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# Extraction of quercetin and rutin from fortified spreadable processed cheese

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*A functional spreadable processed cheese (SPC) containing quercetin or rutin was produced by melting the ingredients at 85 °C for 2 min, and the retention rate of both flavonoids and antioxidant activity of the SPC samples assessed. Ultrasound-assisted extraction of quercetin and rutin from SPC to methanol (99.9% and 50%, v/v) for 30 min, respectively, was found to be optimal extraction procedure. Whereas a high recovery rate (92 %) for quercetin was achieved, only 64 % of rutin was recovered from the SPC sample. The results have indicated that rutin was thermally unstable during the melting process. SPC with quercetin or rutin exhibited effective radical-scavenging activity and possessed strong ferric reducing antioxidant potential. These results suggest that spreadable processed cheese may serve as the functional product.*

**Keywords:** Processed cheese; Flavonols; Recovery; Antioxidant activity

## 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

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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 [1].

Processed cheeses are good source of proteins, fat, minerals, and vitamins in the diet [2]. Although various cheese types have been identified as a good source of bioactive peptides [3], 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 results in improvement of nutritional value and sensory attributes, decreasing the deterioration process of quality parameters in various cheeses [4−8]. Polyphenols are the main compounds of interest among plant-based materials representing the principal antioxidants in human diet. There are a limited number of studies regarding the evaluation of the effect of the individual phenolic compounds on the antioxidant capacity of cheeses [9−11]. 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. Krumov and co-workers [6] improved the microbial quality of processed cheese by the addition of natural spice extracts and carrot paste [8] and apricot pulp [7] were used to prepare the processed cheese analogues.

Quercetin (3,5,7-trihydroxy-2-(3,4-dihydroxyphenyl)-4Hchromen-4-one) and rutin (quercetin-3-rutinoside) are the dietary flavonoids occurring in plants. Both flavonoids are well-known for their therapeutic potential in various diseases like cancer, coronary artery, asthma, and diabetes [12]. Due to the health-promoting effects of quercetin and rutin, an increased interest about their utilization in food systems has arisen [13−16].

The aim of the present study was to find the optimal extraction conditions, as well as to establish the possibility of improving the antioxidant capacity of spreadable processed cheese using quercetin and rutin.

## Materials and methods

### Processed cheese manufacturing

The composition of the raw materials (Eidam cheese blocks ≈ 50 % (w/w) dry matter content, 30 % (w/w) fat in dry matter content, 8-week maturity; butter ≈ 84 % (w/w) dry matter content; and water) for the processed cheese spread sample production was designed to achieve the final products with 37 % (w/w) dry matter content and 50 % (w/w) fat in dry matter content. The total concentration of emulsifying salts was 2.3 % (w/w; the amount was calculated on the total weight of the melt) and the following phosphates (Fosfa PLC, Břeclav-Poštorná, Czech Republic) were used: monosodium dihydrogen-phosphate (19 % rel.; the ratio calculated when the total amount of emulsifying salts was 100 %), disodium

hydrogenphosphate (37 % rel.), tetrasodium diphosphate (22 % rel.), and sodium salt of poly-metaphosphate (22 % rel.). Two additions of flavonoids were applied to improve the functional properties of SPC; rutin hydrate (≥ 94% purity) and quercetin hydrate powders (≥ 95% purity) at 0.50 % (w/w) and 0.18 % (w/w), respectively. Quercetin could not be used at the same amount as rutin because of undesirable organoleptic changes of finished SPC (data not shown). The amount of butter and water applied were adjusted according to the above mentioned additions in order to maintain constant values of dry and fat moieties in the dry matter contents, respectively. Control samples (without rutin and/or quercetin) were also prepared.

To manufacture the model processed cheese samples, the Stephan UMC-5 equipment (Stephan Machinery; Halmen, Germany) was used devised with indirect heating. Firstly, Eidam block cheese and butter were cut into small pieces (approx. 2×2×2 cm), put into the kettle and minced for 30 s at 3000 rpm. Subsequently, water, the mixture of emulsifying salts and oil, rutin and/or quercetin were added into the blend. The total amount of a batch was approximately 1130–1180 g. The mixture was heated up to 85 °C at a constant agitation (1500 rpm) and kept for 2 min at this temperature. Finally, samples were poured into 100 g polystyrene doses with sealable lids. The packed samples were cooled down and stored (6 ± 2) °C prior to analysis.

