Extraction, Identification, and Health Benefits of Anthocyanins in Blackcurrants (Ribes nigrum L.)

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Featured Application: The in-depth discussion on the extraction methods of anthocyanins from blackcurrants and the molecular mechanisms behind its health benefits improves the utilization of blackcurrant anthocyanins in academic and industrial fields.

Abstract: The fruit of the blackcurrant (Ribes nigrum L.) is round-shaped, dark purple, bittersweet, and seed-containing edible berries. The blackcurrant has been used as a traditional medicine in both Asia and European countries. It is known as a rich source of antioxidants, largely due to its high content of phenolic compounds, especially anthocyanins. Studies on anthocyanins from blackcurrants have adopted different extraction methods and a panel of anthocyanins has been identified in them. Research on the health benefits of blackcurrant anthocyanins has also grown. To present a general overview of research in blackcurrant anthocyanins, this review focuses on the extraction methods of anthocyanins from blackcurrants and the molecular mechanisms underlying their health benefits.

Keywords: blackcurrant; anthocyanins; extraction; identification; health benefits

1. Introduction

The blackcurrant (Ribes nigrum L.) is a branched shrub with dark purple, bittersweet, and seed-bearing berries that can grow to about 1 cm diameter [1]. All parts of the plant, including the fruits (skin, flesh, and seeds) and leaves, can be utilized. The fruit is often used to make dark violet dyes and can be eaten raw or in processed forms like juice, jams, and jellies. Blackcurrant fruit waste has also been studied for its application in renewable hair dyes [2]. The leaves of the plant have been used as a flavor enhancer in soups due to their strong scent. Oil extracted from the seeds is often used in cosmetics [3,4]. The fruits and leaves of the blackcurrant have a long history as a traditional medicine in both Asia and Europe [5,6]. Nowadays, blackcurrant extract capsules have been commercialized as a dietary supplement and marketed as an immunity booster.

Fresh blackcurrant fruit contains a variety of functional and bioactive compounds such as soluble sugar, organic acid, diverse vitamins, multi-amino acids, diverse minerals, elements, and unsaturated fatty acids [7–9]. One of the most studied groups of bioactive compounds in blackcurrants are anthocyanins. Anthocyanins exist not only in blackcurrant berries but also in its seeds and leaves [10,11].

The anthocyanin presents up to 2–4 g/kg of fresh weight in blackcurrants [12]. It contributes to many sensorial properties, such as color, aroma, taste, and astringency.
The basic chemical structure of anthocyanins is composed of a sugar conjugate and a phenolic aglycon [13]. Both are known to affect the bioavailability and metabolism of anthocyanins [14]. The number of articles studying anthocyanins in blackcurrants have increased considerably over the decades (Figure 1). Research has found that blackcurrant anthocyanins (BCA) exert a wide range of biological functions including antioxidant, anti-inflammatory, and phytoestrogenic activities, as well as an improvement of vision and neuroprotective effects [1,15]. Since the vast majority of studies focus on the fruits of blackcurrants, and blackcurrant fruits contain a much higher amount of anthocyanins than the leaves [16], in this review, “blackcurrants” refers to the blackcurrant fruit, unless otherwise mentioned.

A variety of extraction methods have been applied to blackcurrants for anthocyanin extraction. However, it is difficult to compare the extraction yields among different methods since many factors, such as cultivars and storage conditions, have an impact on the anthocyanin yield [8,17]. These discrepancies could give confusion to the people utilizing blackcurrants in academic and industrial fields. Thus, this review analytically organized the available information and aimed to facilitate a better understanding regarding BCA extraction methodologies, as well as the health benefits and related molecular mechanisms of BCA. With an emphasis on the molecular pathways regulated by BCA, we wanted to make an in-depth discussion on the health-promoting role of BCA.

2. Extraction Methods

Solid-liquid extraction is a classic approach used to extract anthocyanins from plant tissues, such as blackcurrants. The polarity of anthocyanins facilitates their dissolution into polar solvents. Besides traditional solvent methods, new technologies have been tested for a better extraction yield of anthocyanins. These unconventional technologies include microwave-assisted extraction, ultrasonic-assisted extraction, enzyme-assisted extraction, and pulsed electrical field extraction [18].

