LWT - Food Science and Technology 89 (2018) 164–170

Contents lists available at ScienceDirect

LWT - Food Science and Technology

journal homepage: www.elsevier.com/locate/lwt

Impact of *in-vitro* gastro-pancreatic digestion on polyphenols and cinnamaldehyde bioaccessibility and antioxidant activity in stirred cinnamon-fortified yogurt

Ahmed Helal[a], Davide Tagliazucchi[b,*]

[a] Department of Food and Dairy Sciences and Technology, Faculty of Agriculture, Damanhour University, 22516, Damanhour, Egypt
[b] Department of Life Sciences, University of Modena and Reggio Emilia, Via Amendola, 2 - Pad. Besta, 42122 Reggio Emilia, Italy

ARTICLE INFO

Keywords:
Functional yogurt
Cinnamon
Phenolic compounds
Radical scavenging activity
Bioaccessibility

ABSTRACT

In this study, cinnamon powder was supplemented into yogurt as a functional ingredient. The total phenolic compounds, individual phytochemicals and radical scavenging activity of the yogurts were measured and compared with a cinnamon water extract treated in the same way as the fortified yogurt. Cinnamon-fortified yogurt displayed higher total phenolic content ($P < 0.05$) and higher radical scavenging activity ($P < 0.05$) compared to plain yogurt. Phenolic acids, flavonols and cinnamaldehyde were identified in the cinnamon-fortified yogurt. Results showed that only the 34.7% of the total phenolic compounds present in the cinnamon water extract were found in the cinnamon-fortified yogurt, the remaining being bound to milk proteins. A low recovery was also found for the individual phytochemicals. However, *in-vitro* digestion of the cinnamon-fortified yogurt resulted in the release of phenolic compounds from milk proteins so that at the end of the digestion the amount of phenolic compounds recovered in the cinnamon-fortified yogurt was higher than that found in the digested cinnamon water extract ($P < 0.05$). These results clearly showed that yogurt matrix enhance the gastro-intestinal stability and the bioaccessibility of cinnamon polyphenols. Cinnamon-fortified yogurt can be considered an important source of dietary bioaccessible polyphenols.

1. Introduction

Developing of functional foods with health promoting natural ingredients has increased in the past decade (Granato, Nunes, & Barba, 2017). The development of new products with potentially positive effect on health using traditional herbs and food, which are known to be safe from the toxicological standpoint, is generally desirable since there is an increasing interest among consumers to look for healthier and natural food (Granato et al., 2017). Traditional herbs and food used to improve the functionality of food are normally chosen because rich in phenolic compounds, which possess strong antioxidant activity and show protective effects against chronic diseases including diabetes, cardiovascular diseases and cancer (Del Rio et al., 2013). In the Middle East and Arab countries, cinnamon powder is a well-known and commonly used food and traditional herbal medicine. Cinnamon showed several beneficial health properties such as anti-tumoural, cardiovascular, cholesterol lowering, and antioxidant activities (Gruenwald, Freder, & Armbruester, 2010; Hlebowicz, Darwiche, Bjorgell, & Almer, 2007; Hlebowicz et al., 2009). Cinnamon polyphenols mainly consist of condensed tannins (oligomeric and polymeric procyanidins) and monomeric phenolic compounds such as flavonols and phenolic acids (Gu et al., 2004; Helal, Tagliazucchi, Verzelloni, & Conte, 2014). Cinnamaldehyde is also a major component in cinnamon bark, which exhibits several biological effects such as anti-tumoural, pro-apoptotic and anti-inflammatory activities (Chao et al., 2008; Roussel, Hininger, Benaraba, Ziegenfuss, & Anderson, 2009).

