BIO Web of Conferences **59**, 02011 (2023) https://doi.org/10.1051/bioconf/20235902011
ICBB 2023

A review of the physiological functions of blueberry anthocyanins and their applications in food

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Abstract. Anthocyanins are a class of water-soluble natural pigments, which are widely used in the food field because of their high nutritional value and various physiological functions. The extraction and purification technology of blueberry anthocyanins is one of the hot spots of research, and new techniques to improve the extraction rate and utilization have been emerging in recent years. This paper reviews the chemical structure, types, extraction and purification techniques, physiological functions and applications of blueberry anthocyanins in the food field, and gives an outlook on the application prospects, in order to provide scientific guidance and theoretical reference for the development and technical improvement of blueberry related products.

1. Introduction

Blueberry (Vaccinium spp.) is a small berry rich in anthocyanins, vitamin C, ellagic acid, myricetin, Ca, Fe, P, Mg and other mineral elements [1]. Because of its health-nurturing effects, blueberry has been listed by the Food and Agriculture Organization of the United Nations as one of the top five health foods for human beings and has been selected as the third generation of fruits in the world [2]. Blueberry anthocyanins are a class of pure natural pigments extracted from blueberries, with antioxidant, anti-inflammatory, anti-diabetic, and vision improvement effects [3], which has important research value and application prospects in the field of food and health products. This paper reviews the chemical structure and types of blueberry anthocyanins, their physiological functions, extraction and purification techniques, as well as applications in the food field, in order to provide theoretical references for the study of the biochemical properties of blueberry anthocyanins and the development of new blueberry anthocyanin functional foods.

2. Chemical structure and type of blueberry anthocyanins

Anthocyanins have a 3,5,7-trihydroxy-2-phenylchroman cationic structure, and when the benzene ring is replaced by different substituents at each carbon position, it will display different properties [4]. The qualitative and quantitative analysis of anthocyanin species in blueberry by HPLC and HPLC-ESI-MS techniques revealed that there are mainly five anthocyanin glycosides present in blueberry and the contents were mallow pigment > cornflower pigment > delphinidin > petunidin > peonidin [5]. In addition, external influences such as growing environment, soil condition and harvesting period may also cause the difference of anthocyanin species and quality in blueberry fruits [6].

3. Physiological functions of blueberry anthocyanins

3.1 Antioxidant effect

Blueberry anthocyanins have been promoted and utilized as natural antioxidants. The scavenging ability of blueberry anthocyanins on DPPH radicals, FRAP, $ABTS^{\bullet+}$ and superoxide anions is significantly higher than that of ascorbic acid [7]. Based on the above findings, related processes have been developed to improve the antioxidant properties of blueberry anthocyanins, such as acylating blueberry anthocyanins with n-valeric acid, n-decanoic acid and myristic acid. They find that the inhibition of β-carotene bleaching and the reduction of malondialdehyde by the acylated blueberry anthocyanins are stronger than the original anthocyanins [8]. In summary, blueberry anthocyanins are a class of pure natural antioxidants with good anti-aging effects on human body, which can be used as functional components of health and beauty products. Through the relevant technology, the antioxidant properties of blueberry anthocyanins can be protected and further improved during the processing and

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utilization process, thus improving the product performance.

3.2 Prevention of cardiovascular and cerebrovascular diseases

Cardiovascular disease has become one of the most important sources of threat to human health. In a meta-analysis of six studies, higher anthocyanin intake was found to be associated with a reduced risk of all-cause mortality, primarily due to the declined incidence of cardiovascular disease [9]. Studies testing the effects of anthocyanins on central arterial stiffness and central blood pressure in older adults found that short-term anthocyanin intake had a favourable reduction effect in both factors [10], which confirmed the pharmacological effects of anthocyanins in cardiovascular disease. Recent studies have attempted to combine the pharmacological effects of anthocyanins with food science and found that blueberries attenuate the deleterious effects of energy-dense high-fat/high-sugar meals consumed over a 24-hour period, such as acute cardiometabolic burden [11]. Therefore, processing blueberries into post-meal snacks or beverages may be a starting point for developing related products.

