	-1.0	-0.4	1.1	-0.1	-0.9	-0.3	0.9	-0.2	-1.0	-0.3	1.1	-0.1	-0.8	-0.3	0.9	0.7	-0.9	-0.4	1.2	-0.4	-0.8	-0.2	0.9
Et211	0.2	-1.0	-0.4	1.1	-0.2	-0.9	-0.3	1.0	-0.3	-1.0	-0.4	1.1	-0.2	-0.8	-0.3	0.9	0.6	-0.9	-0.4	1.1	-0.5	-0.8	-0.2	0.9
Et221	0.1	-1.1	-0.4	1.2	-0.3	-1.0	-0.3	1.1	-0.5	-1.1	-0.3	1.2	-0.3	-0.9	-0.3	1.0	0.4	-1.0	-0.3	1.2	-0.6	-0.9	-0.2	1.1
Et222	-0.1	-1.0	-0.4	1.1	-0.5	-0.9	-0.3	1.0	-0.6	-1.0	-0.3	1.2	-0.5	-0.8	-0.3	1.0	0.3	-0.9	-0.3	1.0	-0.8	-0.8	-0.2	1.1
Et212	0.1	-1.3	-0.4	1.3	-0.3	-1.2	-0.3	1.2	-0.4	-1.3	-0.4	1.4	-0.3	-1.1	-0.3	1.2	0.5	-1.2	-0.4	1.3	-0.6	-1.0	-0.3	1.2
Et311	0.2	-0.7	-0.3	0.8	-0.2	-0.6	-0.2	0.6	-0.3	-0.7	-0.2	0.8	-0.2	-0.5	-0.2	0.6	0.6	-0.6	-0.3	0.9	-0.5	-0.4	-0.1	0.7
Et321	0.3	-0.6	-0.3	0.7	-0.1	-0.5	-0.2	0.5	-0.2	-0.6	-0.2	0.7	-0.1	-0.4	-0.2	0.5	0.7	-0.5	-0.3	0.9	-0.4	-0.3	-0.1	0.5
Et322	0.3	-1.1	-0.5	1.2	-0.1	-1.0	-0.3	1.0	-0.2	-1.1	-0.4	1.2	-0.1	-0.9	-0.4	1.0	0.7	-1.0	-0.4	1.3	-0.4	-0.8	-0.3	1.0
Et312	0.3	-0.9	-0.4	1.0	-0.1	-0.8	-0.3	0.8	-0.2	-0.9	-0.3	0.9	-0.1	-0.7	-0.3	0.8	0.7	-0.8	-0.3	1.1	-0.4	-0.6	-0.2	0.8

