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ADDITION OF RUTIN/QUERCETIN MIXTURE TO SPREADABLE PROCESSED CHEESE: ANTIOXIDANT AND TEXTURAL CHARACTERISTICS

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ABSTRACT

Spreadable processed cheese is a traditional product made from a mixture of cheese, fat, water and emulsifying salts.The aim of this research was prepared spreadable processed cheese with new functional properties. Spreadable processed cheese enriched with the mixture (1:1) of rutin and quercetin (1.0 g.100g^{-1}) was prepared at two melting temperature (80°C and 90°C) for three holding times (1, 5 and 10 min). The effect of melting temperature and holding time on the quercetin and rutin content was assessed using liquid chromatography with UV detection after ultrasonic-assissted extraction to methanol. The corresponding antioxidant characteristics were determined using spectrophotometric assays for total phenolics (TPC) and radical scavenging activities DPPH and ABTS. The extraction yield for quercetin varied from 45.8 to 66.4% and from 12.8 to 40.8% for rutin. The level of quercetin significantly descrased with the increase of holding time, while rutin content has increased with the increase of melting temperature. TPC values ranged from 10.8. to 14.8 mg GAE·g^{-1} in SPC sample enriched with rutin/quercetin mixture, and the increase of melting temperature resulted in the decrease of TPC values. DPPH and ABTS assays did not reveal any statistically significant pattern using Kruskal-Wallis ANOVA. The addition of the mixture of flavonoids into the processed cheese significantly reduced the complex modulus in comparison with the control sample (without flavonoids). This indicate that the structure of enriched SPC sample was more flexible than those in control processed cheese samples. Both melting temperature and holding time increased the complex modulus. Spreadable processed cheese are scarcely used as a carrier of flavonoids in scientific researches probably due to very complex matrices. Our research proved that spreadable processed cheese containing rutin/quercetin mixture can be used as a functional food.

Keywords: processed cheese; flavonoid; antioxidant; technology; rheometry

INTRODUCTION

Quercetin (3,5,7-trihydroxy-2-(3,4-dihydroxyphenyl)-4H-chromen-4-on) and rutin (quercetin-3-rutinoside) are major dietary flavonoids examined in functional food. Buckwheat, as a natural source of those substances, is usually used in preparation of various bakery **(Krejzová et al., 2017; Lin and Zhou, 2018; Wang et al., 2017)** and pasta products **(Cho and Lee, 2015; Jambrec et al., 2015)**. Both flavonoids has a potential to be beneficial to human health. Quercetin and its derivates may act as antioxidant and anti-inflammatory agents **(Lesjak et al., 2018)** when consume in quercetin-rich diet. Pharmacological potential of rutin including vasoprotective, antidiabetic or neuroprotective effects has been extensively studied **(Rauf et al., 2017)**. Enrichment of food with plant extracts or pure chemical substances was investigated in order to develop new functional food products. Cheese is one of the promising carrier of health-related compounds. Cheese prepared using various lipophilic compounds such as retinyl palmitate, α-tocopherol, CoQ_{10} **(Stratulat et al., 2017)** and soy phytosterols **(Giri et al., 2014)** were examined. Phenolics such as catechin and epigallocatechin were frequently used as functional ingredients for manufacturing cheese with enhanced antioxidant properties **(Rashidinejad et al., 2016; Lamothe et. al, 2016; Han et al., 2011)**. Quercetin and its inclusion compounds with cyclodextrins were added to fresh cheese enhancing its nutraceutical properties **(Pereira et al., 2017)**. However, low-fat cheese or ripened cheese were assessed in above cited research papers. Processed cheese spread is scarcely used in experiments, probably due to the high content of fat and high temperature of processing. Spreadable processed cheese are multicomponent mixture comprising of cheese, fat, water and emulsifying salts. This mixture is stirred and melted at temperatures ranged from 85°C to 110°C for

holding times from 1 to 10 minutes. In our recent work, the content of quercetin in spreadable processed cheese (SPC) decreased with the increase of holding time but stable at different melting temperature. On the other hand, the level of rutin incorporated into the SPC showed decreasing trend with the increase of melting temperature **(Přikryl et al., 2018)**. In this research, SPC samples with mixture of rutin/quercetin was prepared and the effect of processing condition on the recovery of quercetin and rutin, antioxidant properties and textural characteristics was determined.

