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Review article

Bioactivity of Aronia Products, and The Promising Use of Aronia in Dairy Industry

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Abstract

In recent years, foods are not only valued in terms of taste and nutritional value, but also preferred in terms of post-consumption health effects. In particular, the trend towards functional foods that offer health benefits beyond their nutritional value to consumers has increased. Dairy products have a very important place among functional foods, and fruit-added dairy products like fermented milk, kefir and yogurt etc. lead the way due to the consumption preferences. Aronia (*Aronia melanocarpa*) is a forest fruit originating from North America, but it is grown in many parts of Europe lately. The fruit is very rich in proanthocyanins, anthocyanins, procyanidin, phenolic acids, flavonols and flavanones. It is known as the fruit with the highest antioxidant activity, and shows antidiabetic, anti-inflammatory, antiviral, antibacterial, hypotensive, cardioprotective, hepatoprotective and anticarcinogenic properties. The fruit is mostly used as ingredient in wine, marmalade, fruit juice, tea, extract and as dietary supplement or food colorant. The health-promoting effects of aronia due to its bioactivity are enhanced with the functional dairy products, and lately aronia-added yogurt and kefir are industrially manufactured and marketed for dairy consumers. This review focuses on the health effects of aronia and the promising use of the fruit in dairy products.

Keywords: Aronia, Bioactivity, Antioxidant, Yogurt, Kefir.

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INTRODUCTION

During the last few decades, dietary preferences of consumers have changed in terms of nutritional, sensory and functional characteristics of food. The demand for functional foods offering health benefits has increased. Functional foods are defined as food products that have positive impact on health, state of mind, or physical performance beyond their nutritional value (Rincón-León, 2003). They are consumed for the regulation of the immune system, control of mental and physical disorders, prevention of and recovery from specific diseases, and slowing down the aging process. They are natural or processed foods enriched with biologically active compounds (Terpeu et al., 2019) with antimicrobial, antioxidative, antihypertensive, antidiabetic, immunomodulator, antithrombotic, anticarcinogenic and anti-inflammatory activities. In this way, daily intake of functional foods seems to be the easiest and the most economic way of sustainable well-being.

Among functional foods, fruit-added dairy products lead the way due to consumption preferences and improved bioactivity. Especially berries such as blackberry, raspberry, black currant, blueberry and elderberry are delicious, low-energy, rich in fiber and phenolic compounds (Ozdemir & Ozkan, 2020), and included in formulations of dairy products like fermented milks, kefir and yogurt. Aronia (*Aronia melanocarpa*), also named as chokeberry, belongs to the *Rosacea* family. It is a forest fruit originating from North America where it has been used for the treatment of cold by native Americans (Tolić, Jurčević, Krbavčić, Marković & Vahčić, 2015), and has been cultivated in Europe and Russia since 1900s. It is a 2-3 m tall shrub, flowering in clusters in May to June and ripened berries (6-13 mm, 0.5-2.0 g) turn to black to be harvested between August and September (Kulling & Rawel, 2008). The most popular cultivars are Nero and Viking. It has an increasing popularity due to its resistance to mechanical harvesting, cold storage, frost and transportation (Bakir, 2019). The berries are commonly used for syrup, fruit juice, marmalade, jam, extract, wine and tea production, and has also been used as herbal medicine in Russia and East Europe countries for its antihypertensive and anti-artherosclerotic effects (Kokotkiewicz, Jaremicz & Luczkiewicz, 2010). However, utilization of aronia as a food or ingredient is challenging because of its sensory properties (Laaksonen, Knaapila, Niva, Deegan & Sandell, 2016).

There has been growing interest in aronia supported by scientific research, in the last two decades especially. Several articles examined the composition, beneficial health effects and clinical trials of the fruit.

