

ANTIOXIDANT PROPERTIES OF PROCESSED CHEESE SPREAD AFTER FREEZE-DRIED AND OVEN-DRIED GRAPE SKIN POWDER ADDITION

Michaela Frühbauerová, Libor Červenka, Tomáš Hájek, Richardos Nikolaos Salek, Helena Velichová, František Buňka

ABSTRACT

Processed cheese spread (PCS) is a popular product with high nutritional value and containing protein, fat and minerals. Grape skin is waste from winery processing plants that still has phenolic substances with significant antioxidant activity that could be used for valorisation of processed cheese and increasing the content of nutrients, phenolics and overall antioxidant properties. Both oven-dried (OD) and freeze-dried (FD) grape skin (GS) powder was characterised by the principal ingredients, the content of phenolic compounds and antioxidant capacity. Similarly, the influence of the addition of OD-GS and FD-GS powders on processed cheese spread (PCS) at 1% and 2% (w/w) levels were examined. The OD-GS and FD-GS powders were characterised by protein content, fat content, moisture and dietary fibre, thus showing that drying technique did not affect those parameters. The OD-GS powder exhibited higher content of rutin, (+)-catechin, (-)-epicatechin and total flavonoid content (TFC), while higher total phenolic content (TPC) and ABTS radical cation were observed for freeze-dried GS powder. Fortification of PCS with 1% and 2% (w/w) of GS powder increased protein content. An ANOVA procedure revealed that addition of FD-GS powder to processed cheese spread was superior to TPC values together with rutin, (+)-catechin, and (-)-epicatechin contents. The higher phenolic contents reflected the higher antioxidant capacity of PCS samples fortified with FD-GS powder. Freeze-dried grape skin powder was the better choice for valorisation of processed cheese spread.

Keywords: grape; valorisation; processed cheese; antioxidant; chromatography

INTRODUCTION

Processed cheese spread (PCS) is a multi-component mixture made from water, cheese, fat, and emulsifying salts (phosphates or citrates). This mix is processed by stirring and melting in temperatures ranging from 85 to 110 °C for up to 20 min (Černíková, et al., 2018). The obtained hot mixture is poured into cups, cooled down and stored at refrigeration temperature. Processed cheeses are products with extended shelf life that deliver bioactive proteins, fats, minerals and vitamins to consumers (Henning, et al., 2006). Despite the high nutritional value, various types of cheese have been enriched by addition of herb or medicinal plant extracts during their preparation, for instance, the addition of rosemary leaves to ripened semi-hard cheese (Marinho, et al., 2015). The fortification of frequently used food products may enhance the consumption of various health-promoting substances and might be helpful for human health (Rashidinejad, et al., 2015). The authors found that hard low-fat cheese fortified by catechin maintained its antioxidant activity after *in vitro* digestion experiment.

Grape berries (*Vitis vinifera L.*) are used in the winemaking industry to produce alcoholic beverages by pressing berries and subsequent fermentation of liquid. The press residues constitute 20% (w/w) of the total grapes used for wine production (Teixeira, et al., 2014). Grape pomace from white grape varieties is an excellent source of phenolic compounds (for example gallic acid, catechin, epicatechin and procyanidins) (Genova, Tosetti and Tonutti, 2016); therefore, it can be used for the valorisation of various food products. Grape skin powder or grape flour has been successfully incorporated into bread (Šporin, et al., 2018) or yoghurts (Karnopp, et al., 2017). Only a limited number of studies regarding the enrichment of processed cheese, probably due to the higher processing temperature and high-fat content, exist. In our recent studies, we described the effect of processing parameters on the antioxidant properties of processed cheeses fortified by quercetin or/and rutin (Příkryl, et al., 2018). Functional processed cheese spreads have been prepared with the addition of tomato paste (Mehanna, et al., 2017), carrot paste (Mohamed, Shalaby and Gafour,

2016) and pulp from apricots (Mohamed and Shalaby, 2016). In a study of Torri et al. (2016), grape skin powder was added to cow's milk curd before ripening process to produce Robiola (soft-ripened cheese), focusing on optimisation of enrichment using sensory evaluation. The authors found that the amount of powders exceeding 0.8% and 1.6% (w/w) for Barbera and Chardonnay powder, respectively, negatively affected the acceptability of cheeses. In a recent study, various fruit and vegetable by-products were added into the curd to produce Primosale cheese (Costa, et al., 2018). They found wine pomace to be an excellent source of polyphenolic compounds with antioxidant activity. The main aim of this study is to determine the effect of the addition of grape skin powder on principal ingredients and antioxidant properties of processed cheese. We assumed that the addition of freeze-dried grape skin powder would enhance the functional properties of enriched processed cheese spread.

Scientific hypothesis

Freeze-dried grape skin powder enhances the antioxidant status of fortified processed cheese spread more than the oven-dried grape skin powder does.

MATERIAL AND METHODOLOGY

All solvents for extraction, chromatographic analysis and chemicals used for determination of antioxidant activity were purchased from Sigma-Aldrich (Prague, Czech Republic).

Preparation of grape skin powder

Grapes of the white variety 'Müller Thurgau' were harvested from the Prostřední Hory (Bzenec, Czech Republic) vineyard track during September 2017. After pressing the grape berries to obtain a liquid for wine manufacturing, a portion of grape pomace was immediately stored at $-20\text{ }^{\circ}\text{C}$ in an evacuated plastic package. Before processing, grape pomace was thawed and the needles and seeds were removed using an analytical sieve (mesh size $0.5 \times 0.5\text{ cm}$). Grape skins (GS) were dried at the following conditions: oven-drying (OD) was performed in laboratory air-forced oven (HS62A, Chirana, Brno, Czech Republic) at $46\text{ }^{\circ}\text{C}$ for 24 h. Freeze-dried (FD) samples were prepared at $-40\text{ }^{\circ}\text{C}$ (12 Pa) for 48 h (CoolSafe 100-4, Trigon Plus, Čestlice, Czech Republic). Dried grape skins were milled at 5000 rpm for 10 s with a Grindomix GM 200 (Retsch GmbH, Haan, Germany) and sieved to obtain particles $<800\text{ }\mu\text{m}$. Both GS-OD and GS-FD powders were stored in a tightly sealed plastic pack at $-20\text{ }^{\circ}\text{C}$ until use. The contents of crude protein (Method 960.52), fat (Method 920.39), moisture (Method 934.01), ash (Method 930.05) and total dietary fibre (Method 985.29) were determined according to AOAC (Horwitz, 2000) procedure in duplicate. The total content of saccharides was calculated from differences.