	W222				W212				W311				W321				W322				W312				
	ΔL*	Δa*	Δb*	ΔE*	ΔL*	Δa*	Δb*	ΔE*	ΔL*	Δa*	Δb*	ΔE*	ΔL*	Δa*	Δb*	ΔE*	ΔL*	Δa*	Δb*	ΔE*	ΔL*	Δa*	Δb*	ΔE*	
Et111	-0.2	-0.8	-0.2	0.8	0.4	-0.7	-0.3	0.8	-0.3	-0.9	-0.2	1.0	0.0	-1.1	-0.3	1.1	-0.2	-1.2	-0.5	1.3	-0.4	-0.8	-0.3	1.0	
Et112	-0.2	-0.7	-0.3	0.7	0.4	-0.6	-0.3	0.8	-0.3	-0.8	-0.3	0.9	0.0	-1.0	-0.4	1.0	-0.1	-1.1	-0.5	1.2	-0.3	-0.7	-0.3	0.9	
Et121	-0.1	-0.7	-0.2	0.7	0.5	-0.6	-0.3	0.8	-0.2	-0.8	-0.2	0.9	0.0	-1.0	-0.3	1.0	-0.1	-1.1	-0.5	1.2	-0.3	-0.8	-0.3	0.9	
Et122	0.0	-0.8	-0.3	0.9	0.6	-0.8	-0.3	1.0	-0.1	-0.9	-0.3	1.0	0.1	-1.1	-0.4	1.2	0.0	-1.2	-0.5	1.3	-0.2	-0.9	-0.3	1.0	
Et211	-0.1	-0.8	-0.3	0.9	0.5	-0.7	-0.3	0.9	-0.2	-0.9	-0.3	1.0	0.0	-1.1	-0.4	1.2	-0.1	-1.2	-0.5	1.3	-0.3	-0.9	-0.3	1.0	**ΔE* 1.0**
Et221	-0.3	-0.9	-0.3	1.0	0.3	-0.9	-0.3	1.0	-0.4	-1.0	-0.3	1.1	-0.1	-1.2	-0.4	1.3	-0.2	-1.3	-0.5	1.4	-0.4	-1.0	-0.3	1.1	
Et222	-0.4	-0.8	-0.3	1.0	0.2	-0.8	-0.3	0.8	-0.5	-0.9	-0.3	1.1	-0.2	-1.1	-0.4	1.2	-0.4	-1.2	-0.5	1.4	-0.6	-0.9	-0.3	1.1	
Et212	-0.2	-1.1	-0.3	1.2	0.4	-1.0	-0.4	1.1	-0.4	-1.2	-0.3	1.3	-0.1	-1.4	-0.4	1.5	-0.2	-1.5	-0.6	1.6	-0.4	-1.2	-0.4	1.3	
Et311	-0.1	-0.5	-0.2	0.6	0.5	-0.4	-0.2	0.7	-0.3	-0.6	-0.2	0.7	0.0	-0.8	-0.3	0.9	-0.1	-0.9	-0.4	1.0	-0.3	-0.6	-0.2	0.7	
Et321	0.0	-0.4	-0.2	0.4	0.6	-0.3	-0.2	0.7	-0.2	-0.5	-0.2	0.6	0.1	-0.7	-0.3	0.8	0.0	-0.8	-0.4	0.9	-0.2	-0.5	-0.2	0.6	
Et322	0.0	-0.9	-0.3	1.0	0.6	-0.8	-0.4	1.1	-0.2	-1.0	-0.4	1.1	0.1	-1.2	-0.5	1.3	0.0	-1.3	-0.6	1.4	-0.2	-1.0	-0.4	1.1	
Et312	0.0	-0.7	-0.3	0.8	0.6	-0.6	-0.3	0.9	-0.1	-0.8	-0.3	0.9	0.1	-1.0	-0.4	1.1	0.0	-1.1	-0.5	1.2	-0.2	-0.8	-0.3	0.9	

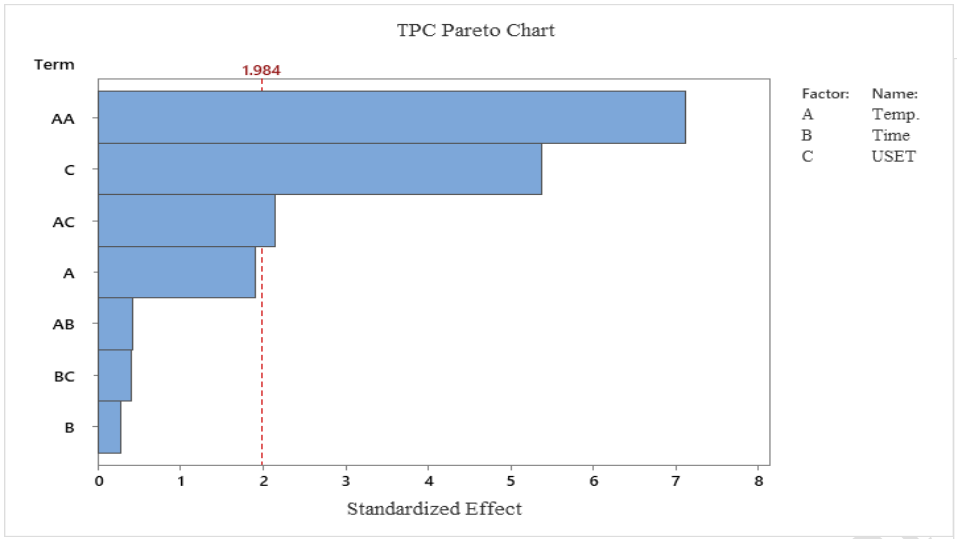
Table A13. summary of results obtained from color evaluation of Aronia pomace extracts extracted with 50 % glycerol with 1% citric acid, and 100 % water with 1% citric acid.