COMPOSITION OF ARONIA BERRIES

The composition of the berries is reported as 15.6-28.8% dry matter, 4.4-5.8 g ash/kg, 6.2-10.8% sugar, 0.14% fat, 0.7-6.0% protein, and 5.62% fiber (Tanaka & Tanaka, 2001; Kulling & Rawel, 2008; Sójka, Kołodziejczyk & Milala, 2013). The main fatty acids are linoleic acid (47.8-57.2%), oleic acid (26.4-28.4%) and palmitic acid (11.0-15.5%) (Tanaka & Tanaka, 2001; Merdzhanov et al, 2013; Bakir, 2019). Relatively high amounts of potassium and zinc, and 25-90 µg vitamin B_1, 25–110 µg vitamin B_2, 30-85 µg vitamin B_6, 5-100 mg vitamin C, 50-380 µg pantothenic acid ve 100–550 µg niacin is determined in 100 mL fresh aronia juice. Fresh berries and pulps are good sources of dietary fiber containing cellulose, hemicellulose and lignin (Borycka & Stachowiak, 2008). Mainly defined organic acids are malic, citric and quinic acids (Bakir, 2019), and berries are characterized with relatively low acidity with a pH 3.3-3.9. The bitter almond flavor of the fruit comes from amygdalin, which is 5.8 $mg\cdot100g^{-1}$ in fresh aronia juice (Kulling & Rawel, 2008), and 7-185 $mg\cdot100g^{-1}$ in pomace mostly in seed fractions (Sójka, Kołodziejczyk & Milala, 2013). Amygdalin is a cyanogenic compound perceived as potentially harmful, because of the formation of poisonous hydrogen cyanide when it is decomposed. Therefore, the elimination of seeds in dietary applications and thus controlling the amount of amygdalin in the product should be taken into consideration (Sójka, Kołodziejczyk & Milala, 2013). Benzaldehyde, benzaldehyde cyanohydrin and hydrocyanic acid are the main volatile compounds (Hirvi & Honkanen, 1985). Benzaldehyde (52.3-2972.9 $\mu g\cdot kg^{-1}$), hexanal (56.6-412.8 $\mu g\cdot kg^{-1}$), butane-1-ol (18.5-1445.6 $\mu g\cdot kg^{-1}$), *cis*-3-hexenol (22.5-166.0 $\mu g\cdot kg^{-1}$), ethanol (20.2-322.2 $\mu g\cdot kg^{-1}$), phenylmethanol (425.4-1114.7 $\mu g\cdot kg^{-1}$), hexan-1-ol (59.1-694.1 $\mu g\cdot kg^{-1}$), methanol (17.5-139.7 $\mu g\cdot kg^{-1}$), pentan-1-ol (10.9-48.7 $\mu g\cdot kg^{-1}$) and ethyl-ethanoate (10.2-283.0 $\mu g\cdot kg^{-1}$) were reported in different cultivars of aronia (Butorová, Vítová & Polovka, 2016).

BIOACTIVITY OF ARONIA AND PRODUCTS

Aronia extracts are marketed as dietary supplements for the curement of hypertension, stress, diabetes and cancer for a while. Many studies have shown potent protective and beneficial effects of aronia fruit and extract, pulp, juice, powder, tea, yogurt, kefir and fermented milk on human health based on their antioxidant, anti-inflammatory, antidiabetic, anticarcinogenic, antimutagenic, antibacterial, hypolipidemic, cardioprotective and hepatoprotective properties (Table 1). Among these properties, total phenolic content (TPC) and antioxidant activity of aronia is considered to be outstanding.

Phenolic Content of Aronia and Related Antioxidant Activity

As a result of the body's energy metabolism or due to various environmental factors (radiation, smoking, various drugs, and environmental pollution), free radicals (reactive oxygen and nitrogen species, ROS and RNS) can be produced. When they are over-produced or not eliminated, oxidative stress and as a result severe chronic and degenerative diseases such as cancer, autoimmune diseases, aging, cardiovascular disorders, Alzheimer's disease and Parkinson's disease occur (Davies, 2000; Sayre, Perry & Smith, 2008). Antioxidants like phenolic compounds is significant in oxidative stability of foods and in the prevention of such diseases. The term antioxidant is defined as any substance that considerably prevents oxidation of a substrate which is easily oxidized, particularly in low amounts compared to the substrate (Halliwell & Gutteridge, 2015). An antioxidant is acting as preventive or chain-breaking. The preventive ones act by blocking the formation of ROS and RNS, and the scavenging ones remove ROS and RNS quickly before they bind to biologically essential molecules. Phenolic compounds have strongly scavenge free radicals and oxidants (Denev, Kratchanov, Čížž, Lojek, & Kratchanova, 2012), and they also block the pro-oxidative enzymes (Bräunlich et al., 2013) and increase antioxidative enzyme activity (Francik et al., 2014).

Aronia berries gain attention by strong antioxidative activities due to their high content of phenolic compounds (Jakobek et al., 2007; Kapci, 2013; Tolić et al., 2015; Du & Myracle, 2018). Phenolic compounds like proanthocyanins, anthocyanins, flavonols, flavanols and phenolic acids, are the most prominent components of aronia berries (Kähkönen et al., 2001; Slimestad et al., 2005; Kulling & Rawel, 2008; Denev et al., 2012; Bakir, 2019; Rodríguez-Werner et al., 2019). Total phenolic compounds (TPC) of the berries has been reported to be more than 20 mg gallic acid equivalents (GAE)·g-1 (Kähkönen et al., 2001), and 1494 mg GAE·100 g-1 for aronia tea (Tolić et al., 2015). TPC of the aronia fruit, powder and nutraceutical syrup was found to be 1294.86-3946.49 mg GAE·100 g-1 and 50.23-78.92 mg GAE·100 g-1 for aronia tea brewed for 3 to 15 min. (Bakir, 2019). Proanthocyanidins (condensed tannins) cause the astringent taste of the berries with a drying and contracting mouthfeel (Bajec & Pickering, 2008). The proanthocyanidin level is determined approximately 8192 mg·100 g-1 (Oszmiański & Wojdylo, 2005), found more in pulp than fruit juice. Proanthocyanidins are becoming the focus of attention in nutrition and food industry due to their potent antioxidant activity and possible preventive health effects (Santos-Buelga &Scalbert, 2000). Procyanidins (PCs), a sub class of proanthocyanins, were identified as the main phenolic compounds in aronia (Oszmiański & Wojdylo, 2005). PCs are in general oligomeric and polymeric epicatechins (Kulling & Rawel 2008, Denev et al., 2012). Almost half of the antioxidant activity of aronia is based on PCs (Malik et al., 2003). The antioxidative effects of PCs may lessen the incidence of cancer (Bagchi et al., 2000), and cardiovascular diseases (Reed, 2002).