Scientific hypothesis

Both melting temperature and holding time significantly affect the level of rutin and quercetin as well as antioxidant and textural properties of enriched spreadable processed cheese.

MATERIAL AND METHODOLOGY

All solvent and chemicals were purchased from Sigma-Aldrich (Prague, Czech Republic).

Processed cheese manufacturing

Processed cheese was manufactured from the following ingredients per 100g of final product: Eidam cheese (Kromilk PLC, Kroměříž, Czech Republic, 8-week maturity, 50 g), butter (Madeta PLC, České Budějovice, Czech Republic, 84 g), water, emulsifying salts (Fosfa PLC, Břeclav, Poštorná, Czech Republic, 2.3 g) and the mixture of quercetin hydrate/rutin hydrate (1.0 g, 1:1, w/w). The amount of butter and water was adjusted in order to obtain product with 37 $g.100g^{-1}$ dry matter content and 50 $g.100g^{-1}$ of fat in dry matter content. Control sample (without quercetin/rutin mixture) was also prepared.

The SPC samples were prepared according to **Přikryl et al. (2018)** at two melting temperatures (80°C and 90°C) and three holding times (1, 5 and 10 min).

The preparation of extracts

SPC samples (1.0 g) was extracted by 10.0 mL of methanol in ultrasonic bath Sonorex TK52 (Bandelin Electronic, Berlin, Germany) for 30 min. After centrifugation at 1400 × g for 10 min (Vintrum NF400, Nűve, Ankara, Turkey), a clear supernatant was filtered using 0.45 μm syringe polytetrafluoroethylene membrane filter (Labicom, Olomouc, Czech Republic).

Determination of antioxidant characteristics

Antioxidant assays

The total phenolic assay (TPC) using Folin-Ciocalteau reagent was applied according to Santos et al. (2012). The results were expressed as amount of gallic acid per gram of SPC sample measured at 765 nm (DU 530, Beckman Coulter Inc., Brea, CA, USA). The extraction solvent used instead of SPC sample extract served as a blank.

The DPPH (2,2-diphenyl-1-picrylhydrazil) and ABTS (2,2´-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid) radical scavenging assays were performed as described by Mišan et al. (2011). The increase of absorbance was measured at 515 and 734 nm for DPPH and ABTS radical scavenging assay, respectively. The scavenging activity was plotted against various concentration of Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) and expressed as Trolox antioxidant capacity ($TEAC_{DPPH}$ and $TEAC_{ABTS}$) in amount of Trolox per g of SPC sample.

HPLC analysis of quercetin and rutin

Agilent 1100 Series (Agilent Technologies, Santa Clara, CA, USA) equipped with a quarternary pump, a degasser, an autosampler, a termostetted column compartment, and UV-MS detector Agilent 1100 Series LC/MSD Trap SL. A Gemini 5 μm column (150 × 3.0 mm) was used (Phenomenex®, Torrance, CA, USA). The gradient flow rate 0.7 mL/min (formic acid: acetonitrile from 900 mL:100 mL to 500 mL: 500 mL for 0 – 15 min) was applied. Formic acid (0.21% v/v) was acidifed to pH 3.05.

