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Incorporation of strawberries preparation in yoghurt: Impact on phytochemicals and milk proteins

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ABSTRACT

An immediate decrease in the total antioxidant activity (23%) and total phenolic content (14%) was observed after addition of strawberry preparations to yoghurt. The total anthocyanin content did not change immediately, but decreased 24% throughout the yoghurt shelf-life.

The individual compounds, (+)-catechin (60%), (−)-epicatechin (60%), kaempferol (33%) and quercetin-3-rutinoside (29%) decreased after 24 h in the yoghurt made with the strawberry preparation. During the remaining period of storage these compounds increased by 47%, 6%, 4% and 18%, respectively. Pelargonidin-3-glucoside decreased 49% after 28 d.

Immediately after the addition of the strawberry preparation to yoghurt, β-lactoglobulin decreased to values lower than the limit of detection and α-lactalbumin by approximately 34%, and was reduced further slowly throughout yoghurt self-life. An immediate interaction between the carrageenan present in the strawberry preparation and β-LG was observed. The variations of both polyphenols and protein in the presence of carrageenan and the potential interactions were discussed.

1. Introduction

Yoghurt is a very popular fermented milk that is produced all over the world by acid coagulation of milk without drainage (Sodini, Remeuf, Haddad, & Corrieu, 2004). It is considered by nutritionists as having high nutritional value and positive bioactive effects, usually reinforced by the addition of prebiotic ingredients and probiotic bacteria. Fruit yoghurt is among the most common fermented dairy products consumed around the world (Saint-Eve, Lévy, Martin, & Souchon, 2006). To increase the functionality and antioxidant capacity of these dairy products, food ingredients such as strawberry fruit is commonly added (Coïsson, Travaglia, Piana, Capasso, & Arlorio, 2005; Trigueros, Pérez-Alvarez, Viuda-Martos, & Sendra, 2011).

Yoghurts are made mainly from cow milk, whose proteins are composed by *ca.* 80% caseins (α_{s1}-, α_{s2}-caseins, β-casein and *k*-casein) and *ca.* 20% whey protein formed by four major soluble proteins: β-lactoglobulin (β-LG), α-lactalbumin (α-LA), blood serum protein (BSA) and immunoglobulins (Igs). These proteins represent 50%, 20%, 10% and 10% of the whey proteins fraction, respectively (Farrell et al., 2004; Jovanovic, Barac, Macej, Vucic, & Lacnjevac, 2007; Ruprichová, Dračková, Borkovcová, & Vorlová, 2012). The whey proteins can bind with many kinds of endogenous and exogenous agents such as dietary polyphenols (Xiao, Mao, et al., 2011).

Whey proteins when exposed to high temperatures (>65 °C) irreversibly denature and coagulate, as opposed to caseins, which do not coagulate when subjected to a high heat treatment (Jovanovic et al., 2007). Caseins micelles aggregate through isoelectric precipitation brought about by the action of lactic acid bacteria or organic acids. The casein strands can be broken and the size of the aggregates decreased. The rearrangement and syneresis of the acid induced casein network in yoghurt occurs during storage (Everett & McLeod, 2005).

Polyphenols have a significant affinity for proteins that lead to the formation of soluble complexes, which can grow in size and even form sediments. Most of the models suggest that protein–polyphenol complexes are formed by multiple weak interactions (mainly hydrophobic) between the amino acid side chains and the polyphenol aromatic rings, indicating that the association of polyphenols with proteins is mainly a surface phenomenon.

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Sometimes these interactions could be complemented by hydrogen bonding, which would play an important role in reinforcing and stabilizing the complexes (Charlton et al., 2002).

The formation of protein–polyphenol complexes is deeply influenced by the nature of the protein (i.e. number of proline residues), the nature of the polyphenol (i.e. number of aromatic rings), the temperature of the system and the presence of other components (i.e., sugars, polysaccharides) that can affect the interaction (Prigent et al., 2003).These interactions may have a detrimental effect on the *in vivo* solubility of both phenolic and proteins. The majority of the published studies discuss pure proteins interactions with specific polyphenols (Charlton et al., 2002; Kanakis et al., 2011; Viljanen, Kylli, Hubbermann, Schwarz, & Heinonen, 2005; Xiao, Mao, et al., 2011).

The potential decrease in the solubility of the complexes formed between protein and polyphenol, does not necessarily alter the bioavailability of such polyphenols (Ferruzzi, Bordenave, & Hamaker, 2012). Nielsen and Leufven (2008) and Neilson et al. (2007) observed that polyphenol–protein complexes formed in milk with cocoa chocolate present in the gut were effectively disrupted by normal digestion, having a minimal impact in flavan-3-ols bioaccessibility *in vivo*.

Until now little is known about the interactions of milk proteins in the yoghurt model with fruit polyphenols and the formulation components as a whole.

Strawberry formulations were used for this study because they are rich in phenolic compounds, in particular anthocyanins, and are the leading fruit used in European fruit enriched yoghurts.

The aims of this study were (i) to evaluate the protein fraction of the yoghurt before and after the addition of strawberry preparations, (ii) to evaluate the antioxidant properties and fruit phytochemical content in the final strawberry yoghurt format and (iii) to establish the possible interactions between the strawberry preparation components and the milk proteins or other preparation compounds.