Table 1. Studies regarding bioactivity of aronia and products

Studied aronia product	Bioactivity	Reference
Berry and extract	Antioxidant	Kähkönen, Hopia & Heinonen, 2001; Wu, Gu, Prior & McKay, 2004; Osmiańzski & Wojdylo, 2005; Jakobek, Šeruga & Krivak, 2011; Kapci, 2013; Tolić et al., 2015; Szopa et al. 2017; Dąbrowska et al., 2019; Denev, Číž, Kratchanova & Blazheva, 2019; Rudic et al., 2022
	Antibacterial	Bräunlich et al., 2013; Denev et al., 2019; Daoutidou, Plessas, Alexopoulos & Mantzourani, 2021; Deng et al., 2021
	Anti-inflammatory	Ohgami et al., 2005; Martin et al., 2014; Kang et al., 2017; Lee et al., 2018; Jang, Lee, Choi & Yim, 2020; Ali et al., 2021; Li et al., 2022
	Antidiabetic	Bhaswant, Shafie, Mathai, Mouatt & Brown, 2017; Jeon et al., 2018; Jeong & Kim, 2019; Chen, Zhu & Meng, 2020; Mu et al., 2020
	Hypolipidemic	Xie et al., 2017; Kim et al., 2018; Jeong & Kim, 2019; Tasic et al., 2021
	Cardioprotective	Bell & Gochenaur, 2006; Naruszewicz, Laniewska, Millo & Dłuzniewski, 2007; Olas et al., 2008; Ćujić et al., 2018; Istas et al., 2019
	Anticarcinogenic	Malik et al., 2003; Lala et al., 2006; Kędzierska et al., 2013; Choi et al., 2018a; Thi & Hwang, 2018; Bakir, 2019; Wei, Yu, Hao, Fan, & Gao, 2020, Gill et al., 2021; Yu et al., 2021
	Antimutagenic	Gasiorowski et al., 1997
	Immunomodulatory	Gajic et al., 2020; Ali et al., 2021; Bushmeleva et al., 2021
Pulp	Antioxidant	Oszmiański & Wojdylo, 2005; Rodríguez-Werner, Winterhalter & Esatbeyoglu, 2019
	Antibacterial	Tamkutė et al., 2021
Juice	Antioxidant	Oszmiański & Wojdylo, 2005; Kardum et al., 2014a; Kardum et al., 2014b; Skarpańska-Stejnborn, Basta, Sadowska & Pilaczyńska-Szcześniak, 2014; Bontsidis et al., 2021; Yaneva, Dinkova, Gotcheva & Angelov, 2021
	Antidiabetic	Milutinović et al., 2019
	Anti-inflammatory	Handeland, Grude, Torp, & Slimestad, 2014; Valcheva-Kuzmanova, Kuzmanov, Kuzmanova & Tzaneva, 2018
	Hypolipidemic	Simeonov et al., 2002; Skoczyñska et al., 2007; Daskalova, Delchev, Vladimirova-Kitova, Kitov & Denev, 2021
	Cardioprotective	Stojković et al., 2021
	Hepatoprotective	Valcheva-Kuzmanova, Borisova, Galunska, Krasnaliev & Belcheva, 2004
	Anticarcinogenic	Bermúdez-Soto et al., 2007; Choi et al., 2018b
	Immunomodulatury	Stojković et al., 2020
Powder	Antioxidant	Bakir, 2019; Song, Park & Kim, 2019
Tea	Antioxidant	Tolić et al., 2015; Bakir, 2019
Yogurt	Antioxidant	Catalkaya, 2015; Nguyen & Hwang, 2016; Cuşmenco & Bulgaru, 2020; Dimitrellou, Solomakou, Kokkinomagoulos & Kandylis, 2020
Kefir	Antioxidant	Du & Myracle, 2018
Fermented milk	Antioxidant	Szajnar, Pawlos & Znamirowska, 2021

