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Phenolic Extracts from Bilberry (*Vaccinium myrtillus* L.) Residues as New Functional Food Ingredients

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Abstract – Bilberry residues were investigated as a potential source of phenolic antioxidants. Polyphenol extraction was carried out in a mechanically stirred device at 40 °C and 300 rpm using aqueous ethanol (60% v/v) as solvent. The polyphenol content of the resulting extract was 81.69 ± 6.12 mg GAE/g dw and the antioxidant activity was 7.58 ± 0.27 mmol TE/g dw. Bilberry extracts were incorporated in varying amounts into a natural drinking yogurt and a condensed milk. Depending on the added amount of extract, the antioxidant activity of the fortified drinking yogurt ranged from 1.69 to 3.27 µmol TE/mL and that of the condensed milk from 12.16 to 23.16 µmol TE/g.
The results obtained demonstrate that bilberry residues can be used as a valuable source of phenolic antioxidants and support the possibility of using the phenolic extracts to produce new functional food products with high antioxidant capacity. Using an agro-industrial waste as a source of polyphenols could contribute to reduce its environmental impact and provide economic benefits to the producers.

Keywords: Bilberry, Phenolic compounds, Antioxidant activity, Functional foods, Waste valorization.

Introduction

Polyphenols are a class of plant secondary metabolites exhibiting high antioxidant activity and other important properties such as induction of apoptosis, inhibition of DNA synthesis and modulation of signal transduction pathways [1]. The antioxidant activity of these compounds is related to their ability to quench reactive oxygen species (ROS) and to stimulate the endogenous defense system [2]. For these reasons, polyphenols are recognized as important phytonutrients capable of protecting cellular components from oxidative damage and contributing to the prevention of oxidative stress-related degenerative diseases [3]. Since humans cannot synthesize polyphenols and their dietary intake is often inadequate, in recent years much effort has been directed towards the development of foods enriched with polyphenols. Fruit smoothies fortified with olive leaf extracts [4] and polyphenol-enriched soybean flour [5] are just two examples of the products that have been investigated for this purpose.

This study was aimed at assessing the suitability of phenolic extracts obtained from bilberry residues as an ingredient for the production of new functional foods. Bilberry (*Vaccinium myrtillus* L.) is a perennial shrub native to Northern Europe and cultivated for fruit production in many parts of the world. From the industrial processing of bilberries into juices, jams and purees, a solid waste consisting mainly of the fruit skins and seeds is generated. Bilberry skins are a very rich source of polyphenols, particularly anthocyanins and flavonoids [6], which can be recovered from the plant material by solid-liquid extraction followed by solvent evaporation [7]. Here we show that dry phenolic extracts obtained from bilberry processing waste have valuable functional properties and can be easily incorporated into common milk products to produce new functional foods with high antioxidant capacity.

Experimental

Chemicals and plant material
Ethanol, methanol, hydrochloric acid and sodium carbonate were obtained from Carlo Erba (Milano, Italy). Gallic acid, DPPH (2,2-Diphenyl-1-picrylhydrazyl), Trolox (6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) and the Folin-Ciocalteu reagent were purchased from Sigma-Aldrich (Milano, Italy). All chemicals were reagent grade and used without further purification.
Bilberry press residues were obtained from "Rigoni di Asiago SRL" (Asiago, VI, Italy). The material consisted mainly of the fruit skins and was stored in plastic bags at –20 °C. When needed, an aliquot of the frozen waste was thawed in air at room temperature.

Analytical Methods
Moisture content was determined by drying to constant weight. Measurements were made using an electronic moisture analyzer (model MAC 50/1, Radwag, Poland).
Total phenolics were determined by the Folin-Ciocalteu method with some modifications [8]. The results were expressed as gallic acid equivalents (GAE) per unit weight of solid, or unit volume of liquid, using a calibration curve obtained with gallic acid standards.
Antioxidant activity was determined by the DPPH assay, according to the procedure described in [9]. The results were expressed as Trolox equivalents (TE) per unit weight of solid, or unit volume of liquid, using a calibration curve obtained with Trolox standards.

Production of Dry Bilberry Extracts
A cylindrical glass vessel (1.5-L working volume) provided with a mechanical stirrer and a thermostated water jacket was used to extract polyphenols from bilberry skins (Fig. 1). Forty grams of the plant material and 1 L of aqueous ethanol

(60% v/v) were loaded into the extractor and stirred at 40 °C and 300 rpm for 150 min. After this time, the vessel was emptied and the suspension was filtered on paper. The liquid was evaporated in a Rotavapor (R-215, BÜCHI Labortechnik AG, Switzerland) equipped with a vacuum controller and a diaphragm pump. Evaporation was carried out at 40 °C and 15–20 mbar. The resulting dry extract was weighed and assayed for total phenolics and antioxidant activity.

