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

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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.

Keywords: processed cheese; flavonoids; melting condition; antioxidants

Chemical compounds studied in this article: Quercetin (PubChem CID: 5280343); Rutin (PubChem CID: 5280805)

Conflict of interest: none

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 minutes). 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 Miglioranza, 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, Shalaby & Gafour, 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, Hassan, El-Messery & Mohamed, 2017). However, authors usually studied the nutritional characteristics of cheese samples in 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 (Černíková, Salek, Kozáčková, Běhalová, Luňáková & Buňka, 2017; Swenson, Wendorff & Lindsay, 2000).

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, ≥ 94% purity) and SPC/quercetin (contains quercetin hydrate powders, ≥ 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 5µm 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×c+4.72 (R^2=0.998) and Y=4.79×c-2.88 (R^2=0.999), respectively. An ion trap mass spectrometry detector with an ESI source was used to confirm the presence of both flavonoids. ESI mass spectra were measured in the range of m/z 200–1000 in negative-ion mode. The concentration of both flavonoids was expressed in μg per g of sample. Retention of flavonoids was calculated according to the following equation:

$$R\ (\%) = (\text{flavonoid found (mg/g)}/\text{flavonoid added (mg/g)}) \times 100 \quad (1)$$

2.5 Determination of antioxidant activity of spreadable processed cheese

The total phenolic assay (TPC) was adopted from Santos, Shetty, Cecchini & da Silva Miglioranza (2012). A reagent mixture containing extraction solvent instead of the sample extract served as the blank. The results were expressed as the amount of gallic acid per ml of extract.

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 of Mišan et al. (2011). Both DPPH• and $ABTS^{\bullet+}$ scavenging activities *I* were calculated using the formula:

$$I(\%) = (1 - A_1/A_0) \times 100 \quad (2)$$

where A_0 is the absorbance of blank solution; A_1 is the absorbance of radicals with sample extract. DPPH• and $ABTS^{\bullet+}$ scavenging activities *I* were then plotted against various

concentration of Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) and the results were expressed as Trolox equivalent antioxidant capacity ($TEAC_{DPPH}$ and $TEAC_{ABTS}$) in μg Trolox/ml of the extract. Each extract was examined in duplicate for its antioxidant activity.

2.6 Rheological properties

Rheological properties of model processed cheese were measured according to Černíková et al. (2017). Briefly, a dynamic oscillatory shear rheometer (RheoStress 1, Haake, Bremen, Germany) at 20.0 ± 0.1 °C with a plate-plate geometry (diameter 35 mm, gap 1 mm) were used. The complex modulus (G*) at reference frequency 1 Hz were calculated based on values of storage (G´) and loss (G´´) moduli:

$$G^* = \sqrt{(G´)^2 + (G´´)^2} \quad (3)$$

With increasing values of the complex moduli (G*) of processed cheese, the consistency become more rigid and the gel strength rises (Černíková et al., 2017).

2.7 Statistical analysis

The results represented the average means with standard deviation (SD) of repeated measurements (N = 4). Nonparametric statistical methods were used in this study. Two-factor Kruskal-Wallis analysis of variance (ANOVA) was applied in order to determine the effect of melting temperature (factor A) and holding time (factor B) on the content of flavonoids and antioxidant properties. Multiple comparison procedure among means was performed using the Tukey's method. Spearman correlation coefficients (r) were calculated to describe the mutual

associations between variables. All the statistical methods were done at the probability level of $P = 0.05$ (Statistica CZ, StatSoft CR s.r.o., Prague).

3. Results and discussion

3.1 The effect of processing conditions on the content of quercetin and rutin in processed cheese spreads

As can be seen from Fig. 1, both quercetin and rutin were successfully extracted after manufacturing of processed cheese spreads using pure and aqueous methanol (water: methanol, 1:1), respectively. When quercetin or rutin were mixed with the finished SPC sample (control), the extraction efficiency and subsequent LC determination of quercetin and rutin exhibited 96.0 ± 4.0 and 91.0 ± 5.0% retention, respectively.

