LWT - Food Science and Technology 62 (2015) 393–399

Contents lists available at ScienceDirect

LWT - Food Science and Technology

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

Total phenolic content and antioxidant properties of hard low-fat cheese fortified with catechin as affected by *in vitro* gastrointestinal digestion

A. Rashidinejad [a, b, *], E.J. Birch [a], D. Sun-Waterhouse [c], D.W. Everett [a, b]

[a] University of Otago, PO Box 56, Dunedin 9054, New Zealand
[b] Riddet Institute, Private Bag 11 222, Palmerston North 4442, New Zealand
[c] Plant and Food Research, Private Bag 92169, Auckland Mail Centre, Auckland 1142, New Zealand

ARTICLE INFO

Article history:
Received 22 January 2014
Received in revised form
21 December 2014
Accepted 29 December 2014
Available online 6 January 2015

Keywords:
Catechin
Cheese ripening
In vitro recovery
Phenolic content
Simulated digestion

ABSTRACT

The total phenolic content (TPC) and antioxidant activity (AA) of a low-fat hard cheese fortified with different concentrations of catechin was examined over 90 days of ripening after *in vitro* digestion of cheese samples. TPC and AA increased in cheese after manufacturing and over the ripening period at 8 °C. Prior to analysis, the cheese samples were subjected to a two-stage *in vitro* digestion with gastric and intestinal phases to simulate human digestive conditions. The AA, measured as both ferric reducing antioxidant power and oxygen radical absorbance capacity, showed a high degree of correlation ($R^2 > 0.89$) with the TPC results. The *in vitro* recovery fraction of catechin after 90 days of ripening was 0.607, 0.628 and 0.752 for the cheeses fortified with catechin at 125, 250 and 500 mg kg^{-1}, respectively. This study shows the feasibility of incorporating phenolic antioxidants into a protein-rich food, such as cheese, and maintaining antioxidant activity after digestion.

1. Introduction

Catechins are a class of flavonoids with demonstrated health benefits attributed to antioxidant activity (AA). These benefits include anti-allergic, anti-microbial and anti-carcinogenic effects (Yamamoto & Gaynor, 2001) and mitigation of heart disease (Hertog, Feskens, Hollman, Katan, & Kromhout, 1993). The efficacy of catechins is associated with the dietary quantity. Consumption of five cups or more of green tea is required to show the beneficial effect of catechins (Kuriyama, 2008). In practice, most people do not drink such a large volume of green tea on a daily basis due to reasons such as the high caffeine content (Chu & Juneja, 1997), therefore, catechins are packaged in different dietary forms, such as supplements, and incorporated into functional foods to increase the amount consumed (Ferruzzi & Green, 2006; Najgebauer-Lejko, Sady, Grega, & Walczycka, 2011; O'Connell, Fox, Tan-Kintia, & Fox, 1998; Pattono et al., 2009; Wegrzyn et al., 2008). As an example, Arts, Hollman, Feskens, Bueno, and Kromhout (2001) reported the mean daily catechin intake from a normal diet in the Dutch population was 50 mg. Tea was the main source of catechin in all age groups. Chocolate was the secondary source for children whereas apples and pears were the secondary source for adults and the elderly. In a population where tea consumption is low, consuming this amount of catechin from a delivery vehicle, such as cheese, would be easily achievable without overconsumption. Concentrations of catechin 1–2 g kg^{-1} could be added to cheese such that even a small portion consumed daily (25–50 g) should be able to provide a typical amount of catechin for an adult.

Most measurements of the amount and AA of phenolic antioxidants in dairy products have employed methanol extraction. This type of extraction can result in an overestimation of the actual value in food products during digestion because the solubility of the phenolics in methanol may be higher than in the digesta. Furthermore, the stability and extractability of catechins is affected by interactions with components of the food matrices, such as with the protein phase (Rashidinejad, Birch, Sun-Waterhouse, & Everett, 2013; Xiao et al., 2011). There is a need for an extraction solvent system that simulates the actual human physiological conditions for evaluating both the intrinsic and fortified phenolics in food products. A gastrointestinal digestion model containing digestive enzymes serves this purpose.