Processed cheese manufacturing

The composition of raw materials, including Eidam cheese (dry matter $\approx 50\text{ g}\cdot 100\text{ g}^{-1}$ and fat in dry matter $\approx 30\text{ g}\cdot 100\text{ g}^{-1}$, 8-week maturity), butter (dry matter $\approx 84\text{ g}\cdot 100\text{ g}^{-1}$ and fat in dry matter $\approx 98\text{ g}\cdot 100\text{ g}^{-1}$) and

water, was adjusted to obtain processed cheese with dry matter $\approx 37\text{ g}\cdot 100\text{ g}^{-1}$ and fat in dry matter $\approx 50\text{ g}\cdot 100\text{ g}^{-1}$. A ternary mixture of monosodium dihydrogenphosphate (19%), disodium hydrogen phosphate (37%), tetrasodium diphosphate (22%) and the sodium salt of polyphosphate (22%) was used in a total concentration of $2.8\text{ g}\cdot 100\text{ g}^{-1}$. For the preparation of functional processed cheese spread (PCS), OD-GS and FD-GS powders were added to produce PCSOD-GS and PCSFD-GS samples. Both grape skin powders were added at 1.0 and 2.0% (w/w) levels. Processed cheese without grape skin powder served as a control. Model processed cheese was manufactured in Stephan UMC-5 (Stephan Machinery GmbH, Halmen, Germany) equipment with indirect heating as is described in the flow chart below (Figure 1). Eidam block cheese and butter were cut into small pieces, put into the kettle and minced for 30 s. Then water, emulsifying salts and GS powder were added into the blend. The mixture was heated at $90\text{ }^{\circ}\text{C}$ for 13 min at a constant agitation of 1500 rpm. Samples were poured into 80 g polystyrene doses with caps. The packages were cooled down to $6\text{ }^{\circ}\text{C}$ and stored at $-20\text{ }^{\circ}\text{C}$ to avoid deterioration. Dry matter (DM), fat and protein contents were evaluated according to Method 969.19, Method 2001.14 and Method 960.52 as described in Horwitz (2000), respectively, in three repetitions.

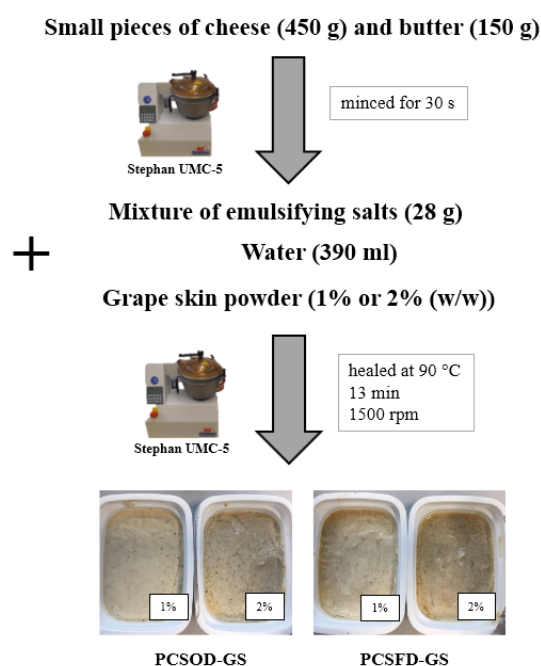


Figure 1 Flow chart of the cheese-making process. PCSOD-GS = processed cheese spread with oven-dried grape skin powder, PCSFD-GS = processed cheese spread with freeze-fried grape skin powder.

Extracts preparation

A glass test tube with 1.0 g of dried GS sample and 10.0 mL of 50% methanol solution was put in ultrasonic bath Sonorex TK52 (Bandelin Electronic, Berlin, Germany) for 30 min. A clear supernatant after centrifugation at 4100 rpm for 5 min (Universal 320, Hettich, Tuttlingen, Germany) was obtained.

Extract of PCS samples was obtained according to the procedure described in **Přikryl et al. (2018)**, i.e. 1.0 g of frozen PC sample was extracted into 10.0 mL of 50% methanol solution in an ultrasonic bath for 30 min. Subsequently, elimination of proteins and salts was performed using the procedure of **Khalifa, Omar and Mohamed (2017)** with a slight modification; the pH of extract was adjusted to 4.0 using HCl (2 M) and precipitated proteins were removed by centrifugation at 6000 rpm for 10 min. Then, the pH of clear supernatant was adjusted to 7.0 using NaOH (1 M) followed by centrifugation at 6000 rpm for 10 min to remove remaining proteins and salts. Supernatants were kept refrigerated and used for antioxidant assays and HPLC analysis. Two extracts per sample were prepared, and each extract was measured in duplicate, resulting in a sample size $N = 4$.

Chromatographic analysis of the extracts (HPLC analysis)

First, GS were screened for the presence of the following phenolic compounds: quercetin, rutin, (+)-catechin, (-)-epicatechin, resveratrol, caffeic acid, p-cumaric acid and ellagic acid. Secondly, phenolic substances identified in GS extracts were determined in PCSOD-GS and PCSFD-GS extracts. Before injection, each extract was filtered through a syringe filter (0.45 μm , Labicom, Olomouc, Czech Republic). Samples were injected into an Agilent 1290 Infinity (Agilent Technologies, Santa Clara, CA, USA) equipped with a degasser, an autosampler, a binary pump, a thermostated column compartment and DAD detector. A Zorbax Elipse Plus 1.8 μm C18 (50 \times 2.1 mm; Agilent Technologies) column thermostated at 40 °C was used. For the analysis, 2 μL of the sample were injected. A mixture of 0.01 M ammonium acetate adjusted to pH 3.1 using formic acid (solution A) and acetonitrile (solution B) was used as mobile phase with a flow rate 0.6 mL.min⁻¹. The gradient for solution B was 0-3 min at 3%; 10 min at 20% and 20 min at 80%. The signal was detected at 280 nm. The identification of each peak in chromatograms of the extracts was carried out by comparing retention time and absorption spectrum against a pure standard. Quantitative determinations were done using calibration plots of a selected external standard.