3.3 Anti-inflammatory effect

Inflammation is a physiological response triggered by external bacterial and viral infections in the human body, and blueberries are able to produce certain anti-inflammatory and analgesic effects on the organism. In an experiment using acrylamide to induce neurotoxicity in rats, blueberry anthocyanin extract was found to have a protective effect on rats. The reason for this is that blueberry anthocyanins were able to significantly reduce the production of malondialdehyde while increasing the levels of glutathione and antioxidant enzymes and inhibiting the expression of pro-inflammatory cytokines, thus preventing acrylamide-induced oxidative stress and neuroinflammation [12]. In addition, some studies found that blueberry anthocyanins can inhibit the expression of related inflammatory proteins COX-2 and NF-κBp65, and produce anti-inflammatory function on RAW 264.7 cells through NF-κB mechanism, which is another mechanism of anti-inflammatory effect of blueberry anthocyanins on the organism [13]. Based on the anti-inflammatory function of blueberry anthocyanins, they may be used to repair and improve male reproductive dysfunction caused by pathological changes, thus providing new therapies for patients [14].

3.4 Anti-diabetic effect

The latest foreign research confirms that blueberry anthocyanins have a certain therapeutic effect on diabetes. Regular intake of blueberries can reduce the levels of fructosamine and glycated hemoglobin in the blood and provides good control of blood sugar. These results, as well as lower triglyceride concentrations, indicate improvements in liver enzymes, AST and ALT, thus benefiting the cardiometabolism of patients with type II diabetes [15]. Related domestic studies have also indicated that blueberry anthocyanins can repair and improve liver and kidney function impairment caused by type II diabetes, and the therapeutic effect increases along with concentration. Therefore, blueberries can be a potential functional food for the prevention of diabetes and its complications [16].

3.5 Immune regulation effect

Blueberry yeast fermented powder can reduce cyclophosphamide-induced splenocyte viability in cell lines, increase natural killer cell activity and cytokine production. Therefore, this powder is expected to serve as a natural product-derived immunostimulator and can be a good supplement for patients suffering from immune diseases [17]. Another experiment found that flavonoids of blueberry leaves can exert immunoregulatory effect in lipopolysaccharide- induced RAW 264.7 cells by suppressing TNF-α via the NF-κB signal pathway, thus providing a plausible mechanism for the immunomodulatory mechanism of blueberry anthocyanins [18]. In the post-epidemic era, this effect is getting more and more attention. Therefore, how to ensure that the content of anthocyanins added to food can meet the immune needs while reducing the cost as much as possible is worth the attention of product development.

3.6 Other effects

In addition to the above functions, in recent years, scholars have also found that blueberry anthocyanins can improve non-alcoholic fatty liver and treat gastric ulcers by reducing dyslipidemia and intestinal microbial dysbiosis [17-18]. As the research and experiments on blueberry anthocyanins continue, other physiological activities of blueberry anthocyanins will be further discovered.

4. Extraction and purification of blueberry anthocyanins

4.1 Extraction of blueberry anthocyanins

Commonly used blueberry anthocyanin extraction techniques are mainly divided into direct extraction method and auxiliary extraction method. Since anthocyanins are soluble in polar solvents, the direct extraction method usually uses ethanol and acetone as extraction solvents, and a small amount of hydrochloric acid, citric acid or trifluoroacetic acid solution is added to improve the extraction rate. This technique is easy to operate set up, but the extraction rate is low, solvent consumption is high and often mixed with impurities. [21]. Auxiliary extraction methods include enzymatic and ultrasonic-assisted extraction methods. Enzyme extraction can break down the cellulose in the plant cell wall, thus promoting the release of anthocyanins. The extraction rate of blueberry anthocyanins using cellulase and pectinase was as high as (118.93±1.56) mg/100 g and (56.64±4.66) mg/100 g, respectively [22], but this method may affect the purity and recycling rate of the product. The ultrasonic-assisted continuous method leads to instantaneous rupture of both the cell wall and the whole blueberry. At the same time, the vibration effect can enhance the release, diffusion and dissolution of intracellular substances, thus significantly improving the extraction efficiency. Therefore, this scheme is able to achieve (205.13 ± 0.45) mg/100 g of anthocyanin extraction, which is higher than the results obtained by enzymatic method [23]. It is also worth noting that during the extraction process, the ultrasound power and time have a great impact on the product; if the power is too high, it will warm up the system and lead to the thermal degradation of anthocyanins [24].

