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Biotransformation in Temperate Climate Fruit: A Focus on Berries

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Abstract Berry fruits, cultivated in temperate zones, are popularly used in the human diet. With the increase in production and consumption, the development of food industry is oncoming in the sense of developing new products that can meet market needs, in addition to the development new formulations, enhancing the industrial waste and bio-transforming it to generate high value-added products is fundamental. The use of fermentation processes to increase the level of functional compounds and the production of enzymes, antibiotics and flavorings is a good example.

Keywords: *bioactive compounds, flavour, antibiotics, enzymes, solid-state fermentation*

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1. Introduction

Berry fruits, cultivated in temperate zones, such as blueberry, blackberry, raspberry, strawberry and cranberry are popularly used in human diet [1].

The cultivation of these fruits is becoming more popular in many countries. Brazil has a privileged climatic condition for such cultivation since it has temperate regions in four states. Thus, the country has potencial production potential for all year. In addition, another economically important aspect is that the Brazilian production of fruits occurs from December to February, the off-season in the United States and the European Union that are the main consumer centers.

Currently most of the Brazilian production is consumed *in natura* or exported. However, in other countries where the consumer has already entered these fruits in his daily diet, several products are processed and the waste generated by industries has been used as fermentation substrate obtaining a lot of value-added by-products.

This review aimed to present the main characteristics of strawberry, blackberry, raspberry, cranberry and blueberry, regarding the growing potential in Brazil, the biologically active compounds present and the use of industrial waste of these fruits as a substrate for biotransformation and production of phenolic compounds, enzymes, antibiotics, organic acids and flavorings.

2. Berries

The *small fruit* designation is used in the international literature to refer to several crops like strawberry, blackberry, raspberry, blackcurrant, blueberry, etc., and is limited to the name – *e.g*. berries - and to the size of the crop [2].

According to reference [3], the cultivation of berries has attracted the attention not only of consumers, but also the interest of producers and traders. In general, the cultivation of these species is characterized by low cost of deployment, affordable cost of production for small producers, economic return, adaptation to socio-economic conditions and the local environment, unskilled manpower demand, possibility of cultivation in an organic system and greater demand than supply [4]. The profitability of this type of fruit per hectare is high, exports have grown in recent years, as well as the planted area and in many regions constitute an important alternative for both small farms as well as for large companies.

The great marketing appeal of these fruits is due to the high content of antioxidants and anti-cancer substances that attract the consumer, as in the case of the blueberry, which is regarded as one of the richest in natural antioxidants among small fruits [5,6].

Berries are a natural source of substances which have antioxidant activity. Extracts of various fruits such as blackberry, cherry, blackcurrant, blueberry and raspberry have been found to be effective in combating free radicals [7]. Recently, due to association of the small fruits to bioactive properties such as high content of antioxidants and anti-cancer substances, fruit consumption demand by the population has increased, in search of food supplementation from diet diversification based on fruits.

The changes in the eating habits of population, observed in recent years, coupled with the increased purchasing power of the low income population, has provided ample market opportunity for the production of fresh and industrialized fruits in Brazil, with the southern states, São Paulo and Minas Gerais standing out, especially considering that climate conditions in these regions allow the supply of temperate fruit species at different times of the year [2,8].

2.1. Strawberry

Strawberry is a perennial, creeping, herbaceous belonging to the Rosacea family and genus *Fragaria* [9]. The edible part is the strawberry, which is a non-climacteric pseudofruit [10] of bright red coloring, engaging odor, texture and slightly acidified taste [11]. The color of strawberry is due to anthocyanins, and its unique flavor is due to the citric and malic acids, and sugars [12].

The strawberry is the most popular and consumed type of berry fruit for its sweet flavour. There are more than 600 strawberry cultivars; they differ in flavour, size and texture. In addition to cultivated strawberries, some cultivars grow wild or come from natural breeding. These can be usually smaller in size, but feature a more intense flavor [13].

2.2. Cranberry

Cranberry is a red fruit belonging to the Ericaceae family native of the United States [14]. Due to the great bitter taste, the fruit is not consumed *in natura*. According to data from the National Agricultural Statistic Service (NASS) [15] the fruit is marketed in three basic categories: fresh (5%); sauce, concentrated and various value-added applications (35%); and juice (60%) [14]. There are no reports yet, about cultivation of this species in Brazil.