Determination of antioxidant properties

Total phenolic content (TPC) was determined by measuring the complex of antioxidants with Folin-Ciocalteu reagent at 765 nm (DU 530, Beckman Coulter Inc., Brea, USA) using the procedure described in **Přikryl et al. (2018)**. The results were expressed as gallic acid (GAE) equivalents (mg.g⁻¹ of dry matter (DM)). Total flavonoid content (TFC) was determined using aluminium chloride assay (**Denni and Mammen, 2012**). The increase of absorbance at 415 nm was proportional to the increase in the content of flavonoids. Results were reported as quercetin (QUE) equivalents (mg.g⁻¹ DM).

ABTS (2,2'-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid) radical scavenging activity assay was adopted from our previous study (**Červenka, et al., 2018**). The reaction between ABTS^{•+} and antioxidants was monitored at

734 nm, and the results were reported as Trolox equivalents antioxidant capacity (TEAC) in mg.g⁻¹ DM.

Reducing power (RP) of extracts was determined via the formation of Prussian blue at 700 nm (ferric-ferrous complex) according to the procedure of **Pavithra and Vadivukkarasi (2015)**.

Statistical analysis

Order statistic methods for small sample size were used throughout this study. The mean and its deviation were calculated according to Horn's procedure (**Horn, 1983**) and were expressed as pivot half sum (P_L) and pivot range (R), respectively. Nonparametric statistical methods were used in this study. The sign test was applied for pair-wise comparisons between means for grape powder samples. As far as processed cheese is concerned, the pair-wise comparison procedure was performed using Tukey's method. A two-factor Kruskal-Wallis analysis of variance (ANOVA) was applied to determine whether the drying method of GS powder (factor A) and the amount added to PCS sample (factor B) influenced the antioxidant properties in fortified PCS samples. To determine associations among variables, Spearman rank-order correlation coefficients (r) were assessed. All the statistical treatments were done at the probability $P = 95\%$ (Statistica CZ, 12.0, StatSoft CR s.r.o., Prague).

RESULTS AND DISCUSSION

Composition of grape skin powder

As can be seen from Table 1, there were no statistical differences between the contents of crude protein, fat, total dietary fibre and ash for oven-dried and freeze-dried GS powders. On the other hand, the type of drying process affected the TPC and antioxidant properties of samples. Freeze-dried GS powder has a higher level of TPC (19.97 \pm 1.60 mg GAE.g⁻¹ DM) and more than twice as high TEAC_{ABTS} value (127.10 \pm 27.28 mg Trolox.g⁻¹ DM) in comparison with the oven-dried GS samples. It has been previously concluded that the freeze-drying process is superior for the preservation of antioxidant compounds, and probably higher efficiency of extraction due to pronounced disruption of plant cells via the formation of ice crystals (**Kamiloglu, et al., 2016, Kamiloglu and Capanoglu, 2014**). However, such a release of flavonoid compounds from plant cells may cause their pronounced exposure to oxygen. As was described in a study of **Nunes et al. (2016)**, oven drying of guava powder released more soluble flavonoids than the freeze-drying technique. In the chromatogram (Figure 2) of dried grape skin powder extract, fourteen well-resolved peaks were observed. However, from eight selected polyphenolic compounds, only the presences of (+)-catechin, (-)-epicatechin and rutin were confirmed in grape skin powder samples using retention time and absorption spectra in the current study. There was no difference in the number of peaks/compounds in oven-dried and freeze-dried GS powder extracts. The chromatograms differed in the heights of the peak showing that oven-dried GS powder extracts contained more polyphenolic substances.

Table 1 The main composition and antioxidant properties of grape skin powders (pivot half sum (P_L) \pm pivot range (R), $N = 2$).

	Oven-dried	Freeze-dried
Main composition (g.kg⁻¹)		
Protein	157.5 \pm 7.0 ^A	150.5 \pm 4.2 ^A
Fat	74.2 \pm 5.1 ^A	72.3 \pm 4.5 ^A
Moisture	55.0 \pm 4.3 ^A	59.5 \pm 6.4 ^A
Ash	13.2 \pm 2.0 ^A	14.6 \pm 3.1 ^A
Total saccharides	701.2	703.5
Total dietary fibre	228.8 \pm 8.5 ^A	231.7 \pm 9.1 ^A
Phenolic content (mg.g⁻¹ DM)		
(+)-catechin	1712.5 \pm 1.2 ^B	1450.0 \pm 1.5 ^A
(-)-epicatechin	1383.3 \pm 3.0 ^B	1023.4 \pm 2.2 ^A
Rutin	221.8 \pm 1.0 ^B	112.0 \pm 1.2 ^A
Total phenolics (mg GAE.g ⁻¹ DM)	10.1 \pm 0.93 ^A	19.97 \pm 1.60 ^B
Total flavonoids (mg QUE.g ⁻¹ DM)	0.73 \pm 0.01 ^B	0.52 \pm 0.02 ^A
Antioxidant activity		
TEAC _{ABTS} (mg Trolox.g ⁻¹ DM)	54.44 \pm 2.44 ^A	127.10 \pm 27.28 ^B
Reducing power (absorbance unit)	1.140 \pm 0.010 ^A	1.136 \pm 0.012 ^A

Note: DM, dry matter; GAE, gallic acid; QUE, quercetin; TEAC_{ABTS}, Trolox equivalent antioxidant capacity using 2,2'-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid. Values sharing the same superscript letters in row (^{A-B}) are not statistically significant different from each other (the sign test, $p < 0.05$).