The concentration of quercetin ranged from 4.17 ± 0.15 to 2.39 ± 0.02 mg/g in SPC/quercetin samples. Significantly higher content of quercetin was determined in SPC/quercetin samples manufactured at 80°C for 1 min ($P<0.01$), and the lowest content was obtained after thermal treatment at 90°C for 10 min ($P<0.01$). Significant decrease of quercetin content with the increase of holding time was observed at both melting temperatures. Extraction of SPC/rutin samples to aqueous methanol and subsequent determination of rutin by LC-MS method resulted in its considerably lower amount. Rutin levels ranged from 1.92 ± 0.04 to 1.90 ± 0.02 mg/g after manufacturing of processed cheese spreads at 80°C. A lower level of rutin was observed when SPC samples were prepared at 90 °C showing significant differences within the holding times. Both quercetin and rutin were considered as thermally unstable compounds particularly in alkali conditions and in the presence of oxygen in previous studies (Buchner, Krumbein, Rohn & Kroh, 2006; Barnes, Foss & Schug, 2013). After LC analysis, only peaks

corresponded to quercetin (t_R=11.33 min) and rutin (t_R=7.31 min) were detected at 360 nm under the given experimental conditions for all the SPC samples (Suppl. 1A, 1B). No interference peaks occurred when control SPC sample was processed. The LC/MS spectrum of quercetin peak showed two fragment ions at m/z 300.9 and 600.3, the first corresponded to quercetin molecule, the latter can indicate the presence of a dimmer (Suppl. 2). Quercetin dimmer was identified as a product of the oxidation of quercetin molecule (Pham, Bortolazzo &White, 2012). Rutin peak gives only one fragment ion at m/z 609.1 (not shown). Thermal degradation of quercetin and rutin was extensively studied by Buchner, Krumbein, Rohn & Kroh (2016) and Barnes, Foss & Schug (2013) using mass spectrometry techniques. In general, quercetin was more stable at acidic pH, and the degradation rate increased with the increase of pH and temperature. Barnes, Foss & Schug (2013) identified degradation products of quercetin after heating its solution (pH 5.9) at 85°C for >9.6 min. Their experimental conditions are close to those used in our experimental procedure. The pH of SPC samples have been measured in our study and ranged from 5.80 to 5.92. At the similar pH (5.0), rutin was found to be more stable than quercetin during heating of aqueous solutions at 100°C for 300 min (Buchner, Krumbein, Rohn & Kroh, 2016). While the results of thermal degradation studies of quercetin and rutin in solution are consistent, contradictory conclusions have arisen from the experiments on foodstuffs. For instance, quercetin remained constant during the cooking of blueberry filling at 90°C (Rodriguez-Mateos, Cifuentes-Gomez, George & Spencer, 2014) whereas decrease after steaming of onion for > 10 min was reported (Harris, Brunton, Tiwari & Cummins, 2015). In a study of Vallverdú-Queralt, Regueiro, de Alvarenga, Torrado & Lamuela-Raventos (2014), quercetin decreased more abruptly during the cooking of tomato sauce than rutin. They explain the higher stability of rutin towards the oxidation by the presence of the sugar moiety in the 3-hydroxy-function at the C-ring, whereas in quercetin it remained unoccupied. Quercetin seemed to be more stable during

manufacturing of processed cheese spread with clear pattern regarding the melting temperature and holding time (see Fig. 1). The higher stability of quercetin in our study is in agreement with the study of Vogrincic, Timoracka, Melichacova, Vollmannova & Kreft (2010) who found quercetin more stable than rutin during the bread rising and baking process. The low retention of rutin after manufacturing of SPC sample can be explained by its interaction with L-amino acids, particularly with arginine or lysine via hydrophobic interactions. The stability of such a molecular complex has increased with the increase in temperature (Biçer & Őzdemir, 2014). The complexation of rutin with protein was also described (Cui, Kong, Chen, Zhang & Hua, 2014). Since processed cheese is rich source of protein and amino acids (Buňka, Hrabě & Kráčmar, 2004), we may imply that such complexes were formed during manufacturing of processed cheese spreads and was not able to be extracted to aqueous methanol. In addition, rutin was found to be unstable in aqueous solutions when sonicated. This phenomenon is known as acoustic cavitation, and is attributed to the formation of highly reactive hydroxyl radicals during the passage of ultrasonic wave through the bubbles of water (Chua, 2013). The degradation rate of rutin by hydroxyl radicals was dependant on the temperature of extraction, liquid height, ultrasound intensity and pulse length. The sonication process during the extraction of rutin from SPC samples was found to be acceptable for our purposes since it gave high retention of rutin (91.0 ± 5.0%).

