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Effects of industrial and home-made spread processing on bilberry phenolics

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Delphinidin 3-glucoside (PubChem CID: 443650)
Chlorogenic acid (PubChem CID: 1794427)
Protocatechuic acid (PubChem CID: 72 and 8468)
Caffeic acid (PubChem CID: 689043)
Gallic acid (PubChem CID: 370)
(+/−)-Catechin (PubChem CID: 107957)
(−)-Epicatechin (PubChem CID: 72276)
Myricetin (PubChem CID: 5281672)
Quercetin (PubChem CID: 5280343)

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ABSTRACT

Bilberries processed into spreads represent an important source of anthocyanins if these remain rich in the final product. The effects of thermal processing were studied with non-ground and ground bilberries processed into spreads according to industrial and home-made procedures. Samples were analysed by LC–DAD–MS/MS and LC–MS. The spreads had 28–60% less total phenolics, 4–62% less anthocyanins, and 1-fold to 2-fold more phenolic acids and total flavonols than the bilberries, but approximately equal flavanols. The home-made spread from ground bilberries had *ca.* 26% higher antioxidant activity. Delphinidin 3-glucoside and cyanidin 3-glucoside were taken through the two spread procedures, with their degradation to gallic acid (38–57%), protocatechuic acid (1–2%) and 2,4,6-trihydroxybenzaldehyde determined. The amounts of gallic and protocatechuic acids did not reflect well for anthocyanin degradation. The industrial spread procedure with non-ground bilberries is a more suitable procedure to maintain the final content of anthocyanins.

1. Introduction

Many consumers are asking for food products like fruit spreads that have low calorie intake, and higher fruit and reduced sugar content. Fresh bilberries (*Vaccinium myrtillus* L.) are rich in bioactive compounds, and especially in phenolics (Giovanelli & Buratti, 2009). They are particularly high in anthocyanins, which represent over 90% of their total phenolics, and include other flavonoids (flavanols and flavonols), phenolic acids, and stilbenes (Može et al., 2011). As such, they can be used for fruit spreads. The antioxidant capacity of anthocyanins has been shown in *in vitro* and *in vivo* studies (Bornsek et al., 2012; Ziberna et al., 2010, 2012).

Fruit spreads can represent important constant sources of anthocyanins in the human diet throughout the year only if their phenolics remain rich in anthocyanins and/or other antioxidants. However, anthocyanins are commonly known to be unstable molecules, so it is necessary to investigate how fruit-spread processing affects their content. Thermal degradation is primarily caused by oxidation, cleavage of covalent bonds, or enhanced oxidation (Patras, Brunton, O'Donnell, & Tiwari, 2010), and these processes can lead to a variety of different products, which will depend on

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the severity and nature of the heating (Holzwarth, Korhummel, Kammerer, & Carle, 2012). To date, there have only been a few studies on this topic. It has been shown that anthocyanin levels can decrease through the processing of bilberry jam (Šavikin et al., 2009) due to their hydrolysis to aglycones and their further degradation (Fig. 1) to phenolic acids and 2,4,6-trihydroxybenzaldehyde (THBA) (White, Howard, & Prior, 2011). Alternatively, the content of some flavonols and phenolic acids does not change during jam production, as was reported for nine different types of berries by Amakura and colleagues (Amakura, Umino, Tsuji, & Tonogai, 2000). In contrast, a study has shown that with jam processing from red raspberries, while the phenolic acids increase, the flavonols decrease (Zafrilla, Ferreres, & Tomás-Barberán, 2001).

The purpose of the present study was to investigate how different thermal processing affects anthocyanin stability and antioxidant activity in bilberry spreads, and to determine the major thermal degradation products in the spreads. We also investigated whether the degradation acids from two anthocyanins, delphinidin 3-glucoside and cyanidin 3-glucoside, can be used as markers of anthocyanin degradation.

