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Euterpe oleracea juice as a functional pigment for yogurt

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Abstract

The juice of *Euterpe oleracea* Mart. fruit (Arecaceae), known as Açai in the Brazilian Amazon region, is dark purple with a high anthocyanin and phenolic content. The antioxidant and anti-radical properties of *E. oleracea* juice are well known; the chemical characterisation of its phenolic composition as well as its potential use as food ingredient and natural pigment have been previously studied. Cyanidin 3-*O*-glycoside, and various hydroxy-benzoic and hydroxy-cinnamic acids were detected in *E. oleracea* juice. The radical scavenging properties, measured as the mean of DPPH radical tests, were similar to those obtained by a common commercial bilberry juice. Therefore, novel natural colorants from *E. oleracea* juice could be considered as "functional" ingredients for their anti-oxidant and anti-radical activity. Yogurt is a typical fermented dairy product consumed all around the world; the yogurt flavouring is obtained both by means of natural ingredients like fruit juices and also by synthetic aromas. The aim of this work was to evaluate the use of *E. oleracea* fruit juice as a natural colorant for yogurt. The results obtained showed that yogurt enriched with *Euterpe* juice (10%, w/w) showed characteristics similar to those of typical commercial yogurt with bilberry juice. Aggregation of milk proteins in the *E. oleracea* containing yogurt was measured by SDS-PAGE. The protein profile of the *E. oleracea* containing yogurt was essentially identical to the untreated control yogurt. In conclusion, we suggest that *E. oleracea* juice could be used as a natural functional pigment for flavouring and colouring yogurt.

Keywords: *Euterpe oleracea*; Yogurt; Anthocyanins; Cyanidin-3-*O*-glycoside; Phenolics

1. Introduction

The juice of *Euterpe oleracea* Mart. fruit (Arecaceae), known as *Açai* in the Amazonian region, is dark purple with a high anthocyanins and phenolics content (Muñiz-Miret, Vamos, Hiraoka, Montagnini, & Mendelsohn, 1996). The juice is obtained by cold pressing the thin pulp of the ovoidal fruit (berry); the stone is about the 80% of the volume of the fruit (Rogez, 2000). The chemical composition of *Euterpe* fruit pulp and juice are quite different, as expected, the main quantitative difference is represented by the water content (Rogez, 2000).

UV–Vis spectrometry and HPLC analysis of the Açai juice confirmed a significant cyanidin-3-*O*-glycoside (kuromanin) content. Cyanidin and its glycosides represent one of the major groups of naturally occurring anthocyanins and have been shown to have antioxidant and potential health-promoting properties. The bioactive properties of anthocyanins have been demonstrated using in vitro (isolated systems and cell models) as well as in vivo (both rat and human intervention studies) studies (e.g., Galvano et al., 2004).

The principal bio-activities of anthocyanins are antioxidant, anti-radical and anti-proliferative. Recently the inhibition of the lipid peroxidation in a cell in vitro model was demonstrated (Bianchi et al., 2001). Many other in vitro cellular models have been optimized to study the

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bio-activity of anthocyanin and proanthocyanidin fractions (Galvano et al., 2004). Primarily because of it's commercial availability, cyanidin (aglycone), cyanidin 3-*O*-glycoside and related cyanidin glycosides have been the most commonly investigated anthocyanins, i.e., anti-oxidant activity, metabolism and bioavailability (Amorini et al., 2001; Lee, 2002). Concerning the real in vivo bio-activity of these compounds, their bioavailability is the critical point, and recent studies have shown that their absorption and metabolism may be considerably higher than originally assumed (e.g., Felgines et al., 2003; Talavéra et al., 2003).

Some hydroxy-benzoic and hydroxy-cinnamic acids were also detected in the *Euterpe* juice; the main phenolic acid was vanillic acid. Also chlorogenic and *p*-hydroxybenzoic acids were detected in the fresh juice (Coïsson, Capasso, Piana, Travaglia, & Arlorio, 2002). Some of these phenolic acids were also reported as powerful anti-oxidant in vitro (Shahidi & Naczk, 2003).

The radical scavenging properties of this pigmented juice, evaluated by means of stabilized radical by in vitro studies using the DPPH radical test and expressed as Percent of Inhibition after 15 min of reaction (IP%), were similar to those measured for bilberry juice (Coïsson et al., 2002). The colour of *E. oleracea* juice is stable in the pH range of natural white yogurts (as well as in the pH range of ice-creams). In fact, the colouring properties of the Açai juice (not-pasteurized and not-processed with other thermal industrial practices) are stable in the range of pH 3–5.

