

REVIEW ARTICLE


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Vaccinium myrtillus L. Fruits as a Novel Source of Phenolic Compounds with Health Benefits and Industrial Applications - A Review



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Abstract: Consumers' demand for healthier foods with functional properties has had a clear influence on the food industry and in this sense, they have been attaching natural sources of bioactive ingredients into food products. *Vaccinium myrtillus* L. (bilberry) is known to be a functional food, presenting its fruits in the form of a small dark blueberry. This coloration is due to its high content in anthocyanin, being also associated with bilberries' beneficial health effects. In the bilberry industry, there is a very high annual loss of this fruit due to the less aesthetic shape or appearance, in which they cannot be considered suitable for sale and are therefore disposed of as biological waste. Therefore, it is of great importance to valorize this fruit and this review aimed to completely characterize the fruits of *V. myrtillus* in order to comprehend the relationship between their consumption and the beneficial effects regarding consumer's health. Thus, this review provides a description of the nutritional and bioactive compounds present in bilberry fruits, followed by their beneficial health effects. An overview of the natural pigments present in these fruits was also explored, focusing particularly in the anthocyanins composition, which represents the most widely studied class of bioactive compounds of *V. myrtillus* fruits. Finally, industrial applications of these fruits and by-products, as an efficient approach to the production of value-added products with economical and environmental impact, were also discussed.

In general, *V. myrtillus* is a rich source of micronutrients and phytochemical compounds, such as organic acids, sugars, vitamins, fibers and phenolic compounds (anthocyanin and non-anthocyanin compounds), with nutritional and functional properties, that justify the growing interest in these berries, not only for food applications, but also in the pharmaceutical industry.

Keywords: *Vaccinium myrtillus* L., phenolic compounds, natural pigments, health benefits, anthocyanin, vitamins.

ARTICLE HISTORY

Received: November 25, 2019
Accepted: March 8, 2020

DOI:

10.2174/138161282666200317132507



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1. INTRODUCTION

Bilberry (*Vaccinium myrtillus* L.) is a dark blue fruit that belongs to the genus *Vaccinium*, family *Ericaceae*, which comprises around 450 species of trees, shrubs, sub-shrubs and hemiphytes distributed all over the world [1]. These fruits are usually consumed in the fresh form, however, due to their short shelf life, they are also frozen, dried or processed in the form of jams, juices and wines or liqueurs [2]. Bilberries have been conventionally consumed and used in traditional medicine since ancient times, being harvested from wild bushes, although currently the cultivation of these fruits is commonly performed in northern and eastern Europe, and also in northern Africa [3].

These fruits are described as being an important source of phenolic compounds and carotenoids, also containing moderate levels of other micronutrients such as vitamins. Nevertheless, it is due to their high levels of anthocyanins that these fruits are recognized for their bioactive properties [4]. Anthocyanins, besides being responsible for the blue color of bilberries, are the major group of flavonoids in these berries and have been associated to many beneficial health effects, such as prevention or treatment of cancers, cardiovascular diseases, obesity, diabetes, aging diseases, urinary tract infections and periodontal diseases [5, 6]. The high content of these flavonoids has also highlighted these fruits as interesting sources of

coloring compounds for food and pharmaceutical applications [7]. According to the literature, the anthocyanin profile in bilberry consists of fifteen main compounds, derived from five aglycones (delphinidin, cyanidin, petunidin, peonidin, and malvidin) linked to different sugar moieties (galactose, glucose and arabinose) [8, 9]. Consumers are increasingly concerned about choosing foods labeled as healthier and more natural. In this sense, the food industry has been exploring various natural sources in order to enrich different products [10]. Currently, there are many products in the market with the incorporation of berries, namely bilberry, highlighting their beneficial effects, usually their antioxidant potential [11].

In this review, the nutritional characterization and phenolic composition of *V. myrtillus* L. fruits, and also their beneficial health effects, are discussed. Natural pigment exploitation and industrial application are also the main focus of this review.

2. NUTRITIONAL CHARACTERIZATION

Due to their reduced environmental adaptability, it is extremely difficult to cultivate bilberries. Moreover, there are still few producers of these fruits because of their low productivity, justified by the small size of the fruits when compared to similar blueberries (*V. corymbosum* L.), which have a larger fruit size. Most of the available bilberries (*V. myrtillus* L.) are mainly obtained from wild plants that grow in northern and southern Europe [12], while highbush blueberries (*V. corymbosum*) are originated from North America [13]. Sometimes bilberry is also called blueberry because both have a similar appearance and are close relatives, but the true blueberry is native to the United States [14]. According to Coudun & Gégout [15], there are several environmental factors, such as climatic con-

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ditions, soil type or cultivation conditions, among others, which may affect the plant growth and consequently, fruit productivity [15]. In the case of bilberries plants, some authors report that light availability influences plant development and consequently, fruit yield [16]. Additionally, it is found that weather conditions can also influence the nutritional and chemical composition of the fruits. This was proven through studies performed from several authors, which demonstrated that bilberries fruits growing in northern latitudes present higher phenolic contents than those from southern latitudes [17, 18]. There are more than 70 registered ethnomedical and food uses of 36 *Vaccinium* species, being *V. myrtillus*, the species with the highest number of described uses [5]. The American Herbal Products Association classified *V. myrtillus* as a Class 1 product category assigned when considering it as safe to consume [19]. The fruits of this plant are usually consumed as food, while the leaves or aerial parts are commonly associated with their medicinal use [5, 20]. *V. myrtillus* berries can be consumed fresh, frozen and dried, as well as in their processed forms, such as juices, jams or food supplements [14].

The interesting nutritional and functional properties described for bilberries justify the growing interest in these berries. The fruits of *V. myrtillus* are rich sources of micronutrients and phytochemical compounds with health benefits, such as organic acids [21-23], sugars [21, 22], vitamins [24], fibres [25], and phenolic compounds [20, 26]. The organoleptic properties of these fruits are clearly influenced by the different content of the mentioned compounds, namely sugars and organic acids [27]. Since sugars and organic acids are considered the main soluble constituents of berries, some authors describe their content as a direct effect on fruit taste and ripeness and consequently, on consumer's acceptability [26, 28]. According to Michalska & Łysiak [29], besides the taste of the bilberry fruits, the vitamin C content is important in the consumption of these fruits because 100 g of the fruit provides, on average, 10 mg of ascorbic acid, which is equal to 1/3 of the daily recommended intake [29]. However, Uleberg *et al.* [21] indicated that cool temperatures and genetic factors influence the taste of bilberry fruits, which explained the sweeter taste of fruits cultivated in Northern areas in comparison to those from Southern areas [21].