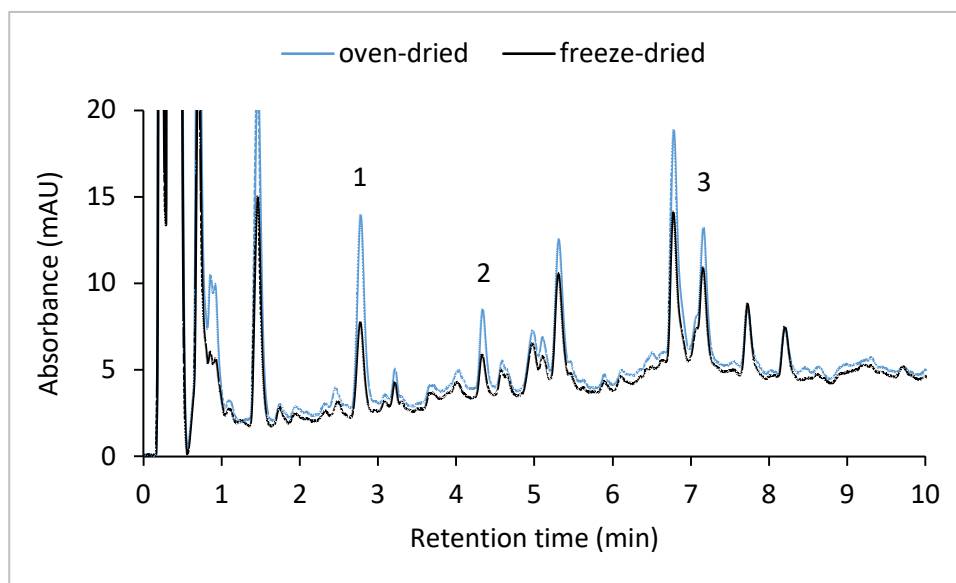


Figure 2 Separation of phenolic compounds in oven-dried and freeze-dried grape skin powder extracts using HPLC/DAD, detection at 280 nm; (+)-catechin (1), (-)-epicatechin (2) and rutin (3).

As shown in Table 1, significantly higher contents of (+)-catechin, (-)-epicatechin and rutin were observed in oven-dried GS powders, which corresponded to TFC values.

Properties of processed cheese spread containing grape skin powder

Table 2 shows the differences in the main composition of fortified processed cheese. The addition of grape skin powders significantly increased the protein content in processed cheese samples when added in 2% (w/w) levels, i.e. from 112.5 \pm 5.0 g.kg⁻¹ (in control) to 128.4 \pm 5.7 and 129.7 \pm 2.3 g.kg⁻¹ for processed cheese enriched with freeze-dried and oven-dried GS powders, respectively

($p < 0.05$). Since we used the same amount of ingredients (cheese, butter, polyphosphate salts) for the preparation of all of the processed cheese samples, the variation in protein content was due to the addition of grape skin powder. Although the values of dry matter content increased linearly with the level of addition of grape skin powder, the effect of fortification was recognized as insignificant ($p > 0.05$). On the contrary, **Khan et al. (2018)** found that fortification of Gouda cheese with the mango kernel did not significantly change the protein content.

Pair-wise comparison tests revealed that only processed cheese with 2% (w/w) of oven-dried GS powder had significantly higher dry matter content than that found in

Table 2 The main composition of processed cheese spread (PCS) fortified with oven-dried (OD) and freeze-dried (FD) grape skin powder (GS). Pivot half sum (P_L) \pm pivot range (R) ($N = 3$).

Ingredient (g.kg ⁻¹)	PCS control	PCS fortified with OD-GS powder (%, w/w)		PCS fortified with FD-GS powder (%, w/w)	
		1.0	2.0	1.0	2.0
		Protein	112.5 \pm 5.0 ^A	120.3 \pm 4.3 ^A	129.7 \pm 2.3 ^B
Fat	191.0 \pm 3.1 ^A	188.5 \pm 2.7 ^A	187.4 \pm 3.0 ^A	189.3 \pm 4.2 ^A	186.9 \pm 1.8 ^A
Dry matter	368.2 \pm 14.0 ^A	394.6 \pm 6.2 ^{AB}	419.0 \pm 18.0 ^B	387.3 \pm 7.0 ^{AB}	395.9 \pm 4.8 ^{AB}
Ash content	39.5 \pm 1.8 ^A	40.8 \pm 2.2 ^A	42.1 \pm 1.4 ^A	40.9 \pm 1.1 ^A	42.4 \pm 2.0 ^A

Note: Values sharing the same superscript letters in row (^{A-B}) are not statistically significant different from each other (Tukey's pair-wise comparison test, $p < 0.05$).

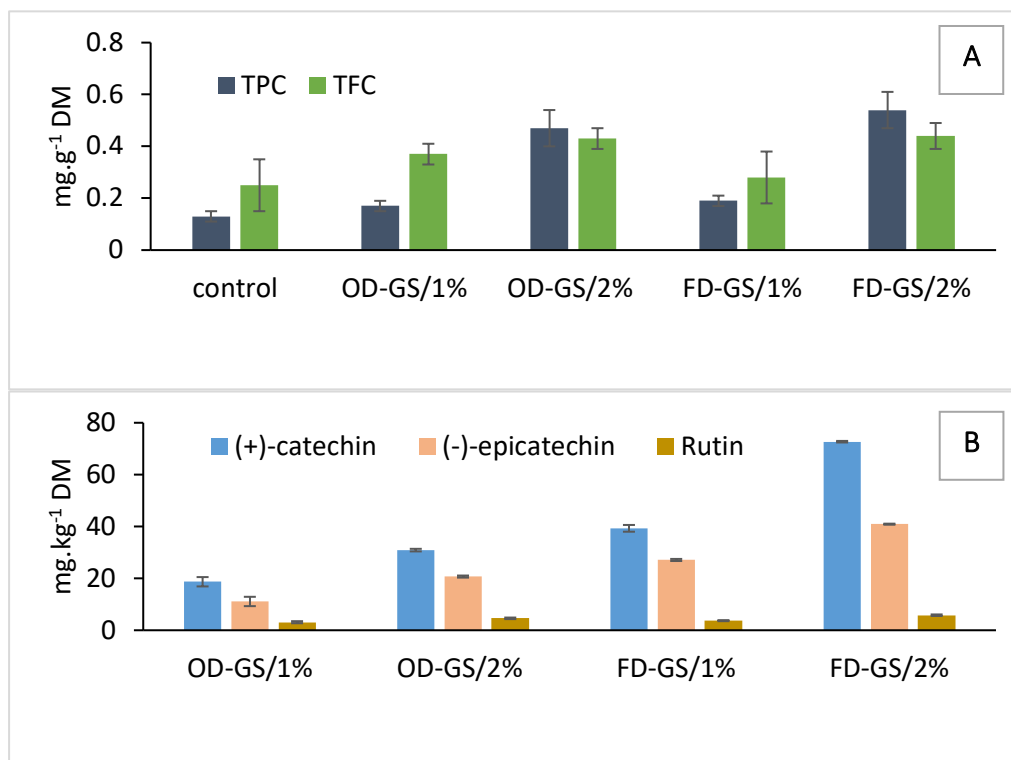


Figure 3 The content of **A**) total flavonoid (TFC), total phenolic contents (TPC), and **B**) phenolic individuals in processed cheese spread supplemented with oven-dried (OD) and freeze-dried (FD) grape skin (GS) powder at 1% and 2% (w/w) levels. Results are expressed as gallic acid and quercetin equivalents for TPC and TFC, respectively.

control ($p < 0.01$). Fat and ash contents remained similar for all the processed cheese samples ($p > 0.05$).