2.1. Solvent Extraction for Blackcurrant Anthocyanins

The polarity of the targeted compound is the most critical factor for extraction reagent choice. Given their polarity, anthocyanins are usually extracted by methanol, ethanol, acetone, and/or water [19]. Ethanol and methanol are more efficient at extracting anthocyanins from blackcurrants than water [20]. A mixture of different solvents showed a better extraction ability than a single solvent. In total, 50% methanol showed a better extraction ability than water and methanol alone [21]. When 40%, 60%, and 96% aqueous ethanol were investigated for their extraction capacity, 60% ethanol showed the highest anthocyanin extraction efficiency [22].
Although acetone and methanol are effective extraction reagents, their application in foods is restricted due to their toxicity. Ethanol is more suitable for food applications but is more difficult to eliminate during the purification process [23].

Additionally, the presence of acid improves anthocyanin extractability due to its ability to liberate those bioactive compounds from blackcurrant pomace and skin [24,25]. It is worth noting that hydrochloric acid may hydrolyze acylated anthocyanins [26]. To avoid the breakdown of acylated anthocyanins, certain organic acids, such as formic, acetic, citric, or tartaric acids, whose elimination is easier during the anthocyanin concentration process, are preferred [24].

To concentrate anthocyanins furthermore, liquid–liquid extraction was additionally performed [27]. In a sequential extraction of blackcurrant juice residues that used water followed by methanol, anthocyanin detection increased approximately 2-fold in the methanol extract. [28].

2.2. Non-Conventional Extraction

The major challenges of conventional solvent extraction include longer extraction time, the demand for costly and high purity solvent, evaporation of a large amount of solvent, low extraction selectivity, and the thermal decomposition of thermolabile substances [29]. To overcome these limitations, new and promising extraction techniques have been introduced. These techniques are considered non-conventional extraction techniques. Some of the promising techniques include ultrasound-assisted extraction, enzyme-assisted extraction, microwave-assisted extraction, and pulsed electric field assisted extraction [30]. Those techniques reduce solvent consumption and shorten the extraction time, while the extraction yields of anthocyanins are similar to or even higher than those procured with conventional methods. However, no single method is considered as standard for extracting bioactive phytochemicals. Due to the involvement of multiple factors in the non-conventional extraction method, factorial designs and response surface methodologies (RSM) are often used to optimize the extraction conditions. The anthocyanins extracted using RSM are mainly affected by solvent concentration, extraction time, and solvent/solid ratio [31]. The studies that used non-conventional extraction methods to extract anthocyanins from blackcurrants are summarized in Table 1.

Table 1. Non-conventional extraction methods of blackcurrant anthocyanin.

| Method          | Condition                                                                 | Efficiency 1 | Reference |
|-----------------|---------------------------------------------------------------------------|--------------|-----------|
| Microwave-assisted | pH 2, 10 min extraction time, 700 W microwave power                      | ↑ 20%        | [32]      |
| Ultrasonic-assisted | 40 kHz frequency, 10 min extraction time, 70% amplitude                  | ↑ 20% from freeze-dried samples, → from frozen or over-dried samples | [33] |
| Ultrasonic-assisted | 45 kHz frequency, 15 min extraction time, 90 °C                        | →            | [34]      |
| Ultrasonic-assisted | Ultrasonic bath, 40 min extraction time                                | →            | [35]      |
| Enzyme-assisted  | Pectinolytic enzyme preparations, 30 min treatment, 60 °C               | → or ↑       | [36]      |
| Enzyme-assisted  | Pectinolytic enzyme preparations or protease, 10 min treatment, 100 °C  | ↓ or →       | [37]      |
| Pulsed electrical field | 1318 V/cm and 315 pulses                                           | ↑ 6%         | [38]      |

1 Compared to the conventional extraction method performed in each study. ↑ Indicates increased extraction yield. → Indicates no change of extraction yield. ↓ Indicates decreased extraction yield.
2.2.1. Microwave-Assisted Solvent Extraction

Microwave-assisted solvent extraction (MASE) is a technique that combines microwave and traditional solvent extraction. Microwaves heat up the molecules using ionic conduction and dipole rotation. The heating up of moisture inside plant cells due to the microwave effect results in evaporation and generates enormous pressure on the cell wall, which then ruptures. This process increases the yield of phytoconstituents [39].