The addition of quercetin or rutin to the finished SPC sample was also performed in our laboratory in order to assess the effect of the melting temperature on the quercetin/rutin level. An appropriate amount of quercetin or rutin was added to 1.0 g of processed cheese sample at 0.18 and 0.50 % (w/w), respectively. The mixtures were vigorously stirred using a stainless-steel spatula and left in refrigerator overnight. Recovery rate (RR) of flavonoids was calculated according to the following equation:

$$RR\,[\%] = \frac{flavonoid\ found\ [\mathrm{mg\ g^{-1}}]}{flavonoid\ added\ [\mathrm{mg\ g^{-1}}]} \times 100 \qquad (1)$$

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 an ultrasound bath (model Sonorex TK52; Bandelin Electronic, Berlin, Germany) for various time (5, 15 and 30 min). Distilled water, ethanol (96%, Lach:ner, Neratovice, Czech Republic), 50% ethanol (v/v), methanol (99.9% purity, Sigma-Aldrich, Darmstadt, Germany) and 50% methanol (v/v) were used as the extraction solvents. A clear supernatant was obtained after agitating at 4000 rpm for 10 min in a centrifuge (model Vintrum NF400, Nüve, Ankara, Turkey) followed by the filtration using a syringe PTFE membrane filter (pore diameter of 0.45 μm; Labicom, Olomouc, Czech Republic). Acetate cellulose filters were used in case of distilled water extract. Two extracts were prepared for each trial.

## HPLC analysis of rutin and quercetin

Rutin and quercetin were analyzed using SpectraSYSTEM™ HPLC P2000 equipped with a UV detector SpectraSYSTEM™ UV3000 and an autosampler Spectra Series AS100 (both Thermo Separation; Waltham, MA, USA). A Kinetex XB-C18 column (150 × 4.6 mm, particle size 2.6 μm) was used (Phenomenex®; Torrance, CA, USA). Mixture of deionized water acidified with formic acid to pH 3.0 (solution A) and acetonitrile (solution B) was used as a mobile phase at a gradient flow rate 0.7 mL $min^{-1}$ (0–100 % of solution B (v/v) for 0–20 min). Injection volume was set to 20 μL. The proper analysis was performed at 40 °C and the peaks of rutin and quercetin were detected at 275 nm. The contents of rutin and quercetin were determined by calibrating the peak area (PA) vs. concentration (c) of analytes with the regression equations $PA = 62434\ c + 14402$ for rutin and $PA = 57878\ c + 19394$ for quercetin, respectively.

## Determination of antioxidant activity of spreadable processed cheese

All the chemicals were purchased from Sigma-Aldrich (Prague division, Czech Republic). The DPPH (2,2-diphenyl-1-picrylhydrazil) and ABTS (2,2´- azino-bis-3-ethylbenzthiazoline-6-sulphonic acid) radical-scavenging activity assays were adopted from the experimental procedure by Mišan and co-workers [17]. A portion (5.0 mL) of methanolic solution containing the DPPH radical at the concentration of 25 μg $mL^{-1}$ was mixed with 0.5 mL of processed cheese extract solution, and left to stand for 20 min in the dark. The decrease of the absorbance at 517 nm was measured against methanol using a DU-530 UV/VIS spectrophotometer (Beckman Coulter; Brea, California, USA). As a blank sample, 0.5 mL of appropriate extraction solvent was used instead of the sample extract in the reaction mixture.

ABTS radical cation ($ABTS^{\bullet+}$) was prepared from ABTS solution (5.0 mL, 50 mg $L^{-1}$) and 100 μL of potassium persulphate (64 mmol $L^{-1}$). The mixture was stored in a dark for 12–16 hours at laboratory temperature before use. After addition of 0.5 mL of processed cheese sample extract to 3.0 mL of $ABTS^{\bullet+}$ solution, the absorbance of the mixture was measured against the blank after 50 min of reaction in the dark. Extraction solution (0.5 mL) combined with 3.0 mL of $ABTS^{\bullet+}$ served as a negative control. Both DPPH• and $ABTS^{\bullet+}$ scavenging activities $I$ were calculated using the formula:

$$I\,[\%] = 1 - \frac{A_1}{A_0} \times 100 \tag{2}$$

where $A_0$ is the absorbance of blank solution; $A_1$ is the absorbance of radicals with sample extract.

A Ferric Reducing Antioxidant Power (FRAP) method was selected according to the procedure of Benzie and Szeto [18]. An aliquot (5.0 mL) of reaction mixture was combined with 0.5 mL of processed cheese extract, and a formation of blue colour was examined after 50 min at ambient temperature. A reaction mixture with 0.5 mL of extraction solvent instead of the sample extract was used as the negative control. The formation of blue color was monitored at the wavelength 593 nm.

DPPH• and ABTS•$^{+}$ scavenging activities *I*, and the absorbance at 593 nm for FRAP method were plotted against various concentrations of Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid). The antioxidant activities of processed cheese extract in a sample were expressed in mmol Trolox/g. Each extract was examined in duplicate for its antioxidant activity.