Preparation of Polyphenol-Enriched Milk Products

A natural drinking yogurt (Danone SA, France) and a condensed milk (Nestlé SA, Switzerland) were used as a base for the production of high antioxidant capacity functional foods. Dry bilberry extracts obtained as described in the previous section were incorporated by hand at room temperature in the two products. Three degrees of enrichment were considered: 1, 2 and 4 mg GAE/mL for the drinking yogurt; 6.7, 13.4 and 26.8 mg GAE/g for the condensed milk. These values were selected so as to obtain comparable amounts of antioxidants per serving of each product. The enriched milk products were assayed for total phenolics and antioxidant activity.

Results and Discussion

Functional foods are usually defined as foods that, by virtue of the presence of physiologically active components, can provide health benefits beyond basic nutritional functions [10]. They can be produced by adding one or more health-promoting components, by removing harmful components or by modifying the nature or the bioavailability of specific components [11]. In this study we have investigated the possibility of using the phenolic extract obtained from bilberry residues as an ingredient to fortify two common milk products, a drinking yogurt and a condensed milk.

Bilberry extracts obtained after solvent evaporation had a moisture content of about 10% (w/w) and a polyphenol content of 81.69 ± 6.12 mg GAE/g dw. The antioxidant activity, expressed as Trolox equivalents, was 7.58 ± 0.27 mmol TE/g dw.

Fortified milk products were prepared by adding 100 to 400 mg of dry extract to 100 mL of drinking yogurt or 15 g of condensed milk. The appearance of the fortified milk products can be seen in Fig. 2, while their characteristics are reported in Tables 1 and 2. The antioxidant activity of the drinking yogurt ranged from 1.69 to 3.27 µmol TE/mL and that of the condensed milk from 12.16 to 23.16 µmol TE/g, depending on the added amount of bilberry extract. In terms of serving size (100 mL for the drinking yogurt and 15 g for the condensed milk), the above values correspond to an average intake of 0.7 to 1.35 mmol TE.

It may be interesting to evaluate the extent to which the two fortified products could contribute to total dietary antioxidant capacity (TDAC). In a recent study on a healthy Italian population, the TDAC was estimated as 6.61 mmol Trolox/person/day [12]. This value is close to that found for a Spanish population [13] and can be considered as representative of the Mediterranean diet. It follows that one serving size of the two products could contribute to approximately 10–20% of TDAC, depending on the enrichment degree. This is a significant contribution, considering that the overall intake of fruits and vegetables contributes at most to 15–20% of TDAC [13].

Another point to be made is that the polyphenols incorporated in the milk products are expected to be highly bioavailable, having been extracted and separated from the plant matrix components. It is known, in fact, that polyphenols in plant tissues are either bound to cell wall components or confined in the vacuoles [14]. In order to be absorbed by the organism, the food matrix in which they are entrapped must be degraded and the phenolic-polysaccharide bonds cleaved. Finally, the highest level of polyphenols in the enriched products (about 2 mg GAE/g) was significantly lower than the organoleptic threshold values determined for beverages enriched with polyphenols from berry fruits [15], yerba mate/black currant [16] and olive vegetation water [17]. Accordingly, although an accurate sensory evaluation should be performed on the fortified milk products, their sensory quality can be expected to be largely preserved.

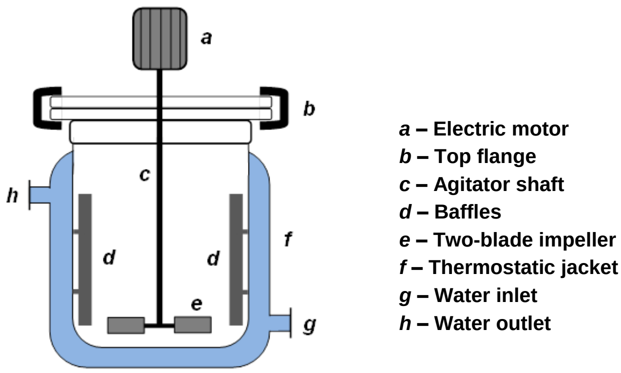

Fig.1. Schematic diagram of the apparatus used to extract polyphenols from bilberry residues.

TABLE 1. Characteristics of the functional products obtained by adding different amounts of dry bilberry extract to drinking yogurt (DY). q_{EXT} is the added amount of extract per volume of product, c_{POL} is the phenolic content of the final product, expressed as gallic acid equivalents (GAE), and AC is the antioxidant capacity, expressed as Trolox equivalents (TE).