Bilberries have different organic acids in their composition (malic, citric, gallic, chlorogenic, ascorbic and quinic acids), being citric, malic and quinic acids the main ones. Fructose and glucose are described as the highest group of free sugars in bilberry, although the presence of sucrose has also been reported as a relevant constituent [21, 22, 26].

The mineral content in fruits and vegetables is very important, contributing to their nutritional value. However, it is necessary to balance these mineral elements in the human diet, because trace elements can be toxic when consumed at a higher amount than the recommended intake. Some wild fruits, including bilberry, have been explored and identified as interesting sources of minerals [30, 31]. Regarding mineral composition, these fruits have three main macroelements (Ca, P, and Mg) and seven microelements (Fe, Ba, Na, Mn, Cu, Sr, and Zn) [16]. Comparative studies between wild and commercial fruits have shown that wild bilberry has higher concentrations of minerals than commercial fruits [16]. As mentioned above, these differences may be justified by the cultivation conditions, namely the soil composition [32].

Regarding the proximate composition, the fresh fruits contain around 84 % of water, 9.7 % of carbohydrates, 0.6 % of proteins, 0.4 % of fats, and 3-3.5 % of fibres, with an estimated energetic value of 192 kJ [29].

3. BIOACTIVE COMPOUNDS

Phenolic compounds are a group of secondary plant metabolites recognized for their health-protective action, namely as an antioxidant [2, 33], anti-inflammatory [34] and antihypertensive [35], antimicrobial [36] and anticancer agents [37]. The fact that *V. myrtillus*

has a high content of phenolic compounds may account for the growing demand for this fruit. *Vaccinium myrtillus* L. is rich in polyphenols, with anthocyanins flavan-3-ols, flavonols and phenolic acids as the main group of compounds identified by several authors [2, 13, 42, 43, 46, 91, 106] (Fig. 1). Flavonoids, such as flavan-3-ols (catechins and proanthocyanidins) and flavonols (*i.e.*, kaempferol, quercetin, myricetin), phenolic acids (mainly hydroxycinnamic and hydroxybenzoic acids) and derivatives of stilbenes, are the major non-anthocyanin polyphenols present in *V. myrtillus* fruits [20, 29, 38].

The flavan-3-ols are usually found with varying concentrations in commonly consumed foods such as fruits, legumes, vegetables and nuts. They are natural antioxidants that may contribute to prevent rancidity due to the oxidation of unsaturated fats and stabilize food colours, as well as being involved in chemoprevention against a variety of diseases [39]. The most common flavan-3-ols are procyanidins, consisting of (epi)catechin oligomers [39, 40] and can be classified into A-type and B-type, depending on the stereo configuration and linkage between monomers. B-type procyanidins are the most abundant, with procyanidins B1, B2, B3 and B4 occurring most frequently [39]. Table 1 shows the contents of the main phenolic compounds determined in bilberries in recent studies by several authors. Data are expressed in different units and as a dry or fresh weight basis, as reported by the authors. Regarding flavan-3-ols, the concentrations of catechin, epicatechin, procyanidin dimers and trimers, and gallocatechin are collected, as well as total flavan-3-ol contents. As it can be seen, only catechin was identified and quantified in all the represented studies.

The individual and total contents of flavonols in fruits of *V. myrtillus* are also presented in Table 1. All the reported studies identified kaempferol, quercetin and myricetin glycosides as the main compounds, although Zorenc *et al.* [2] also reported laricitrin, syringetin and isorhamnetin glycosides [2].

Phenolic acids are phenols that possess one carboxylic acid function and include two main groups: the hydroxycinnamic and hydroxybenzoic acids [41]. Caffeic, *p*-coumaric, vanillic, ferulic, and protocatechuic acids are widely present in many plants, whereas gentisic and syringic acids have a more restrictive distribution, being reported in *V. myrtillus* by Değirmencioğlu *et al.* [42] and Tumbas *et al.* [43] (Table 1).

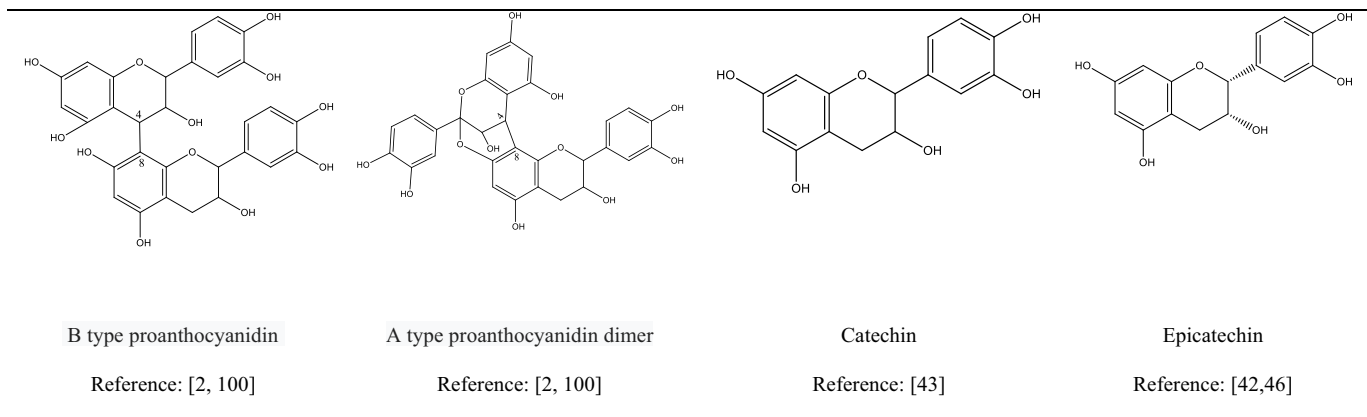
Regarding stilbenes, there are few studies that reveal its presence in bilberry fruits, however, Ehalá, Vaher, & Kaljurand [44] and Može *et al.* [13] identified and quantified *trans*-resveratrol in concentrations ranging 97.8 % and 0.3 mg/100 g fw, relating their presence to the antioxidant activity of the fruit [13, 44].

Anthocyanins are pigments commonly present in plants, where they are responsible for the characteristic blue, red or purple colour. Actually, anthocyanins are considered the most important water-soluble pigments in plants, being particularly relevant in flowers and berries, namely bilberry, cherry or blackcurrant [45]. Many studies describe anthocyanins as the most abundant polyphenol class in *V. myrtillus* fruits, being responsible for the characteristic dark blue colour of bilberry. Glucosides, galactosides and arabinosides of delphinidin, cyanidin, petunidin, peonidin and malvidin characterize the anthocyanin profile of bilberry [46]. Owing to their interest and relevance in the sensory and health properties of fruits, a more profound discussion regarding this group of compounds is made in the following section.

