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BIO-FUNCTIONALITY OF BRINED CHEESES: COMPARISON BETWEEN CURD COOKED AND UNCOOKED TYPES

SUMMARY

The cooking temperature of cheese affects the degree and pattern of proteolysis due to the residual activity of chymosin, leading to variations between different cheese varieties. The main objective of this research is to investigate the chemical and biochemical changes in brined cheeses produced with and without cooking, in order to control and enhance cheese quality. Brined cheeses made with ewe's milk combined with different technologies of curd treatment (cooked-C vs uncooked -N), were studied for their total free amino acid (TFAA) content, water-soluble nitrogen (WSN), 12% trichloroacetic acid-soluble nitrogen (TCA-SN) and angiotensin-converting enzyme-inhibition activity (ACE-i). (N) cheeses had the highest pH, fat, and salt value than (C) cheese. Also, the higher TFAA mg Leu/kg, DPPH (2,2-diphenyl-1-picrylhydrazyl), and ABTS (2,2-azinobis, 3-ethylenebenzothiazoline-6-sulfonic acid) were conducted in the uncooked cheese. Cooked curd cheese was the most active with an ACE-i activity of 93.1%. On the contrary, the same cheese showed the lowest antioxidant activity (88.1%). The RP-HPLC profiles show that the formation rate of smaller peptides was increased in cheese made without heat treatment than in heat treated one probably due to the heat deactivation process of proteolytic enzymes. Maillard reaction during cooked (C) cheeses has implicated that volatile formation is caused through reactions in higher temperature treatment.

Keywords: Cooked cheese, proteolysis, enzyme-inhibition activity (ACE-i), peptide profile, volatiles

INTRODUCTION

The city of Tetovo lies in the Polog region, North Macedonia, at the base of the Sharr Mountain on a surface of 1,080 km^2 at an altitude of 460-500 m, in the course of the river Pena. Polog's lowland lies in the southeast-northwest direction, at a distance of 55 km and a width of 8-10 km. It has a semi-continental climate, with warm and relatively humid summers, cold and snowy winters, and autumn falls are frequent rainfall. Since it is surrounded by mountains on all sides and due to the appearance of hot and cold air streams, in the Polog area comes the

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emergence of the inversion process. Natural conditions, such as climate, topography, and geological composition, have allowed numerous springs of running water to emerge on the outskirts of Tetovo. This makes Tetovo one of the few towns in Macedonia with access to drinking water, as well as water for industrial and irrigation purposes (Abdii and Xhulaj, 2016). In the Tetovo district, the sheep population is approximately 20035, which includes 17251 Sharplaninka, 1,224 Ovčepolka, 201 Karakaçnanska, 463 Witendberg, 6 Crnagllava, and 6 Avasi, among others. The goat population consists of 1,695 total goats, including 327 Alpina, 75 Saanen, 660 Balkan, 218 Alpine hybrids, 13 hybrids of Saanen species, and 390 others. The local dairy community comprises 30 cheese producers from the Sharr Mountain, Dry Mountain, and Zheden Mountain regions, with the grazing and milking season beginning between June 1st and June 10th (SSO, 2015-2020).White cheese is a brined variety of cheese with either soft or semihard texture, white colored and with close texture, pickled flavour commonly made from cow milk, sheep milk and ripened in brine for 60 days. Traditionally, has been produced by farmers using raw milk and traditional techniques using only elementary equipment. According to North Macedonian Regulation, there are following groups of cheeses: cheeses with ripening, cheeses with molds, cheeses in brine, cheeses with steamed dough and cheeses without ripening (Official Gazette of Republic of Macedonia, 2011). Cheeses in brine are cheeses in which ripening and storage until consumption takes place in brine. It is produced from raw milk and also pasteurized milk in industrial conditions in the Balkan region (Sulejmani and Selimi, 2022). The composition type of cheeses varies by many factors such as milk composition, pre-treatment and ripening requirements. Protein hydrolysis is considered to involve the polypeptide formation to enhance the biological value or functional properties, enhance flavor quality, and provide a way for new protein synthesis (Sulejmani and Hayaloglu, 2020). Pešić *et al.* (2014) conducted that treatment for 10 min at 90 ° C incorporates whey proteins (more than 95%) as complexes of all three caseins (κ-, b- and aS2). The presence of whey proteins significantly enhances the nutritional value of cheese due to their excellent amino acid composition. Traditional brined cheeses from North Macedonia are highly valued products known for their intense flavors. However, there is a lack of comprehensive data on comparative studies regarding the impact of cheese curd heat treatment on their bioactivity. This investigation aims to provide insights into the proteolysis status and volatile compounds present in brined cheese produced under various curd conditions. The quality of cheese can be greatly influenced by the heat treatment of the curd. Therefore, this study focuses on evaluating the technological processes involved in curd treatment for brined cheese production and how these processes affect the biofunctional properties of the cheese.

