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## Review

# Fermented black chokeberry (*Aronia melanocarpa* (Michx.) Elliott) products – A systematic review on the composition and current scientific evidence of possible health benefits

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## ABSTRACT

Black chokeberry (*Aronia melanocarpa* (Michx.) Elliott) is recognized for its potential health benefits, largely attributed to its high phenolic content. However, many phenolic compounds possess a low bioavailability, potentially limiting their beneficial effects. Fermentation of chokeberry has been proposed as a method to improve bioavailability, bioactive composition, sensory qualities, and nutritional value. This systematic review provides an overview of fermented chokeberry products, including compound composition, sensory attributes, and health benefits observed in *in vivo* and *in vitro* studies. While sensory evaluations highlighted diverse flavour profiles and acceptability, human intervention studies suggested potential benefits for glucose-dependent insulinotropic peptide increase. Animal models indicated anti-obesity and immunomodulatory properties, while *in vitro* studies demonstrate antioxidant, anti-melanogenesis, and anti-diabetic effects. Despite some promising findings in human and animal trials, challenges such as participant adherence and dosing inconsistencies force further protocol improvements. Through continuous scientific research, fermented chokeberry products may emerge as functional foods contributing to human health.

## 1. Introduction

For several decades, evidence suggests a strong correlation between higher consumption of fruits and vegetables and decreased mortality rates, particularly in relation to cardiovascular diseases and cancer, which are the leading causes of globally deaths (Wang et al., 2014; WHO, 2020). Given their widespread impact across diverse populations and regions, combating these diseases necessitates global efforts in prevention, early detection, diagnosis, and treatment. Addressing associated risk factors like unhealthy diets and sedentary lifestyles is crucial for reducing disease outbreaks (Aune et al., 2017; Hartley et al., 2013). Berries, renowned for their antioxidant and anti-inflammatory properties (Basu et al., 2010; Khan et al., 2023; Muraki et al., 2013), offer additional health benefits when incorporated into the diet, further improving preventive measures against chronic diseases (Derrick et al., 2021; Kalt et al., 2020; Yang & Kortessniemi, 2015). Thus, advocating for increased consumption of fruits is crucial for promoting overall health

and well-being and reducing the risk of degenerative diseases.

Among the diverse array of berries, black chokeberry (*Aronia melanocarpa* (Michx.) Elliott) gained attention for its potential health-promoting properties (Kasprzak-Drozd et al., 2021). Originally native to the eastern regions of North America, black chokeberry is now also cultivated in various regions of Europe. Being part of the *Rosaceae* plant family, black chokeberry is a deciduous shrub that typically reaches heights of 0.5–1 m. Its leaves, characterized by fine teeth, have a medium green colour, and lack any hair, instead featuring raised glands along the midrib's top. Fruit formation begins in mid to late summer, with the pea-sized berries maturing to a purplish-black shade. These berries, classified as pomes, naturally fall from the plant post-ripening. Despite their juicy nature, they tend to shrivel after reaching full ripeness, with both their juice and seeds displaying a deep purple colour (USDA, 2009).

Like many other berries, black chokeberries contain significant levels of phenolic compounds, especially flavonoids. As anticipated, they have

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demonstrated notable antioxidant potential, a characteristics that aligns with the inherent chemical composition of phenolic compounds (Wu et al., 2004). From a biological point of view, anti-inflammatory effects were described in numerous studies (Jurendić & Ščetar, 2021; Ren et al., 2022). Additionally, black chokeberries have been associated with positive effects on blood sugar regulation, lipid metabolism, and blood pressure, being crucial factors in preventing metabolic disorders such as diabetes and obesity (Christiansen et al., 2022; Hawkins et al., 2021; Rahmani et al., 2019). Incorporating black chokeberries into the diet, whether consumed fresh, dried, or as juice, offers a convenient and delicious way to harness their potential health advantages.

However, and as already quite often shown for other fruits and vegetables, bioavailability of phenolic compounds is crucial for assessing their health benefits. Despite their abundance, various factors impact their absorption in the human body, including chemical structure and interactions with other food components during the course of digestion. Anthocyanins and (polymeric) procyanidins, both being quite prominent in chokeberries, are poorly absorbed in the small intestine, but are undergoing significant microbial transformation in the large intestine (Mattioli et al., 2020). Additionally, acylation of flavonoids with hydroxycinnamic acids limits their absorption efficiency even more (Denev et al., 2012). Overall, bioavailability of chokeberries is low.

For overcoming the challenge of a low bioavailability, fermentation emerges as a promising method to enhance the absorption of phenolic compounds (Adebo & Medina-Meza, 2020). During the fermentation process of polyphenol-rich plant substrates, microorganisms produce enzymes such as glycosidases, esterases, decarboxylases, reductases, and tannases to counteract the potential toxicity of the xenobiotic phenolic compounds (Rodríguez et al., 2009). Furthermore, selected microorganisms exploit the sugar moiety of glycosides for energy gain and cell growth (Plamada & Vodnar, 2022). These enzymes convert phenolic compounds into degradation products, some of which with a pronounced higher antioxidant capacity and bioavailability. For instance, lactic acid fermentation of cherry juice and broccoli puree resulted in the conversion of caffeic acid into the more potent antioxidant dihydrocaffeic acid. Similarly, chlorogenic acid in broccoli puree was hydrolysed into caffeic acid during fermentation, which is comparatively better absorbed in the gastrointestinal tract (Filannino et al., 2015). Fermentation of soybean flour led to the conversion of isoflavone glucosides into aglycones, which are absorbed more efficiently (Landete et al., 2015). In an indirect manner, fermentation also leads to an improved digestibility and enhanced absorption of nutrients through the metabolic conversion of the complex matrix of polysaccharides and proteins, which are mainly hydrolysed to smaller compounds (Sivapragasam et al., 2023), in parallel more easily releasing bound phenolic compounds (De Montijo-Prieto et al., 2023).

Besides compound transformation, fermented products contain different microorganisms such as *Saccharomyces* yeasts and lactic acid bacteria, some of which are probiotics, i.e. beneficial bacteria that support gut health and contribute to a balanced microbiota (Bell et al., 2018). Consuming fermented fruits and their related products regularly may help improve digestion, boost immune function, and even alleviate certain gastrointestinal disorders (Patel et al., 2023).

Fermented black chokeberry products emerge as a novel strategy to increase the content of beneficial compounds and potentially enhance their bioavailability. Incorporating fermented black chokeberry products into the diet could offer a convenient and effective means of maximizing the intake of bioactive compounds and potentially maximizing their physiological effects. In this context, the objective of this study was to conduct a systematic literature survey for getting an overview on food chemical and nutritional aspects of fermented products derived from black chokeberry or those incorporating fermented black chokeberry as a key ingredient. This systematic review sought to elucidate the composition, the potential biological activities, and health benefits associated with fermented black chokeberry products and their impact on various aspects of human health. Additionally, this review

aimed at identifying gaps in the current research and propose future directions for investigating the role of fermented black chokeberry products in promoting optimal health outcomes.

## 2. Black chokeberry (*Aronia melanocarpa* (Michx.) Elliott)

### 2.1. Composition of the raw berries

The composition and nutritional value of chokeberries are depending on factors such as variety, maturity, and ecophysiological conditions (Sidor et al., 2019). Studies reported the dry weight of chokeberry fruits, juice, and pomace, with varying percentages of soluble substances (Table 1) (Sidor & Gramza-Michałowska, 2019). Chokeberry contains carbohydrates in the form of simple sugars, with varying amounts of fructose and glucose, along with minor levels of sucrose (Šnebergrová et al., 2014). Additionally, sorbitol is present in relatively large quantities. The fruit's dietary fiber content, primarily found in the pomace, is notably high, constituting about 70 % of its dry matter. The majority of this fiber is insoluble, consisting of lignin, cellulose, and hemicelluloses (Wawer et al., 2006).

Protein content in chokeberries is low. However, they contain a variety of free amino acids, including indispensable ones like histidine, lysine, and threonine, as well as non-essential ones such as alanine, arginine, asparagine, cysteine, glutamic acid, serine, and tyrosine. Especially, the pomace is notably rich in amino acids, but the absolute amount of protein still remains relatively low (Piesza et al., 2015). While the overall lipid content in fresh chokeberry fruit is low, the seeds and skin fractions contain higher amounts, particularly in the form of triacylglycerols with unsaturated fatty acids such as linoleic and oleic acids, sterols (campesterol,  $\beta$ -sitosterol, stigmasterol, isofucosterol), and phospholipids as well (Dulf et al., 2012; Sójka et al., 2013).

Chokeberries contain various vitamins and minerals, including vitamins B, C, and K, carotenoids, and tocopherols. Additionally, they are rich in ash, comprising different essential minerals (most abundant are potassium, calcium, and phosphorus, followed by the magnesium and sodium) and trace elements (zinc, iron, selenium, copper, molybdenum, chromium). It has been also reported that chokeberries contain some toxic elements, such as lead, cadmium, mercury, arsenic. However, these are depending on the geographic origin, respectively the soil, in which the plants were grown in, and the concentrations of these metals are below the maximum permissible limits in foods (Pavlovic et al., 2015).

Black chokeberries are abundant in different phenolic compounds, including phenolic acids, anthocyanins, flavonols, and flavan-3-ols with procyanidins as the main oligomeric and polymeric derivatives of them. The levels of phenolic compounds in chokeberries vary with reported amounts as high as 37,600 mg/kg of dry mass (Hudec et al., 2006; Oszmiałski & Wojdyło, 2005). The dark blue colour of chokeberries is due to its high concentration of anthocyanins, primarily cyanidine derivatives such as cyanidine-3-O-glucoside (Cy3G), -3-O-galactoside, -3-O-xyloside, and -3-O-arabinoside (Wu et al., 2004). Flavonols in chokeberries are mainly quercetin derivatives (such as isorhamnetin, quercetin-3-O-glucoside, -3-O-galactoside, -3-O-rutinoside, -3-O-robinobioside, and -3-O-vicianoside). Minor flavonols are myricetin and kaempferol derivatives (Mikulic-Petkovsek et al., 2012; Tian et al., 2017). Procyanidins in chokeberries are characterized by a high degree of polymerization, with varying mean procyanidin polymerization degrees reported across different studies (Sidor & Gramza-Michałowska, 2019). Phenolic acids such as chlorogenic acid and neochlorogenic acid are predominant in chokeberries, along with other derivatives like 4-hydroxybenzoic acid, caffeic acid and its further derivative cryptochlorogenic acid, ellagic acid, ferulic acid, *p*-coumaric acid, protocatechuic acid, salicylic acid, syringic acid, and vanillic acid (Li et al., 2012).

Analysing the aroma profile of a fruit is crucial for determining the overall quality of the fruit in question. The identified volatile



**Table 1**

Nutritional profile of black chokeberry (*Aronia melanocarpa*). Adapted from Jurendić & Šćetar (2021) and Christiansen et al. (2022). Total phenolic content (TPC), total anthocyanins content (TAC), cyanidine-3-O-glucoside (Cy3G), gallic acid equivalents (GAE).

