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## Impact of pumpkin seed oil and coffee treatment on the characteristics of semi-hard cheese

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### ABSTRACT

Semi-hard cheese was made from fresh domestic cow milk and treated with pumpkin seed oil and coffee during its ageing. The energy value and composition of the cheese were examined by determining the content of dry matter, minerals, proteins, fat, caffeine and fatty acids. During the ageing period, the cheese was dipped into a sweet, freshly-prepared, high-quality coffee drink and coated with a mixture of milled coffee grains and cold pressed pumpkin seed oil to improve the sensory properties of the cheese, but also to improve its nutritional value. It is considered that treated cheese could have greater nutritional and pro-health properties than untreated cheese, due to nutritional and healing properties of pumpkin seed oil and coffee used during the maturation period. Pumpkin seed oil contains carbohydrates, minerals, proteins, and important unsaturated fatty acids, while coffee is rich in antioxidants and helps in the prevention of type 2 diabetes, Parkinson's and Alzheimer's disease, and high cholesterol. To determine the impact of the treatment, the fatty acid and caffeine contents were determined by the GC-FID and the UPLC-DAD method. The cheese treated with pumpkin seed oil and coffee had a higher amount of unsaturated fatty acids (UFA) and a lower amount of saturated fatty acids (SFA) than the untreated cheese. The proportions of long-chain UFA, such as the C18:2n6c (Omega 6) and the C18:3n3 (Omega 3), were higher in the treated cheese than those in the untreated cheese, as well as the C20:1, the C22:2 and the C24:1, which were not detected in the untreated cheese. Caffeine concentration in the treated cheese was 33.08 mg/L.

## Introduction

High fat and protein content in cheese makes it an energy-rich and nutritious dairy food with a long history in the human diet. In particular, it is a rich source of proteins, fat, vitamins, and minerals. In addition to their nutritional role, cheese components have also shown to exert important health benefits (López-Expósito et al., 2017). Some fatty acids present in cheese, such as conjugated linoleic acid (CLA), have shown to display anticarcinogenic and antiatherogenic properties (Lee et al., 2005; Battacharaya et al., 2006). CLA refers to a group of polyunsaturated fatty acids that exist as positional and stereo isomers of conjugated dienoic

octadecadienoate (C18:2). The fatty acid profile and the CLA content of cheese are influenced by several factors, such as farming practices, genetic and physiological aspects related to the animals (Collomb et al., 2006), and cheesemaking technology (heat treatment of milk and/or curd, starter cultures, ripening). Therefore, recent studies have focused on increasing the CLA content of cheese by supplementing the diet of the animals with vegetable oils rich in polyunsaturated fatty acids and/or by adding CLA dairy starter producers during cheese manufacture (López-Expósito et al., 2017). The implementation of oils rich in polyunsaturated fatty acids in the cheesemaking process could also present great potential for increasing the CLA level in cheese.

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Pumpkin seed oil contains around 98% of fatty acids, approximately 70% of which are unsaturated fatty acids. The main fatty acids in pumpkin seed oil are linoleic (18:2; 35.2–60.8%), oleic (18:1; 21.0–46.9%), stearic (18:0; 3.1–7.4%), and palmitic acid (16:0; 9.5–14.5%) (Murkovic and Pfannhauser, 2000). Also, some traces of other highly unsaturated acids, like linolenic acid (18:3; 0.2%) and nervonic acid (24:1; 0.3%), have been found (Cert et al., 2000). The remaining 2% consist of a number of hydrocarbons, fatty alcohols, carotenoids, pigments, tocopherols and tocotrienols, phytosterols, phenolic compounds, and minor glyceridic compounds (Ryan et al., 2007). Some reports stated the potential of pumpkin seed oil in the prevention of hyperplasia of the prostate, making it useful in the management of benign prostatic hyperplasia (Gossell-Williams et al., 2006). This oil has been related to the inhibition of the progression of hypertension and arthritis, as well as to reducing the levels of breast, gastric, colorectal, and lung cancer (Stevenson et al., 2007).

Coffee is a complex beverage containing more than 1000 compounds responsible for its pleasant flavour and aroma. Methylxanthines (caffeine, theobromine, theophylline) are among the many bioactive compounds present in coffee. Caffeine concentration varies among different food products, with coffee having the highest concentration in general, compared to tea, soft drinks, energy drinks, shot drinks, and solid foods (de Mejia and Ramirez-Mares, 2014). It is considered that habitual coffee consumption has several health benefits (Cano-Marquina et al., 2013), including lower risks of Parkinson's and Alzheimer's disease, a favourable effect on liver function, a possible role in weight loss (increased metabolic rate, energy expenditure, lipid oxidation, and lipolytic and thermogenic activities), and a decreased risk of developing certain cancers (endometrial, prostatic, colorectal, liver) (O'Keefe et al., 2013).