Antioxidant properties of processed cheese spread containing grape skin powder

The content of phenolic individuals is depicted in Figure 3B and show their higher content in processed cheese fortified with freeze-dried grape skin powder at both levels. Total phenolic content of processed cheese samples was determined using Folin-Ciocalteu's assay (Figure 3A). Significant increase of TPC values was obtained for processed cheese supplemented with GS powder at 2% (w/w) level from 0.13 ± 0.02 mg GAE.g⁻¹ DM (control sample) to 0.47 ± 0.07 and 0.54 ± 0.07 mg GAE.g⁻¹ DM for oven-dried and freeze-dried GS powder, respectively. The addition of OD-GS powder to processed cheese spread resulted in the increase in TFC values from 0.25 ± 0.10 mg

QET.g⁻¹ DM (control sample) to $0.37 - 0.43$ mg QET.g⁻¹ DM without respect to GS level. Freeze-dried GS powder enhanced processed cheese samples with flavonoids at a higher level (2%, w/w). It is interesting to note that even the control sample exhibited TFC value that then slightly increased with the addition GS powder. It has been reviewed that antioxidant properties of milk and milk products are due to the presence of sulphur-containing amino acids, vitamins, enzymes, peptides and oligosaccharides (Khan, et al., 2019; Usta and Yilmaz-Ersan, 2013; Atmaca, 2004; Egger and Ménard, 2017; Alenisan, et al., 2017). Although oven-dried GS powder exhibited higher levels of (+)-catechin, (-)-epicatechin and rutin, their content in PCS samples with OD-GS powder decreased in comparison with PCS fortified with FD-GC powder. TPC values showed very high correlation with the content of all phenolic constituents ($0.746 < r < 0.826$, $p < 0.01$) while low correlation coefficients have been

observed for TFC values showed values where ($r = 0.610$, $p < 0.05$).

Antioxidant capacity of processed cheese samples in terms of TEAC_{ABTS} significantly increased with the increase of GS level ($p < 0.05$). Surprisingly, TEAC_{ABTS} values were approximately tenfold higher (0.31 – 0.72 mg Trolox.g⁻¹ DM) for processed cheese with 1% (w/w) of GS in comparison with that of the control, followed by an additional tenfold increase (5.22 – 6.69 mg Trolox.g⁻¹ DM) when 2% (w/w) of GS powders were added (Figure 4). Such an increase may be attributed to the higher temperature used for melting the processed cheese in this study (90 °C for 13 min). It was previously published that pasteurization enhanced antioxidant properties of grape juice by releasing some polyphenolic compounds that were previously bound to other molecules (Genova, Tosetti and Tonutti, 2016; Fuleki and Ricardo-da-Silva, 2003). For instant, general increase in ABTS scavenging was detected in fresh and technologically harvested grape juices followed by pasteurization at 78 °C for 30 min (Genova, Tosetti and Tonutti, 2016).

In our previous work, the antioxidant properties of processed cheese fortified by rutin or quercetin increased with the increase of melting temperature as measured by ABTS^{•+} assay (Přikryl, et al., 2018). In addition, polyphenolics contributed differently to antioxidant capacity measured by ABTS^{•+}, as was observed in a study of Lingua et al. (2016). They found that (-)-epicatechin, peonidin-3-glucoside and peonidin-3-acetylglucoside positively correlated with the assay, while pigment A and siringetin-3-glucoside had a negative effect. Similarly, strong positive correlation was observed for TEAC_{ABTS} values and the content of (+)-catechin, (-)-epicatechin and rutin ($r = 0.955$, $p < 0.001$; $r = 0.739$, $p < 0.01$ and $r = 0.951$, $p < 0.001$, respectively). Considering that high correlation coefficient reflects the strong association between variables, we may conclude that TEAC_{ABTS} was

mainly influenced by (+)-catechin and rutin contents followed by TPC values ($r = 0.877$, $p < 0.01$).

The addition of GS powder had influence on reducing the power of processed cheese samples (Figure 4), where an increase was observed with the increase of GS powder level. Even though oven-dried and freeze-dried GS powders had similar RP values (see Table 1), processed cheese samples enriched with freeze-dried GS powder showed significantly higher RP values than in the case of incorporation of oven-dried GS powder, particularly at the 2% level (w/w) ($p < 0.05$). Those discrepancies can be explained by the formation of new compounds, which may have enhanced the antioxidant capacity of the samples. Although the masking of antioxidant properties of various plant-based extracts by the addition of milk or whey proteins is common in literature, an interaction of protein and polyphenolic compounds may also increase antioxidant capacity. For instance, the mixing of α -casein or β -casein with epigallocatechin gallate led to the increase of inhibition of ABTS^{•+} during storage (Almajano, Delgado and Gordon, 2007). In a study by Sęczyk, Świeca and Gawlik-Dziki (2017), the addition of green coffee extract into soymilk significantly elevated ABTS radical scavenging ability (3.5-fold) and reducing power (13.8-fold). The study of interactions between polyphenolic compounds and β -conglycinin revealed that antioxidant capacity increased after the formation of the protein-phenolic complex (Zhao, et al., 2018; Murray, 2002). The authors also demonstrated the increase of antioxidant capacity of the protein-phenolic mixture after heating at 90 °C for 30 min. Thus, we suppose a formation of new products during the preparation of enriched process cheese with enhanced antioxidant activity towards ABTS^{•+}. Both TPC and TFC values had the greatest influence on reducing power. Strong positive associations have been found for RP vs. TPC ($r = 0.97$, $p < 0.001$) and RP vs. TFC content ($r = 0.944$, $p < 0.001$).