ID	q_{EXT} (mg/100 mL)	c_{POL} (mg GAE/mL)	AC (µmol TE/mL)
DY-1	100	0.081 ± 0.007	1.693 ± 0.059
DY-2	200	0.162 ± 0.009	2.513 ± 0.092
DY-3	400	0.317 ± 0.013	3.275 ± 0.123

TABLE 2. Characteristics of the functional products obtained by adding different amounts of dry bilberry extract to condensed milk (CM). q_{EXT} is the added amount of extract per weight of product, c_{POL} is the phenolic content of the final product, expressed as gallic acid equivalents (GAE), and AC is the antioxidant capacity, expressed as Trolox equivalents (TE).

ID	q_{EXT} (mg/15 g)	c_{POL} (mg GAE/g)	AC (µmol TE/g)
CM-1	100	0.537 ± 0.015	12.165 ± 0.704
CM-2	200	1.157 ± 0.039	17.097 ± 1.082
CM-3	400	2.055 ± 0.092	23.165 ± 1.176

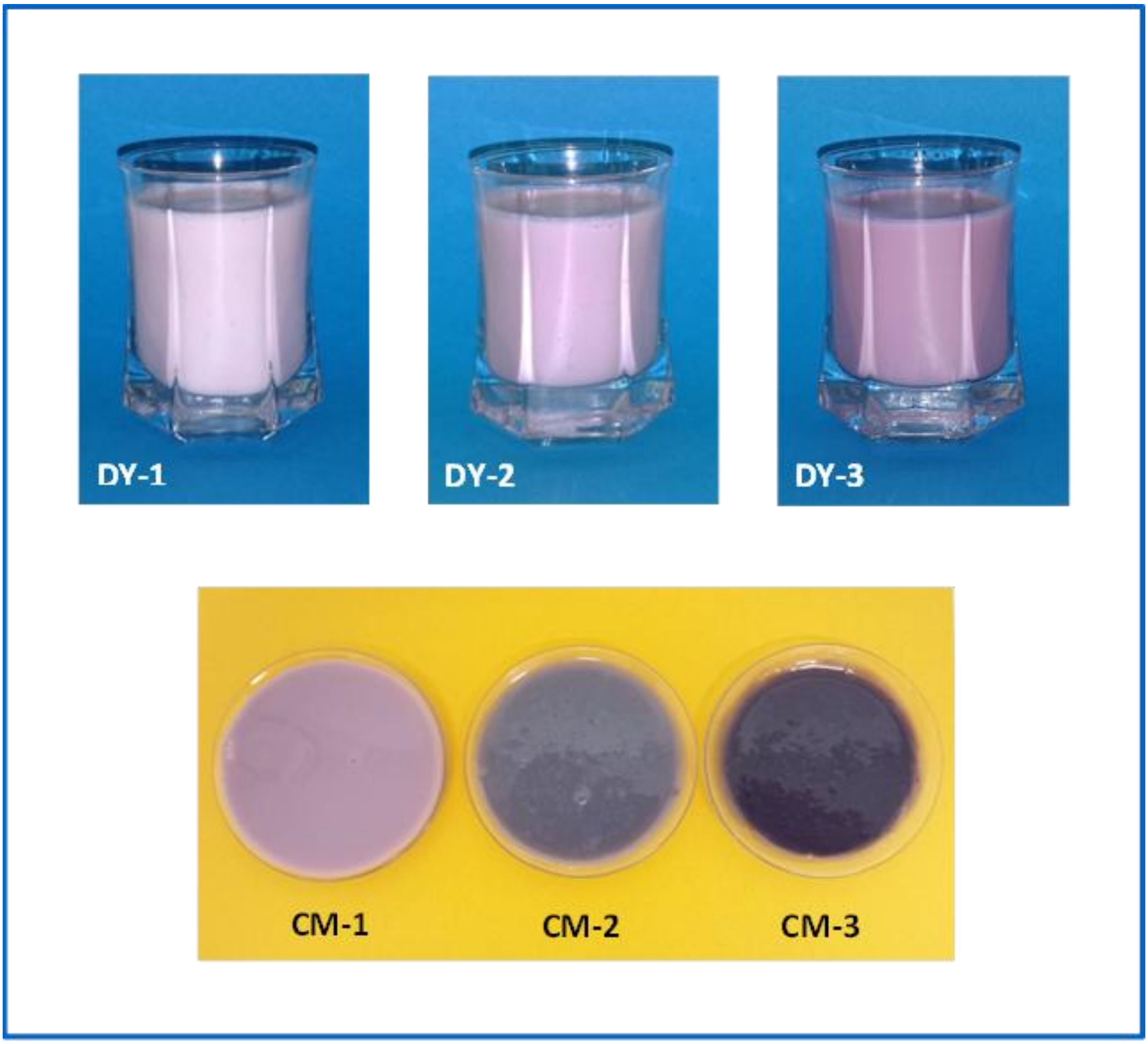

Fig.2. Fortified milk products obtained by incorporation of varying amounts of dry bilberry extracts (see Tables 1 and 2) into drinking yogurt (DY) and condensed milk (CM).