MATERIAL AND METHODS

The study was conducted in a local sheep farm in Tearce village (42°07`50.00"N, 21°05`32.20"E) located 12 km to the northeast of Tetovo with an altitude of 500 meters, northwestern part North Macedonia. The sheep graze in the field grass and polygonal plants of the meadows. Geographical region,

milking season and animal feed influence the variable quality of milk used to produce these traditional cheeses.

Figure 1. Map of the traditional brined cheese production area (arrows)

Fresh raw milk from 180 sheep was used during milking in the morning collecting 85 litres of milk. From 35 litres of milk, 7 kg of (N, uncooked) cheese and from 50 liters of milk, 12 kg of (C, cooked) cheese was produced (Fig 2). The dry matter, protein, salt, fat and total free amino acid (TFAA) content in the brined cheeses were determined according to Ardo and Polychroniadou (1999). The water-soluble nitrogen (WSN) and 12% trichloroacetic acid-soluble nitrogen (TCA-SN) as % of total nitrogen of the cheeses were determined by the methods as described by Kuchroo and Fox (1982). Total free amino acids (TFAAs) were determined on the water-soluble fraction of the cheeses by the cadmium-ninhydrin method as described in Folkertsma and Fox (1992). pH meter with combined electrode (Orion, USA) was used for determination of the pH of the cheeses. Reversed-phase high-performance liquid chromatography (RP-HPLC) and static solid-phase microextraction (SPME) method using a gas chromatography mass spectrometry (GC-MS) system (Shimadzu, Kyoto, Japan) were used for analysis of peptide profile and volatiles in cheeses, respectively (Sulejmani and Hayaloglu, 2018).

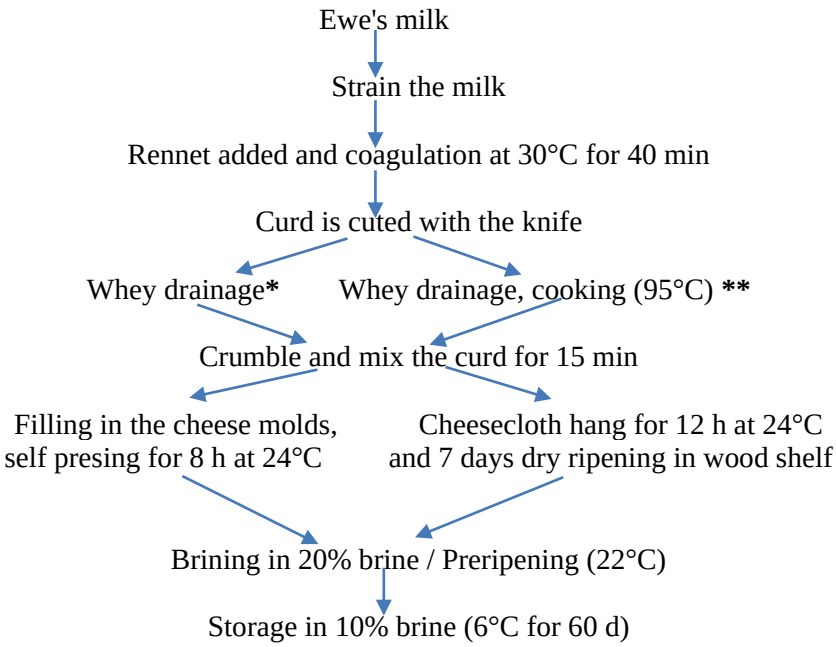


Figure 2. Technological process of production cooked (C)** and uncooked (N)* brined cheeses