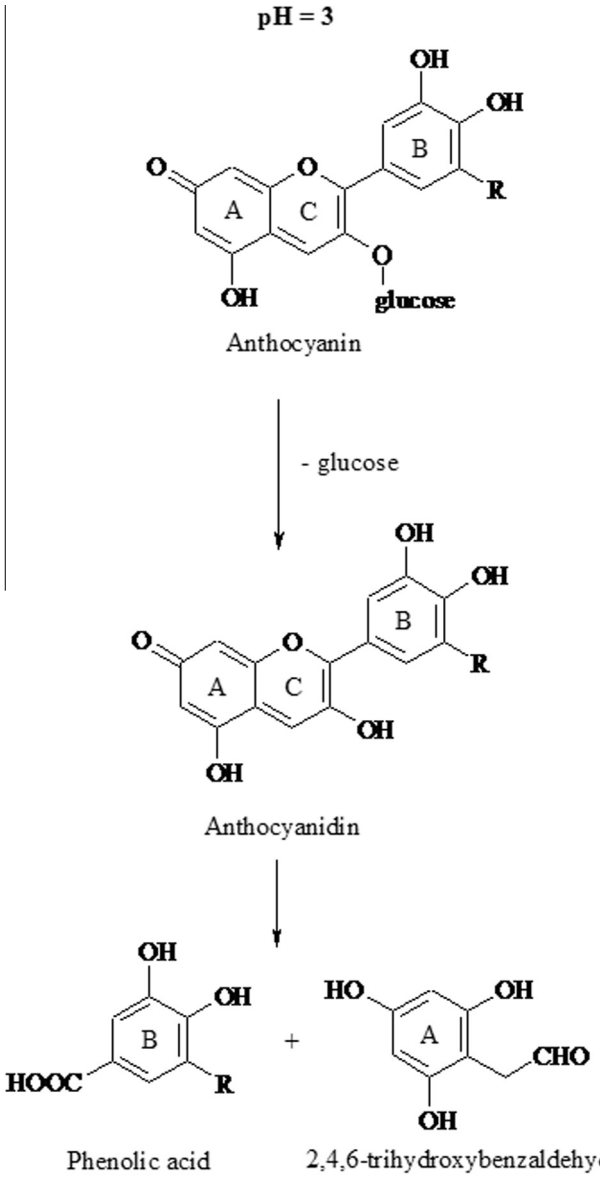

Fig. 1. Anthocyanin degradation at pH 3. R = H for cyanidin 3-glucoside and protocatechuic acid, and R = OH for delphinidin 3-glucoside and gallic acid.

2. Materials and methods

2.1. Samples

Bilberries (*V. myrtillus* L.) were collected at the full ripe stage in the second half of June 2011, in the woods of Lučarjev Kal in Slovenia. The ripe bilberries were immediately stored at −20 °C, with the spreads or the bilberry extracts prepared about 1 month later.

2.2. Chemicals

Pectin was from Danisco (Copenhagen, Denmark), and the industrially produced sucrose was from Mercator (Ljubljana, Slovenia). Cyanidin 3-glucoside was from Polyphenols Laboratories AS (Sandnes, Norway), and delphinidin 3-glucoside from Extrasynthese (Genay Cedex, France). Folin–Ciocalteu reagent, 2,2-diphenyl-1-picrylhydrazyl (DPPH), chlorogenic acid (as the 3-caffeoilquinic acid isomer), (+/−)-catechin, (−)-epicatechin, myricetin, quercetin, THBA, and protocatechuic, salicylic, vanillic, caffeic, ellagic and gallic acids were from Sigma Aldrich (Steinheim, Germany). All of the other reagents used were chemically pure, and all of the solvents were of HPLC purity. Aqueous solutions were prepared using Milli-Q water (Millipore, Bedford, USA).

2.3. Spread preparation

Two types of bilberry spreads were prepared, as one from whole, non-ground bilberries (spreads A), and the other from ground bilberries (spreads B), by both of two procedures: industrial processing and home-made preparation.

2.3.1. Industrial procedure

Both of these spreads were prepared using a UMC 5 Electronic Stephan Universal Machine (Hameln, Germany).

The first of the industrial spreads was made with non-ground bilberries (IndA). Here, 420 g of previously thawed bilberries were mixed with 200 g sucrose, 0.75 g ascorbic acid, and 1.75 g citric acid, and heated with mixing at 300 rpm using a stirrer, thus causing little damage the bilberries. After the temperature reached 77 °C, the mixture was maintained at this temperature for 13 min, with slow mixing at 300 rpm under 80% vacuum. Then the vacuum was released, and the mixture was maintained at 77 °C for a further 10 min. During this time, 15 g sucrose supplemented with 3.5 g pectin (as the gelling agent) was added to the mixture, and gently mixed in. The spread was weighed and then immediately hot-filled as aliquots in 150 g glass jars. The jars were covered, cooled, and stored at 6 °C in the dark for one day, before the extracts were prepared.

The second industrial spread B (IndB) was made with ground bilberries, with 420 g previously thawed bilberries initially ground for 90 s (20 s at 300 rpm; 20 s at 600 rpm; 20 s at 900 rpm; and finally 30 s at 1200 rpm) using a Waring blendor, to obtain a puree. Then 200 g sucrose, 0.75 g ascorbic acid, and 1.75 g citric acid were added, with mixing at 300 rpm. Then the same procedure was followed as described above for the IndA preparation, with the jars of IndB spread also stored at 6 °C in the dark for one day, before the extracts were prepared.

2.3.2. Home-made procedure

The two 'home-made' bilberry spreads were HomeA, with non-ground bilberries, and HomeB, with ground bilberries. These were prepared in a standard 1-L metal saucepan. As for the industrial spreads, 420 g previously thawed bilberries were used either without grinding (HomeA) or with grinding (HomeB; see above for details), and mixed with 200 g sucrose, and heated to boiling