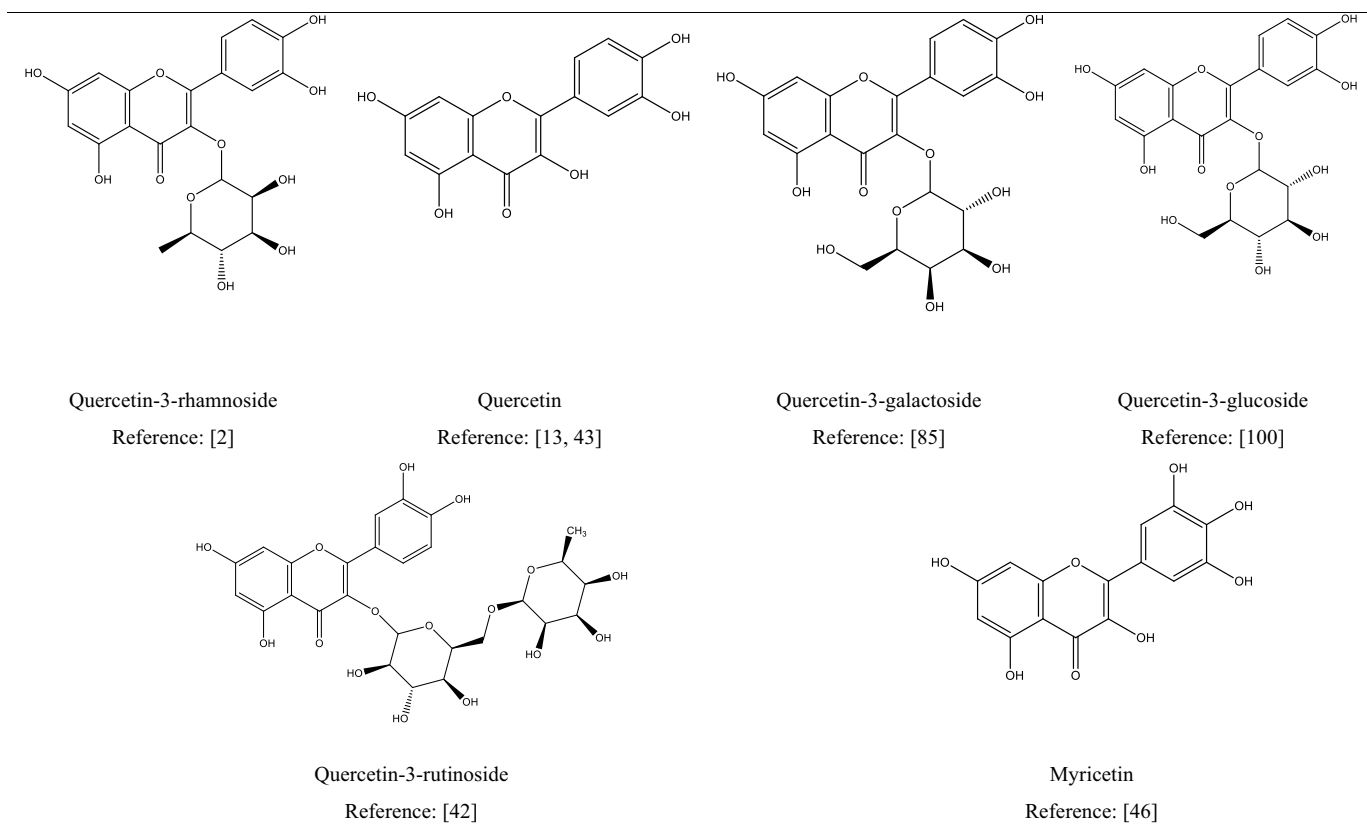
4. NATURAL PIGMENTS

Textile, food, painting, cosmetic, and pharmaceutical industries are some of the sectors where colour plays a very important role. Research on natural pigments is of enormous importance as it has a direct impact on environmental, economic and human health safety [47]. Natural pigments generally extracted from different parts of plants, namely seeds, fruits, vegetables or roots, have played an

Flavan-3-ols



Flavonols



Phenolic acids

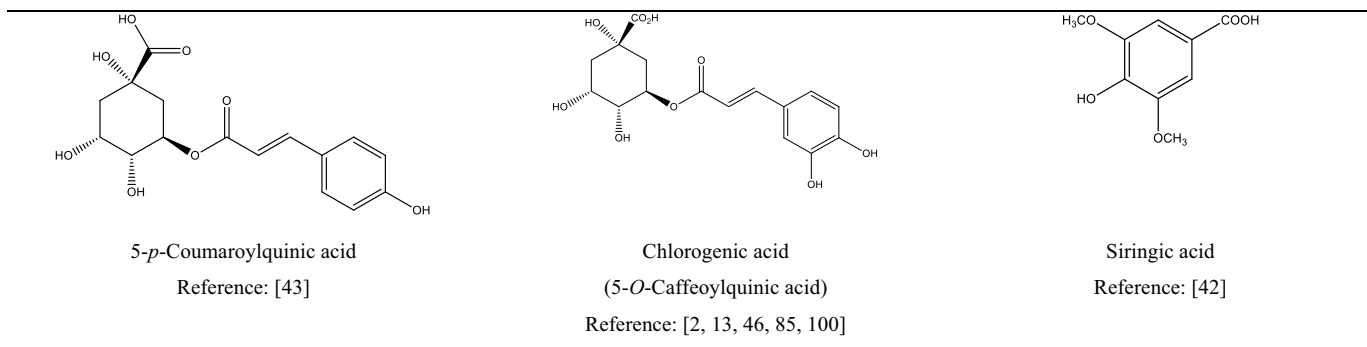
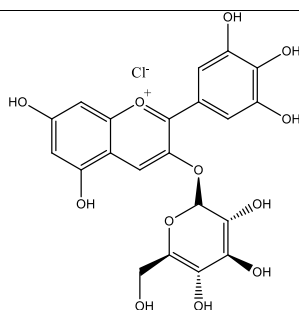


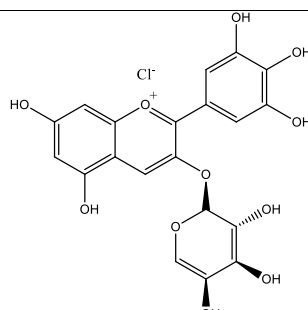
Fig. (1) Contd....