4.2 Purification of blueberry anthocyanins

The extracted blueberry anthocyanins contain protein, pectin and other impurities, thus affecting the applications blueberry anthocyanins. At present, the commonly used techniques for the purification of blueberry anthocyanins include resin purification, high-speed counter-current chromatography and solid phase extraction [25]. The principle of resin purification method is using special sorbent to selectively absorb the active ingredients and simultaneously sieve out the invalid ingredients. This is the main method for blueberry anthocyanins purification since the products own high purity and non- absorbability [26]. In terms of resin selection, DM28 has the highest adsorption capacity and DM21 has the fastest adsorption speed [27]. Based on this method, introducing dextran gel LH-20 technology is able to increase the adsorption rate of blueberry to 97.73% and desorption rate to 81.52%, while the anthocyanin purity increases from 4.58% to 90.96% [28]. However, there are also disadvantages of resin purification, such as the poor performance of domestic resins which are rigid and easy to break, and there is a lack of standardized technical requirements for the production and application [29]. In addition to resin purification and its derivative fields, high-speed counter-current chromatography is often used in conjunction with other techniques for the purification of anthocyanins. Since both its stationary and mobile phases are liquids, there is no irreversible adsorption. Therefore, the purity of anthocyanins obtained by this method was 43.64% in an optimal solvent system, and this result could be increased to 90.61% by combining it with semi-preparative liquid chromatography [30]. The innovation of the existing technology to improve the efficiency of anthocyanin extraction and purification is important to improve the utilization of blueberry anthocyanins.

5. Blueberry anthocyanins in food applications

Blueberry anthocyanins have multiple functions such as antioxidant, anti-inflammatory and cardiovascular protection, and are widely used in the field of food research and development because of their abundant sources, safety and non-toxicity. At present, the utilization of blueberry anthocyanins is mainly divided into the following two ways: first, using the anthocyanin extraction and purification process to obtain high-purity anthocyanins from blueberries, and then add the anthocyanins to the products in the form of food additives; second, directly using blueberries as raw materials for product processing, the food products made contain a certain amount of blueberry anthocyanins. In view of the current blueberry anthocyanin extraction and purification process is not perfect, the relevant processing technology is still in the primary stage, the current market of blueberry anthocyanin products is mainly the second, and has involved fruit wine, fruit juice, jam and other major fields.

5.1 Application of blueberry anthocyanins in fruit wines

Blueberry wine is rich in anthocyanins. When acid-tolerant yeast YM1-1 and ester-producing yeast T1 were used as the experimental strains, the anthocyanin content in blueberry wine could reach 368.5 mg/L under the best fermentation process conditions, while the product was mellow and fruity [31]. At present, the blueberry wine on the market mainly includes compound fruit wine and blueberry beer. A new health fruit wine developed with blueberry and wolfberry can improve human immunity and relieve back pain caused by sedentary session, which is expected to become a new tonic health food in the post-epidemic era [32]. In addition, using concentrated blueberry juice, beer wort, brewer's yeast, etc. as the main raw materials, selecting low-temperature fermentation technology can brew blueberry beer, which has the characteristics of beer, but also add blueberry flavour and nutritional value [33].

Although the anthocyanin content of blueberry wine is higher than that of ordinary fruit wine, the utilization of anthocyanins is actually not high, and more than 42% of anthocyanins and more than 15% of polyphenols are stored in blueberry pomace [34]. Therefore, realizing the effective utilization of blueberry pomace can not only protect the environment and improve the utilization of anthocyanins in blueberry, but also bring economic benefits. In addition, the current share of blueberry wine in the domestic market is still small and needs to be developed. The vigorous development of blueberry wine industry can help improve the dietary structure and health of the residents, as well as increase the utilization rate of blueberry and drive agricultural development and rural revitalization.

5.2 Application of blueberry anthocyanins in juice drinks

Blueberry flesh is tender and juicy, rich in nutrients. There are a variety of blueberry juice on the market using blueberry flesh as raw materials, adding sweeteners, flavours and other food additives to make blended juice. Related research points out that the best formula of blueberry juice is 22.88% of raw blueberry juice, 10.80%

of sugar, 0.15% of citric acid, 0.10% of xanthan gum and sodium carboxymethyl cellulose, the prepared drink has a strong blueberry flavour, and the blueberry anthocyanin content is 4.96 mg/L [35]. In addition, fermenting blueberry juice with saccharomyces cerevisiae and lactic acid bacteria can improve the content of flavour substances in blueberry juice. The best flavour of fermented blueberry juice was obtained after continuous fermentation at 34℃ for 18h when the inoculum of brewer's yeast was 2‰ and the inoculum of Lactobacillus plantarum and Lactobacillus case was 1.5‰ [36].

During the processing and storage of blueberry juice, heat treatment, sterilization methods, and package translucency can affect anthocyanin stability, juice colour and nutritional value [37]. Therefore, relevant studies have been devoted to reduce anthocyanin loss during processing. It was found that blueberry juice pre-treated with steam can reduce anthocyanin decomposition and increase antioxidant activity of the solution compared to hot water bath [38]. In addition, blueberry juice at pH = 2.1 retained better total anthocyanin content, and the anthocyanin content of blueberry juice stored at 4°C was about 56% higher than that at 25°C [39] . Therefore, acidification and refrigerated storage of blueberry juice are effective methods for anthocyanin retention.