When compared with conventional solvent extraction, MASE increased the anthocyanin extraction yield from blackcurrant marc by more than 20% [32,40]. A group of factors, including microwave power, marc/solvent ratio, pH, and time, showed a significant effect on the yields of anthocyanin extraction. Microwave power and time were the most dominant [32,40].

2.2.2. Ultrasonic-Assisted Extraction

Ultrasonic-assisted extraction (UAE) is performed using a high-intensity region of ultrasound (10–1000 W/cm²). Sonication and acoustic cavitation take place in the main body for UAE effects [41]. Sonic waves form gas bubbles by creating areas of alternating pressure in the medium. At the critical points of these gas bubbles, rapid condensation occurs and the energy inside the bubbles release. This creates shock waves with a large amount of heat and energy, which then physically affect the sample [42,43]. UAE facilitates solvent penetration and mass transfer. It therefore reduces the solvent usage and extraction temperature and it increases extraction rate and yield [44]. Compared to conventional extraction methods, UAE leads to a higher amount of and more stable polymeric anthocyanins in anthocyanin-rich fruit extracts [45].

Ultrasound pretreatment mixed with conventional extraction showed different results depending on the blackcurrant sample types: frozen, lyophilized, and over-dried [33]. Ultrasonic treatment increased total anthocyanins yield by 20% from freeze-dried samples but showed no effect on frozen or over-dried samples [33]. Additionally, UAE did not show an enhanced extraction efficiency of anthocyanins from blackcurrants in another two studies, which used frozen samples [34,35].

2.2.3. Enzyme-Assisted Extraction

Cell wall degrading enzymes (e.g., cellulases, pectinases, proteases, and α-amylase) can degrade and break down cell structure, and are thereby used to facilitate the extraction of intracellular contents, including anthocyanins [46].

Enzyme treatment with a panel of pectinolytic enzyme preparations and protease significantly enhance plant cell wall breakdown of blackcurrants [37]. A variety of pectinolytic enzyme preparations were used upon blackcurrant pulp samples during the maceration step and some of them significantly enhanced the anthocyanin yields in blackcurrant juice, while others showed no effect [36]. Inconsistent findings were reported by another group of researchers. A panel of pectinolytic preparations showed no effect on or decreased the extraction yields of anthocyanins from pomace remaining from blackcurrant juice [37]. Given the different types of samples used in the two studies (pulp vs. pomace), the enzymes might have worked differently [36,37].

2.2.4. Pulsed Electric Field

Pulsed electric field (PEF) treatment is a non-thermal technology that exposes a sample to repetitive short voltage pulses at relatively low energy and moderate intensity. The application of PEF in plant tissues improves the permeabilization of the cell membranes which thus enhances the release of liquid and targeted compounds from the cells [47].

To the best of our knowledge, there has been only one study on the effect of PEF treatment on BCA extraction. It reported that under optimum treating conditions an increment of 6% for total monomeric anthocyanins extracted from blackcurrants was found [38]. Given the limited study on PEF treatment, the effect of PEF on BCA extraction needs further investigation.
The adoption of non-conventional extraction methods did not show a remarkable increase in the extraction yield of anthocyanins from blackcurrants. However, the reduced extraction time and solvent consumption were significant. For example, MASE greatly reduced the extraction time from 300 min to 10 min and reduced the solvent to sample ratio from 40:1 to 20:1 [32]. UAE also reduced the extraction time from 60 min to 10 min [33]. In addition, the studies on enzyme-assisted extraction showed they should be carried out with great caution given the possible negative effect of cell wall degrading enzymes on BCA extraction.