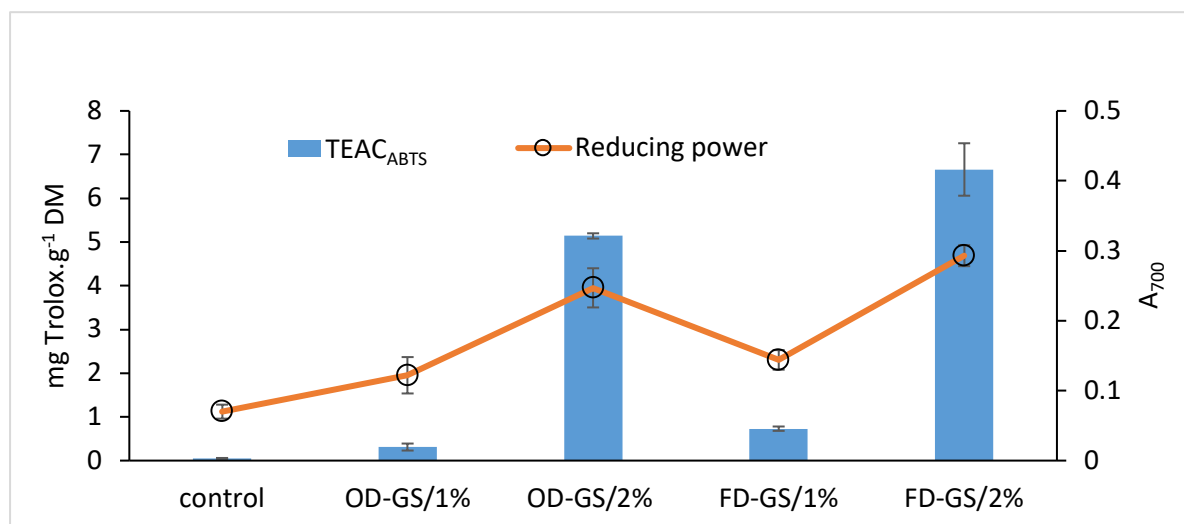


Figure 4 Trolox equivalent antioxidant capacity using ABTS (TEAC_{ABTS}, left y-axis) and reducing power (right y-axis) of processed cheese spread supplemented with oven-dried (OD) and freeze-dried (FD) grape skin (GS) powder at 1% and 2% (w/w) levels.

Rutin, (+)-catechin and (-)-epicatechin also showed strong positive correlations with reducing power ($p < 0.001$) of processed cheese enriched with GS powder, i.e. $r = 0.859$; 0.842 and 0.917 , respectively.

Two-factor Kruskal-Wallis ANOVA was applied to study the effect of grape skin powder levels and type of drying used for their preparation (oven-dried vs. freeze-dried). As can be seen from Table 3, the drying technique has a significant effect on the TPC values, all the phenolic individuals, and reducing power ($p < 0.01$) in processed cheese spreads where the addition of freeze-dried GS powder assured their higher values. Total flavonoids and TEAC_{ABTS} values were not influenced by the drying technique. The effect of GS powder level was found to be significant for all parameters except for (-)-epicatechin content. As expected, the addition of GS powder at a higher level was reflected in higher values of TPC and TFC values, rutin and (+)-catechin contents, and antioxidant capacities in terms of TEAC_{ABTS} and reducing power.

Table 3 The effect of drying technique (Factor A) for preparation of grape skin powder and its amount (Factor B) added to preprocessed cheese spread using two-factor Kruskal-Wallis ANOVA procedure.

	Factor A	Factor B
Total phenolics	$p < 0.05$	$p < 0.001$
Total flavonoids	$p < 0.05$	$p < 0.01$
(+)-catechin	$p < 0.01$	$p < 0.001$
(-)-epicatechin	$p < 0.01$	$p < 0.05$
Rutin	$p < 0.001$	$p < 0.01$
TEAC _{ABTS}	$p < 0.05$	$p < 0.001$
Reducing power	$p < 0.01$	$p < 0.001$

Note: TEAC_{ABTS}, Trolox equivalent antioxidant capacity using 2,2'-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid.

CONCLUSION

Grape skin powder, a waste product from wine production, can be used as an ingredient for the production of processed cheese with enhanced properties. Grape skin powder samples have a high content of protein ($150.5 - 157.5 \text{ g.kg}^{-1}$) and total dietary fibre ($228.8 - 231.7 \text{ g.kg}^{-1}$). Freeze-dried grape skin powder possessed higher total phenolic content and the ability to scavenge ABTS^{•+}, but lower total flavonoid content as well as the levels of rutin, (+)-catechin, and (-)-epicatechin. The incorporation of grape skin powder at 2 % (w/w) levels into the processed cheese significantly increased the protein content to 128.4 and 129.7 g.kg^{-1} for freeze-dried and oven-dried grape skin powders, respectively. Addition of freeze-dried grape skin powder into processed cheese was beneficial to antioxidant capacity in terms of reducing power. Higher contents of rutin, (+)-catechin and (-)-epicatechin, as well as total phenolic content, were achieved through the incorporation of freeze-dried GS powder, as was determined using two-factor ANOVA procedure. Based on the chemical analysis, we may conclude that using freeze-dried grape skin powder for the valorisation of processed cheese spread is the better choice in comparison with oven-dried grape skin powder. The hypothesis of this research was confirmed.