Anthocyanins



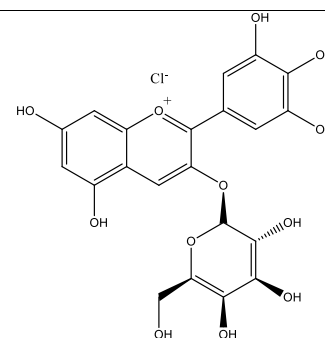
Dephinidin-3-glucoside

Reference: [85, 103]



Delphinidin-3-arabinoside

Reference: [21, 101]



Delphinidin-3-galactoside

Reference: [21, 22, 95, 102, 104]

Fig. (1). Structural formulas of the main bioactive compounds found in bilberry.

Table 1. Content levels of flavan-3-ols, flavonols and phenolic acids in fruits of *Vaccinium myrtillus* L.

Phenolic Compounds	[2]	[43]	[85]	[100]	[13]	[42]	[46]
Catechin	29.67 mg/100 g (dw)	15.04 µg/100 g (dw)	91.64 mg/g (dw)	96 mg/100 g (dw)	0.2 mg/100g (fw)	2.72 mg/kg (fw)	13.9 mg/kg (dw)
Epicatechin	59.14 mg/100 g (dw)	nd	nd	7.9 mg/100 g (dw)	2 mg/100g (fw)	28.54 mg/kg (fw)	255 mg/kg (dw)
Procyanidin dimers	72.10 mg/100 g (dw)	nd	nd	117 mg/100 g (dw)	nd	nd	nd
Procyanidin trimers	59.33 mg/100 g (dw)	nd	nd	109 mg/100 g (dw)	nd	nd	nd
Gallocatechin	35.72 mg/100 g (dw)	nd	nd	nd	nd	6.66 mg/kg (fw)	nd
Total flavan-3-ols	244.09 mg/100 g (dw)	15.04 µg/100 g (dw)	91.64 mg/g (dw)	329.9 mg/100 g (dw)	2.2 mg/100g (fw)	37.92 mg/kg (fw)	268.9 mg/kg (dw)
Myricetin hexoside	29.61 mg/100 g (dw)	nd	28.60 mg/g (dw)	nd	nd	nd	nd
Myricetin pentoside	3.34 mg/100 g (dw)	nd	nd	nd	nd	nd	nd
Myricetin-3-glucuronide	2.23 mg/100 g (dw)	nd	nd	nd	nd	nd	nd
Myricetin-3-rhamnoside	2.12 mg/100 g (dw)	nd	nd	nd	nd	nd	nd
Laricitrin-3-galactoside	0.10 mg/100 g (dw)	nd	nd	nd	nd	nd	nd
Laricitrin-3-glucoside	4.33 mg/100 g (dw)	nd	nd	nd	nd	nd	nd
Laricitrin-3-glucuronide	0.34 mg/100 g (dw)	nd	nd	nd	nd	nd	nd
Myricetin	6.20 mg/100 g (dw)	40.66 µg/100 g (dw)	nd	nd	0.4 mg/100g (fw)	nd	36.9 mg/kg (dw)
Syringetin-3-galactoside	8.69 mg/100 g (dw)	nd	nd	nd	nd	nd	nd
Syringetin-3-glucoside	1.01 mg/100 g (dw)	nd	nd	nd	nd	nd	nd
Isorhamnetin-3-arabinoside	nd	nd	nd	6 mg/100 g (dw)	nd	nd	nd
Isorhamnetin-3-galactoside	5.27 mg/100 g (dw)	nd	nd	nd	nd	nd	nd
Isorhamnetin-3-glucoside	36.60 mg/100 g (dw)	nd	nd	nd	nd	nd	nd
Quercetin-3-arabinoside	nd	nd	38.14 mg/g (dw)	nd	nd	nd	nd
Quercetin-3-rhamnoside	67.36 mg/100 g (dw)	51.80 µg/100 g (dw)	nd	6 mg/100 g (dw)	nd	nd	nd

(Table 1) Contd....