	GL111				GL112				GL121				GL122				GL211				GL221			
	ΔL*	Δa*	Δb*	ΔE*	ΔL*	Δa*	Δb*	ΔE*	ΔL*	Δa*	Δb*	ΔE*	ΔL*	Δa*	Δb*	ΔE*	ΔL*	Δa*	Δb*	ΔE*	ΔL*	Δa*	Δb*	ΔE*
W111	1.4	-0.7	-0.3	1.6	2.4	0.4	0.0	2.5	2.3	0.9	0.4	2.4	1.1	0.4	0.2	1.2	0.4	0.3	0.0	0.5	0.2	0.7	0.3	0.8
W112	1.8	-0.8	-0.4	2.0	2.8	0.3	-0.1	2.9	2.7	0.8	0.3	2.8	1.5	0.3	0.1	1.5	0.8	0.2	-0.1	0.8	0.6	0.6	0.2	0.9
W121	1.9	-0.7	-0.3	2.1	2.9	0.4	-0.1	3.0	2.8	0.9	0.4	2.9	1.6	0.4	0.2	1.6	0.9	0.3	0.0	0.9	0.7	0.7	0.2	1.0
W122	1.8	-0.8	-0.4	2.0	2.8	0.3	-0.1	2.8	2.6	0.7	0.3	2.7	1.4	0.3	0.1	1.5	0.8	0.1	0.0	0.8	0.6	0.5	0.2	0.8
W211	1.0	-0.8	-0.3	1.3	2.0	0.3	0.0	2.1	1.9	0.8	0.4	2.1	0.7	0.4	0.2	0.8	0.0	0.2	0.0	0.2	-0.2	0.6	0.3	0.7
W221	2.1	-0.9	-0.4	2.3	3.1	0.2	-0.2	3.1	2.9	0.6	0.2	3.0	1.7	0.2	0.1	1.8	1.1	0.0	-0.1	1.1	0.9	0.5	0.1	1.0
W222	1.7	-0.8	-0.4	2.0	2.8	0.3	-0.1	2.8	2.6	0.7	0.3	2.7	1.4	0.3	0.1	1.4	0.7	0.1	-0.1	0.7	0.5	0.5	0.2	0.8
W212	1.1	-0.9	-0.4	1.5	2.2	0.2	-0.1	2.2	2.0	0.6	0.3	2.1	0.8	0.2	0.2	0.8	0.1	0.0	0.0	0.1	-0.1	0.4	0.2	0.5
W311	1.9	-0.7	-0.4	2.0	2.9	0.4	-0.1	2.9	2.7	0.8	0.3	2.8	1.5	0.4	0.1	1.6	0.8	0.2	0.0	0.8	0.7	0.6	0.2	0.9
W321	1.6	-0.5	-0.3	1.7	2.6	0.6	0.0	2.7	2.4	1.0	0.4	2.7	1.2	0.6	0.2	1.4	0.5	0.4	0.1	0.7	0.4	0.8	0.3	1.0
W322	1.7	-0.4	-0.2	1.8	2.7	0.7	0.1	2.8	2.6	1.1	0.5	2.8	1.4	0.7	0.4	1.6	0.7	0.5	0.2	0.8	0.5	0.9	0.4	1.1
W312	1.9	-0.8	-0.3	2.1	2.9	0.3	-0.1	3.0	2.8	0.8	0.3	2.9	1.6	0.4	0.2	1.6	0.9	0.2	0.0	0.9	0.7	0.6	0.2	1.0