REFERENCES

- Almajano, M. P., Delgado, M. E., Gordon, M. H. 2007. Changes in the antioxidant properties of protein solutions in the presence of epigallocatechin gallate. *Food Chemistry*, vol. 101, no. 1, p. 126-130. <https://doi.org/10.1016/j.foodchem.2006.01.009>
- Alenisan, M. A., Alqattan, H. H., Tolbah, L. S., Shori, A. B., 2017. Antioxidant properties of dairy products fortified with natural additives: A review. *Journal of the Association of Arab Universities for Basic and Applied Sciences*, vol. 24, no. 1, p. 101-106. <https://doi.org/10.1016/j.jaubas.2017.05.001>
- Atmaca, G. 2004. Antioxidant effects of sulfur-containing amino acids. *Yonsei Medical Journal*, vol. 45, no. 5, p. 776-788. <https://doi.org/10.3349/ymj.2004.45.5.776>
- Černíková, M., Salek, R. N., Kozáčková, D., Buňka, F. 2018. The effect of different agitations and temperature maintainings on viscoelastic properties of full-fat processed cheese spreads. *LWT - Food Science and Technology*, vol. 89, p. 244-247. <https://doi.org/10.1016/j.lwt.2017.10.054>
- Costa, C., Lucera, A., Marinelli, V., Del Nobile, M. A., Conte, A. 2018. Influence of different by-products addition on sensory and physicochemical aspects of Primosale cheese. *Journal of Food Science and Technology*, vol. 55, no. 10, p. 4174-4183. <https://doi.org/10.1007/s13197-018-3347-z>
- Červenka, L., Hájek, T., Salek, R. N., Černíková, M., Velichová, H., Buňka, F. 2018. Addition of rutin/queretin mixture to spreadable processed cheese: antioxidant and textural characteristics. *Potravinárstvo Slovak Journal of Food Sciences*, vol. 12, no. 1, p. 129-134. <https://doi.org/10.5219/872>
- Denni, M., Mammen, D. 2012. A critical evaluation on the reliability of two aluminum chloride chelation methods for quantification of flavonoids. *Food Chemistry*, vol. 135, no. 3, p. 1365-1368. <https://doi.org/10.1016/j.foodchem.2012.05.109>
- Egger, L., Ménard, O., 2017. Update on bioactive peptides after milk and cheese digestion. *Current Opinion in Food Science*, vol. 14, p. 116-121. <https://doi.org/10.1016/j.cofs.2017.03.003>
- Fuleki, T., Ricardo-da-Silva, J. M. 2003. Effects of cultivar and processing method on the contents of catechins and procyanidins in grape juice. *Journal of Agricultural and Food Chemistry*, vol. 51, no. 3, p. 640-646. <https://doi.org/10.1021/jf020689m>
- Genova, G., Tosetti, R., Tonutti, P. 2016. Berry ripening, pre-processing and thermal treatments affect the phenolic composition and antioxidant capacity of grape (*Vitis vinifera L.*) juice. *Journal of the Science of Food and Agriculture*, vol. 96, no. 2, p. 664-671. <https://doi.org/10.1002/jsfa.7138>
- Henning, D. R., Baer, R. J., Hassan, A. N., Dave, R. 2006. Major advances in concentrated and dry milk products, cheese, and milk fat-based spreads. *Journal of Dairy Science*, vol. 89, no. 4, p. 1179-1188. [https://doi.org/10.3168/jds.S0022-0302\(06\)72187-7](https://doi.org/10.3168/jds.S0022-0302(06)72187-7)
- Horn, P. S. 1983. Some easy t statistics. *Journal of the American Statistical Association*, vol. 78, no. 384, p. 930-936. <https://doi.org/10.1080/01621459.1983.10477042>
- Horwitz, W. 2000. *Official methods of analysis of AOAC International*. 17th ed. Gaithersburg, Maryland, USA: AOAC International, 2200 p. ISBN-13 978-093558467-7.
- Kamiloglu, S., Capanoglu, E. 2015. Polyphenol content in figs (*Ficus carica L.*): Effect of sun-drying. *International Journal of Food Properties*, vol. 18, no. 3, p. 521-535. <https://doi.org/10.1080/10942912.2013.833522>
- Kamiloglu, S., Toydemir, G., Boyacioglu, D., Beekwilder, J., Hall R. D., Capanoglu, E. 2016. A review on the effect of