3.2 Antioxidant properties of processed cheese spread

Folin-Ciocalteau´s assay was used to determine the total phenolic content (TPC) of SPC samples. Prior to analysis of SPC samples containing flavonoids, the control samples (without addition of quercetin and rutin) at each processing condition were screened for the TPC (Suppl. 3). As can be seen, TPC values ranged from 58.2 ± 0.5 to 65.7 ± 0.5 µg gallic acid/ml when extracted to methanol and from 27.2 ± 1.9 to 41.6 ± 1. µg gallic acid/ml, when extracted

to aqueous methanol. FC assay was primarily designed for the determination of amino acid tyrosine containing phenol group (Apak, Özyörek, Güçlü & Çapanoğlu, 2016). The amount of this amino acid in processed cheese spread was estimated in the range from 10.8 to 5.9 g/kg in the study of Buňka, Kříž, Veličková, Buňková & Kráčmar (2009), therefore we may assume that it may also react with the FC reagent in our study. Antioxidant properties were measured in terms of DPPH and ABTS assays, however only latter gave positive results with the extracts of control SPC samples (Suppl. 3). The antioxidant properties of cheese was previously attributed to the content of bioactive peptides (Meira et al., 2012) and free amino acids, mainly tyrosine, methionine and tryptophan (Bottesini et al., 2013). The corresponded $TEAC_{ABTS}$ values for control SPC samples were in the range from 68.4 ± 3.0 to 78.0 ± 4.7 μg Trolox/ml of methanol extract and from 47.0 ± 0.4 to 76.4 ± 4.2 μg Trolox/ml when extracted to aqueous methanol. In order to evaluate the effect of quercetin or rutin content on the antioxidant activity of SPC samples, the results of TPC and ABTS assays were corrected for corresponded values obtained in control SPC samples.

The TPC values for the extract of SPC/quercetin were determined in the range from 251.7 ± 0.5 to 263.2 ± 0.5 μg gallic acid/ml at 80 °C and from 285.8 ± 0.2 to 318.6 ± 1.4 μg gallic acid/ml at 90 °C (Table 2). Antioxidant properties of SPC/quercetin extract measured in terms of DPPH assay showed increasing values of $TEAC_{DPPH}$ from 157.4 ± 19.7 to 263.5 ± 19.7 μg Trolox/ml at 80 °C with the increase in time. On the other hand, a decrease of $TEAC_{DPPH}$ values from 329.9 ± 30.7 to 216.0 ± 15.8 μg Trolox/ml with the increase of processing time at 90 °C was examined. Concerning the results of $ABTS^{\bullet+}$ assay, slight increase in $TEAC_{ABTS}$ values was observed with the increase of processing time from 5.0 to 10.0 min at each temperature.

TPC values for SPC/rutin extracted to aqueous methanol were shown in Table 2. The decrease from 88.2 ± 1.4 to 54.7 ± 2.9 μg gallic acid/ml with the increase of time was observed in

samples manufactured at 80 °C and the increase has occurred when the processing time increased from 1.0 to 5.0 min at 90 °C (from 47.3 ± 1.9 to 70.6 ± 0.5 μg gallic acid/ml).