Chemical composition	Berries	Juice	Pomace
<i>g/100 g or mL of fresh weight</i>			
Dry matter %	1.5–3.1	1.1–1.7	4.5–5
Total sugars	6.8–15.8	11–14.3	8.4
Glucose	1.1–4	3.2–4	2.2
Fructose	1.4–4.2	3–3.9	2.4
Sorbitol	4.4–7.6	4.8–6.4	3.8
Fat	0.14	<0.1	2.9–13.9
Proteins	0.7	0.2	4.9–24.1
Fiber	5.6	0.3	63–78
Minerals	0.4–0.6	0.5	1.4–3.9
<i>mg/100 g or mL of fresh or dry (*) weight</i>			
TPC (measured spectrophotometrically, GAE)	603–2340	281–558	6310
TPC (measured chromatographically)	247–2773.41	99.63–1,123.74	8044–24,447.77*
TAC	209–1019.80	2.39–217.95	1684.60–11,573.19*
Cyanidin-3-O-galactoside	101–636	1.62–149.84	1119.70–7961.70*
Cy3G	3.41–46.20	0.05–12.01	21.0–220.06*
Cyanidin-3-O-arabinoside	94.18–299.40	0.66–50.90	454–3116.02*
Cyanidin-O-xyloside	9.92–38.20	0.06–5.20	89.9–275.41*
Phenolic acids	98.50–333.70	38.30–293.21	253.20–2367.04*
Chlorogenic acid	61–218	10.30–138.90	33.20–1192.69*
Neochlorogenic acid	37.5–115.70	28–154.31	220–1174.35*
Flavonols (quercetin, quercetin-3-O-galactoside, quercetin-3-O-glucoside, quercetin-3-O-rutinoside, etc.)	21.2–71	16.5–21.3	57.0–126.80*
Procyanidins	663.7–1645.64	224.9–392.62	6201.73–13,492*

compounds in chokeberries comprise aldehydes, alcohols, esters, and ketones (Butorová et al., 2016; Romani et al., 2016). Alcohols are the most abundant compounds identified, comprising 40–52.6 % of the volatile compounds; Ethanol, methanol, butan-1-ol, hexan-1-ol, and phenylmethanol were found. Aldehydes constitute 13–33.3 % of the volatile compounds identified in chokeberry. Some of the aldehydes are benzaldehyde, ethanal, hexanal, nonanal, octanal, pentanal, propanal, and *trans*-2-hexenal. Esters constitute 11.8–22.7 % of the volatile compounds, including ethylbutyrate, ethyldecanoate, ethylethanoate, and ethylpentanoate. Ketones make up to 13.3 % of the volatile compounds in chokeberries, including heptan-2-one, pentan-2-one, and propan-2-one (Butorová et al., 2016). Each of these compounds contribute to the distinct aroma characteristics of chokeberries.

## 2.2. Traditional black chokeberry products

Due to its high total phenolic content, black chokeberries have a bitter taste and are rarely consumed raw. However, they are widely utilized in the food industry to produce various products, as being processed into juices, nectars, syrups, jams, preserves, wines, infusions, and dietary supplements. Nectars contain additional sugars or sweeteners or other fruit juices for enhancing the flavour (Teneva et al., 2022). Syrups are concentrated liquids from sweetened chokeberry juice, commonly used as toppings or flavourings (Kawa-Rygielska et al., 2019). Jams and preserves are thick spreads made by cooking chokeberries with sugar, used for spreading on toast or incorporating into pastries (Kmiećik et al., 2001). Chokeberry wines are alcoholic beverages produced through fermentation with residual sugar contents similar to wines made from grapes (Čakar et al., 2018). Infusions are made by extraction of powdered chokeberries in hot water, however they are often blended with other herbs or fruits (Tolić et al., 2015). Additionally, chokeberry extracts or powders are utilized as dietary supplements for their potential health benefits, available in various convenient forms such as capsules or tablets (Tasić et al., 2021). Even though black chokeberry products include a wide variety, approximately 90 % of the berries are utilized for juice production, while the remaining portion is allocated to the manufacturing of infusions, dietary supplements, and other products (Sójka et al., 2013).

The chokeberry market was valued at 356.9 million dollars in 2023

and is anticipated to grow at a compound annual growth rate of 3.4 %, being forecasted to exceed 498.7 million dollars by the year 2033. Major countries driving demand include the USA, China, Canada, Germany, India, the United Kingdom, and Australia. Germany holds a market share of approximately 26.9 % (FMI, 2023).

## 3. Fermented black chokeberry

### 3.1. The process of fermentation of black chokeberry products

Various microorganisms such as *Acetobacter* spp., *Brettanomyces* spp., *Kluyveromyces* *lactis*, *Lactobacillus* spp., *Monascus purpureus*, *Rhizopus oligosporus*, and *Saccharomyces* spp. (Table 2), are utilized to ferment black chokeberry fruits, juice, pomace, or extracts of the aforementioned. The temperature during fermentation exhibits a broad range from approximately 20 °C to potentially exceeding 40 °C, contingent upon the particular microorganism used or the nature of the fermentation process. The duration of fermentation extended from brief periods, such as a few hours (e.g., 8 h), to more extensive spans, encompassing multiple days (e.g., 10 d), or even weeks (e.g., 2 weeks), contingent upon the microorganism utilized and the desired outcome of the fermentation process. pH levels within the fermentation environment varied, typically falling within the range favourable to microbial growth and fermentation activity, typically between pH 4 and pH 6, with certain instances specifying values like 4.6 or 5.5. Nonetheless, explicit pH measurements were not consistently provided for all fermentation protocols (Čakar et al., 2018; Du & Myracle, 2018a; Kim et al., 2023; Yaneva et al., 2022).

Black chokeberries fermentation leads to the breakdown of the plant cell walls, releasing non-covalently and covalently bound polyphenols, which then significantly contribute to the physicochemical characteristics of fermented black chokeberries products (Zhao et al., 2021). This process is facilitated by different microorganism enzymes, such as  $\beta$ -glucosidase, decarboxylases, esterases, hydrolases, and reductases play critical roles in catalysing reactions that breakdown complex carbohydrates to simple sugars that are then substrates for releasing organic acids and generating several further different metabolites (Yang et al., 2024). However, the extent of enzymatic breakdown varies among the different microorganism strains.

After conducting a systematic survey across three databases

**Table 2**A summary of the *in vitro* and *in vivo* investigations involving the fermented products derived from black chokeberry (*Aronia melanocarpa*).

Functional matrix	Microorganism used for fermentation; Fermentation conditions	Quality characteristics	Experimental model/Biological activity assessed	Intervention	Observed biological effects	Reference
<i>In vivo</i> studies						
Fermented chokeberry pulp (FAE)	Unspecified fermentation microorganism	Snack bars, macronutrient content: 3.7 g fats, 43.2 g carbohydrates, 2.5 g proteins, 9.2 dietary fibers, 234.6 kcal.	Triple-blinded, triple-crossover, 8-week study in subjects with T2DM	Snack bars: 34 g of fermented chokeberry pulp (893 mg anthocyanins), 55 g of raisins, and 3 g of coconut oil	↑ Glucose-dependent insulinotropic peptide, ↑ HDL-C, ↓ TC, ↔ BP, ↔ adiponectin, ↔ hs-CRP	(Christiansen et al., 2023a, 2023b)
Fermented chokeberry berry extract	<i>Saccharomyces kluyveri</i> DJ 97 KCTC 8842P and <i>Acetobacter</i> sp. HJK 9-1; 48 h at 25 °C, followed by 7 days at 30 °C, pH = 4	ND	High-fat diet (HFD)-induced C57BL/6J obese mice	100 mg/kg body weight/rat of fermented chokeberry extract	↓ BW, ↓ TAG, ↓ FBG, ↓ insulin	(Kim et al., 2018)
Fermented chokeberry juice extract	<i>Lactobacillus plantarum</i> EJ2014; 9 days at 30 °C	ND	<i>In vitro</i> (RAW 264.7 cells) and <i>in vivo</i> (BALB/c mice) experiments	125, 250, or 500 mg/kg of Fermented chokeberry juice extract	Anti-inflammatory effects, modulates immune response	(Ali et al., 2021)
Chokeberry vinegar	<i>Acetobacter pasteurianus</i> SRCM 101341; 12 days at 25 °C	↑ Total phenolic, flavonoid, anthocyanin, and organic acid contents after fermentation	<i>In vitro</i> (RAW 264.7 and 3T3-L1 cell lines) and <i>in vivo</i> C57BL/6 mice	50, 100, or 150 µL of chokeberry vinegar	Antioxidant activity, anti-inflammatory, anti-lipogenesis, and anti-obesity activities in RAW 264.7 and 3T3-L1 cells; ↓ BW, ↓ BF, ↓ lipid profile	(Lim et al., 2022)
<i>In vitro</i> studies						
Kombucha-fermented chokeberry berries	Consortium of microorganisms referred to as kombucha (SCOBY); 10/20 days at 25 °C	↑ biologically active compounds, free radical scavenging capacity, positive effect on skin cell viability and metabolism, probiotic activity	<i>In vitro</i> and cellular models to evaluate antioxidant properties, cytotoxicity, and protective effects against oxidative stress		Minimal cytotoxic effects; ↑ antioxidant activity (DPPH IC <sub>50</sub> = 722 µg/mL, ABTS <sup>+</sup> IC <sub>50</sub> = 126 µg/mL); Reduction of oxidative stress in human and yeast cells (30–300 µg/mL); Efficacy dependent on concentration and fermentation time; Potential in cosmetic industry	(Ziemlewska et al., 2023)
Fermented chokeberry berry extract (FA)	<i>Monascus purpureus</i> ; 14 days at 30 °C	ND	B16F10 melanoma cell line		Melanin inhibitor potential (500 µg/mL)	(D. H. Kim et al., 2023)
Kefir containing chokeberry berry fruit juice	<i>Lactococcus lactis</i> , <i>L. cremoris</i> , <i>L. diacetylactis</i> , <i>Lactobacillus acidophilus</i> , <i>Saccharomyces cerevisiae</i> , and <i>Kluyveromyces lactis</i> ; 24 h at 23 °C	↑ Bioaccessibility, ↑ α-glucosidase inhibition potential	Antioxidant capacity assessment (DPPH assay) and α-glucosidase inhibition potential		↑ antioxidant activity (DPPH IC <sub>50</sub> = 12.01–24.07 mg per part), ↑ α-glucosidase inhibitory activity (IC <sub>50</sub> = 152.53 mg/mL kefir)	(Du & Myracle, 2018b)
Chokeberry wine	<i>Saccharomyces cerevisiae</i> Lievito Secco EZ FERM, <i>S. cerevisiae</i> ICV D254; 7–10 days at 20 °C, subsequently kept 6 months at 12 °C	↑ phenolic compounds	α-glucosidase inhibition potential		↑ α-glucosidase inhibition potential (IC <sub>50</sub> = 28 µg/mL)	(Çakar et al., 2018)
Pale ale chokeberry-infused beer	<i>Saccharomyces cerevisiae</i> ; 2 weeks 18 °C	↑ EBC color rating, ↑ TPC, ↑ antioxidative activity, unchanged pH, increased attenuation	Antioxidant capacity assessment (DPPH assay)		↑ antioxidant activity (11.2–84.1 µg AAE/mL)	(Jahn et al., 2020)
Chokeberry pomace	<i>Aspergillus niger</i> and <i>Rhizopus oligosporus</i> ; 12 days at 30 °C, pH = 5.5	↑ phenolic compounds and antioxidant activities, ↓ anthocyanins, ↑ lipid content with high linoleic acid	Antioxidant capacity assessment (DPPH and ABTS radicals)		↑ antioxidant activity (DPPH 54–60 %, ABTS <sup>+</sup> 406–488 mmol TE/g DW)	(Dulf et al., 2018)
Probiotic oat beverage enriched with chokeberry berry juice	<i>Lactobacillus plantarum</i> Pro; 8 h at 37 °C	↑ sensory acceptance (appearance, color, aroma, texture, flavor, sweetness, aftertaste) of beverage supplemented with 20 % chokeberry berry juice	Antioxidant capacity assessment (DPPH and FRAP assays)		↑ antioxidant activity (DPPH 0.38–3.15 mM TE/cm <sup>3</sup> , FRAP 0.45–3.24 mM TE/cm <sup>3</sup> )	(Yaneva et al., 2022)

