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Influence of curd heating on proteolysis and volatiles of Kashkaval cheese

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ABSTRACT

Kashkaval is the most popular hard cheese in Macedonia and other countries of Balkan peninsula. The aim of this research is to assess the differences of heat treatments effect (60, 70 and 90 °C for 5 min) in several biochemical and technological characteristics of Kashkaval cheese. Proteolysis was observed to take place at a faster rate in the Kashkaval cheeses made using the lower heat treatment. The residual enzyme activity of cheeses averaged 67.7, 43.7 and 8.4% for the cheeses heated at 60, 70 and 90 °C, respectively. Acids and esters constituted the main chemical class of the cheeses during ripening (mean abundances of these were 57.1% and 26.8% w/w of total volatiles, respectively). The colour (L^*) and meltability values decreased significantly during ripening. In conclusion, powerful correlations were observed between extents of the heat treatment and levels of residual coagulant activity, breakdown of proteins and formation of volatiles.

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1. Introduction

Kashkaval is a pasta-filata type of cheese which has a very large popularity after white-brined cheese (Belo sirenje) in Macedonia (Sulejmani, Hayaloglu, & Rafajlovska, 2014). Its production dating back to the eleventh and twelfth centuries; however, historical references suggest that Kashkaval has an even older tradition (Santa & Srbinovska, 2014). The cheese also manufactured in different countries such as Kashkaval, Kashkavalo (Russian types), Kaskaval Balkan, Kaskaval Preslav (Balkan types), Kasseri (Greek type), Caciocavallo (Italian type) and Kashar (Turkish type) (Hayaloglu, 2009). It possesses a hard (120 days-old Kashkaval) or semi-hard texture (1st day-old Kashkaval). In 2014, the total hard cheese production including Kashkaval cheese in Macedonia was 4 632 megagrams per annum.

During storage of cheese; glycolysis, proteolysis, and lipolysis are the main primary reactions throughout cheese ripening. Secondary events lead to the creation of volatile compounds and pathways for the production of flavour compounds from fatty acids and amino acids (McSweeney, 2004). Proteolysis is the most complex biochemical event that take place during ripening and among these, the primary proteolysis is the most significant which was defined as the alterations in caseins (α_{s1}-, α_{s2}-, β- and κ-casein) due to residual coagulant or native enzymes (Onipchenko, 2012). The degrees of both primary and secondary proteolysis in pasta-filata cheeses can differ excessively depending on the extent to which the coagulant and starter culture are heat inactivated during stretching (Feeney, Fox, & Guinee, 2001). The functional features such as meltability and colour attributes are important characteristics that impact the quality of the cheese. The rheological behavior of cheese is related to its composition, microstructure, physicochemical state of components and its macrostructure (Pereira et al., 2016). Moreover, a food product's quality depends on permanent consumer expectations with respect to the sensory attributes, thus it is necessary to evaluate the relevant attributes for acceptance and purchase intent of a food product (Gaze et al., 2015).

Only few is known for the manufacturing technology and the gross compositional characteristics of Kashkaval cheese produced in Macedonia (Mijačević & Bulajić, 2004; Santa & Srbinovska, 2014). There aren't studies on peptide profiles, urea-PAGE patterns, volatile profiles and residual coagulant activity of the cheese. Moreover, this research provides initial characterization of the volatile composition and proteolysis of Kashkaval cheese. Colour parameters are very significant for a whole characterization of the cheese. The purpose of this work was to define the chemistry, proteolysis, residual coagulant activity, volatiles and meltability of Macedonian Kashkaval cheese. Since, its manufacture process consists of different heat treatments (e.g., 60, 70 and 90 °C), which influence all ripening characteristics of the cheese that define the

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final quality. As a result, it was essential to understand the effect of stretching temperatures (60, 70 and 90 °C) on overall quality characteristics.

2. Materials and methods

2.1. Cheese manufacture and sampling

Kashkaval cheese was manufactured in duplicate using a 5400 L of cow's milk (pasteurized at 66 °C for 25 min) in a local dairy plant ("Eko Sharr" in Poroj village, Tetovo, Macedonia). After heat treatmen, the milk was cooled to 32 °C and the mixture of commercial freeze-dried culture (*Lactococcus lactis* subsp. *lactis*, *L. lactis* subsp. *cremoris*, *Streptococcus thermophilus*, *Lactobacillus delbrueckii* subsp. *bulgaricus*, *L. casei*) (0.01%) (Genesis, Sofia, Bulgaria), non-animal rennet (Chy Max® Liquid Plus, Chr. Hansen, Denmark) at a level of 6 mL 100 L^{-1} and 1444 g $CaCl_2$ (Sartorius, Italy) were added. After milk coagulation at 33 °C for 60 min, the cheese curd was cut and mechanically stirred at 35 °C for 60 min. Following the stirring phase, the curd was pressed (5 bar) and rest for curd fermentation (for 40–60 min) until the required pH level of 4.95. The ripened curd was mechanically sliced (milled), stretched in a hot brine (18% NaCl, w/v) at 60 (sample K60), 70 (sample K70) and 90 (sample K90) °C for 5 min. Following the texturizing and kneading phase, the curd was molded into plastic squares and transferred into ventilated areas for 3–4 days at 4 °C. The curd was pre-ripened for 15 days at 15 °C and 80–85% relative humidity. During this period, the curd gained a specific yellow colour due to proportional increasing of β-carotene, especially at the surface of cheese. The cheeses were ripened for 120 days at 5 ± 1 °C and samples were analyzed after 1, 30, 60, 90 or 120 days of ripening for some physical characteristics, proteolysis and volatile analysis.