Aronia is one of the richest sources of anthocyanins (357-1790 mg 100 g^{-1}), mainly cyanidin 3-O-galactoside (C3G), 3-O-glucoside, cyanidin 3-O-arabinoside and cyanidin 3-O-oxyloside, which are the reason of the purple and blue color of the berries (Benvenuti et al., 2004; Jakobek et al., 2007; Denev et al., 2012; Markkinen et al., 2019). They are considered safe, since they have been consumed in fruits and vegetables for centuries without any health risk. Anthocyanins in aronia represent nearly 25% of TPC (Osmiańszki & Wojdylo, 2005). Cyanidin 3-arabinoside has the strongest antioxidant activity, and it is a strong pro-oxidative enzyme inhibitor (Bräunlich et al., 2013). *In vivo* experiments show that anthocyanins of aronia reduce lipid peroxidation (Faff & Frankiewicz-Jóźko, 2003; Kowalczyk et al., 2004). Aronia berries have significantly higher anthocyanin and phenolic content than other berries like blueberry, cranberry, raspberry and lingonberry (Kähkönen et al., 2001; Kapci, 2013; Bakir, 2019). The anthocyanin level of the fruit, pulp and fruit juices are between 880-1970 mg 100 g^{-1} (Osmiańzski & Wojdylo, 2005). In another study, the anthocyanin levels of aronia tea and neutraceutical syrup was determined 21.28-1658.81 mg Cyanidin Equivalents (CyE)·100 g^{-1} (Bakir, 2019).

Two phenolic acids, chlorogenic and neochlorogenic acids are also noticeable components of the berries, which have antioxidant, antimutagenic and anticarcinogenic activities (Tolić et al., 2015; Bakir, 2019). Aronia fruit, powder, neutraceutical syrup and tea include 90.28-493.20 mg chlorogenic acid·100 g^{-1} and 42.55-274.34 mg neochlorogenic acid·100 g^{-1} (Oszmiański & Wojdylo, 2005; Bakir, 2019). In addition, 307.20- 634.57 mg quercetin·100 g^{-1}, 70.13-182.46 mg quercetin-3-O-glucoside·100 g^{-1}, 48.93-143.92 mg kaempferol-3-rutinoside·100 g^{-1} and 38.45-63.56 mg kaempferol-3-O-glucoside·100 g^{-1} has been determined as major flavanols and glycosides. Quercetin is a strong antioxidant and ensures the oxidative stability of the related products (Bakir, 2019). Epicathecin with antioxidant activity is also found in aronia berries (47-84 mg·100 g^{-1}, Rop et al., 2010). Chlorogenic acid, quercetin and catechin in aronia products were found to be highly effective anticarcinogens too (Bakir, 2019). Furthermore, minerals like selenium, zinc, and copper, β-carotene, the vitamins E and C content of the aronia berries contribute to the antioxidative activity.

In vitro experiments of aronia products indicates very significant antioxidant activity. Aronia fruit has the highest antioxidant capacity measured with ORAC, among berries and other fruits (Wu et al., 2004). In a research, DPPH radical inhibition levels were determined between 96,55 and 291,34 μmol Trolox Equivalents (TE)·100 g^{-1}, for aronia tea, fruit and neutraceutical syrups (Bakir, 2019). The total antioxidant capacity (TAC, 12.09-191.31 mmol TE·100 g^{-1}) and reducing power (RP, 13.50-79.86 mmol TE·100 g^{-1}) of different aronia products (dried berries, aronia tea and powder) possessed high antioxidant capacity (Tolić et al., 2015). In another study, the antioxidant activity of aronia products was determined 279.38 and 439.49 μM Trolox·100 g^{-1} fresh fruit, 127.45 and 314.05 μM Trolox·100 g^{-1} aronia juice, and 301.89 and 779.58 μM Trolox·100 g^{-1} pulp, for DPPH and ABTS radicals respectively (Oszmiański & Wojdylo, 2005). The antioxidant activity of dried aronia extracts were found to be higher from other fruit preparations (Banach et al., 2020). Also, lipid peroxidation inhibition and anti-inflammatory activities were investigated by lipopolysaccharide (LPS)-stimulated RAW 264 cells. It was found that dried aronia extract decreased the amount of inflammation and lipid peroxidation markers *in vitro*. Moreover, it was approved that extracts did not display any cytotoxic effect.

Especially *in vivo* antioxidant activity of aronia berries that correlates with its high phenolic content is remarkable (Wu et al., 2004; Kulling & Rawel, 2008). Studies conducted on both healthy subjects and those suffering from non-infectious diseases have confirmed the antioxidant activity. Daily consumption of 150 mL aronia juice for 8 weeks increased the antioxidative capacity of the plasma (Skarpańska-Stejnborn et al., 2014). Twenty-nine healthy female subjects were served aronia juice for 12 weeks, and thiobarbituric acid-reactive substances values decreased (Kardum et al., 2014a). It is reported that supplementation of 100 mL of aronia juice 3 times a day for 90 days to 25 healthy women, enhanced the antioxidative enzyme activities in the red blood cells (Kardum et al., 2014b). Another research expressed that cyanidin derivatives of aronia are important antioxidants of DPPH radical, prevent gastric mucosal damage and have antiulcerative activity on rats (Matsumoto et al., 2004). Aronia extract improved the enzymatic antioxidant barrier of the Winstar rats exposed to cadmium, and prevented oxidative damage (Dąbrowska et al., 2019). And in a few studies, it was revealed that aronia anthocyanins decrease lipid peroxidation and enhance the antioxidant defence system enzyme activity (Faff & Frankiewicz-Jóźko, 2003; Kowalczyk et al., 2004).