(continued on next page)



Table 2 (continued)

Functional matrix	Microorganism used for fermentation; Fermentation conditions	Quality characteristics	Experimental model/Biological activity assessed	Intervention	Observed biological effects	Reference
Probiotic oat beverage enriched with chokeberry fruit extract and lyophilizate	<i>Lactobacillus plantarum</i> DKK 003 strain; 20 h at room temperature	Changes in TPC during fermentation, a high lactic acid bacteria count, a low pH (approximately 4.15), no microbiological contamination	Antioxidant capacity assessment (TEAC assay)		↓ antioxidant activity (0.489–0.638 mg GAE/L)	(Marchwinska et al., 2023)
Yogurt-style product enriched with probiotic-fermented Chokeberry berry juice	<i>Lactobacillus plantarum</i> ATCC 14917; 5–5.5 h at 40 °C, pH = 4.6	↑ physicochemical properties, consistent stability and viability of probiotic strain, distinctive fruity flavor, lower levels of syneresis indicating improved water retention and texture stability	Antioxidant capacity assessment (DPPH assay)		↑ antioxidant activity (69.05 μmol TE/100 g)	(Plessas et al., 2024)
Fermented yogurt supplemented with chokeberry berry juice	<i>Lactobacillus acidophilus</i> , <i>L. casei</i> , <i>L. rhamnosus</i> , <i>L. lactis</i> , and yeast; 24 h at 37 °C	↑ total acidity, ↓ lightness and yellowness, ↑ LAB with longer incubation time and higher levels of chokeberry juice, ↑ polyphenol and flavonoid contents	Antioxidant capacity assessment (DPPH, ABTS, and FRAP assays)		↑ antioxidant activity (DPPH = 68.50–77.87 %, ABTS <sup>+</sup> = 59.98–70.90 %, FRAP = 29.55–29.86 %)	(Nguyen & Hwang, 2016)
Fermented emmer-based beverage fortified with Chokeberry berry fruit juice	<i>Lactobacillus plantarum</i> 2035; 2 h at 30 °C	↑ red colour and total phenolic content, high apparent viscosity and water-holding capacity, probiotic effect	Antioxidant capacity assessment (DPPH assay)		↑ antioxidant activity (94–136 μmol TE/100 g)	(Dimitrellou et al., 2021)
Fermented chokeberry berry fruit juice	<i>Lactobacillus plantarum</i> , <i>Lactobacillus acidophilus</i> , and <i>Lactobacillus rhamnosus</i> ; 48 h at 37 °C	↑ total phenolic and total flavonoid contents, ↓ reducing sugar content, ↓ pH value, dark red color, ↑ flavor profile	Antioxidant capacity assessment (DPPH, ABTS, and FRAP assays)		↑ antioxidant activity (DPPH = 69.84–92.70 %, ABTS <sup>+</sup> = 94.33–94.95 %, FRAP = 1.48–1.82 mg/L)	(Wang et al., 2024)
Fermented chokeberry berry fruit juice	<i>Lactobacillus paracasei</i> SP5; 48 h at 30 °C, pH = 4	Probiotic effect, fruity and floral notes, ↑ phenolic content, absence of ethanol, ↑ shelf-life	Antioxidant capacity assessment (FRAP and TEAC assays)		↑ antioxidant activity (FRAP = 50–70 mmol Trolox/L, TEAC = 7.5–11 mmol Fe <sup>2+</sup> /L)	(Bontsidis et al., 2021)
Kefir containing chokeberry berry fruit juice	<i>Lactococcus lactis</i> , <i>L. cremoris</i> , <i>L. diacetylactis</i> , <i>Lactobacillus acidophilus</i> , <i>Saccharomyces cerevisiae</i> , and <i>Kluyveromyces lactis</i> ; 24 h at 23 °C	↑ sensory acceptability score (6.3), ↑ phytochemical composition (total phenolic compounds, anthocyanins)	Antioxidant capacity assessment (DPPH assay)		↑ antioxidant activity (IC <sub>50</sub> = 27.59–28.84 mg kefir/mL)	(Du & Myracle, 2018a)
Water kefir produced from chokeberry berry fruit juice and pomace	<i>Lactobacillus paracasei</i> , <i>L. hilgardii</i> , <i>L. nagelii</i> , <i>Saccharomyces spp.</i> , and <i>Brettanomyces spp.</i> ; 72 h at 35 °C	↑ phenolic, flavonoid, and anthocyanin contents, no significant difference in overall acceptability, taste, aroma/odor, and turbidity	Antioxidant capacity assessment (DPPH and CUPRAC assays)		↑ antioxidant activity (DPPH = 49.0–58.9 mg TE/100 mL, CUPRAC = 5.57–6.68 mg TE/100 mL)	(Esatbeyoglu et al., 2023)
Chokeberry wine	<i>Saccharomyces cerevisiae</i> 7–10 days at 20 °C, subsequently kept 6 months at 8 °C	↑ phenolic compounds content and composition by microvinification	Antioxidant capacity assessment (DPPH and FRAP assays)		↑ antioxidant activity (FRAP=85.56–94.55 mmol/L Fe <sup>2+</sup> , DPPH IC <sub>50</sub> = 1.26–1.56 %)	(Çakar et al., 2016)
Chokeberry wine	<i>Saccharomyces cerevisiae</i> and <i>S. bayanus</i> Fermentation at 20 °C, subsequently kept 10 months either at 4 or 25 °C	Different alcohol content depending on the storage temperature and the type of yeast used for fermentation, stable pH values and total acidity, color parameters according to the type of yeast used for fermentation and the storage temperature, sensory qualities (taste, aroma, colour, consistency, and general assessment) according to temperature consumed	Antioxidant capacity assessment (ABTS and FRAP assays)		↑ antioxidant activity (ABTS <sup>+</sup> = 1.73 mmol Trolox/100 mL, FRAP = 1.38 mmol Trolox/100 mL)	(Lachowicz et al., 2017)

Abbreviations: High-density lipoprotein cholesterol (HDL-C), total cholesterol (TC), blood pressure (BP), high-sensitivity C-reactive protein (hs-CRP), body weight (BW), body fat (BF), triacylglycerol (TAG), fasting blood glucose (FBG), ascorbic acid equivalent (AAE), dry weight (DW), Trolox equivalents (TE), gallic acid equivalents (GAE), lactic acid bacteria (LAB).



(PubMed, Scopus, and Web of Science) until 15th June 2024, a diverse range of fermented products based on or incorporating black chokeberries has been identified, including beer, juice, kefir, kombucha, fermented extracts, oat- and emmer-based beverages, pomace, vinegar, and wine (Supplementary Fig. 1). The production processes of these products encompass four main and prominent types of fermentation used in traditional food technology: lactic acid fermentation, alcohol fermentation, acetic acid fermentation, and solid-state fermentation (SSF).

In lactic acid fermentation, black chokeberries are utilized in various forms: the berries can be either squeezed to juice and after that fermented or they can be added to beverages or yogurt, and then undergo fermentation together with the other ingredients (Bontsidis et al., 2021; Dimitrellou et al., 2021; Du & Myracle, 2018a; Esatbeyoglu et al., 2023; Wang et al., 2024). This fermentation process involved inoculation with lactic acid bacteria such as *Lactobacillus plantarum*, *L. paracasei*, *L. acidophilus*, and *L. rhamnosus* contributing to the tangy flavor profile characteristic of lactic acid fermentation.

Alcohol fermentation also plays a significant role in the production of fermented black chokeberry products. For example, in the process of beer production, the whole black chokeberries are integrated at various stages of the brewing process to infuse some valuable compounds and their distinctive flavor into the final product (Jahn et al., 2020). Additionally, fermented chokeberry extract is produced through alcohol fermentation using *Saccharomyces kluyveri*. Furthermore, chokeberry wine production involves fermentation by *S. cerevisiae* and other yeast strains, producing a complex array of flavors and aroma that develop over time during further maturation like as in other prominent alcoholic beverages (6 months at 12 °C) (Čakar et al., 2018; Čakar et al., 2016; Lachowicz et al., 2017).

Acetic acid fermentation is done in the production of black chokeberry vinegar. Here, specific bacterial strains are introduced into sterile chokeberry juice supplemented with ethanol or chokeberry wine as the basis. Over the course of about 12 d, ethanol is transformed into acetic acid, resulting in the characteristic sharp acidity of a non-grape fruit vinegar (Lim et al., 2022).

Lastly, SSF or substrate fermentation is used as well to process chokeberries. For example, black chokeberry pomace, the solid remains after juice extraction, was subjected to fungal SSF (*Aspergillus niger* and *Rhizopus oligosporus*) with the goal to liberate and recover phenolic compounds and lipids from it (Dulf et al., 2018), while ethanolic extracts of chokeberry were fermented with *Monascus purpureus* in order to study its inhibitory effect on melanogenesis (Kim et al., 2023).

Overall, the manufacturing processes involving lactic acid fermentation, alcohol fermentation, acetic acid fermentation, and SSF highlight the versatility of black chokeberries in creating a diverse array of fermented products, each offering distinct flavors, textures, and health benefits.

### 3.2. Composition of fermented black chokeberry products

#### 3.2.1. Carbohydrates

In fermentation, facilitated by microbial activity, carbohydrates – simple sugars as well as complex ones – undergo transformation into diverse end products, including organic acids, alcohol, and gases. The final level of carbohydrates, often mainly less complex sugars in the final fermented products represents a crucial aspect of the fermentation process (Yaa'ri et al., 2024). This residual sugar content does not only influence the taste, texture, and sensory attributes of the fermented product, but also reflects the extent of sugar utilization by fermenting microorganisms. Low levels of residual sugars indicate efficient fermentation, where the majority of sugars have been converted into desired end products such as organic acids or alcohol. On the other hand, high levels of residual sugars may suggest incomplete fermentation, possibly due to factors such as insufficient microbial activity, suboptimal fermentation conditions, or the presence of inhibitors. Therefore, evaluating the residual sugar content in black chokeberry is essential for

ensuring product consistency, quality, and safety.

Black chokeberries contain various sugars, primarily in the form of simple sugars such as glucose, fructose, and sucrose. They can also contain the sugar alcohol sorbitol. During the fermentation process (at 30 °C for 48 h) of chokeberry juice with *L. paracasei* SP5 and subsequent storage (at 4 °C for 4 weeks), there were fluctuations in sugar concentrations. Initially, there was an increase within the first 48 h, followed by a slight decrease, as noted by Bontsidis et al. (2021). However, although there was a decline in glucose and fructose concentrations, sorbitol content increased from 20.1 g/L to 24.3 g/L during the 48 h of fermentation with *L. paracasei* SP5. After that, the level of sorbitol remained constant throughout the four-week storage period. The increase in sorbitol content during fermentation and its stability during storage can be attributed to microbial activity, which converted glucose and fructose through various enzymatic pathways into sorbitol (Fig. 1), and the inherent stability of sorbitol itself (Bontsidis et al., 2021). In the case of chokeberry juice fermentation involving *L. plantarum* (JYLP-375), *L. acidophilus* (JYLA-16), and *L. rhamnosus* (JYLR-005), the levels of reducing sugars significantly decreased by almost 65 % (Wang et al., 2024). This decrease can be attributed to the consumption of sugars by lactic acid bacteria for their growth and conversion into lactic acid. It should be mentioned that neither of fermentation protocols of chokeberry juice did not used added sugar to facilitate the fermentation process. The initial sugar content of unfermented chokeberry juice varied between 28.8 and 103 g/L.