Yogurt is the most popular fermented dairy product and is highly appreciated for its nutritional value and good digestibility (Saint-Eve, Levy, Martin, & Souchon, 2006). Recently, numerous studies underlined the health benefits of yogurt consumption in terms of enhancement of the immune system, improvement of bowel function, protection against colon cancer and *Helicobacter pylori* infection (El-Abbadi, Dao, & Meydani, 2014). The health benefits of yogurt have been ascribed to the presence of bioactive peptides and probiotics (Rutella, Tagliazucchi, & Solieri, 2016). However, it is not considered a source of phenolic compounds and therefore traditional herbs or food such as spices, fruit juices and grape seed or extract had been used to enhance the phenolic content of yogurt (Chouchouli et al., 2013; Illupapalayam, Smith, & Gamlath, 2014; Karraslan, Ozden, Vardin, & Turkoglu, 2011; Oliveira et al., 2015). Yogurt matrix seems to be an excellent delivery

* Corresponding author.
E-mail address: davide.tagliazucchi@unimore.it (D. Tagliazucchi).

http://dx.doi.org/10.1016/j.lwt.2017.10.047
Received 7 August 2017; Received in revised form 13 October 2017; Accepted 22 October 2017
Available online 24 October 2017
0023-6438/

vehicle for plant-derived phenolic compounds. The low pH increase the stability of phenolic compounds during storage (Chouchouli et al., 2013), whereas the presence of proteins or large peptides and fat should maintain the integrity of phenolic compounds during digestion increasing their bioaccessibility (Tagliazucchi, Helal, Verzelloni, & Conte, 2012; Lamothe, Azimy, Bazinet, Couillard, & Britten, 2014). Bioaccessibility is defined as the amount of a specific compound solubilized in the small intestine and available for the subsequent absorption. The bioaccessibility definition comprises the release of compounds from food matrices and their stability under the gastro-intestinal condition (Tagliazucchi, Verzelloni, Bertolini, & Conte, 2010). This latter point is of paramount importance since only the compounds released from the food matrix and stable in the gastro-intestinal condition are potentially bioavailable and in condition to exert their beneficial effects on the gastro-intestinal tract.

The main objective of the present study was to fortify the phenolic content of yogurt, using cinnamon powder and to evaluate the bioaccessibility of phenolic compounds and cinnamaldehyde and the antioxidant activity during simulated gastro-pancreatic digestion of the cinnamon-fortified yogurt.

2. Materials and methods

2.1. Materials

Dano® full cream milk powder was obtained from Arla Foods Ingredients (Viby J, Denmark). YOFLEX® commercial yogurt starter culture of *Streptococcus thermophilus* and *Lactobacillus delbrueckii* ssp. *bulgaricus* were obtained from Chr. Hansen, (Hoersholm, Danmark). Cinnamon bark powder (*Cinnamomum cassia*) was purchased from local market (Damanhour, Egypt). Enzymes and reagents for the *in-vitro* digestion, radical scavenging activity analysis as well as phenolic standards were supplied by Sigma (Milan, Italy).

2.2. Preparation of stirred yogurts and cinnamon water extract

Yogurt preparation and experimental strategy are summarized in Fig. 1.

Stirred yogurt was manufactured according to the instructions of Illupapalayam et al. (2014) with some modifications. Briefly, plain yogurt was prepared by heat-treating reconstituted full-fat milk powder (12% w/v) at 95 °C for 5 min followed by cooling to 45 °C. For the preparation of plain yogurt with sucrose, 7.5% (w/v) of sucrose was added to the milk powder and treated as reported above. The cinnamon-fortified yogurt was prepared by adding 1.5% (w/v) of cinnamon powder to the reconstituted milk powder following by the same heat-treatment as reported above. In the cinnamon fortified yogurt with sucrose, an amount of 7.5% of sucrose was also added before the heat-treatment. All the treatments were then filtered using stainless-steel mesh to remove the insoluble materials, inoculated with starter culture and incubated at 45 °C until the pH reached 4.4 (~8 h). Cooling to 5 °C was done to halt further acidification. The yogurt was manually stirred during the cooling using stainless-steel kitchen whisker. The stirred samples were transferred into yogurt cups aseptically and stored in refrigerator at 5 °C for one day.

A control (named cinnamon water extract) with cinnamon powder (1.5% w/v) but without milk powder was also prepared and heat-treated, inoculated, stirred and cooled as the cinnamon fortified yogurt.