	GL222				GL212				GL311				GL321				GL322				GL312				
	ΔL*	Δa*	Δb*	ΔE*	ΔL*	Δa*	Δb*	ΔE*	ΔL*	Δa*	Δb*	ΔE*	ΔL*	Δa*	Δb*	ΔE*	ΔL*	Δa*	Δb*	ΔE*	ΔL*	Δa*	Δb*	ΔE*	
W111	0.3	0.7	0.3	0.8	0.2	0.6	0.3	0.7	0.3	0.6	0.3	0.7	0.5	0.6	0.3	0.9	0.2	0.6	0.3	0.7	0.5	0.5	0.3	0.7	
W112	0.7	0.6	0.2	0.9	0.6	0.5	0.2	0.801	0.7	0.5	0.2	0.9	0.9	0.5	0.2	1.1	0.6	0.5	0.2	0.8	0.9	0.4	0.2	1.0	
W121	0.8	0.7	0.2	1.1	0.7	0.6	0.2	0.963	0.9	0.6	0.2	1.1	1.0	0.6	0.3	1.2	0.7	0.6	0.2	0.9	1.0	0.5	0.2	1.1	
W122	0.6	0.5	0.2	0.9	0.6	0.5	0.2	0.763	0.7	0.4	0.2	0.9	0.9	0.5	0.2	1.0	0.6	0.4	0.2	0.7	0.8	0.4	0.2	0.9	**ΔE* 1.4**
W211	-0.1	0.6	0.2	0.7	-0.2	0.5	0.3	0.611	0.0	0.5	0.2	0.6	0.1	0.5	0.3	0.6	-0.2	0.5	0.2	0.6	0.1	0.4	0.2	0.5	
W221	0.9	0.5	0.1	1.1	0.9	0.4	0.1	0.974	1.0	0.4	0.1	1.1	1.2	0.4	0.2	1.3	0.9	0.4	0.1	1.0	1.2	0.3	0.1	1.2	
W222	0.6	0.5	0.2	0.8	0.5	0.4	0.2	0.709	0.7	0.4	0.2	0.8	0.8	0.5	0.2	1.0	0.5	0.4	0.2	0.7	0.8	0.4	0.2	0.9	
W212	0.0	0.4	0.2	0.5	-0.1	0.4	0.2	0.428	0.1	0.3	0.2	0.4	0.2	0.4	0.3	0.5	-0.1	0.3	0.2	0.4	0.2	0.3	0.2	0.4	
W311	0.7	0.6	0.2	1.0	0.6	0.6	0.2	0.871	0.8	0.5	0.2	1.0	0.9	0.6	0.2	1.1	0.6	0.5	0.2	0.8	0.9	0.5	0.2	1.0	
W321	0.4	0.8	0.3	1.0	0.4	0.7	0.3	0.887	0.5	0.7	0.3	0.9	0.7	0.8	0.3	1.1	0.4	0.7	0.3	0.9	0.6	0.7	0.3	1.0	
W322	0.6	0.9	0.4	1.2	0.5	0.8	0.4	1.06	0.6	0.8	0.4	1.1	0.8	0.9	0.5	1.3	0.5	0.8	0.4	1.0	0.8	0.8	0.4	1.1	
W312	0.8	0.6	0.2	1.0	0.7	0.5	0.2	0.902	0.8	0.5	0.2	1.0	1.0	0.5	0.3	1.2	0.7	0.5	0.2	0.9	1.0	0.4	0.2	1.1	

ANNEX B FIGURES:

A.