Antibacterial and Anti-Inflammatory Activities of Aronia

Aronia and products have strong antibacterial and anti-inflammatory activities. Aronia berries exhibit antibacterial activity against *Escherichia coli* (*E. coli*), *Bacillus cereus*, *Staphylococcus aureus* (*S. aureus*) and *Pseudomonas aeruginosa* (Bräunlich et al., 2013; Liepina et al., 2013). Aronia extracts showed bacteriostatic activity against *E. coli* and *S. aureus* (Kim & Shin, 2020). Another study investigated the antibacterial effects of aronia extract, and effective results against *S. aureus, E. coli* and *Streptococcus pyogenes* have been obtained (Daoutidou et al., 2021). Also aronia anthocyanins possessed strong antibacterial effect against *E. coli* (Deng et al., 2021).

Consumption of aronia is also effective on the bacteria responsible for urinary tract infections (Handeland et al., 2014). Two groups of people suffering from urinary tract infections were served aronia juice for 3 months. Antibacterial activity determined increased with a dose-dependent manner, and the incidence of infection decreased by 55% at 156 mL juice serving. Also, antibiotic intake decreased in parallel. Aronia extract supplementation to mice with dextran sulfate sodium-induced colitis, inhibited prostoglandin E2 production and decreased the interleukin-6 (IL-6), nitric oxide (NO) and tumor necrosis factor (TNF) levels (Kang et al., 2017). In another research, inflammation in lipopolysachharide-induced uveitis has been down-regulated (Ohgami et al., 2005). Aronia extract inhibited IL-6 and up-regulated interleukin-10 formation in mouse spleens (Martin et al., 2014) and decreased IL-6, NO and TNF formation in macrophages and microglia (Lee et al., 2018). These anti-inflammatory effects were based on quercetin and cyanidin-3-arabinoside (Martin et al., 2014). Fermented aronia fruit extract fortified with γ-aminobutyric acid (GABA) including bioactive compounds exerted anti-inflammatory effects on female BALB/c mice (Ali et al., 2021). C3G was extracted from aronia and applied to mice which were exposed to PM10 (particules below 10 μM) for the investigation of anti-inflammatory activity (Cui et al., 2021). Administration of C3G was found to help the treatment of inflammation in mice with PM10-induced pulmonary. As a result of these studies, it can be judged that aronia may be beneficial for preventing and treating bacteria induced immunological disorders.

Anticarcinogenic, Antimutagenic and Immunomodulatory Activities

In recent years, several gene, vaccines, hormonal, biological, targeted therapies are developed besides the usual surgical methods, radiotherapy and chemotherapy for the cure of cancer. Antioxidative herbal supplements have gained importance besides chemotherapeutic drugs. Based on *in vitro* and *in vivo* studies, aronia berries and aronia extracts are found to have antiproliferative or protective effects against colon cancer. Aronia extract has been reported to induce programmed cell death for HT-29 colon cancer cells, as well as inhibit their growth (Malik et al., 2003). Aronia-fermented catechol was found to inhibit human breast cancer stem cells in a dose-dependent way (Choi et al., 2018b). Also triterpene acid with the same inhibitory effects had been isolated from methanolic extracts of aronia (Choi et al 2018a). Another study showed that aronia juice inhibited Caco-2 cell proliferation (Bermúdez-Soto et al., 2007). Compared with untreated cells, exposure of Caco-2 cells to 2% and 5% digested aronia juice is reported to result in ~40% and ~70% inhibition, respectively. This anticarcinogenic effect is verified with an animal study, where Fischer male rats were treated with azoxymethane (Lala et al., 2006). Anthocyanin-rich extract from aronia reduced the rate of colonic epithelial cell proliferation. In a study conducted on 47 aged women with breast cancer, aronia extract decreased the plasma protein modifications of the patients (Kędzierska et al., 2013).

It has also been determined that phenolic compounds isolated from aronia berries exhibit antimutagenic activity (Gasiorowski et al., 1997). Anthocyanins isolated from aronia significantly inhibited the mutagenic activity of 2-aminofluororene and benzo(a)pyrene. In an animal study where rats were served with aronia daily, cadmium toxicity and accumulation in the kidney and liver was reduced (Kowalczyk et al., 2002). Healthy mice were fed with aronia extract for 7 days, and the results implied that aronia extract stimulated pro-inflammatory properties in immunity (Gajic et al., 2020). Aronia extracts promoted rapid immune system recovery in Wistar rats, normalized the leukocyte count, and improved monocyte and neutrophil phagocytic indicators (Bushmeleva et al., 2021).