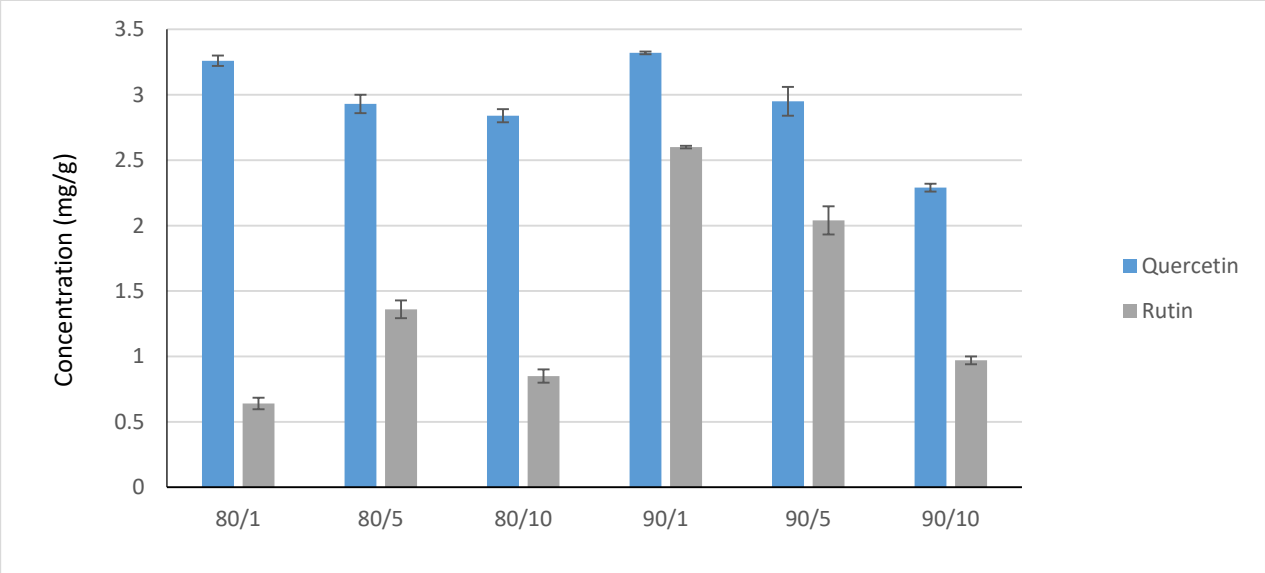

Figure 1 The effect of melting temperature and holding time (80/1 means 80°C for 1 min, 80/5 means 80°C at 5 min, etc.) on the quercetin and rutin levels extracted from spreadable processed cheese. Average mean ± standard deviation (n = 4).

The analysis was performed at 40°C and both peaks of quercetin a rutin were detected at 360 nm. Quantification was based upon the separation of standard solution of flavonoids from 1 to 100 μg.mL^{-1} and plotting the peak area against concentration.

Determination of rheologial properties

A dynamic oscillatory shear rheometer (RheoStress 1, Haake, Bremen, Germany) with a plate-plate geometry (diameter 35 mm, gap 1 mm) were used. The complex modulus (G^*) at reference frequency 1 Hz was calculated based on the values uf storage (G´) and loss (G´´) moduli:

$$G^* = \sqrt{(G')^2 + (G'')^2} \quad (1)$$

Increasing values of the complex moduli (G*) indicated rigid consistency of processed cheese and rising gel strength **(Černíková et al., 2017).** All rheological measurements were done at 20.0°C.

Statisic analysis

All the measurements were performed in four repetitions (n = 4) and the average mean with standard deviation (SD) was calculated. Non-parametric Kruskall-Wallis ANOVA was used for determination of the effect of melting temperature and holding time on the content of quercetin/rutin and antioxidant properties. Spearman correlation coefficients (r) were calculated in order to find mutual associations between variables. All the statistical procedures were done at the probability level of p = 0.05 (Statistica 12, StatSoft CR s.r.o., Prague, Czech Republic).

RESULTS AND DISCUSSION

Rutin and quercetin content in SPC samples

Extraction of flavonoids from solids into alcohols and aqueous-alcoholic solutions belongs to the frequently used methods in plant-based material prior to their determination. As can be see from **Figure 1**, higher concentrations (p <0.001) of quercetin were determined in SPC samples extracted to 100% methanol in comparison with rutin levels. The extraction yield for quercetin ranged from 45.8 to 66.4% and from 12.8 to 40.8% for rutin. As was expected, higher levels of quercetin than rutin was recovered from SPC samples extracted to methanol. This is probably due to the higher hydrophobic properties of quercetin. It should be noted that cheese is a very complex matrix and various biochemical reaction may occur between phenolics and cheese proteins as was suggested in a study of **Rashidinejad et al. (2016)**. The recovery of green tea catechins from full-fat cheese varied from 3.2 to 29.4% in their experiments. In our previous work **(Přikryl et al., 2018)**, we prove that quercetin and rutin added separately to the formulation of SPC samples, may undergo thermal degradation or chemical reaction with the constituents of cheese during melting. Quercetin was more sensitive to holding time whereas rutin level decreased with the increase of melting temperature. In this research, the level of quercetin significantly descreased with the increase of holding time (p = 0.013). Kruskal-Wallis ANOVA procedure revealed that rutin concentration was influenced by the melting temperature but in the opposite manner in comparison with our previous research **(Přikryl et al., 2018)**; i.e. rutin level was higher in SPC samples processed at 90°C than 80°C (p = 0.025). Rutin level usually decreased with the increase of processing temperature. For instance, **Chaaban et al. (2016)** determined the degradation kinetics of rutin in solution at various temperatures and found that half life time for rutin has decreased from 19.25 to 1.99 h at 70°C and 90°C, respectively. **Krejzová et al. (2017)** found that the amount of rutin in buckwheat flour and seeds substantially decreased when thermall treatment exceeded 150°C. On the other hand, rutin content significantly increased with the elevated temeparture during baking of Tartary buckwheat enriched bread **(Wang et al., 2017)**. However, rutin was released from the cell wall of buckwheat during baking procedure in this research, which should not be expected in SPC samples enriched with rutin/queretin. We