3.3 The results of Kruskal-Wallis ANOVA and correlation analysis

In order to determine the effect of melting temperature and holding time, Kruskal-Wallis ANOVA procedure was applied to all the variables. It is evident that both quercetin and rutin content decreased with the increase in temperature and time (Table 3). The degradation of quercetin was significantly enhanced by the increasing of processing time ($P<0.01$) than by the temperature. On the contrary, temperature caused significant loss of rutin ($P<0.01$) than the increasing time. For SPC/rutin sample extracts, both processing temperature and time did not significantly change TPC and antioxidant capacities. Processing temperature significantly affected the TPC ($P<0.01$) and $TEAC_{ABTS}$ ($P<0.05$) values in SPC/quercetin samples, however increasing trend in TPC and decreasing trend in $TEAC_{ABTS}$ values were obtained with the elevated melting temperature. The processing time was not significant factor. The Pearson's correlation coefficients as shown in Table 4 were performed to elucidate the trend of association between quercetin and rutin contents, TPC and antioxidant capacities. Quercetin content was weakly negative correlated with total phenolic content and antioxidant capacity (DPPH and ABTS). Weak positive correlation was observed between TPC and $TEAC_{DPPH}$, whereas negatively correlated with $TEAC_{ABTS}$ ($p=-0.813$; $P<0.01$). The increase of total phenolic content associated with the decrease of antioxidant properties measured by $ABTS^{\bullet+}$ assay was explained by the hindrance of steric accessibility of phenolic groups to the $ABTS^{\bullet+}$ site, particularly in heterocyclic polymeric polyphenols (Apak, Özyürek, Güçlü and Çapanoğlu, 2016). Similar results were obtained for medicinal plant extracts of *Saraca asoca* (Ghatac et al., 2015) and *Centella asiatica* (Chew et al., 2011). In addition, Buchner, Krumbein, Rohn & Kroh (2016) reported the increase of antioxidant activity even after the

decrease of quercetin content in solution during thermal treatment. They proved the formation of new substances (degradation products) with higher antioxidant activity. Based on our results and literature cited, we may hypothesize that new compounds were formed during the manufacturing of SPC/quercetin samples, which possessed antioxidant activity but were not detectable under our experimental conditions. The effect of processing time and temperature on both $TEAC_{DPPH}$ and $TEAC_{ABTS}$ values of SPC/rutin sample extracts was not confirmed by Kruskal-Wallis ANOVA (Table 3). Nevertheless, rutin content in SPC/rutin samples positively correlated with the TPC values ($r=0.807$; $P<0.01$) and $TEAC_{DPPH}$ values ($r=0.747$; $P<0.01$). TPC showed strong positive correlation with $TEAC_{DPPH}$ ($r=0.622$; $P<0.05$).These findings indicate that antioxidant properties of processed cheese spread was influenced by the presence of rutin molecule.

3.*3 The results of rheological properties*

The results of the complex modulus (G*, the meaning was explained in part 2.6) of model processed cheeses manufactured under different agitation and melting temperature were displayed in Table 5. The values of G* significantly increased ($P<0.05$) with the increase of holding time. Elevated temperature of melting also caused the increase of G* ($P<0.05$). The higher levels of observed processing parameters led to development of denser net structure and therefore the model processed cheese became more rigid (Černíková et al., 2017).The addition of rutin or quercetin influenced the consistency of model processed cheeses ($P<0.05$; Table 5). It could be hypothesed that the latter mentioned antioxidants could disrupt slightly the protein network. The effect of both of added substances on rheological properties of samples were practically similar ($P\geq0.05$; Table 5).

4. Conclusions

Processed cheese spreads were not frequently used for the development of functional food probably due to the adverse conditions during manufacturing process. This paper describes the effect of melting temperature and holding time on the content of rutin and quercetin, and on the antioxidant properties of processed cheese spread. The results showed that both flavonoids decreased during the cheese processing. While quercetin content decreased with the increase of holding time, rutin degradation was pronounced at elevated processing temperature. Rutin content affected the antioxidant capacity of processed cheese samples showing strong positive correlation with total phenolic content and DPPH scavenging activity whereas quercetin content did not exhibit apparent association towards antioxidant capacity. Both rutin and quercetin significantly decreased the gel strength of the samples. We used chemical substances for the preparation of functionalized processed cheese spread in order to facilitate the experimental design and for subsequent interpretation of results. For practical purposes, the addition of plant extracts rich in quercetin/rutin or other polyphenolic substances should be further examined. Processed cheese spread fortified with rutin or quercetin has a potential to be a functional food and contribute to health when it is consumed.

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Figure 1 The effect of processing temperature and time (80/1 means 80 °C for 1 min, 80/2 means 80 °C for 5 min, etc.) on the quercetin (white column) and rutin (grey column) levels extracted from processed cheese spread. Methanol and aqueous methanol (water: methanol, 1:1) were used for extraction of quercetin and rutin, respectively. Statistical differences in quercetin and rutin levels is indicated by different small and capital letters, respectively ($P<0.05$). Average mean ± standard deviation (N=4)

420 Table 1 Formulation of the processed cheese samples with and without added antioxidants manufactured at different melting temperature and
421 holding times
422

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

423 * Dutch-type semihard cheese, 8-week maturity; ** Composition of the mixture of emulsifying salts: monosodium dihydrogenphosphate (19 %
424 rel.; the ratio calculated on the total amount of emulsifying salts = 100 %), disodium hydrogenphosphate (37 % rel.), tetrasodium diphosphate (22
425 % rel.) and sodium salt of polyphosphate (22 % rel.)

