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Why Blueberries?

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Abstract

Small berry fruits, like blueberries, are popular due to their appearance, are easy to eat and have a unique taste. Blueberries belong to the *Cyanococcus* section of the *Ericaceae* family. They are known to be one of the richest sources of natural antioxidants. Blueberries are a flavorful and colorful fruit, known for their health benefits attributed to numerous bioactive compounds with various therapeutic effects. The research aims to understand the metabolic and genetic factors that influence the nutritional and sensory qualities of blueberries. They can be consumed as either fresh or processed such as snacks, desserts, fruit salads, and with ice cream, yogurt or processed products such as frozen berries into conserves, purees and juices, dried fruit. They are rich in organic acids phenolic compounds that protect human against a wide variety of diseases since blueberry has high antioxidant activity. In this review, blue-berry worldwide production trend and the effect of blueberry nutrient content on human health will be discussed. And there are also some studies carried out in recent years to determine the chemical composition such as sugars, organic acids and phenolic compounds by advanced methodologies in blueberry fruits. This review could be beneficial for future studies will be conducted on blueberry production and its nutritional content.

Keywords

Blueberries, Superfoods, Biochemical Properties, Health Benefits, Nutrient Composition

1. Introduction

As a member of berries blueberries belong to the *Vaccinium* spp. and *Ericaceae* family. Blueberries are classified into four species based on morphological classification: rabbieye blueberry (*V. virgatum*), northern high shrub (*V. corymbosum*), southern high shrub (*V. formosum*), and low shrub (*V. angustifolium*). Among *Vaccinium* species, blueberries (*Vaccinium myrtillus* L.) and lingonber-

ries (*Vaccinium vitis-idaea*) are popular in the human diet. Due to having deep blue fruit color it's called blueberries and its production and consumption trend is increasing year by year recently in all over the world. Regarding its popularity, it has been known that it is not only preferable with its high value crop globally requested by the national and international market and a good health pack for healthy life, also. It is one of the five healthiest fruits blueberry varieties including no-chill to high chill ones. Consumer demand for products rich in phytochemicals is increasing as a result of greater awareness of their potential health benefits. Furthermore, blueberries have too many uses either fresh or processed such as frozen, beverages, drinking's as tea, wines, pastry, cakes, jellies, smoothies, ice cream, dried, freeze-dried jam, syrups, marmalades, fermented, yoghurt, cosmetic and pharmaceutical purposes. Compared to other berries, blueberry fruits are relatively suitable for export and have longer storage potential and are suitable for transportation. Recently, its production and consumption trend increased to more than one million tons. China and the United State was the world's largest and main blueberry producer. Therefore, the present work aimed to compile the existing evidence regarding the various potential benefits of blueberry production and consumption. Blueberries have a high profit per cropped area and offer higher incomes to the small farmers. Both berries are now seeing a continuous annual growth of market demands that are driving the expansion of the cultivation area. Blueberry world production has increased significantly over the last few years due to increasing demand for its health-promoting properties. For this reason, blueberry production has spread to some regions especially in the Mediterranean countries with non-optimal soil conditions (soil pH > 5.5). In the Mediterranean region, due to earliness advantages, shortage of suitable low-pH soils, high profit and benefits small farmers tend to grow blueberries in soilless cultivation under high plastic tunnels. Although the pH buffering capacity of growth media in soilless systems is generally relatively low, controlling the pH in those systems can be challenging.

2. Health Promoting Properties

According to epidemiological studies, the consumption of fruits has been systematically associated with a reduction of the risk of many diseases because phenolic compounds are present abundant in such fruits [1] [2]. Consumption of berry fruits has a vital importance for healthy life and prevents the risk of many chronic diseases such as cardiovascular and neurodegenerative diseases [3]. Blueberries are called süperfrüits since they are abundant in phenolic compounds as contribute to health promotion and are rich in flavonoid and antioxidant capacity. Blueberry fruits are rich in water and consist main glucose and fructose sugars and some varieties have also galactose and rhamnose sugars in low level and those sugars moieties associated with phenolic compounds. Addition to sugars, blueberries have a relatively high amount of organic acids mainly citric acid, minerals e.g. phosphorus, potassium and magnesium and fiber, particularly pec-