* Corresponding author. University of Otago, PO Box 56, Dunedin 4778, New Zealand. Tel.: +64 3 479 9198; fax: +64 3 479 7567.
E-mail address: ali.rashidinejad@otago.ac.nz (A. Rashidinejad).

http://dx.doi.org/10.1016/j.lwt.2014.12.058
0023-6438/

Cheese contains a small amount of phenolic compounds of limited importance (O'Connell & Fox, 2001) due to low AA (Han et al., 2011; Huvaere et al., 2011). Catechins are unstable at high pH values, high temperatures and over long time storage (Lun Su, Leung, Huang, & Chen, 2003). The relatively high levels of protein and calcium in cheese, the low pH and storage temperature, along with widespread consumption, suggest that this would be a good vehicle for the incorporation of antioxidant compounds into a food product with high nutritional value. To this end, we examined the release and recovery of catechin added to low-fat cheese after *in vitro* gastrointestinal digestion. To the best of our knowledge, the effect of incorporated catechins on the antioxidant properties of cheese under digestive conditions has not been reported.

2. Materials and methods

2.1. Chemicals

Food grade liquid polyethylene glycol (PEG), porcine bile, (+)-catechin (≥98% purity), Trolox, gallic acid monohydrate, and 2,4,6-tris (2-pyridyl)-s-triazine (TPTZ) were purchased from Sigma-Aldrich (Auckland, New Zealand). Fluorescein was obtained from Eastman Kodak (Kingsport, TN, USA). Folin–Ciocalteu's phenol reagent and pancreatin were purchased from Merck (Darmstadt, Germany). Purified porcine pepsin was provided from Applichem (Darmstadt, Germany). 2,2′-Azobis(2-amidinopropane) dihydrochloride (AAPH) was purchased from Cayman (Ann Arbor, MI, USA). All chemicals used in this study were of analytical reagent grade.

2.2. Experimental design

A completely randomized design was employed, and four replications were used for each treatment. The treatments in this study included: 1) Control 1 (Con 1) – pasteurized skim milk added with the same amount of PEG but without catechin, 2) Control 2 (Con 2) – pasteurized skim milk without adding either catechin or PEG, 3) Cheese (125 mg kg^{-1}) – cheese made from pasteurized skim milk with added 125 mg kg^{-1} catechin in PEG, 4) Cheese (250 mg kg^{-1}) – cheese made from pasteurized skim milk with added 250 mg kg^{-1} catechin in PEG, 5) Cheese (500 mg kg^{-1}) – cheese made from pasteurized skim milk with added 500 mg kg^{-1} catechin in PEG. Con 1 was set up to eliminate the effect of PEG as a variable. Twenty vats of cheese were made over five sessions with four vats of cheese in each cheese manufacturing session.

2.3. Cheese manufacture and analysis

Catechin was dissolved in PEG (at a concentration of 10 g per 100 g), and the catechin-PEG mixture (in glass bottles covered with aluminium foil) was stirred, then homogenised using a Microfluidizer (Microfluidics, Newton, MA, USA) at a pressure of 100 MPa for five strokes circulation. Low-fat hard cheese was made from pasteurized skim milk (0.1 g of fat per 100 g milk) in 500 mL vats as previously described (Rashidinejad et al., 2013). Cheese from each vat was weighed, divided into three parts and vacuum packed in air-tight foil pouches (Audio Vac, Weesp, The Netherlands). One cheese sample was transferred to a −80 °C freezer as the day 0 sample immediately after production for later analysis whilst the other two samples were ripened at 8 ± 2 °C for 30 and 90 days, respectively. Sub-samples were withdrawn randomly at different locations within each cheese sample for analysis.