426 Table 2 The effect of melting temperature and holding time on the antioxidant properties of processed cheese spread with 0.5 g/100 g of
427 quercetin or rutin.

	80 °C			90 °C		
	1 min	5 min	10 min	1 min	5 min	10 min
	Extracted to methanol					
TPC	^{a}251.7 ± 0.5	^{b}263.2 ± 0.5	^{a}255.1 ± 1.4	^{e}318.6 ± 1.4	^{d}305.4 ± 1.0	^{c}285.8 ± 0.2
$TEAC_{DPPH}$	^{a}157.4 ± 19.7	^{bd}241.2 ± 4.0	^{bef}263.5 ± 19.7	^{cf}329.9 ± 30.7	^{ade}227.6 ± 1.0	^{ade}216.0 ± 15.8
$TEAC_{ABTS}$	^{b}1352.4± 23.1	^{b}1346.1 ± 3.4	^{c}1471.9± 58.5	^{a}1186.7± 57.3	^{a}1226.3 ± 14.3	^{b}1340.5 ± 29.2
	Extracted to aqueous methanol (1:1)					
TPC	^{f}88.2 ± 1.4	^{d}76.4 ± 0.5	^{b}54.7 ± 2.9	^{a}47.3 ± 1.9	^{de}70.6 ± 0.5	^{ce}69.9 ± 0.5
$TEAC_{DPPH}$	^{d}106.2 ± 1.1	^{c}92.5 ± 5.0	^{cd}97.6 ± 3.3	^{b}74.7 ± 1.0	^{cd}99.8 ± 2.0	^{a}56.8 ± 3.0
$TEAC_{ABTS}$	^{a}756.1± 148.0	^{a}808.0± 139.0	^{a}780.0 ± 4.3	^{a}751.4 ± 9.2	^{a}854.5 ± 7.8	^{a}802.6 ± 18.0

428 Average mean ± standard deviation (N=4); TPC, total phenolic content (µg gallic acid/ml); $TEAC_{DPPH}$, Trolox equivalent antioxidant capacity
429 using 2,2-diphenyl-1-picrylhydrazil assay (µg Trolox/ml); $TEAC_{ABTS}$, Trolox equivalent antioxidant capacity using 2,2´- azino-bis-3-
430 ethylbenzthiazoline-6-sulphonic acid assay (µg Trolox/ml); significant difference between means in row is indicated by different small letters in
431 superscript ($P<0.05$)

Table 3 The results of Kruskal-Wallis ANOVA on the effect of melting temperature (T) and holding time (t) on the quercetin and rutin contents, total phenolic content (TPC) and antioxidant capacity of processed cheese samples.

Parameter	T	t
Quercetin	n.s.	$\downarrow^{**}$
TPC	$\uparrow^{**}$	n.s.
$TEAC_{DPPH}$	n.s.	n.s.
$TEAC_{ABTS}$	$\downarrow^{*}$	n.s.
Rutin	$\downarrow^{**}$	n.s.
TPC	n.s.	n.s.
$TEAC_{DPPH}$	n.s.	n.s.
$TEAC_{ABTS}$	n.s.	n.s.