Phenolic Compounds	[2]	[43]	[85]	[100]	[13]	[42]	[46]
Quercetin-7-rhamnoside	nd	nd	nd	48 mg/100 g (dw)	nd	nd	nd
Quercetin-3-galactoside	4.03 mg/100 g (dw)	nd	45.78 mg/g (dw)	nd	nd	nd	nd
Quercetin-3-glucuronide	2.82 mg/100 g (dw)	nd	nd	nd	nd	nd	nd
Quercetin-3-glucoside	4.03 mg/100 g (dw)	nd	nd	236 mg/100 g (dw)	nd	nd	nd
Quercetin-3-(6''-acetyl) glucoside	nd	nd	nd	97 mg/100 g (dw)	nd	nd	nd
Quercetin-3-pentoside	nd	nd	29.29 mg/g (dw)	64 mg/100 g (dw)	nd	nd	nd
Quercetin-3-rutinoside	nd	51.80 µg/100 g (dw)	27.03 mg/g (dw)	196 mg/100 g (dw)	0.2 mg/100g (fw)	3.84 mg/kg (fw)	nd
Quercetin	nd	243.30 µg/100 g (dw)	4.40 mg/g (dw)	146 mg/100 g (dw)	0.8 mg/100g (fw)	1.62 mg/kg (fw)	2.2 mg/kg (dw)
Kaempferol	nd	15.64 µg/100 g (dw)	nd	30 mg/100 g (dw)	nd	1.72	nd
Kaempferol-3-glucoside	nd	nd	nd	nd	nd	nd	nd
Kaempferol-3-glucuronide	2.82 mg/100 g (dw)	nd	nd	nd	nd	nd	nd
Kaempferol-3-(6''-acetyl)glucoside)	nd	nd	nd	8 mg/100 g (dw)	nd	nd	nd
Kaempferol-3-rhamnoside	nd	nd	nd	10.3 mg/100 g (dw)	nd	nd	nd
Total flavonols	173.03 mg/100 g (dw)	403.2 µg/100 g (dw)	173.24 mg/g (dw)	847.3 mg/100g (dw)	1.4 mg/100g (fw)	57.82 mg/kg (fw)	39.1 mg/kg (dw)
Dicaffeoylquinic acid	nd	nd	nd	31.6 mg/100g (dw)	nd	nd	nd
4-O-Caffeoylquinic acid	nd	nd	nd	37 mg/100g (dw)	nd	nd	nd
<i>trans</i> 5-O-Caffeoylquinic acid	818.39 mg/100 g (dw)	nd	nd	649 mg/100g (dw)	nd	nd	nd
<i>cis</i> 5-O-Caffeoylquinic acid	31.84 mg/100 g (dw)	nd	nd	nd	nd	nd	nd
Caffeic acid derivatives	66.83 mg/100 g (dw)	nd	nd	nd	nd	nd	nd
Caffeic acid	4.24 mg/100 g (dw)	15.33 mg/100 g (dw)	6.26 mg/g (dw)	nd	0.3 mg/100g (fw)	5.46 mg/kg (fw)	3.1 mg/kg (dw)
Chlorogenic acid	nd	21.0 mg/100 g (dw)	6.40 mg/g (dw)	nd	23.1 mg/100g (fw)	1.25 mg/kg (fw)	1320 mg/kg (dw)
3- <i>p</i> -Coumaroylquinic acid	nd	nd	nd	7.3 mg/100g (dw)	nd	nd	nd
4- <i>p</i> -Coumaroylquinic acid	nd	nd	nd	3.5 mg/100g (dw)	nd	nd	nd
5- <i>p</i> -Coumaroylquinic acid	3.04 mg/100 g (dw)	57.87 mg/100 g (dw)	nd	29.2 mg/100g (dw)	nd	nd	nd
<i>p</i> -Coumaric acid hexoside	3.99 mg/100 g (dw)	nd	nd	nd	0.3 mg/100g (fw)	7.63 mg/kg (fw)	1.20 mg/kg (dw)
Coumaroyl iridoid isomers	31.68 mg/100 g (dw)	nd	nd	82.5 mg/100g (dw)	nd	nd	nd
Coumaric acid derivatives	3.18 mg/100 g (dw)	nd	nd	243.7 mg/100g (dw)	nd	nd	nd
Ellagic acid	nd	9.99 mg/100 g (dw)	nd	nd	1.2 mg/100g (fw)	nd	nd
Ferulic acid	4.84 mg/100 g (dw)	22.76 mg/100 g (dw)	nd	7.3 mg/100g (dw)	0.4 mg/100g (fw)	10.60 mg/kg (fw)	0.44 mg/kg (dw)
Gallic acid	nd	7.24 mg/100 g (dw)	nd	nd	6.2 mg/100g (fw)	19.19 mg/kg (fw)	33.3 mg/kg (dw)
Gallic acid derivatives	nd	nd	nd	36 mg/100g (dw)	nd	nd	nd
Protocatechuic acid	nd	19.41 mg/100 g (dw)	7.66 mg/g (dw)	nd	nd	nd	nd
Syringic acid	nd	27.43 mg/100 g (dw)	nd	nd	nd	637.43 mg/kg (fw)	nd
Vanillic acid	nd	nd	nd	nd	nd	532.97 mg/kg (fw)	nd
Total phenolic acids	968.02 mg/100 g (dw)	181.03 mg/100 g (dw)	20.32 mg/g (dw)	1127.1 mg/100 g (dw)	31.5 mg/100 g (fw)	1214.53 mg/kg (fw)	1358.04 mg/kg (dw)

Abbreviations used: nd- Not detected, fw- Fresh weight, dw- Dry weight

important role since ancient times and particularly, in textile dyeing [48]. For its part, the food industry routinely applies pigments to foods to restore colour losses or make them more attractive to consumers. Food colorants can be classified as artificial or natural. It is estimated that around 31 % of the colorants used by the food industry are obtained from natural sources, such as plants, animals and microorganisms [49].

Currently, due to several published scientific studies that associate the consumption of certain artificial additives with potential adverse health effects, an aversion has been created to this type of colorants. This has put pressure on the industry that is looking for natural alternatives capable of performing the same functions as artificial colorants and additionally offering bioactive properties [50].

Several studies have shown that extracts rich in anthocyanins could be used not only as colorants but also as potential functional food ingredients and/or dietary supplements due to their biological properties [21]. Anthocyanins are classified in the USA as natural food colourings in the fruit (21 CFR 73,250) and vegetable (21 CFR 73,260) category, and in the EU, they are included as additives under code E163 [51].

Anthocyanins are the most abundant and widely studied class of bioactive compounds in the fruits of *V. myrtillus*. The anthocyanin profile in bilberry is characterized by fifteen main compounds, among which delphinidin glycosides constitute the best represented ones, as it is shown in Table 2, where the concentrations determined by different authors are collected.

The type and quantity of anthocyanins are affected by several internal and external factors, such as genetic factors (varietal and regional), environmental variables (light intensity, temperature, humidity, the use of fertilisers), fruit size, ripening stage, pre-harvest environmental conditions and storage. The influence of regional factors on the anthocyanin content in bilberry fruits has been shown by Uleberg *et al.* [21], which demonstrated differences between anthocyanin content in samples collected in northern and southern Finland, with higher anthocyanin and total polyphenol contents in the northern region.

The use of extracts obtained from natural sources rich in anthocyanins has gained prominence, in view of their use by the food and pharmaceutical industries, with different purposes. The low stability of these compounds requires the utmost care. In most cases, water and alcoholic solutions are used as extraction solvents under acidic conditions, so as to maintain the stability of flavylium ion and increase the intensity of the red hue of the anthocyanin [52]. Anthocyanins, in addition to making products more attractive, play a key role as bioactive compounds with putative healthy effects. Studies developed by Prior *et al.* [53] and Fernandes *et al.* [54] suggest the potential of anthocyanins to be used as nutraceuticals and functional food ingredients to fight obesity and type 2 diabetes. Fernandes *et al.* [54], in a study carried out with mice, showed the hypoglycemic effects of cyanidin-3-glucoside, reducing blood glucose levels and enhancing insulin sensitivity by the downregulation of the retinol binding protein-4 expression. Prior *et al.* [53] found that purified anthocyanins from *V. myrtillus*, as well as blueberry juice, could prevent dyslipidemia and obesity in the same animal model. Moreover, Yamaura *et al.* [55] showed that cyanidin-3-glucoside and a quercetin fraction from bilberry have beneficial dermatological effects and inhibit inflammation of injured skin, correcting the Th1/Th2 balance and reducing IL-17.

Not only individual anthocyanins from *V. myrtillus* present beneficial effects, but Cooke *et al.* [56] demonstrated that one extract with 40 % anthocyanins, containing the main fifteen bilberry compounds, possessed chemopreventive effects for colorectal cancer in rats.