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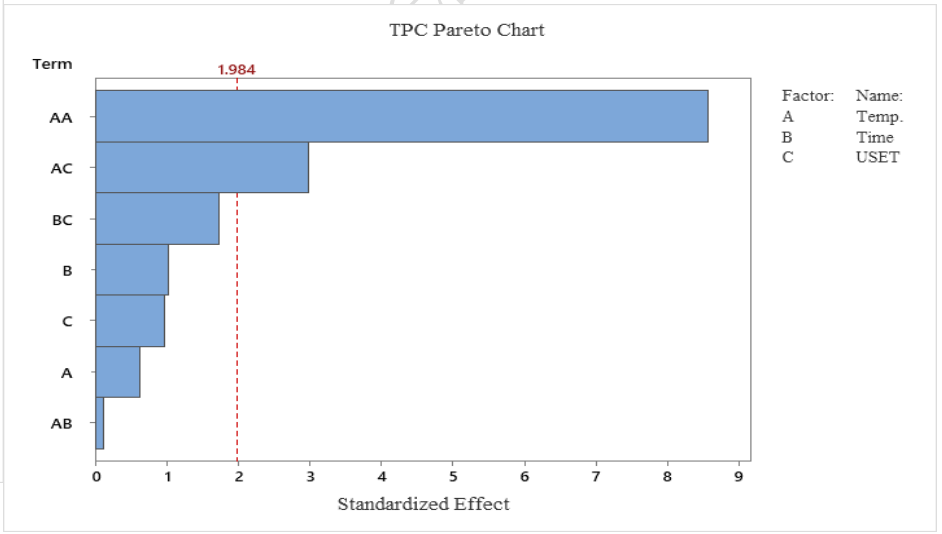

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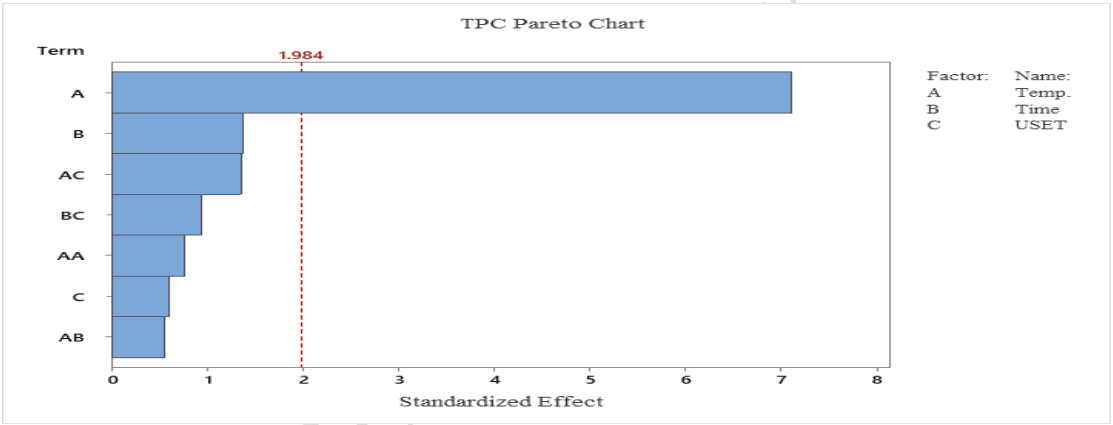
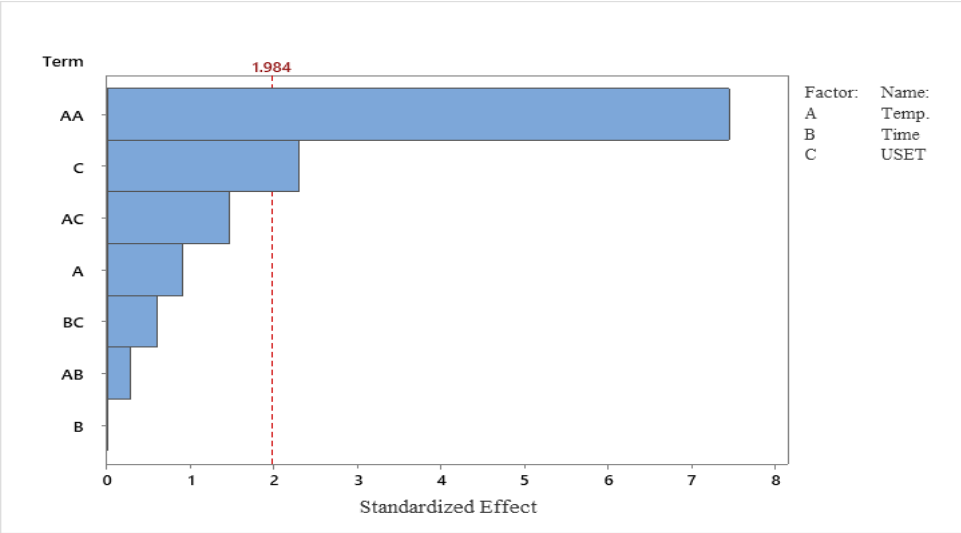