Conclusion

At present, there is a growing consumer demand for functional foods containing fruit-derived ingredients because of their perceived naturalness and recognized health benefits. The results of this study demonstrate that common food products such as drinking yogurts and condensed milk can be easily fortified with phenolic extracts obtained from bilberry residues. Enrichment of food products with bilberry polyphenols could represent a simple means of increasing the dietary intake of antioxidants and provide beneficial health effects such as reduced incidence of oxidative stress-related. In addition, the use of an agro-industrial waste as the source of polyphenols could contribute to reduce its environmental impact and provide economic benefits to the producers.

Future research should evaluate the storage stability of the fortified products and the characteristics of the products obtainable by replacing bilberry residues with other polyphenol-rich agro-industrial wastes.

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References

[1] Dai, J., and Mumper, R. J., 2010, "Plant phenolics: extraction, analysis and their antioxidant and anticancer properties," Molecules, 15, pp. 7313–7352.

[2] Landete, J. M., 2013, "Dietary intake of natural antioxidants: Vitamins and polyphenols," Crit. Rev. Food Sci. Nutr., 53, pp. 706–721.

[3] Vauzour, D., Rodriguez-Mateos, A., Corona, G., Oruna-Concha, M. J., and Spencer, J. P. E., 2010, "Polyphenols and human health: Prevention of disease and mechanisms of action," Nutrients, 2, pp. 1106–1131.

[4] Kranz, P., Braun, N., Schulze, N., and Kunz, B., 2010, "Sensory quality of functional beverages: Bitterness perception and bitter masking of olive leaf extract fortified fruit smoothies," J. Food Sci., 75, pp. S308–S311.

[5] Roopchand, D. E., Kuhn, P., Rojo, L. E., Lila, M. A., and Raskin, I., 2013, "Blueberry polyphenol-enriched soybean flour reduces hyperglycemia, body weight gain and serum cholesterol in mice," Pharm. Res., 68, pp. 59–67.

[6] Manganaris, G. A, Goulas, V., Vicente, A. R., and Terry, L. A., 2014, "Berry antioxidants: Small fruits providing large benefits," J. Sci. Food Agric., 94, pp. 825–833.

[7] Zuorro, A., and Lavecchia, R., 2014, "Recovery of phenolic antioxidants from the peel fraction of bilberry (*Vaccinium myrtillus* L.) processing waste," Biotechnology: An Indian Journal, 9(7), pp. 273–279.

[8] Zuorro, A., and Lavecchia, R., 2013, "Influence of extraction conditions on the recovery of phenolic antioxidants from spent coffee grounds," American Journal of Applied Sciences, 10(5), pp. 478–486.

[9] Zuorro, A., 2014, "Response surface methodology analysis of polyphenol recovery from artichoke waste," American Journal of Applied Sciences, 11(9), pp. 1463–1471.

[10] Bigliardi, B., and Galati, F., 2013, "Innovation trends in the food industry: The case of functional foods," Trends Food Sci. Technol., 31, pp. 118–129.

[11] Gupta, S., and Abu-Ghannam, N., 2012, "Probiotic fermentation of plant based products: Possibilities and opportunities," Crit. Rev. Food Sci. Nutr., 52, pp. 183–199.

[12] Di Giuseppe, R., Arcari A., Serafini, M., Di Castelnuovo, A., Zito F., and De Curtis, A., 2012, "Total dietary antioxidant capacity and lung function in an Italian population: A favorable role in premenopausal/never smoker women," Eur. J. Clin. Nutr., 66, pp. 61–68.

[13] Saura-Calixto, F., and Goñi, I., 2006, "Antioxidant capacity of the Spanish Mediterranean diet," Food Chem., 94, pp. 442–447.

[14] Bohn, T., 2014, "Dietary factors affecting polyphenol bioavailability," Nutr. Rev., 7, pp. 429–452.

[15] Jaeger, S. R., Axten, L. G., Wohlers, M. W., and Sun-Waterhouse, D., 2009, "Polyphenol-rich beverages: Insights from sensory and consumer science," J. Sci. Food Agr., 89, pp. 2356–2363.

[16] Orjuela-Palacio, J. M., Zamora, M. C., and Lanari, M. C., 2014, "Consumers' acceptance of a high-polyphenol yerba mate/black currant beverage: Effect of repeated tasting," Food Res. Int., 57, pp. 26–33.

[17] Servili, M., Rizzello, C. G., Taticchi, A., Esposto, S., Urbani, S., Mazzacane, F., Di Maio, I., Selvaggini, R., Gobbetti, M., and Di Cagno, R., 2011, "Functional milk beverage fortified with phenolic compounds extracted from olive vegetation water, and fermented with functional lactic acid bacteria," Int. J. Food Microbiol., 147, pp. 45–52.