DEVELOPMENT OF ARONIA BASED VEGAN NUTRITIONAL SUPPLEMENTATION

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ABSTRACT

DEVELOPMENT OF ARONIA BASED VEGAN NUTRITIONAL SUPPLEMENTATION

Functional foods have gained great importance for the global consumer base and the food industry, especially with the changing living standards after the Covid-19 epidemic. *Aronia melanocarpa* is a fruit with high bioactive substance content, antioxidant content and rich nutritional value, which has become widespread all over the world and is increasing its popularity in Turkey. In the study carried out, it was aimed to design a functional food product that could be effective on the major and serious problems that humanity has to deal with, with this rich and beneficial content, which is one of the reasons why *Aronia melanocarpa* was chosen as a super fruit. The physical, chemical, microbiological, shelf life and sensory properties of the prepared products were analyzed, and the Aronia smoothie product was also examined in terms of *in vitro* digestion and ACE inhibitor activity. The protein level of this functional product was increased with the protein powder extracted from red lentils, and a protein content of 23.7% for the Aronia powder mix product and 3.74% for the Aronia smoothie product was obtained. After *in vitro* digestion applied to the Aronia smoothie product, an increase in the phenolic and antioxidant properties of the product was observed. ACE inhibitor activity of the Aronia smoothie product, which has a positive effect on hypertension and heart diseases, which are serious chronic health problems worldwide, was determined to be 22%. As a result of sensory analysis, the overall acceptability score of the Aronia smoothie product was obtained as 3.65.

ÖZET

ARONYA İÇERİKLİ VEGAN BESİN TAKVİYESİ GELİŞTİRİLMESİ

Fonksiyonel gıdalar, özellikle Kovid-19 salgını sonrasında değişen yaşam standartlarıyla birlikte küresel tüketici kitlesi ve gıda sektörü için büyük önem kazanmıştır. *Aronia melanocarpa*, biyoaktif madde içeriği yüksek, antioksidan içeriği ve zengin besin değeri olan, tüm dünyada yaygınlaşan ve bununla birlikte Türkiye'de de giderek yaygınlaşma hızını artıran bir meyvedir. Gerçekleştirilen çalışmada *Aronia melanocarpa*'nın süper meyve olarak seçilmesi nedenlerinden olan bu zengin ve faydalı içerik ile insanlığın uğraşmak zorunda kaldığı büyük ve ciddi sorunlara etkili olabilecek bir fonksiyonel gıda ürünü tasarlamak amaçlanmıştır. Hazırlanan ürünlerin fiziksel, kimyasal, mikrobiyolojik, raf ömrü ve duyusal özellikleri analiz edilmiş ve bunun yanında Aronya smoothie ürünü *in vitro* sindirim ve ADE engelleyici aktivite açılarından da incelenmiştir. Bu fonksiyonel ürünün kırmızı mercimekten ekstrakte edilen protein tozu ile protein seviyesi artırılmış ve Aronya toz karışım ürünü için %23,7, Aronya smoothie ürünü için ise %3,74 protein içeriği elde edilmiştir. Aronya smoothie ürününe uygulanan *in vitro* sindirim sonrası ürünün fenolik ve antioksidan özelliklerinde artış gözlenmiştir. Aronya smoothie ürününün dünya genelinde uğraşılan ciddi kronik sağlık sorunlarından olan hipertansiyon ve kalp hastalıkları üzerinde olumlu etkisi bulunan mekanizma ADE engelleyici aktivitesi %22 olarak tespit edilmiştir. Duyusal analiz sonucunda Aronya smoothie ürününün genel kabul edilebilirlik puanı 3,65 elde edilmiştir.

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LIST OF ABBREVIATIONS

A	: *Aronia melanocarpa*
AS	: Aronia Smoothie
A + PP	: *Aronia melanocarpa* + Protein Powder
A + PP + BM	: *Aronia melanocarpa* + Protein Powder + Black mulberry
ACE	: Angiotensin-I converting-enzyme
ACE-I	: Angiotensin-I converting-enzyme inhibition
FOSHU	: Foods for Specified Health Uses
HHL	: Hippuryl-L-Histidyl-L-Leucine
PCA	: Plate Count Agar
PDA	: Potato Dextrose Agar
PP	: Protein Powder
TPC	: Total Phenolic Content
TTA	: Total Titratable Acidity
UHT	: Ultra High Temperature
VRBA	: Violet Red Bile Agar
WSI	: Water Solubility Index
WHC	: Water Holding Capacity

CHAPTER 1

INTRODUCTION

1.1. Functional Food

The positive effects of plants, fruits and vegetables on human health have been accepted from past to present and have been used as natural treatment methods. In recent years, especially after the Covid-19 pandemic, people's expectations from food products have started to change gradually. Changing life and especially nutritional preferences have also closely influenced the food industry and turned the development line of the sector in this direction. As a result of people's increasing interest and desire for research on health and quality of life, the connection between nutrition type and chronic epidemics has also increased. Consumers no longer see food as just a tool that gives a feeling of fullness, but rather as a nutrient that has a direct effect on health and prevents chronic diseases when used regularly and correctly by supplementing the necessary nutrients, as well as having a positive effect on physical and mental health (Siró et al. 2008). In fact, it was very common to use food for medicine not only today but also centuries ago. In fact, 2500 years ago, Hippocrates said "food should be used as medicine and medicine should be used as food" and showed how it is not correct to refer to food as a product that only makes you feel full (Ghevariya and Singh 2023). Increasing life difficulties and health problems after the twentieth century have led scientists working on nutrition and food to design new, innovative and healthy foods in order to prevent nutritional deficiencies, which are one of the biggest effects of these negative situations on health, and to produce something that has a supporting effect on human life physically and mentally (Kaur and Das 2011). At this point, the functional food perspective emerges, arguing that food is not just a tool that provides satiety and that it should improve the life of the consumer with at least one positive effect on human health in addition to the nutritional values it provides.

The term functional food, which means foods that have at least one positive effect on human health in addition to their basic nutritional values, help certain body

functions, and have a positive effect on some chronic diseases, first appeared in Japan in the 1980s and was later pioneered by the United States, Europe and Canada continued to develop (Villaño et al. 2016). The Japanese coined the term Foods for Specified Health Use (FOSHU), making them the only country to date to have established a specific approval process for functional foods (Kaur and Das 2011). Apart from this, there is no international organization established for functional foods and there is no definitive definition. For this reason, it is possible to encounter a wide variety of definitions for the term functional food developed by different food, nutrition and health organizations. The definitions of the term functional food determined by different organizations of different countries are brought together in Table 1. Although the definitions differ from each other, they all follow a general line. However, in today conditions, the lack of an accepted and approved definition of such an important term for the food industry and humanity and the lack of a legal framework are among the major challenges faced by the sector.

Table 1. Different definitions for functional food determined by different organizations.

Organization	Func. Food Definition	References
European Food Safety Authority (EFSA)	"A functional food is a food that, in addition to providing basic nutrition, positively impacts specific functions in the body, resulting to an improved state of health and well-being or a lower risk of disease." It could be a natural food or one that has been altered through technological or biotechnological processes, such as the addition or removal of certain components. To be designated as a functional food, it must demonstrate its therapeutic effects in quantities typical of a regular diet."	Martirosyan and Singharaj, 2016
Functional Food Center (FFC)	"Adequate & non-toxic amounts of natural or processed foods containing either identified or unidentified biologically active components that have been scientifically demonstrated to offer health benefits for the prevention, management, or treatment of chronic illnesses are recommended."	Martirosyan and Miller, 2018
Institute of Food Technologists (IFT)	"Foods and food components which offer benefits to health beyond basic nutrition are products that provide essential nutrients in quantities that frequently exceed normal requirements for normal functioning, health, and development." They also include other bioactive components that are beneficial to general health."	MacAulay and Petersen, 2005
International Life Sciences Institute (ILSI)	“Foods that provide health benefits beyond basic nutrition due to the presence of physiologically active dietary components.”	Crowe and Francis, 2013

Functional foods are divided into two different categories: traditional functional foods and modified functional foods (Essa et al. 2023). All these subcategories include the requirement to find at least one health effect in addition to the nutritional effect given by the general term functional foods. Traditional functional foods are foods that have inherent positive health effects, such as dairy products, fruits, vegetables, meat products and grains, without any modification of the existing food (Essa et al. 2023). These foods can directly positively affect human health with the active ingredients they contain when consumed regularly and in a controlled manner. It will be possible to divide traditional functional foods into two subheadings according to their types: plant-based traditional foods and animal-based traditional foods (Ghevariya and Singh, 2023). Modified functional foods, on the other hand, are foods obtained by enriching an existing food with the addition of an external nutrient in order to increase its health benefits, giving that food its own properties as well as positive effects on health (Essa et al. 2023). Modified functional foods are obtained by enriching the food externally with bioactive substances such as vitamins, minerals, antioxidants and phenolics, and active substances such as omega-3, plant fibers and probiotics, thus giving the food a health effect or increasing the existing effect.

Bioactive components are one of the most important ingredients that provide health benefits in a food, especially for functional foods (Martirosyan and Miller 2018). If we look at the definition of bioactive substances in order to understand this important effect on functional foods more clearly, we can see that bioactive substances are the name given to chemical components that contribute to the regulation of biological mechanisms. In fact, when we look at the food source in general, bioactive substances constitute a very small part, but they play one of the main roles when the regulating mechanism of action on human health is taken into consideration. Even though they have such a positive and effective structure, bioactive substances can negatively affect human life by causing toxicity when not consumed consciously and correctly (Martirosyan and Miller 2018). For this reason, bioactive ingredients integrated into functional foods are designed by conscious and expert food scientists in accordance with the limits. With regular and conscious consumption, functional foods have a positive effect on many physical and psychological health problems such as diabetes, cardiovascular diseases, neurological disorders, blood pressure, obesity and cancer, and become an important alternative to drugs with the help of bioactive substances they contain. Moreover, while doing this, its side effects are much more limited and minimal

compared to drugs. Antioxidants, phenolic compounds, carotenoids, flavonoids, phenolic acids, lignans and phytosterols are among the most important bioactive substances and are involved in the solution mechanism of many health problems. In this sense, it is possible to design functional foods with effective mechanisms to prevent many different diseases by using these ingredients.

While functional food products stood out with products that were generally enriched in vitamins and minerals and were a solution to a single health problem when the sector was first born, with its structure that has developed over time, it has now become a product that supports a single health problem while being enriched with different beneficial bioactive substances, fibers, vitamins and minerals. Rather, it has turned into products that aim to have protective properties for more than one health problem with one product (Siró et al. 2008). Although they are generally shaped and customized according to the demands of the consumer base, functional foods can be designed by adapting them to almost any food type. If we classify them in the context of their intended use, it will be possible to divide them into 3 types. The first of these, although it is difficult to define it so specifically in terms of behavior, cognitive and psychological aspects, is a specific one such as improving regular stomach and colon functions with the biomarker "it adds goodness to your life" or improving their learning and behavioral abilities with the phrase "improves the lives of children". These are foods that regulate vital functions. Another group of functional foods are foods designed to reduce and prevent existing health risks such as high blood pressure, high sugar, high cholesterol or cancer. Finally, it will be possible to classify, for example, lactose-free products and gluten-free foods as functional foods that "make life easier" for consumers in the face of existing sensitivities (Siró et al. 2008). Although it can generally be integrated into all types of food products, if we adapt it to foods in the sector, probiotics, prebiotics, functional beverages, cereals, meats, eggs, bakery products, teas, spices, nutritional supplements, nutraceuticals and snacks are functional food classes with intense product diversity.

Due to the current position of the lifestyle of the consumer mass, their concern for human life and their preference for a healthy lifestyle have steadily increased the development of functional foods, especially after the pandemic. With this perspective update, the functional foods sector is now among the strategies taken to improve and protect human life and public health for country administrators (Sun-Waterhouse 2011). As a result of all these, the functional foods sector has become a dynamic market within

the world food sector. Functional foods, which have a high interest and development worldwide, continue to develop very rapidly over time in our country. One of the major reasons why the market has become so popular is that it offers a food option that will make people who have adopted and want to adopt a private lifestyle feel safe. The functional foods market, which is constantly increasing its development momentum, is expected to reach a value of at least 91 billion dollars in the global market in the near future (Essa et al. 2023). According to Siró et al. (2008), the largest segment of the functional foods market is the United States, followed by Europe and Japan. Although different economic values are assessed by different studies, it is certain that the functional foods market occupies an important place in the food industry and this economic value is increasing day by day. These three dominant markets, which control the majority of the food industry, such as the USA, Europe and Japan, contribute to more than 90% of total sales (Siró et al. 2008). According to countries, the market share of functional food is directly proportional to the legal limits of that country in the food sector, and as can be seen from here, the USA is in a more favorable position than other countries in this regard. It is not surprising that Japan, the third country dominating the market, is in this position as it is the birthplace of the functional food industry. Again, according to the data given by Siró et al. (2008), more than 1700 functional food products were introduced to the market in Japan between 1988 and 1998, and a turnover of approximately 14 billion dollars was reached as of 1999. In addition, it launched more than 500 functional food products with the FOSHU label in 2005, probably with the widest and most innovative functional products in the world (Siró et al. 2008 ; Kaur and Das, 2011). In addition to these three leading countries, Germany, France, the United Kingdom and the Netherlands are also among the important countries in the functional food sector in Europe. With all this, it can be seen that the global consumer interest in functional foods is generally high and is gradually increasing. The existence of regional and national differences also shows us that consumer lifestyle and openness to development directly affect this market. Studies show that the European market is generally heterogeneous and the market share of functional foods varies regionally. For example, while the interest of the consumer base in Northern and Central European countries in functional foods is that consumers adopt natural, fresh and innovative foods in terms of their perspective and lifestyle and find them beneficial for health, in Mediterranean countries, this situation indicates the opposite (Siró et al. 2008).

Under the functional foods sector, products rich in bioactive substances such as antioxidants and phenolics, foods with rich fiber content, food products developed using probiotic properties and herbal sweeteners such as stevia are rapidly being introduced to the market (Kaur and Das 2011). According to the information provided by Kaur and Das, (2011), Canada has an international market share of approximately 32% with its companies such as Harmonium International Inc., which works on probiotics, CV Technologies Inc., which deals with natural healthy foods, Lassonde Industries Inc., which deals with functional juice products, and Ocean Nutrition Canada Ltd., which deals with omega-3 fatty acids. has achieved significant growth and development. For the last 20 years, the European functional food market has gained a different perspective in this race in the sector with innovative functional products such as Unilever's (UK) 'Flora Pro-Active' and Nestle's (Switzerland) 'Petit' drinking yoghurt. Raisio Food Group for Finland, which is an important pawn for the functional food sector on a European basis, Danone of France and Iparlat companies of Spain are among the important organizations that direct this sector (Kaur and Das 2011). Functional beverages rank first in the market with 58%, followed by dairy products and grain products (Kaur and Das 2011). According to Kaur and Das, (2011) data, nutraceuticals, consisting of food supplements, vitamins and minerals, which were worth 44 billion INR as of 2009, are estimated to reach 95 billion INR as of 2013, and this represents a significant growth on a sector basis.

There are some factors that limit the development and impact of functional foods, which have such a positive impact on the food industry and consumer base. Although it is the starting point of a mindset dating back centuries, the lack of a clear definition of the term functional foods that has been finalized and declared at the international level and a supporting official institution has a slowing effect on the development of this sector. Since it does not have any molded and approved structure, legal obstacles and ethical differences create serious resistance on the sector (Sun-Waterhouse 2011). Since it is a new and developing field, more academic and sectoral research should be carried out and scientists who will be pioneers in this perspective should be trained. Some stereotypical pioneers and producers of the food industry should be informed on this issue and made open to development in order not to become an obstacle. In addition, consumer resistance, which cannot be separated from the traditional food term, is one of the important factors slowing down the growth in the sector. More specific organizations should be developed on a sector basis and larger

budgets should be used. Since it is a sector that directly affects human health and its health effects need to be proven, more clinical studies should be carried out. In order to obtain more innovative and functional products, more technological budget is needed, and since these are innovation-based studies, cost is again the most important restrictive parameter on this sector. The presence of high-level technological requirements and the difficulty of procuring this technology also have a restrictive effect. Serious regulations should be made and the sector should be supported in every sense to eliminate these factors, which have such a positive impact on health and economy and have a serious limiting effect on a sector that is open to development.

1.2. Aronia Melanocarpa

Nutrition with natural products of plant, fruit and vegetable origin is a method that has a positive effect on health, prolongs the quality of life and lifespan, and has a preventive and resistant effect against diseases. This subject, on which thousands of studies have been carried out for many years, shows that foods with a rich structure such as berries, fruits, vegetables and plants have a positive effect on human health and are used as a preventive against chronic problems such as cancer, heart diseases, neurological diseases, obesity and blood pressure which is an important supporter for the functional food field (Platonova et al. 2021). For all these reasons, the food industry and food scientists are now concentrating on designing functional foods that eliminate existing health problems on humanity, improve the quality of life and make life easier, by using these natural plant-based, fruit and vegetable products and innovative technologies.

Aronia melanocarpa Elliot fruit, also known as black chokeberry, is a valuable fruit originating from the Rosaceae family (Teneva et al. 2022). *Aronia melanocarpa* fruit is a small, dark blue, berry-like fruit that originates from the Eastern part of the North America (Platonova et al. 2021). *Aronia melanocarpa* fruit is a highly sustainable fruit species that can grow up to 2-3 m in height and blooms with white tiny flowers that yield high yields generally in May and June (Lee, Shin, and Choi 2018). The color of Aronia fruit varies between red (*Aronia arbutifolia*), purple (*Aronia prunifolia*) and black (*Aronia melanocarpa*) depending on the species (King and Bolling, 2020 ; Lee et

al. 2018). Although it varies by country and region, the most commonly produced, used and known types are Viking, Nero, Aron, Galichanka and Hugin (Platonova et al. 2021). *Aronia melanocarpa* fruit is a popular fruit that is widely used both in medicine and in the food industry worldwide (Zhao et al. 2022). *Aronia melanocarpa* fruit, whose popularity increased in the twentieth century and later, began to spread rapidly in countries such as Denmark, Bulgaria, Poland, Serbia, Germany and Russia and became an important food and medicinal product (Platonova et al. 2021). Poland has gained production leadership by undertaking approximately 90% of the global *Aronia melanocarpa* production. *Aronia melanocarpa* fruit, which was initially known and produced only in certain countries, is now produced in every region and country, and its fame has spread rapidly. Its development and production are increasing rapidly day by day. Although it is widely used in the food industry both in its natural fresh fruit form and in processed form, its use alone is more limited due to consumer acceptability due to the astringent taste it leaves in the mouth in its fresh and natural form. Aronia fruit, which can be integrated into all kinds of processes in the food industry, can be used in many products by drying and turning it into powder, especially fruit juice, snacks, chocolate, bakery products, dairy products, jam, canned food, wine, syrups and jellies. In addition to being preferred when designing innovative and functional foods with its rich content and the effect it leaves on the consumer, it is also widely preferred as a colorant in the food industry and other sectors due to its intense color pigmentation.

Aronia melanocarpa fruit has a very rich content in terms of micro and macro nutrients. It is a good source of ingredients such as dietary fiber, protein, vitamin B, caratoneids, niacin, pantothenic acid, folic acid, vitamin C, tocopherols, vitamin K, Na, Ca, Mg, K, P, Zn, Cu, Mn (Teneva et al. 2022 ; Platonova et al. 2021). *Aronia melanocarpa* is a plant rich in polyphenols, especially anthocyanin, which gives the *Aronia melanocarpa* fruit its dark blue color (Nour 2022). Aronia fruit contains four main anthocyanins: cyanidin-3-O-galactoside (67.5%), cyanidin-3-O-arabinoside (24.8%), cyanidin-3-O-glucoside (3.8%), and cyanidin-3-O- xyloside (4%) is dominant (Jiao et al. 2021). In addition, Aronia fruit, which has a significantly higher antioxidant content than other berry and fruit types, thanks to the high ascorbic acid, proanthocyanidins, and hydroxycinnamic acids it contains, is a valuable source of nutritional content (Nour 2022). Apart from its polyphenol content, *Aronia melanocarpa* is also a source of glucose, fructose, sucrose, sorbitol and pectin (Platonova et al. 2021). Thanks to all this high nutritional content, it has been selected as 'super fruit' in Russia

and is used in the medical and food sectors. When evaluated from a nutritional point of view, Aronia fruit is low in calories (84 kcal per 100 g), low in sugar (19 g per 100 g), low in fat (0.13 g per 100 g), pectin (0.3-0.6%), protein (1.4 g per 100 g), provides vitamin B groups, vitamin C (13-270 mg/kg) and minerals (4.4-5.8 g/kg as ash value) (Lee, Shin, and Choi 2018). Due to all its rich content, Aronia fruit has made a serious impact in the food and medicine industry worldwide and has many positive effects on health with various products.

1.2.1. Health Benefit Constituents of *Aronia melanocarpa*

Aronia melanocarpa fruit consists of many important complex biologically active components that have positive effects on health. Even though they all have this richness of ingredients, of course, there are differences between the types of ingredients due to the same species growing under different growing conditions and being exposed to different climatic conditions and external factors. It has important contents such as high antioxidants, phenolic compounds, anthocyanins, procyanidins, flavonols, flavanols and phenolic acid (Zhang et al. 2021). All these beneficial components have a positive effect on health when consumed. For this reason, *Aronia melanocarpa* fruit is used as a positive effect mechanism in various serious health problems with these rich contents.

1.2.1.1. Polyphenols

Aronia melanocarpa has a richer content than many fruits, vegetables and herbal products, including berries, in terms of the polyphenols it contains. *Aronia melanocarpa* fruit has a chemically rich structure consisting of polyphenols that form aromatic rings by combining many hydroxyl groups. The content of these rich phenolic rings contains many phenolic composite subgroups such as flavonoids, phenolic acids, simple phenols, stilbens and lignans, originating from the different structures formed by the rings (Zhang et al. 2021). While these rich polyphenols in its content have a positive effect on

many health problems, the astringent and bitter taste of *Aronia melanocarpa* fruit also comes from the polyphenol group it contains (King and Bolling 2020).

1.2.1.2. Flavonoids

Aronia melanocarpa, with its rich flavonoid structure, has a good effect on many problems such as antioxidant, antimicrobial, anticancer, antidiabetic and cardiovascular, which have a positive effect on human health. Polyphenolic compounds containing two aromatic rings connected to each other with a 3-carbon structure are called flavonoids and consist of subgroups including flavones, flavan-3-ols, anthocyanidins, flavanones, isoflavones, dihydroflavonols and flavan-3,4-diols (Zhang et al. 2021).

1.2.1.3. Flavanols

In a study conducted by Zhang et al. (2021), the most important flavanols found in chokeberry fruit are procyanidins, while according to the data given by King and Bolling, (2020), the most dominant flavanols in Aronia fruit are quercetin. Flavanols are found in lower concentrations in Aronia than proanthocyanidins and anthocyanins (King and Bolling 2020).

1.2.1.4. Anthocyanins

The second highest concentration of phenolics found in *Aronia melanocarpa* is anthocyanins (Zhang et al. 2021). The differences and intensities of colors in fruits and vegetables occur thanks to anthocyanins, and the reason for this intense color pigmentation of *Aronia melanocarpa* fruit is its intense anthocyanin and pigment content (King and Bolling 2020). When evaluated on a fresh weight basis, Aronia berries are the fruit with the richest anthocyanins content (King and Bolling 2020).

1.2.1.5. Phenolic Acids

Aronia fruit has a phenolic acid profile consisting mainly of neochlorogenic and chlorogenic acids, but also contains small amounts of vanillic, ferulic, syringic and gallic acid phenolic acids (King and Bolling 2020).

1.2.1.6. Astringent Compounds

Although aronia fruit is a highly preferred fruit in the food industry due to its health effects, its consumption in its natural and fresh form is limited by the consumer due to the astringent taste it leaves in the mouth. Amygdalin and proanthocyanidins are the main structures that contribute to the astringent and bitter taste of Aronia fruit, and some hydroxycinnamic acids such as vanillic and syringic acid can also cause astringency (King and Bolling 2020). In order to eliminate this taste problem of *Aronia melanocarpa* fruit, different auxiliary products can be used and this situation can be solved by applying different processes. Despite its astringent taste problem, due to its special content and thousands of positive effects, *Aronia melanocarpa* fruit is increasingly among the favorites of both the consumer and the food industry, especially in terms of functional foods.

Aronia melanocarpa, with its rich and beneficial content, has preventive effects on a wide range of health problems such as anticancer, antiatherogenic, anti-inflammatory, anti-tumor, gastroprotective, hypotensive, lipid lowering, anticarcinogenic, diabetes, neuroprotective and cardioprotective (Teneva et al. 2022). In addition, due to its high anthocyanin content, it regulates functions such as lowering blood sugar, lowering blood lipids, protecting the liver and preventing Alzheimer's disease (Deng et al. 2022). Many studies have shown that Aronia has a positive potential on regulating obesity, obesity induced inflammation and other related diseases with its high anthocyanin content (Jiao et al. 2021). More specialized studies show that *Aronia melanocarpa* fruit also has a positive anti-aging effect, and in addition, it is among the data obtained as a result of the studies that it has an effect on improving the learning capacity and keeping the memory sharp (Daskalova et al. 2019).

1.2.2. Health Benefits of Aronia melanocarpa

Aronia melanocarpa fruit has been popular for many years and has been among the fruits that contribute to herbal treatment methods due to its effect on human health. Thanks to its rich content, it has a positive effect on many current and common health problems such as heart diseases, neurological problems, obesity, blood pressure, sugar and cancer, while also having psychological regulatory effects. Additionally, its anti-aging effect is one of the important effects that can be given among its existing benefits. In order to support these health effects, *in vitro* tests are also carried out in addition to *in vivo* studies and their clinical effects are monitored (Zhang et al. 2021). Using the preventive and regulatory effects of *Aronia melanocarpa* and its products on diseases will be a good strategy for the food industry and medicine.

1.2.2.1. Anticancer Activity

Many studies have shown that *Aronia melanocarpa* fruit has an anti-tumor effect on humans and animals, thanks to its rich polyphenol content (Zhang et al. 2021). In order to use this effect, *Aronia melanocarpa* fruit is transformed into many different food products and offered to the consumer. According to Zhang et al. (2021), as a result of some studies, it has been observed that black chokeberry fruit is used to treat breast cancer, bowel cancer and leukemia. Another study showed that Aronia fruit inhibited the development of colon cancer (Zhang et al. 2021).

1.2.2.2. Antidiabetic Activity

Diabetes is one of the most challenging and widespread health problems that humanity is struggling with globally in today conditions, with carbohydrates being heavily included in humanity's daily diet. As a result of clinical studies and *in vitro* studies, the effect of Aronia consumption on reducing insulin resistance has been observed (King and Bolling 2020). In a study, it was observed that a drinking water

product enriched with chokeberry reduced blood glucose levels. Many studies have been conducted due to the positive effects of *Aronia melanocarpa* on numerous health problems, and within the scope of these studies, the antidiabetic effect of *Aronia melanocarpa* fruit has been proven by both *in vivo* and *in vitro* studies.

1.2.2.3. Cardio-protective Effects

Cardiovascular problems are one of the most important health problems and causes of death faced by humanity. People's poor nutrition, low physical activity, alcohol, smoking, intense life stress and irregular lifestyles are situations that support heart diseases and increase the risk (King and Bolling 2020). *In vitro* studies with its rich content, especially phenolics, have shown that *Aronia melanocarpa* fruit can have a preventive effect on several cardiac diseases (Zhang et al. 2021). According to Zhang et al. (2021) data, it has been observed that chokeberry juice extract reduces cardiovascular problems by reducing lipid activity with the intense amount of niacin it contains. With the supporting data of the studies conducted on all these effects, the effect of *Aronia melanocarpa* fruit in reducing the risk of heart problems has been concretely proven.

As can be seen, *Aronia melanocarpa* fruit has a preventive mechanism against many health problems, thanks to the dense and beneficial constituents and bioactive substances it contains. It has been the subject of many studies and these situations have been concretely proven by *in vitro* and clinical studies as well as *in vivo* studies. While an important issue such as health is a situation that people need to be so sensitive about, it is an important point that the studies are carried out in a sound, scientific and accurate manner, supported by clinical studies. In a study conducted by Jiao et al. (2021), the inhibitory effect of the cyanidin-3-O-galactoside component found in *Aronia melanocarpa* on high fat diet and obesity was observed in mice. In another study conducted by Zhao et al. (2022), *Aronia melanocarpa* fruit proved to be a good method to treat liver fibrosis by improving the intestinal microbiota on mice. In a study conducted by Platonova et al. (2021), she demonstrated the curative effect of *Aronia melanocarpa* juice on mice on mice, while also improving locomotor functions and showing its neuroprotective effect by increasing the density of nerves in the

hippocampal perforin pathway. In an *in vitro* and *in vivo* study conducted by Park et al. (2013) on the antiviral activity of *Aronia melanocarpa*, it was observed that it showed anti-influenza properties with the help of ellagic acid and myricetin it contained and protected mice against the deadly influenza threat.

As a result of all these health effects, it can be seen that *Aronia melanocarpa* fruit is one of the very important food sources that should be consumed by humanity. This important fruit species, revealed after thousands of studies, has a very important position for the medicine and food industry. Table 2 includes some of the scientific studies on *Aronia melanocarpa* fruit.

Table 2. Some scientific literature studies on *Aronia melanocarpa*.

Name of the Article	**Aim of the Article**	**Conclusion of the Article**	**Reference**
Growth Performance and Fatty Acid Profiles of Ducks Fed a Diet Supplemented with Aronia (*Aronia melanocarpa*) Powder	To assess the fatty acid compositions and growth performance of ducks fed diets containing Aronia powder.	Addition of Aronia powder to duck fed has been observed to improve weight gained and feed: gain ratio relative to control conditions.	Lee, Shin, and Choi 2018
Effect of supplementation with chokeberry juice on the inflammatory status and markers of iron metabolism in rowers	To examine how supplementing with Aronia juice affects the levels of hepcidin, pro-inflammatory cytokines, and specific markers of iron metabolism in rowers who have just completed a hard workout.	It has been observed that supplementing the rowers with Aronia juice significantly reduces the TNF-alpha level and increases the antioxidant activity of the plasma.	Skarpańska-Stejnborn et al. 2014
Addition of Medicinal Plants Increases Antioxidant Activity, Color, and Anthocyanin Stability of Black Chokeberry (*Aronia melanocarpa*) Functional Beverages	To investigate the effect of adding herbs such as Lady's mantle, lavender, roseship and meadowsweet to ready-to-drink Aronia nectar obtained after pasteurization and 4 months of storage on parameters such as chemical composition, color stability and antioxidant activity.	It was observed that the addition of the herbs mentioned during the processing of Aronia fruit successfully increased the properties of the functional beverages such as antioxidant activity, polyphenol content, color and anthocyanin stability.	Teneva et al. 2022
Black chokeberry (*Aronia melanocarpa*) extracts in terms of geroprotector criteria	To give a general overview of the effects of Aronia extracts in both in vitro and *in vivo* conditions in order to identify any potential anti-aging properties that would make them qualified as geroprotectors.	It has been observed that Aronia extract exhibits geroprotective activities such as improving glucose and lipid metabolism, ameliorating neurodegenerative disorders, prolonging lifespan, antiviral and antibacterial activity, protection of the gastrointestinal tract, and antiproliferative activity.	Platonova et al. 2021

Table 2. (cont.). Some scientific literature studies on Aronia melanocarpa.