2.2. Methods

2.2.1. Compositional analysis, assessment of proteolysis and residual coagulant activity

Cheese samples were analyzed at the first day of ripening for moisture by the oven drying method at 102 °C, salt by $AgNO_3$ titration, total protein by Kjeldahl, fat by van Gulik methods as described by Ardo and Polychroniadou (1999). Titratable acidity (as percentage of lactic acid) and pH of cheese were determined at the first day of ripening as described by Hayaloglu, Guven, Fox, and McSweeney (2005). Total nitrogen (TN) content, the levels of water-soluble nitrogen (WSN, as % of TN), 12% trichloroacetic acid soluble nitrogen-soluble (TCA-SN, as % of TN) and total free amino acid (FAA) were determined at five different stages of ripening (1, 30, 60, 90, and 120 days) as described by Ardo and Polychroniadou (1999). All analyses were performed in triplicate. The water-insoluble fractions of the cheeses after WSN fractionation were freeze-dried and analyzed at four different stages of ripening (30, 60, 90, and 120 days) by urea polyacrylamide gel electrophoresis (urea-PAGE) using Protean II XI vertical slab gel unit (Bio-Rad Laboratories Ltd., Watford, UK) according to the method of Andrews (1983). The obtained gels were stained by the method of Blakesley and Boezi (1977) with Coomassie Brilliant Blue G-250. After destaining with pure water, gel slabs were scanned (HP ScanJet software, ScanJet G4010, Hewlett Packard, Palo Alto, CA). Scans of the electrophoretograms were used to quantify bands using densitometric software (Image Master Total-Lab Phoretix 1D Pro software, Keel House, Newcastle upon Tyne, UK). The caseins were determined quantitatively by integration of peak volumes using the densitometer. The water–soluble nitrogen fractions of the cheeses were also freeze-dried for determination of peptide profiles. The analysis were realized by reverse-phase high performance liquid chromatography (RP-HPLC) using a Shimadzu LC 20 CE Prominence HPLC system (Shimadzu Corporation, Kyoto, Japan) (Hayaloglu et al., 2005). Residual coagulant activity (RCA) in Kashkaval cheese was determined at three different stages of ripening (1, 30, and 120 days) by a RP-HPLC (Shimadzu, model LC-20CE Prominence HPLC, Shimadzu Corp., Kyoto, Japan) system consisting of diode array detector model SPD-M20A as described by Hurley, O'Driscoll, Kelly, and McSweeney (1999). The results were expressed as following formula:

$$RCA = \frac{Peak\ area\ of\ product}{Peak\ area\ of\ substrate\ in\ blank} \times 100$$

2.2.2. SPME/GC–MS analysis of volatile compounds

Analysis of the volatiles were performed at three different stages of ripening (1, 30, and 120 days) by a static solid phase micro-extraction (SPME) method using gas chromatography-mass spectrometry (GC–MS) system (Shimadzu Corporation, Kyoto, Japan) as described by Hayaloglu, Tolu, Yasar, and Sahingil (2013). The identifications were based on comparing mass spectra of unknown compounds with those in Wiley 7 (7th edition, 7th ed.; John Wiley & Sons Inc., 2005) and NIST/EPA/NIH 02 (http://www.nist.gov) mass spectral library. Identifications were also confirmed by comparing retention times with reference standards when it was available. The concentrations were calculated by comparing of the peak areas of the internal standards containing mixture of 100 ppm 2-methyl-3-heptanone, 300 ppm 2-methyl-1-pentanoic acid and 278 ppm ethyl heptanoate in methanol (Sigma-Aldrich Co., St. Louis, MO, USA). Each compound was expressed as μg/100 g of cheese. The analyses were performed in triplicate.

2.2.3. Assessment of meltability

Meltability of the cheese samples were established at four different stages of ripening (30, 60, 90, and 120 days) as described by Hayaloglu, Karatekin, and Gurkan (2014). In this method, cheese disks with 30 mm diameter and 15 mm height were placed in the centre of a glass Petri dish and heated at 105 °C for 60 min in a hot air oven. After cooling for 30 min on a smooth table, the diameter of the melted cheese circles was measured using a micrometer. The average of the six readings of the diameter at different places on the melted circles was recorded as mm.

2.2.4. Colour analysis

Colour parameters of cheese samples were quantitatively determined at four different stages of ripening (30, 60, 90, and 120 days) using a Minolta Chroma meter (model CR-5, Konica Minolta, Osaka, Japan) with 10° standard observer and D_{65} illuminant. Measurements were performed in triplicate for each treatment on non-overlapping areas of the cheese. The following colour parameters were determined: lightness (L^*), redness (a^*:+red, −green) and yellowness (b^*:+yellow, −blue). The measurements were repeated at five randomly selected locations on each sample and average data were reported.

2.2.5. Statistical analysis

The results were statistically analyzed by analysis of variance (ANOVA). A randomized complete block design of three treatments (cheeses K70, K90 or K60), five ripening periods (1, 30, 60, 90, and 120 days) and two blocks (trials) were used to analyze the response variables of volatiles data. Duncan's multiple-comparison test was used as a guide for paired comparisons of treatment means. The level of significance of differences between treatments was considered at $P < 0.05$. The relationship between parameters in volatiles was assessed by the principal component

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