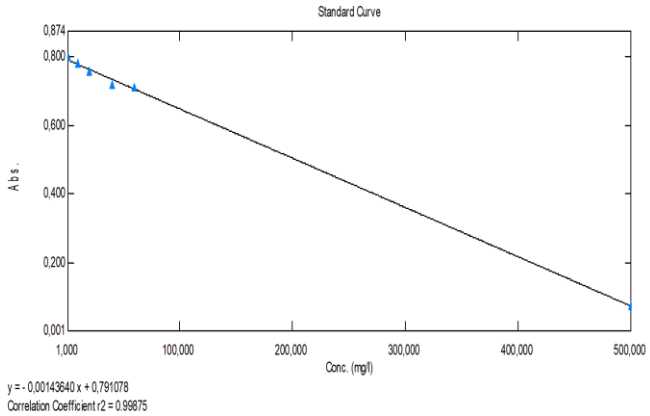
To evaluate DPPH free radical scavenging ability (RSA), ABTS•+ radical scavenging activity, and ACE inhibitory activity of salted cheeses, the methods of Brand-Williams *et al.* (1995), Re *et al.* (1999) and Sahingil *et al.* (2014) were used, respectively (Fig 3 a,b,c). Samples were chemically analysed in triplicate or more on the 1st week of ripening (fresh).

For the statistical analyses in this study, SPSS (SPSS Inc., USA) was used. All results are expressed as mean ± standard deviation. For statistical differences between cheeses (C) and (N) in the type of salted cheese, the independent T-test was used at the level of significance of differences ($P < 0.05$).

RESULTS AND DISCUSSION

The chemical compositions of the brined cheese samples are given in Table 1. The pH value affects the growth of microorganisms and the activity of enzymes. The higher pH value was in (N) cheese (5.16), than in (C) cheese (5.12) ($P<0.05$). The higher pH in (N) brined cheeses occurs due to the intensive proteolysis or proteins breakdown and formation of ammonia and metabolism of lactic acid (Sulejmani *et al.*, 2014). The protein of the (C) cheese was higher than uncooked (N) cheese ($P<0.05$). The content of protein in cheese depends on the amount of casein in raw milk and cheese production techniques. The results showed that the (N) cheeses have the lower dry matter and fat values compared to the cooked brined cheese ($P<0.05$). The salt content of the brined cheeses is similarly with the most varieties of brined cheese (Bintsis and Papademas, 2018).

a)



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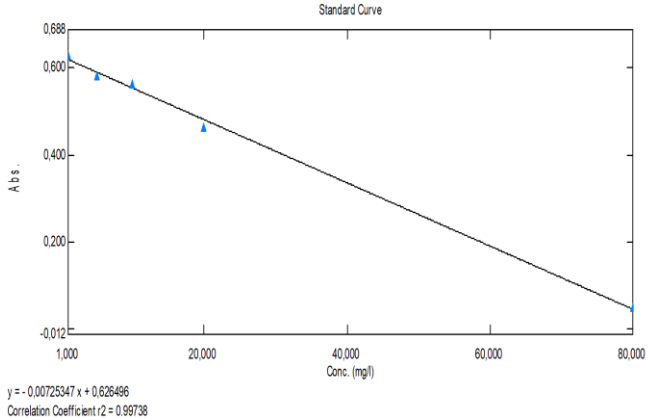

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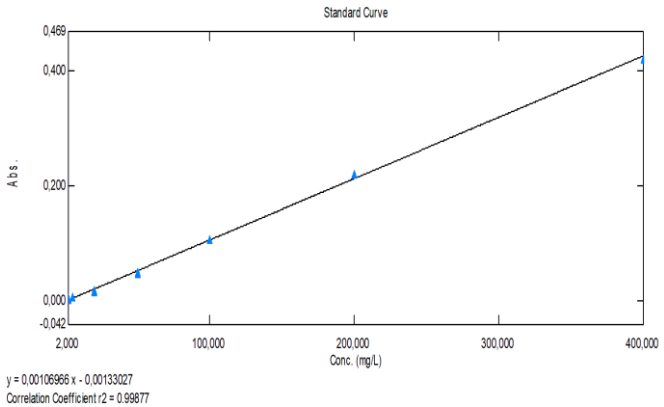

Figure 3. Calibration curve of the DPPH (a), ABTS (b) and TFAA (b) solutions

The dry matter of the brined cheeses are higher than that reported for fresh Turkish Beyaz cheese (Turkish Food Codex: 2015/6). The values for proteolysis respectively soluble nitrogen (WSN), expressed as % of TN, total free aminoacids, and peptide profile in brined cheeses made from cooked and

uncooked curd are shown in Table 1 and Figure 4. The action of milk and rennet proteases is expressed through indicators respectively parameters of soluble nitrogen compounds (SN) in cheese as an indicator of the extent of proteolysis (Kalit *et al.*, 2005).