Figure B1. Standardized Pareto Charts for TPC (A is 50% ethanol with 1% citric acid, B is 50% glycerol with 1% citric acid, C is 100% water with 1% citric acid)

A.



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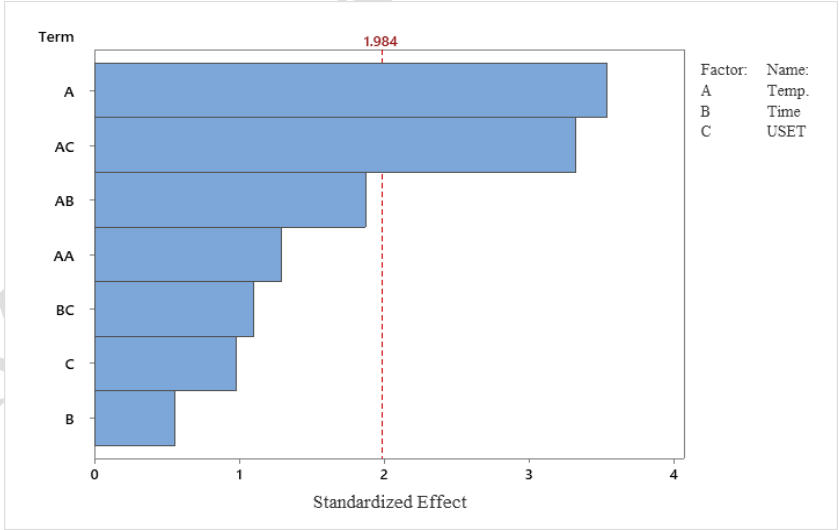