Name of the Article	Aim of the Article	Conclusion of the Article	Reference
Quality Characteristics, Anthocyanin Stability and Antioxidant Activity of Apple (Malus domestica) and Black Chokeberry (*Aronia melanocarpa*) Juice Blends	To investigate apple juice's potential as a new functional food by adding Aronia juice as a health-promoting component.	Adding Aronia juice to apple juice has made the emerging new functional food product a juice enriched with anthocyanins and with extraordinary health benefits. According to the results of the sensory analysis obtained, it was observed that adding Aronia improves the color of the product and increases the flavor and overall acceptability of the juice.	Nour 2022
Proteomic analyses revealed the antibacterial mechanism of *Aronia melanocarpa* isolated anthocyanins against Escherichia coli O157: H7	To investigate changes in the proteome of Escherichia coli O157:H7 after AMAs (*Aronia melanocarpa* anthocyanins) treatment using the TMT quantitative proteomics method.	It has been observed that AMAs have antibacterial activity against E. coli O157:H7 and cause morphological changes and cytoplasmic aggregation of E. coli O157:H7 cell.	Deng et al. 2022
Chokeberry Reduces Inflammation in Human Pre-adipocyte Cells	In order to investigate the impact of chokeberry juice extract, Cyanidin 3-O-galactoside (C3Gal), and Cyanidin 3-glucoside (C3G) on palmitate as PA or lipopolysaccharide (LPS) induced inflammation as evaluated by interleukin-6 (IL-6).	Palmitate (PA) induces inflammation in human primary subcutaneous pre-adipocyte cells, and this inflammation is reduced by the addition of Aronia extract, and isolated anthocyanins such as C3-galactoside or glucoside have been observed to have partial inhibition.	Brunelle et al. 2022
Antibacterial mechanisms of *Aronia melanocarpa* (Michx.), Chaenomeles superba Lindl. and Cornus mas L. leaf extracts	To analyze the in vitro antibacterial effects of *Aronia melanocarpa*, Chaenomeles superba, and Cornus mas leaf extracts in respect to pathogenic bacteria and meat deterioration.	It has been observed that extracts of the leaves of A. melanocarpa, C. superba, and C. mas cause significant morphological impacts on bacterial cells, and these extracts restrict DNA synthesis by diminishing DNA gyrase activity.	Efenberger-Szmechtyk et al. 2021
Chokeberry (*Aronia melanocarpa*) fruit extract abrogates melanoma progression through boosting up IFN-γ-producing cells	To investigate the effects of oral consumption of Aronia extract on tumor development using murine B16 melanoma cells to induce tumors in C57BL/6 mice.	Aronia extract has been observed to exert a significant anti-tumor effect in the melanoma model by increasing the frequency of IFN-γ-producing tumor-fighting cells in the TME, as well as inhibiting the infiltration of tumor-promoting MDSC.	Gaji´c et al. 2022
Aronia melanocarpa (Michx.) Elliot fruit juice reveals neuroprotective effect and improves cognitive and locomotor functions of aged rats	To analyze the impact of Aronia juice on the behavioral and locomotor functions in aged rats, as well as how these effects correlate to morphological changes in the hippocampus and brain AChE activity.	It has been observed that Aronia juice significantly improves the ability to learn tasks and locomotor functions of aged mice. It is also concluded that the behavioral and memory changes achieved are consistent with the increased number of nerve fibers in the perforating pathway in the hippocampus, which may serve as an indicator of increased functional activity of cholinergic neurons.	(Daskalova et al. 2019)

Table 2. (cont.). Some scientific literature studies on *Aronia melanocarpa*.

Name of the Article	**Aim of the Article**	**Conclusion of the Article**	**Reference**
Protective effects of *Aronia melanocarpa* juices either alone or combined with extracts from Rosa canina or Alchemilla vulgaris in a rat model of indomethacin-induced gastric ulcers	To analyze the protective effect of pure Aronia juices and Aronia-based blend juices in a rat model of indomethacin-induced gastric ulcer.	Pure Aronia juices and Aronia juices combined with Rosa canina or Alchemilla vulgaris extracts have been observed to reduce the severity of indomethacin-induced gastric injury.	Valcheva-Kuzmanova et al. 2019
Edible film production using *Aronia melanocarpa* for smart food packaging	Film production using bio-sourced Aronia fruit and naturally-sourced starch to produce smart packaging and investigation of their use in active packaging systems.	It has been observed that the edible film made from Aronia and starch can be utilized in food packaging as a colorimetric deterioration indicator, and the results can be seen visually without the use of any equipment.	Ozcan and Kandirmaz, 2022
Histopathological evaluation of the effects of *Aronia melanocarpa* fruit juice on myocardium and coronary arteries in rats with diet-induced metabolic syndrome	Histopathological investigation of the effects of Aronia fruit juice on myocardial and coronary vasculature in MS rats.	It has been observed that nutritional therapy with Aronia juice reduces histopathological changes in coronary and myocardial vessels in rats with diet-induced MS. As a result of these data, the cardio and vasoprotective effects of feeding with Aronia fruit juice can be verified.	Reyzov et al. 2022
Effect of Aronia Extract on Collagen Synthesis in Human Skin Cell and Dermal Equivalent	To analyze the effect of *Aronia melanocarpa* extract on human skin condition.	Aronia extract appears to regulate collagen synthesis in dermal fibroblasts, with activation of COL1A1 transcription and a reduction in MMP-mediated type I collagen degradation.	Lee et al. 2022
Aronia melanocarpa polysaccharide ameliorates liver fibrosis through TGF-b1-mediated the activation of PI3K/AKT pathway and modulating gut microbiota	To analyze the anti-hepatic fibrosis effect of Aronia polysaccharide on TAA-induced liver fibrosis mice and the mechanism of this effect, as well as changes in intestinal flora *in vivo*.	*Aronia melanocarpa* polysaccharides (AMP) appear to be a serious and effective method to treat liver fibrosis by improving the gut microbiota.	Zhao et al. 2022

With its increasing popularity day by day, it is preferred especially for innovative, functional food products with important health benefits. It is integrated into many different food products and commercial product diversity is gradually increasing. It is also understood from the scientific studies in the table that Aronia melanocarpa super fruit is at the very beginning of its adventure worldwide and is an important field

of study within the rapidly developing food industry. With its wide range of research directions and subjects, this fruit, where scientists have obtained different results and developments by conducting studies from different fields, is in the serious focus of both the scientific world and the industry.

Table 3. Some commercial products with *Aronia melanocarpa* on market.

Name of Product	**Ingredients**
Eclectic Institute, Freeze Dried Fresh, Aronia Berry, 450 mg, 90 Veg Caps	Freeze dried organic Aronia berries (*Aronia melanocarpa*), Hypromellose (vegetarian capsule)
Garden of Life, Kids, Organic Elderberry Immune Syrup with Aronia Berry and Vitamin C, 3.9 fl oz (116 ml)	Purified water, organic glycerin from non-GMO corn, organic strawberry flavor, organic lemon juice concentrate, mandarin peel flavor (to preserve freshness), organic stevia extract (leaf), organic citrus extract, adds a trivial amount of sugar.
Pure Original Ingredients Aronia Berry Powder (8 oz) Fruit Supplement Extract, Always Pure, No Additives or Fillers	Aronia berry extract powder
Powbab Dried Aronia Berries from 100% USA Grown Organic Aronia Chokeberry.	Organic aronia berries
Powbab Aronia Berry Powder from 100% USA Grown Organic Aronia Chokeberry.	Organic aronia berries
Aronia Berry Unsweetened Powder, No Pesticides, NOT Aronia Cherry from Overseas but Aronia Berry USA Grown, Aronia Black Chokeberry, Pure & Simple with No Additives	Aronia (*Aronia melanocarpa*)
Pure Original Ingredients Aronia Berry, (100 Capsules) Chokeberry Extract, Always Pure, No Additives or Fillers	*Aronia melanocarpa* extract (fruit)
Prof. Dr. Orhan Şen Form Superfood Aronia Tea	Aronia extract, Hibiscus leaf, Rosemary leaf, Sage, Green tea, Heather leaf, Mate leaf, Senna leaf, Fennel, Thyme, Rosehip, May chamomile, Ginseng.
Hekimhan Aronia Berry Mixed Herbal Tea	*Aronia melanocarpa*, prunus avium, zea mays, Catherine wheel, rosa canina, esuriet foneum
Chocolate-plum-bar	40% plums, chocolate 25% (crystal cane sugar, cocoa mass, cocoa butter), raisins, apricots, almonds, oatmeal. Cocoa: 60% minimum in the dark chocolate.
Aronia Original Organic Aronia Dried Berries Covered with Dark Chocolate	Dark chocolate 59% (cocoa paste, sucrose, cocoa butter), Aronia berries dried 39%, coating: gum arabic (2%) from controlled organic cultivation
Aronia Super Food Aronya Tea	Aronia (*Aronia melanocarpa*), Rosemary (Rosmarinus Officinalis), Heather (Calluna vulgaris), Sage (Salvia Sclarea), Okra (Hibiscus Sabdariffa), Thyme (Thymus) sp.), Cassia (Cassia Acutifolia), Mate Leaf (llex paraguariensis), May Chamomile (Matricaria recutita), Fennel (Feniculum Vulgare)

As can be seen, dried Aronia berries, Aronia powder, chocolates made with Aronia, herbal teas, syrups for children, Aronia fruit extracts, nutritional supplements and bars containing Aronia are among the food products commercially available in the markets (Table 3). However, this diversity and the popularity of *Aronia melanocarpa* continues to increase day by day.

1.3. Plant-based Vegan Diet

Vegan nutrition, that is, plant-based diet, is a concept that has become increasingly popular lately and is especially preferred by the young consumer group. Veganism, also called vegetarianism, can be generally defined as the dietary preference of the consumer not to consume any animal food along with meat and meat products. Consumers who have adopted a vegan diet generally prefer a diet based on grains, plants, nuts, legumes, fruits and vegetables. It can be understood from all these definitions that the vegetarian consumer group who prefers a vegan diet is a consumer group that has adopted a special lifestyle, and the vegan diet is considered a healthy, therapeutic and healing type of nutrition. It has been seen from the results obtained from the studies that the vegan diet has positive effects in the treatment and prevention of diseases that humanity is seriously struggling with, such as obesity, diabetes, cancer, cardiovascular diseases, blood pressure and metabolic problems (Glick-Bauer and Yeh 2014). In a study conducted by Craig, (2009), the results of a survey conducted by Harris Interactive are given, and according to the results of this survey conducted across America, approximately 1.4% of the American population adopts a vegan diet. In addition to living a healthy life, veganism represents the duty of living with positive effects on the Earth, such as taking good care of the environment and environmental resources, protecting animals and acting ethically in this regard, protecting from diseases caused by animals, and protecting cultural and social values (Craig 2009). Of course, people who have adopted this special type of nutrition need to complete their daily intake of nutrients and should be careful not to suffer from nutritional deficiencies instead of adhering to this healthy lifestyle. Vegan nutrition content is rich in magnesium, phytochemicals, folic acid, dietary fiber, zinc, calcium, vitamin C and vitamin E, and the richness of these protective nutrients minimizes the intake of

nutrients that are actively involved in a wide range of chronic health problems (Craig 2009). Especially in today's conditions, where people's interest in health has hardened after the Covid-19 pandemic, the interest in veganism is increasing, pushing the food industry to act in this line. While the group of producers and researchers aiming to produce innovative, functional foods targets a special audience, they are increasingly designing and producing various alternatives for a special consumer group that has adopted a vegan diet.

1.4. High Protein Diet

Protein is one of the most important basic macronutrients for human life and nutrition. In today's conditions, the consumer mass prefers diets with high protein content and follows a diet that is low in fat by providing most of their daily nutritional needs from protein. Although fat nutrients are also an important type of nutrients, when consumed without care, they cause serious chronic health problems, weight gain and life-limiting effects. For this reason, it will be possible to complete the remaining fat macronutrient taken in an effective and healthy way with a protein-rich diet. Protein is considered the main nutrient, especially for the consumer group who has made sports a lifestyle, and the amount of protein to be taken varies from person to person depending on the degree of sports performed. According to a study by Antonio et al. (2014), the amount of protein consumed by an average adult daily in the US is 91 g, or 1 g/kg based on ideal body weight. Due to this important type of nutrition that appeals to special audiences, there is a wide range of products in the food market and its diversity is increasing day by day. In addition to the nutritional value it provides, it is a type of food that is preferred and added to foods, especially in functional food design and production, due to its positive effects on health. According to the legal rules, foods can be divided into classes such as protein added or high protein content, according to the amount of protein they contain, and these special terms can be included on the labels. Purchasing such products is also largely preferred by the private consumer group. At the same time, a high protein diet is also a positive diet type for weight loss. According to Pesta and Samuel, (2014), in a protein-rich diet, the feeling of fullness will increase when the body consumes more protein, thus less fat and carbohydrate intake will occur,

which directly affects weight loss. In this way, high-protein diet becomes a type of nutrition that attracts the attention of consumers who want to lose weight, as well as people who have made sports a part of their life.

1.4.1. Plant-based high protein diet

Protein-based diets, which are a common type of nutrition, are divided into two directions which plant-based and animal-based protein-containing diets.

Plant-based protein sources are the ones that are gradually increasing their mass and gaining popularity globally. Protein, which is already a valuable nutritional source, is obtained entirely from plant-based raw materials. Plant sources contain much more protein than is known. In addition to being healthy and rich in nutrients, plant-based proteins are more accessible in terms of cost than animal-based proteins. For this reason, instead of animal-based protein sources, which are more laborious and have high economic value, plant-based proteins, which are more accessible to the consumer, have begun to be popularized and introduced. Plant-based protein content, which is an important alternative for the vegan diet type that has become widespread with today changing lifestyles, also eliminates the protein deficiency that occurs when the consumer base that adopts this diet type cannot consume animal based food products. The emergence of plant proteins as an alternative to protein from animal-derived meat will not only eliminate the negative environmental effects of meat, but will also provide a nutritious and healthy food source with high nutritional value for approximately 9 billion consumers by 2050 (Heydari, Najib, and Meda 2022). In addition to all these benefits, it can be obtained from a wide variety of plants. Plant-based products include legumes, which consist of products such as lentils, peanuts, soy, and broad beans; grains such as rice, corn, wheat and oats; it can be divided into groups as nuts such as almonds, walnuts, pistachios, hazelnuts and other plant-based products such as sunflower, quinoa, sesame and hemp (Guidi, Formica, and Denkel 2022). For people who cannot consume the necessary nutrients due to their busy daily life tempo, nutritional supplement products rich in protein obtained from plant sources help to easily complete this deficiency and also affect the healthy nutrition of consumers.

1.4.1.1. Lentil

Lentil, also known as *Lens culinaris M.*, is an important legume species belonging to the Leguminosae family (Saricaoglu 2020). Lentils, which have a low-fat content as well as a high source of protein and fiber, attract people's attention as a type of food rich in many essential vitamins and minerals (Saricaoglu, 2020 ; Lee et al. 2021). Depending on its genetic type, growing conditions and region, lentils are a rich source of protein in the range of 22% - 31% (Saricaoglu 2020). Despite its rich content, abundance of production and affordable price, lentils are not widely used. Over time, as the plant-based diet becomes more widespread, its preference increases as alternatives to existing protein sources are sought, and its rich content also comes to the fore. According to the data given in the study conducted by Liu et al. (2022), lentil production reached 57 million tons worldwide in 2019, and Canada assumed the title of leading producer by undertaking approximately 40% of this production. Lentils are a plant food source with a low glycemic index, with low starch digestion ability, which makes it a very important effect in providing glycemic and insulin control (Liu et al. 2022). Thus, lentils become of great importance in the fight against very important global health problems such as obesity, cardiovascular problems and diabetes. Approximately 80% of the storage proteins in lentils are in the cotyledons, of which 70% are globulins, 16% albumins, 11% glutelin and 3% prolamins (Lee et al. 2021). These proteins contained in the lentil legume are of great importance for improving the physical and chemical properties of foods such as solubility, foaming, emulsification (Lee et al. 2021). With its positive effects on food, it affects the production, quality, shelf life, consumption and sensory properties of foods. Lentils, which positively affect both the quality and consumption of food and have many positive effects on health, have become an important raw material for the functional food sector, especially as a protein source for the consumer segment that does not consume animal products, especially with the direction of the changing food trend recently.

Among the lentils, there are red and green lentil types globally, but the most preferred and widely used commercially is the red lentil (Aydemir and Yemenicioğlu 2013), with a ratio of 2/3 worldwide. In addition, red lentils, which are widely consumed, are the leading lentil type in terms of production and preferability in Turkey. Considering its functional and nutritional content, production capacity and low-cost

ability, red lentil is definitely a plant that should be integrated more into the food industry. With all these positive effects, red lentils, which are a legume with a significant value and an important impact, both in terms of sustainability and the positive quality effects they add to food products, have an important place in terms of comparison with other plant protein sources.

1.5. Plant-based Protein Powder in Food Products

In order to use the existing protein from plant sources, which are rapidly spreading as an alternative to animal protein sources, it is necessary to isolate the proteins. Designing new and functional foods by integrating isolated proteins into foods is becoming increasingly common in the food industry. The protein content of foods is a very important factor in terms of the functionality, physical and chemical structure of that food. The high protein content of food provides processed foods with effects that improve the structure of the food, such as gelation, solubility, foaming, water absorption and emulsification (Joshi et al. 2011). For this reason, performing protein extractions from plant sources and obtaining protein isolates and adding them to foods to give them functional properties are among the methods used in the food industry. There are different methods used to extract protein from plants. In order to store and use the obtained protein isolates for a longer period of time and to integrate them into food products more easily, the isolates obtained from the raw material plant used are brought into dry powder form. Various drying methods are also used to turn the isolates obtained after the extraction process into dry powder. These different methods used affect the efficiency of the amount of protein taken from the raw material. For this reason, a lot of literature studies have been done and experiments have been carried out with different methods.

Powdered protein isolate obtained from red lentils can be applied to various foods with different formulations in order to increase the functional properties and protein content of foods. The resulting dry powder protein isolates can be added to powdered food mixtures, bakery foods, pasta, beverages and snack food products, thus increasing both the protein amount and functionality of the product, while at the same time providing an important alternative protein source for consumers who do not

consume animal foods and eat a vegan diet. In addition, athletes with a special lifestyle also significantly prefer these protein-enriched foods. These types of food products provide both ease of life for a special consumer group and can provide a significant amount of nutritional value they need. With the ease of use and effective nutritional content they offer, these types of functional food products are highly preferred today.

1.6. Nutritional Supplement

Nutritional supplement, also known as dietary supplement, is used for products rich in special ingredients such as vitamins, minerals, proteins, herbals, antioxidants and amino acids, which are used to complete the diet as a complement, supplement and nutritional supplement in addition to a nutritional regime. Supplements are generally valuable products that are produced for special purposes and offered to the consumer, appealing to a special consumer group. Sports drinks, energy bars, meal replacement foods and multi-purpose powder mixtures are used to complement the diet as an addition and contribution to the normal nutritional routine, especially by adding functionality (Maughan, Depiesse, and Geyer 2007). Dietary supplement products are available in tablet, capsule, liquid, chewable preparations and powder forms (Rautiainen et al. 2016). Although these types of foods are definitely not medicine, they are preferred in the daily routine to fill the remaining deficiencies in the diet or to get more needed food. In today's conditions of evolution to a healthy lifestyle, its importance is increasing, especially after the pandemic, and such products are combined with functionality and gain a serious development in the food industry. This product group, which has influenced special audiences, also produces a positive effect on many health problems with regular use with its richly designed contents. In the market study conducted by Djaoudene et al. (2023), it was emphasized that there has been a significant increase in the use of nutritional supplements in the last 20 years, and the reflection of this can be detected in economic sales and the increasing product diversity in the market. The nutritional supplement market in the global market has been determined to be approximately 152 billion dollars in 2021, and according to the latest market reports, this share is expected to reach 300 billion dollars by 2028 (Djaoudene et al. 2023). Dietary supplements have also come under the scrutiny of the scientific

world, and studies and scientific publications have continued to increase recently. Especially after the covid-19 pandemic, the sales number of products in this category increased by approximately 50% between 2018 and 2020 (Djaoudene et al. 2023). Based on all these, it is understood that due to today's evolving living conditions, the global nutritional supplement food market has increased its popularity and is expected to continue increasing its momentum day by day.

1.7. Smoothie Type Food Product

After global change and transformation, people prefer functional foods that have positive health effects as well as nutritional values when consuming food. Fruit and vegetable products are among the preferred raw materials for functional foods. Fruits and vegetables can be consumed directly or processed and used through different processes. Fruits and vegetables, which can be integrated into various food products, are a very important input source for beverages. Smoothie type beverage product can be defined as a type of blended beverage obtained using fruit, fruit juice, yoghurt, milk, honey and fruit and vegetable powders and particles (Nunes et al. 2016a). The smoothie product was first introduced to America in 1960 (Stan and Popa, 2019a). Smoothies are generally used by consumers as snacks and are widely available on market shelves. Smoothies, which are healthy, satisfying, have rich nutritional content and low fat and energy levels, are now a popular food type for consumers of all ages, as they are generally prepared with intense fruit and vegetable content and enriched with superfoods. In addition to their health and nutritional effects, smoothies with various fruits in very interesting colors, tastes, sensory properties and appearance are produced to make the product more attractive to the consumer. As seen in the study conducted by Walkling-Ribeiro et al. (2010) and in the vast majority of literature reviews specifically for this product, the Brix parameter for smoothie products is very important for the quality of the product and generally the possible Brix value of smoothies varies between 12-17. The amount of nutrients it contains and its functional properties vary depending on the fruits, vegetables and raw materials used in the smoothie product.

1.8. Freeze Drying of Food Product

Drying unit operation is used as a method designed to increase the amount of dry matter in food by significantly reducing the free water in foods. While drying methods are traditionally used in many countries as sun drying, with the advancement of technology, different current types such as vacuum drying, spray drying, hot air drying and freeze drying have developed and are developing (Oyinloye and Yoon 2020). Drying food products and especially powdering them and integrating them into different food designs is widely used as an important basic process in the industry. While doing this, it will be necessary to act without forgetting the degree of acceptability of a food by the consumer. For this reason, during the drying process, it is necessary to evaporate the water on the food and obtain a more concentrated dry matter, without exposing the physical appearance, nutritional and textural structure and holistic structure of that food to any negative effects. Drying a food and reducing the amount of water in it means making that food longer-lasting, stable and less susceptible to microbial load. The reason for this is that the more water the food contains, the more likely it is that the food will spoil and become a more habitable environment for microorganisms. In this sense, drying foods has positive effects in terms of usability, shelf life and quality characteristics of that food. At the same time, according to Oyinloye and Yoon, (2020), since the surface area decreases while drying the food, the shrinkage of the package also provides a great advantage for post-production operational activities such as storage and transfer fees. When applying different traditional and technological drying methods on food, the most important parameter to consider is how all these processes will affect the final product quality of the food. Generally, technological methods are preferred rather than traditional when drying high quality and expensive food products with rich and valuable nutritional content. Especially for food products that are more sensitive to heat, the freeze-drying method is more commonly used. Freeze drying process is also known as lyophilization and is highly preferred when producing delicate and valuable powdered and solid foods. Since the process is carried out at low temperatures and under vacuum, the process is completed without damaging the valuable nutrients in the food and without any change in the quality and structure of the product. It is widely preferred to keep loss to a minimum, especially in foods containing sensitive and valuable bioactive substances such as antioxidants, phenolics and anthocyanins.

However, since the drying process applied to food will affect important quality parameters such as color, smell, taste, texture, water activity and physical appearance of that food, the most appropriate method should be chosen according to the type of food used. The freeze-drying process, which basically works with sublimation, is carried out by direct dehydration of frozen products. The freeze-drying process consists of three basic stages. The first of these is the initial freezing phase, and the freezing phase here includes the formation of ice nuclei. The second step is primary drying, and at this stage the ice separated from the dissolved phase is removed from the environment by sublimation. Then, the final stage, the secondary drying stage, begins after all the frozen water has been removed by sublimation and can therefore be facilitated by increasing the temperature of the product (Roy and Nath Gupta 2004). In the freeze-drying process, 99% dehydration occurs compared to the beginning (Oyinloye and Yoon 2020). Although the freeze-drying process is a long-term and expensive method, it is widely preferred in the food industry. The reason for this is that, compared to other drying processes, it is a process with more effective water removal capacity, less water activity, higher final product quality and more sensitive to valuable substances in the nutritional content (Bhatta, Janezic, and Ratti 2020). Freeze drying sector has been increasing its share and capacity in the market in recent years. While it can be applied to all food industry subcategories, it is especially preferred for foods in powder form. According to a report by Oyinloye and Yoon, (2020), global dried fruit and vegetable products are reported to have an annual growth market share of 3.3%. It is significantly preferred in fruits and vegetables, beverages, snacks, flour, bakery products, teas, natural colorants, additives and especially powder products. At the same time, when extracting protein from plant-based protein sources and obtaining isolates, the lyophilization process is used and then the protein powders are obtained by grinding and turning them into powder. However, the drying process is also of great importance for powder products. Water activity should be minimized by choosing the method to obtain the most effective product with the highest dry matter ratio. However, the product should not compromise on its quality features and should lose its valuable and rich nutritional content to a minimum. Achieving this high quality in powdered foods depends on the product having low water activity and minimum loss of bioactive substances and ingredients. When these conditions are met, the produced and designed powdered product can remain stable throughout its shelf life. Based on all these, it will be possible to say that the freeze-drying technique is an effective method for drying food products, especially

when obtaining powdered end products. The freeze-drying process, which is also one of the common methods used to dry and powder extracts, is of great importance for the production of functional foods that are produced in this way. While the structural, quality and stable properties it provides make the product usable in the long term, it also helps to protect the sensitive components in its content to a great extent.

1.9. Angiotensin-I Converting Enzyme Inhibitor Activity (ACE-I Activity)

Cardiovascular problems are one of the most important diseases for humanity, which have existed from past to present and have been seriously dealt with recently. Hypertension, which has a very high impact mechanism on cardiovascular disorders, is one of the diseases that is becoming increasingly common and needs to be seriously controlled (Guerrero et al. 2012). Diet and controlling the foods consumed are among the most effective and least affected ways of health problems that face a serious challenge for humanity. However, it is also necessary to make an effort to change the lifestyle and make it healthy.

Angiotensin converting enzyme, or ACE for short, has the ability to increase blood pressure by catalyzing the conversion of decapeptide angiotensin I to the vasoconstrictor angiotensin II. ACE, which provides this mechanism, has a serious vital effect on the regulation of blood pressure in the human body. ACE inhibitor peptide, which is a competitive inhibitor for the active site of the angiotensin converting enzyme, has the effect of inhibiting the conversion of angiotensin I to angiotensin II, which is the mechanism that increases blood pressure (Liu et al. 2021). In addition to having this amazing effect, ACE inhibitor peptide can also have a blood pressure-lowering effect by preventing the degradation of the vasodilator bradykinin, ensuring that it causes normal vasodilation. Considering all these magnificent effects, it is possible for blood pressure patients, which constitute a large mass globally, to have a positive impact on their lives by making changes in their nutritional routines.

In this important mechanism, drugs have been made with components that provide ACE inhibitor activity and are used against this common chronic disease. However, considering the synthetic structure and serious side effects of the drugs, by

using the diet and the foods consumed consciously, correctly and regularly, rather than being exposed to high doses of drugs after becoming ill, we can naturally take these ingredients into the body in the long term, in lower doses, and develop a resistant mechanism against these chronic diseases. It is possible to show. It is possible to see more ACE inhibitor activity in foods with high and strong protein content.

CHAPTER 2

MATERIALS AND METHODS

2.1. Materials

Aronia melanocarpa fruit was purchased from local growers in the Bursa region. It was first dried and then powdered by applying the freeze-drying process within Martin Brothers Food. The black mulberry powder used to improve the taste was also allocated within Martin Brothers Food.

Red lentils used to obtain plant-based protein powder were purchased from local markets. It was extracted and turned into protein powder within the Izmir Institute of Technology.

Ultra-high temperature (UHT) oat milk was purchased from local markets to be used in smoothie.

All chemicals used were allocated from the laboratory of Izmir Institute of Technology, Food Engineering Department.

2.2. Methods

The preparation stages of the designed functional foods and their specific physical, chemical and microbiological analysis methods are given.

2.2.1. Preparation of Powder Mixture

The preparation stages of the Aronia melanocarpa-based powdered nutritional supplement consist of freeze-drying the Aronia melanocarpa fruit, protein extraction from red lentils and obtaining the final product, the powder mixture.

2.2.1.1. Freeze Drying of *Aronia Melanocarpa*

10 kg of fresh *Aronia melanocarpa* fruits, obtained from local growers, were subjected to the freeze-drying process within Martin Brothers Food. The process was carried out using a PIGO-FD85 type freeze dryer and was exposed to temperatures of -35 to -40 ℃ for approximately 30 hours. After the necessary conditions were completed, the fruits were dried and ready to be used.

2.2.1.2. Protein Extraction of Red Lentil

Red lentil proteins were obtained by making minor changes to the isoelectric point-based precipitation method in (Moghadam et al. 2020).

First of all, red lentils were taken as grains and turned into flour with the help of a grinder. A 10% w/v aqueous solution of red lentil flour was prepared with distilled water and mixed with the help of a magnetic stirrer at 300 rpm for 10 minutes. Then, in the alkaline extraction stage, the pH value of the solution was adjusted to 9.0 using 1 M NaOH and continued stirring at 300 rpm for 2 hours at 25 °C. Then the solution was centrifuged at 9000 rpm, 4 ℃ for 30 minutes. The supernatant obtained after centrifugation was collected and precipitation was separated. The pH value of the collected supernatant was brought to the isoelectric point of 4.5 with the help of 1 M HCl and mixed with a magnetic stirrer at 300 rpm for 30 minutes. Subsequently, the solution was centrifuged at 9600 rpm, 4 ℃ for 30 minutes, under the same conditions as the first centrifugation process.

The resulting supernatant was separated and the remaining precipitation was collected (Figure 1).

The resulting precipitation was collected in centrifuge tube and stored at -20 ℃ until the lyophilization stage (Figure 2). After all the samples were collected, lyophilization was performed and the extracts obtained were then turned into powder. After lyophilization, the dull red lentil proteins were ground to the desired size powder using a laboratory grinder.

Figure 1. Final supernatant before lyophilization.

Figure 2. Collected supernatant and stored -20 ℃ before lyophilization.

2.2.1.3. Preparation of Powder Mix

Freeze-dried fruits were pulverized with the help of a grinder. Since the main ingredient of the product consists of *Aronia melanocarpa* powder, the highest input rate was achieved by using this fruit powder.

Then, in order to keep the protein amount of the product high, vegetable protein powder, extracted and lyophilized into powder, was added.

Experiments have been made with various ratios and different auxiliary powders in order to adjust the flavor balance, which is an important factor in the preferability of food products. In order to capture the flavor of the product, which does not contain any additives or sugar, and to break the sour taste of *Aronia melanocarpa*, recipes were created using banana, apple, black mulberry, strawberry and fig powders in freeze-dried form and taste tests were conducted. Black mulberry powder was selected from these auxiliary powders and added to the recipe of the product in freeze dry powder form.

Finally, experiments were made with all these input powders in different ratios, and with subsequent tastings, the most correct ratio for the product was decided.

The powders were added to the product in these proportions, made homogeneous with the help of a mixer, and stored in airtight aluminum packages.

2.2.1.4. Preparation of Extracts for Antioxidant Properties and Total Polyphenol Content Analyses

For the extraction of bioactive substances in the Aronia powder mixture, the method applied by Van de Velde et al. (2022), was used with some modifications. 1 g freeze dried Aronia powder mixture was diluted using 9 ml extraction solvent (80% ethanol: 20% distilled water) and vortexed for 1 minute. Then, ultrasonication was applied for 15 minutes. After sonication, the samples were centrifuged at 5000 rpm, 4 °C for 5 minutes. After the centrifugation process was completed, the supernatant was collected in falcons and dilution, ultrasonication and centrifugation processes were performed two more times, 3 times in total, using 9 ml of extraction solvent on the remaining pellet. After the entire process was completed, the collected supernatants were stored at -20 °C until used for the detection of bioactive substances.