↑, increasing trend; ↓, decreasing trend; $^{*}P< 0.05$; $^{**}P< 0.01$; n.s., not significant ($P>0.05$); TPC, total phenolic content (μg gallic acid/ml); $TEAC_{DPPH}$, Trolox equivalent antioxidant capacity using 2,2-diphenyl-1-picrylhydrazil assay (μg Trolox/ml); $TEAC_{ABTS}$, Trolox equivalent antioxidant capacity using 2,2´- azino-bis-3-ethylbenzthiazoline-6-sulphonic acid assay (μg Trolox/ml)

Table 4 Pearson´s correlation coefficient between the content of quercetin, rutin, total phenolic content (TPC) and antioxidant capacities in processed cheese samples

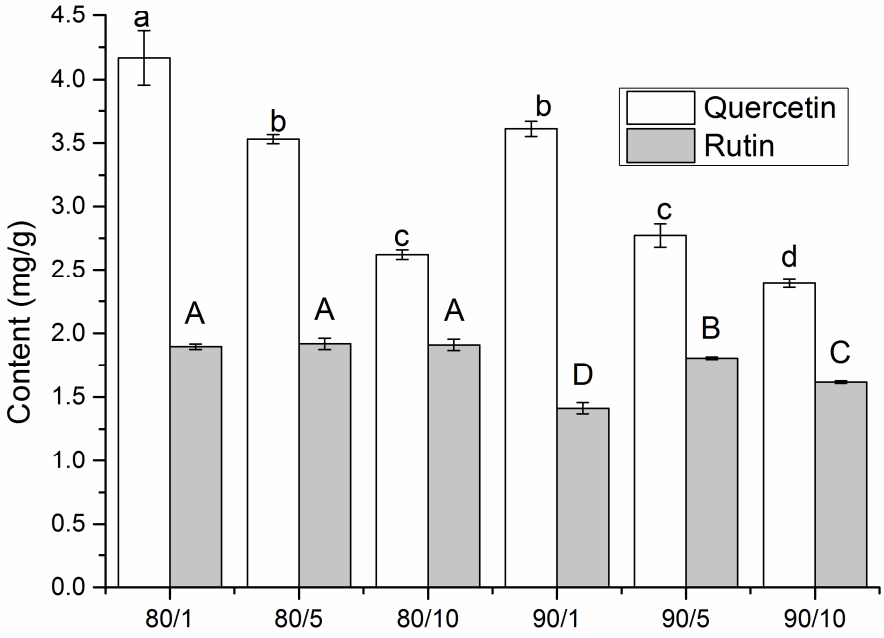
	TPC	$TEAC_{DPPH}$	$TEAC_{ABTS}$
Quercetin	-0.158	-0.030	-0.223
TPC		0.484	-0.813^{**}
$TEAC_{DPPH}$			-0.147
Rutin	0.807^{**}	0.747^{**}	0.123
TPC		0.622^{*}	0.266
$TEAC_{DPPH}$			0.014

* $P<0.05$; $^{**}P<0.01$; TPC, total phenolic content (μg gallic acid/ml); $TEAC_{DPPH}$, Trolox equivalent antioxidant capacity using 2,2-diphenyl-1-picrylhydrazil assay (μg Trolox/ml); $TEAC_{ABTS}$, Trolox equivalent antioxidant capacity using 2,2´- azino-bis-3-ethylbenzthiazoline-6-sulphonic acid assay (μg Trolox/ml)

446 Table 5 Values of the complex modulus at the reference frequency of 1 Hz (G*; kPa) of the
447 model processed cheese with and without added antioxidants manufactured at different
448 melting temperature and holding times.

Samples with	Melting temperature (°C)	Holding time (min)		
		1	5	10
Control	80	3443 ± 138 ^{a}A	6748 ± 412 ^{a}B	9168 ± 494 ^{a}C
	90	4401 ± 190 ^{a}A	8499 ± 541 ^{a}B	12038 ± 601 ^{a}C
Rutin	80	2829 ± 126 ^{b}A	6481 ± 358 ^{a}B	8338 ± 330 ^{b}C
	90	3945 ± 216 ^{b}A	9014 ± 368 ^{a,b}B	11124 ± 536 ^{b}C
Quercetin	80	2814 ± 145 ^{b}A	6482 ± 358 ^{a}B	9288 ± 361 ^{a}C
	90	3677 ± 177 ^{b}A	9404 ± 521 ^{b}B	11055 ± 585 ^{b}C

449 The values were expressed as mean ± standard deviation (N=4); significant difference
450 between means in column is indicated by different superscript letters ($P<0.05$); the means
451 within a row followed by capital letters differ ($P<0.05$).



- Processed cheese spread as a functional food
- Quercetin and rutin as functional ingredients
- Higher retention of quercetin than rutin
- Rutin more sensitive to melting temperature, quercetin to holding time