The use of *V. myrtillus* fruits as a source of natural pigments has also been evaluated by some authors. Thus Camire *et al.* [57] compared breakfast cereals coloured with an extract rich in anthocyanin from *V. myrtillus* and an extract obtained from grape juice. The results concluded that bilberry concentrate possessed much higher anthocyanin content (125.4 mg/100 g, fresh weight) than the grape juice extract (28.3 mg/100 g, fresh weight) and storage over a period of three months did not change the anthocyanin content in bilberry extrudates.

Although the external appearance of food is the organoleptic characteristic that gains the first attention of consumers, it is necessary to assess acceptability. Pasqualone, Bianco, & Paradiso [58] evaluated the acceptability of consumers to biscuits added with anthocyanin extracts obtained from bilberry fruits. This study revealed that most consumers, while detecting differences in colour when compared to biscuits without anthocyanin incorporation, accepted the anthocyanin-enriched product satisfactorily. Another study revealed that the incorporation of *V. myrtillus* in ice-cream increased the levels of some major and minor essential elements, such as K, Se, Mn and Zn, and presented a good score by the panelists. Therefore, the addition of *V. myrtillus* extracts to ice cream was recommended as a natural source to increase the nutritional value and improve physicochemical properties, thus increasing the added value of the product [59].

5. HEALTH BENEFITS

The berries of *V. myrtillus* are popular worldwide and consumed since ancient times, constituting an important part of the usual diet, as well as used in several popular medicines [9]. These fruits are recognized as rich natural sources of polyphenols and other bioactive compounds with health benefits. Among others, antioxidant, anti-obesity, anti-proliferative, cardioprotective, anti-inflammatory, ocular, hypoglycemic and antibacterial effects have been associated with consuming bilberry fruits [60-62]. The numerous scientific studies that support the beneficial effects associated with these fruits explain the increase in the production and consumption of novel products and dietary supplements containing bilberry [63]. However, some studies have warned that high dose consumption of this berry may cause some unwanted effects, including possible interactions with concurrently and subsequently administered medicinal products [64]. It is important to consider that there is still a long way to go in order to confirm the relationship between the consumption of this type of berries and the bioactive properties, since most of the scientific research carried out in this field has been carried out *in vitro*. In this section, the principal health benefits associated with the consumption of berries or product development from *V. myrtillus* are reviewed.

5.1. Antioxidant Properties

Antioxidants are thought to prevent chronic complications in part through their interactions with reactive oxygen species (ROS) and their ability to scavenge free radicals [38]. The antioxidant properties of polyphenols have been strongly related to their ability to act as reducing agents [65, 66].

Drózdź, Šežienė, & Pyrzynska [67] demonstrated that fruits of *V. myrtillus* exhibited high antioxidant activity as good electron donors, and their extracts were able to reduce copper (II)-neocuproine chelate, as well as to quench 2,2-diphenyl-1-picrylhydrazyl radicals (DPPH•). Veljković *et al.* [68], in studies in rats, found relevant EC₅₀ values for the DPPH radical scavenging activity and inhibition of lipid peroxidation in liposomes (0.202 ± 0.008 mg/mL and 0.33 ± 0.01 mg/mL respectively) for bilberry fruits. The same authors, in assays with Wistar albino rats, showed significantly decreased lipid peroxidation (MDA) and protein oxidation (AOPP) levels ($p < 0.001$) in the groups in which *V. myrtillus* was supplemented than in the control group [68].

Table 2. Contents of individual anthocyanins in *Vaccinium myrtillus* fruits.

Origin	Anthocyanins															References
	Cy-3-arab	Cy-3-gal	Cy-3-glu	Dp-3-arab	Dp-3-gal	Dp-3-glu	Mv-3-arab	Mv-3-gal	Mv-3-glu	Pn-3-arab	Pn-3-gal	Pn-3-glu	Pt-3-arab	Pt-3-gal	Pt-3-glu	
Finland Southern (mg/100 g fw)	37.0	34.0	41.0	57.5	45.7	54.6	2.6	13.3	16.6	nd	2.1	9.3	nd	10.0	25.9	[21]
Finland Northern (mg/100 g fw)	44.0	59.5	50.9	87.8	98.9	76.4	9.6	34.2	46.8	nd	4.8	17.7	nd	26.3	45.3	[21]
Slovenia (mg/kg fw)	161.92	542.45	194.47	640.31	700.46	381.11	42.45	109.97	149.63	9.66	19.42	108.09	78.31	213.63	416.06	[22]
Poland (mg/g dw)	936	375	396	741	1060	1247	295	127	506	15	107	198	340	581	541	[104]
Italy Northern (mg/100 g fw)	37.1	56.3	55.0	97.4	127.1	119.0	nd	19.7	56.1	nd	nd	24.8	20.1	33.1	65.9	[95]
Slovenia (mg/100g fw)	110.6	122.6	13.04	152.3	167.1	169.1	28.24	21.66	24.89	22.99	17.82	20.75	19.98	14.76	16.72	[13]
Finland (mg/kg fw)	450	493	488	632	629	562	91	124	350	20	34	187	137	167	359	[101]
Mustasaari, Finland (mg/kg fw)	220	300	220	340	360	300	53	100	150	18	36	75	84	120	170	[102]
Milan, Italy (mg/g extract)	32.18	40.03	41.81	49.06	49.07	54.21	8.75	13.23	40.15	1.75	4.34	15.90	13.44	17.92	38.49	[79]
Bulgaria (mg/100 g dw)	78.13	244.81	563.38	101.57	91.54	733.01	27.65	17.80	46.65	15.42	14.39	57.61	38.67	27.74	17.53	[103]

Abbreviations used: dp- delphinidin; cy- cyanidin; pt- petunidin; pn- peonidin; mv- malvidin; gal- galactoside; glc- glucoside; arab- arabinoside; nd- not detected; fw- fresh weight; dw- dry weight.

5.2. Anti-obesity Effects

The anti-obesity mechanisms for berries may include a reduction in lipid absorption, a decrease in differentiation and proliferation of preadipocytes, a decrease in lipogenesis, an increase in lipolysis, and the inhibition of pro-inflammatory adipokine secretion [69, 70]. Studies carried by Kowalska *et al.* [69], demonstrated the capability of *V. myrtillus* to diminish lipid accumulation with a concomitant down-regulation of peroxisome proliferator-activated receptor gamma (*PPARγ*), enhancer-binding protein alpha (*C/EBPα*) and sterol regulatory element binding transcription factor 1 (*SREBP1c*) in mouse embryo 3T3-L1 adipocytes, as well as to suppress the expression of adipocyte fatty acid-binding protein (*aP2*) and resistin [71].