Hepatoprotective and Cardioprotective Activities

The hepatoprotective effect of aronia juice in carbon tetrachloride (CCl_4)-exposed rats has been observed, and aronia juice prevented CCl_4-induced lipid peroxidation increase in rat liver and plasma (Valcheva-Kuzmanova et al., 2004). It is reported that aronia fruit can positively affect various risk factors for cardiovascular diseases. Vasoprotective and antiplatelet effects have been observed in porcine coronary arteries (Bell & Gochenaur, 2006; Olas et al., 2008). It has been suggested that aronia can be used as a possible supplement for the prevention of ischemic heart disease in post-myocardial infarction patients (Naruszewicz et al., 2007). Spontaneously hypertensive rats (SHRs) were fed with freeze-dried aronia berries and decrease in blood pressure was obtained compared to the SHRs fed with normal diet (Yamane et al, 2017). Four week supplementation of dried aronia extract to hypertensive rats significantly reduced systolic pulse pressure compared to control group (Ćujić et al., 2018). Significant decrease of plasma and erythrocytes TBARS (thiobarbituric acid reactive substances formed as a byproduct of lipid peroxidation) was also found. Daily supplementation of aronia extracts to patients with metabolic syndrome for 4 weeks significantly lowers the systolic and diastolic blood pressure (Tasic et al, 2021).

Antidiabetic and Hypolipidemic Activities

Several studies confirm that aronia juice may be beneficial in prevention and the cure of obesity, diabetes and hypercholesterolemia. The human hepatocellular HepG2 cells and mouse myoblast cells were treated with aronia anthocyanin extracts, and the results indicated that aronia anthocynanin extract could improve insulin sensitivity (Chen et al., 2020). Male rats were exposed to high fat and carbohydrate diet supplemented with anthocyanins from aronia, for 16 weeks (Bhaswant et al., 2017). Systolic blood pressure and total body fat mass were decreased; liver and cardiovascular functions, and glucose tolerance are improved; total cholesterol and triacylglycerols were reduced compared to control. In hyperglycemic rats and ICR mice, antidiabetic effects were observed after oral administration of aronia extract (Jeon et al., 2018). Supplementation of aronia-based functional beverages to healthy rats for 3 months decreased the total cholesterol and triglycerides values, and significantly increased HDL-cholesterol levels (Daskalova et al., 2021). Twenty-one patients with non-insulin dependent diabetes were served 200 mL of aronia juice for 3 months, which affected the lipid and total cholesterol levels, and lowered fasting blood glucose levels (Simeonov et al., 2002). In men with mild hypercholesterolemia, 250 mL of daily aronia juice intake for 6 weeks resulted in an important reduction in triglyceride, LDL cholesterol and total cholesterol levels, and on the contrary increased HDL cholesterol (Skoczyñska et al., 2007). Futhermore, statistically significant decrease in serum glucose concentrations have been reported.

ARONIA BASED FOOD PRODUCTS

Fresh aronia berries are rarely preferred due to their astringent, sour and bitter taste (Duffy et al., 2016; Andrade et al., 2021), however aronia products such as wine, juice, tea, syrup, jam and dietary supplements are widely commercialized and consumed (Rodríguez-Werner et al., 2019). The berries are mixed in formulations with other fruits like apple, cranberry and pomegranate for consumer preferences.

Fermentation of fruits is a beneficial way of changing the undesirable sensory properties (Duffy et al., 2016). It is noted that good taste and flavour can be achieved with aronia fermentation (Balcerek & Szopa, 2002). Additionally, fermentation may be an efficient way of increasing the bioavailability of the phenolic compounds, since the digestibility of them is difficult (Braga et al., 2018; Du & Myracle, 2018). During fermentation, bound phenolic compounds can be released resulting in an increase in the antioxidative activity (Kwaw et al., 2018). Moreover, the anthocyanins of aronia act as prebiotics, stimulating the growth of probiotic bacteria both during fermentation and after consumption in the intestine (Braga et al., 2018; Du & Myracle, 2018; Oh et al., 2020).

A few studies have focused on aronia fruit, juice and beverage fermentation. Aronia fruit was fermented with *Lactiplantibacillus plantarum* EJ2014 by yeast extract and monosodium glutamate, at 30 °C for 9 days (Ali et al., 2021). Significant amount of GABA, minerals, polyphenols and flavonoids are formed after fermentation. Fermented aronia fruit extract treatment on RAW 264.7 cells and female BALB/c mice resulted in inhibiting proinflammatory cytokines in RAW 264.7 cells and modulating immune response in mice, underlining the use of the product as a promising functional food for immunity disorders.