2.2.1.5. pH Determination

To define the pH values of *Aronia melanocarpa* powder mixture, the method described by Kha et al. (2010), was used with some modifications. 1 g of powder mix and 25 ml of deionized water were mixed with a heated magnetic stirrer at 45 ℃ for 5 minutes. The pH value of the resulting mixture was measured using a pH meter, Hanna HI 2211.

2.2.1.6. Determination of Total Titratable Acidity (TTA)

Determination of the titratable acidity value in the samples, the basic analysis method created by Nielsen, (2017b), was applied with minor modifications. First, 1 g of powder mix samples were diluted using 20 ml of distilled water and stirred for 10 min at 45 ℃ with a heating magnetic stirrer for better dissolution of powder particles. Then, approximately 3-4 drops of phenolphthalein indicator were dropped onto this prepared mixture and stirred. Then, 100 ml of NaOH was prepared to be 0.1 N and added to the burette. Finally, the prepared NaOH solution was added to the samples until their color turned to pink.

After the analysis procedure was applied, the following formula was used to calculate the titratable acidity values of the samples (Yuan et al. 2018;Nielsen 2017c).

$$\%\ Acid = \frac{\text{V of NaOH (ml)}\times\text{N of base in}\frac{\text{mEq}}{\text{ml}}\times Eq.wt.of\ acid\ (\frac{mg}{mEq})}{\text{sample volume (g)}\times 1000(\frac{\text{mg}}{\text{g}})} \times 100 \qquad (1)$$

where:

N = normality of titrant which is a NaOH

Eq. wt. = equivalent weight of predominant acid which is a citric acid and given as a 64.04 in Nielsen, (2017b).

As a result of all of this, the results are expressed as a percentage of citric acid for the sample.

2.2.1.7. Protein Analysis

The protein content of the Aronia powder mixture was determined using the Kjeldahl AOAC basic method with some modifications (Sáez-Plaza et al. 2013). Protein analysis for the samples was carried out by Izmir Institute of Technology Biotechnology and Bioengineering Application and Research Center. 20 ml sulfuric acid, antifoaming agent and two catalyzer tablets were added to 1 g of sample. This mixture which is a prepared in Kjeldahl tubes was then heated at 450 ℃ for 5 hours. After the degradation stage, organic nitrogen content was converted to ammonium sulfate. In the distillation phase, which took approximately 4 minutes, 70 ml NaOH and 100 ml distilled water were added for neutralization and ammonia formation, and then 70 ml boric acid was added. During the titration step, ammonia was ionized to bind with HCl (0.1M) with titration and distillation unit (Vapodest 50s, Gerhardt GmbH & Co). As a result of the literature review, the generally used nitrogen to protein conservation factor 6.25 was taken.

Percentage of protein value was calculated using the following formulas.

$$\%\ Protein = \frac{\text{Protein factor} \times 1.4007 \times \text{T} \times \text{F}}{\text{Content (g)}} \quad (2)$$

where:
T = molarity of HCl (titer)
F = factor (consumption (ml) – blank value (ml))

2.2.1.8. Moisture Content

To determine the moisture content of *Aronia melanocarpa* powder mixture, the method obtained by Nielsen, (2017) was used with some modifications. 1 g of powder sample was dried in the oven at 105 ℃ for 6-7 hours until it reached a constant weight.

Then, final weighing was made and moisture content was calculated according to the following formula (Nielsen 2017a).

$$\%moisture = \frac{(wt\ of\ wet\ sample+pan)-(wt\ of\ dried\ sample+pan)}{(wt\ of\ wet\ sample+pan)-(wt\ of\ pan)} \times 100 \quad (3)$$

2.2.1.9. Ash Content

The method determined by Marshall (2010), was applied to define the ash content of the aronia powder mix samples. The samples were kept at 575 ± 25 ℃ for 8 hours using a muffle oven until they reached a constant weight. Calculations were made using the following formulas and ash contents were calculated (Marshall 2010).

$$\%ash\ (dry\ basis) = \frac{(wt\ after\ ashing)-(tare\ wt\ of\ crucible)}{(original\ sample\ wt) \times (dry\ matter\ coefficient)} \times 100 \quad (4)$$

where: dry matter coefficient = %solids/ 100

2.2.1.10. Water Solubility Index (WSI)

Some minor modifications were made to the method used by Sadowska et al. (2019), to determine the water solubility index value of the Aronia powder mixture. 0.5 g powder diluted with 6 ml of distilled water, then it was kept in 37 ℃ water bath for 30 min. After that, sample centrifuged for 20 min at 5000 rpm, 4 ℃. The resulting supernatant was oven dried at 105 °C for approximately 6 hours until it reached a constant weight and then reweighed. Water solubility index value was calculated as a percentage by comparing the amount of dried supernatant(g) relative to 0.5 g of powder.

2.2.1.11. Water Holding Capacity (WHC)

Water holding capacity value is measured based on the method used by (Sadowska et al. 2019) with minor modifications. 1 g Aronia powder mix mixed with 50

ml distilled water and vortexed for 1 min to obtain a homogeneous mixture. Then, centrifuged at 5000 rpm for 20 min, 4 ℃. The resulting supernatant was decanted and the remaining solid weighed again. As a result, the WHC value was defined in g water/g d. m.

2.2.1.12. Mineral Analysis

Inductively coupled plasma optical emission spectrometry (ICP-OES, 5110) was used by Izmir Institute of Technology Environmental Development Application and Research Center to identify the mineral content of Aronia powder mixture samples. After the deformation phase of the samples was completed, it was carried out under high pressure and temperature in a closed container using 2 ml hydrogen peroxide and 10 ml nitric acid. After all these processes, the content of the product in terms of Ca, Fe, K, Mg, Na, P and Zn minerals is defined in mg/ kg.

2.2.1.13. Color Analysis

The color properties of the Aronia powder mix sample were determined using a colorimeter (Konica Minolta, CR400) calibrated with a standard calibration apparatus. The color properties of the samples were defined using the CIE L*, a*, b* color parameters. Among these parameters, the L* value defines the darkness and lightness properties of the samples, the a* value defines the greenness (-) and redness (+), while the b* value defines the blueness (-) and yellowness (+) (Taskin 2020).

2.2.1.14. Total Phenolic Content Analysis

The total phenolic content of the Aronia powder mixture was determined by making some changes on the basic Folin-Ciocalteu method applied by Singleton et al. (1999). The extracts prepared and kept at -20 C. 500 µl of extracted sample was added

to 2 ml of Folin-Ciocalteu phenol reagent and kept in the dark for 5 minutes. Then, 1 ml of 7.5% sodium carbonate was added to the samples and left in the dark for 55 minutes. Later, when the samples obtained became cloudy, affecting the spectrometric reading, they were centrifuged at 5000 rpm, 4 ℃ for 10 minutes to eliminate this situation. The resulting clear supernatant was measured against ethanol at 765 nm using a UV-visible spectrophotometer (Shimadzu, UV-1601). In addition, control samples were created by adding the extraction solution (80% ethanol: 20% distilled water) as a sample used in the extraction stage.

For calculations, a reference curve was created using different concentrations of gallic acid as a standard and the results were defined in mg gallic acid (GAE)/L (Tirla et al. 2023).

2.2.1.15. Antioxidant Activity (DPPH, ABTS) Assay

Two different analysis methods were used to define the antioxidant content of the Aronia powder mixture.

ABTS radical stock solution was prepared based on the basic method given by (Re et al. 1999). ABTS was dissolved in distilled water to 7 mM and potassium persulfate was added to 2.45 mM. The resulting mixed solution was kept in the dark and at room temperature for 12-16 hours before using. The absorbance value of the prepared ABTS solution was adjusted to be read between 0.68 and 0.72 by dissolving it using ethanol. This assay was used with some modifications to the methods applied by Re et al. (1999) and İzli, Yildiz, and Berk, (2022). 60 µl of the sample distilled with extraction solution (80% ethanol: 20% distilled water) at a ratio of 1:5 was added to 3 ml of the prepared ABTS solution. The absorbance value of the resulting mixture was measured against ethanol at 734 nm after 3 minutes with a UV-visible spectrophotometer (Shimadzu, UV-1601). The same amount of extraction solution (80% ethanol: 20% distilled water) was used as a control sample.

In order to determine the free radical scavenging activity of Aronia powder mixture, the DPPH (2,2-diphenyl-1-picrylhydrazyl) method applied by Toupal and Coşansu, (2023), was used with some modifications. 0.05 mM DPPH solution was prepared by dissolving it with ethanol. 100 µl of extracted sample was added directly to

2900 µl of prepared DPPH solution without any dilution. The mixture was left in the dark and at room conditions for 30 minutes. Measurement was made with a UV-visible spectrophotometer (Shimadzu, UV-1601) and the absorbance value was set as 517 nm against ethanol. Extraction solution (80% ethanol: 20% distilled water) was used as a control sample.

Trolox standards were dissolved in ethanol using the same methods and absorbance values. The results obtained are given in µmol Trolox/ ml.

2.2.1.16. Microbiological Analyses

Bacterial counting studies for aronia powder mixture samples were carried out using the spread plate method, including total viable detection with Plate Count Agar, coliform detection using Violet Red Bile Agar, and yeast and mold detection with Potato Dextrose Agar.

2.2.1.17. Shelf-life Analyses

Shelf-life studies for aronia powder mixture are planned monthly. For this product in powder form, microbiological analyzes (PCA, PDA, VRBA), pH, color, moisture, total phenolic content and antioxidant (ABTS, DPPH) content were regularly monitored throughout its shelf life.

Since the product is in powder form, it is recommended to store it in airtight aluminum packages and in the refrigerator.

2.2.1.18. Statistical Analysis

The averages and standard deviations of all data obtained were calculated using the Excel application. Statistical significance was defined using one-way analysis of variance (ANOVA). Comparison of the data was made using the Tukey test with the help of Minitab 17 Statistical Software ($\alpha = 005$).

2.2.2. Smoothie

A functional and vegan smoothie drink is being designed using the Aronia melanocarpa based nutritional supplement end product.

2.2.2.1. Smoothie Preparation

The produced Aronia mix powder and oat milk were tried in different proportions and structure and taste tests were carried out, and after trying approximately 5 different recipes, the most suitable form for smoothie was obtained. As a result of the trials, a smoothie prepared using 12% aronia mix powder was determined as the final product recipe. 12% aronia mix powder was added to the oat milk and homogenized with the help of a homogenizer for a total of 4 minutes, 15000 rpm for the first 2 minutes and then 12000 rpm. Afterwards, the homogeneous mixture was bottled and pasteurized at 70 °C for 20 minutes.

2.2.2.2. Preparation of Extracts for Antioxidant Properties and Total Polyphenol Content Analyses

The extraction process for the determination of bioactive substances from smoothie samples does not differ except that the number of dilutions is doubled. A total of 2 dilutions were made using 9 ml of extraction liquid (80% ethanol: 20% distilled water) and all other procedures were performed as given in section 2.2.1.4. (Van de Velde et al. 2022).

2.2.2.3. pH Determination

To measure the pH values of smoothie samples, measurements were made using a digital pH meter (Hanna, HI 2211).

2.2.2.4. Determination of Titratable Acidity (TTA)

To determine the smoothie sample percentage titratable acidity value, the method mentioned in section 2.2.1.6. were used with a slight difference at a part of dilution. For smoothie sample, 1 g of smoothie sample were diluted using 20 ml of distilled water without any stirring and heating part. Then, same procedure and formula was applied with *Aronia melanocarpa* based nutritional supplement powder samples.

2.2.2.5. Protein Analysis

Determination of the protein content for smoothie samples, the method which is the described in section 2.2.1.7. is used.

2.2.2.6. Moisture Content

The method and steps specified in method section 2.2.1.8. were used to determine the moisture content of smoothie samples.

2.2.2.7. Ash Content

The methods and steps specified in method section 2.2.1.9. were used to determine the ash content of smoothie samples.

2.2.2.8. Determination of Brix Value

The Brix value of the *Aronia melanocarpa* based functional, vegan smoothie sample was determined by using a digital refractometer (Mettler Toledo, RE50) device

at room temperature. Measurements were performed in three replicates and two parallel measurements.

2.2.2.9. Mineral Analysis

The method which is the described in section 2.2.1.12. was used for the determination of mineral content for smoothie samples.

2.2.2.10. Color Analysis

The color characteristics of smoothie samples were defined using methods mentioned in section 2.2.1.13.

2.2.2.11. Total Phenolic Content Analysis

Total phenolic content of smoothie samples determined using method which is mentioned in section 2.2.1.14. without any differences.

2.2.2.12. Antioxidant Activity (DPPH, ABTS) Assay

For antioxidant determination of smoothie samples, ABTS and DPPH methods were applied in exactly the same way as mentioned in section 2.2.1.15.

2.2.2.13. *In vitro* Digestion

The digestion protocol determined by Brodkorb et al. (2019) was applied to determine the bioaccessibility properties of functional, vegan Aronia based smoothie

samples. *In vitro* digestion procedure was performed for the final consumed product which is Aronia based smoothie sample, and separately for red lentil protein and oat milk, *Aronia melanocarpa* powder and oat milk, *Aronia melanocarpa* powder, red lentil protein powder and oat milk to understand their interactions with each other.

First, three different digestive fluids were prepared: salivary, gastric and intestinal. All of these liquids were prepared by adding them to the previously prepared stock solutions in the proportions specified in the protocol. The *in vitro* digestion procedure consists of three important stages: oral, gastric and intestinal.

Simulated salivary fluids (SSF) prepared in the oral phase consist of 15.51 mmol/ L KCl, 3.7 mmol/ L KH_2PO_4, 13.6 mmol/ L $NaHCO_3$, 0.15 mmol/ L $MgCl_2(H_2O)_6$, 0.06 mmol/ L $(NH_4)2CO_3$, 1.1 mmol/ L HCl and 1.5 mmol/L $CaCl_2$ solutions. The pH value of the prepared SSF is adjusted to 7 and kept at 37 ℃ for 2 minutes. The saliva mixture concentration was raised to 75 U/ml by adding α-amylase to the saliva stock solution.

Simulated gastric fluids (SGF) prepared in the gastric phase consist of 6.9 mmol/ L KCl, 0.9 mmol/ L KH_2PO_4, 25 mmol/ L $NaHCO_3$, 47.2 mmol/ L NaCl, 0.12 mmol/ L $MgCl_2(H_2O)_6$, 0.5 mmol/ L $(NH_4)_2CO_3$, 15.6 mmol/ L HCl and 0.15 mmol/L $CaCl_2$ solutions. The pH value of the prepared SGF is adjusted to 3. Pepsin enzyme was added to the gastric fluid to increase the concentration of the gastric mixture to 2000 U/ml. The samples were shaken from bottom to top at 60 rpm for 2 hours at 37 °C.

Simulated intestinal fluids (SIF) prepared in the intestinal phase consist of 6.8 mmol/ L KCl, 0.8 mmol/ L KH_2PO_4, 85 mmol/ L $NaHCO_3$, 38.4 mmol/ L NaCl, 0.33 mmol/ L $MgCl_2(H_2O)_6$, 8.4 mmol/ L HCl and 0.6 mmol/L $CaCl_2$ solutions. The pH value of the prepared SIF is adjusted to 7. In addition, bile salt and pancreatin enzyme were added to the intestinal mixture. The prepared mixture was mixed from top to bottom at 60 rpm for 2 hours at 37 °C. The pH of the mixtures was monitored throughout the determined digestion period. The reason for this is that the lipase enzyme loses its activity and becomes inactive if the pH value of the environment drops to 5.7.

The prepared samples, whose digestion stages were completed, were stored at -20 ℃ until measurements were taken. Finally, total antioxidant content, total phenolic content and ACE inhibitor activity values of the post-digestion products were measured.

2.2.2.14. ACE Inhibition (ACE-I) Activity Assay

Samples to be used in ACE-Inhibitor analyzes were centrifuged at 10000 rpm at 4 ℃ for 20 minutes. After centrifugation, supernatants were collected by filtering on filter paper and stored at -20 ℃ until analysis. The pH values of the samples obtained were adjusted to 8.3 with the help of 2.5 M NaOH before analysis.

ACE Inhibitor Activity analysis was based on the method applied by Pihlanto et al. (2008), with minor changes. Hippuryl-L-Histidyl-L-Leucine (HHL) and ACE substrates were dissolved in 100 mM borate buffer containing 0.3 M NaCl and 1M HCl to make the pH value 8.3 with vortexed 2 min. The experiment, consisting of 250 µl HHL solution, 50 µl ACE solution and 50 µl sample, with a total reaction volume of 350 µl, was carried out in microcentrifuge tubes. While the sample and substrate solution were mixed at 37 °C for 10 minutes, ACE was incubated under the same conditions. Following ten minutes, ACE was added to the sample and substrate mixture, and the reaction proceeded for thirty minutes at 37 °C. The reaction mixture was shaken every 10 minutes. After 30 minutes, the reaction was stopped by adding 100 µl of 1 M HCl and filtered through a 0.20 mm pore filter. HPLC analysis was performed using Diode-Array Detector (DAD) and a symmetry C18 (3.0×150 mm, 5µm, Walters) column was used. HA and HHL were detected at 228 nm. The column used was eluted at a constant flow rate 0.5 ml/ min with a system of two solvents which are 0.05% Trifluoroacetic acid (TFA) in water and 0.05% TFA in acetonitrile. When the analysis temperature is 30 ℃, the injection volume is 50 µl. HA standard samples were prepared daily during the experiment to plot a standard curve and check the results. ACE-Inhibitory activity was calculated with the following equation (Pihlanto, Akkanen, and Korhonen 2008):

$$\%inhibitor\ activity = \frac{(HA\ control)-(HA\ sample)}{(HA\ control)} \times 100 \quad (5)$$

where $HA_{control}$ represents the conversion of peak areas into hippuric acid in mm according to the standard curve created in the absence of samples, and HA_{sample} represents in the presence of samples. These analyses were done by Integrated Research Centers in Izmir Institute of Technology.

2.2.2.15. Microbiological Analyses

All bacterial counting methods given in section 2.2.1.16. were applied in the same way for smoothie samples.

2.2.2.16. Shelf-life Analyses

Shelf-life studies for the Aronia smoothie product are planned as 21 days weekly. Regular monitoring of microbiological analyzes (PCA, PDA, VRBA), pH, total titratable acidity, color, brix, total phenolic content, antioxidant (ABTS, DPPH) activity and ACE-Inhibitor activity at 0th, 7th, 14th and 21st days throughout the shelf-life studies has been made.

The Aronia based smoothie product should be kept closed in the refrigerator throughout its shelf life.

2.2.2.17. Sensory Analysis

Sensory analyzes were carried out in the Sensory Analysis Laboratory of Izmir Institute of Technology, Department of Food Engineering, with the participation of 20 experienced and inexperienced people. Participants were questioned whether they were allergic to the Aronia based smoothie product or any of the raw materials it contained, and product information and project information were clearly stated in the pre-prepared sensory form. The prepared Aronia smoothie samples were evaluated on a 5-degree hedonic scale (5- extremely like and 1- extremely dislike). In the prepared sensory form, the product was presented for evaluation in terms of color, odor, texture, consistency/viscosity, taste and general acceptability parameters. All conditions in the sensory analysis laboratory have been prepared in such a way that participants can make reliable and accurate evaluations and decisions.

2.2.2.18. Statistical Analysis

The averages and standard deviations of all data obtained were calculated using the Excel application. Statistical significance was defined using one-way analysis of variance (ANOVA). Comparison of the data was made using the Tukey test with the help of Minitab 17 Statistical Software ($\alpha = 005$).

CHAPTER 3

RESULTS AND DISCUSSION

3.1. Preparation of The Products

The preparation of aronia powder mix and aronia smoothie products and the results obtained are discussed in this section.

3.1.1. Preparation of Aronia Powder Mix

As a means to preserve the maximum number of bioactive substances in its content, especially its high antioxidant and phenolic properties, *Aronia melanocarpa* fruit was dried with the freeze-drying technique and then turned into powder. The purpose of the designed product is to produce a vegan, protein-rich and functional nutritional supplement using *Aronia melanocarpa* fruit as a base. In this context, it is very important for the designed product that the contents of the raw materials used are affected by the process applied in a minimum amount. One of the most important goals is to design a product that is completely free of additives and added sugar and that the consumer can enjoy in terms of taste as well as its health effects. For this reason, in order to get rid of the astringent taste that *Aronia melanocarpa* fruit leaves in the mouth when consumed alone, an attempt was made to achieve taste balance by using powders of different freeze-dried fruits. Protein powder obtained from red lentils was added to the mixture by the lyophilization method and 10 different recipes were obtained by using different auxiliary fruits in different proportions. Following the taste tests on the recipes, the actual product, Aronia powder mix, was given until its formulation as given Table 4. The final structure of the Aronia powder mix nutritional supplement product is as shown in Figure 3.

Table 4. Final Aronia powder mix nutritional supplement recipe.

Ingredient	**Percentage (%)**
Aronia melanocarpa Powder	40-70
Red Lentil Protein Powder	20-40
Black mulberry Powder	10-35

The product was kept in air- and light-proof aluminum packages and in the refrigerator for analysis. The shelf life has been determined as 2 years, based on the shelf life of equivalent products.

Figure 3. Final powder form of Aronia powder mix product.

The packaging to be presented to the consumer is designed to contain 30 chassis in a box of 20 g, as seen in Figures 4 and 5. The most important reason for this will be to demonstrate to the consumer audience in a more applicable way that the effects of this product, which will be presented to the consumer as a nutritional supplement, will be seen with regular consumption. By using one sachet every day, they will consume the

product in a regular period of 1 month and will be able to see its effects in the maximum way.

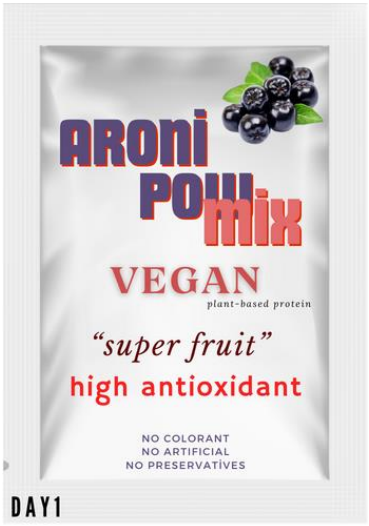

Figure 4. 20 g Aronia powder mix sachets (daily).

Figure 5. 30 x 20 g Aronia powder mix box (monthly).

Since Aronia powder mix product is a nutritional supplement in powder form, it is recommended to the consumer that it be consumed with products such as water, milk, yoghurt, smoothie, breakfast cereal, pancakes, etc. rather than consumed alone.

3.1.2. Preparation of Aronia Smoothie

Aronia smoothie product was designed in order to experience the Aronia powder mix product, which is recommended to be used directly on its own as well as to be included in meals as a by-product, with smoothie, which is the most preferable and applicable product type, and to perform sensory analyzes and conduct *in vitro* digestion trials. Aronia smoothie product, just like the Aronia powder mix product, is prepared with plant-based oat milk in order to maintain its vegan feature as seen in Figure 6. In order to decide the amount of Aronia powder mix, samples were prepared in different proportions and the 12% ratio was determined by taste test. The smoothie has been pasteurized to limit the microbial load. After reviewing equivalent products and literature, the shelf life for Aronia smoothie was determined as 21 days. The product has been stored in the refrigerator, away from air and light.

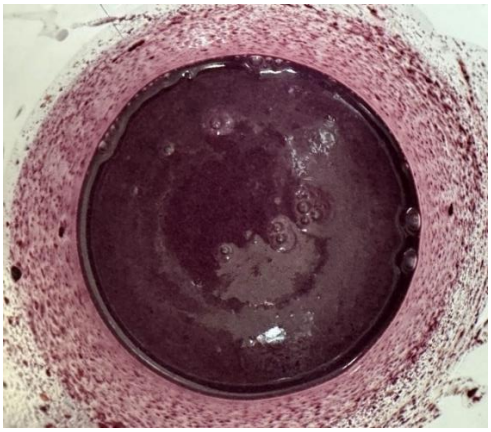

Figure 6. Prepared Aronia smoothie sample after homogenization.

3.2. pH Determination

pH control was carried out for Aronia powder mix and Aronia smoothie products throughout their shelf life. pH control is an important quality control parameter for food products. Especially in fruit and vegetable products, some criteria affect the pH value of the products. It is possible to list these criteria, which have a significant impact on the *Aronia melanocarpa* fruit, such as the region where the product is grown, soil type,

climate conditions, harvesting conditions, maturity level of the product when it is collected and the types of processes applied to the product (Petković et al. 2021). The main characteristic pH value of the fruit is in its fresh state and this level can increase or decrease with each process applied. In his study, Petković et al. (2021), reported the pH value of the fresh *Aronia melanocarpa* fruit as 3.40-3.43. As can be seen from this value, *Aronia melanocarpa* fruit has a low pH value, that is, a high acidity level.

pH control was performed monthly for the Aronia powder mix product throughout its shelf life (Figure 7). The pH value started at 3.72 from the moment of production and then increased regularly until it reached 3.85. The pH value of the product was found to be slightly higher than the pH value of the fresh *Aronia melanocarpa* fruit. This change is an expected effect due to the processes applied to the product and other inputs in the powder mixture. Other powder ingredients in the product reduced the acidity of the product to some extent and increased its pH value. In fact, the most important factor in designing the product as a powder mixture is to break the astringent taste that comes from the high acidity of the *Aronia melanocarpa* fruit, and this can be seen with the pH values. These added inputs increased the pH value of the product and reduced its acidity to some extent. When we look at the pH value obtained throughout the shelf life, it can be said that there is generally a regular and very small increase. This change, which was observed over a period of approximately one year, is so small that it can be ignored. The biggest effect in concluding that the product has achieved stability in its properties throughout its shelf life is the very low amount of water in the product and the application of very careful storage conditions.

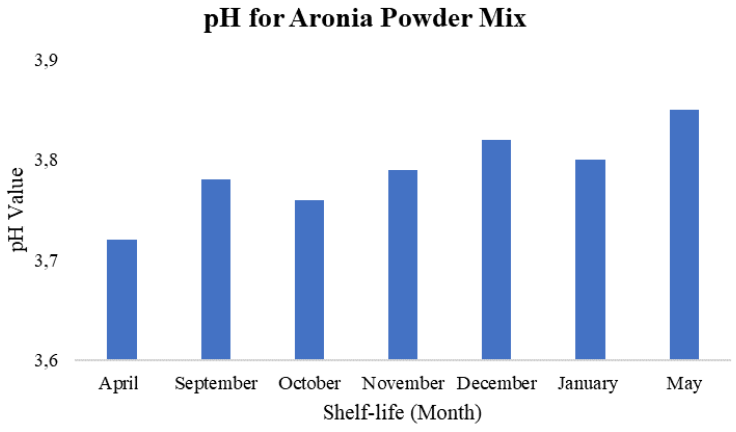

Figure 7. pH values for Aronia powder mix throughout the shelf-life.

In the study conducted by Taskin, (2020), on different types of freeze-dried *Aronia melanocarpa* fruit, pH values were given in the range of 3.54-3.92. These results, obtained similarly with the Aronia powder mix product, also prove that the drying process has a small effect on the pH value of the product. Considering the effect of the region where the product is grown, the conditions and other effective parameters on the pH value, the difference between the results is too small to be taken seriously. In the research conducted by Tolic et al. (2015), on different types of food products obtained using *Aronia melanocarpa* fruit, the pH value of powdered *Aronia melanocarpa* products was obtained in the range of 4.02-4.13. These results show that the products in this study have a higher pH value, that is, lower acidity. However, the difference is still not very high. It should be noted that this low difference may come from the growing conditions of the fruit, its type or process conditions, and this is an expected situation.

For the Aronia smoothie product, pH control was carried out weekly throughout its shelf life (Figure 8). The pH value of the product has been measured as 4.98-5.15 from the moment of production. An increase, although not significant, was observed over time during shelf life. This low increase is an expected analysis result. The Aronia smoothie product, which has a higher pH value and lower acidity compared to the Aronia powder mix product, is due to the oat milk added to the recipe. Added oat milk reduced the acidity of the product and increased its pH value.

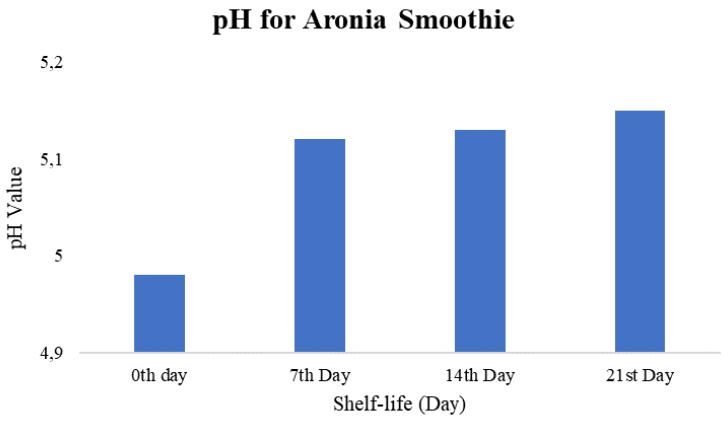

Figure 8. pH values for Aronia smoothie product throughout the shelf-life.

No literature source could be found on the pH values of a smoothie product made directly from *Aronia melanocarpa* fruit. In general, there are studies on the chemical and physical properties of fruit juices made with *Aronia melanocarpa*. In a study conducted by Yi et al. (2022), pH was monitored on Aronia berry juice throughout its 5-week shelf life. Except for the control sample that was not subjected to heat treatment, no significant change was observed in the pH values of fruit juices produced with heat treatment throughout their shelf life. The pH values of heat-treated Aronia berry juice products increased and changed within the range of 3.65-3.70 throughout their shelf life. As can be seen, this increase is too low to be taken seriously, just like the Aronia smoothie product. One of the most important reasons why this change in the unheated control sample is continuous and high is that microbial life is not limited by heat treatment. The microbial load, which continues to develop over time throughout its shelf life, also causes an increase in the pH value of the product. Aronia berry juice product has a low pH value and high acidity level. Although comparing it with the Aronia smoothie product does not give a very effective result in this case, it will be good to understand the situation of a product in a beverage category that has been heat treated throughout its shelf life. However, the pH value of oat milk, which is used in the Aronia smoothie product and contains a large portion of the product, is given as approximately 5.92 in a study conducted by Krishi Vidyapeeth et al. (2020). Inspired by these pH values given separately for Aronia berry juice and oat milk products, it is an expected result that the pH value of the Aronia smoothie product, which has common ingredients, is around 5 on average. It is considered a possible situation that these results may occur as a result of combining the Aronia powder mix product, which has a high acidity level, with the oat milk product, which has a lower acidity level.

3.3. Determination of Total Titratable Acidity (TTA%)

Total titratable acidity is another important criterion that gives an idea about the quality of food products. The concept of TTA, which has a parallel relationship with pH, is one of the parameters that should be taken into consideration for foods. Apart from pH, TTA value gives an idea about the acidity value of the products, while also being an indicator of how this acidity rate reflects on the taste of the product. While TTA analysis

was performed for Aronia powder mix, TTA analysis for Aronia smoothie product was examined throughout its shelf life.

The %TTA value of Aronia powder mix product is calculated as 2.69%. This is a high acidity level and the astringent taste of the product supports this value. In a study conducted by Tolic et al. (2015), %TTA values of products from different categories made using *Aronia melanocarpa* fruit were given. The %TTA values of the products in powder form were obtained in the range of 1.67-2.30%. As can be seen, powder products generally have high %TTA values, just like the Aronia powder mix product. It is also possible to say that this high acidity value is due to the very low amount of water in the products.