Similarly to chokeberry juice fermented with *L. paracasei* SP5, sucrose had varying levels in water kefir derived from pomace and juice at the start of fermentation. However, for water kefir made from aronia pomace, the elevated sucrose content was because the total soluble solids were initially too low (6.00 °Brix) and to compensate, additional sugar was added, raising the level to 10.4 °Brix. Therefore, pomace-based water kefir displayed a higher sucrose content (7.75 g/100 mL) compared to juice-based kefir (3.19 g/100 mL). Sucrose was undetectable after 72 h in both types of kefir. The utilization of sucrose within the initial 24 h of fermentation positively correlates with the production of ethanol by yeasts equipped with invertase enzymes, which break down sucrose into fructose and glucose. Subsequently, these sugars are primarily utilized by lactic acid and acetic acid bacteria. While chokeberry pomace-based kefir showed an increase in reducing sugars, chokeberry juice kefir exhibited a diminishing. Fructose content increased significantly in pomace-based kefir (from 0.52 g/100 mL to 1.94 g/100 mL), but decreased slightly over 72 h in juice-based kefir (from 1.72 g/100 mL to 1.56 g/100 mL) (Esatbeyoglu et al., 2023). Moreover, glucose content slightly rose in pomace-based kefir, but decreased in juice-based kefir.

#### 3.2.2. Organic acids

Besides other microorganisms, the process of lactic fermentation involves lactic acid bacteria (LAB) converting simple and complex carbohydrates into various organic acids, such as propionic, formic, acetic, and lactic acid. These acids enhance food flavour and prevent spoilage. The concentration of organic acids produced varies depending on factors like strain, pH, and temperature (Punia Bangar et al., 2022).

During fermentation (30 °C for 48 h) and storage (4 °C for 4 weeks) of chokeberry juice using *L. paracasei* SP5 strain, lactic acid and acetic acid were detected in the final product. Lactic acid levels notably increase during fermentation and remain high (>9.0 g/L) throughout the 4-week period. Acetic acid, detected in trace amounts after the second week, likely originates from citric acid, as observed in previous studies on lactic acid fermentation of fruit juices. Malic acid, which is the primary organic acid in chokeberry, decreases over time due to metabolism by the probiotic strain into lactic acid through malolactic fermentation (Bontsidis et al., 2021).

Fermented plant-based beverages represent a significant option as alternatives to dairy products, addressed to individuals seeking plant-based options due to dietary preferences, lactose intolerance, or vegan



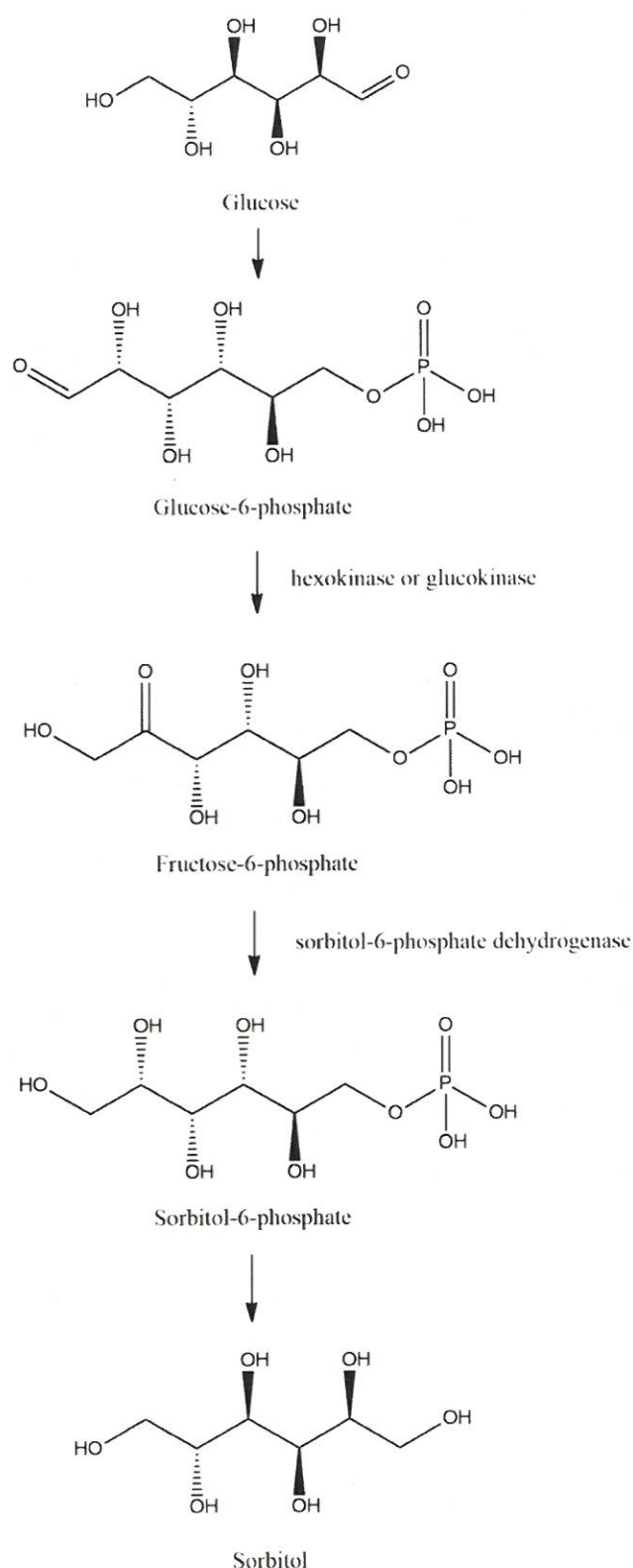


Fig. 1. Bioconversion of sorbitol from glucose by *Lactobacillus plantarum*. and Adapted from Ladero et al. (2007) Jan et al. (2017).

lifestyles. Yaneta et al. (2022) developed a functional oat beverage by enriching with chokeberry juice (5–30 %) and fermenting with *L. plantarum* Pro (8 h at 37 °C). The initial acidity levels of functional oat beverage, enriched with varying concentrations of chokeberry juice, ranged from pH 5.88 for the control beverage (no juice) to pH 4.10 for the highest concentration of 30 % chokeberry juice (Yaneva et al., 2022). The final acidity of all beverages was pH 4.02. This decrease in acidity was attributed to the low acidity of pure chokeberry juice (pH 3.74), containing organic acids. Dimitrellou et al. (2021) developed a functional emmer beverage by enriching with chokeberry juice (20 %) and fermenting with *L. plantarum* 2035 (2 h at 30 °C), and stored 4 weeks at 4 °C. Following the addition of chokeberry juice, the pH of the beverage decreased to 4.69. The pH remained stable during the 2 h fermentation process, suggesting that this pH level is optimal for the growth of *L. plantarum* 2035 (Dimitrellou et al., 2021). Throughout fermentation, *L. plantarum* strains produce diverse organic acids, such as lactic acid, acetic acid, tartaric acid, malic acid, succinic acid, and citric acid (Hu et al., 2019; Szczerbic et al., 2022).

The process of acetic fermentation involves acetic acid bacteria converting ethanol, sugars and polyols into various organic acids, aldehydes and ketones via oxidative fermentation (Hata et al., 2023). Lim et al. (2022) analysed the organic acid composition of chokeberry vinegar, produced by fermenting chokeberry juice supplemented with 5 % ethanol (95 %) using the strain *Acetobacter pasteurianus* SRCM 101341 at 25 °C for 12 d. Their findings revealed that the proportion of organic acids in chokeberry and commercial vinegars were different: chokeberry vinegar contained 6.2 mg/mL of acetic acid, 2.7 mg/mL of formic acid, and 0.04 mg/mL of fumaric acid, while commercial vinegar contained 13.0 mg/mL of acetic acid and 18.1 mg/mL of citric acid (Lim et al., 2022). The differences in organic acid proportions between chokeberry vinegar and commercial vinegar can be attributed to variations in starting materials, microbial strains, fermentation conditions, substrate availability, fermentation duration, and post-fermentation processing.

### 3.2.3. Phenolic compounds

3.2.3.1. Spectrophotometric evaluation of total anthocyanin content (TAC), total flavonoid content (TFC), and total phenolic content (TPC). Total anthocyanin content (TAC), total flavonoid content (TFC), and total phenolic content (TPC) are commonly employed sum parameters for evaluating different phenolic fractions and their fate during processing. In a study conducted by Wang et al. (2024), TAC notably decreased post-fermentation of black chokeberry juice, dropping from 101 to 65.7 mg/100 mL (fermented with *L. plantarum* (JYLP-375), *L. acidophilus* (JYLA-16), and *L. rhamnosus* (JYLR-005)). However, TPC and TFC increased after fermentation, particularly for black chokeberry juice fermented with *L. acidophilus*, with TPC and TFC rising to 168.4 and 65.9 mg/100 mL, respectively. In the same manner, Bontsidis et al. (2021) showed that the application of lactic acid fermentation by *L. paracasei* SP5 led to a remarkable increase of TPC in black chokeberry juice.

Moving from black chokeberry juice fermentation to solid-state fermentation (SSF) applied to chokeberry pomaces, Dulf et al. (2018) investigated the transformative effects of *Rhizopus oligosporus* and *Aspergillus niger* activity on the profile of bioactive compounds. During the growth period, TPC increased significantly. With *R. oligosporus* fermentation, TPC content gradually increased until day 2, followed by a rapid surge to 1.8-fold from the initial value of 951 mg gallic acid equivalents (GAE)/100 g dry weight (DW) to 1779 mg GAE/100 g DW at day 6, before declining and stabilizing at day 12. For SSF with *A. niger*, TPC levels exhibited a sigmoid growth curve, peaking at 1704 mg GAE/100 g DW at day 9, representing a more than 1.7-fold increase. TFC amounts showed similar trends as TPC, increasing by 1.5-fold to 264 mg QE/100 g DW at day 9 with *A. niger* SSF and by 1.6-fold to 277 mg QE/100 g DW at day 6 with *R. oligosporus* SSF before decreasing gradually.



TAC contents increased approximately 1.2-fold during the first two days of SSF, reaching 610 mg Cy3G/100 g DW for *R. oligosporus* and 590 mg Cy3G/100 g DW for *A. niger*, before decreasing and stabilizing for the remaining fermentation period (Dulf et al., 2018). Moreover, Lim et al. (2022) assessed the concentrations of TPC, TFC, and TAC in chokeberry vinegar (based on chokeberry juice) fermented with *A. pasteurianus* SRM 101341. TPC and TFC in the vinegar were 15 mg GAE/mL and 2.9 mg quercetin equivalents (QE)/mL, respectively. Additionally, anthocyanin content in chokeberry vinegar was 7.6 mg Cy3G/L. This indicates a significant presence of bioactive compounds in the vinegar, highlighting that *A. pasteurianus* converted the sugars present in chokeberry juice into acetic acid, leading to the formation of vinegar. Further, the microbial transformation contributed to the development of the unique flavor profile of black chokeberry vinegar (c.f. to subchapter 3.2.4).