3. Identification of Blackcurrant Anthocyanins

Four major anthocyanins in blackcurrants were analyzed using high-performance liquid chromatography with a diode-array detector (HPLC-DAD), with mass spectrometry (HPLC-DAD-MS), or with tandem mass spectrometry (HPLC-DAD-MS/MS) (Figure 2). Over 70% of the antioxidant capacity of blackcurrants came from those anthocyanins [22,48]. Four dominant anthocyanins—cyanidin-3-O-glucoside, cyanidin-3-O-rutinoside, delphinidin-3-O-glucoside, and delphinidin-3-O-rutinoside—accounted for 90% of the total anthocyanin content in various cultivars of blackcurrants [48,49]. They were also the major anthocyanins discovered in the buds, leaves, and seeds of blackcurrants [10,50]. Among the four anthocyanins, delphinidin-3-O-rutinoside was the most prevalent followed by cyanidin-3-O-rutinoside, delphinidin-3-O-glucoside, and cyanidin-3-O-glucoside [48,49].

![Figure 2. The structures of the major anthocyanins in blackcurrants including cyanidin-3-O-glucoside, cyanidin-3-O-rutinoside, delphinidin-3-O-glucoside, and delphinidin-3-O-rutinoside.](image)

The other minor anthocyanins that were identified in various blackcurrant cultivars using HPLC-DAD-MS included the 3-O-glucosides and the 3-O-rutinosides of pelargonidin, cyanidin, peonidin, delphinidin, petunidin, malvidin, cyanidin 3-O-arabinoside, and delphinidin 3-O-(6′′-p-coumaroylglucoside)s of cyanidin and delphinidin [22,51].

The bioavailability of anthocyanins in blackcurrants were also studied. After consuming a 20% blackcurrant juice (250 mL), the abundance of consumed anthocyanins in urine was $0.021 \pm 0.003\%$ of delphinidin glycosides and $0.009 \pm 0.002\%$ of cyanidin glycosides over a 24 h period [52]. In another study, the recoveries of delphinidin-3-O-rutinoside and cyanidin-3-O-rutinoside in urine samples were $0.040 \pm 0.011\%$ and $0.048 \pm 0.016\%$, respectively, over a period of 48 h after blackcurrant juice consumption [53]. Although anthocyanins in blackcurrants present a low bioavailability, their metabolites may contribute to the in vivo protective effects observed.
4. Health Benefits of Blackcurrant Anthocyanins and Related Molecular Mechanisms

We have previously discussed the health benefits of blackcurrants [1]. However, we wanted to focus on the anthocyanins in this review; therefore, only studies that used blackcurrant extracts rich in anthocyanin are included. Extracts containing more than 20% anthocyanins are included in the following discussion. In addition, some of the extracts used in certain studies were commercially purchased from fruit extract companies. The concentrations of anthocyanins in those extracts may not have been determined and listed in pertinent articles. Furthermore, the underlying molecular mechanisms through which BCA exerts the health benefits are also discussed.

4.1. Antioxidant and Anti-Inflammatory Activities

BCA was widely studied for its antioxidant and anti-inflammatory activities. BCA was shown to alleviate the reactive oxidative species (ROS) production induced by both menadione and \( \text{H}_2\text{O}_2 \) in SH-SY5Y human neuroblastoma cells [54,55]. In addition, a clinical trial showed that consumption of BCA prior to exercise facilitated recovery from exercise-induced oxidative stress [56].