Only a few studies on *E. oleracea* juice have been reported (Araujo et al., 2004; Bobbio, Bobbio, Oliveira, & Fadelli, 2002; Del Pozo-Insfran, Brenes, & Talcott, 2004). Data on the functional properties of this matrix are still lacking.

The concept of "functional food" involves and requires the use of positive bio-active ingredients or the presence of natural healthy bio-active molecules in foods. The European consensus document (Bellisle et al., 1998) used the following definition: "A food can be regarded as "functional" if it is satisfactorily demonstrated to affect beneficially one or more target functions in the body, beyond adequate nutritional effects in a way that is relevant to either an improved state of health and well-being and/or reduction of risk of disease".

Under the technological point of view, these "functional" ingredients must show positive action for the texture, the stability or the consistency of the complex food too. Novel natural pigments such as *E. oleracea* juice, containing potential health-promoting bio-active phenolics, could be considered as "functional" ingredients for their anti-oxidant and anti-radical activity. The Açai pulp can also be considered as a good "supplement" for foods and dietetics, because its protein content is about 3% w/w (similar to cow's milk), its calcium content is 1180 mg kg^{-1}, it has a good vitamin content (vitamin C, tocopherols, vitamin A) and finally it has a high anthocyanin content (cyanidin 3-*O*-glycoside; about 400 mg kg^{-1}) (Rogez, 2000).

Yogurt is typical fermented milk consumed all around the world. This "biotechnological" food is considered by nutritionists as having high nutritional value (namely because they lack lactose and have a significant concentration of Ca^{++}) and positive bio-active effects (namely in products added with pre-biotic ingredients and pro-biotic bacteria) (Lourens-Hattingh & Viljoen, 2001). The "natural" plain yogurt is produced by concentrating milk (often by using ultrafiltration processes) and adding lactic bacteria that increase the lactic fermentation. The yogurt is also produced and consumed in flavoured and supplemented forms. In this case, the flavouring is done by addition of natural ingredients (for example juice of plant species) or by addition of synthetic flavour compounds. Yogurt is typically pigmented and flavoured adding fruit juices or pulps of pear, apricot, peach, apple, plum, but mainly using "berry" fruits like bilberry, strawberry, raspberry, bilberries, blackberry, blackcurrant and cherry. Many of these fruits are well known as very good sources of anthocyanins.

In order to evaluate the production of novel dairy foods, we have studied the effect of pigmentation and the use of *E. oleracea* juice as functional ingredient to colour the white yogurt from whole milk, a dairy product consumed in all European countries.

The aims of this study were: (i) to evaluate the use of the *Euterpe* juice (Açai) to flavour the natural white yogurt; (ii) to evaluate the change of yogurt pH after the addition of *Euterpe* juice (natural and juice clarified by centrifugation); (iii) to evaluate the protein fingerprints of yogurt before and after the addition of the juice and (iv) to evaluate the antiradical scavenging properties of this novel food by mean of in vitro DPPH test, in comparison to a commercial bilberry-enriched yogurt.

2. Materials and methods

2.1. Samples and chemical characterization

E. oleracea juice was kindly provided by Soul Food (Rivoli, Italy). White natural plain yogurt (cow's milk, % fat: 3.9%) and bilberry-enriched yogurt were purchased in a local shop. Protein, water, fat, total phenolic content, total anthocyanins, pH, density and other chemical parameters were measured using previously described methods (Coïsson et al., 2002).

2.2. Protein analysis

Protein analysis was performed using a standard SDS-PAGE protocol (Laemmli, 1970). Samples were dissolved in 10 ml of Tris–glycine (0.01 M, pH 8.3) and urea (6 M) buffer, stirred for 2 h at 25 °C and loaded in the gel diluted 1:1 with Laemmli sample-buffer.

2.3. Antiradical and antioxidant activity

Antiradical properties were evaluated by measuring the scavenging of the DPPH radical and expressed as Inhibition Percent (IP%), as described in Brand-Williams, Cuvelier, and Berset (1995). Analysed samples were obtained by dilution of 1 g of sample with 100% methanol to a final volume of 10 ml.