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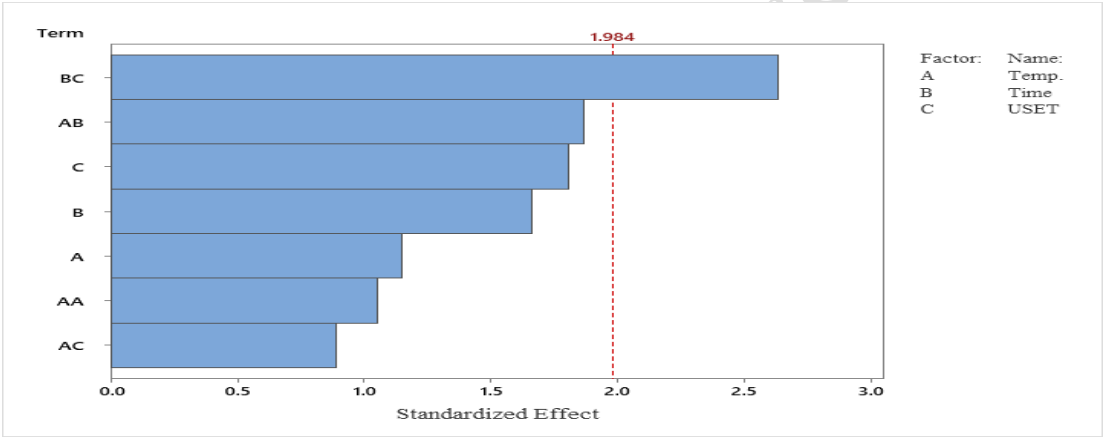
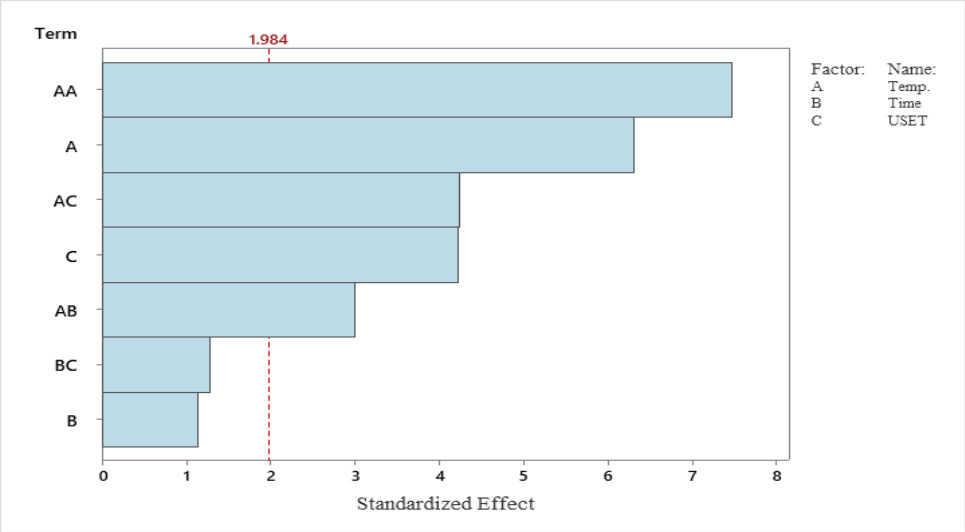


Figure B2. standardized Pareto Charts for FRAP (A is 50% ethanol with 1% citric acid, B is 50% glycerol with 1% citric acid, C is 100% water with 1% citric acid).

A.



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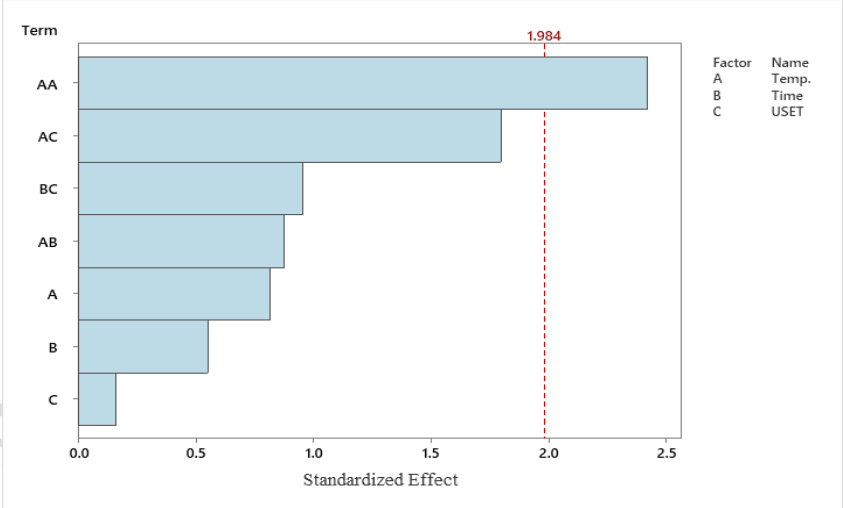