Furthermore, researchers characterized the bioactive compounds in yogurt-style products enriched with probiotic-fermented chokeberry juice and extracts obtained from dried chokeberries. For instance, Plessas et al. (2024) conducted an evaluation of the TPC in such yogurt-style products. Their study revealed that the TPC was higher in yogurt samples inoculated with chokeberry juice (fermented with *L. plantarum*). Specifically, TPC values were 16 µg GAE/g for yogurt inoculated with chokeberry juice fermented with *L. plantarum* and 26 µg GAE/g for yogurt inoculated with yogurt starter culture and chokeberry juice fermented with *L. plantarum*. Moreover, Nguyen and Hwang (2016) analysed TPC and TFC in yogurt fortified with varying concentrations of chokeberry juice following a 24-hour incubation period with *L. acidophilus*, *L. casei*, *L. rhamnosus*, and *L. lactis*. Yogurts enriched with 3 % chokeberry juice exhibited the highest TPC with 54 mg GAE/g dry weight, whereas the control yogurts had the lowest TPC with 16 mg GAE/g dry weight. Notably, TPC increased by 2.5- and 3.3-fold in yogurts containing 2 % and 3 % chokeberry juice, respectively, compared to the control. Regarding TFC, the control yogurts had 118 mg CE/g, while yogurts with 1–3% chokeberry juice showed higher flavonoid levels, ranging from 122 to 152 mg CE/g. Relative to the control, yogurt containing 2 % and 3 % chokeberry juice exhibited 1.2- and 1.3-fold increases in total flavonoid content, respectively. Incorporating chokeberry juice into yogurt led to elevated TPC and TFC, underscoring the potential health benefits of such fortification. Lastly, Ali et al. (2021) studied the impact of *L. plantarum* EJ2014 fermentation on TPC and TPC in chokeberry extract. Over the fermentation period from day 0 to day 9, there was an increase in TPC by 46.2 % and flavonoid content by 11.4 % in fermented chokeberry extract.

The transformations of phenolic compounds in black chokeberry during fermentation reveals a multifaceted process influenced by various enzymatic activities. Enzymes such as  $\beta$ -glucosidase, decarboxylases, esterases, hydrolases, and reductases play pivotal roles in catalyzing reactions that modify these compounds. Additionally, the breakdown of black chokeberry cell walls during fermentation facilitates the release of phenolic compounds, enhancing their extractability. Furthermore, fermentation promotes the mobilization and release of phenolics from their glycosides or otherwise bound forms, thereby augmenting their availability, which may potentiate their physiological effects (Adebo & Medina-Meza, 2020).

While fermentation processes generally can enhance the bioavailability of phenolic compounds of some black chokeberry products, contrasting effects have been observed with the use of kefir grains. Kefir grains are complex microbial communities consisting of a dextran matrix primarily composed of  $\alpha$ -D-glucopyranosyl residues with connected side chains. These grains are composed of a diverse composition of microorganisms, including *Lactobacillus* and *Leuconostoc* species responsible for generating the dextran structure (Gökirmaklı & Güzel-Seydim, 2022). The microbial composition of kefir grains varies depending on factors such as origin, culture methods, and fermentation conditions, leading to variability in the composition of the grains and the resulting fermented beverage. Fermentation with kefir grains resulted in a decline of the TPC, TFC, and TAC levels in both water kefir made from

chokeberry pomace or juice (Esatbeyoglu et al., 2023). Before fermentation, TPC and TFC levels were higher in water kefir made from chokeberry pomace and juice compared to levels after the fermentation (72 h). Specifically, TPC only slightly decreased from 7.3 GAE mg/100 mL to 7.2 GAE mg/100 mL in water kefir made from chokeberry pomace, while it significantly decreased from 7.2 GAE mg/100 mL to 5.8 GAE mg/100 mL in water kefir made from chokeberry juice. Similarly, TFC decreased from 1.7 to 1.5 mg QE/100 mL in water kefir from pomace and from 1.6 to 1.3 QE mg/100 mL in water kefir from juice. Consistently, TAC decreased after fermentation as well. Water kefir made from chokeberry pomace exhibited a decrease from 18 mg/100 mL at the beginning to 13.3 mg/100 mL after 72 h, while water kefir made from juice decreased from 7.3 mg/100 mL at 0 h to 5.3 mg/100 mL at 72 h (Esatbeyoglu et al., 2023).

The decrease of phenolic compounds observed during fermentation with the use of kefir grains can be attributed to several factors. The specificity of fermenting microbial strains towards phenolic compounds and their metabolism could result in their utilization or transformation, decreasing their levels (Adebo & Medina-Meza, 2020). In this sense, Özcelik et al. (2021) reported a decrease in TPC in water kefir made from hawthorn, cornelian cherry, roship, red plum, and pomegranate juices. Additionally, microbial enzymes may catalyze the decomposition of phenolic compounds, breaking them down into simpler forms (aglycones or smaller phenolic acids) or metabolites (conjugated with other compounds or decomposed to small phenolic compounds without an aromatic ring structure) (Adebo & Medina-Meza, 2020).

**3.2.3.2. Advanced analytical techniques (AATs).** Besides simple and fast methods which only act as sum parameters for estimating fate of phenolic compounds, advanced analytical techniques are utilized to thoroughly explore the chemical composition of different products and identify specific bioactive compounds (Ciesla & Moaddel, 2016). Relying solely on overall parameters is now considered somewhat outdated. However, they can still be regarded as indicative values. So ideally, both determinants – sum and detailed composition – are always analyzed and presented.

Several studies utilized advanced analytical methods, often as hyphenated techniques, to elucidate the chemical composition of fermented chokeberry products and identify bioactive compounds associated with their potential health benefits. For example, Ziemlewska et al. (2023) applied high-performance liquid chromatography (HPLC) to analyse secondary plant metabolites, including phenolic acids and flavonoids, in chokeberry-enriched kombucha. They observed significant fluctuations in the concentrations of several biologically active compounds during a 20-day fermentation period compared to the initial extract. Notably, gallic acid levels increased from 1.48 µg/mL in the initial extract to 3.19 µg/mL after 10 days of fermentation, remaining consistent thereafter. Protocatechuic acid concentrations also rose during fermentation, from 0.13 µg/mL initially to 0.2 µg/mL after 10 and 20 days. Levels of neochlorogenic, chlorogenic acid, and quercetin glucosides decreased initially, then increased slightly (Ziemlewska et al., 2023). Moreover, Kim et al. (2023) revealed that the content of gallic acid was significantly increased in *M. purpureus* fermented chokeberry extract compared to that of unfermented one. These variations underscore the dynamic nature of the fermentation process and its impact on the composition of bioactive compounds in the kombucha and fermented chokeberry extract.

Moreover, fermentation of chokeberry juice using four different strains of *L. plantarum* (DSM 16365, DSM 20174, DSM 10492, DSM 100813) had a significant impact on the reduction of total hydroxycinnamic acids, evaluated using high-performance liquid chromatography with diode array detection and electrospray ionization tandem mass spectrometry (HPLC-DAD-ESI-MS/MS) (Markkinen et al., 2019). Specifically, strain DSM 10492 exhibited notably higher efficacy for fresh juice, while the difference was not statistically significant in enzyme-



treated juice. Main hydroxycinnamic acids neochlorogenic acid and chlorogenic acid were affected by the fermentation, leading to a decrease in caffeic acid content and no significant change in quinic acid content. Fermentation also resulted in a decrease of flavonol glycosides, with DSM 10492 showing a significant impact in fresh juice and DSM 100813 and DSM 10492 in enzyme-treated juice. Despite differences in TAC among samples fermented with different strains, the anthocyanin's profile remained similar. Those authors concluded that the influence of fermentation on phenolic compounds appears to vary significantly based on the specific strain used.

Lachowicz et al. (2017) conducted an analysis of phenolic compounds in chokeberry wine using ultra-performance liquid chromatography with photodiode array detection and fluorescence detection (UPLC-PDA-FL), studying variations before and after fermentation and storage with yeasts, *S. cerevisiae* and *S. bayanus*. Phenolic compounds quantified included anthocyanins (mainly Cy3G, cyanidin-3-O-galactoside, -3-O-arabinoside, -3-O-xyloside), phenolic acids (mainly chlorogenic and neochlorogenic acid), flavonols & flavones (mainly quercetin and isorhamnetin derivatives), and flavan-3-ols (as monomers, dimers, and polymeric procyanidins). Results showed that the phenolic content in chokeberry wine was predominantly composed of phenolic acids, followed by flavan-3-ols, anthocyanins, and flavonols & flavones. There were no significant changes observed in the concentrations of phenolic acids during production and storage. However, the content of flavan-3-ols slightly decreased after storage at 25 °C for 6 months, while anthocyanin content decreased significantly. Flavonols and flavones content also decreased after storage. The phenolic content in chokeberry wine, including flavan-3-ols, anthocyanins, flavonols, and flavones, declined after storage, primarily due to factors such as temperature and chemical reactions. Flavan-3-ols decreased as a result of breakdown and polymerization, while anthocyanins degraded, leading to color changes. Flavonols and flavones decreased particularly due to storage temperature (Lachowicz et al., 2017).

Liquid chromatography with tandem mass spectrometry (LC-MS/MS) analysis of fermented chokeberry extracts revealed the presence of various bioactive compounds (Ali et al., 2021). Among these, the most significant and prevalent compounds included phenolic acids (neochlorogenic acid and chlorogenic acid), anthocyanins (cyanidin-3-O-galactoside, cyanidin-3-O-arabinoside, and cyanidin-3-O- $\beta$ -glucopyranoside), and flavonol glycosides (quercetin-di-hexoside, quercetin-3-O-vicianoside, quercetin-3-O-robinobioside, quercetin-3-O-rutinoside, quercetin-3-O-galactoside, quercetin-3-O-glucoside). The bioconversion of anthocyanins involves a series of metabolic processes (Fig. 2). Initially, anthocyanins are degraded into smaller phenolic compounds of lower molecular weight (Braga et al., 2018). The proposed pathway for anthocyanin bioconversion involves the formation of phenolic acids, particularly through chalcones (Faria et al., 2014). Cy3G and cyanidin-3-O-rutinoside are converted into phenolic acids like protocatechuic acid, dihydrocaffeic acid, and caffeic acid (Xie et al., 2016), which can also undergo conversions among themselves with minor modifications, for instance, caffeic acid can be transformed into protocatechuic acid (Braga et al., 2018). These mechanisms occur concurrently with the decomposition of sugar moieties during the early stages of fermentation. Moreover, this metabolism also been observed in *in vivo* studies, where gut microbiota play a significant role in the degradation of anthocyanins (Xie et al., 2016). Spontaneous degradation by bacterial action leads to the cleavage of glycosidic linkages and breakdown of anthocyanidin heterocycles. Major human metabolites of cyanidin glucosides include protocatechuic acid (Vitaglione et al., 2007).