For the Aronia smoothie product, %TTA values were examined weekly throughout its shelf life (Figure 9). No significant change in acidity values was observed throughout the shelf life of the product. The smoothie product, which had a TTA value of 0.48% for two weeks after the first production moment, was later measured as 0.38% and 0.39%, respectively. Although there is a slight decrease over time, there is not enough change to be taken seriously, as in pH values. The pH value of the same product increases in very small amounts over time during its shelf life. By combining these two values, it is possible to conclude that the acidity of the product decreases slightly over time.

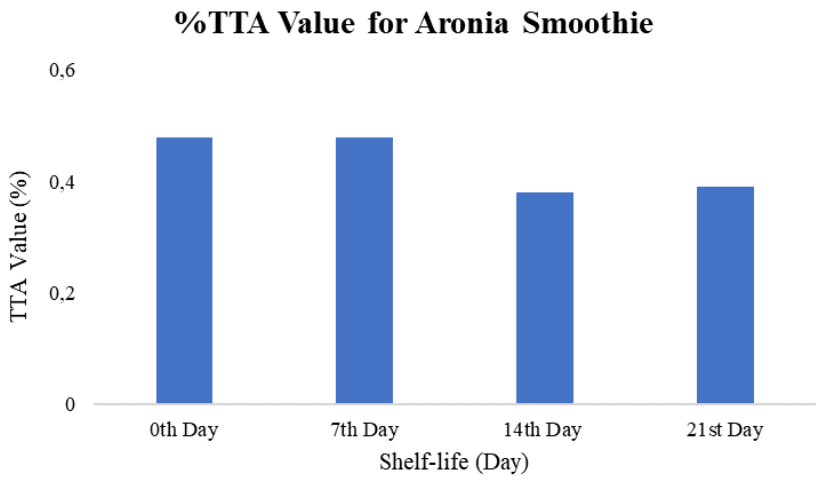

Figure 9. %TTA values for Aronia smoothie product throughout the shelf-life.

According to Nunes et al. (2016), the change in %TTA values of smoothie-type drinks is monitored throughout the 21-day shelf life. In all results, acidity values were obtained in the range of approximately 0.6-0.8%. This change during shelf life is very low. This situation is also observed in the Aronia smoothie product, in a way that is not significant. It can be concluded that stability in terms of acidity can be achieved in the products.

If Aronia powder mix and Aronia smoothie products are compared, the pH value of the product in powder form is lower and the %TTA value is higher. The reason for this is that the water content of the powder product is very low and the oat milk added while turning the product into a smoothie greatly breaks and reduces the acidity of the powder product. Considering that the taste of the products also supports this situation, it can be concluded that the %TTA value seriously affects the quality characteristics of food products and is directly reflected in the taste of the final product.

3.4. Protein Analysis

Considering today nutritional conditions, functional foods with high protein content have gained serious importance, especially for the consumer group who has determined a healthy diet. People want to get the daily nutritional content they need with practical, healthy and effective foods in the pace of their daily lives, and protein is one of the most important nutrients. Foods with high protein content, healthy, functional, yet easy to use and practical to use in daily routine are preferred by a special consumer group, namely athletes, people with active and busy lives, and people who care about healthy nutrition.

In general, fruits and fruit-containing products are foods that are low in protein (Aderinola 2018). As stated by Pop et al. (2022), Aronia fruit is a very low fruit in terms of protein content, with an amount of 0.7 g / 100 g. Due to the necessity of the designed food to be functional and to appeal to a special consumer group, protein powder extracted from red lentils of plant origin was added to the products, making them rich in protein content. Thus, the deficiency of fruit powder and smoothie products, which are the most preferred product groups by consumers, has been eliminated and products that will contribute to people's daily protein needs have been obtained.

Aronia powder mix and Aronia smoothie products designed in line with these explanations will be among the products preferred by consumers with their high protein content. Protein analysis for Aronia powder mix and Aronia smoothie products was carried out in three parallel and two repetitions. According to the results obtained, the protein amount of Aronia powder mix product was obtained as 23.7%. As stated in the Turkish Food Codex Nutrition Communiqué, if the protein amount of a food is over 20%, it can be labeled as 'high protein', and if the amount of protein is at least 12%, it can be labeled as 'protein added'. The Aronia powder mix product developed in this context can be offered to the consumer as a high-protein dietary supplement. This shows that Aronia powder mix product can be a very important nutritional source to meet the amount of protein required for this type of consumer.

For the Aronia smoothie product designed using Aronia powder mix, the amount of protein was obtained as 3.74%. To prepare a smoothie, 12% Aronia powder mix is added to the mixture. In the calculation made using these ratios, the amount of protein coming from Aronia powder mix to the Aronia smoothie product is approximately 2.84%. The remaining protein comes from oat milk. In the study conducted by Krishi Vidyapeeth et al. (2020) on oat milk, it was stated that the protein content of oat milk was 0.966%. This ratio is consistent when the recipes of Aronia powder mix and Aronia smoothie products are taken into consideration and calculations are made.

In a study conducted by Bratosin et al. (2024), a healthy and innovative bar was developed using Aronia powder. The protein content of the bar product containing Aronia was obtained as 12.20%. Likewise, Zuber Gluten-Free Protein Bar, a bar commercially available in the market, contains 28.6% protein. This rate is a high value compared to its equivalents and can be declared on the label. When we look at the protein content of the Aronia powder mix product, it is close to this level with 23.7% and contains more protein than many products in its own category.

According to Savas and Akan, (2021), the protein content of a dairy beverage product made using raspberry and oats was 3.20-3.94%. In a study conducted by Mehta et al. (2017), it was aimed to make an alternative smoothie to be consumed at high protein breakfasts. The protein content of the smoothie product designed for this purpose was 3.48%. Aronia smoothie product has a very similar and high value with 3.74% protein content.

Pınar Protein Milk, which is commercially available on market shelves, will be one of the best examples. According to the data given on Pınar Protein milk, 1 glass

(250 ml) of Pınar Protein milk provides approximately 26% of the daily protein amount. These values, of course, vary depending on gender, weight and living conditions. As seen in Table 5, the necessary calculations are given according to living conditions, gender and body weight in the calculations made by Pınar. Calculations were made on the daily protein intake amounts for Aronia powder mix and Aronia smoothie products, taking Pınar Protein Milk product as a reference. Aronia powder mix product will be offered to the consumer in 20 g sachets and a 30-day box. In this context, 20 g (1 sachet) of Aronia powder mix product contains 23.7% protein and meets 10% of the average daily protein intake.

Table 5. Recommended daily protein intake calculated by Pınar.

	Daily Protein Need		
	Daily Recommended Amount of Protein	**Calculated as an Example**	
		Man (70 kg)	**Woman (55 kg)**
Daily Routine Activity	0.8 g protein per 1 kg body weight	56 g	44 g
Endurance Sports: endurance and continuity	1.2-1.4 g protein per 1 kg body weight	84-98 g	67-77 g
Power Sports: strength, endurance and speed	1.7-1.8 g protein per 1 kg body weight	119-126 g	94-99 g

The amount of sachet to be consumed can be determined by the consumer according to parameters such as living conditions, gender and weight, and the amount of protein desired to be consumed. According to the calculations made on Aronia smoothie, 1 glass (250 ml) of Aronia smoothie product will meet 19% of the average daily protein intake. In the high protein smoothie development study conducted by Mehta et al. (2017), it was stated that the final product obtained met approximately 18.83% of the daily protein amount with 3.48% protein content. As can be seen from the parallelism of these results, Aronia powder mix and Aronia smoothie products will take their place among the preferable food products rich in protein content.

3.5. Moisture Content

Moisture content can be explained as a term used to describe the amount of water in a food and an analytical measurement method (Park 1996). The amount of moisture in the food is a very important parameter in terms of the stability and quality of that food. Moisture content analysis was carried out for both Aronia powder mix and Aronia smoothie products.

Since moisture content is a very important parameter in terms of quality for powdered food products, moisture content analysis was carried out for the Aronia powder mix product throughout its shelf life. Moisture content is an important parameter that affects the characteristics, stability and quality of powdered food products especially during their shelf life (Eisinaitė et al. 2020). The amount of moisture in the product is an important factor that affects the chemical reactivity of the products, such as lipid oxidation, and the growth and development level of microorganisms (Eisinaitė et al. 2020). The moisture content of a food affects the strength of its resistance to spoilage (Nielsen 2017b). Food products with low moisture content have higher resistance to spoilage and microorganisms. This is due to the fact that due to the low water content, the appropriate environment for these developments and formations cannot be provided. Storage conditions, starting from the production of the product throughout its shelf life, are the most important factors that directly affect the amount of moisture. The moisture content of the Aronia powder mix product was measured as 1.2% after the first production. The product was then stored in airtight aluminum zip lock packages under refrigerator conditions. Due to some problems, the moisture content of the product, which was continued to be checked after 5 months, was obtained as 1.28%. Since this negligible difference in moisture value after 5 months indicates that there is no negative or major change on the product, it was decided to continue the analysis of the product on a monthly basis in the future. Afterwards, moisture content was regularly achieved as 1.32%, 1.45%, 1.5% and 1.48% during October, November, December and January (Figure 10). As a result of these, since no major changes were observed, it was decided to conduct the next analyzes in May, just before the delivery of the study, and the moisture content of the product was measured as 1.55% in May. A slight change and increase in the moisture content of the product was observed during this one-year shelf-life follow-up approximately from its production. One of the most

important reasons for ensuring this shelf-life stability can be explained as paying careful attention to the storage conditions of the product and storing it in refrigerator conditions in an airtight manner. In a study conducted by Eisinaitė et al. (2020), black chokeberry pomace product was turned into powder. The moisture value of this product, which is in powder form, was obtained as 1.74% on average.

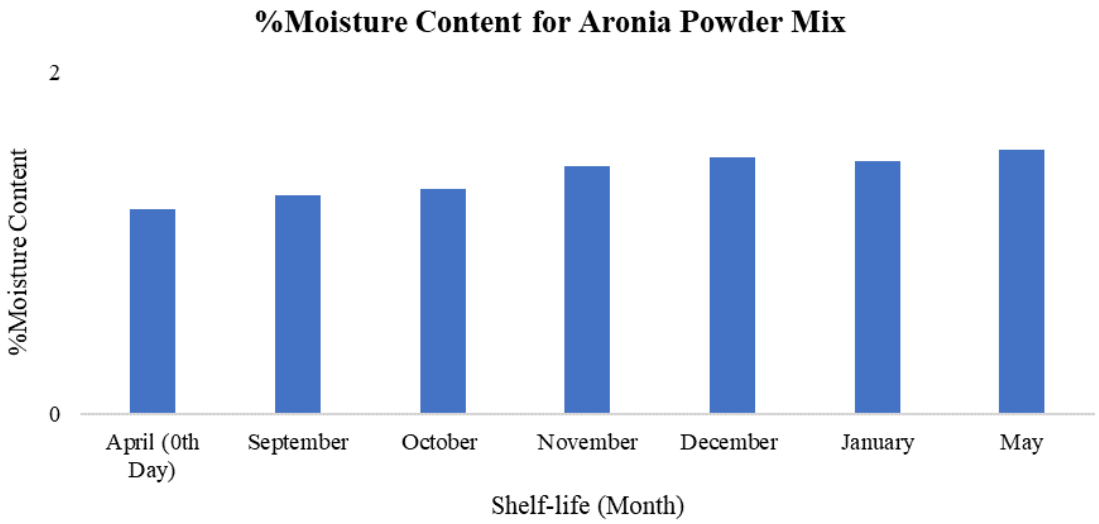

Figure 10. %Moisture content for Aronia powder mix throughout the shelf-life.

The moisture content value of the product is suitable for powder products. The results are consistent with the amount of moisture obtained throughout the shelf life of the Aronia powder mixture nutritional supplement product. For powder products, it would be correct to conclude that the lower the moisture content, the better the product quality and stability. For this reason, the moisture content of these products is very suitable for their product categories and shows that the product will be more resistant to microbial deterioration and development. In another study conducted by Lazăr et al. (2020), the moisture value of a product dried and powdered with the freeze dry technique using *Aronia melanocarpa* pomace was measured as 3.53% on average.

The moisture content of the Aronia powder mixture product dietary supplement end product throughout its shelf life is lower than the product in this study. Both products have a good dry matter content for powder products. In another literature study, the dry basis moisture content for the instant green smoothie product pulverized with the freeze dry technique was given as 95.18% and it was said that this moisture

content was very low and increased the shelf life of the product (Dilrukshi and Senarath 2021). When compared to Aronia powder mixture, it can be concluded that it has a lower moisture content throughout its shelf life and in this case, it has more positive effects in terms of product stability, microbiological properties and shelf life (Dilrukshi and Senarath 2021). If the moisture content of a powdered food product is less than 5% on a wet basis, it seriously affects the shelf life and consumer acceptance properties of that product (Tahsiri et al. 2017).

In a study conducted by Ghendov-Mosanu et al. (2022), the moisture content of more than one Aronia-based powder product was measured on the 1st and 50th days throughout its shelf life. It was noticed that the differences between these two measurements made during the shelf life were almost non-existent and therefore there was no need to make very frequent measurements. This situation led to the conclusion that, similarly for the Aronia powder mix product, since the measurement differences during its shelf life are very small, the measurement deficiencies can be compensated for. It has been seen that this stability, which was achieved in the first place with low moisture content, was kept constant with careful storage conditions and did not adversely affect the quality and microbial load of the product.

The moisture content for Aronia smoothie product is measured as 79.99%. In a study conducted by Uzodinma et al. (2020), analyzes were carried out on different smoothie samples. Moisture contents of all samples studied were obtained in the range of 65.15 – 73.68%. Aronia smoothie has a similar moisture content in terms of moisture content. Of course, the amount of milk added to the product due to the recipe directly affects this result. However, obtaining similar results when compared in the same product category is important for the confirmability of the product and the recipe. In another literature study conducted by Alake et al. (2022), different smoothie samples were studied. Among the products examined, the lowest moisture content was 63.27%, while the highest moisture content was 85.12%.

Aronia based smoothie product is within this range in terms of moisture content. In another study conducted on smoothie products, it is concluded that the amount of solid raw material added directly affects the moisture content of the product, although it depends on the recipe of the product, and the moisture content of the products examined in this study is in the range of 49-79% (Aderinola 2018). The Aronia based smoothie product, which falls within the range in its own product category, also depends on the

product recipe inputs, but shows that the smoothie product group products remain within the range and are suitable in terms of moisture content.

3.6. Ash Content

Defining the ash number of foods is very important in determining and defining their nutritional profile, and ash analysis is used as an important parameter that gives insight into the quality characteristics of foods (Ghannadiasl and Nourani 2019). The ash content of the product can be defined as the sum of mineral and inorganic components found in the food (Anggarani et al. 2019). The ash content of a food is analyzed by identifying the inorganic substances left behind after the burning of the organic substances in that food. The high ash content of a food is directly proportional to the mineral content of that product (Anggarani et al. 2019). Ash analysis was carried out for Aronia powder mixture and Aronia based smoothie products. According to the analysis results, Aronia powder mix contains 2.53% ash, while Aronia smoothie product has 0.33% ash content (Figure 11). Based on the results obtained from Aronia powder mixture nutritional supplement and vegan and functional Aronia based smoothie products, we can say that the amount of ash can give different results depending on the structure and nutritional content of the product.

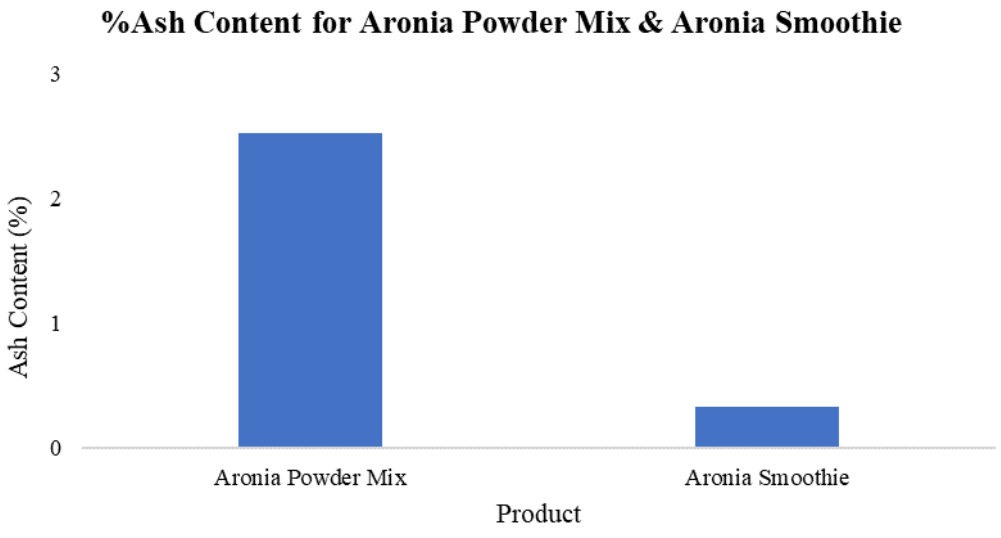

Figure 11. %Ash content for Aronia powder mix and Aronia smoothie products.

In a study conducted by Perring and Tschopp, (2019), the chemical and physical properties of milk-based powder products were examined. In this examination conducted on a powder product, it was observed that the ash content of the products varied between 2.16-4.66%.

In the analysis of a lyophilized pomegranate-based powder product, which is also in powder form, the ash content of these products was found to be in the range of 2.5-2.75 (Viuda-Martos et al. 2012). Similar results were obtained with the ash values of the products in these literature studies, whose product structures and applied process stages were found to be similar to the Aronia powder mixture product. It is possible that small differences between products in terms of results may arise from the differences in the raw materials, contents, process and storage conditions of the products. In a study conducted by Waszkiewicz et al. (2023), two different smoothie-type products were examined.

The ash content of these products with similar Brix and dry matter amounts was obtained as 0.35 and 0.30. Aronia smoothie has very similar ash content to ash values. In a literature study conducted by Habschied et al. (2023), the ash value of a beverage type food product containing chokeberry juice was calculated as 0.25%. Again, in this study, it had a similar ash content value to the Aronia smoothie product due to its product category.

In addition, a study was conducted by Bilge et al. (2016), proving that there is a correct proportion between the mineral content and ash content of the products. In the literature study, different flour samples were examined in terms of ash content and mineral content. As can be seen from the results, the mineral contents of samples with high ash content are lower than those with low ash content. When the Aronia powder mixture and Aronia based smoothie samples are examined, it is seen that the mineral content of the Aronia powder mixture end product with a high ash content is higher than the Aronia based smoothie product with a low ash content.

As can be seen from the results and the literature reviews, there is a direct proportion between the ash content and mineral content of a food product. Functional and vegan Aronia powder mixture nutritional supplement and Aronia based smoothie products, designed with functional features, also yield results in parallel with this direct dependency.

3.7. Water Solubility Index (WSI) & Water Holding Capacity (WHC)

Water holding capacity and water solubility index analyzes were carried out only for the Aronia powder mix product. Water holding capacity and water solubility index values are important parameters that affect the quality and physical structure of the products. Water holding capacity analysis was performed to measure the total water absorption ability of the Aronia powder mix product per gram. Again, water solubility index analysis was performed to determine how much solubility of the same product is in water per amount. There is a subtle nuance between the two analyses. While water holding capacity shows how much water a dry powder product can absorb, water solubility index shows how effectively this product can dissolve in water. Water holding capacity and water solubility index properties may vary depending on the nutritional content, dry matter amount and physical structure of the product. The water holding capacity value of the Aronia powder mix product was obtained as 1.89 g water/g d.m. In addition, the water solubility index value of the product was measured as 58.86% (Figure 12).

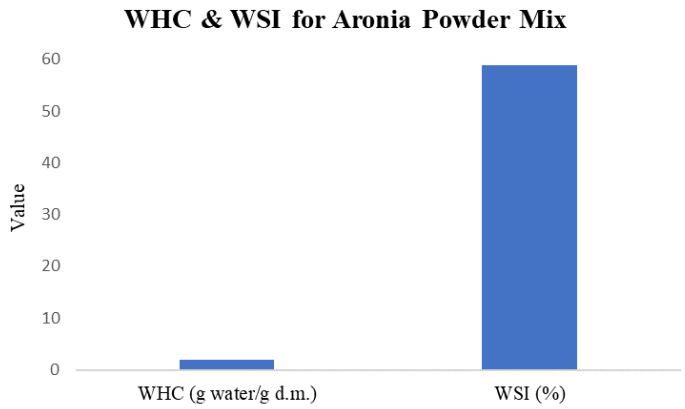

Figure 12. Water holding capacity and water solubility index values for Aronia powder mix.

In the study conducted by Sadowska et al. (2019), WHC and WSI values were given for the chokeberry powder product. WHC and WSI values measured in products with different moisture contents and different drying methods varied. Chokeberry

powder, which is dried with the freeze dry technique and has a dry matter content of 99.18%, has a water holding capacity value of 2.94 g H_2O/ g d.m. and a water solubility index value of 57% (Figure 13). It has been observed that the WHC and WSI values of this chokeberry powder product, whose dry matter content is close to the Aronia powder mix product and dried with the same drying method, have similar results. Although there is a small difference between the results, it is possible to explain this difference because the two products have different raw materials and inputs, are produced under different conditions, have different nutritional contents and, of course, have physical and structural differences.

Figure 13. WSI measurement samples after oven drying.

3.8. Mineral Analysis

Mineral analysis was carried out for Aronia powder mix and Aronia smoothie products. Profiles of both products in terms of Ca, Fe, K, Mg, Na, P and Zn minerals were obtained. Ca, P, K and Mg minerals are macro elements that must be taken into the body and are responsible for the control and regulation of metabolism. Although these minerals do not enter the tissue structure, they play an important role in protecting the health of the organism due to these effects. Microelements such as Fe and Zn are minerals that are of vital importance in our body, especially for enzymes and protein

structures (Pavlovic et al. 2015). All these minerals have daily doses and limits that must be taken into our body.

The most dominant mineral in the nutritional profile of Aronia powder mix product was K in the amount of 5353.55 mg/kg. Then, it was followed by P with 4297.15 mg/kg, Ca with 1148.21 mg/kg, Mg with 622.43 mg/kg, Na with 249 mg/kg, Fe with 137.01 mg/kg and the least abundant mineral Zn with 19.09 mg/kg (Table 6). In the literature analysis conducted by Pop et al. (2022), to determine the mineral structure of *Aronia melanocarpa* fruit, the mineral concentration of Aronia fruit was compiled from different studies. In this study, the mineral with the highest concentration in the *Aronia melanocarpa* fruit can be determined as K with 6790 mg/kg. This is followed by Ca mineral with 3220 mg/kg. These are followed by P with a concentration of 956 mg/kg and Mg with a concentration of 669 mg/kg. However, as in the Aronia powder mix product, the minerals with the lowest concentrations were Fe with 14.2 mg/kg and Zn with 8.40 mg/kg. Since the high concentration of Aronia powder mix functional product consists of *Aronia melanocarpa* fruit, it is expected that the literature review with the Aronia-based powder mix product will have parallel results. In the study conducted by Lazăr et al. (2020), for the *Aronia melanocarpa* powder product, its profile was analyzed in terms of Na, K, Ca and Mg minerals. The highest mineral content was potassium at 294.0 mg/kg. While the Ca content was determined in the range of 119.0-133.0 mg/kg, the magnesium concentration was measured in the range of 67.0-69.0. Iron mineral was found in the range of 13.0-18.0 mg/kg, as in the Aronia powder mix product. In the literature study, the value of *Aronia melanocarpa* pomace powder product in terms of Zinc concentration is approximately 1.0 mg/kg. When all these results are taken into consideration, it can be seen that when *Aronia melanocarpa* fruit and these fruit-based powder products are analyzed in terms of mineral composition, the results obtained are similar in terms of species and concentration amounts.

Table 6. Mineral profiles for Aronia powder mix.

	Ca (mg/kg)	**Fe (mg/kg)**	**K (mg/kg)**	**Mg (mg/kg)**	**Na (mg/kg)**	**P (mg/kg)**	**Zn(mg/kg)**
Aronia Powder Mix	1148.21	137.01	5353.55	622.43	249.00	4297.15	19.09

In the mineral analysis made for the Aronia smoothie product obtained by blending oat milk with Aronia powder mix, the most dominant mineral was Ca with 1471.56 mg/kg, followed by K with 841.21 mg/kg. This was followed by P with 610.16 mg/kg, Na with 383.80 mg/kg, Mg with 112.17 mg/kg, Fe with 14.70 mg/kg, and the least mineral content was Zn with 3.41 mg/kg (Table 7). In a study conducted by Pavlovic et al. (2015), on the Aronia juice product, the mineral content of the product was analyzed. The most dominant mineral found in Aronia juice products was K with 2123.5 mg/kg. In the Aronia smoothie product, the most dominant mineral after Ca was found to be K. The reason for the predominance of the Ca mineral may be the high Ca value of the oat milk used in the smoothie product. Since the type of product analyzed in the literature study was fruit juice, no dairy raw materials were used (Pavlovic et al. 2015). In the study conducted by Pavlovic et al. (2015), the K mineral found in the Aronia juice product is followed by Ca with 658 mg/kg, P with 596.75 mg/kg, Mg with 353 mg/kg, Na with 38.7 mg/kg, Fe with 14.73 mg/kg, according to concentration ratio and finally Zn with 2.5 mg/kg. In the literature review, it was seen that mineral analysis was not performed directly on a smoothie containing Aronia, and therefore a commercial fruit juice product in a similar category was examined. After the examination, it was concluded that Aronia smoothie and Aronia juice products in the study had consistent values in terms of mineral content.

Table 7. Mineral profiles for Aronia smoothie.

	Ca (mg/kg)	**Fe (mg/kg)**	**K (mg/kg)**	**Mg (mg/kg)**	**Na (mg/kg)**	**P (mg/kg)**	**Zn(mg/kg)**
Aronia Smoothie	1471.56	14.70	841.21	112.17	383.80	610.16	3.41

Differences were observed in the mineral values of Aronia powder mix and Aronia smoothie products. In general, there was a decrease in the mineral amounts of Aronia smoothie compared to Aronia powder mix. As can be seen in Figure 14, while the amount of Ca and Na increased, the mineral amounts of Fe, K, Mg, P and Zn decreased. This decrease is an expected result. The reason for this can be explained as the value per mg/kg during mineral analysis was made into a product by adding milk to the smoothie product and was measured in a more dilute way. Since the structure and

especially the dry matter amount of the two products produced are different, the amount of minerals per dry matter in the samples taken during this analysis decreased. In fact, adding milk to the powdered product while producing smoothies diluted the product, and therefore a decrease in the mineral content of the product was detected. Contrary to this general decrease, the increase in the amount of Ca and Na can be understood when the nutritional content of oat milk used in smoothie production is examined. The oat milk used contains 1.2 mg/ml Ca mineral. This increase in Ca concentration as a result of mineral analysis becomes significant when compared to the amount of oat milk used in the product recipe. Likewise, the increase in the amount of Na becomes significant when compared to the amount of milk used by looking at the nutritional label of the oat milk used. In the calculations made to determine the decrease rate of the falling minerals, it was obtained that Fe 89%, Mg 82%, K 84%, P 85% and Zn mineral decreased by 84%. Considering that all the decreasing minerals are in the range of approximately 82-89%, these close values will support the reason explained above. The amount of oat milk added to the product reduced all the decreasing minerals at approximately the same rate, and the reason for the increase in the increased minerals is due to the nutritional content of the added oat milk.

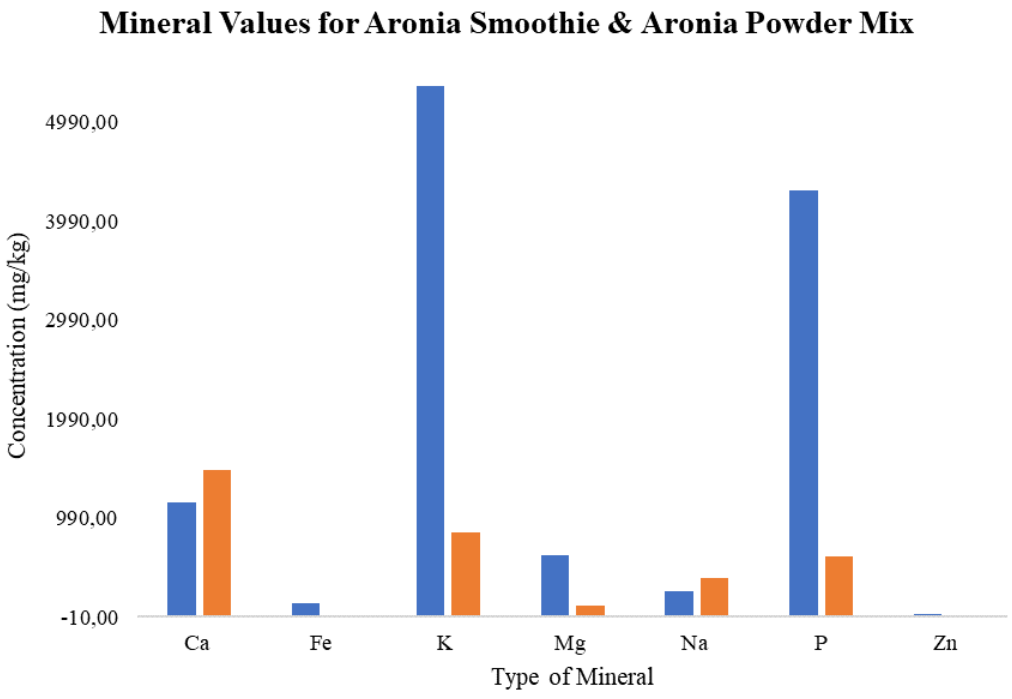

Figure 14. Mineral profiles for Aronia smoothie and Aronia powder mix.

In the literature study carried out by Pavlovic et al. (2015), different types of commercial products containing Aronia were used and the mineral profiles of these products were determined. In the study, when the situation between Aronia berries and Aronia juice products is examined based on the changes in minerals, it is possible to capture a situation similar to the relationship between Aronia powder mix and Aronia smoothie products. When looking at the mineral content of the Aronia juice product, a decrease was observed in the concentration of K, Ca, Mg, P and Zn minerals. When the ratio is determined by dry matter calculation compared to Aronia berry products, it is expected that the fruit juice sample will have a lower mineral content. The data in this literature review also supported the study Pavlovic et al. (2015). Differently, there was an increase in Na and Fe minerals in the Aronia fruit juice product compared to the Aronia berry product. The reason for this can be explained as the Ca mineral coming from the oat milk used in the smoothie study, as well as the fact that these minerals come from other raw material inputs used while making fruit juice.

Dietary Reference Intake values for mg/200 g serving of Aronia based smoothie product are calculated for children aged 4-8, adolescents aged 14-18 and adults aged 19+. As a result of the calculations made based on the study conducted by Pop et al. (2022), the data in Table 8 was obtained.

When compared to the Aronia fruit juice examined in the study conducted by Pop et al. (2022), the Aronia smoothie product has a higher content in terms of Ca, Na, Fe and Zn minerals, while it has a slightly lower content in terms of K. However, Mg and P minerals have a similar concentration value for Aronia smoothie and Aronia juice products.

As a result of all these, Aronia powder mix and Aronia smoothie products have similar and generally more intense mineral content when compared to product groups in the same segment. Calculations based on Dietary Reference Intakes values can also be concluded that it has a higher mineral supply feature than other commercial Aronia products.

When compared to the Aronia fruit juice examined in the study conducted by Pop et al. (2022), the Aronia smoothie product has a higher content in terms of Ca, Na, Fe and Zn minerals, while it has a slightly lower content in terms of K. However, Mg and P minerals have a similar concentration value for Aronia smoothie and Aronia juice products.

Table 8. Mineral concentrations of Aronia smoothie product compared to Dietary Reference Intakes.