Cranberries, like other small fruits, are rich in phenolic phytochemicals, such as phenolic acids (especially ellagic acid), tannins and flavonoids. The consumption of this fruit has historically been associated with health benefits, especially decreases in the incidence of urinary tract infections and intestinal disorders caused by *E. coli* O157:H7, its consumption has also been associated with the ability to inhibit peptic ulcer associated with *Helicobacter pylori* bacteria. The processing of this fruit to elaborate juices generates pomace as a byproduct. The pomace is mainly composed of peel, pulp and fruit seed. This is high in fiber and has relatively little amount of carbohydrates and protein [1,14,16].

2.3. Blackberry

The blackberry belongs to *Rosaceae* family and the *Rubus genus* that contains about 740 species divided according to some authors, into 12-15 subgenres [17,18]. This fruit has an erect or creeping posture, which produces aggregated fruit formed by minidrupes of about four to seven grams of black color and a sweet to sweet-acid flavor [19]. There are numerous blackberry cultivars known and marketed, but currently the two most important are the Brazos and Tupi [20].

In Brazil there are five native species of blackberries - *R. urticaefolius*, *R. erythroclados*, *R. brasiliensis*, *R. sellowii* and *R. imperialis* - white, pink, red or black colors. None of the Brazilian species was domesticated. The cultivars of blackberries used in the country are the result of introductions, crosses and selections of American cultivars [8]. Rio Grande do Sul state is Brazil's main producer, but the fruit has a high potential for cultivation in temperate regions such as Santa Catarina, Paraná, São Paulo and Minas Gerais [18].

2.4. Raspberry

The raspberry belongs to the family Rosaceae, genus Rubus and subgenus Idaeobatus. This subgenus comprises about 200 species, distinguished by the ease with which their mature fruits are separated from the receptacle. Many of these species have been improved, but only the red and black raspberry are produced on a large scale [8,21].

The raspberry is native to central and northern Europe and parts of Asia [8]. However the *Idaeobatuss* subgenre, occurs on five continents, but has its distribution centered mainly in the Northern Hemisphere, with a focus on Asia, Europe and North America [22]. In Brazil, the raspberry crop was introduced in the region of Campos do Jordão - SP, and the major producing states are Rio Grande do Sul, São Paulo and Minas Gerais [3].

2.5. Blueberry

Blueberries are a small fruit native of the United States, belonging to the genus *Vaccinium*, and the *Ericaceae* family. According to statistics from the Food and Agriculture Organization (FAO), from 1970 to 2011, the world production of blueberries increased approximately 7 times [23]. According to the National Agricultural Statistics Service (NASS) of the United States Department of Agriculture (USDA), the United States is the leading producer of blueberries, which makes the blueberry the second most produced and marketed small fruit in the country, only behind strawberry [24].

In South America, blueberry cultivation (*Vaccinium* spp.) is booming in countries like Chile, Argentina and Uruguay [25]. In Brazil, the cultivation of blueberries has not been well known. However, Brazil has the potential to produce blueberries throughout allyear. So, an important economic aspect is to produce Brazilian blueberries at least from December through February, which is between harvest seasons in the main consumer centers in the USA and European Union [26].

In the world there are three main groups of blueberries commercially produced: the lowbush, the highbush and the rabbiteye [8].

3. Bioactive Compounds

Fruit extracts showed antioxidant capacity, which helps slow down or prevent the development of several degenerative diseases, due to their abilities to react with free radicals. This protects the tissues in the human body against oxidative stress and pathologies due to cancers, cardiovascular diseases and inflammatory processes [27,28]. These beneficial effects are most probably related to the presence of bioactive compounds, such as carotenoids, tocopherols, vitamin C and phenolic compounds [29,30].

A large number of *in vitro* and *in vivo* studies have shown that berries stand out as fruits with high anticarcinogenic properties, which in turn are attributed to the high content of bioactive compounds such as flavonoids (anthocyanins, flavonols and flavanols), tannins (proanthocyanidins, ellagitannins and gallotannins) and phenolic acids (hydroxybenzoic acids, hydroxycinnamic and derivatives) (Figure 1) [31]. However the reference [26] found small amounts of carotenoids and ascorbic acid, along with elevated levels of tocopherols in blueberries, which was also reported by others [18,32] in fruits such as blackberry, blueberry and goldenberry (*Physalis peruviana*). So, in other study, [6] verified that compounds other than phenolics can affect the antioxidant activity of blueberries.