### 3.2.4. Volatile organic compounds

Volatile organic compounds are a broad range of chemical constituents that contribute to the flavor of fermented products (van Wyk, 2024). These compounds include small molecules such as simple alcohols, esters, acids, ketones, and aldehydes, among others, which are formed through the metabolic activities of microorganisms during

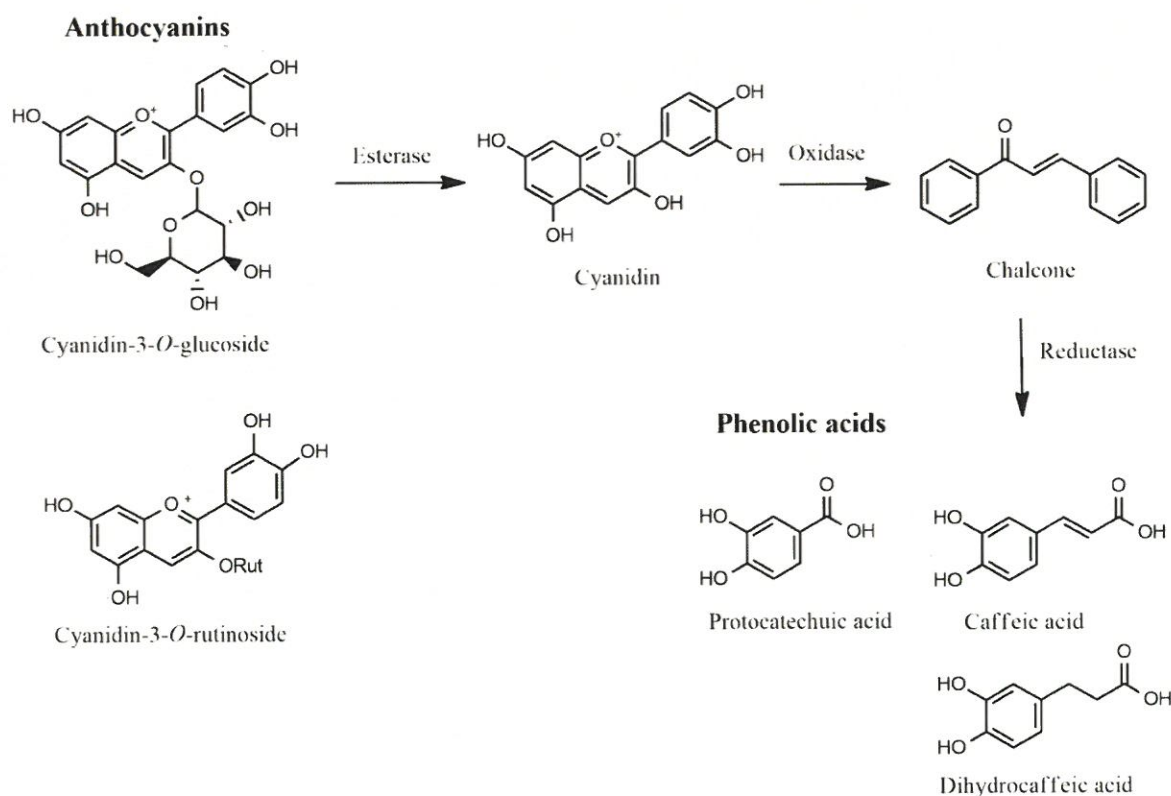


Fig. 2. Bioconversion of anthocyanins into simpler phenolic compounds involving Lactobacilli and Bifidobacteria.



fermentation. They play a crucial role in shaping the sensory profile of fermented chokeberry products, contributing to fruity, floral, and spicy characteristics of the products.

Qualitative analysis with gas chromatography–ion mobility spectrometry (GC–IMS) of volatile compounds in black chokeberry juice fermented by three lactic acid bacteria strains during 48 h revealed the presence of almost 40 volatile organic compounds (VOC), categorized into four alcohols, seven aldehydes, six esters, two ethers, six ketones, one pyrazine, and 14 unidentified compounds. Many of these VOC have previously been reported in chokeberry juices or fruits, as well as in other foods fermented by lactic acid bacteria or produced through lactic acid fermentation (Wang et al., 2024). Moreover, Bontsidis et al. (2021) reported that following a 48-h fermentation period of chokeberry juice with *L. paracasei* SP5, a total of 55 compounds were identified, comprising 23 alcohols, four aldehydes, 13 ketones, four esters, five organic acids, and six further compounds. Notably, esters and alcohols emerged as the predominant flavour volatile compounds detected in the fermented juices at 4 °C. In contrast, the unfermented juice contained 78 compounds, consisting of 28 alcohols, seven aldehydes, 18 ketones, seven esters, six organic acids, and 12 other compounds.

Esatbeyoglu et al. (2023) evaluated the volatile compounds present in water kefir beverages produced from chokeberry juice and pomace. A total of 99 volatile compounds were identified in total, before and after fermentation. After fermentation, ethanol became the primary volatile component in both types of kefir, followed by other alcohols and esters. Fermentation significantly increased the alcohol and ester contents in both groups. Conversely, the total aldehyde and ketone contents decreased significantly after fermentation. Wang, Zhang & Lei (2022) also reported that ketones and aldehydes decreased in fermented pear juices. This decrease in aldehydes and ketones may result from their breakdown or conversion to alcohols or acids via the metabolic activities of lactic acid bacteria. Notably, the absence of 3-penten-2-one, a lipid oxidation product, after fermentation further suggests an improvement in the quality of the kefir beverages. Laureys & De Vuyst (2017) similarly noted that aldehydes like hexanal, furfural, and benzaldehyde, which were present before fermentation, were absent in water kefir after the fermentation. Overall, the production of water kefir from chokeberry juice and pomace using the kefir starter culture resulted in significant compositional changes in volatile compounds. GC–MS analysis revealed that there were 10 different types of volatile compounds in chokeberry vinegar, while only six types were identified in commercial vinegar. The primary components in chokeberry vinegar were 2-(2-methoxyethoxy) ethyl acetate, isothiocyanic acid, and 2,2,4-trimethyl-1,3-pentandiol diisobutyrate. On the other hand, commercial vinegar mainly consisted of 2-(2-methoxyethoxy)ethyl acetate, succinic anhydride, and 6-azabicyclo[3.2.1]octane. Several compounds including cyclohexanone, 3,3,5-trimethylcyclohexanol, dihydro-3-methylene-5-methyl-2-furano ne,  $\delta$ -hexalactone, 2-ethyl 3-hydroxyhexyl 2-methylpropanoate, and 3,4-dihydroxystyrene were found in chokeberry vinegar, but were absent in commercial vinegar (Lim et al., 2022). These compounds are typically found in vinegars made from various fruits such as pineapple (*Ananas comosus* (L.) Merr.), mango (*Mangifera indica* L. cv. Kent), riberry (*Syzygium luehmannii* (F.Muell.) L. A. S. Johnson), and sour cherry (*Prunus cerasus* L.), each of which possesses unique secondary plant metabolites and compounds contributing to their distinct flavor and aroma profiles (Bonneau et al., 2016; Elss et al., 2005; Noé et al., 2019; Özen et al., 2020). Their absence in commercial vinegar underscores its production from concentrated fermented alcoholic solutions.

### 3.3. Sensory qualities of black chokeberry fermented products

The most important characteristic of the newly developed chokeberry products for their success on the market is their sensory profile. VOC profiles of fermented chokeberry products play a crucial role in shaping their sensory profile. These compounds are formed during

fermentation through the metabolic activities of microorganisms. They contribute significantly to the fruity, floral, and spicy characteristics of fermented chokeberry products. Overall, the sensory profile encompasses various aspects, including appearance, aroma, taste, texture, and overall mouthfeel, which collectively contribute to the consumer's perception and enjoyment of the product. By assessing the sensory characteristics of fermented black chokeberry products, researchers try to align with more conscious and dynamic consumer preferences.

Water kefir, a non-dairy alternative to traditional milk kefir, is made of sucrose with or without added fruit, fermented using kefir grains. Unlike its dairy counterpart, water kefir appeals to a broader consumer base, including vegans and those with lactose intolerance. Its distinct flavor profile arises from self-carbonation and concurrent lactic acid and alcoholic fermentations. In the study described by Esatbeyoglu et al. (2023) the sensory attributes of water kefir beverages infused with chokeberry juice and pomace was evaluated before and after fermentation, considering factors such as colour, turbidity, attractiveness (both visual and gustatory), aroma, sweetness, acidity, carbonation, and overall acceptability. Notable findings included consistent evaluations of color, turbidity, visual attractiveness, and carbonation across all tested groups. The pomace-based kefir exhibited lower acidity levels pre-fermentation compared to chokeberry juice-based kefir, which registered the highest acidity post-fermentation. While both pomace and juice scored highest for sweetness, fermented varieties scored lower due to reduced sugar levels post-fermentation. Chokeberry juice-enriched kefir garnered the highest scores for aroma and gustatory attractiveness, leading to its designation as the most preferred group, although other variants remained within acceptable limits. Despite kefir's inherent sourness and frothy texture, chokeberry juice and pomace present promising opportunities for producing fruit-based kefir beverages with health benefits, serving as a potential avenue for diversifying the market for kefir-like products.

Moreover, in the study described by Du and Myracle (2018a), three chokeberry kefir products with different sweeteners were evaluated. Participants equally accepted the color of all products, but significant differences were observed in other attributes. Sucrose-sweetened kefir was preferred for sweetness, followed by stevia addition. Consumers noted unpleasant aftertastes with non-nutritive sweeteners, impacting product acceptance. Flavor was best in sucrose-sweetened kefir, while texture was as well superior in the sucrose-sweetened variant, exhibiting a thicker consistency. Overall, sucrose-sweetened chokeberry kefir received the highest acceptability score.

Another product from the dairy, namely yogurt-style products enriched with probiotic-fermented *A. melanocarpa* berry juice, received high acceptance scores and favourable impressions overall, without any unpleasant flavours noted (Plessas et al., 2024). Samples made with fermented chokeberry juice containing *L. plantarum* were particularly notable for their exceptional aroma and fruit flavor, likely due to the abundance of phenolic compounds found in the juice. The colour of fermented milk samples was significantly influenced by the addition of fermented chokeberry juice. The presence of phenolic compounds in samples enriched with probiotic fermented *A. melanocarpa* berry juice, derived from the initial juice content, enhanced the flavor of fermented milk by accentuating its unique aroma qualities. Additionally, volatile phenols produced during the metabolic processes of probiotic or yogurt cultures contributed to a more complex and enriched flavour and aroma profile. Moreover, Nguyen and Hwang (2016) conducted a study evaluating sensory scores of yogurt samples fortified with varying amounts of chokeberry juice. Fortifying yogurt with chokeberry juice significantly affected sensory parameters such as colour and taste. Control yogurt had the lowest color and taste scores, with scores increasing as chokeberry juice content increased. However, there were no statistically significant differences observed in flavour, mouthfeel, consistency, or overall acceptance between the control yogurt and the yogurt containing chokeberry juice. Despite chokeberry's typically bitter or astringent taste, even 3 % juice in yogurt did not negatively impact sensory



attributes, therefore addition of 1–3 % chokeberry juice into yogurt was recommended for improvement of yoghurt qualities.

Three formulations of oat-chokeberry beverages were tested by Yaneva et al. (2022), with the addition of 10 %, 15 %, and 20 % chokeberry juice, alongside a fermented control oat drink. Sensory analysis using a 9-point hedonic scale assessed consumer acceptance. The beverages were sweetened with a natural, non-caloric sweetener (*Stevia dulce*) added after fermentation. Enrichment with chokeberry juice correlated positively with sensory attributes, particularly colour and overall acceptance. The 10 % chokeberry beverage had the lowest colour score, while the 15 % and 20 % versions, exhibiting a purple-reddish hue due to anthocyanins, scored higher. The 20 % chokeberry beverage received the highest colour score. A strong relationship was found between overall acceptance and sensory attributes, particularly colour, aroma, and sweetness. Sensory tests indicated that all formulations were well-liked by untrained panellists, with scores ranging from ‘like very much’ to ‘like moderately.’ While the 20 % chokeberry beverage scored highest, the differences between the 15 % and 10 % versions were not significant, suggesting all formulations were successful based on sensory analysis.

### 3.4. Concluding remarks on fermentation and composition of chokeberry products

As it could be noticed, the fermentation process of black chokeberry products is a multifaceted and dynamic phenomenon that significantly impacts their bioactive composition, sensory attributes, and nutritional value. Through fermentation, various microorganisms contribute to the breakdown of plant cell walls, enzymatic actions, and metabolic activities, leading to the release, modification, and synthesis of bioactive compounds such as phenolic compounds, organic acids, and flavour-relevant volatile compounds. The fermentation process influences the flavour, texture, and sensory properties of black chokeberry products, resulting in a diverse choice of fermented beverages and foods with distinct flavours and health benefits. Some studies showed that fermentation can both increase and decrease the levels of valuable compounds, depending on factors such as microbial strains, fermentation conditions, and processing techniques. However, even though there are a wide range of chokeberry fermented products, a majority part of consumers is unfamiliar with them, suggesting higher promotion might be achieved with greater familiarity with these products (Du & Myracle, 2018a). Nevertheless, despite the availability of a diverse array of chokeberry fermented products, a significant portion of consumers remains unaware of them. This indicates that increased promotion efforts could enhance consumer familiarity and acceptance of these products.