Aronia Smoothie			Dietary Reference Intakes (DRI)			% of DRI for 200 g portion of Aronia Smoothie		
Mineral	**mg/kg**	**mg/200 g portion**	**Children (4-8 y)**	**Adolescents (14-18 y)**	**Adults (19+)**	**Children (4-8 y)**	**Adolescents (14-18 y)**	**Adults (19+)**
Ca	1471.56	294.3	1000	1300	1000	29.43	22.6	29.43
Na	383.80	76.76	1000	1500	1500	7.68	5.12	5.12
K	841.21	168.2	2300	3000	3400	7.3	5.6	4.9
Mg	112.17	22.4	130	410	420	17.2	5.5	5.3
P	610.16	122	500	1250	700	24.4	9.8	17.4
Fe	14.7	2.94	10	11	8	29.4	26.7	36.8
Zn	3.41	0.68	5	11	11	13.6	6.2	6.2

As a result of all these, Aronia powder mix and Aronia smoothie products have similar and generally more intense mineral content when compared to product groups in the same segment. Calculations based on Dietary Reference Intakes values can also be concluded that it has a higher mineral supply feature than other commercial Aronia products.

3.9. Color Determination

Color is a very important quality parameter for food products. The color of the product directly reflects the external appearance of the product and affects the acceptability level of the product to consumers (Petković et al. 2021).

Aronia melanocarpa fruit is a purple berry class fruit with very high color pigmentation. Due to its anthocyanins content, Aronia extracts are used as a very important natural dye source in the manufacture of confectionery and food industry (Ghendov-Mosanu et al. 2022). This high colorant content of Aronia fruit is at a level that will cause the product to be used directly as a colorant, and therefore color is a very important parameter that must be monitored in Aronia-based products.

Although the CIE L*a*b* color analysis method is one of the most preferred color measurement methods, the L* value determines the lightness feature of the

product, the a* value determines the redness measure and the b* value determines the yellowness measure (Yuan et al. 2018).

Color analyzes for Aronia powder mix and Aronia smoothie products were carried out throughout their shelf life.

Color measurements were carried out on a monthly basis for the Aronia powder mix product. No significant change was observed in the L*, a*, b* values of the product in these monthly measurements made throughout its shelf life (Figure 15). The L* value, which represents the lightness degree of the product, was measured in the range of 39.53 – 40.25 throughout its shelf life. While the a* value, which determines the degree of redness, varied between 8.69 and 8.96, insignificant decreases occurred during the shelf life. The b* value, which determines the yellowness degree of the product, showed a non-significant change in the range of 8.24 – 8.45. In a study conducted by Petković et al. (2021), in which bread was made using *Aronia melanocarpa* powder, the average L*, a*, b* values obtained were around 85, 1.5 and 16, respectively. Compared to the Aronia powder mix product, the resulting bread product has a darker, less red and more yellow color sequence. In a study conducted by Horszwald, Julien, and Andlauer (2013), the color properties of Aronia powder products obtained with different drying methods were compared. The L*, a*, b* values of the powder product obtained by the freeze-drying technique were measured as 24.35, 22.48 and 4.50, respectively. Compared to the Aronia powder mix product obtained by the freeze-drying method, this product has brighter, redder and less yellow properties.

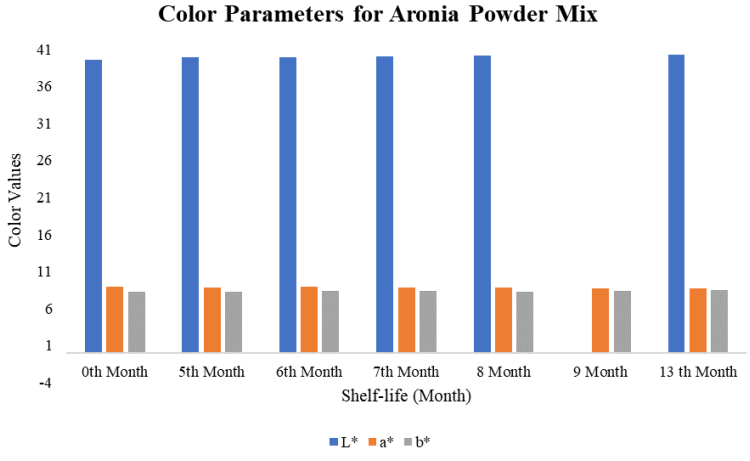

Figure 15. L*, a*, b* color parameters for Aronia powder mix throughout the shelf-life.

The biggest reason why the Aronia powder mix product is more yellow is that the red lentil protein powder it contains has an orange color. If we look at the effect of different drying techniques on the color properties of the product in this study conducted by Petkovic et al. (2021), it is observed that the oven vacuum drying method produces darker products than the spray and freeze-drying methods. Again, the products obtained by the oven vacuum drying method have less red and less yellow characteristics than spray and freeze-drying products. If the spray and freeze-drying methods are compared, there is no significant difference in color properties between the two.

Color measurements were carried out on a weekly basis for the Aronia smoothie product. In these weekly measurements made during the 21-day shelf life, no significant change was observed in the L*, a*, b* values of the product (Figure 16).

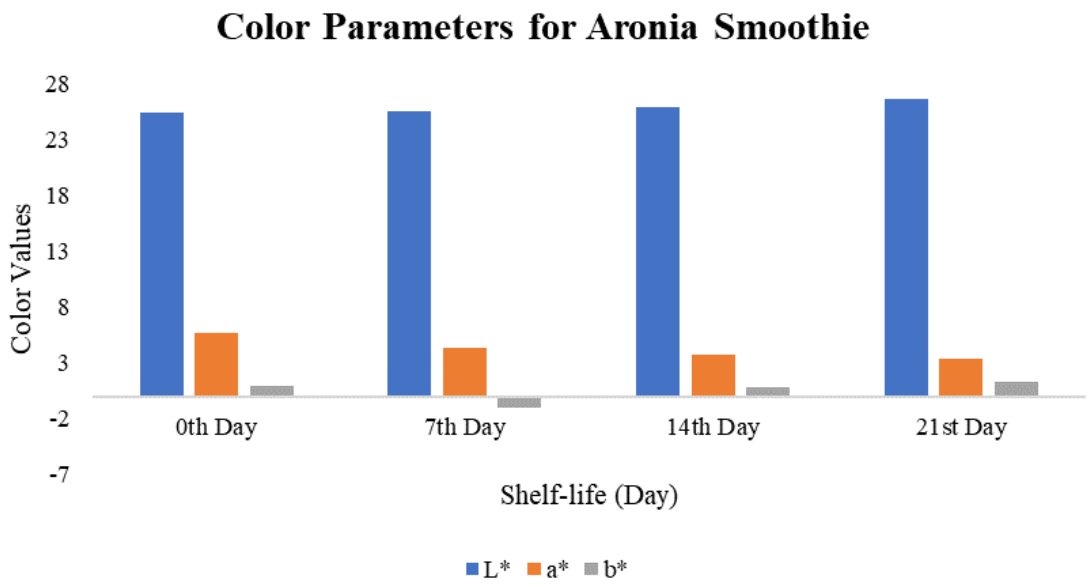

Figure 16. L*, a*, b* color parameters for Aronia smoothie throughout the shelf-life.

The L* value, which represents the lightness level of the Aronia smoothie product, was obtained in the range of 25 - 27, the a* value, which determines the redness amount, was obtained in the range of 3 - 6, and the b* value, which determines the yellowness value, was obtained in the range -1 - 1. No significant change in color measurements was observed during the shelf life of the product. Color measurements were carried out throughout the shelf life of the smoothie product made from different

fruits, including chokeberry, made by Škegro et al. (2021a). No significant change was observed in L*, a*, b* values during the 21-day shelf life of the product under high pressure conditions. Average L*, a*, b* values were measured as 45, 14.6 and 17.6, respectively. Compared to the Aronia smoothie product, a product with brighter, redder and more yellow color features was obtained. According to Yi et al. (2022), color analysis was carried out for the Aronia juice product stored in the refrigerator throughout its 24-week shelf life. It was observed that the product did not show any significant change during its shelf life. L*, a*, b* values were measured as an average of 35, 2 and -0.40, respectively. Compared to the Aronia smoothie product, the juice product is brighter, less red and has approximately the same level of yellowness.

When the color analyzes of Aronia-based products in similar categories are examined, it is observed that the results differ from each other. The fact that the genetic types and growing conditions of the Aronia fruits used are different and the applied processes and external factors are different explains this situation (Taskin 2020).

3.10. Total Phenolic Content Analysis

Polyphenols are one of the most important compounds that should be taken for human nutrition (Jurendi´c et al. 2021). *Aronia melanocarpa* fruit is one of the fruits with the highest phenolic compounds among the berry species, including its leaves, and has been the subject of many studies in this context. It has been observed that the total phenolic content of Aronia fruit, which is the same product, varies from study to study and from product to product. As stated in the study conducted by Benvenuti et al. (2004), the total phenolic content of the product can be affected by many factors such as environmental factors such as temperature, region, humidity, product growing time and harvest time (maturity status), amount of light exposure, applied process type and time, and storage conditions.

Total phenolic content controls were carried out for both Aronia powder mix and Aronia smoothie products throughout their shelf life. The data obtained were given on the Gallic acid standard curve and the calculations were carried out according to this standard.

As a result of the experiments, it was observed that the Aronia powder mix product has a high content of polyphenol. One of the biggest reasons why this level is higher than literature reviews is that, in addition to *Aronia melanocarpa* fruit, other freeze dry raw material inputs used in the recipe also have high phenolic content. As seen in Figure 17, although the results obtained are stable enough that no significant change can be considered, an increase in the phenolic content is generally observed throughout the shelf life. There was no significant change in the phenol content during the shelf life of the product.

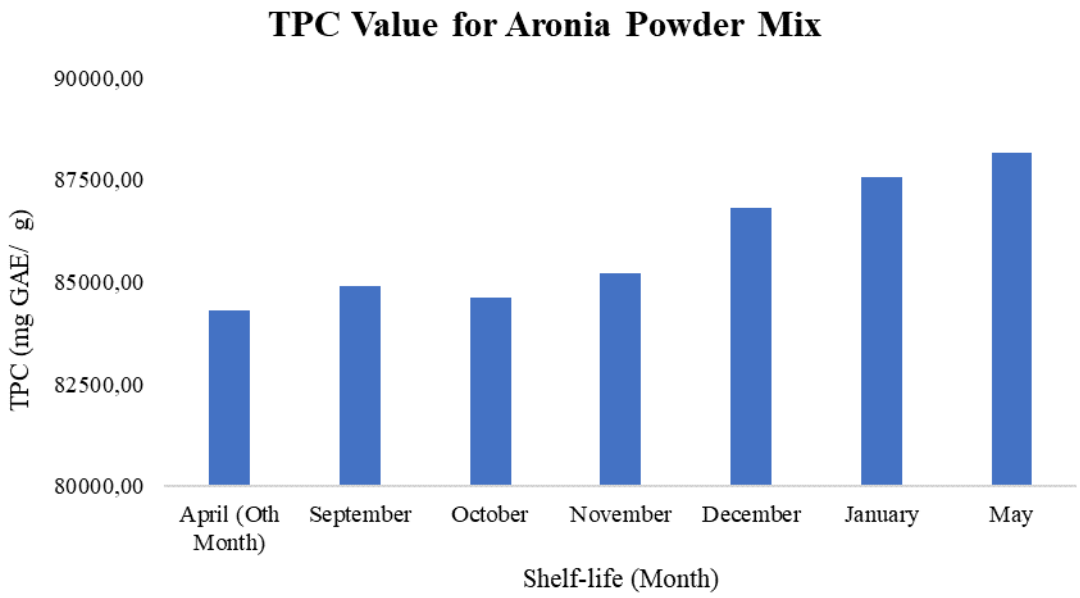

Figure 17. TPC values for Aronia powder mix throughout shelf-life.

One of the most important reasons for this situation can be explained as keeping the product in the refrigerator, without light and air, throughout its shelf life. In the same study conducted by Benvenuti et al. (2004), the total phenolic contents of different berry species, including Aronia, were examined. According to the data obtained, *Aronia melanocarpa* was the berry containing the highest phenol with 690.2 mg / 100 g total phenol content. In a study conducted by Tokuşoğlu, (2018) on Aronia-based new food products and the shelf life of these products, the total amount of polyphenols contained in fresh Aronia berries was reported as 1012.67 mg GAE / 100 ml. In this study conducted on different Aronia-based food products, it was calculated that Aronia

powder products dried and powdered using the freeze-drying technique also contained 444.72 mg GAE / 100 ml phenol. When the total phenol amount of this product, which has the same process and is very similar to the Aronia powder mix product, is compared, it will be possible to state that the Aronia powder mix product contains approximately twice as much phenol. One of the most important factors affecting the total phenol content in the processed product is the process type, and the basic process applied to the Aronia powder mix product is the drying process using the freeze dry technique. In a study conducted by Thi and Hwang, (2016), the powder product obtained using Aronia was examined in terms of its polyphenol content, and the drying process applied until it became powder was tested with different methods and results were obtained on how the difference in the drying process affected the total phenol content of the product. Sun-dried, oven-dried and freeze-dried drying processes were studied and the highest total phenol content was found in products produced using the freeze-drying technique, with 919.7 mg GAE / g. Again, in a study where the powder product obtained using Aronia was examined with different drying techniques, fluidized bed jet drying, freeze drying, vacuum drying and convection drying techniques were used. In this study conducted by Sadowska et al. (2019), the highest total phenol content was found in the products dried by the fluidized bed jet drying method, with 2484.60 mg GAE/100 g. This is followed immediately by the product dried with the freeze-drying technique, with a phenol content of 2255.86 mg GAE/100 g. As can be seen from the results, the difference between these two different drying techniques is not significant and, in this case, the freeze-drying technique still remains highly preferable. In a study making bread using chokeberry powder, the effect of drying on the polyphenol content of Aronia powder is shown. In this study conducted by Petković et al. (2019), fresh chokeberry has a very high phenol content of 5222.54 mg GAE/ 100 g, while chokeberry products dried at different temperatures of 50, 60 and 70 ℃ contain phenol in the amount of 1918.79, 1346.35 and 1169.39 mg GAE/ 100 g, respectively. As can be understood from here, the total phenol content of a product is highly affected by the process and parameters applied to the product.

Aronia smoothie product, although not as much as Aronia powder mix, has a very high polyphenol content among the products in its category. Total phenol content monitored throughout shelf life increases regularly, as shown in Figure 18. However, as can be seen from the results, these changes are not significant. The biggest reason for this consistency in the product values throughout its shelf life is that it is stored in light-

proof, airtight containers under appropriate refrigerator conditions. In a study conducted by Denev et al. (2018) on functional food design using *Aronia melanocarpa* fruit, the total phenol content of the products was obtained in the range of 1022-1795 mg / 100 g. Total phenol content in Aronia fruit juices made with different extraction types carried out by Labur et al. (2017), was calculated in the range of 761.11 – 955.03 mg/ L. The change in the total phenolic content of the smoothie product obtained by mixing different fruit types, including chokeberry, during its 21-day shelf life is given (Škegro et al. 2021b).

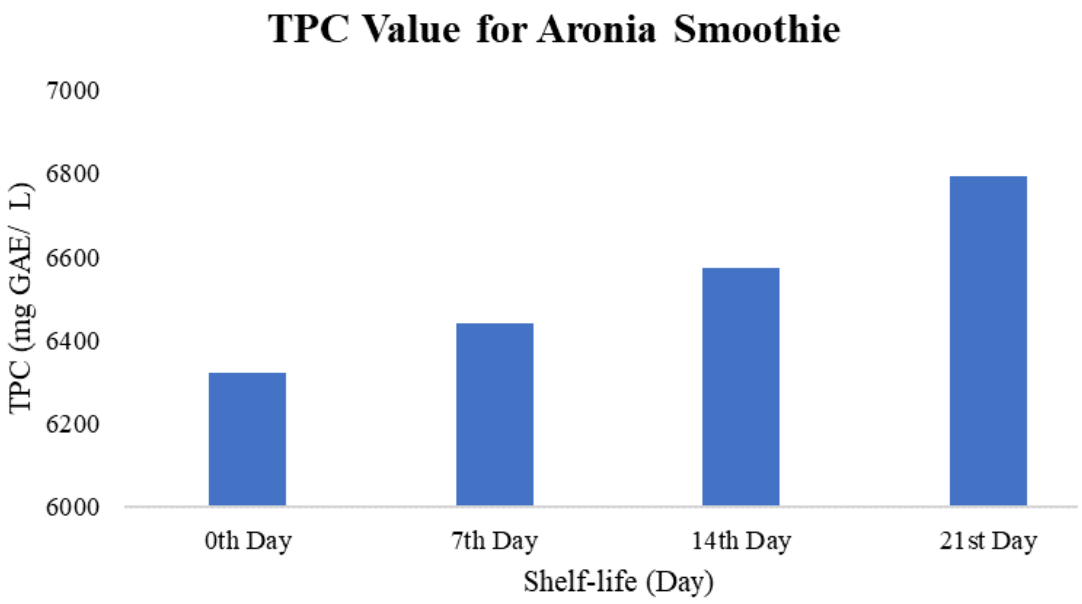

Figure 18. TPC values for Aronia smoothie throughout shelf-life.

In this study conducted by Škegro et al. (2021), although there was a general decrease in polyphenol content, no regular increase or decrease was observed, and while a decrease was observed for a while, a small increase was observed in the last week. In the designed Aronia based smoothie product, although there was no significant change in the total phenol content throughout its shelf life, a regular increase was observed.

In a study conducted by Nguyen and Hwang, (2016), total phenol content was examined on yoghurt enriched by adding different amounts of Aronia. Among these yoghurts, the product with the highest total phenol content was the yogurt with the highest Aronia concentration (54.05 mg GAE/ g), while the product with the lowest

polyphenol content was the control sample without Aronia (16.34 mg GAE/ g). This serious difference in the total phenol content of samples with and without Aronia clearly proves that *Aronia melanocarpa* fruit is a serious source of polyphenols. Since the main purpose of the study was on yoghurt, a small amount of Aronia was added compared to Aronia powder mix and Aronia based smoothie products, and yet these high values were obtained. Considering that *Aronia melanocarpa* fruit is the base ingredient for Aronia powder mix and Aronia smoothie products, it would be possible to find these high phenol contents meaningful. As studied by Tolic et al. (2015), the total phenol contents of different Aronia-based food products such as juice, powder, capsules, fruit tea and dried berry were examined. The average total phenol content of these products was calculated as 4415, 4539, 4901, 2217 and 2210 mg GAE/ 100 g, respectively. As can be seen from these results, different polyphenol contents were detected in different products. The reasons for this situation include differences in analysis methods, species differences of *Aronia melanocarpa* fruit, growing conditions, storage conditions of the product, different process applications and process parameters (Tolic et al. 2015).

According to the results obtained from all these examined different food products based on *Aronia melanocarpa* and fresh Aronia fruit polyphenol studies, it can be said that the number and complexity of the processes applied to a product have a decreasing effect on the total phenol content. However, it is observed that many external factors affect the total phenol content of the product, and it is noteworthy that this value is especially high in *Aronia melanocarpa* based food products.

3.11. Antioxidant Activity (DPPH, ABTS) Assay

Antioxidants are one of the leading bioactive substances found especially in fruits and vegetables (Dilrukshi and Senarath 2021). Regular consumption of fruits, vegetables and other foods rich in antioxidant properties has positive effects on health and also helps protect against chronic diseases (Jurendi´c et al. 2021). In general, berries are fruits with high bioactive substance content, but especially *Aronia melanocarpa* fruit has a significantly high concentration on the basis of all bioactive and especially in terms of antioxidant content. The most common assay types used to detect antioxidant activity are DPPH (2,2-diphenyl-1-picrylhydrazyl) and ABTS (2,2-azino-bis (3-

ethylbenzothiazoline-6-sulfonate)) radicals (Jurendi´c et al. 2021). It can be easily seen from literature reviews that plant foods and fruits are richer in terms of total phenol and antioxidant contents than other foods, and it is also among the results given that there is a good correlation between the concentrations of these two bioactive substances in food (Denev et al. 2018).

Antioxidant activities of both Aronia powder mix and Aronia smoothie products were examined throughout their shelf life by DPPH and ABTS assay methods.

It was observed that the antioxidant activity of the Aronia powder mix product, measured on a monthly basis, decreased regularly in both ABTS and DPPH assay types. As seen in Figure 19 and Figure 20, these decreases for both assays are so low that they are not significant and are an expected effect as a result of literature searches.

In a study by Bratosin et al. (2024), the antioxidant contents of fresh Aronia and freeze dried Aronia fruits were compared. While Fresh Aronia fruits have an antioxidant content of 4140.1 µM Trolox/ 100 g fresh weight, the antioxidant content of freeze dried Aronia, obtained by drying the same product with the freeze-drying process, was found to be 10621.67 µM Trolox/ 100 g dry weight. As a result of this situation, it will be possible to say that the freeze-drying process increases the antioxidant content of the product by making it more concentrated (Bratosin et al. 2024).

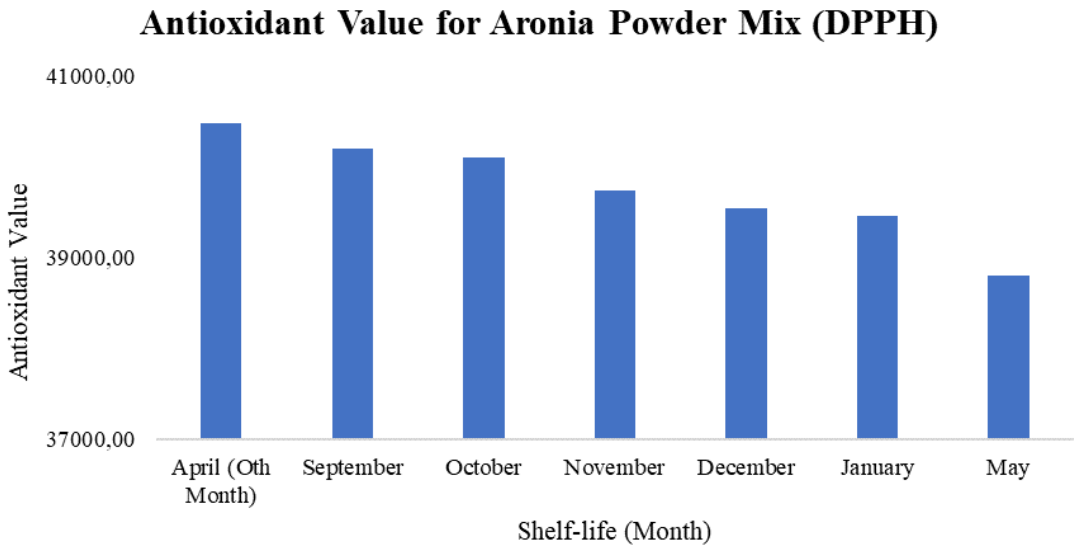

Figure 19. Antioxidant activity for Aronia powder mix by DPPH assay throughout the shelf-life.

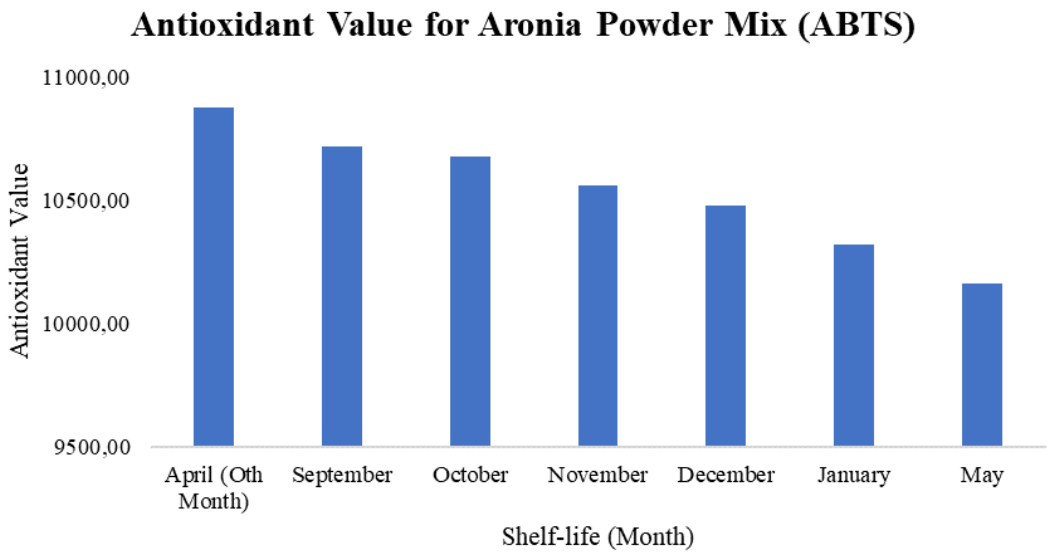

Figure 20. Antioxidant activity for Aronia powder mix by ABTS assay throughout the shelf-life.

As reported in a study by Dilrukshi and Senarath, (2021), the loss of antioxidants in the freeze-drying process is very low compared to other processes, approximately 14.12%. Supporting this, in a study conducted by Thi and Hwang (2016), the antioxidant properties of *Aronia melanocarpa* fruit were examined through different drying processes such as sun drying, oven drying and freeze drying. Among these drying types, the strongest bioactive substance protection was observed in chokeberry products dried with the freeze-drying method. In a study conducted by Horszwald et al. (2013), it was examined how different drying processes affected the antioxidant capacities of the Aronia powder product. Compared to the traditional oven drying method, the results obtained were much greater with freeze-dried powder products. Antioxidant contents of aronia powders were analyzed by both ABTS and DPPH methods. According to the results obtained, freeze dried Aronia powder was found to contain 180.45 µmol Trolox/100 mg antioxidant by ABTS, while it contained 24.68 µmol Trolox/100 mg antioxidant by DPPH method (Horszwald, Julien, and Andlauer 2013). According to the data obtained, just like in the Aronia powder mix product, non-significant decreases in antioxidant content occurred for both assays throughout the shelf life (Figure 21 & Figure 22).

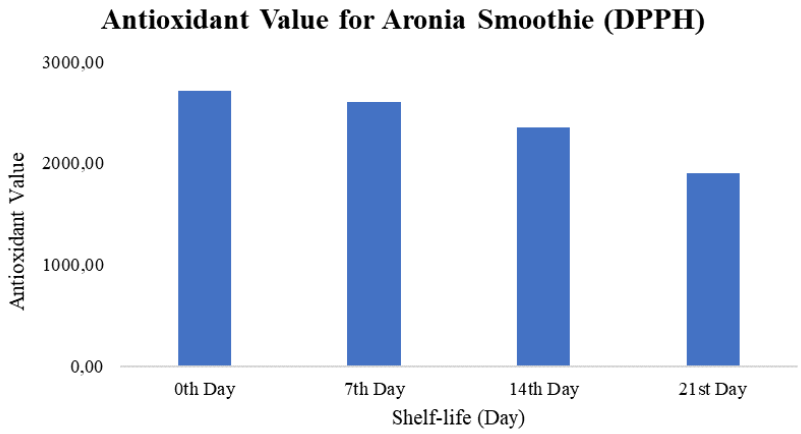

Figure 21. Antioxidant activity for Aronia smoothie by DPPH assay throughout the shelf-life.

In a literature study conducted by Denev et al. (2018), the antioxidant activities of functional drinks obtained using *Aronia melanocarpa* fruit were analyzed. According to the results obtained, juice products contain 56542.3 – 92654.8 mmol TE/ L antioxidant activity, while nectar products contain 27562.3 – 39254.4 mmol TE/ L antioxidant activity. ABTS and DPPH assay types and antioxidant contents of dairy drinks obtained by Savas and Akan, (2021), using raspberry were examined during the 21-day shelf life.

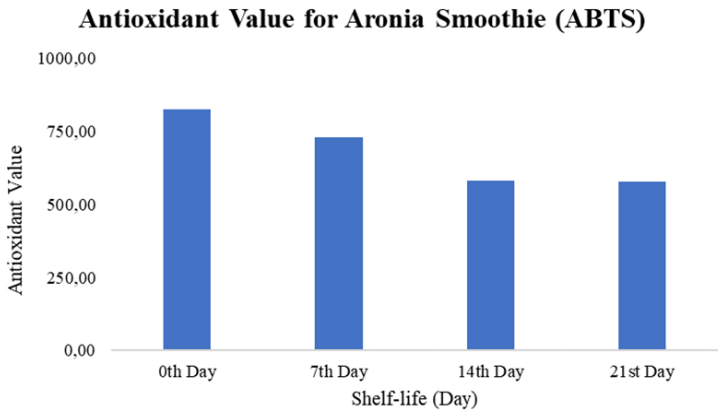

Figure 22. Antioxidant activity for Aronia smoothie by ABTS assay throughout the shelf-life.

According to the data obtained, statistically non-significant decreases in the antioxidant content of the products were observed during the shelf life of both methods, just like in Aronia smoothie and Aronia powder mix products. While the antioxidant content of the dairy drinks designed in the study was obtained as 1.50-5.22 mg Trolox/100 ml with the ABTS method, the results were calculated as 57.11-66.54% with the DPPH method. Bioactive substances, especially the antioxidants contained in the products, are sensitive and light-sensitive components. For this reason, it is very important to preserve the produced products correctly throughout their shelf life in order to minimize the content loss of the product. While the Aronia powder mix product was stored in the refrigerator in air- and light-proof aluminum packages, Aronia smoothie products were also stored in the refrigerator out of light. It would be possible to comment that these careful storage conditions had a positive effect on the decrease in the antioxidant contents of both products by making them less than significant.

It can be seen from all the results obtained and the literature sources examined that different results were obtained from different methods used to determine antioxidant activity. It can also be seen from the Aronia powder mix and Aronia smoothie samples that the antioxidant content analyzed by DPPH assay was higher than the results obtained by ABTS. In the study conducted by Tirla et al. (2023), to design a new functional sports drink containing *Aronia melanocarpa*, the antioxidant contents examined by both ABTS and DPPH assays were obtained more in the DPPH method than in ABTS. Since the antioxidant contents of the products are affected by environmental factors, the degree of maturity of the product, agricultural history, type, structure, genetic factors, applied processes and protocols used in analysis methods, it would not be correct to compare each result with each other. If each of them can be said to be the same in terms of their own processes and stages, it will be possible to make comparisons.

3.12. Microbiological Analysis

Microbiological analyzes were carried out for Aronia powder mix and Aronia smoothie products throughout their shelf life. Total viable count, yeast, mold and total coliform counts were performed for both products throughout their shelf lives. It was

decided which counts to be made for the products by conducting literature searches on the basis of product categories and examining the Turkish Food Codex Microbiological Criteria Regulation. It is of great importance to carry out microbiological control and analysis for foods. It is necessary to carry out all necessary microbiological analysis of food products that directly affect people's health at every stage and before the final product is released to the market. Maximum count limits have been determined for each food product type on the basis of the Turkish Food Codex Microbiological Criteria Regulation, and each product must comply with these criteria.

For the Aronia powder mix product, total viable counts, yeast, mold and total coliform counts were carried out from the moment of production throughout its shelf life. Since the water content of this product, dried with the freeze dry technique, is very low, the possibility of microbial development is seriously limited. The reason for this is that microbial organisms need nutrients and water in the environment to thrive. No formation or development was observed in any of the counts made during the shelf life of the Aronia powder mix product. Serious attention has been paid to the hygienic and careful production and storage of the product, from the moment of production to the storage conditions until the analyzes carried out on the last day. Microbiological analyzes show that microbiological stability has been achieved for the Aronia powder mix product. In a study conducted by Lazăr et al. (2020), microbiological analyzes of the *Aronia melanocarpa* pomace product in lyophilized powder form were performed. Yeast, mold, *Enterobacteriaceae*, *Escherichia coli* and *Salmonella* counts were made for the product. While no occurrence was observed in the *Salmonella* counts, less than 10 counts were made in the counts made for other living species. Since this amount is negligibly small, it does not cause any problems for the product. In this case, it can be concluded that microbiological stability is provided for this product. As can be seen from the study, the water activity of the product is very low, in the range of 0.285-0.315. The effect and importance of such a low water activity in providing microbial stability for the product can be based on its importance.