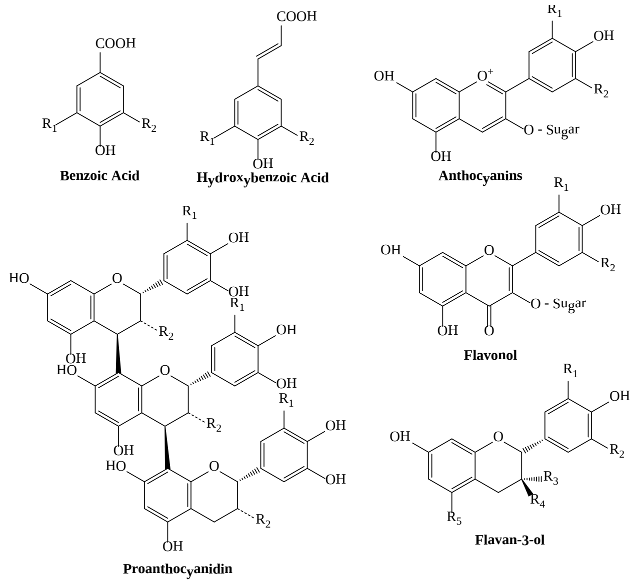

Figure 1. Structure of flavonoids found in Berries

Among the bioactive compounds present in berries, phenolic compounds stand out, which comprise more than 8,000 substances that have already been identified [33]. These compounds are classified according to the structure of the aromatic ring, the number of carbon atoms substituting the ring and connecting it together. Thus, the metabolic changes that phenolic compounds undergo during the metabolic processes in plants make them show a structural and functional diversity and can be hydroxylated and methoxylated in various positions [34].

Table 1 presents the content of phenolic phytochemicals in strawberry, raspberry, blackberry, cranberry and blueberry.

With regard to other types of pigments that can be found, in a study by [39] determining the content of lutein, zeaxanthin, lycopene, β-carotene, α-carotene and β-cryptoxanthin in strawberry, raspberry, blackberry and blueberry, it was found that blackberry had the highest carotenoid content (440 $\mu g.100g^{-1}$), followed by raspberry (370 $\mu g.100g^{-1}$) and blueberry (290 $\mu g.100g^{-1}$), the strawberry had the lowest levels (26 $\mu g.100g^{-1}$). However, when the carotenoid content of berries is compared to other fruits, the values are considered low, this is because in fruits whose main compounds belong to the anthocyanin class, berries for example, the carotenoid content is reduced during ripening [40].

As for the tocopherol content, in a study of 33 fruits by [41] it was found that berries (strawberry, raspberry, blueberry, blackberry and cranberry) had amounts greater than most fruits. However, it is important to note that while the tocopherols and tocotrienols are found in photosynthetic organisms the presence of these compounds in plant foods occurs mainly in dark green vegetables, in oil seeds, vegetables oils and wheat germen [42].

Table 1. Phenolic compounds values in strawberry, cranberry, raspberry, blackberry and blueberry ($mg.100g^{-1}$ of fresh fruit)

Compound	Strawberry	Cranberry	Raspberry	Blackberry	Blueberry
Ellagic acid	34.4 - 63.0 [35,36]	12.0 PS [36]	150 [36] PS	ND [18]	2.7 [26]
Galic acid	0.5 - 4.4 [36]	ND [36]	1.9 - 3.8 [36]	350.5 [18]	58.7 [26]
Ferulic acid	0.0 [36]	ND [36]	0.3 - 1.7 [36]	35.7 [18]	9.8 [26]
Caffeic acid	<0.05 -1.4 [36]	ND [36]	0.6 - 1.0 [36]	15.4 [18]	0.7 - 7.7 [26]
Chlorogenic acid	*	0.5 [37]	*	*	186 - 208 [38]
p-coumaric acid	0.7-17.0[35,36]	0.0 - 2.0 [36,37]	0.7 - 2.0 [36]	ND [18]	0 - 3.9 [18,26,37]
p-hydroxybenzoic	1.0 - 3.6 [36]	ND [36]	1.5 - 2.7 [36]	0.01 [18]	0.03 [26]
Quercetin	0.3 - 3.9 [35]	7.3 - 25.0 [36]	2.9 [36]	20.2 [18]	2.2 - 15.6 [26,35]
Myricetin	ND [36]	1.1 - 7.7 [36]	0.0 [36]	ND [18]	0.8 - 36.0 [26,35]
Kaempferol	0.2 - 9.9 [35]	0.0 - 0.3 [36]	<0.01 [36]	ND [18]	6.2 [26]
Catechin	*	41.700 DM [36]	*	ND [18]	ND [26]
Epicatechin	*	44.700 DM [36]	*	120.2 [18]	ND [26]

DM: Dry Matter; * Not found in literature; - ND: Not Detected

In a study by [26] with pulp, peel and the whole fruit of six blueberry cultivars, it was found that the peeling of the fruit has 10-58 times more tocopherols than the fruit flesh itself. Similar results were found for carotenoids, vitamin C, anthocyanins, flavonols, flavanols and phenolic acids (with the exception of gallic acid), all found in larger quantities in the peel of the fruit, resulting in a higher antioxidant activity of peel.