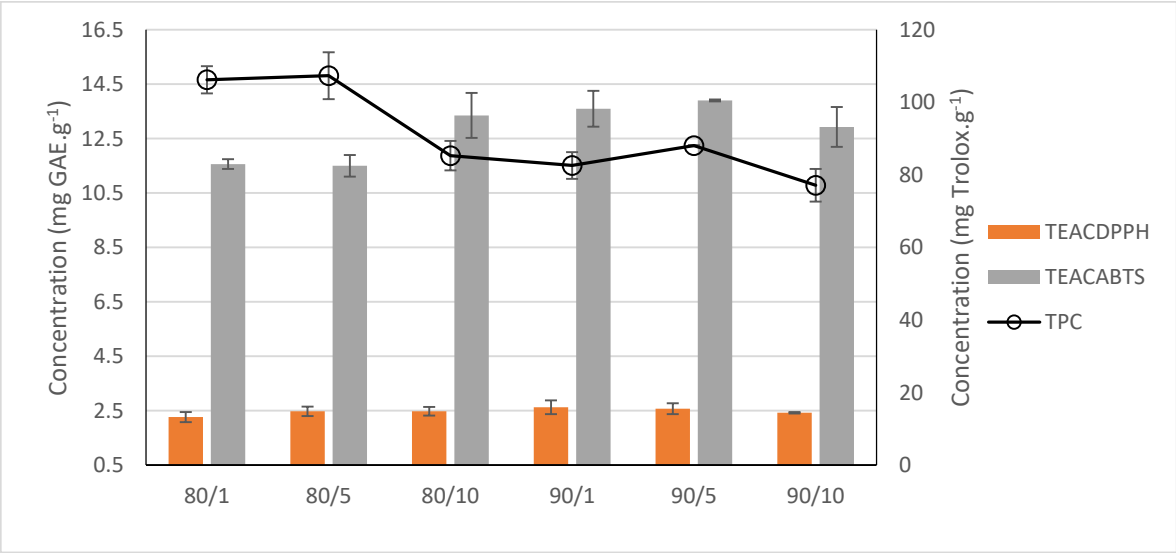

Figure 2 The effect of melting temperature and holding time (80/1 means 80°C for 1 min, 80/5 means 80°C at 5 min, etc.) on antioxidant properties of spreadable processed cheese enriched with quercetin and rutin. Average mean ± standard deviation (n = 4).

may hypothesise that different rheological properties of processed cheese prepared at 90°C could enhanced the extraction yield of rutin.