The usage of potentially probiotic strains for juice fermentation may lead to an increase of antioxidant activity (Wu et al., 2020), due to the high phenolic biotransformation of *Lactobacillus* strains with various enzymatic activities such as decarboxylase (Li et al., 2021). Aronia juice was fermented with *Lactobacillus paracasei* SP5 at 30 °C for 48 h (Bontsidis et al., 2021). The antioxidant activity of the juice fermented increased significantly in all the storage periods, compared with the unfermented sample. The antioxidant acitivity and TPC of the aronia juice were enhanced by the breakdown of anthocyanins and other larger-in-size phenolic compounds through fermentation, and aromatic desirable volatiles were produced.

Different concentrations of aronia juice (5-30%) and oat beverages were subjected to lactic acid fermentation with *Laciplantibacillus plantarum* Pro to accomplish a new probiotic oat-aronia beverage with high antioxidant activity, sufficient probiotic cell viability, good texture and sensory properties (Yaneva et al., 2021). Probiotic bacteria growth was satisfactory up to 20% aronia juice. The antioxidant activity increased with aronia juice concentration, and was higher when the aronia juice was subjected to fermentation with oat beverages.

USE OF ARONIA IN DAIRY PRODUCTS

Fermented dairy products such as yogurt, kefir and fermented milk have been part of the human diet for long, and known as appropriate foods for the delivery of probiotic bacteria. Since it was well stated above that lactic acid fermentation of aronia leads to valuable functional products, using aronia as an ingredient in fermented dairy products is a promising practice increasing the nutritional and benefical properties. However, aronia addition during production may be challenging because of the acidity of the fruit, the probability of the antibacterial compounds, and the changes in sensory and textural properties. Aronia utilization may stimulate or limit the growth of the probiotic bacteria, thus all these factors should be considered in the production process.

There has been limited number of research on the use of aronia in the production of dairy products. The studies done were on yogurt, kefir, and fermented milk. For the preparation of aronia yogurt, 1-3% aronia juice and a culture consisting of *Lactobacillus acidophilus (L. acidophilus)*, *Lactobacillus casei*, *Lactobacillus rhamnosus (L. rhamnosus)* and *Lactobacillus lactis* were inoculated to milk and incubated (Nguyen & Hwang, 2016). The pH values of aronia yogurt samples were lower (4.25-4.29) compared to the control, in an aronia juice dose-dependent manner. Also, the number of lactic acid bacteria increased proportionally, and 2% and 3% aronia juice added yogurts had counts of 10.51 and 10.67 log cfu$\cdot$mL^{-1} after 12 h incubation, respectively. TPC of yogurts increased with aronia juice addition, with 16.34 mg GAE$\cdot$g^{-1} in control and 54.05 mg GAE$\cdot$g^{-1} in %3 aronia juice added yogurts. Total flavonoid contents increased in the same way, 1.2-1.3 fold (122.40-152.10 mg CE$\cdot$g^{-1}) for aronia added yogurts compared to control (117.71 mg CE$\cdot$g^{-1}). In another study, aronia juice addition during the production of goat milk yogurt decreased the time of milk coagulation (32 min. earlier) and the acidity (Boycheva et al., 2011). After two days of cold storage, aronia juice added sample's acidity slowly increased, affecting the stability of yogurt in a positive way. Aronia juice supplementation increased the number of starter culture (79.6%) due to the stimulative effect of bioactive compounds (amino acids, vitamins etc.) found in the berry. Aronia added yogurts had less syneresis than the control samples, but the difference disappeared after 24 and 48 h of storage. Also, unsaturated fatty acid amounts of the yogurt was higher in aronia juice supplemented sample. In another study, goat's milk was supplemented with 10% aronia, peach, raspberry, strawberry and apple pulp and fermented with *Lactococcus lactis* subsp. *lactis* biovar diacetylactis added yogurt culture at 37 °C for 6 h (Cușmenco & Bulgaru, 2020). The pH of the aronia yogurt (4.65) was below the control. Total number of lactic acid bacteria (7.16 log cfu$\cdot$mL^{-1}) slightly decreased compared to the control. Among all the yogurts, the highest phenolic content (187.15 mg GAE$\cdot$100 g^{-1}) and anthocyanin content (56.45 mg$\cdot$100 g^{-1}) belonged to the aronia yogurt. Set type yogurts were produced with 2-6% aronia juice concentrate, and stored for 21 days (Olika, 2022). The yogurt with 2% concentrate addition was recommended for commercializing as a fruit yogurt with functional properties, but the bioactive compounds were found to be higher in 6% concentrate added yogurt.