tins [4] [5] [6]. Kalt, W. [7] reported that according to the previous results on human clinical studies, and from animal and *in vitro* research health benefits of blueberries interests continue to grow. The same authors implied that blueberries contain a large number of phytochemicals, and most of them are anthocyanin pigments. Blueberries are rich in endogenous phenols, which are the most beneficial components in blueberries and include anthocyanins, tannins, pterostilbene and phenolic acids and are utilized as natural colorants and preservatives in foods [8]. As the most abundant compound, various anthocyanins compounds up to fifteen different ones such as monoarabinosides, monoglucosides and monogalactosides of cyanidin, peonidin, delphinidin, petunidin and malvidin previously reported in blueberries by the several authors [9] [10] [11]. It has been previously reported that gallic, syringic and vanillic acids and five different cinnamic acids as chlorogenic, the major derivative present, caffeic erulic, o- and p-coumaric acid, catechin, epicatechin, myricetin, kaempferol and quercetin. compounds also associated with flavonoids [12] [13] [14]. According to the previously published papers anthocyanins antioxidant activity has been associated not only with a direct quenching of reactive species but also with an upregulation of antioxidant and de-toxifying [15] [16]. Among the phenolic compounds anthocyanins are the main phenolics and because of anthocyanins rich phytochemicals probably blueberry make the greatest impact on health functionality. However, its positive health effect can not be seen when it consume rarely because of this the consumption of regular, moderate intake of blueberries can reduce the risk of cardiovascular disease, death, and type 2 diabetes, and with improved weight maintenance and neuroprotection. Kalt, W. [7] implied that these findings are supported by biomarker based evidence from human clinical studies and suggested to consume regularly. They can be employed as nutritional supplements and a daily intake of blueberries (about 1/3 cup) can prevent the onset of chronic diseases, such as obesity, diabetes, atherosclerosis, e.g. [17]. Due to having rich nutritional content, blueberry has been developed into many nutritional supplements for disease prevention and dietary regulation, such as blueberry lutein eye care tablets, blueberry dietary supplements as blueberry powder, pterostilbene capsules e.g. Because of its great importance some of the authors reviewed on the progress of extraction, purification and analysis methods of phenolic compounds from blueberry, recently [8].

3. Economic Importance

Blueberry growing is highly profitable that its trend increases day by day all over the world. Growers should consider the impact that a particular enterprise such as a blueberry planting can have on the overall financial stability of the farm business. In the previous papers, many economic analyzes have been revealed and this provides aware different farmers and gave rise to increasment of production [18] [19]. Asănică, A. [20] reported that compared to other valuable species, blueberries are still very expensive but highly appreciated fruits in the

market due to the well-known health benefits and magnific taste. The author implied that the plantation may run for decades and in this regard, even the investment is significant, revenue and exploitation time reward a lot. According to the economic analysis for the three production systems of blueberry, it was stated that whether the normal intensive culture system in beds, the super-intensive culture system in pots or the super-intensive culture system in pots and plastic-coated ones, they all have high profitability. The analysis of the cost and profitability of blueberry has been done according to the crop system taking into consideration several elements such as: the number of plants per hectare, the total duration of the exploitation, the value of the investment, the yield the cost of production e.g. The same author reported that although the initial investment is higher in the super intensive container system, at the end, this system turns out to be more profitable, bringing about a 10 times higher return than the intensive system. Under the plastic, the production system adds a cost to investment and maintenance, but it is about 2 times more profitable than outdoor pot system. However, yield for a mature farm will vary with cultivar or variety grown, soil type, and management practices, also. In conclusion, awareness of its health effects and high profitable traits trends in blueberry production increases year by year all over the world. Indoor pot system in comparison to outdoor system of blueberries in raised beds reveals that even the exploitation costs are higher, the system is about 2 times more profitable. The higher value of the earnings allows a faster depreciation of the investment and a lower recovery time for the super intensive system (2 years) compared to the intensive one on the raised beds (6 years). Even the initial investment in the pot system is higher, during the exploitation period, it proved to be more efficient up to 10 times more than system with raised beds.

4. Biocemical Analysis

Blueberries are rich in bioactive compounds, especially flavones and other polyphenolic compounds [21], which provide powerful antioxidant properties and are highly beneficial to human health [22]. The most important flavor components of blueberries are sweetness and sourness produced by sugar and acid. In addition, sugar and acid types and their relative proportions affect the taste of the fruit. The most abundant sugars in berries are glucose, fructose and the most abundant organic acids are malic acid and citric acid [23] [24]. The effect of biochemical substances in fruit on flavor is quite different. Sweet fruit taste is positively correlated with glucose, sucrose and total sugars, and negatively correlated with oxalic acid, citric acid, quinic acid and total acids [25]. Fruit acidity is one of the most important substances affecting the organoleptic quality of blueberries. It is the organic acid content that determines the acidity of the blueberry fruit [26]. Some of the studies carried out in recent years have been summarized to determine the sugars, organic acids and phenolic compounds and their amounts in blueberry fruit.