Total viable counts, yeast, mold and total coliform counts were performed weekly for the Aronia smoothie product produced with Aronia powder mix using oat milk, throughout its 21-day shelf life. All counts were carried out in 2 parallel and 3 repetitions. After ensuring that the product was produced under hygienic conditions, it was pasteurized at 70 °C for 20 minutes. This heat treatment was applied to limit the microbial growth that may occur during the shelf life of the product and keep it below

the determined limit. In the counts made after pasteurization, no viable growth was observed for yeast, mold and total coliform counts. In the total viable count, viable formation was observed in the petri dishes made from only one parallel side of the product throughout its shelf life, but it remained below the determined limit. Since no living organisms were observed in the other parallel of the same product, it revealed the possibility of a contamination that was not taken into consideration for the product jar in that parallel during production. In a study conducted by Škegro et al. (2021), microbiological analyzes were performed for a smoothie product produced using chokeberry. Aerobic mesophilic bacteria, *Enterobacteriaceae*, *L. monocytogenes*, *Salmonella*, yeast and mold counts were carried out during the 21-day shelf life of the product. It was examined both throughout its shelf life and a control sample without heat treatment was also analyzed to determine the effect of heat treatment. Growth of aerobic mesophilic bacteria, *Enterobacteriaceae*, yeast and mold were observed in the control sample without heat treatment. However, no bacterial formation or growth was observed in the samples of the same product that were subjected to heat treatment by pasteurization during the 21-day shelf life. This situation reveals that it is necessary to apply heat treatment in order to ensure microbial stability in food products such as beverages.

3.13. Determination of Brix Value

Brix value was monitored only for the Aronia based smoothie product, not for the Aronia powder mixture nutritional supplement. Brix values of *Aronia melanocarpa* smoothie product were examined weekly throughout its shelf life (0th, 7th, 14th, 21st day). Measurements were made in 2 parallel and 3 repetitions. As can be seen in Table 9 and figure 23, no significant change was observed in the results over time throughout the shelf life.

The product has an average Brix value of 14.48 throughout its shelf life. Although the Brix value obtained on the day of production was 14.45, the Brix value of the product was measured as 14.6 on the 21st day, which is the last day of its shelf life.

Table 9. Brix values for Aronia-based smoothie product throughout the shelf-life.

	0th Day	**7th Day**	**14th Day**	**21st Day**
Aronia Smoothie	14,45	14,41	14,47	14,60

A slight increase in Brix value was observed. It is thought that the reason for this may be the deterioration of the homogeneity of the powder sample in the product mixture and a slight precipitation during the shelf-life follow-up. Shaking the product before consumption eliminates this problem. Since this change did not occur to a large extent, it was concluded that it did not cause a problem in the consistency quality criterion of the product. In the literature review for different smoothie products, it was observed that although they differed depending on the fruit content used, in general, parallel values were obtained with the study conducted.

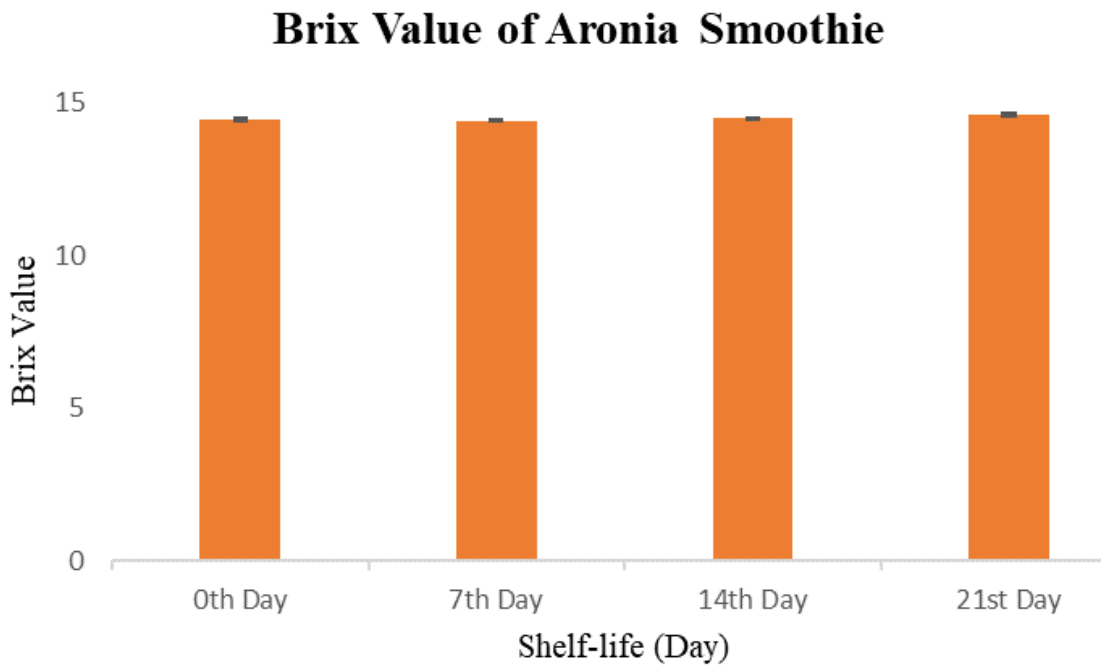

Figure 23. Brix values for Aronia-based smoothie product throughout the shelf-life.

In the study conducted by Stan and Popa, (2013), the Brix values of different smoothie samples in the same segment were examined for the smoothie product that would be made using different fruits without adding any preservatives, and it was concluded that the Brix values of the products were between 13.2 - 15.2. In this study conducted on multiple different smoothie products, the range given indicates a consistent retention throughout the shelf life for the Aronia-based smoothie product in our study. In another literature study conducted by Balaswamy et al. (2013), similar results were observed. In the study, products were obtained by using different raw materials in different proportions and the different smoothies obtained were analyzed. The obtained Brix values are in a wide range of values such as 6 - 26. The reason for this wide Brix range is the determination of products by creating a wide product mixture range with many different types of raw materials. It was observed that the Brix value, where the results were concentrated especially for fruit-based products, was in the range of 12-16. Additionally, measurements were made at the 0^{th} and 6^{th} months to see the effect of shelf life on the Brix value of the products. The results obtained were in the form of minor changes in all products that cannot be taken seriously. As a result of all these, it is possible to conclude that the Aronia-based smoothie product produced in the study gives a result compatible with literature studies in terms of Brix value, and this is supported by consistent changes throughout its shelf life.

3.14. *In- vitro* Digestion

In vitro digestion analysis was carried out for the Aronia smoothie product. It was aimed to determine how the antioxidant (DPPH, ABTS) and total phenolic (TPC) contents of the product are affected after digestion. The average value of the results examined throughout the shelf life was taken as pre-digestion, and the result obtained after completing the total digestion process, which took 2 minutes in the mouth, 2 hours in the stomach and 2 hours in the intestine, was taken as post-digestion. Along with these results, the state of the product before and after digestion is given in Figure 24. As seen in Figure 24, Aronia smoothie product showed an increase in both antioxidants and total phenolics after *in vitro* digestion. While a significant increase in antioxidant properties is observed after digestion in terms of ABTS, a smaller change occurs in

DPPH and TPC results. As a result of DPPH, the original antioxidant value of the product was approximately 2391 µmol Trolox/ 100, while after the entire digestion was completed, the DPPH value was obtained as 2738 µmol Trolox/ 100. As can be seen, the antioxidant content of Aronia smoothie increased in terms of DPPH, although it was not significant. As a result of the ABTS procedure, the antioxidant content before digestion was 677 µmol Trolox/ 100, and when the intestinal digestion was completed after the *in vitro* digestion protocol, the post-digestion ABTS value was obtained as 2437.48 µmol Trolox/ 100.

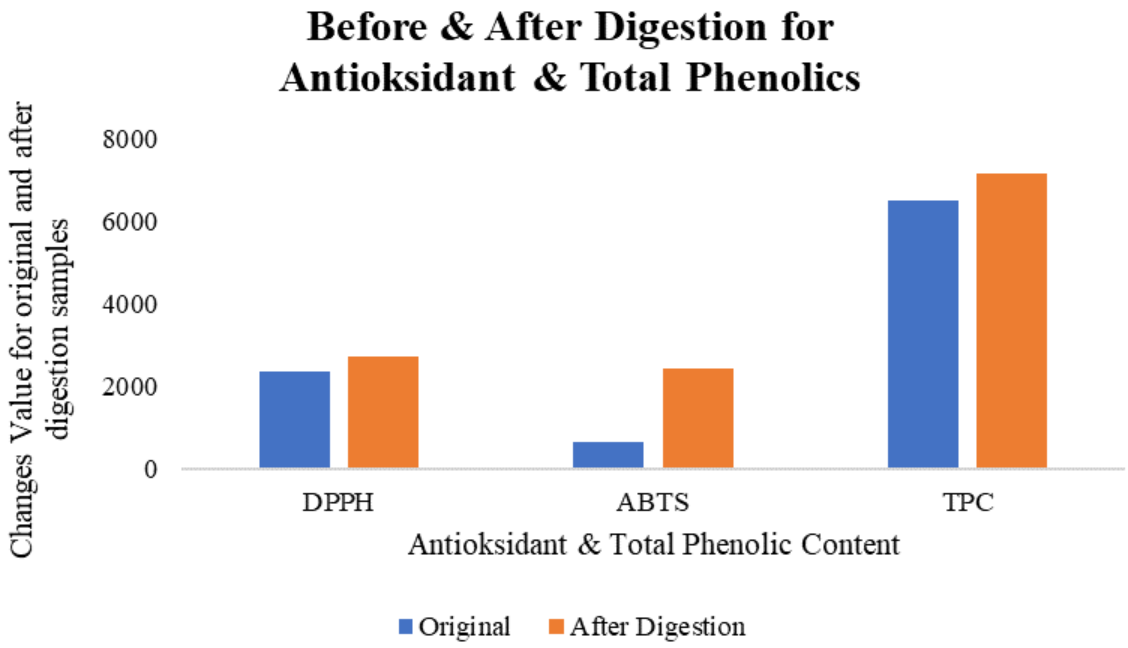

Figure 24. Change of Aronia smoothie product in terms of antioxidants and total phenolics before and after *in vitro* digestion.

It can be seen from here that the Aronia smoothie product increased its antioxidant content with a significant increase in terms of ABTS antioxidant content after digestion. If the total phenolic content amount before and after digestion is compared, an increase was observed here as well. While the original (before digestion) product had a TPC value of 6532 mg GAE/ 100, after digestion was completed, the total phenolic content of the Aronia smoothie product reached 7168.03 mg GAE/ 100. Although no significant increase as ABTS is observed, Aronia smoothie product achieves a positive effect after *in vitro* digestion in terms of total phenolics. It can be

seen from the results obtained that the Aronia smoothie product prepared using the Aronia powder mix product, which has high antioxidant and total phenolic substance content, also has high antioxidant and phenolic content before digestion. Aronia smoothie product was positively affected by digestion in terms of both antioxidants and phenolics and became more efficient in terms of antioxidants and phenolics.

In a study conducted by Chen et al. (2014), experiments were carried out on the changes in the antioxidant and phenolic substance contents of 33 different fruit products before and after digestion. With the DPPH method, the antioxidant content of the studied fruits generally increases in all fruits compared to their original state after digestion. As for the antioxidant content measured by the ABTS method, it is observed that while there is an increase in the antioxidant content of all fruits after digestion, this increase is lower than the increase obtained with the DPPH method. In the same study, the post-digestive total phenolic content of 33 different fruits was evaluated. It is concluded that all studied fruits resulted in a very serious increase in total phenolic substances compared to their pre-digestion, that is, their original state. Post-digestion antioxidant and phenolic substance contents obtained for Aronia smoothie product are consistent. From these results, it is possible to conclude that the digestive conditions prepared create an environment that will positively affect antioxidants and phenolics for these fruits and the Aronia smoothie product.

In another study conducted by Wootton-Beard et al. (2011), antioxidant and phenolic contents of vegetable juice products on 23 different commercial market shelves were monitored before and after *in vitro* digestion. After intestinal digestion, the DPPH antioxidant value increased compared to the original sample antioxidant content. When we look at the number of antioxidants that changed after digestion, an increase was observed in all products after digestion. Total phenolic content also shows a significant increase after digestion compared to before digestion for all products. These results are similar to the Aronia smoothie product in terms of the type of change.

According to the data obtained from the analysis results of the designed Aronia smoothie product and the literature studies, it was concluded that the antioxidant and phenolic contents of the products after *in vitro* digestion developed and increased after ABTS, DPPH and TPC analyses. According to Kamiloğlu, (2019), the reason for these increases after discounting is that, as a result of the enzymatic hydrolysis that occurs as a result of the prepared digestive conditions, phenolic substances and antioxidants originating from the structure of these foods are released and an increase in their

numbers may be observed. It can be interpreted that the post-digestion increases in antioxidants and phenolics in the *in vitro* digestion results means an increase in the number of bioaccessible antioxidants and phenolics that can be obtained from these products (Kamiloğlu 2019).

In addition to the antioxidant and phenolic contents of the final product, Aronia smoothie, before and after *in vitro* digestion, mixtures were prepared with only *Aronia melanocarpa* powder, only protein powder and Aronia plus protein powder with oat milk. In this way, it was aimed to monitor how the antioxidant and phenolic contents of individual ingredients are affected after *in vitro* digestion.

As seen in Figure 25, although the protein powder extracted from red lentils has a lower amount than other samples in terms of total phenolic content, it still seems to have a rich content around 2407 mg GAE/ 100. Afterwards, *Aronia melanocarpa*, Aronia plus protein powder and Aronia smoothie products are listed in order of richness in terms of content.

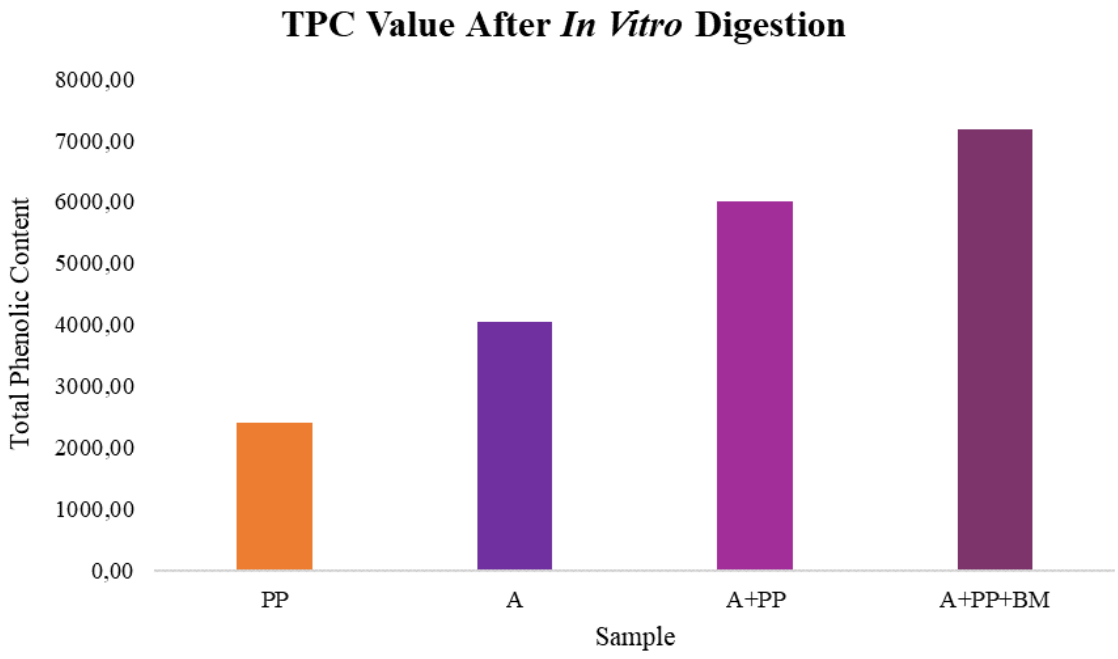

Figure 25. *Aronia melanocarpa*, protein powder and Aronia plus protein powder total phenolic content after *in vitro* digestion.

As can be seen from here, when products with phenolic content were added to each ingredient, the Aronia smoothie product had the highest content in total. In

addition to the Aronia plus protein powder product, it is observed that there is also black mulberry in the smoothie mixture and the phenolics from black mulberry have an increasing effect on the phenolic content of the Aronia smoothie product. Again, the antioxidant contents of these products after *in vitro* digestion are given in Figures 26 and 27 using ABTS and DPPH methods.

With the DPPH method, the lowest antioxidant sample is protein powder obtained from red lentils, as expected. The antioxidant content obtained from the mixture obtained by combining protein powder with *Aronia melanocarpa* fruit showed less antioxidant activity than the sample prepared with only *Aronia melanocarpa* fruit, albeit with a small difference. The reason for this may be that an interaction between protein powder and *Aronia melanocarpa* fruit may have a reducing effect on antioxidant activity. When we look at the final product, Aronia smoothie, it is observed that it has the highest antioxidant content after digestion. Since black mulberry was added to the Aronia plus protein powder mixture and black mulberry is a fruit rich in antioxidants, it had an increasing effect on the antioxidant content of the final product despite the antioxidant-lowering effect between *Aronia melanocarpa* and protein powder.

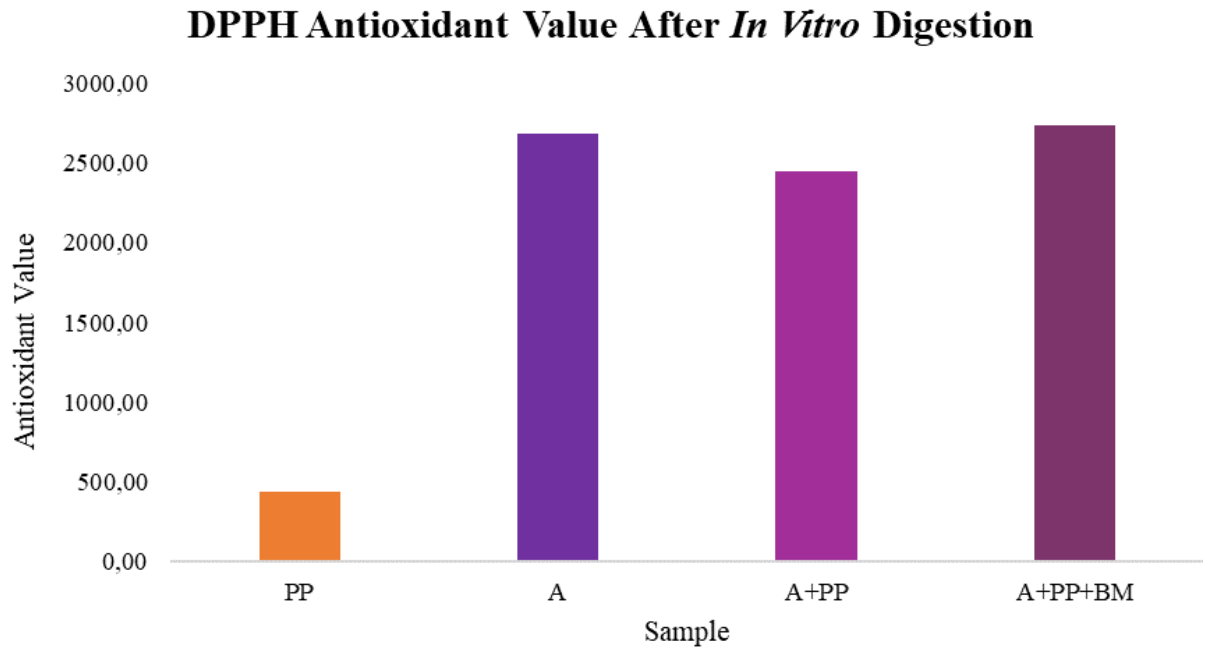

Figure 26. *Aronia melanocarpa*, protein powder and Aronia plus protein powder DPPH antioxidant content after *in vitro* digestion.

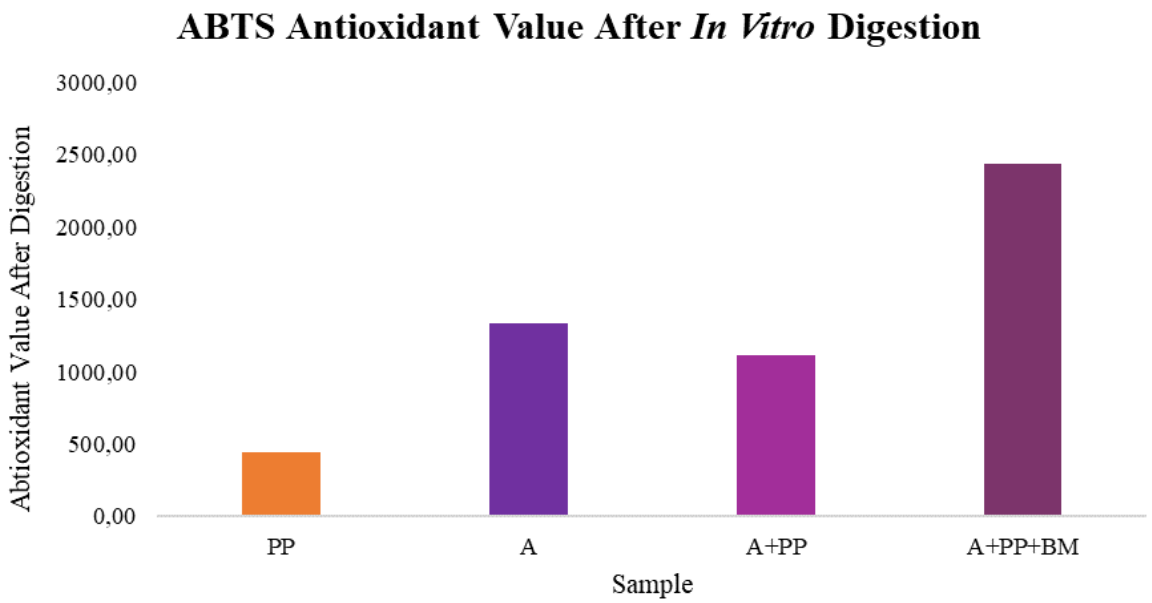

Figure 27. *Aronia melanocarpa*, protein powder and Aronia plus protein powder ABTS antioxidant content after *in vitro* digestion.

Similar results were obtained when the antioxidant contents of the products taken by applying the ABTS method on the same samples were compared. As expected, protein powder obtained from red lentils has a low antioxidant content. While *Aronia melanocarpa* showed higher antioxidant content than the mixture with protein powder alone, as in DPPH, the highest antioxidant content was obtained in the final product, Aronia smoothie, with the effect of black mulberry.

3.15. ACE Inhibition (ACE-I) Activity Assay

Aronia smoothie product, with its protein powder content extracted from red lentils, was also examined in terms of ACE inhibitor activity after *in vitro* digestion. It was thought that red lentils might have such an effect due to their high protein content, and since ACE inhibitory activity shows its effect after the food is taken into the body and digested, analyzes were carried out after the digestive process in the intestine. While the Aronia smoothie product was examined for its ACE inhibitor effect after *in vitro* digestion, separate ingredients of the product and mixtures with oat milk were

prepared and analyzed in the same way in order to observe their interaction with each other. Samples were prepared with only red lentil protein powder, only *Aronia melanocarpa* and Aronia plus protein powder, and analysis was carried out after *in vitro* digestion, and the results are shown in Figure 28.

The ACE inhibitor effect of the protein powder extracted only from red lentils after *in vitro* digestion was obtained with the highest effect among all samples, at 55%. This is an expected result due to its high protein content. The mixture prepared only with *Aronia melanocarpa* fruit has the lowest ACE inhibitor activity, with an inhibitory effect of 10%. Although *Aronia melanocarpa* fruit is a very strong fruit in terms of phenolic and antioxidant content, this may have different effects depending on the detailed phenol and antioxidant types and profiles.

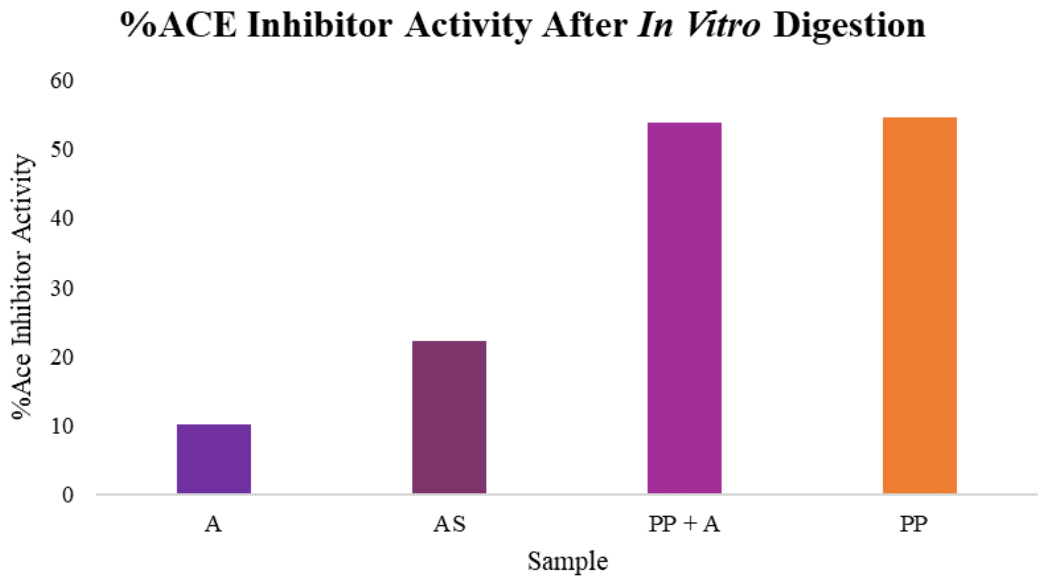

Figure 28. %ACE Inhibitor Activity of *Aronia melanocarpa*, Aronia smoothie, protein powder and Aronia plus protein powder samples after *in vitro* digestion.

When we look at the ACE inhibitor mechanism of the mixture prepared with aronia plus protein powder, it can be said that it has a high inhibitory mechanism with a rate of 54%. Considering the individual effects of *Aronia melanocarpa* and red lentil protein powder and the results after mixing, it can be concluded that the ACE inhibitor activity that the two inputs bring together is positively affected by the interaction. However, in the Aronia smoothie product, which is the final product obtained by adding

black mulberry in addition to Aronia plus protein powder, there was a decrease in ACE inhibitor activity and reached 22%. Here, it is observed that the interaction resulting from the combination of black mulberry, *Aronia melanocarpa* and red lentil protein powder has a reducing effect on ACE inhibitor activity. Although black mulberry fruit is rich in phenolic and antioxidant content, there is a need to know its detailed profile and subspecies. As a result of this detailed profile, detailed extraction of the components that affect ACE inhibitor activity positively or negatively can be made and a clear conclusion can then be reached. However, these effects can be achieved by making superficial inferences based on the results given by separate inputs.

In a study conducted by Gammoh et al. (2018), it was observed that protein and phenolic interactions in wheat flour product directly affected ACE inhibitor activity. The effects of albumin, glutelin, prolamin and globulin proteins, which are rich in protein content, on phenolics were examined and it was concluded that each of them had different ACE inhibitor effects within themselves and even according to their molecular weight properties. While differences were also observed between the free protein ends and the ends bound to phenolics, it was observed that these interactions and therefore the ACE inhibitor activity had different effects even with the process conditions. When all the results were evaluated, it was found that low molecular weight fractions of wheat proteins showed higher ACE inhibitory properties than high molecular weight fractions.

In another study, it was stated that different types of phenolic contents had different effects on ACE inhibitor activity using different plant samples (Al Shukor et al. 2013). As Al Shukor et al. (2013) stated, while tannic acid provides a high degree of ACE inhibitory activity, when caffeic acid and ferulic acid phenolics were evaluated, it was determined that they showed a mechanism to reduce the ACE inhibitory effect.

It should be understood from these literature reviews and the data obtained that, although it is generalized that the phenolic content positively affects the ACE inhibitor activity, the phenolic and protein contents of the food studied need to be extracted and known in more detail. Different protein and phenolic types may have different positive or negative effects on ACE inhibitor activity. Again, the interactions of these protein and phenolic structures with each other can lead to different results depending on the content of the product and the phenolic and protein types it contains. Therefore, making general comments on this mechanism without knowing detailed phenolic and protein profiles does not give clear results.

3.16. Shelf-life Analysis

Shelf life for food products is an important criterion that must be taken into consideration, and each type of product has its own shelf-life period. Shelf life is a period of time that starts from the date a food product is produced and continues until the last day the product can be consumed. In order to determine the shelf life of a newly produced food product, a parallel date can be determined by looking at other products in the same product group, and in addition, an accelerated shelf-life test is performed.

A shelf life of 2 years has been determined for the Aronia powder mix product, based on other freeze dry powder mix products in the same category. The reason for such a long shelf life is that the water content of the product is very low and its physical, chemical and microbiological activity is stabilized. Storing the product in appropriate and correct conditions is also one of the important factors associated with its shelf life. In the context of this determined period, it has been determined that the necessary analyzes for the Aronia powder mix product will be carried out on a monthly basis. Microbiological, total phenolic content, antioxidant activity, moisture, pH and color analyze were carried out monthly for the Aronia powder mix product throughout its shelf life. Analyzes were carried out at the time of first production, but due to some problems, analyzes could not be carried out for 4 months and after the analysis of the product produced in April, another analysis was carried out in September. Since no serious change was observed in the analyses, it was thought that this period would not have a significant effect and the analyzes continued to be carried out monthly. Shelf life analyzes continued to be carried out monthly for 5 months, including January. Since no significant and unexpected changes were observed in the analysis results after all this time, the analyzes were not continued due to the limited sample amount. Before the final study was delivered, the control analyzes were repeated for the last time in May and the changes over a 14-month period from the moment of production were revealed. The product was stored in the refrigerator at +4 ℃ in air and light-proof aluminum packages throughout its shelf life.

For the Aronia smoothie product, literature searches were conducted based on products in the same category and a shelf life of 21 days was decided as the most appropriate period. Microbiological, antioxidant activity, total phenolic content, pH, color, Brix and TTA analyzes of the product were carried out every week for 21 days.

Although no negative situation or change is observed, the appropriate shelf-life period has been determined for the product. The product was stored in unopened sterile jars in the refrigerator at +4 °C throughout its shelf life.

3.17. Sensory Analysis

Aronia smoothie product, which is one of the product types recommended for use because the *Aronia melanocarpa* powder product which is a nutritional supplement cannot be consumed directly on its own, is designed to be evaluated from a sensory perspective. For this reason, sensory analysis was carried out only for the Aronia based smoothie product. In the analysis carried out in the professional sensory laboratory with 20 experienced and inexperienced participants, the Aronia smoothie product was examined in terms of color, smell, texture, consistency and flavor properties and presented for evaluation. The overall score given to the product, which was evaluated out of 5 points in total, as the total evaluation of all these features, was evaluated as overall acceptance (Figure 29).

Aronia smoothie product received an average of 4.45 points from the panelists based on its color. Following the comments, the majority of participants liked the color of the product. Due to the high coloring and pigmentation properties of *Aronia melanocarpa* fruit, the smoothie product obtained has resulted in a striking and appetizing product in purple color.