### Statistical analysis

The results represented the average means with standard deviation (SD) of the repeated measurements ($N = 4$). Multiple comparison procedure among means was performed using the Tukey's method. Statistical treatments of the data were done at the probability level of $p = 0.05$ (OriginPro 9.0; Origin Lab. Corp., Northampton, MA, USA).

## Results and discussion

### Extraction efficiency of rutin and quercetin from spreadable processed cheese

The extraction process of the target compounds to water, ethanol (96% and 50%, v/v) solutions encountered complications. Water extract was not filterable after centrifugation and thus being not able to be injected into the HPLC system. Ethanol and ethanol/water supernatants were clear after performing centrifugation and filtration; however, a significant turbidity has occurred within an hour of storage at laboratory temperature. Additional centrifugation/filtration did not improve the stability of the extract. Therefore, water, 96% and 50% (v/v) ethanol were not further used in the experiment. Methanol (100%, 50%, by volume) were found to be suitable for the extraction of quercetin and rutin prior to HPLC analysis. The extracts were stable within 8 h of storage in terms of clear appearance. The effect of methanol and methanol/water extraction solvents and the time of extraction in ultrasound bath is depicted in Fig. 1. Higher areas of peak of rutin in 50% methanol extracts were determined after 5 and 30 min of extraction ($p < 0.05$) in comparison with those in methanol. The area of peak of rutin significantly increased from $(6.6 \pm 1.3)\times10^3$ mAU s to $(19.7 \pm 1.0)\times10^3$ mAU s with the prolongation of extraction time from 5 min to 30 min, respectively

($p < 0.001$), when extracted to 50% methanol (Fig. 1, image A). Quercetin, a less polar substance than rutin, appeared to have a greater affinity to methanol (Fig. 1, image B). Significantly higher areas of peak of quercetin were found in 99.9% methanol than in 50% methanol extracts at each extraction time ($p < 0.001$). Ultrasound extraction of SPC sample into pure methanol for 30 min gave rise to the highest peak area of quercetin ($(44.0 \pm 3.0) \times 10^3$ mAU s).

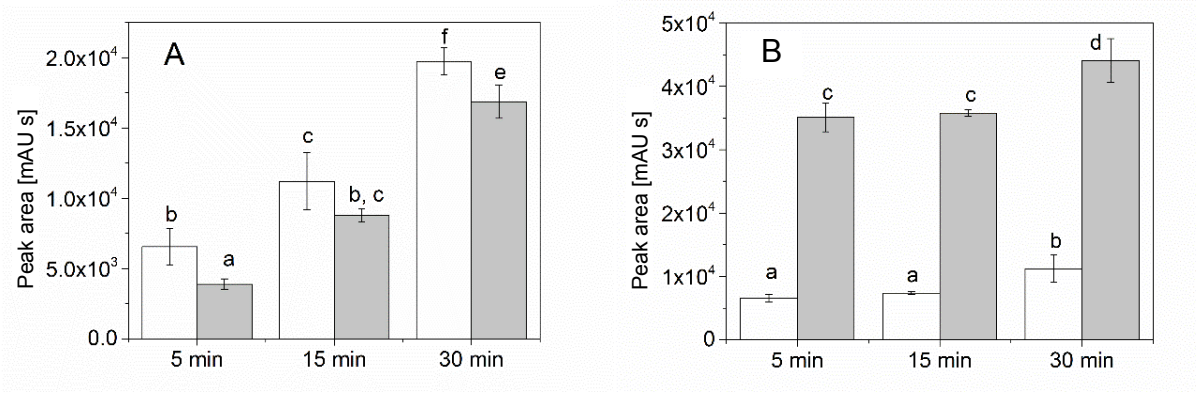


**Fig. 1** *The peak areas of rutin (A) and quercetin (B) after ultrasound-assisted extraction of spreadable processed cheese containing rutin (0.50 %, w/w) and quercetin (0.18 %, w/w) to methanol solutions (white columns 50%; grey columns 99.9%)*

Means with the same letter are not statistically different ($p > 0.05$).