3. Results and discussion

The complete chemical characterization of the Açai (*Euterpe* juice) has been reported in a previous study and the composition of the *E. oleracea* juice used as functional ingredient in this study was similar (Coïsson et al., 2002). Data obtained in the present study confirmed the presence of cyanidin 3-*O*-glycoside, in a concentration of more than 1600 mg kg^{-1} in fresh well-conserved juice (transport and storage of the juice were at −20 °C), similar to the level previously described by Del Pozo-Insfran et al. (2004). It is well known that anthocyanins are widely distributed in nature, giving colour from red through purple to blue in many vegetables and fruits. Their health promoting effects, together with those of other class of natural anti-oxidant molecules (proanthocyanidins, phenolic acids, vitamin C, vitamin E, vitamin A) are well established. The singular properties of the *Euterpe* juice are positively correlated to the high anthocyanin content. The water content of the *Euterpe* juice was 87.3%; dry matter: 12.7%; protein content: 1.09% ($N \times 6.25$, Kjeldhal method).

The mean of the pH value of the natural centrifuged juice from *E. oleracea* (measured immediately after the defrosting, at 25 °C) was 4.90; the mean of the pH of white yogurt was 3.66. After the addition of the *Euterpe* juice to the whole white yogurt, the colour was compared to the commercial bilberry-enriched yogurt. The intensity of the colour as well as the colour of the new product was similar to those typical of diluted bilberry in white yogurt, with a smooth note of brown (visual subjective evaluation, no instrumental analyses were performed). Detailed oflactometry and rheology analyses will be done in the near future.

After the addition of the Açai juice to the white plain yogurt (10% w/w, a typical dose used by industries, and chosen to take into consideration the viscosity of this juice) the pH did not change significantly (pH: 3.71 versus 3.66), as reported in Table 1. The analysis of commercial bilberry-enriched yogurt (fruit juice content declared: 8.6%) showed a pH of 3.70. The evolution of the acidity of the yogurt (not further packaged) was similar to those typical of bilberry-enriched yogurt (data not shown).

Table 1
pH values of different samples

Sample	pH
Natural yogurt (white, from cow's milk)	3.66
Commercial yogurt with bilberry	3.70
Euterpe juice	4.90
Euterpe centrifuged juice	4.92
Yogurt + *Euterpe* natural juice (10% w/w)	3.71
Yogurt + *Euterpe* centrifuged-juice (10% w/w)	3.71

The colour of the *Euterpe*-enriched yogurt was similar to commercial bilberry yogurt. The total anthocyanin content was determined spectrophotometrically at 520 nm; the enriched-yogurt was diluted in water 1:10, v/v. The results were 0.124 ± 0.003 and 0.105 ± 0.004 (mg kg^{-1}) for commercial bilberry-enriched yogurt and *Euterpe*-enriched yogurt, respectively. The colouring properties of *Euterpe* juice (Açai) were stable for 2 days at 4 °C, in presence of air (visual evaluation of the superficial colouring modifications).

According to our previous study, the colouring properties of *E. oleracea* are seriously affected by the oxidation (mainly by polyphenoloxidase enzymes) and the consequent polymerization of some phenolic constituents (enzymatic browning). As in the case of other natural pigmented plant juices, the colour is strictly related to the freshness of the product.

Any coagulation-precipitation of proteins of white yogurt was noted after the addition of the juice. More specific data of the protein pattern was obtained by evaluating the protein fingerprints by SDS-PAGE analysis. The protein composition of the white yogurt did not show any significant differences after the addition of the natural fresh *Euterpe* juice (as well as the centrifuged juice without particles-fibre fraction of the juice, Fig. 1).

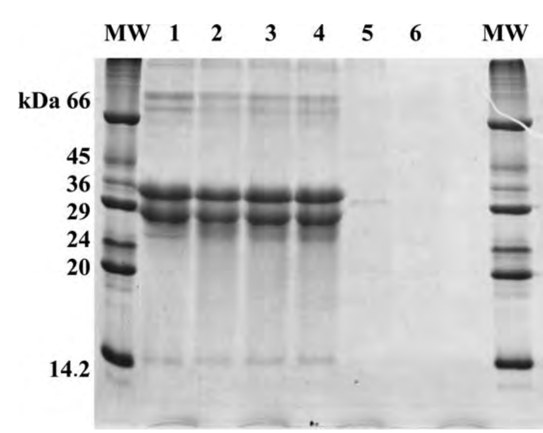

Fig. 1. SDS-PAGE fingerprints of proteins from samples analysed. 1, white yogurt; 2, bilberry-enriched yogurt (8.6% declared); 3, *Euterpe*-enriched yogurt (10%); 4, *Euterpe*-enriched yogurt (10%, centrifuged before the use and before the analysis); 5, *Euterpe* juice; 6, *Euterpe* juice (before the analysis); MW, molecular weight.