The objective of this research was to determine and compare specific characteristics of cheese treated with coffee and pumpkin seed oil and untreated cheese, their microbiological stability, as well as the composition and content of fatty acids and caffeine content.

## Materials and methods

### Cheese

The cheeses used for the analysis were produced from fresh domestic cow milk, which was first pasteurized and then inoculated with the freeze-dried lactic starter culture Choozit™ MTD 41 LYO (4-8 DCU/100 L of vat milk) composed from *Lactococcus lactis subsp. lactis*, *Lactococcus lactis subsp. cremoris*, *Lactococcus lactis subsp. lactis biovar. diacetylactis*, and *Streptococcus thermophilus*. Marzyme® 150 MG (2600 - 3300

IMCU/100 L of milk), a protease of the *Rhizomucor miehei* origin, was used as a microbial coagulant. The cheeses were pressed in moulds at the pressure 170 kN/m<sup>2</sup> to 200 kN/m<sup>2</sup>, then dry-salted and placed into the cheese cave for 5 weeks of ageing (temperature 14 - 12 °C, relative humidity: 85 - 80%). During the ageing period, the cheeses were turned daily, and half of them were dipped into a sweet, freshly-prepared, high-quality coffee drink and coated with a mixture of milled coffee grains and cold pressed pumpkin seed oil once a week. After the 5-week ageing period, the cheeses were cold stored in vacuumed plastic bags and placed in a refrigerator at 6 °C, until the determination of their chemical characteristics, microbiological stability, as well as the composition and content of fatty acids and caffeine. The coffee and pumpkin seed oil coat was manually removed from the cheese surface immediately before analysis. Untreated cheese was used as a control.

### Pumpkin seed oil

Pumpkin seed oil was used for cheese treatment during ageing. The pumpkin seed oil was provided by a small family oil mill plant that is producing cold pressed oil from ecological pumpkin seeds. The energy value of 100 mL of the used pumpkin seed oil is 3406 kJ (829 kcal) and it contains 92 g of fat (15 g corresponds to saturated fatty acids, SFA). The used seed oil did not contain carbohydrates, proteins, or salt.

### Coffee grains

Pascucci extra bar mild coffee (80% Arabica 20% Robusta blend) with 1.80% of caffeine was used for the coffee treatment of cheese during ageing. It is an intense, sweet coffee, with a clean and fresh taste, rich in aromatic nuances, full-bodied and creamy, composed of natural and washed coffee.

## Methods

### Determination of cheese characteristics

Both cheeses were analysed to determine their mineral, protein, fat, dry matter, and salt content. All the methods are approved by the International Organization for Standardization (ISO) and listed in Table 1.

### Microbiological analysis

The microbiological analysis was conducted in accordance with the Croatian Guide for the Microbiological Criteria for Food. Certified methods for determining the control microorganisms in cheese are listed in Table 2.

**Table 1.** Standard methods for cheese determination

Content in cheese	Determination method	
mineral	<b>AOAC 935.42</b>	Official Ash of Cheese Gravimetric Method
protein	<b>ISO 1871:2009</b>	The reference method for the determination of nitrogen by the Kjeldahl method
fat	<b>ISO 1735:2004</b>	The reference gravimetric method for the determination of fat content
dry matter	<b>ISO 5534:2004</b>	The reference method for the determination of the total solids content of cheese and processed cheese
salt	<b>ISO 5943:2006</b>	The potentiometric titration method for the determination of the chloride content of cheese and processed cheese products

**Table 2.** Methods for the detection and enumeration of microorganisms in tested cheese

Tested microorganism	Method	
<i>Salmonella spp.</i>	<b>EN ISO 6579-1</b>	Horizontal method for the detection, enumeration, and serotyping of <i>Salmonella</i>
<i>Escherichia coli</i>	<b>EN ISO 16649-2</b>	Horizontal method for the enumeration of beta-glucuronidase-positive <i>Escherichia coli</i>
<b>Coagulase-positive <i>Staphylococci</i> /<i>Staphylococcus aureus</i></b>	<b>EN ISO 6888-1</b>	Horizontal method for the enumeration of coagulase-positive staphylococci ( <i>Staphylococcus aureus</i> and other species)
<b>Sulphite-reducing clostridia</b>	<b>EN ISO 15213</b>	Horizontal method for the enumeration of sulphite-reducing bacteria growing under anaerobic conditions
<i>Listeria monocytogenes</i>	<b>EN ISO 11290-1</b>	Horizontal method for the detection and enumeration of <i>Listeria monocytogenes</i> and of <i>Listeria spp.</i>

#### Determination of fatty acids content

The fatty acids content was determined by Gas Chromatography on the Zebron TM ZB-WAX. GC Cap. column 30 m x 0.25 mm x 0.25 µm, with a Polyethylene Glycol (PEG) phase and the 3 mL/min Constant Flow Helium. The injection temperature was 260 °C, and the FID detection temperature was 250 °C (Phenomenex GC Application. ID No.: 16320). For that purpose, the fatty acids were transformed into volatile fatty acid methyl esters with a potassium methoxide solution. The stock solution Sigma-Aldrich Supelco 37 Component FAME Mix was used as the standard for fatty acid methyl esters.