5.3. Anti-proliferative Properties

Polyphenols, which are plentiful in *V. myrtillus*, are among the most promising anti-carcinogenic agents in plants. Demirel Sezer *et al.* [72] found that quercetin and kaempferol, the most abundant non-anthocyanin polyphenols present in *V. myrtillus* extracts, showed strong cytotoxic, antioxidant and apoptotic effects. *V. myrtillus* anthocyanins have also demonstrated the ability to upregulate tumor suppressor genes, induce apoptosis in cancer cells, repair and protect genomic DNA integrity and improve neuronal and cognitive

brain function [73]. Nguyen *et al.* [74] concluded that extracts from *V. myrtillus* were able to inhibit proliferation and induce apoptosis in breast cancer cells through a mechanism that did not involve action in the microtubules or mitosis, although when the concentration of the extracts increases, the organization of the microtubules was affected, leading to the accumulation of cells at mitosis by a direct action on microtubules.

5.4. Cardioprotective Effects

Cardiovascular diseases remain one of the leading causes of death and they are, therefore, a primary focus of research and treatment [75]. Several studies have shown that the intake of berry fruits was associated with a reduced risk of cardiovascular diseases. Ashour *et al.* [76] demonstrated the ability of *V. myrtillus* to protect against DOX-induced cardiotoxicity in rats, which could be attributed, at least in part, to its antioxidant activity. *V. myrtillus* significantly inhibited DOX-induced elevations of LDH, CPK and CK-MB activity in serum, as well as troponin I levels; furthermore, in the histopathological examination, the severity of the histological changes was much lower in sections from rats pre-treated with *V. myrtillus* [76].

Erlund *et al.* [77] studied the effects of *V. myrtillus* berries consumption on platelet function, blood pressure and HDL-cholesterol, and concluded that daily consumption of moderate amounts (100

mg) during two months could contribute to explain the cardiovascular disease protective role of a diet rich in fruits and vegetables. Žiberna *et al.* [78] observed that the administration of a *V. myrtillus* extract (0.01-5 mg/L) to rats increased coronary flow up to 2.5-fold, and decreased lactate dehydrogenase (LDH) release rate during reperfusion by 3.7-fold at 0.1 mg/L and by 6.7-fold at 1 mg/L, compared to controls. It was also effective in the prevention of arrhythmias, whose duration was maximally shortened at 0.1 mg/L to 3.2 ± 0.2 %, and at 1 mg/L to 4.4 ± 0.3 %, in relation to the untreated group.

5.5. Anti-inflammatory Effects

The progression and development of several diseases, such as autoimmune diseases, organ fibrosis, diabetes, obesity, allergies and dysfunction, are influenced by acute and chronic inflammation. Schink *et al.* [62], in a screening of 99 ethanolic plants extracts, found that *V. myrtillus* displayed strong anti-inflammatory activity combined with high cell viability in THP-1, HeLa-TLR4, and HEK-TLR2/HEK-TLR4 cell lines. Anthocyanin-rich extracts from bilberry also showed anti-inflammatory effects against liver inflammation in mice, leading to the suppression of LPS-induced inducible nitric oxide synthase (iNOS), TNF- α , IL-1 β and IL-6 transcripts, and iNOS, TNF- α and NF- κ B protein levels [79]. Furthermore, bilberry also demonstrated the ability to reduce serum C-reactive protein (CRP), IL-6, IL-12 and LPS levels, and downregulate genes associated with the TLR pathway in individuals with metabolic syndrome [62, 80]. All in all, these studies reveal not only the anti-inflammatory potential of *V. myrtillus*, but also its oral effectiveness in humans.

5.6. Hypoglycemic Effects

Postprandial hyperglycemia is a condition that can be improved through lifestyle and diet, preventing the development of cardiovascular diseases and diabetes type 2 [81, 82]. Xu *et al.* [83] showed that the consumption of fruits, like *V. myrtillus*, rich in phenolic compounds, can attenuate postprandial glycemic and insulin responses in young adults, with bilberry fruits having the most insulin lowering effect at 30 min postprandial; this effect was maintained throughout the early postprandial period and was related with the consumed amount of phenolic compounds. Different authors also reported inhibitory effects on α -glucosidase and amyloglucosidase activities of polyphenol-rich extracts from *V. myrtillus* fruits; in particular, phenolic acid-enriched fractions were able to inhibit α -glucosidase *in vitro*, which is considered one of the most effective ways to control type 2 diabetes [60, 84-86].

5.7. Ocular Effects

Eye fatigue, pain, dry eye sensation, excess of tears, blurry vision, glaucoma and cataracts are the most common changes that can impair the quality of vision, especially in individuals with daily work that requires more eye strain [87]. Thus, the development of products which could improve eye health has gained prominence from researchers. Riva *et al.* [88] studied the bioavailability of a standardized *V. myrtillus* extract and its ability to alleviate dry eye symptoms and concluded that it could improve tear secretion in subjects suffering from dry eye symptoms. Other studies described beneficial ocular effects of *V. myrtillus* extracts, namely night vision improvement [89], cataract and glaucoma prevention [90].

5.8. Antimicrobial Effects

Some studies suggest that bilberry may protect against human pathogenic bacteria, due to its composition in phenolic compounds and organic acids [91]. Toivanen *et al.* [92] demonstrated that juices produced from *V. myrtillus* showed potential against pneumococcal infections caused by *Neisseria meningitidis* with a 63 % growth inhibition at a concentration of 10 mg/mL. Huttunen *et al.* [93] studied the inhibitory activity of wild berry juice fractions composed mostly of sugars and some amounts of small size phenol-

ics against *Streptococcus pneumoniae* binding to human bronchial cells and concluded that the highest concentration used in their antimicrobial tests (~86 mg/g) was extremely effective and the growth of *S. pneumoniae* was totally inhibited by *V. myrtillus* extract.

Overall, bilberry has potential to be used in vision improvement, and in the treatment or prevention of conditions associated with dyslipidemia, inflammation, hyperglycemia, increased oxidative stress, cardiovascular disease (CVD), cancer, diabetes, and other age-related diseases, besides their antimicrobial activity.

6. INDUSTRIAL APPLICATIONS

Appearance is a recurrent concern of the food and pharmaceutical industries where colour plays a key role. The demand for the use of natural pigments has increased not only because of the concern with the use of artificial colorants, which is increasingly evident, but also for their nutraceutical properties [10]. The discovery of compounds able to meet the colouring and healthy requirements, with minimal toxicity, has led many researchers to focus on the use of extracts rich in anthocyanins obtained from fruits, including the fruits of *V. myrtillus*, in the development of innovative products.