Table 2
Antiradical activity measured using the DPPH· method

Sample	(v/v in methanol or molarity)	IP%
Natural white yogurt	1:100	9.97
Commercial bilberry-enriched yogurt	1:100	32.66
Yogurt + *Euterpe* juice (10% w/w)	1:100	16.18
Yogurt + *Euterpe* (centrifuged juice, 10% w/w)	1:100	16.62
Caffeic acid	10^{-4} M	94.08
Trolox	10^{-4} M	96.10
Euterpe natural juice	1:250	87.09
Euterpe natural juice	1:500	84.84
Euterpe natural juice	1:1000	22.13

The protein fingerprint of *Euterpe* juice (lanes 5 and 6, Fig. 1) was weak because of the high dilution (protein content: about 1%). Concerning this point, further detailed analysis of the *Euterpe* protein fraction needs to be done after concentration using ultrafiltration techniques. The principal protein fractions in all analysed yogurt (enriched- and not enriched samples) were α_{s1}-casein (apparent molecular weight: 31 kDa), β-casein (MW: 28 kDa), α-lactoalbumin (MW:14.2 kDa), and some casein-aggregates at high MW (Fig. 1).

Using the DPPH radical scavenging method it was shown that the antiradical activity of the sample from the *Euterpe*-enriched yogurt was lower than those observed in commercial yogurt with bilberry juice (Table 2). A dilution of 1:100 of *Euterpe*-enriched yogurt showed about 50% of the activity compared with bilberry-enriched yogurt. There was no significant difference in radical scavenging ability between centrifuged (clarified) and non-centrifuged *Euterpe* juice. Moreover, commercial bilberry-enriched yogurt showed a scavenging property about three fold higher compared with the white yogurt used as reference (Table 2).

However, a comparison of the DPPH radical test with other tests to assess the anti-radical power (ORAC, ABTS, TEAC) could be of value since some assays are better for matrices with a high fat content. Previous studies have assessed some technical differences among the different methods, as well as regarding the interpretation of the results (Arnao, 2000; Lissi, Modak, Torres, Escobar, & Urzua, 1999). Fresh *Euterpe* juice showed a good anti-radical action; a 1:250 dilution (in methanol) was comparable with those obtained with a standard solution of caffeic acid and Trolox (10^{-4} M) (Table 2).

The presence of a single anthocyanin in *Euterpe* juice (compared with the more complex anthocyanin profiles of other berries and fruits) means it has potential as a model matrix to study the hyper- and batho-chromic effects in a natural food. The addiction of some natural anti-oxidant molecules, such as ascorbic acid, could be fundamental to synergistically increasing the anti-oxidant properties of Açai juice alone and in various food matrices.

A key aspect of the health-promoting benefits of *Euterpe* bio-active compounds is their bioavailability in vivo. Recent studies confirmed the absorption of the cyanidin (aglycone) and its glycosides and estimated daily intake of cyanidin 3-*O*-glycoside as 180 mg (Galvano et al., 2004). The principal conventional sources of cyanidin in the European diet are fruit (namely pigmented blood-orange juices, varieties Moro, Tarocco, Sanguinello, blackcurrant and bilberry) and wine. So, the use of *Euterpe* juice as dietary or food supplement could be used to increase the intake of this compound as well as to increase the protection against ROS and NOS bioactive species in vivo.

4. Conclusions

In this work we have evaluated the possible use of the anthocyanin-rich pigmented juice from *E. oleracea* as a functional ingredient in yogurt. Enrichment of yogurt with *Euterpe* juice at 10% w/w showed characteristics similar to those typical of commercial white yogurt that had been supplemented with bilberry juice. We suggest the use of the clarified (centrifuged) or natural anthocyanin-rich *Euterpe* juice for the colouring and the flavouring of the white yogurt to obtain a novel antioxidant-rich dairy food that could be used to increase the dietary intake of cyanidin-3-*O*-glucoside.

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