#### Determination of caffeine content

The caffeine content was determined according to DIN ISO 20481, by Ultra Performance Liquid Chromatography (UPLC, Agilent Infinity 1290 II LC System) with a diode-array detector (DAD) on the Agilent ZORBAX Eclipse Plus, 4.6 x 150 mm, 5 µm (p/n 959993-902), with acetonitrile and 1% formic acid solvent, and 1mL/min flow under the 350 bar pressure limit. All the chemicals were purchased from Sigma/Aldrich, Germany.

## Results and discussion

#### Cheese characteristics

The results of the basic examination of dry matter, fat, protein, mineral, carbohydrates, and salt content, and the energy values are listed in Table 3. Based on the given results, both the treated and the untreated cheese have medium water content (34 – 45%) and according to *Codex Alimentarius* should be classified as hard cheeses, but due to the specific production and maturing conditions, we classified them as semi-hard cheeses. Also, both cheeses can be classified as medium fat, because of the fat content that is higher than 25%. The results of the analysis showed that more than 68% of fat contained in the examined cheese corresponds to saturated fatty acids (SFA), and about 32% to unsaturated fatty acids (UFA). Regarding the similar fat content, both cheeses have similar energy values and protein content. The average salt concentration at the end of ripening was around 1.5%, which was much lower than that described for the majority of cow milk cheeses.

*Microbiological analysis*

Concentrations of all targeted microorganisms are in accordance with the Croatian Guide for Microbiological Criteria for Food, so based on the results given in Table 4, both cheeses are microbiologically stable.

*Fatty acids*

The analysis of fatty acids showed that the treated cheese has a higher amount of unsaturated fatty acids (UFA; mono-, and polyunsaturated fatty acids) and a lower amount of saturated fatty acids than the untreated cheese (Table 5). It is assumed that the higher amount of UFA in the treated cheese comes from the pumpkin seed oil treatment during ageing, so the GC chromatography of fatty acid methyl esters was performed to determine the exact proportion of each fatty acid. The results of the GC chromatography are shown as Figure 1.

The proportions of long-chain UFA, such as the **C18:2n6c** (Omega 6; *linoleic acid methyl ester*) and the **C18:3n3** (Omega 3; *linolenic acid methyl ester*) were higher in the treated cheese than those in the untreated cheese, as well as the **C20:1** (*cis-11-eicosenoic acid methyl ester*), the **C22:2** (*cis-13,16-docosadienoic acid methyl ester*), and the **C24:1** (*nervonic acid methyl ester*), which were not even detected in the untreated cheese. The **C22:1** (*erucic acid methyl ester*) was detected in the untreated cheese in the amount of 0.16%, and none was detected in the treated cheese. There is no significant difference in the SFA content between the treated and the untreated cheese, except for the **C16:0** (*methyl palmitate*) that is lower, and the **C18:0** (*methyl stearate*) which is higher in the treated than in the untreated cheese. The higher amount of the linoleic acid methyl ester, the linolenic acid methyl ester, and the nervonic acid methyl ester corresponds to the pumpkin seed oil fatty acid content that belongs to the oleic-linoleic type of oil (Rabrenović et al., 2014).

**Table 3.** Cheese properties

Specific characteristic	Untreated cheese	Treated cheese
Water (%)	41.29	41.47
Fat (%)	26.39	26.12
SFA (%)	18.06	17.77
SFA (w.m.)	0.6844	0.6805
Protein (%)	23.66	23
Minerals (%)	4.09	3.98
Carbohydrates (%)	4.57	5.43
ABS carbohydrates (%)	4.57	5.43
Sugar (%)	4.57	5.43
Energy value (kcal/100 g)	351.9	348.8
Energy value (kJ/100 g)	1473.2	1460.1
Salt (%)	1.54	1.61

**Table 4.** Results of the microbiological analysis

Tested microorganism	Untreated cheese	Treated cheese
	CFU/g	CFU/g
<i>Salmonella spp.</i>	Not detected in 25 g	Not detected in 25 g
<i>Escherichia coli</i>	< 10 g	< 10 g
<i>Coagulase positive Staphylococci/ Staphylococcus aureus</i>	< 10 g	< 10 g
<i>Sulphite-reducing Clostridia</i>	< 10 g	< 10 g
<i>Listeria monocytogenes</i>	Not detected in 25 g	Not detected in 25 g