## 4. Health benefits of fermented black chokeberry products

### 4.1. Evidence from human studies

The importance of assessing the beneficial effects of fermented chokeberry products rises, as they could represent a promising intervention for enhancing consumer health and well-being, especially considering the already known beneficial effects of unfermented chokeberry. For example, several randomized controlled studies have demonstrated the positive impact of black chokeberry on various health parameters. Tasic et al. (2021) showed significant improvements in blood pressure, heart rate, cholesterol levels, and triglycerides among patients with metabolic syndrome using standardized chokeberry extracts. Similarly, Kardum et al. (2015) observed reductions in blood pressure and triglyceride levels in individuals with high normal blood pressure or grade I hypertension after regular consumption of chokeberry juice. Istas et al. (2019) found enhanced endothelial function and alterations in gut microbiota composition in healthy men consuming chokeberry fruits and extract powders. Meta-analyses by Rahmani et al. (2019) and Hawkins et al. (2021) confirmed these findings, indicating

increases in HDL cholesterol and reductions in total cholesterol, LDL cholesterol, and triglycerides with chokeberry consumption. Furthermore, chokeberry supplementation was associated with decreased systolic blood pressure and total cholesterol, particularly in older adults. These results collectively suggest that black chokeberry supplementation may offer benefits for blood glucose levels, lipid profiles, and blood pressure regulation.

Given that the fermentation process of black chokeberry and the fermentation of products containing black chokeberry can enhance the bioactive composition, sensory characteristics, and nutritional value of the resulting products, it is anticipated that consuming fermented black chokeberry products could positively influence the health parameters of consumers (Table 2). Nevertheless, so far, only two human intervention studies assessed the effects of fermented chokeberry on individuals with type 2 diabetes mellitus (T2DM). The effects of fermented chokeberry pulp on individuals with T2DM were investigated in a study comparing fermented and non-fermented chokeberry pulp with a placebo (Christiansen et al., 2023a). The study utilized a triple-blinded, triple-crossover design with eight-week intervention periods. During these phases, participants consumed two bars per day, each bar containing 34 g of fermented chokeberry pulp, equivalent to 893 mg of anthocyanins, along with 55 g of raisins and 3 g of coconut oil. Results indicated that while fermented chokeberry pulp supplementation led to a higher increase in glucose-dependent insulinotropic peptide (GIP) compared to a placebo. GIP, released by the small intestinal K-cells in response to nutrient intake, plays a role in regulating glucagon-like peptide-1 (GLP-1) (Wang et al., 2021). GLP-1 is an incretin secreted by enteroendocrine L-cells following food ingestion, being crucial for maintaining glucose homeostasis. It enhances insulin secretion in response to glucose stimulation, supports  $\beta$ -cell proliferation and survival, and delays gastric emptying. Moreover, GLP-1 induces feelings of satiety and has been shown to reduce body weight in animal models of obesity as well as in obese individuals (Müller et al., 2019). However, even though fermented chokeberry pulp supplementation led to a higher increase in GIP, no significant effects were noticed in insulin, blood glucose, and GLP-1 levels (Christiansen et al., 2023a). These results are in contradiction with the one reported in the literature. For example, Castro-Acosta et al. (2016) reported that plasma GIP and GLP-1 levels decreased following consumption of 600 mg anthocyanins. The discrepancies could be attributed to variations in the amount, type, and physical form of carbohydrates in the tested meals. In the study described by Christiansen et al. (2023a), the product containing fermented chokeberry extract provided over 40 g of carbohydrates, primarily derived from raisins. It was observed that GIP levels are particularly sensitive to the rate of glucose absorption (Alsalim et al., 2023), which may explain the increase seen in the group consuming fermented chokeberry extract. However, despite the increase in GIP levels among those consuming fermented chokeberry extract, the significance of the GIP increase may be limited clinically, as individuals with T2DM typically demonstrate pronounced resistance to GIP in pancreatic beta-cells (Holst & Rosenkilde, 2020).

Another study done on the same population investigated fermented chokeberry pulp on cardiovascular risk factors (Christiansen et al., 2023b). Despite chokeberry berries' antioxidant properties and potential positive effects on hypertension and dyslipidemia, no significant effects were observed on blood pressure, adiponectin, or high-sensitive C-reactive protein. However, participants supplemented with placebo showed significantly higher increases in total cholesterol and LDL-cholesterol compared to those supplemented with either fermented- or non-fermented chokeberry pulp. The authors suggested that both fermented- and non-fermented chokeberry pulp may have had the potential to prevent the increase in cholesterol levels observed with placebo supplementation. This effect might be due to the capacity of chokeberry to modulate hepatic lipid metabolism and improve antioxidant function in the plasma and liver (B. Kim et al., 2013). Chokeberry anthocyanins, particularly cyanidin derivatives such as Cy3G, have been associated



with a reduction in plasma cholesterol levels. Studies on apoE<sup>-/-</sup> mice have shown that supplementation with anthocyanin extract led to lower total cholesterol levels (Wang, Zhang, et al., 2012). Specifically, apoE<sup>-/-</sup> mice fed Cy3G as part of a cholesterol-rich diet exhibited significantly lower serum total cholesterol compared to controls. This reduction was attributed to increased faecal bile acid excretion, possibly due to the induction of hepatic CYP7A1, a key enzyme in bile acid synthesis (Wang, Xia, et al., 2012).

#### 4.2. Animal model studies

Animal model studies are essential for understanding the pathophysiological impact of different interventions and advancing medical knowledge, thereby playing a crucial role in improving human health and healthcare. Different animal model studies have assessed anti-obesity, immunomodulatory, and antioxidant properties of fermented chokeberry products (Table 2). For instance, Kim et al. (2018) investigated the impact of fermented *A. melanocarpa* using *S. kluyveri* DJ 97 KCTC 8842P and *Acetobacter* spp. HJK 9-1 on male C57BL/6J mice fed a high-fat diet (HFD). They found that fermented chokeberry significantly attenuated weight gain and increase in serum triglyceride levels induced by HFD. Fermented chokeberries also improved glucose tolerance and insulin sensitivity compared to natural chokeberries, suggesting their potential as a dietary supplement for preventing obesity. Moreover, Lim et al. (2022) evaluated the bioactivity and anti-obesity effects of chokeberry vinegar (AV) prepared using *A. pasteurianus* SRCM 101341. AV exhibited higher radical scavenging activity and contained higher levels of polyphenols, flavonoids, and anthocyanins compared to commercial vinegar. Treatment with AV suppressed nitric oxide production in RAW 264.7 cells and reduced lipid accumulation in 3T3-L1 cells. Additionally, oral administration of AV decreased body weight, fat, and serum lipid profile in obesity-induced mice without causing histopathological effects. Lastly, Ali et al. (2021) focused on the immunomodulatory effects of fermented chokeberry extract with *L. plantarum* EJ2014 BALB/c mice. Female BALB/c mice received doses of 125, 250, and 500 mg/kg of fermented chokeberry extract over a period of 21 days. Fermented chokeberry extract administration resulted in increased neutrophil migration and phagocytosis, along with enhanced splenocyte proliferation, CD4<sup>+</sup> and CD8<sup>+</sup> T-cell expression, and lymphocyte proliferation. Moreover, fermented chokeberry extract supplementation led to dose-dependent increases in IFN- $\gamma$ , IL-2, and IL-4 cytokine levels, while decreasing TNF- $\alpha$  and IL-6 levels. In comparison, Zhu et al. (2023) showed that non-fermented black chokeberry polyphenol supplementation of HFD-fed rat model reduced obesity-related symptoms, glucose tolerance, and systemic inflammation by downregulating pro-inflammatory cytokine expression and improving intestinal barrier function, therefore supporting anti-obesity and anti-inflammatory effects of chokeberry. Therefore, these studies suggest that fermented chokeberry products retain the health benefits of their non-fermented counterparts, possessing the same health benefits, including anti-obesity, immunomodulatory, and antioxidant properties. The benefits of fermented chokeberry extracts are correlated with the presence of phenolic acids and anthocyanins, which were the main classes of secondary metabolites present in the fermented extracts (Ali et al., 2021; Kim et al., 2018). Previous studies demonstrated that daily consumption of anthocyanins helps in maintaining and reducing body weight due to their anti-obesity properties, making them promising candidates for dietary supplementation and therapeutic interventions (Azzini et al., 2017).

#### 4.3. In vitro model studies

A lower level of experimental models is studying the effects of fermented chokeberry products in *in vitro* studies, with the aim to bridge the gap between basic research and clinical trials. *In vitro* studies play a crucial role in elucidating the underlying mechanisms of action of

fermented chokeberry products at the cellular and molecular levels. Some *in vitro* studies which focused on fermented chokeberry products assessed antioxidant protection, skin pigmentation modulation, and anti-diabetic properties (Table 2). Ziemlewska et al. (2023) evaluated the effects of kombucha-fermented black chokeberry extracts on yeast cell growth and protection against hydrogen peroxide-induced oxidative stress. A 20-day kombucha-fermented black chokeberry extract inhibited yeast cell growth at concentrations of 0.3 % and completely stopped growth at 0.6 % (Ziemlewska et al., 2023). This inhibition effect might be explained by the presence of significant concentrations of hydroxycinnamic acid derivatives, which can disrupt yeast cell membrane integrity and function, leading to the loss of cellular homeostasis and energy production (Taofiq et al., 2017). In kombucha, further microorganisms are present, as a symbiotic culture of bacteria and yeasts (SCOBY), which is colloquially called “tea fungus” is used. Some of these microorganisms might be resistant towards the impact of hydroxycinnamic acid derivatives, while others, especially some bacteria, might be inhibited or even killed.

Additionally, the same extract described by Ziemlewska et al. (2023) showed protective properties against hydrogen peroxide-induced oxidative stress in both wild-type and *sod1*  $\Delta$  mutant yeast strains. The study also confirmed these observations using the green fluorescent protein (GFP)-YAP1 construct, indicating the activation of cell protection signalling pathways in response to oxidative stress. Moreover, assessment of cell viability indicated no cytotoxic effects on fibroblasts and keratinocytes for the kombucha-fermented black chokeberry extracts tested across all concentrations.

In another study, Kim et al. (2023) investigated the inhibitory effects of fermented *A. melanocarpa* on melanogenesis using the B16F10 melanoma cell line. They found that fermentation with *M. purpureus* effectively inhibited tyrosinase activity and melanogenesis induced by  $\alpha$ -melanocyte-stimulating hormone ( $\alpha$ -MSH). Further analysis revealed that the fermented product downregulated the protein kinase A/cAMP response element-binding protein (PKA/CREB) pathway, leading to decreased levels of tyrosinase, Tyrosinase-Related Protein 1 (TRP-1), and Microphthalmia-Associated Transcription Factor (MITF) proteins. Additionally, the fermented product inhibited MITF transcription by enhancing the phosphorylation of glycogen synthase kinase 3  $\beta$  (GSK3 $\beta$ ) and AKT. These effects were attributed to the increased production of gallic acid, a phenolic compound in *A. melanocarpa* resulting from *M. purpureus* fermentation. Finally, in one of the studies described by Du and Myracle (2018b), it was found that chokeberry kefir exhibited elevated levels of bioaccessible polyphenols and increased antioxidant capacity during digestion. Although digested chokeberry kefir had lower levels of bioaccessible anthocyanins compared to the non-fermented control, it showed stronger inhibitory activity on  $\alpha$ -glucosidase, a digestive enzyme that breaks down carbohydrates into glucose during digestion, thus playing a critical role in regulating blood sugar levels and the management of T2DM (Hossain et al., 2020). This suggests that fermentation may lead to the production of metabolites with enhanced antioxidant capacity and improved  $\alpha$ -glucosidase inhibitory activity. On the other hand, Cakar et al. (2018) studied the  $\alpha$ -glucosidase inhibitory activity of fruit wines made from various berries including blueberry (*Vaccinium myrtillus* L.), black chokeberry, blackberry (*Rubus caesius* L.), raspberry (*R. idaeus* L.), and sour cherry (*Prunus cerasus* L.). They found that all fruit wines exhibited higher bioactivity compared to acarbose, a compound frequently used as positive control in  $\alpha$ -glucosidase inhibition assays. Blueberry and black chokeberry wines showed the highest  $\alpha$ -glucosidase inhibitory activity regardless of the vinification method used. Additionally, chlorogenic and caffeic acids were identified as key bioactive compounds contributing to this activity.