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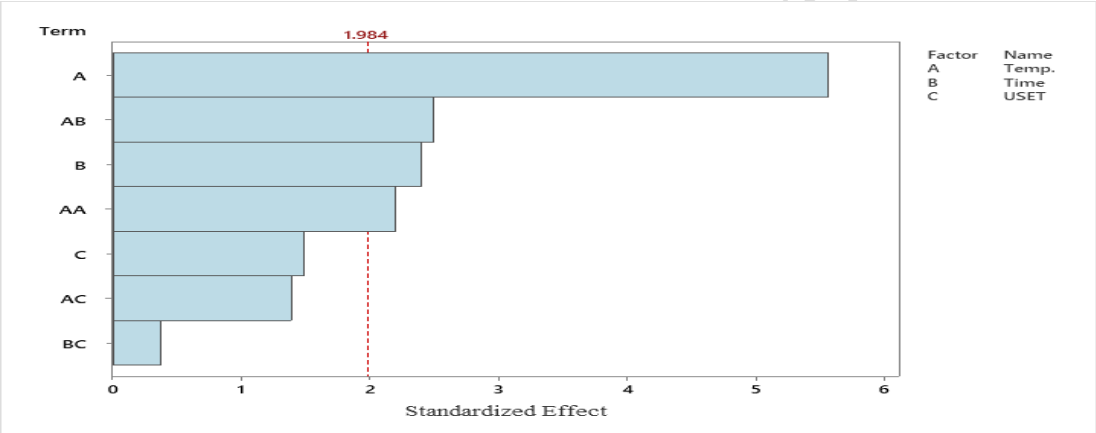

Figure B3. Standardized Pareto Charts for TAC (A is 50% ethanol with 1% citric acid, B is 50% glycerol with 1% citric acid, C is 100% water with 1% citric acid).

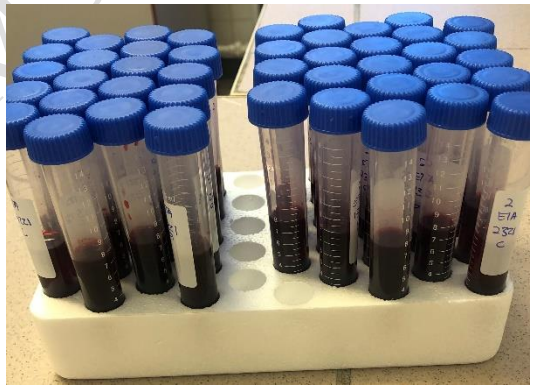

Figure B4. Some photos of laboratory activities.

ANNEX II: STATEMENT

STATEMENT ON CONSULTATION PRACTICES

As a supervisor of Yonas Tega Gebeyehu (Student's name) PBAB3Y (Student's NEPTUN ID), I here declare that the final essay/thesis/master's thesis/portfolio[1] has been reviewed by me, the student was informed about the requirements of literary sources management and its legal and ethical rules.

I recommend/don't recommend[2] the final essay/thesis/master's thesis/portfolio to be defended in a final exam.

The document contains state secrets or professional secrets: yes no*[3]

Place and date: Budapest, 2023 05 03

Internal supervisor
Lilla Szalóki-Dorkó, *PhD*

[1] Please select applicable and delete non-applicable.
[2] Please underline applicable.
[3] Please underline applicable.

ANNEX III: DECLARATION

DECLARATION

on authenticity and public assess of final essay/thesis/mater's thesis/portfolio[1]

Student's name: Yonas Tega Gebeyehu

Student's Neptun ID: PBAB3Y

Title of the document: Optimization of extraction conditions for the extraction of bioactive compounds from Aronia pomace

Year of publication: 2023

Department: Fruit and Vegetable Processing Technology

I declare that the submitted final essay/thesis/master's thesis/portfolio[2] is my own, original individual creation. Any parts taken from an another author's work are clearly marked, and listed in the table of contents.

If the statements above are not true, I acknowledge that the Final examination board excludes me from participation in the final exam, and I am only allowed to take final exam if I submit another final essay/thesis/master's thesis/portfolio.

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Student's signature

[1]Please select the one that applies, and delete the other types.
[2]Please select the one that applies, and delete the other types.