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Antioxidant properties and textural characteristics of processed cheese spreads enriched with rutin or quercetin: The effect of processing conditions

Jakub Přikryl [a], Tomáš Hájek [a], Blanka Švecová [a], Richardos Nikolaos Salek [b], Michaela Černíková [b], Libor Červenka [a, *], František Buňka [b]

[a] Department of Analytical Chemistry, Faculty of Chemical Technology, University of Pardubice, 53210, Pardubice, Studentská 573, Czech Republic
[b] Department of Food Technology, Faculty of Technology, Tomas Bata University in Zlín, 76001, Zlín, nám. T. G. Masaryka, 5555, Czech Republic

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ABSTRACT

Spreadable processed cheese (SPC) with addition of rutin or quercetin (0.5 g/100 g) were prepared at 80 °C and 90 °C for 1, 5 and 10 min. The effect of melting temperature and holding time of melting temperature on the quercetin/rutin retention, total phenolic content (TPC) and antioxidant capacity was studied. It was found that quercetin levels significantly decreased with the increase of holding time ($P < 0.01$) while rutin degradation was more affected by melting temperature ($P < 0.01$). An increase in TPC values and a decrease in antioxidant capacity measured by ABTS assay with the increase in melting temperature were observed in SPC with quercetin. The addition of rutin or quercetin significantly decreases the gel strength of the SPC samples.

1. Introduction

Spreadable processed cheese (SPC) is the multi-component system traditionally made from a mixture of cheeses, fat, water and emulsifying salts (sodium salts of phosphates, polyphosphates or citrates). The mixture of ingredients is stirred and then melted in temperatures ranged from 85 to 110 °C for a certain period of time (usually between 1 and 5 min). The resulted hot mixture is poured into the cups and cooled down below 8 °C (Kapoor & Metzger, 2008).

Processed cheeses are good source of proteins, fat, minerals and vitamins in the diet (Buňka, Hrabě, & Kráčmar, 2004). Although various cheese types have been identified as a good source of bioactive peptides (Korhonen, 2009), the fortification of cheeses with bioactive components has increased in the recent years. Incorporation of dried materials, extracts and essential oils of medicinal herbs into cheeses resulted in improvement of nutritional value and sensory attributes and decreased the deterioration process of quality parameters in various cheeses (Mohamed & Shalaby, 2016; Mohamed, Shalaby, & Gafour, 2016; Mehanna, Hassan, El-Messery, & Mohamed, 2017; Santos, Shetty, Cecchini, & da Silva Maglioranza, 2012). Polyphenols are the main compounds of interest among plant-based materials and they are the principal antioxidants in human diet. There are a limited number of studies regarding the evaluation of the effect of individual phenolic compounds on the antioxidant capacity of cheeses (Faion et al., 2015; Han et al., 2011; Rashidinejad, Birch, Sun-Waterhouse, & Everett, 2014; Stratulat et al., 2014). To the best of our knowledge, SPC or their analogues were scarcely used as the basis for the incorporation of bioactive substances, probably due to the high temperature of processing. Carrot paste (Mohamed et al., 2016) and apricot pulp (Mohamed & Shalaby, 2016) were used for the preparation of processed cheese analogues. In a very recent study, the preparation of functional processed cheese with addition of tomato juice was described (Mehanna et al., 2017). However, authors usually studied the nutritional characteristics of cheese samples in

* Corresponding author.
E-mail address: libor.cervenka@upce.cz (L. Červenka).

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relation to the different amount of bioactive material. To the best of our knowledge, there is no published data that describe the effect of processing conditions on the functional characteristic of processed cheese spreads.

Addition of bioactive compounds could affect not only the taste but also the consistency of processed cheese (Kapoor & Metzger, 2008). On the other hand, the processing parameters such as the agitation speed, melting temperature and holding time of the melting temperature significantly affect the consistency of processed cheese. The latter mentioned factors influence especially intensity of fat emulsification and water hydration processes and therefore the microstructure of processed cheese (Swenson, Wendorff, & Lindsay, 2000; Černíková et al., 2017).

Quercetin (3,5,7-trihydroxy-2-(3,4-dihydroxyphenyl)-4H-chromen-4-one) and rutin (quercetin-3-rutinoside) are the dietary flavonoids presented in plants. Both flavonoids are well-known for their therapeutic potential in various diseases like cancer, coronary artery, asthma and diabetes (D'Andrea, 2015). Due to the health-promoting effects of quercetin and rutin, an increased interest about their utilization in food systems has arisen (Cho & Lee, 2015; Rodriguez-Mateos, Cifuentes-Gomez, George, & Spencer, 2014).

The aim of the present study was to observe the effect of processing conditions (temperature and time) on the content of quercetin and rutin, as well as other functional characteristics of processed cheese spreads.

2. Materials and methods

2.1. Materials

All the solvents for extraction, LC-MS analysis and chemicals for antioxidant assays were purchased from Sigma-Aldrich (Prague, Czech Republic).