BCA exerts cellular antioxidant activity, at least partly, through activating the nuclear factor erythroid 2-related factor 2 (NRF2) pathway. As a transcription factor, NRF2 regulates cellular resistance to oxidants in addition to drug metabolism. It controls the basal and induced expression of a group of antioxidant response element (ARE)-dependent genes, including several drug-metabolizing enzymes (e.g., glutathione S-transferase, NAD(P)H, and quinone oxidoreductase) and a group of antioxidant defense genes (e.g., heme oxygenase 1) [57]. The elevated expressions of NRF2 by BCA treatment was observed in rats with diethylnitrosamine (DENA)-initiated hepatocarcinoma [58]. Another study that used bone marrow-derived macrophages (BMM) from NRF2\(^{+/+}\) mice and NRF2\(^{-/-}\) mice showed that BCA significantly decreased the elevated cellular ROS level in lipopolysaccharides (LPS)-stimulated NRF2\(^{+/+}\) BMM but not NRF2\(^{-/-}\) BMM [59]. This study illustrated the critical role NRF2 plays in the antioxidant capacity of BCA. Besides the antioxidant activity, BCA also exerted an anti-inflammatory effect in various models. Pretreatment with BCA or cyanidin-3-O-glucoside inhibited the LPS-induced secretion of interleukin-6 by U937, a human monoblastic leukemia cell line [15]. A similar inhibition effect of BCA on LPS-stimulated cytokine secretion was also observed in THP-1 cells, another human monocytic cell line [60]. A commercially available BCA product attenuated ovalbumin-induced inflammation in a mouse model of acute allergic lung inflammation [61]. Another commercialized BCA product prevented inflammation in diet-induced obese mice, especially macrophage infiltration in the adipose tissue [62].

The widely studied anti-inflammation effect of BCA is mostly contributed to its suppression of the nuclear factor kappa-light-chain-enhancer of activated B cells (NF-\( \kappa \)B) pathway. NF-\( \kappa \)B, as a transcription factor, regulates many genes involved in inflammation and immune responses. These include chemokines, pro-inflammatory cytokines, adhesion molecules, and inducible enzymes (e.g., inducible nitric oxide synthase (iNOS) and cyclooxygenase-2) [63]. BCA attenuated LPS-induced NF-\( \kappa \)B signaling in multiple macrophage cell lines [59,60]. A recent study showed that consumption of purified anthocyanins isolated from bilberries and blackcurrants inhibited the expressions of proinflammatory genes related to the NF-\( \kappa \)B pathway in whole blood samples from human subjects with metabolic syndrome [64].

4.2. Phytoestrogenic Activity

Estrogens have an impact on the functions of organs and tissues such as bone, blood vessels, skin, and hair. There are two subtypes of estrogen receptor (ER): ER\( \alpha \) and ER\( \beta \). ER\( \alpha \) is mainly present in female reproductive organs, and ER\( \beta \) is expressed all over the body regardless of sex [65]. Phytoestrogens are plant-derived compounds with estrogenic effects. The phytoestrogenic activity of BCA was observed in ovariectomized (OVX) rats and mice. BCA increased the levels of extracellular matrix proteins (e.g., collagen, elastin,
and hyaluronic acid) in the skin of OVX rats [66]. Besides the effect in skin, BCA was also effective in mitigating hair loss in OVX rats and attenuating bone loss and bone resorption activity in OVX mice [67,68].

Some studies put forward that this phytoestrogenic activity of BCA could be mediated through ERα and ERβ [69,70]. The four major BCA all induced ERα and ERβ-mediated transcriptional activities, meanwhile they showed a higher affinity for binding to ERβ than ERα. They also upregulated the downstream target genes of ER [69,70]. BCA upregulated the expression of various estrogen signaling-related genes in the human female skin fibroblast cell line. The predicted upstream regulator was estradiol. Microarray profiling of human female skin fibroblasts showed that ERα was strongly activated after it was exposed to BCA [66].

4.3. Anti-Postprandial Hyperglycemic and Anti-Diabetic Effect

Postprandial hyperglycemia refers to the rapid rising of blood glucose level following a high carbohydrate meal. The regulatory effect of BCA on postprandial hyperglycemia has been previously reported. Both one-time consumption of BCA before a high-carbohydrate meal and 8-day supplementation of BCA reduced postprandial glycemia in humans [71,72]. The α-amylase and α-glucosidase inhibitory activities of BCA were proposed as major reasons [73,74]. The hydrolysis of starch by the digestive enzymes, including α-amylase and α-glucosidase, is a major cause of postprandial hyperglycemia [75]. Besides the effect on postprandial hyperglycemia, BCA also showed an anti-diabetic effect. Dietary BCA significantly improved glucose tolerance in type 2 diabetic mice [76]. Consumption of BCA for 8 weeks alleviated body weight gain and improved glucose metabolism in both low- and high-fat diet-fed mice [77].