4.1. Organic Acids

Jiang *et al.* [26] reported that the effect of salicylic acid (SA), the main metabolite of aspirin, on organoleptic quality and organic acid metabolism in rabbit-eye blueberry (*Vaccinium virgatum*, 'Powderblue') during cold (4 ˚C) storage. The results showed that SA-treated fruit reduced fruit rot and weight loss delayed fruit softening and total soluble solids (TSS) reduction. In the study, the composition and content of organic acids were also analyzed by HPLC and according to Li *et al.* [27]. In that study, the authors implied that XDB-C18 column was used in the analysis (5 mm, 250 mm × 4.6 mm, Agilent, CA, USA). As a mobile phase, 0.01 M KH_2PO_4 buffer (pH = 2.7) prepared with 4% (v:v) methanol was used. During the analysis, the flow rate was 0.6 mL/min and the temperature of this column was set to 25˚C. Wavelength was used as 210 nm in the ultraviolet detector. Organic acid standards were preferred from Sigma-Aldrich (St. Louis, MO, USA). The most abundant organic acid in "Powderblue" blueberry fruit was malic acid, followed by quinic acid, citric acid and succinic acid. Malic acid content in the control fruit decreased from 8.66 $g \cdot kg^{-1}$ to 7.34 $g \cdot kg^{-1}$ in 30 days, while the amount of malic acid In SA-treated fruits did not show a significant change during storage.

Zheng and Akira [28] reported that, sugar and organic acid properties of 11 common blueberry varieties grown in two main producing regions (Weihai and Yingkou) in Northern part of China were investigated. In most of the analyzed blueberry samples, citric acid was the most abundant organic acid, with an average content of close to 80% of total acid. Extraction of organic acids was carried out according to the method reported by [29] with some modifications. The determination was performed using HPLC (LC-10A, Shimadzu, Japan), SPD-10A UV-VIS detector, and a C18 column (Ultimate LP-C18, 4.6 mm × 300 mm, 5 μm, Ultimate, China) according to the method reported by Ma *et al.* [30]. It was measured at 210 nm in the UV detector in HPLC. The operating conditions were programmed as follows: mobile phase, flow rate of 0.01 $mol \cdot L^{-1}$ KH_2PO_4 solution, 0.5 $mL \cdot min^{-1}$; column temperature, 40˚C; and the injection volume is 10 μL. Acid compounds were determined and measured by comparing the relative retention times and peak areas of the standard substances and the results are expressed as $mg \cdot g^{-1}$.

4.2. Sugars

Zheng and Akira [28] studied blueberry sugars and the authors implied that glucose and fructose were the main sugars. Correlation analysis showed that glucose, fructose and sucrose were positively correlated with total sugar content. Zhang *et al.* [27] studied the enzymes involved in the metabolism of sugars and organic acids during the three ripening stages (green, pink and blue) of highbush blueberry (*V. corymbosum*) fruits, they determined that glucose, fructose and sucrose increased gradually from the green period to the blue period. Sugar contents were determined by HPLC, which is one of the reliable and repeatable

chromatographic methods and provide qualitative and quantitative results. The column used in the study was an Agilent Carbohydrate column (5 μm, 4.6 mm × 250 mm), the mobile phase was acetonitrile-water (75:25), and the flow rate was 1.0 mL/min; column temperature was 30˚C and injection volume was 10 μL.

In another study Zhen and Akira studied the sugar profile of blueberries [28], the extraction of sugars was carried out according to a previously reported method by the authors [28]. Sugar determination was carried out using an Ion Chromatograph (ICS-5000, Dionex, USA) with a conductivity detector, a guard column (IonPac AG23, 4 mm × 50 mm, Dionex, USA) and an anion exchange analytical column (Dionex CarboPacTM PA10), 4 mm × 250 mm, Thermo Fisher Scientific, USA) according to a previously reported method [31]. HPLC conditions were as follows mobile phase, 0.2 mol·L^{-1} NaOH solution; flow rate, 1.0 mL·min^{-1}; column temperature, 30˚C; and injection volume, 10 μL. Individual sugars were identified and quantified by comparison with the retention times and peak areas of individual sugar standards (prepared to contain between 5 and 50 mg·L^{-1}). The results were expressed as mg·g^{-1}.

In another study conducted by Okan *et al.* [32] and the authors compared the antioxidant activities, anthocyanin, sugar and phenolic compound contents of blueberry varieties produced in both Turkey and other varieties in the world. Ten standard sugar compounds were analyzed using HPLC-RID to determine the sugar content in blueberry varieties. Seperations were performed on a reverse-phase Nucleosil NH_2 analytical column operating at room temperature with a flow rate of 1 mL·min^{-1}. The sample injection volume was 20 μL. Elution was effected using an isocratic elution of 70% aqueous acetonitrile as a solvent. Compounds were identified by comparing their retention times. According to the previous results, HPLC coupled with RID is one of the most rapid, reliable and repeatable method for identification and quantification of individuals sugars.