Table 1. Physical-chemical and bioactivity properties of fresh brined cheeses

Parameters [a]	**(C)**	**(N)**	*ANOVA**
pH	5.12±0.02	5.16±0.01	*s*
Dry matter	46.52±0.20	45.00±0.32	*s*
Protein	22.84±0.21	16.16±0.33	*s*
Fat	22.25±0.22	26.75±0.34	*s*
Salt	1.76±0.23	2.44±0.35	*s*
WSN/TN (% of TN)	1.7±0.09	4.09±0.37	*s*
TCA/TN (% of TN)	0.72±0.05	0.53±0.31	*s*
TFAA mg Leu/kg	139.9±0.14	173.80±0.16	*s*
%ACE-i	93.05±0.02	82.75±0.05	*s*
DPPH	88.04±0.66	98.29±0.650	*s*
ABTS	17.09±0.50	18.31±0.49	*s*

[a] *Abbreviations: Values are means ± SD; C- cooked, N- uncooked. S-differ significantly (P<0.05). WSN, water-soluble nitrogen TCA;12% trichloroacetic acid-solublenitrogen; TFAA, total free amino acid; angiotensin converting enzyme-inhibition activity (ACE-i).DPPH (2,2-diphenyl-1-picrylhydrazyl), ABTS (2,2-azinobis, 3-ethylenebenzothiazoline-6-sulfonic acid)*

The highest soluble nitrogen (SN) was found in cheese produced without curd cooking. Also enzymes of non-starter cultures act by degrading high and medium molecular weight peptides (Sulejmani and Hayaloglu, 2017). Uncooked cheese had significantly higher antioxidant activity probably due to higher fat content and presence of vitamin E as antioxidant compounds (McSweeney and Sousa, 2000).

In the area of 12–28 min in the chromatogram of (N) cheeses were an increase in peaks of peptides. Whereas, between 32 and 36 min, the peak in (C) cheeses were higher than (N) cheeses. Corresponded peptides eluted on 40–64 min were noted to be higher in (N) cheeses. Changes in production processes (thermal treatment of coagulum), caused changes in the activity of the remaining coagulant and changed the extent of proteolysis. However, degradation of as1- and b-caseins were noticeable lower in (C) cheese than in (N) cheeses due to the high temperature used in the manufacturing of cooked cheeses that indicates lower retention of residual coagulant (Sulejmani and Hayaloglu *et al.*, 2016). At retention times < 15 min, small hydrophilic peptides were detectable in both cheeses. Large hydrophobic peptides seemed between 60–78 min were higher in the (N) cheese which may correlate with the cheese bitterness. The pH also affects the reduction of the aromatic activity and the instability of the anions of the dissociated fatty acids (Alewijn, 2006). The wood used in the drying process of thermally treated cheese contains free extractable compounds of low molecular

weight in its porous structure including volatile and non-volatile organic compounds (Licitra *et al*., 2018).

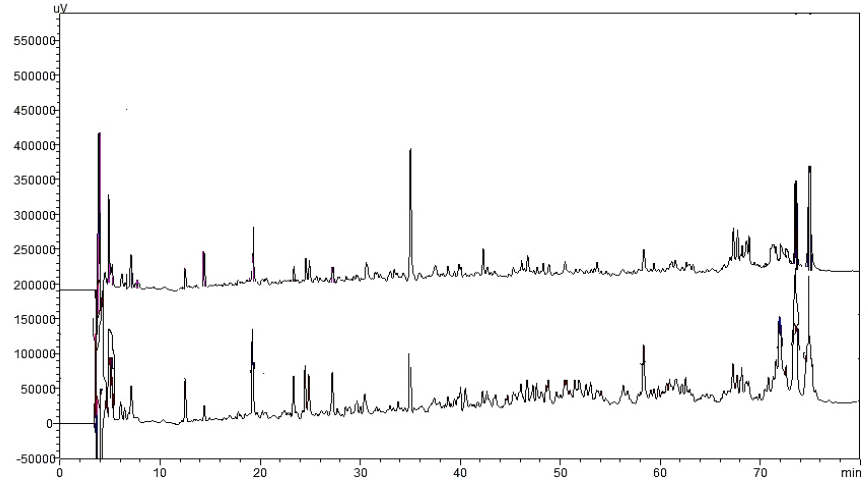

Figure 4. Peptide profile of brined cheeses from cooked (C) and uncooked (N) curd