2.2. Processed cheese manufacturing

The composition of the raw materials is presented in Table 1 and was designed to achieve final products with 37 g/100 g dry matter content and 50 g/100 g fat in dry matter content. The total concentration of emulsifying salts was 2.3 g/100 g (the amount was calculated on the total weight of the melt). Two additions of flavonoids were applied for improving of functional properties of SPC/rutin (contains rutin hydrate, $\geq$ 94% purity) and SPC/quercetin (contains quercetin hydrate powders, $\geq$ 95% purity) at 0.5 g/100 g. The amount of butter and water applied were adjusted due to the above mentioned additions in order to maintain constant values of dry matter and fat in dry matter contents respectively. Control samples (without rutin or quercetin) were also produced.

For the laboratory manufacture of the model processed cheese samples, an equipment Stephan UMC-5 (Stephan Machinery GmbH, Halmen, Germany) with indirect heating was used. Firstly, Eidam block cheese and butter were cut into small pieces (approx. 2 × 2 × 2 cm) and put into the kettle and minced for 30 s (1400 × g). Subsequently, water, the mixture of emulsifying salts and butter, rutin and/or quercetin were added into the blend. The total amount of a batch was approximately 659–676 g. The mixture was heated up at 80 °C and 90 °C at a constant agitation (1500 min^{-1}) and kept for 1, 5 and 10 min at these temperatures. Finally, samples were poured into 80 g polystyrene doses with sealable lids. The packed samples were cooled down and stored (6 ± 2) °C until the analyses were performed. The addition of quercetin or rutin to the finished SPC sample (control) was also performed in our laboratory in order to assess the extraction efficiency. An appropriate amount of quercetin or rutin (0.5 g/100 g) was added to 1.0 g of processed cheese sample. The mixtures were vigorously stirred using stainless steel spatula and left in refrigerator overnight.

2.3. The preparation of the extracts

A glass vial with plastic cap containing 1.0 g of SPC sample and 10.0 mL of extraction solvent was put into the ultrasound bath Sonorex TK52 (Bandelin Electronic, Berlin, Germany) for 30 min. According to PubChem database, XLogP3 (a liphophility index) and TPSA (a polarity index) 1.5/128 and −1.3/266 for quercetin and rutin, respectively, indicate that rutin is more hydrophilic. Therefore, methanol and aqueous methanol (1:1) were used as the extraction solvents for SPC with quercetin or rutin, respectively. A clear supernatant was obtained after centrifugation at 1400 × g for 10 min (Vintrum NF400, Nüve, Ankara, Turkey) followed by the filtration using syringe polytetrafluoroethylene membrane filter (pore diameter 0.45 μm, Labicom, Olomouc, Czech Republic). Two extracts were prepared for each trial.

2.4. HPLC analysis of rutin and quercetin

Rutin and quercetin were analyzed using Agilent 1100 Series (Agilent Technologies, Santa Clara, CA, USA) equipped with a quaternary pump, a degasser, an autosampler, a thermostatted column compartment, a UV and MS detector Agilent 1100 Series LC/MSD Trap SL. A Gemini 5 μm C18 (150 × 3.0 mm) column was used (Phenomenex®, Torrance, CA, USA). Mixture of deionized water acidified with formic acid to pH 3.05 (0.21%, v/v) (solution A) and acetonitrile (solution B) was used as mobile phase at gradient flow rate 0.7 mL/min (formic acid: acetonitrile from 900: 100 mL: mL to 500: 500 mL: mL for 0–15 min). The analysis was performed at 40 °C and peaks of rutin and quercetin were detected at 360 nm. Quantification was based on the separation of standard solutions of quercetin and rutin dissolved in methanol at concentrations from 1 to 100 μg/mL. Peak area (Y) plotted against the concentration (c) of rutin and quercetin gave the calibration equation $Y = 2.26 \times c + 4.72$

Table 1
Formulation of the processed cheese samples with and without added antioxidants manufactured at different melting temperature and holding times.

Raw material	Producer	Dry matter content (g/100 g)	Fat in dry matter content (g/100 g)	Control	With rutin	With quercetin
Edam cheese*	Kromilk PLC, Kroměříž, Czech Republic	50	30	300.0	300.0	300.0
Butter	Madeta PLC, České Budějovice, Czech Republic	84	98	94.0	98.0	98.0
Water	–	–	–	250.0	260.0	260.0
Emulsifying salts**	Fosfa PLC, Břeclav-Poštorná, Czech Republic	>95	–	15.4	15.4	15.4
Rutin	TCI Chemicals, Tokio, Japan	>95	–	–	3.3	–
Quercetin	Sigma-Aldrich, Prague, Czech Republic	>95	–	–	–	3.3

* Dutch-type semihard cheese, 8-week maturity.
** Composition of the mixture of emulsifying salts: monosodium dihydrogenphosphate (19% rel.; the ratio calculated on the total amount of emulsifying salts = 100%), disodium hydrogenphosphate (37% rel.), tetrasodium diphosphate (22% rel.) and sodium salt of polyphosphate (22% rel.).