The preventive effect of BCA on dyslipidemia and hepatic steatosis, which are strongly associated with type 2 diabetes, was also observed in OVX rats [78]. The studies on the molecular mechanisms underlying the anti-obesity effects suggested that modulation of adenosine monophosphate-activated protein kinase (AMPK) and lipid metabolism-associated genes may contribute to these effects [76]. AMPK pathway, as a part of the energy sensing network, is critical at controlling energy expenditure. The activation of AMPK by BCA was observed in multiple animal models, including diabetic mice, nonalcoholic steatohepatitis (NASH) mice, and high-fructose fed rats [76,79,80]. BCA alleviated metabolic syndrome in those mice. It is noteworthy that an intact gut microbiome was found essential for BCA exerting those effects [77]. Therefore, this study suggested the interactions between BCA and the gut microbiome is critical for the anti-diabetic effect of BCA.

4.4. Cardioprotective Effect

BCA was reported effective at maintaining or improving cardiovascular health. A single dose of BCA along with smoking was shown to attenuate the acute endothelial dysfunction induced by smoking and improve the peripheral temperature in young smokers [81]. Short-term BCA intake mitigated central arterial stiffness and central blood pressure in senior subjects [82]. A seven day intake of BCA displayed dose-dependent changes on certain cardiovascular parameters, such as increased cardiac output, increased stroke volume, and decreased total peripheral resistance during supine rest in endurance-trained male cyclists [83]. Six months of BCA consumption also improved the endothelial function in healthy subjects with habitually low fruit and vegetable intake [84].

The underlying pathology for cardiovascular diseases (CVD) is atherosclerosis. This pathology is complex and involves the structural elements of the arterial wall, circulating cells, and some inflammatory cells [85]. Given the complex pathology of atherosclerosis, the cardioprotective effect of BCA also involves a panel of action mechanisms.

Two major initiations involved in the development of CVD are the production of reactive oxidation species (ROS) via vessels and lipid oxidation [86]. Excessive ROS may promote endothelial dysfunction, a contributing factor to atherosclerosis. The molec-
ular mechanisms of BCA combatting excessive ROS are discussed in this review. On the other hand, the oxidation of low-density lipoprotein (LDL) in the vessel wall induced an inflammatory cascade that activates many atherogenic pathways, which led to the formation of foam cells [86]. The accumulation of foam cells results in the formation of a fatty streak, which is the earliest visible atherosclerotic lesion. The anti-inflammatory activity of BCA and related molecular mechanisms was covered in other parts of this review.

Atherosclerotic lesions are initiated through elevated LDL uptake by monocytes and macrophages. The reduction of LDL reduces the risks of CVD [87]. Consumption of anthocyanins from bilberries and blackcurrants decreased LDL-cholesterol levels in dyslipidemic subjects and subjects with metabolic syndrome [88,89]. One of the therapeutic goals to lower circulating LDL-cholesterol is to induce LDL receptor (LDLR) expression and activity in the liver. BCA increased protein levels of LDLR in Caco-2 cells, which led to the enhanced transport of LDL-cholesterol to the apical side of the enterocytes [90]. The BCA-induced increased hepatic LDLR protein expression was also reported in mice fed a high fat and high cholesterol diet [91].

Nitric oxide (NO) is a key cellular messenger in the cardiovascular system [92]. NO maintains vascular integrity through inhibiting platelet aggregation, leukocyte-endothelium adhesion, and proliferation of vascular smooth muscle. Diminished NO bioavailability was shown to cause endothelial dysfunction and increased susceptibility to atherosclerosis [93]. NO is generated by three isoforms of nitric oxide synthases (NOS): neuronal, inducible, and endothelial NOS. BCA activated endothelial NOS in vitro in human endothelial cells and in vivo in blood vessel endothelial cells acquired from OVX rats [94,95]. BCA-treated endothelial cells also showed higher NO levels [95].

Considering the crucial role of inflammation in the pathogenesis of atherosclerosis, the anti-inflammatory activity of BCA also provided a cardioprotective effect.