At present, there are many kinds of compound juice drinks with blueberry as the main ingredient in the market. From the perspective of medicinal food, the combination of blueberry juice concentrate and seven Chinese herbs such as Chinese wolfberry, chrysanthemum and cassia seeds is configured as a compound drink with the medicinal value of relieving visual fatigue [40] . A new small berry complex drink developed from three berries, raspberry, blueberry and indigo, provides a new solution to improve the utilization of small berries [41] . Combining the nutritional value of blueberry anthocyanins with other fruits and vegetables to develop a complex beverage with health benefits is a feasible direction for the development of blueberry products.

5.3 Application of blueberry anthocyanins in jam

Blueberry jam with blueberries as the main raw material, and supplemented with sugar, citric acid, maltose, etc., usually needn't add preservatives and artificial pigments [42]. As the ratio of blueberry pulp 30%, rose 7.5%, oligosaccharides 10%, konjac powder 4% to mix a low-sugar blueberry rose compound health jam, not only combines the flavour of blueberry and rose aroma, but also retains the nutritional value of blueberry anthocyanins, diabetics can eat it in moderation [43]. The blend of prune and blueberry pomace jam allows the product to maintain its blueberry colour while retaining a high anthocyanin content, thus combining the flavour and nutritional value of the product [44].

Anthocyanin losses were also present during the processing of blueberry jam. The change of anthocyanin content in blueberry jam stored at 21℃ for six months under light was determined by spectrophotometric method, and it was found that it decreased from 9.878 g/kg (Jersey variety) and 18.555 g/kg (Nelson variety) to 1.64 g/kg and 3.476 g/kg, respectively. Meanwhile, the average colour reduction rate of blueberry jam was 84.5%, and the reason for the decrease was the decomposition of anthocyanins in the processed products at high temperature [45]. Therefore, reasonable temperature control has a positive effect on the retention of anthocyanins in blueberry jam products.

5.4 Application of blueberry anthocyanins in fruit vinegar

Fruit vinegar is formed by fermentation process with various fruits as raw material, which not only has the taste of traditional vinegar, but also adds fruit flavour and nutrients, so it is often known for its health care function. The preparation of blueberry fruit vinegar is mainly divided into two steps, alcohol fermentation and acetic acid fermentation [46]. Taking blueberry juice and black rice juice as the main raw material, a black rice blueberry fruit vinegar drink made by multi-strain co-immobilization fermentation and mixed fermentation with sweetener and calcium lactate not only has the taste of black rice juice and blueberry juice, but also can realize the reuse of agricultural by-products [47] . Rich selenium blueberry fruit vinegar can let consumers get blueberry taste and nutrients in blueberry, and also moderate amount of selenium which can't be synthesized by human body, further optimize the nutritional value of blueberry fruit vinegar [48].

The loss of blueberry anthocyanins during fruit vinegar making was mainly present during the two fermentations. The anthocyanin content of blueberry fruit vinegar at each stage of fermentation was determined by vanillin-sulfuric acid colorimetric method, and it was found that after two fermentations, the anthocyanin content of blueberry decreased from the original 58.15 mg/dl to 36.16 mg/dl, with a loss rate of 37.78%, especially the loss in the first stage was higher, which might be caused by the dynamic changes of pH and temperature during the fermentation process. The chemical structure of anthocyanins was destroyed, and this conclusion is of great significance for the further development of blueberry fruit vinegar industry [49].

6. Outlook

Blueberry anthocyanins are a class of natural edible pigments, which are not only safe and non-toxic, but also have high nutritional value and pharmacological effects, thus receiving more and more attention in the food field. However, there are still some unfavourable factors limiting the large-scale production and utilization of blueberry anthocyanins, such as the instability of blueberry anthocyanins themselves, and the immature extraction and purification process. At present, most of the direct extraction methods cannot achieve a high extraction efficiency, and the purity needs to be improved. Therefore, it is recommended to combine ultrasonic assisted and enzymatic extraction methods to improve the anthocyanin extraction rate on the basis of existing technologies. In addition, although many foods with blueberry as raw material use the health function of anthocyanin as a publicity point, its health function has not been fully developed. Therefore, it should be combined with market demand, according to the nutritional needs of different age groups to develop more targeted blueberry health products, so as to maximize the nutritional function of blueberries. China's blueberry production areas are all over the north and south, with a complete range of products, which provides raw material support for the development of blueberry anthocyanin-related food industry. Therefore, using advanced technology to extract and purify anthocyanins from blueberries and develop more characteristic blueberry anthocyanin-related products has great potential in China.

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