The moisture, fat, and protein content of cheese samples on day 0 were measured according to AOAC standard method (AOAC, 1984). Cheese yield was calculated as a fraction by dividing the weight of curd by the weight of milk.

2.4. Simulated gastric digestion

A two-stage simulated filtered gastric fluid (SGF) was prepared, based on work carried out by Sarkar, Goh, Singh, and Singh (2009) with some modifications. SGF was prepared by adding NaCl (2 g), HCl (7 mL of 360 g per L) and purified porcine pepsin (3.2 g) in deionized water, diluted to 1 L and adjusted to a pH value of 1.2 using 1.0 mol L^{-1} HCl in deionised water, and finally filtered through a 0.45 μm membrane. The SGF was stored at 4 °C. Gastric lipase was not added as the cheese samples contained a low fat content (<1 g per 100 g of cheese). Gastric lipases are normally present at low concentrations (0.5–1.0 mM) in the human stomach so that the role of gastric lipases in overall lipid digestion in healthy human adults is not very significant except for the people who are suffering from pancreatic lipase deficiency (Bauer, Jakob, & Mosenthin, 2005; Layer & Keller, 2005).

Cheese was grated using a stainless steel grater and 1 g weighed into a 250 mL conical flask (as the gastric model) that was covered with two layers of aluminium foil with a small hole at the top for SGF addition. Each digestion was set up in triplicate. SGF (37 °C, 10 mL) was added to each of the three conical digestion flasks, and placed onto an orbital shaker at 37 °C at 235 rpm for 10 min. The pH of the digestion was adjusted to pH 1.2 before resuming continuous shaking on the orbital shaker at 95 rpm for a further 2 h. After 2 h simulated gastric digestion, a simulated intestinal digestion was applied to the cheese samples.

2.5. Simulated intestinal digestion

Simulated intestinal fluid (SIF) was prepared following the method of Coughlin et al. (2012). Monobasic potassium phosphate (6.8 g) was dissolved in deionized water (250 mL). Sodium hydroxide (77 mL, 0.2 M) was dissolved in deionized water (500 mL) and mixed with the potassium phosphate solution. To this mixture, pancreatin (10 g) and porcine bile extracts (0.05 g) were added. The pH of resulting solution was adjusted to 6.8 using 0.2 mol L^{-1} NaOH, filtered through a 0.45 μm membrane, and stored at 4 °C.

After 2 h of SGF treatment, pre-warmed SIF (36 mL at 37 °C) was added to each gastric digestion sample. The resultant mixtures were incubated on the orbital shaker at 37 °C at 95 rpm for 4 h. The pH was checked after 30 min and maintained at 6.8 by adding 1 mol L^{-1} NaOH if necessary. SIF-digested cheese samples were filtered through a 0.45 μm membrane at 37 °C and the filtrates were kept at −80 °C for further chemical analyses.

2.6. Total phenolic content

The Folin–Ciocalteu method (Singleton, Orthofer, & Lamuela-Raventós, 1999) was used to measure the total phenolic content (TPC) of all the digested cheese samples in six replicates for days 0, 30, and 90 as previously described (Rashidinejad et al., 2013). Results were expressed as mg gallic acid equivalents per kg of cheese. The data of total phenolic content obtained for all digested cheese samples as well as milk samples (taken after addition of catechin and before cheese making) were also used to calculate the retention coefficients in low-fat cheeses after 90 days of ripening (Rashidinejad et al., 2013).

The *in vitro* recovery of catechin in cheese was calculated from the TPC of the corresponding digested cheese after subtracting the TPC of the digested control cheese. The TPC of the corresponding milk was calculated after subtracting the TPC of the control milk. The retention coefficients of catechin in the cheeses were taken into account. As catechin was the only source of phenolic contents added to the control milk, the subtracted TPC values of the control can be considered as the total amount of catechin. Therefore,