Antioxidant properties of SPC samples

SPC samples enriched with the mixture (1:1) of rutin and quercetin at 1.0 g/kg exhibited total phenolics content in the range from 11.9 to 14.8 mg GAE·g^{-1} when melted at 80°C, and from 10.8 to 12.2 mg GAE·g^{-1} when melted at 90°C **(Figure 2)**. According to Kruskal-Wallis ANOVA procedure, increasing melting temperature resulted in significant decrease of TPC values (p = 0.020). Regarding the holding time for each temperature treatment, lower TPC value (11.9 ±0.5 mg GAE·g^{-1}) was obtained after melting SPC sample at 80°C for 10 min whereas constant TPC values were determined at 90°C. As can be seen from **Figure 2**, antioxidant properties of SPC samples measured in terms of ABTS assay showed remarkable higher values (82.5 – 100.5 mg Trolox·g^{-1}) than those obtained using DPPH assay (13.2 – 15.5 mg Trolox·g^{-1}). This discrepanse was maily attributed to the steric hindrance and due to the different mechanism of radical scavenging **(Schaich et al., 2015)**. The overall effect of both melting temperature and holding time on $TEAC_{DPPH}$ and $TEAC_{ABTS}$ values was negligible (p >0.05) but significantly higher $TEAC_{ABTS}$ value (96.4 ±6.2 mg Trolox·g^{-1}) was observed for SPC samples melted at 80°C for 10 min than for 1 and 5 min at the same melting temperature (p <0.01). Correlation analysis revealed both positive and negative weak but non-significant associations between variables. The highest one was for TPC/$TEAC_{ABTS}$ (r = -0.410, p = 0.186). Nevertheless, it is interesting that low TPC values was associated with the high $TEAC_{ABTS}$ value at 80°C and 10 min **(Figure 2)**. The same pattern was determined in tomatoe boiled in water-oil mixture in comparsion with raw counterparts **(Ramírez-Anaya et al., 2015)**. They stated that some hydrosoluble comounds without true antioxidant properties may contribute to the opposite effect. In addition, irreversible higher oxidation products of ABTS and milk protein (particularly with thiol groups) may cause the additional increase of $TEAC_{ABTS}$ **(Çekiç et al., 2015)**.

Rheological properties of SPC samples

The results of the complex modulus G" of processed cheese with quercetin/rutin (1:1) content at different melting temperatures and holding times are presented in Table 2. With the increase of both processing parameters, G" values increased indicating the formation of denser net structure (p <0.05). As can be seen from the differences between control and enriched SPC samples, the addition of quercetin/rutin mixture significantly decreased G" parameters under all the processing conditions (showing negative values). As the complex modulus G" is related to the strenght of the intermolecular interaction among proteins in cheese, our findings confirmed that addition of quercetin/rutin mixture reduced the protein network. This is in agreement with the study of **He et al. (2018)** who found that the addition of quercetin and rutin decreased the cohesivness of Tartary buckwheats starch gel. Different rheological properties of SPC samples manufactured under different conditions may also influence the extractibility of both flavonols. For instance, the Young´s modulus of elasticity (textural characteristic of seed) showed negative correlation with the extraction yield for catechin, epicatechin and procyanidin B_1, but exhibited positive correlation with epicatechin gallate **(Segade et al., 2016)**.

CONCLUSION

The addition of the mixture of rutin/quercetin to the processed cheese spread resulted in its enhanced antioxidant capacity, as was expected. We used rutin and quercetin in combination as these flavonoids ale likely to be occure in plant material together. It is evident from the results that the mixture of rutin and quercetin significantly decreased viscoelastic properties, i.e. enriched processed cheese were more flexible than those in control samples. Increasing melting temperature or holding time increased the complex modulus thus SPC samples became more rigid in texture. The effect of processing parameters on the content of rutin/quercetin and corresponding antioxidant properties was not so unambiguous. While quercetin level decreased with the increase of holding time, rutin levels were affected by melting temperature. However, higher rutin levels were observed in SPC samples prepared at higher temperature. This may be attributed to the changes of consistency and better extractability of rutin, although no correlation was found between rutin content and complex modulus G". Based on the results, we may conclud that scientific hypothesis was not confirmed regarding the impact of processing technology on the antioxidant characteristic but is valid for rheologial properties. Further research is needed to elucidate the effect of rheologial properties on the extractability of phenolic substances from processed cheese matrix.

Table 2 The effect of melting temperature and holding time on the complex modulus at the reference frequency 1 Hz ($G^{"}$, kPa).

	Melting temperature (°C)	Holding time (min)		
		1	5	10
SPC with rutin/quercetin mixture (1:1)	80	2533 ±123^{a} A	6028 ±341^{b} A	7465 ±375^{c} A
	90	3706 ±145^{a} B	7585 ±424^{b} B	11019 ±514^{c} B
Difference from control SPC sample	80	-910***	-720*	-1703**
	90	-695**	-914*	-1019*

Note: Means ±standard deviation (n = 4); significant differences between means for melting temperatures are indicated by capital letters; the means within a row followed by different superscript letters (p <0.05); the differences from control SPC samples are statistically significant at * <0.05, ** <0.01 and *** <0.001.

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