Although the product examined in terms of odor received an average of 3.2 points from the panelists, remaining above average, it remained at a low evaluation compared to other features. As a result of the evaluations made on the feedback, the participants stated that they evaluated the product in this way because it did not have any smell. The product does not have any bad or undesirable odor. It has been reported that they only expect a more appetizing and beautiful smell when it comes together with its color. It can be interpreted that the Aronia smoothie product, which does not have any bad or good smell, is open to improvement in terms of smell. A pleasant scent can be achieved by adding natural ingredients to the product that will make its scent positively attractive.

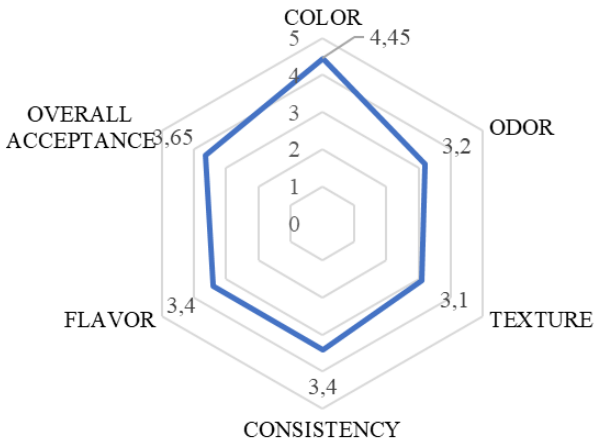

Figure 29. Sensory profile of Aronia smoothie product.

When the Aronia smoothie product was examined in terms of texture and consistency properties, it obtained an average score of 3.1 and 3.4, respectively. When the feedback received was examined, it was said that the consistency of the product was less than that required for a smoothie. They said that they made the evaluation by expecting a texture and density that was more particle and puree-like. However, as a result of the literature searches, as can be seen from the brix section in the analysis section, the consistency of the Aronia smoothie product obtained is consistent with the smoothie references in the literature searches. In general, the expected smoothie consistency of the participants is more suitable for puree and denser consistency products and does not suit the smoothie product in terms of category. Since the consistency that the consumer base in our country expects from the smoothie product does not parallel the structure that it should have under normal conditions, the product achieved a low score even though it remained above the average. In this sense, it is thought that the consumer base should be informed about the smoothie consistency and be accustomed to the normal consistency for this product type.

Finally, in terms of taste, which is one of the most important sensory parameters, the product achieved an average score of 3.4. Since the product is Aronia-based, different freeze dry fruit powders were added to the product in order to eliminate the astringent taste coming from the Aronia fruit, and an attempt was made to achieve a pleasant, preferable and consumable taste without any additives. In fact, through recipe trials, a consumable taste was achieved without the use of additives or added sugar. However, it was understood from the feedback given by the panelists that people expect

more sugary tastes when it comes to smoothies, and since it does not have such a high and intense sugary taste, the product could not exceed 4 points even though it remained above average.

Finally, in the sum of all these parameters, the panelists who evaluated the product in terms of general acceptability gave the product an average of 3.65 points. In a new functional product evaluation out of a total of 5, this score indicates a successful product design. Although the product is open to improvement in terms of smell and taste, consumers should be informed about what should be done in terms of texture on the basis of the smoothie product. Although the Brix value of the Aronia smoothie product is consistent with many smoothie references in the literature, the product received a lot of criticism from the participants in this sense.

It should be noted that Aronia powder mix and Aronia smoothie products are designed as a vegan and herbal product, without containing any additives or added sugar, especially for consumers who have adopted a healthy lifestyle, and the features expected from the product should be evaluated within this framework (Figure 30).

Figure 30. Final Aronia smoothie products after pasteurization.

CHAPTER 4

CONCLUSION

In this study, which was carried out with the aim of designing an *Aronia melanocarpa*-based vegan, high-protein functional nutritional supplement and a functional smoothie product using this supplement, products suitable for healthy alternatives and life orientation trends in the food industry were produced. These designed products are in the class of functional products sought by the consumer, with their rich nutritional content, their positive health effects in addition to their nutritional content, and their content that eliminates these deficiencies for the consumer group who cannot consume animal products and suffer from nutritional deficiencies. At the same time, with the designed usage types, it is both easy to use and has life-making effects. Aronia powder mix product has 23.7% protein content as a high protein product. No significant change was observed in the antioxidant and phenolic substance contents throughout the shelf life of both functional products, which are very rich in antioxidant and phenolic content. Since the raw materials and red lentil protein isolate dried by the freeze-drying process have a very low value in terms of water activity, the quality characteristics of the product remained stable throughout the shelf life of the product and no serious loss of nutritional content was observed due to the freeze-drying effect. Due to the low water activity, the product is less exposed to microbial dangers. Particular sensitivity was shown to the storage conditions throughout the shelf life of the products, and therefore no significant deterioration was encountered in the physical, chemical and microbiological analyses. It was deemed appropriate to experiment with one of the recommended by-products of the Aronia powder mix product and a smoothie drink was designed. For smoothie type beverage products, the Brix value of the product is important in terms of suitability and the Brix value of the product was obtained in the range of 14-15. Aronia powder mix and Aronia smoothie products also have a rich content in terms of minerals. According to the results obtained, it contains primarily K, P, Ca, Mg, Na, Fe and Zn minerals in terms of their density, respectively. Sensory, *in vitro* digestion and ACE inhibitor activity analyzes were carried out for this designed Aronia smoothie product. As a result of the sensory test, the products achieved a good

result, receiving an above average general acceptance score from the panelists. The designed products are rich in antioxidants, phenolics, minerals and proteins and have high functional properties due to the raw materials they contain. There are thousands of positive health effects from both the *Aronia melanocarpa* fruit and the red lentil plant. However, it should not be forgotten that functional products show the expected health effects as a result of regular and conscious consumption. With all these effects, Aronia powder mix and Aronia smoothie products are quality, delicious and do not contain any additives that will be consumed with serious interest by consumers who follow the veganism diet type, athletes and the consumer base who have adopted a healthy lifestyle and pay attention to their nutrition.

As a result, the main purpose of this study is to design a functional nutritional supplement, and an *Aronia melanocarpa*-based, high-protein vegan dietary supplement product that is both nutritionally rich and practical and makes the consumer's life easier, and in the light of this product, the Aronia smoothie product has been created. Especially if we consider the healthy lifestyle style that has emerged globally after the pandemic, the products can attract serious attention of consumers in the market and have a positive effect on various health problems.

REFERENCES

Aderinola, Taiwo Ayodele. 2018. “Nutritional, Antioxidant and Quality Acceptability of Smoothies Supplemented with Moringa Oleifera Leaves.” *Beverages* 4 (4). https://doi.org/10.3390/beverages4040104.

Alake, Abraham, Akinola, T O Fatunmibi, Agboola, and Akua. 2022. “Production and Evaluation of Smoothies Made from Various Fruits Sold in Lagos Market.” *International Journal of Innovative Science and Research Technology*. Vol. 7. www.ijisrt.com642.

Anggarani, M. A., A. D. Ayuningsih, E. Yudianto, and B. A. Prasodi. 2019. “The Composition of Water and Ash of Secang Wood’s Simplicia and Secang Wood Herbal Drink Powder.” In *Journal of Physics: Conference Series*. Vol. 1417. Institute of Physics Publishing. https://doi.org/10.1088/1742-6596/1417/1/012033.

Antonio, Jose, Corey A. Peacock, Anya Ellerbroek, Brandon Fromhoff, and Tobin Silver. 2014. “The Effects of Consuming a High Protein Diet (4.4 g/Kg/d) on Body Composition in Resistance-Trained Individuals.” *Journal of the International Society of Sports Nutrition* 11 (1). https://doi.org/10.1186/1550-2783-11-19.

Aydemir, Levent Yurdaer, and Ahmet Yemenicioĝlu. 2013. “Potential of Turkish Kabuli Type Chickpea and Green and Red Lentil Cultivars as Source of Soy and Animal Origin Functional Protein Alternatives.” *LWT* 50 (2): 686–94. https://doi.org/10.1016/j.lwt.2012.07.023.

Balaswamy, K, PG Prabhakara Rao, A Nagender, G Narsing Rao, K Sathiya Mala, RG Math, A Sat, et al. 2013. “Development of Smoothies from Selected Fruit Pulps/Juices.” *International Food Research Journal*. Vol. 20.

Benvenuti, S., F. Pellati, M. Melegari, and D. Bertelli. 2004. “Polyphenols, Anthocyanins, Ascorbic Acid, and Radical Scavenging Activity of Rubus, Ribes, and Aronia.” *Journal of Food Science* 69 (3). https://doi.org/10.1111/j.1365-2621.2004.tb13352.x.

Bhatta, Sagar, Tatjana Stevanovic Janezic, and Cristina Ratti. 2020. “Freeze-Drying of Plant-Based Foods.” *Foods*. MDPI Multidisciplinary Digital Publishing Institute. https://doi.org/10.3390/foods9010087.

Bilge, Gonca, Banu Sezer, Kemal Efe Eseller, Halil Berberoglu, Hamit Koksel, and Ismail Hakki Boyaci. 2016. “Ash Analysis of Flour Sample by Using Laser-Induced Breakdown Spectroscopy.” *Spectrochimica Acta - Part B Atomic Spectroscopy* 124 (October):74–78. https://doi.org/10.1016/j.sab.2016.08.023.

Bratosin, Bogdan Constantin, Gheorghe-Adrian Martău, Călina Ciont, Floricuța Ranga, Elemér Simon, Katalin Szabo, Sorina Darjan, Bernadette-Emőke Teleky, and Dan Cristian Vodnar. 2024. “Nutritional and Physico-Chemical Characteristics of Innovative Bars Enriched with Aronia Melanocarpa By-Product Powder.” *Applied Sciences* 14 (6): 2338. https://doi.org/10.3390/app14062338.

Brodkorb, André, Lotti Egger, Marie Alminger, Paula Alvito, Ricardo Assunção, Simon Ballance, Torsten Bohn, et al. 2019. “INFOGEST Static in Vitro Simulation of Gastrointestinal Food Digestion.” *Nature Protocols* 14 (4): 991–1014. https://doi.org/10.1038/s41596-018-0119-1.

Chen, Guan Lin, Song Gen Chen, Ying Ying Zhao, Chun Xia Luo, Juan Li, and Yong Qing Gao. 2014. “Total Phenolic Contents of 33 Fruits and Their Antioxidant Capacities before and after in Vitro Digestion.” *Industrial Crops and Products* 57:150–57. https://doi.org/10.1016/j.indcrop.2014.03.018.

Craig, Winston J. 2009. “Health Effects of Vegan Diets.” In *American Journal of Clinical Nutrition*. Vol. 89. https://doi.org/10.3945/ajcn.2009.26736N.

Daskalova, Elena, Slavi Delchev, Mariyan Topolov, Stela Dimitrova, Yordanka Uzunova, Stefka Valcheva-Kuzmanova, Maria Kratchanova, Lyudmila Vladimirova-Kitova, and Petko Denev. 2019. “Aronia Melanocarpa (Michx.) Elliot Fruit Juice Reveals Neuroprotective Effect and Improves Cognitive and Locomotor Functions of Aged Rats.” *Food and Chemical Toxicology* 132 (October). https://doi.org/10.1016/j.fct.2019.110674.

Denev, Petko, Maria Kratchanova, Ivalina Petrova, Daniela Klisurova, Yordan Georgiev, Manol Ognyanov, and Irina Yanakieva. 2018. “Black Chokeberry (Aronia Melanocarpa (Michx.) Elliot) Fruits and Functional Drinks Differ Significantly in Their Chemical Composition and Antioxidant Activity.” *Journal of Chemistry* 2018. https://doi.org/10.1155/2018/9574587.

Deng, Haotian, Yanwen Kong, Jinyan Zhu, Xinyao Jiao, Yuqi Tong, Meizhi Wan, Yang Zhao, Sixu Lin, Yan Ma, and Xianjun Meng. 2022. “Proteomic Analyses Revealed the

Antibacterial Mechanism of Aronia Melanocarpa Isolated Anthocyanins against Escherichia Coli O157: H7." *Current Research in Food Science* 5 (January):1559–69. https://doi.org/10.1016/j.crfs.2022.09.017.

Dilrukshi, S. H.B., and H. P.S. Senarath. 2021. "Development and Quality Evaluation of Freeze-Dried Instant Green Smoothie Powder." *International Journal of Food Science* 2021. https://doi.org/10.1155/2021/6634764.

Djaoudene, Ouarda, Anabela Romano, Yasmine Djedjiga Bradai, Feriel Zebiri, Amina Ouchene, Yasmine Yousfi, Meriem Amrane-Abider, Yasmine Sahraoui-Remini, and Khodir Madani. 2023. "A Global Overview of Dietary Supplements: Regulation, Market Trends, Usage during the COVID-19 Pandemic, and Health Effects." *Nutrients*. Multidisciplinary Digital Publishing Institute (MDPI). https://doi.org/10.3390/nu15153320.

Eisinaitė, Viktorija, Daiva Leskauskaitė, Milda Pukalskienė, and Petras Rimantas Venskutonis. 2020. "Freeze-Drying of Black Chokeberry Pomace Extract–Loaded Double Emulsions to Obtain Dispersible Powders." *Journal of Food Science* 85 (3): 628–38. https://doi.org/10.1111/1750-3841.14995.

Essa, Musthafa Mohamed, Muhammed Bishir, Abid Bhat, Saravana Babu Chidambaram, Buthaina Al-Balushi, Hamdan Hamdan, Nagamaniammai Govindarajan, Robert P. Freidland, and M. Walid Qoronfleh. 2023. "Functional Foods and Their Impact on Health." *Journal of Food Science and Technology*. Springer. https://doi.org/10.1007/s13197-021-05193-3.

Gammoh, Sana, Muhammad H. Alu'datt, Mohammad N. Alhamad, Taha Rababah, Majdi Al-Mahasneh, Ahmad Qasaimeh, Ayman Johargy, Stan Kubow, and Neveen M. Hussein. 2018. "The Effects of Protein-Phenolic Interactions in Wheat Protein Fractions on Allergenicity, Antioxidant Activity and the Inhibitory Activity of Angiotensin I-Converting Enzyme (ACE)." *Food Bioscience* 24 (August):50–55. https://doi.org/10.1016/j.fbio.2018.05.008.

Ghannadiasl, Fatemeh, and Hamed Nourani. 2019. "The Ash Content of Elaeagnus Angustifolia Powder Supplied in Markets." https://www.researchgate.net/publication/339618032.

Ghendov-Mosanu, Aliona, Mădălina Ungureanu-Iuga, Silvia Mironeasa, and Rodica Sturza. 2022. "Aronia Extracts in the Production of Confectionery Masses." *Applied Sciences (Switzerland)* 12 (15). https://doi.org/10.3390/app12157664.

Ghevariya, Hinal H, and Renu Singh. n.d. *1. Functional Foods and Phytochemicals*. https://www.kdpublications.in.

Glick-Bauer, Marian, and Ming Chin Yeh. 2014. "The Health Advantage of a Vegan Diet: Exploring the Gut Microbiota Connection." *Nutrients*. MDPI AG. https://doi.org/10.3390/nu6114822.

Guerrero, Ligia, Julián Castillo, Mar Quiñones, Santiago Garcia-Vallvé, Lluis Arola, Gerard Pujadas, and Begoña Muguerza. 2012. "Inhibition of Angiotensin-Converting Enzyme Activity by Flavonoids: Structure-Activity Relationship Studies." *PLoS ONE* 7 (11). https://doi.org/10.1371/journal.pone.0049493.

Guidi, Sarah, Florian A. Formica, and Christoph Denkel. 2022. "Mixing Plant-Based Proteins: Gel Properties of Hemp, Pea, Lentil Proteins and Their Binary Mixtures." *Food Research International* 161 (November). https://doi.org/10.1016/j.foodres.2022.111752.

Habschied, Kristina, Jelena Nišević, Vinko Krstanović, Ante Lončarić, Kristina Valek Lendić, and Krešimir Mastanjević. 2023. "Formulation of a Wort-Based Beverage with the Addition of Chokeberry (Aronia Melanocarpa) Juice and Mint Essential Oil." *Applied Sciences (Switzerland)* 13 (4). https://doi.org/10.3390/app13042334.

Heydari, Mohamad Mehdi, Tahereh Najib, and Venkatesh Meda. 2022. "Investigating Starch and Protein Structure Alterations of the Processed Lentil by Microwave-Assisted Infrared Thermal Treatment and Their Correlation with the Modified Properties." *Food Chemistry Advances* 1 (October). https://doi.org/10.1016/j.focha.2022.100091.

Horszwald, Anna, Heritier Julien, and Wilfried Andlauer. 2013. "Characterisation of Aronia Powders Obtained by Different Drying Processes." *Food Chemistry* 141 (3): 2858–63. https://doi.org/10.1016/j.foodchem.2013.05.103.

İzli, Gökçen, Gulcin Yildiz, and Senel Ecem Berk. 2022. "Quality Retention in Pumpkin Powder Dried by Combined Microwave-Convective Drying." *Journal of Food Science and Technology* 59 (4): 1558–69. https://doi.org/10.1007/s13197-021-05167-5.

Jiao, Xinyao, Yixiao Shen, Haotian Deng, Qi Zhang, and Jin Zhao. 2021. "Cyanidin-3-O-Galactoside from Aronia Melanocarpa Attenuates High-Fat Diet-Induced Obesity and Inflammation via AMPK, STAT3, and NF-KB P65 Signaling Pathways in Sprague-Dawley Rats." *Journal of Functional Foods* 85 (October). https://doi.org/10.1016/j.jff.2021.104616.

Joshi, M., B. Adhikari, P. Aldred, J. F. Panozzo, and S. Kasapis. 2011. “Physicochemical and Functional Properties of Lentil Protein Isolates Prepared by Different Drying Methods.” *Food Chemistry* 129 (4): 1513–22. https://doi.org/10.1016/j.foodchem.2011.05.131.

Jurendi´c, Tomislav Jurendi´c, Mario Ščetar, Andrée Voilley, and Mia Kurek. 2021. “Aronia Melanocarpa Products and By-Products for Health and Nutrition: A Review.” https://doi.org/10.3390/antiox.

Kamiloğlu, Senem. 2019. “Changes in Green Bean Phenolics, Flavonoids and Antioxidant Capacity during Industrial Freezing Process and in Vitro Gastrointestinal Digestion.” *Akademik Gida* 17 (2): 176–84. https://doi.org/10.24323/akademik-gida.613559.

Kaur, Sumeet, and Madhusweta Das. 2011. “Functional Foods: An Overview.” *Food Science and Biotechnology*. https://doi.org/10.1007/s10068-011-0121-7.

Kha, Tuyen C., Minh H. Nguyen, and Paul D. Roach. 2010. “Effects of Spray Drying Conditions on the Physicochemical and Antioxidant Properties of the Gac (Momordica Cochinchinensis) Fruit Aril Powder.” *Journal of Food Engineering* 98 (3): 385–92. https://doi.org/10.1016/j.jfoodeng.2010.01.016.

King, Erica S., and Bradley W. Bolling. 2020. “Composition, Polyphenol Bioavailability, and Health Benefits of Aronia Berry: A Review.” *Journal of Food Bioactives* 11:13–30. https://doi.org/10.31665/jfb.2020.11235.

Krishi Vidyapeeth, Marathwada, India KS Gadhe, India RP Shaikh, and India Corresponding Author. 2020. “Studies on Quality Evaluation of OAT Milk.” *~ 2275 ~ Journal of Pharmacognosy and Phytochemistry* 9 (1). www.phytojournal.com.

Labur, Jana ic, Nadica Dobričević, Stjepan Pliestić, Ante Galić, Daniela Patricia Bilić, and Sandra Voća. 2017. “Antioxidant Potential of Fruit Juice with Added Chokeberry Powder (Aronia Melanocarpa).” *Molecules* 22 (12). https://doi.org/10.3390/molecules22122158.

Lazăr, Monica-Alexandra, Monica Catană, Luminița Catană, Anda-Grațiela Burnete, Răzvan Ionuț Teodorescu, Adrian Constantin Asănică, and Nastasia Belc. 2020. “Valorisation of Aronia Melanocarpa Pomace for Development of Functional Ingredients With High Nutritional Value And Antioxidant Capacity.”

Lee, Gee Dong, Jung Hee Shin, and In Hag Choi. 2018. “Growth Performance and Fatty Acid Profiles of Ducks Fed a Diet Supplemented with Aronia (Aronia Melanocarpa)

Powder.” *Revista Brasileira de Ciencia Avicola / Brazilian Journal of Poultry Science* 20 (2): 193–96. https://doi.org/10.1590/1806-9061-2016-0445.

Lee, Hui Wen, Yuyun Lu, Yuyu Zhang, Caili Fu, and Dejian Huang. 2021. “Physicochemical and Functional Properties of Red Lentil Protein Isolates from Three Origins at Different PH.” *Food Chemistry* 358 (October). https://doi.org/10.1016/j.foodchem.2021.129749.

Liu, Siyuan, Yikai Ren, Hanyue Yin, Michael Nickerson, Mark Pickard, and Yongfeng Ai. 2022. “Improvement of the Nutritional Quality of Lentil Flours by Infrared Heating of Seeds Varying in Size.” *Food Chemistry* 396 (December). https://doi.org/10.1016/j.foodchem.2022.133649.

Liu, Wen Ying, Jiang Tao Zhang, Takuya Miyakawa, Guo Ming Li, Rui Zeng Gu, and Masaru Tanokura. 2021. “Antioxidant Properties and Inhibition of Angiotensin-Converting Enzyme by Highly Active Peptides from Wheat Gluten.” *Scientific Reports* 11 (1). https://doi.org/10.1038/s41598-021-84820-7.

Marshall, Maurice R. 2010. “Ash Analysis.” In , 105–15. https://doi.org/10.1007/978-1-4419-1478-1_7.

Martirosyan, Danik, and Emma Miller. 2018. “Bioactive Compounds: The Key to Functional Foods.” *Bioactive Compounds in Health and Disease*. Functional Food Institute. https://doi.org/10.31989/bchd.v1i3.539.

Maughan, Ronald J., Frederic Depiesse, and Hans Geyer. 2007. “The Use of Dietary Supplements by Athletes.” In *Journal of Sports Sciences*, 25:103–13. https://doi.org/10.1080/02640410701607395.

Mehta, Dipakkumar, M. H.Sathish Kumar, and Latha Sabikhi. 2017. “Development of High Protein, High Fiber Smoothie as a Grab-and-Go Breakfast Option Using Response Surface Methodology.” *Journal of Food Science and Technology* 54 (12): 3859–66. https://doi.org/10.1007/s13197-017-2841-z.

Moghadam, Maryam, Maryam Salami, Mehdi Mohammadian, Maryam Khodadadi, and Zahra Emam-Djomeh. 2020. “Development of Antioxidant Edible Films Based on Mung Bean Protein Enriched with Pomegranate Peel.” *Food Hydrocolloids* 104 (July). https://doi.org/10.1016/j.foodhyd.2020.105735.

Nguyen, Linh, and Eun Sun Hwang. 2016. “Quality Characteristics and Antioxidant Activity of Yogurt Supplemented with Aronia (Aronia Melanocarpa) Juice.” *Preventive Nutrition and Food Science* 21 (4): 330–37. https://doi.org/10.3746/pnf.2016.21.4.330.

Nielsen, S. Suzanne. 2017a. “Moisture Content Determination.” In , 105–15. https://doi.org/10.1007/978-3-319-44127-6_10.

Nielsen, S. Suzanne. 2017b. “Moisture Content Determination.” In , 105–15. https://doi.org/10.1007/978-3-319-44127-6_10.

Nielsen, S. Suzanne. 2017c. “Standard Solutions and Titratable Acidity.” In , 179–84. https://doi.org/10.1007/978-3-319-44127-6_21.

Nour, Violeta. 2022. “Quality Characteristics, Anthocyanin Stability and Antioxidant Activity of Apple (*Malus Domestica*) and Black Chokeberry (*Aronia Melanocarpa*) Juice Blends.” *Plants* 11 (15). https://doi.org/10.3390/plants11152027.

Nunes, M. Antónia, Anabela S.G. Costa, João C.M. Barreira, Ana F. Vinha, Rita C. Alves, Ada Rocha, and M. Beatriz P.P. Oliveira. 2016a. “How Functional Foods Endure throughout the Shelf Storage? Effects of Packing Materials and Formulation on the Quality Parameters and Bioactivity of Smoothies.” *LWT* 65:70–78. https://doi.org/10.1016/j.lwt.2015.07.061.

Oyinloye, Timilehin Martins, and Won Byong Yoon. 2020. “Effect of Freeze-Drying on Quality and Grinding Process of Food Produce: A Review.” *Processes*. MDPI AG. https://doi.org/10.3390/PR8030354.

Park, Sehee, Jin Il Kim, Ilseob Lee, Sangmoo Lee, Min Woong Hwang, Joon Yong Bae, Jun Heo, Donghwan Kim, Sang Zin Han, and Man Seong Park. 2013. “Aronia Melanocarpa and Its Components Demonstrate Antiviral Activity against Influenza Viruses.” *Biochemical and Biophysical Research Communications* 440 (1): 14–19. https://doi.org/10.1016/j.bbrc.2013.08.090.

Park, Young. 1996. “Determination of Moisture and Ash Contents of Foods. Handbook of Food Analysis.” Marcel Dekker, Inc. https://www.researchgate.net/publication/282135010.

Pavlovic, Aleksandra N., Jelena M. Brcanovic, Jovana N. Veljkovic, Snezana S. Mitic, Snezana B. Tošic, Biljana M. Kaličanin, Danijela A. Kostic, Miodrag S. Eorcrossed D Signevic, and Dragan S. Velimirovic. 2015. “Characterization of Commercially Available

Products of Aronia According to Their Metal Content." *Fruits* 70 (6): 385–93. https://doi.org/10.1051/fruits/2015038.

Perring, L., and A. Tschopp. 2019. "Determination of Ash Content of Milk-Based Powders by Energy Dispersive X-Ray Fluorescence." *Microchemical Journal* 145 (March):162–67. https://doi.org/10.1016/j.microc.2018.10.025.

Pesta, Dominik H., and Varman T. Samuel. 2014. "A High-Protein Diet for Reducing Body Fat: Mechanisms and Possible Caveats." *Nutrition and Metabolism*. BioMed Central Ltd. https://doi.org/10.1186/1743-7075-11-53.

Petković, Marko, Igor Đurović, Nemanja Miletić, and Jovana Radovanović. 2019. "Effect of Convective Drying Method of Chokeberry (Aronia Melanocarpa L.) on Drying Kinetics, Bioactive Components and Sensory Characteristics of Bread with Chokeberry Powder." *Periodica Polytechnica Chemical Engineering* 63 (4): 600–608. https://doi.org/10.3311/PPch.13783.

Petković, Marko, Vladimir Filipović, Jelena Filipović, Igor Đurović, Nemanja Miletić, and Jovana Radovanović. 2021. "Chemical, Antioxidative, and Sensory Characteristics of Wheat Bread Partially Substituted with Black Chokeberry (Aronia Melanocarpa L.) Powder." *Journal of Food Processing and Preservation* 45 (1). https://doi.org/10.1111/jfpp.15027.

Pihlanto, Anne, Sari Akkanen, and Hannu J. Korhonen. 2008. "ACE-Inhibitory and Antioxidant Properties of Potato (Solanum Tuberosum)." *Food Chemistry* 109 (1): 104–12. https://doi.org/10.1016/j.foodchem.2007.12.023.

Platonova, Elena Y., Mikhail V. Shaposhnikov, Hye Yeon Lee, Ji Hyeon Lee, Kyung Jin Min, and Alexey Moskalev. 2021. "Black Chokeberry (Aronia Melanocarpa) Extracts in Terms of Geroprotector Criteria." *Trends in Food Science and Technology*. Elsevier Ltd. https://doi.org/10.1016/j.tifs.2021.06.020.

Pop, Laura, Rui Costa, Adrian Asănică, and Liliana Tudoreanu. 2022. "Mineral Nutritional Value of Products Containing Aronia Fruits and Juices: A Review."

Rautiainen, Susanne, Joann E. Manson, Alice H. Lichtenstein, and Howard D. Sesso. 2016. "Dietary Supplements and Disease Prevention-a Global Overview." *Nature Reviews Endocrinology*. Nature Publishing Group. https://doi.org/10.1038/nrendo.2016.54.

Re, Roberta, Nicoletta Pellegrini, Anna Proteggente, Ananth Pannala, Min Yang, and Catherine Rice-Evans. 1999. “Original Contribution Antioxidant Activity Applying an Improved ABTS Radical Cation Decolorization Assay.”

Roy, Ipsita, and Munishwar Nath Gupta. 2004. “Review Freeze-Drying of Proteins: Some Emerging Concerns.” *Biotechnol. Appl. Biochem*. Vol. 39. www.fda.gov/ora/inspect.

Sadowska, Anna, Franciszek Świderski, Rita Rakowska, and Ewelina Hallmann. 2019. “Comparison of Quality and Microstructure of Chokeberry Powders Prepared by Different Drying Methods, Including Innovative Fluidised Bed Jet Milling and Drying.” *Food Science and Biotechnology* 28 (4): 1073–81. https://doi.org/10.1007/s10068-019-00556-1.

Sáez-Plaza, Purificación, Tadeusz Michałowski, María José Navas, Agustín García Asuero, and Sławomir Wybraniec. 2013. “An Overview of the Kjeldahl Method of Nitrogen Determination. Part I. Early History, Chemistry of the Procedure, and Titrimetric Finish.” *Critical Reviews in Analytical Chemistry* 43 (4): 178–223. https://doi.org/10.1080/10408347.2012.751786.

Saricaoglu, Furkan Turker. 2020. “Application of High-Pressure Homogenization (HPH) to Modify Functional, Structural and Rheological Properties of Lentil (Lens Culinaris) Proteins.” *International Journal of Biological Macromolecules* 144 (February):760–69. https://doi.org/10.1016/j.ijbiomac.2019.11.034.

Savas, Busra Sena, and Ecem Akan. 2021. “Oat Bran Fortified Raspberry Probiotic Dairy Drinks: Physicochemical, Textural, Microbiologic Properties, in Vitro Bioaccessibility of Antioxidants and Polyphenols.” *Food Bioscience* 43 (October). https://doi.org/10.1016/j.fbio.2021.101223.

Shukor, Nadin Al, John Van Camp, Gerard Bryan Gonzales, Dorien Staljanssens, Karin Struijs, Moises J. Zotti, Katleen Raes, and Guy Smagghe. 2013. “Angiotensin-Converting Enzyme Inhibitory Effects by Plant Phenolic Compounds: A Study of Structure Activity Relationships.” In *Journal of Agricultural and Food Chemistry*, 61:11832–39. https://doi.org/10.1021/jf404641v.

Singleton, Vernon L, Rudolf Orthofer, and Rosa M Lamuela-Ravent6s. 1999. “52 Polyphenols And Flavonoids [14] Analysis of Total Phenols and Other Oxidation Substrates and Antioxidants by Means of Folin-Ciocalteu Reagent.”

Siró, István, Emese Kápolna, Beáta Kápolna, and Andrea Lugasi. 2008. “Functional Food. Product Development, Marketing and Consumer Acceptance-A Review.” *Appetite*. https://doi.org/10.1016/j.appet.2008.05.060.

Škegro, Marko, Predrag Putnik, Danijela Bursac Kovacevic, Ana Petra Kovac, Lidija Salkic, Iva Canak, Jadranka Frece, Sandra Zavadlav, and Damir Ježek. 2021a. “Chemometric Comparison of High-Pressure Processing and Thermal Pasteurization: The Nutritive, Sensory, and Microbial Quality of Smoothies.” *Foods* 10 (6). https://doi.org/10.3390/foods10061167.