4. Metabolism of Berries Waste via Semi-Solid Fermentation

The food industry produces large volumes of waste, both solids and liquids resulting from the production of food preparation and consumption. The disposal of such waste results in both economic and environmental problems, because in addition to being highly pollutant, they represent a loss of biomass and valuable nutrients [43,44]. Because of this, the biotransformation of food processing waste has received increased attention as it represents a possible resource of waste conversion into useful and value-added products [44]. These residues have long been used as substrates for solid-state fermentation, production of fertilizers, animal feeds, ethanol production, organic acids and the production of various types of enzymes [14].

4.1. Increased Content of Phenolic Compounds and Antioxidant Activity

Among the strategies used for the enrichment of phenolic antioxidants in fruits, the bioprocessing of waste fruits and vegetables using solid state fermentation with microorganisms that have GRAS (substances generally recognized as safe for use in food) to generate phytochemical profiles that make the increase of health benefits possible [14,43].

The solid state fermentation in small fruits can be done with juice, pulp or by using the resulting byproduct of the extraction juice or production of berry wine [14,45]. There are reports in the literature using byproduct from processing of cranberry juice as substrate for semisolid fermentation using fungi such as *Rhizopus oligosporus* and *Lentinus edodes* in order to increase the production of phenolic compounds and thereby increase the antioxidant action of the fruit [14,16]. These studies have shown that during fermentation there is an increase in the production of phenolic compounds reaching levels found in fruit and juices. [16] found that on 10^{th} day of fermentation of cranberry byproduct, an increase in the production of phenolic compounds occurred resulting in an increase of 11 to 25% in antioxidant capacity compared with the initial values.

The increase of phenolic acids, such as ellagic acid in cranberry-product due to solid state fermentation has also been reported [16,43,46]. This may be due to ellagitannins hydrolysis by enzymes that hydrolyze tannins produced by the fungi used in fermentation. These fungal hydrolases such as glycosidase and fructofuranosidase break the glycosidic bonds existing between the phenolics and sugar, resulting in an increase in antimicrobial activity with *V. parahaemolyticus*, *E. coli* O157:H7 and *H. pylori* being the microorganisms considered to be more sensitive to presence ellagic acid. This sensitivity of micro-organisms could be due to disruption of membrane integrity, blockade of ion channels in the plasma membrane or inhibition of ATP synthesis by inhibiting the flow of electrons through the electron transport chain in the bacterial membrane [16].

The reference [47] found that after 50 days of fermentation using the fungus *Lentinus edodes*, the yield of the free phenolic achieved a maximum 0.5 $mg.g^{-1}$ of byproduct, the authors also identified the major free phenolic through High Performance Liquid Chromatography (HPLC).

4.2. Enzyme Production

Different strains of fungi can be used in solid state fermentation (SSF) process, since the majority can be easily grown on the surface of solid substrates. The objective of the process may be the production of biomass, waste products such as organic acids, production of flavor compounds and flavoring or enzyme production. Since agricultural wastes are rich sources of natural polymers (cellulose, lignin, pectin and starch), large amounts of enzymes are produced [48].

Among the fungi used *Lentinus edodes*, also known as *Shiitake* or Chinese mushroom grows very well in apple pomace and other lignocellulosic waste as a byproduct of processing cranberries or strawberries [43]. This fungus has been used to produce enzymes such as laccase and manganese peroxidase [48] and to produce polygalacturonase [43].

Polygalacturonase is widely used in the food industry, assisting in processing, maceration, liquefaction, extraction, clarification and filtration of juices and fruit wines. For the most common industrial uses, fungal polygalacturonase is more advantageous because it has a higher enzymatic activity. In addition, it presents its optimum activity at low pH, which facilitates its application in processing of fruits and vegetables. Commercial preparations of polygalacturonase used in the food industry are typically derived from fungal sources especially *Aspergillus niger* and the yeast *Kluyveromyces marxianus* [43]. In recent studies the same authors found that high levels of polygalacturonase activity were produced by *L. edodes* during SSF in strawberry pulp. The strawberry pulp was presented as the best substrate for the production of polygalacturonase, while the byproduct of cranberry was not a good substrate.