4.3. Phenolic Compounds

Okan *et al.* studied that [32] phenolic analysis and the authors used seventeen phenolic compound standards to identify and quantify of phenolic compounds by HPLC. HPLC-DAD analysis of the biomass methanol extract was performed according to the method described by Hatipoğlu *et al.* [33], with some changes in the gradient flow of the mobile phase. While doing this analysis, the chromatographic details were as follows; the HPLC-DAD system (Agilent Technology) consisted of quaternary pumps and an auto injector connected to a DAD (diode array detector). An AC-18 reverse phase column (250 mm × 4.6 mm id, 5 μm particle sizes, HICHROM, UK) was used for the analysis which was fixed in the column oven (1260 TCC). The mobile phase was a mixture of solvent A (2% AcOH in water) and solvent B (70:30, acetonitrile/water).

Blueberry fruit is very rich in organic acids (citric, ascorbic, phenolic acids and tannins), phenolic compounds (stilbenoids, tannins and flavonoid compounds including anthocyanin, flavanone, flavanol and quercetin). Blueberry,

which is rich in sugars, mineral substances, vitamins, fibers and pectins, is a berry group preferred by consumers due to its high nutritional value [34]. Bai *et al*. [8] investigated extraction and detection methods of some phenolic compounds in blueberry fruit. They explained each method in detail. Blueberries contain high levels of anthocyanins. There are several methods for extracting anthocyanins from blueberries. Some of these methods are; solvent extraction, ultra-sound-assisted extraction, microwave-assisted extraction (MAE), solvent, enzyme-assisted extraction, extraction using supercritical fluid carbon dioxide. In addition, separation, purification detection and analysis methods are also mentioned. The most commonly used extraction method for anthocyanin is solvent extraction. Some solvent extraction methods may cause partial or complete hydrolysis of anthocyanin and decrease in its activity. SEM can be applied with simple equipment and easily, but it has disadvantages such as long extraction time, low efficiency, high solvent consumption and high temperature demand [35]. Ultrasound-Assisted Extraction (UAE), is a new green extraction method. Zhang *et al*. [36] reported that extraction of anthocyanins from blueberry fruits. The application conditions that it can be extracted optimally were 40˚C, 400 W ultrasonic power. They reported that the extraction time was 40 minutes and the best efficiency was achieved with 108.23 mg/100g DW.

Chromatographic Method; GC requires high temperature and the sample can decompose at high temperatures. GC can be used to separate and analyze small phenolic acid compounds. GC/MS is a useful method for analyzing phenolic acids in plant samples. HPLC is used for the quantitative analysis of phenolic acids in plants and its principle depends on the chemical properties of the components, extraction methods, particle size, storage time and conditions. In HPLC methods, phenolic acids can be determined using different columns, mobile phases, column temperatures and different flow rates. Water methanol and acetonitrile are the most commonly used mobile phases. In addition, mixture of solvents such as formic acid, ammonium acetate and acetic acid can sometimes be added to prevent peak pollution. Microwave-Assisted Extraction (MAE), it is one of the methods used in the extraction of tannins, an important group of phenolic compounds. Zhang *et al*. [36] defined the advantages of MAE as shorter extraction time and less solvent requirement compared to conventional solid-liquid extraction, and the disadvantage of this method is the high cost of equipment required for mass production and thermal degradation of tannins.

In conclusion, according to the previously published results blueberry fruits either fresh or its products are a good source of an antioxidant and potent scavenger of free radicals. In addition, results demonstrated that the quality of blueberry is of multi variate nature, and the analysis requires the aiding of chemometric analysis. HPLC coupled with different detectors such as MS, RID, DAD, UV can be used as the most convenient method for detection of individual sugars, organic acids and phenolic compound analysis.

5. Conclusions

In this paper, general information (botanical classification, production methods, cultivation methods) about the blueberry fruit, which has a rich content in terms of nutrients, is given. The positive effects of blueberry on human health and its economic importance were emphasized. It has been concluded that awareness of the beneficial effects on health and high profitability in blueberry production is increasing every year all over the world. In addition to the antioxidant and polyphenolic characters found in high amounts in the blueberry fruit whose biochemical content was examined, sugar and organic acid compounds, which are two of the most important organoleptic quality in the fruit, are also found. In the previous studies examined, it was determined that the metabolism of these substances (sugar and organic acid) is tightly related to maturation. As a result of the studies investigating the analysis methods of sugar, organic acid and phenolic compounds in blueberry, following results were obtained:

1) Glucose and fructose are the main sugar compounds in blueberry, and the amount of these increases with ripening.

2) Malic, citric and quinic acid are the most common organic acid substances.

3) HPLC, a chromatographic method, is widely used in the analysis of sugars and organic acids.

4) Although the most common method is HPLC to determine phenolic compounds, different methods such as GC, ultrasound-assisted extraction, microwave-assisted extraction, solvent, enzyme-assisted extraction are also used. Each method has advantages and disadvantages over the other.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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