Škegro, Marko, Predrag Putnik, Danijela Bursac Kovacevic, Ana Petra Kovac, Lidija Salkic, Iva Canak, Jadranka Frece, Sandra Zavadlav, and Damir Ježek. 2021b. “Chemometric Comparison of High-Pressure Processing and Thermal Pasteurization: The Nutritive, Sensory, and Microbial Quality of Smoothies.” *Foods* 10 (6). https://doi.org/10.3390/foods10061167.

Stan, Andreea, and Mona Elena Popa. 2013a. “Experimental Research on Active Compounds and Nutrients in New Fruit Smoothie Products.”

Stan, Andreea, and Mona Elena Popa. 2019b. “Research on The Correlation Between Physico-Chemical, Sensory Analysis of Smoothie Type Products and Consumer Preferences.”

Sun-Waterhouse, Dongxiao. 2011. “The Development of Fruit-Based Functional Foods Targeting the Health and Wellness Market: A Review.” *International Journal of Food Science and Technology*. https://doi.org/10.1111/j.1365-2621.2010.02499.x.

Tahsiri, Zahra, / Mehrdad Niakousari, / Gholam, and Reza Mesbahi. 2017. “Effect of Different Drying Techniques on Physicochemical, Micro-Structural and Bioactive Characteristics of Barberry Milk Smoothie Powder.” *International Journal of Food Engineering*. https://doi.org/10.1515//ijfe-2016-0323.

Taskin, Onur. 2020. “Evaluation of Freeze Drying for Whole, Half Cut and Puree Black Chokeberry (Aronia Melanocarpa L.).” *Heat and Mass Transfer/Waerme- Und Stoffuebertragung* 56 (8): 2503–13. https://doi.org/10.1007/s00231-020-02867-0.

Teneva, Desislava, Daniela Pencheva, Ani Petrova, Manol Ognyanov, Yordan Georgiev, and Petko Denev. 2022. “Addition of Medicinal Plants Increases Antioxidant Activity, Color, and Anthocyanin Stability of Black Chokeberry (Aronia Melanocarpa) Functional Beverages.” *Plants* 11 (3). https://doi.org/10.3390/plants11030243.

Thi, Nhuan Do, and Eun Sun Hwang. 2016. “Effects of Drying Methods on Contents of Bioactive Compounds and Antioxidant Activities of Black Chokeberries (Aronia Melanocarpa).” *Food Science and Biotechnology* 25 (1): 55–61. https://doi.org/10.1007/s10068-016-0008-8.

Tirla, Adrian, Adrian Vasile Timar, Anca Becze, Adriana Ramona Memete, Simona Ioana Vicas, Mihaela Simona Popoviciu, and Simona Cavalu. 2023. “Designing New Sport Supplements Based on Aronia Melanocarpa and Bee Pollen to Enhance Antioxidant Capacity and Nutritional Value.” *Molecules* 28 (19). https://doi.org/10.3390/molecules28196944.

Tokuşoğlu, Özlem. 2018. “Aronia Berry Based New Food Products and Shelf Life Stability Studies.” Vol. 1.

Tolic, Mandica-Tamara, Irena Landeka Jurcevic, Ines Panjkota Krbavcic, Ksenija Markovic, and Nada Vahcic. 2015. “Phenolic Content, Antioxidant Capacity and Quality of Chokeberry (Aronia Melanocarpa) Products.” *Food Technolohy Biotechnology*, February, 171–79. https://doi.org/10.17113/ft b.53.02.15.3833.

Toupal, Samin, and Serap Coşansu. 2023. “Antioxidant and Antimicrobial Properties of Freeze-Dried Banana and Watermelon Peel Powders.” *Food and Humanity* 1 (December):607–13. https://doi.org/10.1016/j.foohum.2023.07.008.

Turkish Food Codex Regulation (TFC). Communiqué on Determination of Maximum Levels of Certain Contaminants in Foodstuffs. *Off. Gaz.* 2011.

Turkish Food Codex Regulation (TFC). Communiqué on Nutrition and Health. *Off. Gaz.* 2017.

Uzodinma, E O, Ochulor, I E Mbaeyi-Nwaoha, and E U Onwurafor. 2020. “Chidinma Ezinne Ochulor. Influence of Pasteurization on the Quality of Pineapple, Watermelon and Banana Pulps-Based Smoothie Flavoured with Coconut Milk.” *American Journal of Food Science and Technology*. Vol. 8. www.videleaf.comhttp://pubs.sciepub.com/ajfst/8/3/3.

Velde, Franco Van de, Charito Vignatti, María Paula Méndez-Galarraga, Micaela Gomila, Cecilia Fenoglio, Melisa Donda Zbinden, and María Élida Pirovani. 2022. “Intestinal and Colonic Bioaccessibility of Phenolic Compounds from Fruit Smoothies as Affected by the Thermal Processing and the Storage Conditions.” *Food Research International* 155 (May). https://doi.org/10.1016/j.foodres.2022.111086.

Villaño, D., A. Gironés-Vilapana, C. García-Viguera, and D. A. Moreno. 2016. “Development of Functional Foods.” In *Innovation Strategies in the Food Industry: Tools for Implementation*, 191–210. Elsevier Inc. https://doi.org/10.1016/B978-0-12-803751-5.00010-6.

Viuda-Martos, M., Y. Ruiz-Navajas, A. Martin-Sánchez, E. Sánchez-Zapata, J. Fernández-López, E. Sendra, E. Sayas-Barberá, C. Navarro, and J. A. Pérez-Álvarez. 2012. “Chemical, Physico-Chemical and Functional Properties of Pomegranate (Punica Granatum L.) Bagasses Powder Co-Product.” In *Journal of Food Engineering*, 110:220–24. Elsevier Ltd. https://doi.org/10.1016/j.jfoodeng.2011.05.029.

Walkling-Ribeiro, Markus, Francesco Noci, Denis A. Cronin, James G. Lyng, and Desmond J. Morgan. 2010. “Shelf Life and Sensory Attributes of a Fruit Smoothie-Type Beverage Processed with Moderate Heat and Pulsed Electric Fields.” *LWT* 43 (7): 1067–73. https://doi.org/10.1016/j.lwt.2010.02.010.

Waszkiewicz, Marta, Anna Sokół-Łętowska, Aleksandra Pałczyńska, and Alicja Z. Kucharska. 2023. “Fruit Smoothies Enriched in a Honeysuckle Berry Extract—An Innovative Product with Health-Promoting Properties.” *Foods* 12 (19). https://doi.org/10.3390/foods12193667.

Wootton-Beard, Peter C., Aisling Moran, and Lisa Ryan. 2011. “Stability of the Total Antioxidant Capacity and Total Polyphenol Content of 23 Commercially Available Vegetable Juices before and after in Vitro Digestion Measured by FRAP, DPPH, ABTS and Folin-Ciocalteu Methods.” *Food Research International* 44 (1): 217–24. https://doi.org/10.1016/j.foodres.2010.10.033.

Yi, Tian, Wei Fang, Xiaoqing Xie, Bo Yuan, Mei Lu, and Changmou Xu. 2022. “High Pressure Processing (HPP) Improved Safety and Quality of Emerging Aronia Berry Juice: A Pilot Scale Shelf-Life Study.” *Journal of Food Science and Technology* 59 (2): 755–67. https://doi.org/10.1007/s13197-021-05070-z.

Yuan, Bo, Mary Grace C. Danao, Jayne E. Stratton, Steven A. Weier, Curtis L. Weller, and Mei Lu. 2018. “High Pressure Processing (HPP) of Aronia Berry Purée: Effects on Physicochemical Properties, Microbial Counts, Bioactive Compounds, and Antioxidant Capacities.” *Innovative Food Science and Emerging Technologies* 47 (June):249–55. https://doi.org/10.1016/j.ifset.2018.03.009.

Zhang, Yiwen, Yingchun Zhao, Xinglong Liu, Xueyan Chen, Chuanbo Ding, Ling Dong, Jinping Zhang, et al. 2021. “Chokeberry (Aronia Melanocarpa) as a New Functional Food Relationship with Health: An Overview.” *Journal of Future Foods*. Beijing Academy of Food Sciences. https://doi.org/10.1016/j.jfutfo.2022.01.006.

Zhao, Yingchun, Xinglong Liu, Chuanbo Ding, Yinan Zheng, Hongyan Zhu, Zhiqiang Cheng, Chunli Zhao, and Wencong Liu. 2022. “Aronia Melanocarpa Polysaccharide Ameliorates Liver Fibrosis through TGF-B1-Mediated the Activation of PI3K/AKT Pathway and Modulating Gut Microbiota.” *Journal of Pharmacological Sciences* 150 (4): 289–300. https://doi.org/10.1016/j.jphs.2022.10.001.

APPENDICES

APPENDIX A. %PROTEIN CONTENT RESULTS

Table A. %Protein Values for Aronia powder mix and Aronia smoothie food products.

	Aronia Powder Mix	Aronia Smoothie
% Protein	23.67 ± 3.40[A]	3.74 ± 0.06[B]

* If the same columns are represented by different letters, there is a significant difference for $P < 0.05$.

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0,05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Factor	2	Aronia Powder Mix; Aronia Smoothie

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Factor	1	595,81	595,807	68,72	0,001
Error	4	34,68	8,669		
Total	5	630,49			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
2,94440	94,50%	93,12%	87,62%

Means

Factor	N	Mean	StDev	95% CI
Aronia Powder Mix	3	23,67	4,16	(18,95; 28,39)
Aronia Smoothie	3	3,7367	0,0751	(-0,9831; 8,4565)

Pooled StDev = 2,94440

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Factor	N	Mean	Grouping	
Aronia Powder Mix	3	23,67	A	
Aronia Smoothie	3	3,7367		B

Means that do not share a letter are significantly different.

Figure A. One-way ANOVA & Tukey comparisons result for protein content in Aronia powder mix and Aronia smoothie products.

APPENDIX B. %MOISTURE CONTENT RESULTS FOR ARONIA POWDER MIX

Table B. %Moisture content in Aronia powder mix throughout the shelf-life.

	April (0th Day)	September	October	November	December	January	May
%Moisture	1.20 ± 0.06^{C}	1.28 ± 0.02^{C}	1.32 ± 0.02^{BC}	1.45 ± 0.05^{AB}	1.50 ± 0.03^{A}	1.48 ± 0.01^{A}	1.55 ± 0.05^{A}

* If the same columns are represented by different letters, there is a significant difference for $P < 0.05$.

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0,05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Factor	7	April (0th Day); September; October; November; December; January; May

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Factor	6	0,30916	0,051527	20,93	0,000
Error	14	0,03447	0,002462		
Total	20	0,34363			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0,0496176	89,97%	85,67%	77,43%

Means

Factor	N	Mean	StDev	95% CI
April (0th Day)	3	1,1967	0,0757	(1,1352; 1,2581)
September	3	1,2833	0,0306	(1,2219; 1,3448)
October	3	1,3200	0,0265	(1,2586; 1,3814)
November	3	1,4500	0,0600	(1,3886; 1,5114)
December	3	1,4967	0,0416	(1,4352; 1,5581)
January	3	1,48000	0,01000	(1,41856; 1,54144)
May	3	1,5533	0,0666	(1,4919; 1,6148)

Pooled StDev = 0,0496176

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Factor	N	Mean	Grouping		
May	3	1,5533	A		
December	3	1,4967	A		
January	3	1,48000	A		
November	3	1,4500	A	B	
October	3	1,3200		B	C
September	3	1,2833			C
April (0th Day)	3	1,1967			C

Means that do not share a letter are significantly different.

Figure B. One-way ANOVA & Tukey comparisons result for %moisture content in Aronia powder mix.

APPENDIX C. BRIX RESULTS FOR ARONIA SMOOTHIE

Table C. Brix values for Aronia smoothie throughout the shelf-life.

	0th Day	**7th Day**	**14th Day**	**21st Day**
Brix	14.45 ± 0.03^{B}	14.41 ± 0.01^{B}	14.47 ± 0.04^{B}	14.60 ± 0.02^{A}

* If the same columns are represented by different letters, there is a significant difference for $P < 0.05$.

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0,05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Factor	4	0th Day; 7th Day; 14th Day; 21st Day

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Factor	3	0,061967	0,020656	21,19	0,000
Error	8	0,007800	0,000975		
Total	11	0,069767			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0,0312250	88,82%	84,63%	74,84%

Means

Factor	N	Mean	StDev	95% CI
0th Day	3	14,4533	0,0321	(14,4118; 14,4949)
7th Day	3	14,4067	0,0115	(14,3651; 14,4482)
14th Day	3	14,4667	0,0451	(14,4251; 14,5082)
21st Day	3	14,6000	0,0265	(14,5584; 14,6416)

Pooled StDev = 0,0312250

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Factor	N	Mean	Grouping	
21st Day	3	14,6000	A	
14th Day	3	14,4667		B
0th Day	3	14,4533		B
7th Day	3	14,4067		B

Means that do not share a letter are significantly different.

Figure C. One-way ANOVA & Tukey comparisons result for Brix value of Aronia smoothie product throughout the shelf-life.

APPENDIX D. TOTAL PHENOLIC CONTENT ANALYSIS RESULTS

Table D1. Absorbance values for TPC analysis in Aronia powder mix throughout the shelf-life.

	April (Oth Month)	**Septembe r**	**October**	**Novembe r**	**December**	**January**	**May**
Absorbance (nm)	0.515 ± 0.01^{A}	0.519 ± 0.01^{A}	0.517 ± 0.01^{A}	0.521 ± 0.0^{A}	0.532 ± 0.04^{A}	0.537 ± 0.03^{A}	0.541 ± 0.02^{A}

***** If the same columns are represented by different letters, there is a significant difference for $P < 0.05$.

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0,05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Factor	7	April (Oth Month); September; October; November; December; January; May

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Factor	6	0,002029	0,000338	0,51	0,788
Error	14	0,009208	0,000658		
Total	20	0,011237			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0,0256459	18,05%	0,00%	0,00%

Means

Factor	N	Mean	StDev	95% CI
April (Oth Month)	3	0,51467	0,00723	(0,48291; 0,54642)
September	3	0,51867	0,01242	(0,48691; 0,55042)
October	3	0,51667	0,01159	(0,48491; 0,54842)
November	3	0,52100	0,00529	(0,48924; 0,55276)
December	3	0,5320	0,0461	(0,5002; 0,5638)
January	3	0,5370	0,0401	(0,5052; 0,5688)
May	3	0,5410	0,0223	(0,5092; 0,5728)

Pooled StDev = 0,0256459

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Factor	N	Mean	Grouping
May	3	0,5410	A
January	3	0,5370	A
December	3	0,5320	A
November	3	0,52100	A
September	3	0,51867	A
October	3	0,51667	A
April (Oth Month)	3	0,51467	A

Means that do not share a letter are significantly different.

Figure D1. One-way ANOVA & Tukey comparisons result for total phenolic content analysis absorbance values in Aronia powder mix product.

Table D2. Absorbance values for TPC analysis in Aronia smoothie throughout the shelf-life.

	0th Day	**7th Day**	**14th Day**	**21st Day**
Absorbance (nm)	0.372 ± 0.02^{A}	0.380 ± 0.01^{A}	0.389 ± 0.02^{A}	0.404 ± 0.01^{A}

* If the same columns are represented by different letters, there is a significant difference for $P < 0.05$.

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0,05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Factor	4	0th Day; 7th Day; 14th Day; 21st Day

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Factor	3	0,001717	0,000572	1,72	0,239
Error	8	0,002654	0,000332		
Total	11	0,004371			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0,0182140	39,28%	16,51%	0,00%

Means

Factor	N	Mean	StDev	95% CI
0th Day	3	0,3717	0,0221	(0,3474; 0,3959)
7th Day	3	0,38033	0,01380	(0,35608; 0,40458)
14th Day	3	0,3893	0,0186	(0,3651; 0,4136)
21st Day	3	0,4040	0,0173	(0,3798; 0,4282)

Pooled StDev = 0,0182140

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Factor	N	Mean	Grouping
21st Day	3	0,4040	A
14th Day	3	0,3893	A
7th Day	3	0,38033	A
0th Day	3	0,3717	A

Means that do not share a letter are significantly different.

Figure D2. One-way ANOVA & Tukey comparisons result for total phenolic content analysis absorbance values in Aronia smoothie product.

APPENDIX E. ANTIOXIDANT CONTENT RESULTS

Table E1. Absorbance values for DPPH antioxidant analysis in Aronia powder mix throughout the shelf-life.

	April (Oth Month)	**September**	**October**	**November**	**December**	**January**	**May**
Absorbance (nm)	0.167 ± 0.004^{B}	0.170 ± 0.004^{B}	0.171 ± 0.005^{AB}	0.175 ± 0.003^{AB}	0.177 ± 0.004^{AB}	0.178 ± 0.004^{AB}	0.185 ± 0.003^{A}

* If the same columns are represented by different letters, there is a significant difference for $P < 0.05$.

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0,05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Factor	7	April (Oth Month); September; October; November; December; January; May

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Factor	6	0,000664	0,000111	4,34	0,011
Error	14	0,000357	0,000026		
Total	20	0,001021			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0,0050521	65,01%	50,01%	21,27%

Means

Factor	N	Mean	StDev	95% CI
April (Oth Month)	3	0,16667	0,00473	(0,16041; 0,17292)
September	3	0,16967	0,00503	(0,16341; 0,17592)
October	3	0,17067	0,00666	(0,16441; 0,17692)
November	3	0,17467	0,00379	(0,16841; 0,18092)
December	3	0,17700	0,00529	(0,17074; 0,18326)
January	3	0,17800	0,00529	(0,17174; 0,18426)
May	3	0,18467	0,00404	(0,17841; 0,19092)

Pooled StDev = 0,00505211

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Factor	N	Mean	Grouping	
May	3	0,18467	A	
January	3	0,17800	A	B
December	3	0,17700	A	B
November	3	0,17467	A	B
October	3	0,17067	A	B
September	3	0,16967		B
April (Oth Month)	3	0,16667		B

Means that do not share a letter are significantly different.

Figure E1. One-way ANOVA & Tukey comparisons result for DPPH antioxidant analysis absorbance values in Aronia powder mix product.

Table E2. Absorbance values for DPPH antioxidant analysis in Aronia smoothie throughout the shelf-life.

	0th Day	**7th Day**	**14th Day**	**21st Day**
Absorbance (nm)	0.314 ± 0.008^{C}	0.323 ± 0.004^{C}	0.349 ± 0.007^{B}	0.398 ± 0.001^{A}

* If the same columns are represented by different letters, there is a significant difference for $P < 0.05$.

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0,05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Factor	4	0th Day; 7th Day; 14th Day; 21st Day

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Factor	3	0,013229	0,004410	96,38	0,000
Error	8	0,000366	0,000046		
Total	11	0,013595			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0,0067639	97,31%	96,30%	93,94%

Means

Factor	N	Mean	StDev	95% CI
0th Day	3	0,31133	0,00929	(0,30233; 0,32034)
7th Day	3	0,32300	0,00529	(0,31399; 0,33201)
14th Day	3	0,34933	0,00814	(0,34033; 0,35834)
21st Day	3	0,397667	0,001528	(0,388661; 0,406672)

Pooled StDev = 0,00676387

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Factor	N	Mean	Grouping		
21st Day	3	0,397667	A		
14th Day	3	0,34933		B	
7th Day	3	0,32300			C
0th Day	3	0,31133			C

Means that do not share a letter are significantly different.

Figure E2. One-way ANOVA & Tukey comparisons result for DPPH antioxidant analysis absorbance values in Aronia smoothie product.

Table E3. Absorbance values for ABTS antioxidant analysis in Aronia powder mix throughout the shelf-life.

	April (0th Month)	**September**	**October**	**November**	**December**	**January**	**May**
Absorbance (nm)	0.213 ± 0.004^{A}	0.209 ± 0.008^{AB}	0.208 ± 0.003^{AB}	0.205 ± 0.0^{AB}	0.203 ± 0.005^{AB}	0.199 ± 0.003^{AB}	0.195 ± 0.003^{B}

* If the same columns are represented by different letters, there is a significant difference for $P < 0.05$.

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0,05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Factor	7	April (Oth Month); September; October; November; December; January; May

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Factor	6	0,000697	0,000116	4,16	0,013
Error	14	0,000391	0,000028		
Total	20	0,001087			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0,0052825	64,07%	48,67%	19,15%

Means

Factor	N	Mean	StDev	95% CI
April (Oth Month)	3	0,21300	0,00529	(0,20646; 0,21954)
September	3	0,20933	0,00929	(0,20279; 0,21587)
October	3	0,20767	0,00416	(0,20113; 0,21421)
November	3	0,204667	0,000577	(0,198125; 0,211208)
December	3	0,20300	0,00608	(0,19646; 0,20954)
January	3	0,19867	0,00321	(0,19213; 0,20521)
May	3	0,19500	0,00400	(0,18846; 0,20154)

Pooled StDev = 0,00528250

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Factor	N	Mean	Grouping	
April (Oth Month)	3	0,21300	A	
September	3	0,20933	A	B
October	3	0,20767	A	B
November	3	0,204667	A	B
December	3	0,20300	A	B
January	3	0,19867	A	B
May	3	0,19500		B

Means that do not share a letter are significantly different.

Figure E3. One-way ANOVA & Tukey comparisons result for ABTS antioxidant analysis absorbance values in Aronia powder mix product.

Table E4. Absorbance values for ABTS antioxidant analysis in Aronia smoothie throughout the shelf-life.

	0th Day	**7th Day**	**14th Day**	**21st Day**
Absorbance (nm)	0.147 ± 0.004^{A}	0.123 ± 0.003^{B}	0.086 ± 0.005^{C}	0.085 ± 0.003^{C}

* If the same columns are represented by different letters, there is a significant difference for $P < 0.05$.

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0,05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Factor	4	Oth Day; 7th Day; 14th Day; 21st Day

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Factor	3	0,008194	0,002731	129,03	0,000
Error	8	0,000169	0,000021		
Total	11	0,008363			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0,0046007	97,98%	97,22%	95,44%

Means

Factor	N	Mean	StDev	95% CI
Oth Day	3	0,14700	0,00529	(0,14087; 0,15313)
7th Day	3	0,12333	0,00321	(0,11721; 0,12946)
14th Day	3	0,08633	0,00586	(0,08021; 0,09246)
21st Day	3	0,08500	0,00346	(0,07887; 0,09113)

Pooled StDev = 0,00460072

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Factor	N	Mean	Grouping		
Oth Day	3	0,14700	A		
7th Day	3	0,12333		B	
14th Day	3	0,08633			C
21st Day	3	0,08500			C

Means that do not share a letter are significantly different.

Figure E4. One-way ANOVA & Tukey comparisons result for ABTS antioxidant analysis absorbance values in Aronia smoothie product.

APPENDIX F. TPC, ABTS & DPPH VALUES AFTER *IN VİTRO* DIGESTION

Table F1. TPC absorbance values for protein powder, Aronia melanocarpa and Aronia plus protein powder samples after in vitro digestion.

	PP	**A**	**A+PP**
Absorbance (nm)	0.107 ± 0.004^{C}	0.218 ± 0.005^{B}	0.350 ± 0.003^{A}

* If the same columns are represented by different letters, there is a significant difference for $P < 0.05$.

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0,05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Factor	3	PP; A; A+PP

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Factor	2	0,089281	0,044640	1620,01	0,000
Error	6	0,000165	0,000028		
Total	8	0,089446			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0,0052493	99,82%	99,75%	99,58%

Means

Factor	N	Mean	StDev	95% CI
PP	3	0,10667	0,00473	(0,09925; 0,11408)
A	3	0,21800	0,00656	(0,21058; 0,22542)
A+PP	3	0,35033	0,00416	(0,34292; 0,35775)

Pooled StDev = 0,00524934

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Factor	N	Mean	Grouping		
A+PP	3	0,35033	A		
A	3	0,21800		B	
PP	3	0,10667			C

Means that do not share a letter are significantly different.

Figure F1. One-way ANOVA & Tukey comparisons result about TPC absorbance values for protein powder, *Aronia melanocarpa* and Aronia plus protein powder samples after *in vitro* digestion.

Table F2. DPPH antioxidant absorbance values for protein powder, Aronia melanocarpa and Aronia plus protein powder samples after in vitro digestion.

	PP	**A**	**A+PP**
Absorbance (nm)	0.314 ± 0.005^{C}	0.556 ± 0.007^{A}	0.339 ± 0.006^{B}

***** If the same columns are represented by different letters, there is a significant difference for $P < 0.05$.

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0,05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Factor	3	PP; A; A+PP

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Factor	2	0,105972	0,052986	925,97	0,000
Error	6	0,000343	0,000057		
Total	8	0,106316			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0,0075645	99,68%	99,57%	99,27%

Means

Factor	N	Mean	StDev	95% CI
PP	3	0,31433	0,00643	(0,30365; 0,32502)
A	3	0,55600	0,00872	(0,54531; 0,56669)
A+PP	3	0,33933	0,00737	(0,32865; 0,35002)

Pooled StDev = 0,00756454

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Factor	N	Mean	Grouping		
A	3	0,55600	A		
A+PP	3	0,33933		B	
PP	3	0,31433			C

Means that do not share a letter are significantly different.

Figure F2. One-way ANOVA & Tukey comparisons result about DPPH antioxidant absorbance values for protein powder, *Aronia melanocarpa* and Aronia plus protein powder samples after *in vitro* digestion.

Table F3. ABTS antioxidant absorbance values for protein powder, Aronia melanocarpa and Aronia plus protein powder samples after in vitro digestion.

	PP	**A**	**A+PP**
Absorbance (nm)	0.275 ± 0.002^{A}	0.052 ± 0.004^{C}	0.220 ± 0.002^{B}

***** If the same columns are represented by different letters, there is a significant difference for $P < 0.05$.

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0,05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Factor	3	PP; A; A+PP

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Factor	2	0,080793	0,040396	3748,12	0,000
Error	6	0,000065	0,000011		
Total	8	0,080858			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0,0032830	99,92%	99,89%	99,82%

Means

Factor	N	Mean	StDev	95% CI
PP	3	0,27467	0,00252	(0,27003; 0,27930)
A	3	0,05200	0,00436	(0,04736; 0,05664)
A+PP	3	0,22000	0,00265	(0,21536; 0,22464)

Pooled StDev = 0,00328295

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Factor	N	Mean	Grouping		
PP	3	0,27467	A		
A+PP	3	0,22000		B	
A	3	0,05200			C

Means that do not share a letter are significantly different.

Figure F3. One-way ANOVA & Tukey comparisons result about ABTS antioxidant absorbance values for protein powder, *Aronia melanocarpa* and Aronia plus protein powder samples after *in vitro* digestion.

APPENDIX G. GALLIC ACID STANDARD CURVE FOR TPC ANALYSIS

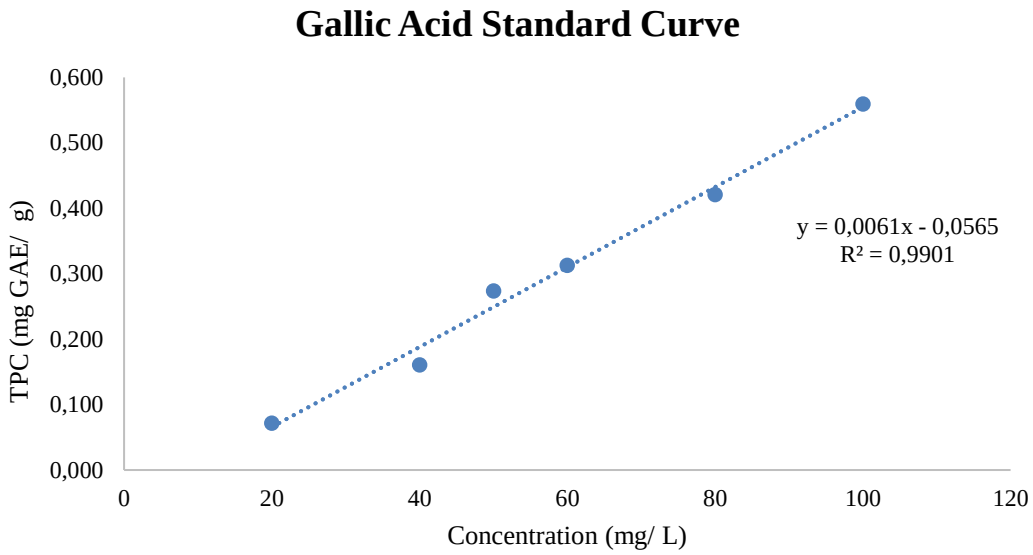

Figure G. Gallic acid standard curve.

APPENDIX H. TROLOX STANDARD CURVE FOR ANTIOXIDANT ANALYSIS

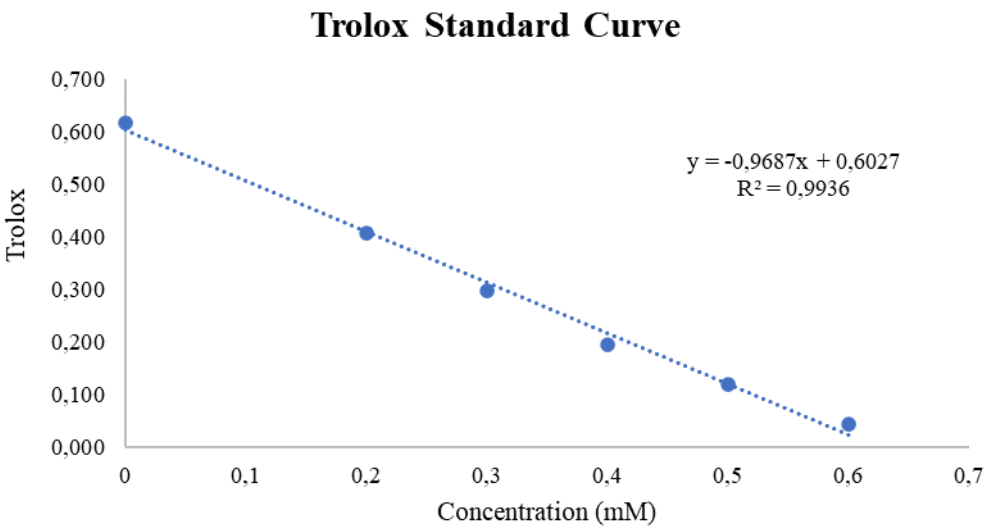

Figure H1. Trolox standard curve for DPPH antioxidant analysis.

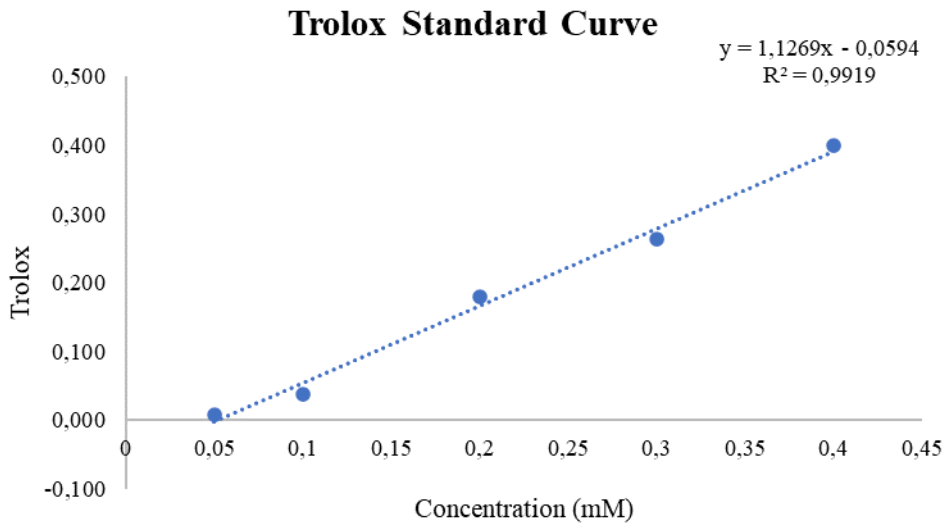

Figure H2. Trolox standard curve for ABTS antioxidant analysis.