**Table 5.** Saturated and unsaturated fatty acid content

Fatty acid content	Untreated cheese	Treated cheese
SFA (%)	68.44	68.05
MUFA (%)	28.73	28.13
PUFA (%)	2.83	3.82

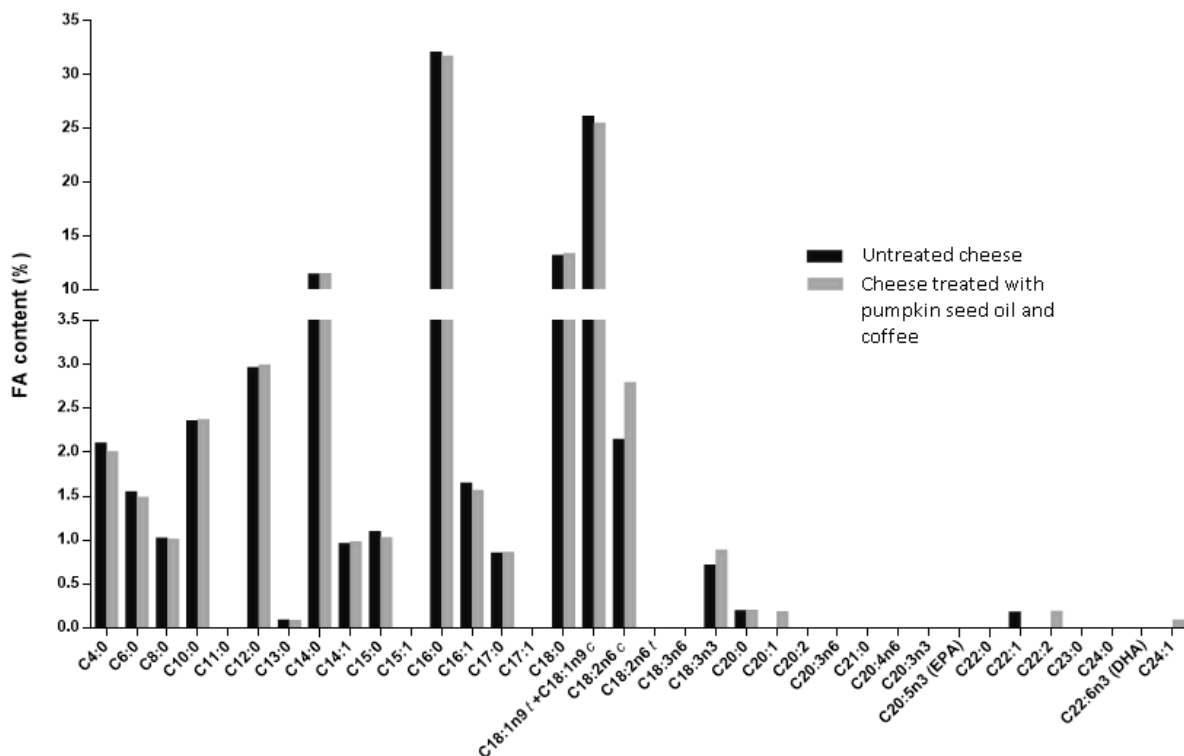


Fig. 1. GC chromatography results of fatty acids for the untreated and the treated cheese

### Caffeine

The treated cheese was tested for caffeine presence to determine the transfer of caffeine from freshly prepared coffee and milled coffee grains to cheese during its ageing. Ultra-performance liquid chromatography (UPLC) showed that the concentration of caffeine in treated cheese was 33.08 mg/L. The caffeine concentration varies among different food products, with coffee having the highest concentration in general. For example, the typical cup of percolated coffee (about 150 mL) contains approximately 85 mg of caffeine (567 mg/L) (Burdan, 2015). If we compare the caffeine content in the treated cheese with the caffeine content of selected beverages and foods, the given result is much lower than others (Mitchell et al., 2014), so the caffeine intake by consuming treated cheese versus coffee is negligible.

### Conclusion

The specific production and maturing process of cheese, during which cheese was treated with pumpkin seed oil and coffee, influenced only the proportion and the composition of fatty acids, and the caffeine content in cheese. While pumpkin seed oil is rich in linolenic acid (C18:2) and stearic acid (C18:0) (it has the linolenic acid content of at least 47%, and the stearic

acid content of at least 8%), and due to the higher level of the C18:0, the C18:2n6c (Omega 6), and the C18:3n3 (Omega 3) in treated cheese, it can be concluded that the treatment with pumpkin seed oil during cheese ripening had a positive impact on the fatty acid content in ripened cheese. Even though the treated cheese contains 33.08 mg/L of caffeine, it is assumed that the treatment with coffee and milled coffee grains had no significant impact on the caffeine content in the treated cheese, but could have had a significant influence on its sensory properties.

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