- drying on antioxidant potential of fruits and vegetables. *Critical Reviews in Food Science and Nutrition*, vol. 56, no. suppl. 1, p. S110-S129. <https://doi.org/10.1080/10408398.2015.1045969>
- Karnopp, A. R., Oliveira, K. G., de Andrade, E. F., Postinger, B. M., Granato, D. 2017. Optimization of an organic yogurt based on sensorial, nutritional, and functional perspectives. *Food Chemistry*, vol. 233, p. 401-411. <https://doi.org/10.1016/j.foodchem.2017.04.112>
- Khalifa, S. A., Omar, A. A., Mohamed, A. H. 2017. The effect of substituting milk fat by peanut oil on the quality of white soft cheese. *International Journal of Dairy Science*, vol. 12, no. 1, p. 28-40. <https://doi.org/10.3923/ijds.2017.28.40>
- Khan, I. T., Nadeem, M., Imran, M., Ajmal, M., Ali, S. 2018. Antioxidant activity, fatty acid characterization and oxidative stability of Gouda cheese fortified with mango (*Mangifera indica* L.) kernel fat. *Journal of Food Science and Technology*, vol. 55, no. 3, p. 992-1002. <https://doi.org/10.1007/s13197-017-3012-y>
- Khan, I. T., Nadeem, M., Imran, M., Ullah, R., Ajmal M., Jaspal, M. H. 2019. Antioxidant properties of Milk and dairy products: a comprehensive review of the current knowledge. *Lipids in Health and Disease*, vol. 18, no. 41, p. 1-13. <https://doi.org/10.1186/s12944-019-0969-8>
- Lingua, M. S., Fabani, M. P., Wunderlin, D. A., Baroni, M. V. 2016. From grape to wine: Changes in phenolic composition and its influence on antioxidant activity. *Food Chemistry*, vol. 208, p. 228-238. <https://doi.org/10.1016/j.foodchem.2016.04.009>
- Marinho, M. T., Bersot, L. S., Nogueira, A., Colman, T. A. D., Schnitzler, E. 2015. Antioxidant effect of dehydrated rosemary leaves in ripened semi-hard cheese: A study using coupled TG-DSC-FTIR (EGA). *LWT - Food Science and Technology*, vol. 63, no. 2, p. 1023-1028. <https://doi.org/10.1016/j.lwt.2015.03.108>
- Mehanna, N. S., Hassan, F. A. M., El-Messery, T. M., Mohamed, A. G. 2017. Production of functional processed cheese by using tomato juice. *International Journal of Dairy Science*, vol. 12, no. 2, p. 155-160. <http://doi.org/10.3923/ijds.2017.155.160>
- Mohamed, A. G., Shalaby, S. M. 2016. Texture, chemical properties and sensory evaluation of a spreadable processed cheese analogue made with apricot pulp (*Prunus armeniaca* L.). *International Journal of Dairy Science*, vol. 11, no. 2, p. 61-68. <http://doi.org/10.3923/ijds.2016.61.68>
- Mohamed, A. G., Shalaby, S. M., Gafour, W. A. 2016. Quality characteristics and acceptability of an analogue processed spreadable cheese made with carrot paste (*Daucus carota* L.). *International Journal of Dairy Science*, vol. 11, no. 3, p. 91-99. <http://doi.org/10.3923/ijds.2016.91.99>
- Murray, B. S. 2002. Interfacial rheology of food emulsifiers and proteins. *Current Opinion in Colloid & Interface Science*, vol. 7, no. 5-6, p. 426-431. [http://doi.org/10.1016/S1359-0294\(02\)00077-8](http://doi.org/10.1016/S1359-0294(02)00077-8)
- Nunes, J. C., Lago, M. G., Castelo-Branco, V. N., Oliveira, F. R., Torres, A. G., Perrone, D., Monteiro, M. 2016. Effect of drying method on volatile compounds, phenolic profile and antioxidant capacity of guava powders. *Food Chemistry*, vol. 197, Part A, p. 881-890. <https://doi.org/10.1016/j.foodchem.2015.11.050>
- Pavithra, K., Vadivukkarasi, S. 2015. Evaluation of free radical scavenging activity of various leaf extracts from *Kedrostis foetidissima* (Jacq.) Cogn. *Food Science and Human Wellness*, vol. 4, no. 1, p. 42-46. <https://doi.org/10.1016/j.fshw.2015.02.001>
- Příkryl, J., Hájek, T., Švecová, B., Salek, R. N., Černíková, M., Červenka, L., Buňka, F. 2018. Antioxidant properties and textural characteristics of processed cheese spreads enriched with rutin or quercetin: The effect of processing conditions. *LWT - Food Science and Technology*, vol. 87, p. 266-271. <https://doi.org/10.1016/j.lwt.2017.08.093>
- Rashidinejad, A., Birch, E. J., Sun-Waterhouse, D., Everett, D. W. 2015. Total phenolic content and antioxidant properties of hard low-fat cheese fortified with catechin as affected by in vitro gastrointestinal digestion. *LWT - Food Science and Technology*, vol. 62, no. 1, Part 2, p. 393-399. <https://doi.org/10.1016/j.lwt.2014.12.058>
- Śęczyk, Ł., Świeca, M., Gawlik-Dziki, U. 2017. Soymilk enriched with green coffee phenolics - Antioxidant and nutritional properties in the light of phenolics-food matrix interactions. *Food Chemistry*, vol. 223, p. 1-7. <https://doi.org/10.1016/j.foodchem.2016.12.020>
- Šporin, M., Avbelj, M., Kovač, B., Možina, S. S. 2018. Quality characteristics of wheat flour dough and bread containing grape pomace flour. *Food Science and Technology International*, vol. 24, no. 3, p. 251-263. <https://doi.org/10.1177/1082013217745398>
- Teixeira, A., Baenas, N., Dominguez-Perles, R., Barros, A., Rosa, E., Moreno, D. A., Garcia-Viguera, C. 2014. Natural bioactive compounds from winery by-products as health promoters: A review. *International Journal of Molecular Sciences*, vol. 15, no. 9, p. 15638-15678. <https://doi.org/10.3390/ijms150915638>
- Torri, L., Piochi, M., Marchiani, R., Zeppa, G., Dinnella C., Monteleone, E. 2016. A sensory- and consumer-based approach to optimize cheese enrichment with grape skin powders. *Journal of Dairy Science*, vol. 99, no. 1, p. 194-204. <https://doi.org/10.3168/jds.2015-9922>
- Usta, B., Yilmaz-Ersan, L. 2013. Antioxidant enzymes of milk and their biological effects. *Journal of Agricultural Faculty of Uludag University*, vol. 27, no. 2, p. 123-130.
- Zhao, M., Jiao, M., Zhou, F. B., Lin, L. Z., Sun, W. Z. 2018. Interaction of beta-conglycinin with catechin-impact on physical and oxidative stability of safflower oil-in-water emulsion. *Food Chemistry*, vol. 268, p. 315-323. <https://doi.org/10.1016/j.foodchem.2018.06.108>

Acknowledgments:

This research was funded by a project from the Faculty of Chemical Technology, University of Pardubice, no. SGS_2019_003.

Contact address:

*Michaela Frühbauerová, University of Pardubice, Faculty of Chemical Technology, Department of Analytical Chemistry, Studentská 573, 532 10 Pardubice, Czech Republic, Tel.: +420466037718, E-mail: michaela.fruhbauerova@student.upce.cz ORCID: <https://orcid.org/0000-0003-1286-7341>

Libor Červenka, University of Pardubice, Faculty of Chemical Technology, Department of Analytical Chemistry, Studentská 573, 532 10 Pardubice, Czech Republic, Tel.: +420466037718, E-mail: libor.cervenka@upce.cz, ORCID: <https://orcid.org/0000-0003-2316-8765>

Tomáš Hájek, University of Pardubice, Faculty of Chemical Technology, Department of Analytical Chemistry, Studentská 573, 532 10 Pardubice, Czech Republic, Tel.: +420466037038, E-mail: tomas.hajek@upce.cz, ORCID: <https://orcid.org/0000-0002-8791-6163>

Richardos Nikolaos Salek, Tomáš Baťa University in Zlín, Faculty of Technology, Department of Food Analysis and Chemistry, nám. T. G. Masaryka 5555, 760 01 Zlín, Czech Republic, Tel.: +420576038087,

E-mail: rsalek@utb.cz,

ORCID: <https://orcid.org/0000-0001-5129-5329>

Helena Velichová, Tomáš Baťa University in Zlín, Faculty of Technology, Department of Food Analysis and Chemistry, nám. T. G. Masaryka 5555, 760 01 Zlín, Czech Republic and College of Business and Hotel Management, Bosonožská 9, 625 00 Brno, Czech Republic, Tel.: +420576031542,

E-mail: velichova@utb.cz,

ORCID: <https://orcid.org/0000-0002-8585-9126>

František Buňka, Tomáš Baťa University in Zlín, Faculty of Technology, Department of Food Analysis and Chemistry, nám. T. G. Masaryka 5555, 760 01 Zlín, Czech Republic, Tel.: +420576033011,

E-mail: bunka@utb.cz,

ORCID: <https://orcid.org/0000-0002-1960-8505>

Corresponding author: *