2. Materials and methods

2.1. Preparation of strawberry formulation

Individually quick frozen (IQF) strawberry (50%) was mixed with sugar (27%), glucose and fructose syrup (8%) in a mixed reactor with jacket heating and cooling. Carrageenan (0.38%), starch (2%), cochineal carmine (0.0095%) and strawberry aroma (0.48%) were dispersed in cold water separately and these ingredients were added to the strawberry. The mixture was pasteurised at 90 °C for 3 min.

2.2. Preparation of strawberry yoghurt samples

Low fat white yoghurts were acquired, at the beginning of their 30 d shelf-life, in a local market and were used to incorporate industrial strawberry preparations under aseptic conditions. Strawberry preparation was added in a proportion of 20% of the yoghurt weight. Fruit preparations are generally added to yoghurt products within the range of 10–20% level in the final product (O'Rell & Chandan, 2006).

The yoghurt-fruit mixture was distributed in 100 mL sterile polypropylene containers and kept during 28 d at 2 °C. The yoghurt and strawberry preparation were stored under the same conditions and were used as control samples. The control samples included one part of yoghurt and strawberry preparation used for the experiment, assuring similar treatments and conditions for all the samples.

Each sample was prepared in triplicate and sampling was performed at days 1, 7, 14, 21 and 28 to prepare extracts for qualitative and quantitative analyses of polyphenols and proteins.

2.3. Extraction of polyphenols for chemical analyses

Strawberry hydrophilic extractions were performed according to Redeuil et al. (2009) with some modifications. Strawberry yoghurt (20 g) was homogenised with 30 mL of methanol acidified with formic acid (9:1 v/v) using an ultra-turrax (IKA Ultra-turrax T18. Wilmington. USA) at 24,000 rpm for 1 min. The homogenised sample was kept at −20 °C during 1 h to allow protein precipitation. The slurry was then centrifuged at 4000×*g* at 4 °C for 10 min and the supernatant filtered through a 3 kDa cutoff membrane (Vivaflow® 50, Sartorius) to remove soluble proteins.

Yoghurt and strawberry control extracts were prepared according to the same procedure described. Water was used as yoghurt fraction substitute for strawberry preparations and as strawberry substitute in white yoghurt control.

A 25-mL aliquot of the extract was evaporated to dryness in a RVC 2-18 speed-vacuum evaporator (Christ. Osterode am Harz. Germany) at 30 °C and the residue was dissolved in 2 mL of methanol. The extract was then filtered through a 0.45-μm cellulose acetate filter (Orange Scientific. Braine-l'Alleud. Belgium) and was used to determine individual phenolic compounds, total antioxidant activity, total phenolic and total anthocyanin content.

The results of each extract determination were reported to the fresh weight of IQF strawberry used in 20% of yoghurt weight. Results as mg per gram of fresh weight were obtained according to Eq. (1).

$$C(\text{mg/g FW}) = \frac{(\text{mg/mL}) * \text{Extract volume (mL)}}{\text{g FW}} \quad (1)$$

2.4. Determination of total antioxidant activity, total phenolic content and total anthocyanin content

The ABTS method is a decolorization assay applicable to hydrophilic antioxidants as described by Giao et al. (2007). To oxidise the colourless ABTS to the blue-green, ABTS radical cation (7 mmol/L) was mixed with potassium persulfate (2.45 mmol/L) and kept for 12–16 h at room temperature in the dark. The ABTS solution was diluted with water to an absorbance of 0.70 (±0.02) at 734 nm. After the addition of 1.0 mL ABTS solution to 10 μL of sample the mixture and the absorbance reading was made after 6 min. The inhibition% of the sample was then compared with a standard curve made from the corresponding readings of ascorbic acid (0.02–0.50 mg/mL) and results expressed as mg ascorbic acid equivalents/g fresh weight.

The total phenolic content was determined using the Folin–Ciocalteu reagent according to the method of Singleton and Rossi (1965) Briefly 50 μL of methanolic extract, 50 μL Folin–Ciocalteau reagent, 1 mL of Na_2CO_3 (75 g/L) and 1.4 mL of ultrapure water were added to 1.5 mL microcentrifuge tubes and the samples were vortexed. Tubes were then left in the dark for 60 min at room temperature. The absorbance of the sample was read at 750 nm using gallic acid (0.015–1.00 mg/mL) as a standard. Results were expressed as mg of gallic acid equivalent/g fresh weight.

Total anthocyanin quantitation was performed by the pH differential method of Lee, Durst, and Wrolstad (2005). Samples were diluted in pH 1.0 buffer (potassium chloride, 0.025 M) and pH 4.5 buffer (sodium acetate, 0.4 M), and then measured at 515 and 700 nm in a Shimadzu 1240 UV–visible spectrophotometer. Total absorbance was expressed by Eq. (2) and converted to milligram of pelargonidin-3-glucoside equivalents per millilitre according to Eq. (3). Results were expressed as mg pelargonidin-3-glucoside equivalent/g fresh weight.