Samples were collected from each treatment at the end of the procedure.

2.3. In-vitro digestion

Yogurt preparations and cinnamon water extract were subjected to *in-vitro* simulated digestion to determine the effect of digestion on phenolic content and radical scavenging activity. The recent standardized digestion method by Minekus et al. (2014) was followed with some modification as reported in Tagliazucchi, Helal, Verzelloni, Bellesia, and Conte (2016). For the gastric step, samples were diluted with simulated gastric fluid stock electrolyte solution (1:1) and homogenized for 2 min in a laboratory blender. The pH was then lowered to 2.5 with 6 mol/L HCl before the addition of 2000 U/mL of pepsin. Samples were incubated for 2 h at 37 °C. The chyme was then subjected to the pancreatic phase of digestion. Simulated intestinal fluid was added and the pH was brought to 7.5 with 20% Na_2CO_3 before adding 0.8 g/L of pancreatin and 10 mmol/L of bile salts. The digestive mixture was incubated in a shaking bath for additional 2 h at 37 °C.

Aliquots of the samples were collected before and after peptic digestion and after pancreatic digestion. The digestions were carried out in triplicate.

2.4. Samples preparation for analysis

Samples from yogurt preparations, cinnamon-water extract and *in-vitro* digestions were centrifuged at 17500*g* for 10 min at 5 °C to eliminate the insoluble material. The clear supernatants were then analysed for the content in total free phenolic compounds, total free tannins and individual free phytochemicals as well as for the radical scavenging activity analysis.

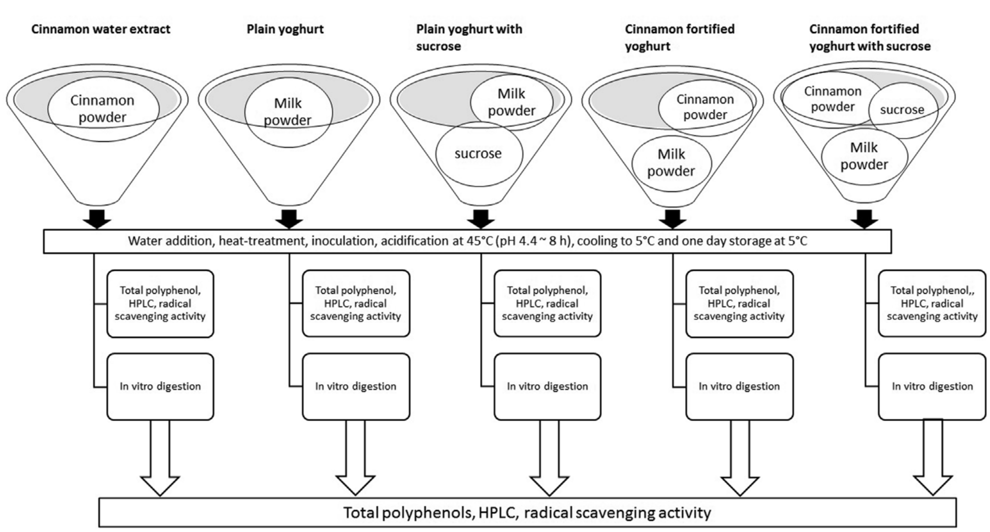

Fig. 1. Experimental strategy for the preparation and characterization of cinnamon-fortified yogurt. This figure details the experimental steps performed for preparing and characterizing cinnamon-fortified yogurt. Milk was formulated starting from full cream milk powder and added at 12% (w/v) concentration. Cinnamon powder was added at 1.5% (w/v) concentration. Sucrose was added at 7.5% (w/v) concentration. Cinnamon water extract was formulated in the same way as the cinnamon-fortified yogurt omitting milk powder from the preparation. After water addition, all the treatments were heat-treated at 95 °C for 5 min followed by cooling to 45 °C and then inoculated with starter culture and incubated at 45 °C until the pH reached 4.4 (~8 h). Abbreviations: HPLC, high performance liquid chromatography.