Daily anthocyanin consumption can range from milligrams to hundreds of milligrams per person, depending on the diet and the sources they are ingested from. The use of anthocyanins as food colourings, especially in more acidic foods, which may favour their stability, has exponentially raised. The exploitation of *V. myrtillus* fruits for medicine and human diet purposes has gained significant attention, being the economically most important wild berries of Northern Europe, widely used by the food industry [94]. This fruit is consumed not only in a fresh manner, but also in processed products (press cake) and derivatives (juice, jam and liqueur) [95, 96]. Fruits drying and its transformation into powder represent a suitable alternative widely used by consumers and the food industry that allow having them available throughout the year for subsequent use as an ingredient in based foods (extruded products, bakeries, sauces, beverages, ice creams, yogurts, and confectionary) [11, 97]. The by-products resulting from the production of bilberries, as well as the fruits that, due to their exterior appearance or size do not meet commercialization standards, may also be used for the preparation of polyphenols extracts and the production of novel foods, conferring them added value and reducing the environmental impact. Some authors showed that the press cake of *V. myrtillus*, a by-product of juice production, can be a suitable and green approach to be used in the preparation of value-added products by the food industry [96, 98, 99]. For instance, Fidaleo *et al.* [33] studied the suitability of phenolic extracts obtained from *V. myrtillus* residues as an ingredient in drinking yogurt and condensed milk with a high antioxidant capacity [33]. There are, already in the market, several companies that have incorporated bilberry in products from several sectors claiming benefits to consumer health. Lusoberry® (<http://lusoberry.com/>), a Portuguese company based in Oliveira do Hospital, is a bilberry producer that began to add value to the fruits using them in the production of oil and wine. The company expounds that bilberry oil does not have as strong taste or acidity as olive oil and that has a higher concentration of magnesium and potassium. For its part, the bilberry wine, not being a novelty in the world, emerges for the first time in the Iberian Peninsula.

Yogurts are an important element of the human diet and often consumed by a large part of the population of all age groups. This may justify the diversity of dairy companies interested in developing bilberry-enriched yogurt. PIA®, a Brazilian company, has developed a yogurt with bilberry preparations, containing 25 % less sugar content than usual (<http://www.pia.com.br/produto/tipo/iogurtes/>). Furthermore, Biedermann® company from Switzerland has developed BioSkyr®, protein-rich fermented milk containing 9.2 % bilberry fruit (<https://biomolkeri.ch>). Both companies have promoted marketing campaigns around these products, highlighting

the health benefits. A Brazilian company dedicated to the preparation of more natural ice cream has launched a claimed healthy product based on bilberry fruit (<http://www.santofruto.eco.br/>).

Another sector that is of great importance in the human diet and which has also been influenced by consumer demand for healthier foods is the bakery industry. Mirtiflor® (<https://www.mirtiflor.pt/>), a family Portuguese company producer of wild fruits, has invested in the development of several products aiming in the full use of these fruits. In the particular case of bilberry, the company, in addition to preparing traditional jams and liqueurs, has launched in the market bilberry cookies. Moreover, the well-known BioGerminal® (<https://www.germinalbio.it/>), which is dedicated to the production of healthy food, has developed an integral spelled pie with bilberry. Moreover, Little Bellies® (<https://bellies.com.au/>) is a well-known children's food brand that has been focusing on launching products labeled as healthier and more natural. In its diversity of choices, there is a snack for children from 9 months of age based on puffed corn with organic bilberry. In addition to being advertised as a natural snack with antioxidant properties, this snack is also gluten-free.

Similarly, the big chocolate companies have also been testing new recipes using powerful fruits to somehow make unhealthy products more attractive to consumers. Thus, Schogetten® (<https://www.schogetten.com>) features chocolate with dehydrated bilberry and muesli and the well-known Guylian® Belgian chocolate (<https://www.guylian.com/>) features a variety of fruit berries topped with its famous chocolate, among them bilberries.

In addition to the various sectors of the food industry, the cosmetic industry has also been focusing on enriching different products with bilberry. Panvel Vert® (<https://www.panvel.com>) has extensive experience in cosmetic products and has developed a body spray with soothing and relaxing properties with bilberry extract enriching the product with vitamins, minerals and other antioxidants. Furthermore, the hair cosmetics industry has been betting on innovation, and brand Loweel® (<http://www.lowell.com.br/>) has developed a hair care kit for repair and nutrition incorporating bilberry antioxidant active agents, claiming the protection from hair aging.

In conclusion, *V. myrtillus* is a widely consumed fruit, with interesting nutritional and therapeutic properties, rich in phytochemical compounds, which can be used in various industrial sectors, not only as such or as derived products, but also taking advantage of the resulting by-products, without losing its claimed beneficial effects.

CONCLUDING REMARKS

The fruits of *V. myrtillus* are appreciated for their nutritional characteristics and their rich content in micronutrients and phytochemicals proven health benefits, including organic acids, sugars, minerals and vitamins, fiber and phenolic compounds, which have been associated to health benefits. Anthocyanins are one of the main compounds present in bilberry fruits composition, responsible for the characteristic colouration of these berries and supposed healthy effects. Thus, they are promising natural colourant molecules, capable of replacing artificial additives. The industry has been exploring the use of bilberries, which has had a huge environmental and economic impact as it makes use of all the fruit, including waste products. Several industrial sectors have been focusing on the production of new products enriched with bioactive compounds obtained from bilberry in order to obtain novel products with the associated beneficial properties. However, at the level of application as a colouring ingredient, there are some further stability studies to be carried out in order to ensure its stability in the final products so as to replace artificial colours in food and textiles.

CONSENT FOR PUBLICATION

Not applicable.

FUNDING

The authors are grateful to the Foundation for Science and Technology (FCT, Portugal) and FEDER under Programme PT2020 for financial support to CIMO (UIDB/00690/2020) and T.C.S.P. Pires grant (SFRH/BD/129551/2017). L. Barros would like to thank the national funding by FCT, P.I., through the institutional scientific employment program-contract. To the project AllNat for the contract of C. Caleja (Project AllNat POCI-01-0145-FEDER-030463). The authors are also grateful to FEDER-Interreg España-Portugal programme for financial support through the project 0377_Iberphenol_6_E. The GIP-USAL is financially supported by the Spanish Government through the project AGL2015-64522-C2-2-R.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

ACKNOWLEDGEMENTS

Declared none.

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