The appropriate selection of extraction conditions was also confirmed by a high recovery rate for both quercetin and rutin when both substances were stirred into the final SPC sample and processed using microwave-assisted extraction for 30 min in 100% (for quercetin) and 50% methanol (for rutin). The recovery rate of quercetin and rutin was (96.0 ± 4.0) and (91.0 ± 5.0) %, respectively. While quercetin also showed a high recovery rate ((92.0 ± 4.0) %) after melting at 85 °C for 2.0 min during manufacturing the processed cheese, rutin loss was significantly higher with a recovery rate at (64.0 ± 2.0) %. It implies that the melting procedure has strongly influenced the rutin extractable amount from the SPC samples. It is not clear whether rutin molecules have undergone decomposition or they have not been bound to other processed cheese components after melting process; hence, being physically unavailable for extraction. The decrease of rutin content with the concurrent increase of quercetin has been explained by the action of degrading enzymes presented in Tartary buckwheat bread [19]; however, this phenomenon cannot be expected in our samples. In a study of Deng et al. [20], the degradation of rutin and formation of quercetin was observed during roasting of Noni leaves at temperature ranged from 100 to 250 °C. It should be noted that peak of quercetin did not appear in chromatograms during HPLC analysis of SPC extracts fortified with rutin in our study. Several studies have reported on the effect of various thermal processing on the rutin

concentration [13,14]. For instance, Cho and Lee [13] found that while frying of buckwheat noodles did not affect the level of rutin, the boiling had resulted in its distinct loss.

On the other hand, the same or higher level of rutin was found in potato tubers after baking, boiling, steaming, and microwave cooking [14]. In the present study, quercetin has been found to be thermally stable in the processed cheese samples, which is in the agreement with other studies examining the impact of higher temperatures on quercetin level. Among others, quercetin level has remained constant during cooking of freeze-dried blueberry powder at 90 °C [15] or heating of *Gingo biloba* up to 70 °C for 24 h [16].

## Antioxidant activity of spreadable processed cheese with quercetin or rutin

After ultrasound-assisted extraction for 30 min, processed cheese with rutin (0.50 %, by mass) and quercetin (0.18 %, by mass) exhibited the antioxidant activity as shown in Table 1. Significantly higher antioxidant activities of SPC/rutin were determined in 50 % (by volume) methanol extracts, whereas SPC/quercetin showed higher antioxidant activities in methanolic extracts using all the antioxidant activity assays.

**Table 1** *Antioxidant activity of spreadable processed cheese (SPC) with rutin (0.50 %, w/w) and quercetin (0.18 %, w/w) after ultrasound-assisted extraction for 30 min*

| Sample | Methanol solution | DPPH assay | ABTS assay | FRAP assay |
|---|---|---|---|---|
| | | Trolox eq. [mmol $g^{-1}$] | | |
| SPC/Rutin | 50% | $^{d}5.00 \pm 0.11$ | $^{d}14.71 \pm 0.16$ | $^{d}12.65 \pm 0.13$ |
| | 100% | $^{c}4.43 \pm 0.14$ | $^{c}12.51 \pm 0.38$ | $^{d}11.45 \pm 0.57$ |
| SPC/Quercetin | 50% | $^{b}3.66 \pm 0.54$ | $^{c}12.97 \pm 0.37$ | $^{c}6.40 \pm 0.54$ |
| | 100% | $^{d}5.84 \pm 0.58$ | $^{e}49.10 \pm 2.98$ | $^{e}16.30 \pm 1.39$ |
| Control | 50% | $^{a}0.03 \pm 0.02$ | $^{b}1.52 \pm 0.01$ | $^{b}0.33 \pm 0.02$ |
| | 100% | $^{a}0.04 \pm 0.02$ | $^{a}0.80 \pm 0.02$ | $^{a}0.23 \pm 0.04$ |

Means with different letters indicate statistical difference in column ($p < 0.05$).

This finding corresponds to the results obtained by HPLC analysis of the processed cheese sample extracts. In general, ABTS and FRAP assays resulted in a higher antioxidant activity expressed as mmol Trolox/g than DPPH assay in our study. Under optimal extraction conditions, SPC/quercetin has always shown higher antioxidant activities than SPC fortified with rutin; probably, due to the steric hindrance [21] in spite of the fact that lower amount of quercetin was added

to the cheese matter (namely: 0.18% by mass of cheese). Interestingly, antioxidant activity was also detected in processed cheese samples without quercetin/rutin (see Table 1).

ABTS radical-scavenging assay of SPC extracts in 100% and 50% (v/v) methanol resulted in the (0.80 ± 0.02) and (1.52 ± 0.01) mmol $g^{-1}$ of Trolox equivalent, respectively, being similar to antioxidant activity of ovine cheeses [22]. Therein, the authors explained the antioxidant activity by the presence of various bioactive peptides and free amino acids.

## Conclusions

The results of this study indicate that addition of both quercetin and rutin offers an improvement of the antioxidant activity of SPC in terms of DPPH- and ABTS-radical scavenging activity, and in FRAP assay. Simple ultrasound-assisted extraction was successfully applied for the recovery of both compounds; however, a significant loss for rutin has been observed in the SPC sample during the manufacturing process. Our findings revealed that rutin was sensitive to the melting temperature used for preparation of the SPC. In our opinion, this approach can be applied in the production of functional processed cheese as the promising source and/or a carrier of some compounds with bioactive properties.

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