Considering other fermented chokeberry products listed in Table 2, it could be concluded that fermentation processes significantly enhance the antioxidant capacity of fermented chokeberry-based products. Various fermentation methods, employing different microorganisms and fermentation conditions, led to increased levels of polyphenols,



especially flavonoids, and other bioactive compounds, thereby enhancing the antioxidant potential of the final products. For example, the fermented yogurt supplemented with chokeberry berry juice, utilizing a combination of *L. acidophilus*, *L. casei*, *L. rhamnosus*, *L. lactis*, and yeast, demonstrated enhanced antioxidant capacity compared to the control product (Nguyen & Hwang, 2016). Similarly, the yogurt-style product enriched with probiotic-fermented chokeberry berry juice, fermented with *L. plantarum* ATCC 14917, demonstrated enhanced antioxidant capacity along with favorable physicochemical properties, including improved water retention and texture stability (Plessas et al., 2024). On the other hand, the chokeberry juices fermented with *L. plantarum*, *L. acidophilus*, and *L. rhamnosus* exhibited increased levels of total phenolic and total flavonoid contents, alongside a dark red color and improved flavor profile, indicating increased antioxidant potential (Wang et al., 2024). Furthermore, the probiotic oat beverage enriched with chokeberry berry juice, fermented with *L. plantarum* Pro, exhibited improved sensory acceptance and increased total antioxidant activity compared to the non-fermented counterpart (Yaneva et al., 2022).

#### 4.4. Probiotic potential of black chokeberry fermented products

When discussing fermentation, one crucial aspect to be considered is the presence of probiotics. These are described as living microorganisms that, when ingested in sufficient quantities, provide health advantages to the host. The levels of lactic acid bacteria during refrigerated storage are closely tied to nutritional and environmental conditions. Different beneficial health advantages could emerge from innovative and non-traditional origins of probiotics, capable of generating bioactive substances that promote health. Studies suggest that an intake of approximately  $10^9$  colony-forming units (CFU) per day constitutes an effective dosage (Latif et al., 2023). Therefore, it is essential to uphold the viability of these strains throughout storage to ensure their effectiveness.

During the fermentation process of *A. melanocarpa* extract by *L. plantarum* EJ2014, the growth of *L. plantarum* was observed to increase overall. Initially, the viable cell count of the *Lactobacillus* starter was  $4.86 \times 10^6$  CFU/mL, which rose to  $1.25 \times 10^9$  CFU/mL on day 1. Subsequently, the count decreased to  $1.55 \times 10^8$  CFU/mL by day 5, remaining steady at  $10^7$  CFU/mL until the end of the fermentation process (Ali et al., 2021). Incorporating chokeberry juice fermented with *L. plantarum* into fermented milk with a commercial yogurt starter culture led to increased counts of lactic acid bacteria during cold storage compared to the commercial yogurt alone. Specifically, higher counts of *S. thermophilus* and *L. bulgaricus*, exceeding  $10^8$  CFU/mL, were observed on the 28th day of storage, while the control yogurt showed significantly lower viability at  $10^7$  CFU/mL. This outcome is likely due to the enrichment of nutrients and prebiotic oligosaccharides provided by the adjunct fermented chokeberry juice (Plessas et al., 2024). Moreover, in the beverage containing 5 % chokeberry juice, the total count of lactic acid bacteria increased from  $6.31 \times 10^6$  CFU/mL to  $7.94 \times 10^{10}$  CFU/mL. With a 10 % concentration of chokeberry juice, lactic acid bacteria count rose from  $3.16 \times 10^6$  CFU/mL to  $7.94 \times 10^{10}$  CFU/mL. At 15 % chokeberry juice concentration, there was a  $3.98 \times 10^3$  CFU/mL increase in lactic acid bacteria count, while at 20 % concentration, the change was estimated at  $5.01 \times 10^2$  CFU/mL. However, a significantly slower growth was observed in the beverage with a 30 % concentration of chokeberry juice, where final concentration of bacteria reached only  $3.16 \times 10^7$  CFU/mL (starting at  $1.26 \times 10^4$  CFU/mL) (Yaneva et al., 2022).

No studies evaluated the probiotic effects of black chokeberry fermented products in *in vitro* or *in vivo* animal or human studies. Nonetheless, given that the effective dosage for observing probiotic effects from ingested foods is approximately  $10^9$  CFU/day, and that the concentrations of lactic acid bacteria in the fermented chokeberry products ranged from  $10^7$  to  $10^{10}$  CFU/mL, this indicates that they may potentially meet the threshold for probiotic efficacy. The beneficial effects and potential mechanisms associated with the probiotic efficacy are

correlated with the modulation of the gut environment and influence of host physiology. For example, fermented chokeberry products containing lactic acid bacteria could also contain short-chain fatty acids (SCFA), namely acetate, propionate, and butyrate. SCFA produced during the fermentation process have been shown to have several health benefits, including promoting gut barrier function, regulating immune responses, and modulating energy metabolism (Canfora et al., 2015; Xiong et al., 2022). These effects are exerted through SCFA interaction with specific receptors, namely G protein-coupled receptor GPR41/FFAR3 and GPR43/FFAR2. It was shown that in the case of fermented foods, SCFA improved hepatic metabolic conditions via FFAR3 in HFD-induced obese mice (Shimizu et al., 2019).

#### 5. Limitations and future perspectives

Fermented black chokeberry products may offer a wide range of health benefits, including anti-obesity effects, immune modulation, antioxidant protection, and potential applications in skincare and diabetes management. These findings highlight the potential of fermented chokeberry products as functional foods with significant health-promoting properties, making them attractive options for consumers looking for natural ways to enhance their well-being.

However, several limitations in the existing studies should be acknowledged to provide a comprehensive understanding of the potential health effects of fermented chokeberry products. Firstly, human clinical trials examining the effects of fermented chokeberry products are limited and the existing ones faced challenges related to participant adherence and retention, leading to high drop-out rates (Christiansen et al., 2023a,2023b). This can introduce biases and affect the reliability of the study results. Moreover, the exact quantity of consumed fermented chokeberry product was inconsistently reported, making it difficult to determine the dose–response relationship and interpret the findings accurately. Furthermore, the microbial strains used to ferment chokeberry berries were not always specified, which is crucial information for understanding the fermentation process and its impact on the composition and bioactivity of the final product. The choice of microbial strain can significantly influence the metabolite profile and health effects of fermented chokeberry products, highlighting the importance of transparency in reporting fermentation protocols. Additionally, in human clinical trials, the concomitant use of medication among participants may confound the study results by introducing additional variables that could have influenced the outcomes being measured. It is essential for researchers to account for medication use and other potential confounding factors in their study design and data analysis to ensure the validity and reliability of the findings.

Moreover, in animal studies investigating the effects of fermented chokeberry products, limitations such as the use of high doses of fermented *A. melanocarpa* extract and/or co-administration with other compounds, such as GABA, may have raised concerns about the extrapolation of findings to human populations (Ali et al., 2021). The interpretation of results from animal studies could also face challenges because of insufficient reporting of dosing information, including whether doses are expressed as absolute amounts or normalized to body weight, which can affect the comparability and reproducibility of the findings (Lim et al., 2022). Overall, while the existing studies provide valuable insights into the potential health effects of fermented chokeberry products, these limitations highlight the need for further research with improved study designs, standardized protocols, and transparent reporting practices to advance the understanding of the therapeutic potential of fermented chokeberry products and their applications in human health and disease.

In this context, it is essential to maintain a critical perspective on the interpretation of research findings. Speculative biochemical pathways based on other research are not conclusive enough to fully elucidate the mechanisms underlying the health benefits of fermented chokeberry products. While existing studies may have provided some insights into



potential pathways, such speculations often lack direct evidence from studies specifically investigating fermented chokeberry products. Moreover, biochemical pathways are complex and interconnected, making it challenging to pinpoint the exact mechanisms by which fermented chokeberry compounds exert their effects. Therefore, the extrapolation of findings from unrelated studies to fermented chokeberry products may introduce biases and inaccuracies. Each fermented product is unique, with its own composition of bioactive compounds and microbial metabolites resulting from the fermentation process. Thus, assumptions about the biochemical pathways based on studies of other foods or compounds may not accurately reflect the biological effects of fermented chokeberry products.

Moreover, a lenticular narrative, which distorts the true purpose of the scientific method, can create a precedent of agreement with spectacularity rather than rigorous analysis (as is the case of the review described by Sivapragasam et al. (2023)). This narrative may prioritize sensationalized outcomes over scientific accuracy, leading to misconceptions about the actual efficacy of fermented chokeberry products. Therefore, it's crucial to approach research findings with scepticism and evaluate them based on robust scientific evidence rather than sensationalized claims.

To address these limitations, more cellular and animal model studies with various disease models and additional human trials are needed. Animal studies can provide valuable insights into the potential mechanisms of action of fermented chokeberry products and help identify promising avenues for further investigation. Additionally, well-designed human trials are essential to evaluate the safety, efficacy, and long-term effects of consuming fermented chokeberry products in diverse populations. As it was shown, the two human clinical trials evaluating the impact of consuming fermented chokeberry products did not show any meaningful results.

## 6. Conclusion

In conclusion, fermented black chokeberry products hold promise as functional foods with significant health-promoting properties. Despite facing various limitations in existing studies, including challenges with participant adherence, inconsistent reporting of consumed quantities, and unspecified microbial strains used in fermentation, the potential of fermented chokeberry products remains evident. While human clinical trials have yet to provide conclusive evidence of their benefits, animal studies and *in vitro* experiments offer valuable insights into their anti-obesity effects, immune modulation, antioxidant protection, and potential applications in skincare and diabetes management.

Nonetheless, it is imperative to address these limitations and pursue future perspectives outlined in this review. Improved study designs, standardized protocols, and transparent reporting practices are necessary to enhance the reliability and validity of research findings. Additionally, further human trials, along with *in vitro* and animal studies, are needed to elucidate the mechanisms of action of fermented chokeberry products and evaluate their safety, efficacy, and long-term effects in diverse populations.

By maintaining a critical perspective on research findings and avoiding speculative conclusions, researchers can advance the understanding of the therapeutic potential of fermented chokeberry products more effectively. With continued scientific rigor and exploration, fermented chokeberry products could emerge as valuable contributors to promoting human health and well-being in the functional food and beverage market.

## CRediT authorship contribution statement

**Oleg Frumuzachi:** Writing – original draft, Methodology, Investigation, Formal analysis, Conceptualization. **Sascha Rohn:** Writing – review & editing, Supervision, Project administration, Conceptualization. **Andrei Mocan:** Writing – review & editing, Supervision, Formal

analysis, Conceptualization.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Data availability

No data was used for the research described in the article.

## Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.foodres.2024.115094>.

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