The cardiovascular effect of BCA was not observed in hyperlipidemic rabbits [96].

4.5. Neuroprotection and Cognitive Improvement

Studies show that BCA administration delayed cognitive deficit and provided a neuroprotective effect. BCA suppressed the neurotoxic effects of rotenone, which can be used for developing a Parkinson’s disease-like phenotype in a primary cell culture model [97]. Long-term supplementation of BCA reduced the spatial working memory deficit in aged APdE9 mice, which were Alzheimer’s disease model mice [54]. An acute BCA supplementation showed a cognitive benefit in healthy young humans [98]. The mechanisms underlying neuroprotective effects of BCA need further investigation. The proposed mechanisms have included modulation of oxidative stress [99]. However, other studies showed that compounds besides anthocyanins in the extracts may account for their neuroprotective effect [98,100].

4.6. Chemoprevention

An anthocyanin-rich fraction of blackcurrant fruit skin extract exhibited a potent cytotoxic effect on HepG2 human liver cancer cells [101]. It also decreased the incidence, total number, and size of preneoplastic hepatic nodules in rats with DENA-initiated phenobarbital-promoted hepatocarcinoma [5].

Further mechanistic studies suggested that BCA exerted chemopreventive actions against DENA-inflicted hepatocarcinogenesis by attenuating oxidative stress through activation of NRF2 pathway along with repressing inflammatory responses through the suppression of the NF-κB pathway [58,102]. NRF2 and NF-κB had a modulating role in cancer pathogenesis and progression [103]. Phase II enzymes, whose expressions are regulated by NRF2, detoxify a body of environmental carcinogens and therefore promote their subsequent excretion. The induction of NF-κB pathway thus contributes to tumorigenesis by transactivating several genes that are connected to tumor promotion [103].
4.7. Vision and Eye Health

The positive effects of BCA on vision and eye health were shown on chicks, healthy human subjects, and glaucoma patients. Oral administration of BCA and intravenous administration of major blackcurrant anthocyanins significantly inhibited the elongation of vitreous chamber depth and axial length caused by negative lenses in chicks [104,105].

In healthy humans, acute ingestion of BCA ameliorated dark adaptation and work-induced transient myopic shift [106]. Administration of BCA for 2 weeks significantly decreased the intraocular pressure in healthy subjects [107].

The effect of BCA on vision was also studied on glaucoma patients. It was found that 24 months of administration of BCA decreased the intraocular pressure in glaucoma patients and delayed the visual field loss and elevated ocular blood flow [108].

The mechanisms of vision benefits of BCA have not been thoroughly studied. One proposed mechanism was mediating plasma endothelin-1 (ET-1) [109]. ET-1, serving as a potent vasoconstrictor, has been suggested to play a role in the local autoregulation of blood flow. However, considering the inconsistent conclusions in regards to ET-1 level change on intraocular pressure [110], this mechanism needs to be further tested.

5. Conclusions

Blackcurrants are rich in color, nutrients, and flavor. The research on blackcurrants is growing. A comprehensive review of extraction and identification of anthocyanins in blackcurrants has provided a better understanding of the application of anthocyanins for promoting health and a thorough discussion on the related molecular mechanisms elucidated the nutritional values of BCA.

Compared with conventional extraction methods, non-conventional extraction techniques include additional factors such as microwaves, ultrasounds, enzymes, and pulsed electric fields, all of which allow the use of less solvents and energy. Not all studies that adopted nonconventional extraction methods significantly increased the extraction yield of anthocyanins from blackcurrants. However, considering the reduced extraction time and solvent usage, non-conventional extraction methods still provided certain advantages for BCA extraction.

The health benefits of BCA suggested the potential for BCA use as a key ingredient of functional food or therapeutic products to treat or prevent various chronic diseases. Its antioxidant and anti-inflammatory activities have been widely studied. They protect against oxidative stress, neuron toxicity, and carcinogens. The phytoestrogenic activity also explains certain anti-aging effects of BCA. In addition, the contribution of the single compound and synergistic effect should be considered for the therapeutic application of BCA.

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