During the fermentation of aronia juice added yogurts, incubation time reduced up to 45 min compared to the control sample, depending on a higher acidification rate (Dimitrellou et al., 2020). Also, post-acidification was observed during storage till 14 days, resulting in similar pH values (4.36-4.39) at the end of storage at day 28. Similar results were obtained for the acidity values. These results could point out that the addition of aronia juice did not affect the fermentation process. The addition of aronia juice increased TPC of the yogurts up to 33%. During storage, a slight decrease was observed (~ 5%). This could be because of the formation of phenolic compounds-milk proteins complexes (Ozdal et al., 2013). The DPPH radical scavenging activity of the yogurts decreased during storage. The viable cell numbers of yogurt starter bacteria were higher than 10^8 cells$\cdot$g^{-1} at the end of storage, meeting the requirement of 10^7 cells$\cdot$g^{-1} (FAO/WHO, 2003). When the fruit juices were added after fermentation, a negative impact has been seen on yogurt starter bacteria due to a possible acid injury (Senaka Ranadheera et al., 2012). At the end of storage, *S. thermophilus* numbers were higher compared to the control sample. This increase in cell numbers could be attributed to phenolic compounds, which are known to advance the viability of bacteria due to their prebiotic effect.

In vitro simulated digestion of anthocyanins from aronia supplemented yogurts was investigated for bioaccessibility and antioxidant activity (Catalkaya, 2015). Aronia berries and pulp were added before or after fermentation. In aronia berry added yogurts TPC, the total anthocyanin content and the antioxidant capacity were higher than the control samples. There were no statistically significant differences between the samples either the fruit was added before or after fermentation for antioxidant capacity. The anthocyanin content was higher in berry added samples than the pulp added ones independent of storage days, but dependent on whether added before of after fermentation. Considering the DPPH radical scavenging activity of the samples, fruit addition after fermentation increased the antioxidant capacity. Yogurt samples were subjected to gastric and intestinal digestion, and higher antioxidant capacity was observed in berry added yogurts. TPC and antioxidant capacity values of the digested samples were higher than undigested ones. After the intestinal digestion, the anthocyanin bioaccessibility significantly decreased and higher amounts of anthocyanins could be recovered in fruit added yogurts.

Aronia juice was also used in kefir production (Du & Myracle, 2018). Commercial kefir culture, sweetener and 13% aronia juice were added to pasteurized milk and left at room temperature for 24 h. Sensory tests were conducted on 100 healthy panelists. Overall acceptability of aronia kefir was better for panelists that consumed kefir before. DPPH values (27.59-28.84 mg$\cdot$mL^{-1}) proved radical scavenging activity. Aronia kefir had high anthocyanin content (16.57-17.22 mg C3G$\cdot$100 g^{-1}), and TPC values were between 40.32 and 43.04 mg GAE·100 g^{-1}. The fermentation of aronia was found to be important for the bioavailability of the phenolic compounds.

Aronia fiber enriched (1.5-3.0%) sheep's milk was fermented with *L. acidophilus* and *L. rhamnosus* (Sjaznar et al., 2021). The decrease in pH value in milk before the fermentation process (with increase in aronia fiber ratio) was attributed to phenolic acids, chlorogenic and neochlorogenic acids, organic acids such as malic acid, and vitamin C content of aronia (Szopa et al., 2017). The pH differences of the fermented milk after fermentation was tought to be based on the fiber dose and the bacterial strain, because *L. acidophilus* is capable of producing more acidic metabolites. The addition of aronia fiber stimulated the growth of both types of bacteria, in a more positive way for *L. acidophilus*. Stimulatory effect of aronia fiber is possibly explained by the phenolic compounds providing energy to the bacteria (Sjaznar et al., 2021), and by the anthocyanins' prebiotic activity (Zhu et al., 2018). According to the texture analysis, samples fermented with *L. acidophilus* were determined harder than samples fermented with *L. rhamnosus* (Sjaznar et al., 2021). In addition, aronia fiber and its dose influenced the hardness of the samples remarkably. Adhesiveness of the samples were higher in *L. acidophilus* fermented and high amount of aronia fiber added milks too. There were no influence of aronia fiber and bacteria on the fermented milks' springiness and cohesiveness. According to sensory evaluation results, the samples with aronia fiber had less intense milky-creamy taste, compared to control samples. Increasing doses of aronia fiber established the sense of sour and additives' taste, and smell. Moreover, a mild off-taste was underlined by the panelists, which is attributed to aronia fiber's procyanidin content with a bitter and astringent taste.

CONCLUSION

This review outlines the bioactivities provided by aronia and products such as antioxidant, antibacterial, anti-inflammatory, anticarcinogenic, antimutagenic, hepatoprotective, cardioprotective, antidiabetic, hypolipidemic, and immunomodulatory activities, and the possible use of aronia in dairy products. *In vitro* and *in vivo* studies clearly verifies the bioactivity and the importance of aronia. Aronia has a great potential to be commercially included in dairy industry, coupled with the above mentioned advantages. Since dairy products are largely consumed and preferred due to their fresh taste and desirable flavor, aronia supplementation of them is supposed to add new products in dairy market and increase their market share. Additionally, dairy products with increased health effects will be obtained. Currently, there is no evidence of any toxic or unwanted effects of aronia berries and products in both experimental animals and human. The only limitation seems to be the astringent taste of aronia, which can be suppressed in fermented dairy products. Further research is recommended to better understand how aronia affects milk macronutrients, micronutrients, and the production process, and in which dairy products aronia can be suited and commercialized.

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