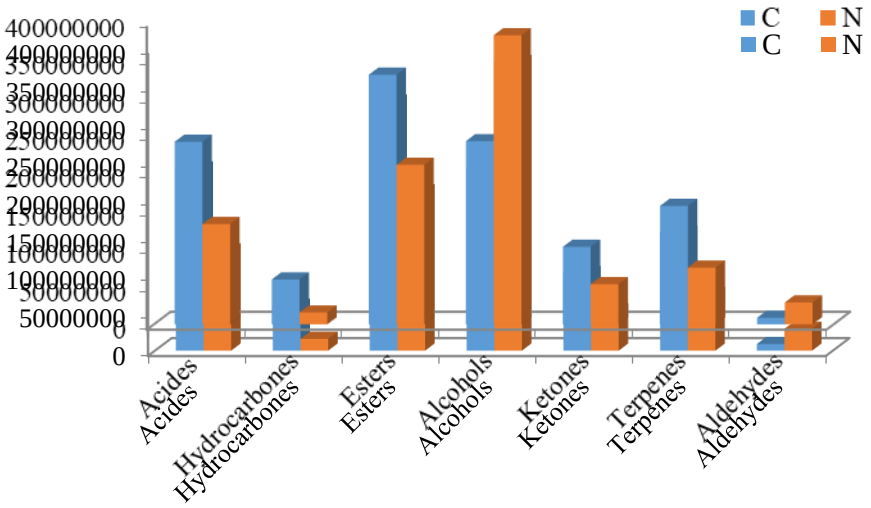

Figure 5. Relationship between average area values of volatile compounds group in cooked (C) and uncooked (N) brined cheeses identified bu GC-MS analysis

In the volatile fraction of the both brined cheeses, the volatile components consisted of 8 ketones, 8 acids, and 17 esters. However cooked brined cheeses

had higher numbers of terpenes (12) and hydrocarbons (13) while uncooked ones had (18) alcohols and (9) aldehydes (Table 3). Cooked cheese is characterized by a higher amount of volatile substances, especially organic acid which can also promote ketone formation (Forss, 1979) (Fig 5; Table 2). The similar results were conducted during characterization of flavor characteristics of cooked cheese were organic acid formation are likely to be due to thermally induced reactions (Sullivan *et al.*, 2023).

Reactions caused by heat treatment including amino acids and their increased catabolism are enabled by cooking through the continuous network of casein (Banks *et al*., 2001). It is highlighted from previous data that dipeptides are sensitive to the Maillard reaction and act as precursors of volatile aromatic compounds (Van Lancker *et al.,* 2010).

Table 2. Types of volatile compounds isolated from two kinds of brined cheese

Compounds	**Cooked**	**Uncooked**
Terpenes	**12**	**5**
Esters	**17**	**17**
Acides	**8**	**8**
Alcohols	**17**	**18**
Ketones	**8**	**8**
Aldehydes	**3**	**9**
Hydrocarbones	**13**	**9**
Total	**78**	**74**

Thermally induced reactions, such as the Maillard reaction and caramelization, affect the concentration of volatile compounds during cooking. These reactions also influence the flavor of cooked cheese by contributing to the formation of specific non-volatile substances within the cheese.

CONCLUSIONS

The activity of enzymes and biochemical processes in brined cheese produced with cooked curds was significantly affected, leading to differences in chemical composition, proteolysis, and volatile compounds. The results of this study indicated that uncooked curds enhanced the production of alcohols and aldehyde volatiles in cheese, with their concentrations being higher in cheeses made from uncooked (N) curds than in those made from cooked (C) curds. These findings suggest that heat-induced changes to the cheese curd are the primary cause of the differences observed in the levels of ACE-inhibiting peptides in the cheeses. The peptide profile revealed a higher formation of smaller peptides in cheese made without heat treatment compared to that made with cooking. The cooking process, which involves high-temperature reactions such as the Maillard reaction, significantly impacts the production of aromatic volatile compounds.

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