4.3. Production of Aromas

In 2012, the world market of aromas, flavors and fragrances saw transactions of 23.4 billion dollars, and there are chances to accelerate this segment by 2017 [49]. Currently, there is a great interest from consumers for these products thus increasing the economic value of bioflavors [44].

Natural flavors are chemicals that have properties that are produced from raw materials of vegetable or animal origin by physical, enzymatic or microbiological treatments [50].

The microbial synthesis of these natural flavorings is generally carried out by submerged fermentation [44]. Its results in high costs in natural flavors, for example, synthetic raspberry ketone costs US $ 58.00 per kilogram while the natural costs US $ 3,000.00 per kilogram (Sigma, 2000). Due to high cost of this technology now used on an industrial scale, there is a need to develop inexpensive processes, which make bioflavors economically viable. This could be achieved by exploiting the metabolic pathways and alternative technologies such as solid state fermentation.

4.4. Antibiotic Production

According to [51], production of antibiotics by fungi have some similar characteristics to the production of other secondary metabolites, such as production of antibiotics in a specific lineage; there is instability in the biosynthetic process, which tends to decrease with successive samplings used for the maintenance of the lines; the production of antibiotics follows fungal growth kinetics associated with a specific culture medium; increased production of a secondary metabolite occurs frequently in the sporulation stage of the microorganism; the structural variety of biosynthesized metabolites can be increased by small variations of culture medium.

New techniques such as solid state fermentation have emerged in order to promote a faster fungal growth and a differentiated production of secondary metabolites [52]. The solid-state fermentation employs a natural substrate as carbon source in the presence of a minimal amount of water. The solid state fermentation for production of antibiotics has been considered very advantageous, with an increased yield and shorter fermentation period on parallel procedures performed in submerged cultures [51].

There are studies that use of raspberry seed powder for production of the antibiotic Neomycin by *Streptomyces marinensis* using solid fermentation [53,54]. This antibiotic is an important aminoglycoside widely used in pharmaceutical preparations because it is effective against gram positive bacteria, gram negative bacteria and mycobacteria [54]. It was first produced by *Streptomyces fradial*, and a mutant strain (*Streptomyces marinensis*) was later developed and identified as a producer of Neomycin, [53].

The reference [53] observed that the concentration of raspberry powder used as substrate has a direct relation with production of Neomycin and may act as a limiting nutrient, since small variations in concentration alter the growth rate of fungi and/or product formation. It was also found that dextrin and raspberry seed powder are key nutrients that control the biosynthesis of the antibiotic.

5. Trends and Prospects

From the studies presented in this work, it was observed that there is a global trend of cultivation of small fruits.

Care with improved food quality is currently on the rise. Many research groups in several fields as food science, biochemistry, medicine and nutrition among others have developed products aiming at the ingestion of larger amounts of bioactive compounds that prevent the development of diseases. The matrices studied, have excellent functional characteristics and can become a good alternative.

The main trends observed are the increasing number of consumers of these fruits and, consequently, the expansion of agricultural area devoted to these crops.

With increasing production and consumption, the development of food industry is urgent in developing new products that can meet market needs. In this regard, many countries already have a variety of juices, jellies, creams, fillings and the like.

In addition to the development of new formulations attractive to consumer, it is necessary to advance in the way of performing the treatment of industrial effluents. In Brazil, the most common is the use of food industry waste in the manufacture of animal feed, ethanol production or fertilizers. However, a challenging thought was presented in this review. This consists of enhancement of the industrial waste, bio-transforming it to generate high value-added products. As an example, the use of fermentation processes to increase the level of functional compounds, making the residue rich in these matrix components much as the fruit itself. Thus, the enhanced residue can be used for other purposes such as the development of concentrated extracts. The use of bio-transformation for the production of enzymes, antibiotics and fragrances also presents a potential market. In short, in Brazil, a major challenge is to develop a new way of seeing the industrial waste, not as a means to increased costs for treatment, but as an alternative of income and development.

This paper presents an important perspective to Brazil, and let taking advantage of its climate potential can become a producer, consumer and exporter of wide range of small temperate fruits. This strategy promotes the development of small agriculture, strengthens the economy and improves the dietary quality of the population.

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