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Changes of organic acids, volatile aroma compounds and sensory characteristics of Halloumi cheese kept in brine

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Abstract

This study examines the changes in organic acids, volatile aroma compounds and sensory characteristics of ovine Halloumi cheese kept in brine (~10% NaCl) at 4 °C for 45 days. The highest total score of the organoleptic assessment was observed at day 1, before storage in brine. During cheese storage, a large reduction of lactose was observed, especially in the first stages of storage while, concurrently, a significantly progressive increase in lactic and acetic acids was observed. Among the volatile aroma compounds determined were alcohols, aldehydes, ketones, acids, esters, hydrocarbons, sulphur compounds, as well as a variety of other compounds in very small quantities. Ethanol and acetic acid were the dominant volatile aromatic compounds and their concentrations increased during storage. The lipolysis of Halloumi cheese during storage was not excessive. The dominant free fatty acids were palmitic, oleic and acetic.

Keywords: Halloumi cheese; Organic acids; Lactose; Volatile aromatic compounds; Lipolysis; HPLC

1. Introduction

Halloumi cheese is a traditional and distinctive cheese of Cyprus. It is widely popular in Cyprus and other countries of the eastern Mediterranean and, more recently, the product has gained international acceptance and recognition. Total exports of Halloumi cheese from Cyprus have risen to ≈2500 metric tonnes (Papademas & Robinson, 2000). The committee for standards of the Cyprus Ministry of Commerce and Industry (1985) established the definition and standards for Halloumi cheese. Large quantities are sold immediately after production and this fresh product has a characteristic aroma, its texture is elastic and compact with no holes, and it is easily sliced. A proportion of the total Halloumi cheese production in Cyprus is preserved in brine. During storage, Halloumi changes markedly in taste and texture and the cheese becomes salty and moderately acidic. However, the fermentation of lactose, the production of organic acids, volatile aromatic compounds, free fatty acids and other compounds during the storage of Halloumi cheese have not been studied up to now. These components, which determine the sensory characteristics of ovine Halloumi cheese, were studied in this work so as to assist the determination of the identity and features of this cheese.

2. Materials and methods

2.1. Milk

Ovine milk from the flock of the Agricultural University of Athens was used.

2.2. Cheese making and sampling

Halloumi cheese was manufactured at the pilot plant of the Dairy Laboratory of the Agricultural University of Athens, following the cheese making procedure described

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by Anifantakis and Kaminarides (1983). Five cheese making trials were conducted. Cheese was subjected to chemical analysis 1 day after preparation and following 15, 30, and 45 days of brine preservation (~10% NaCl) at 4 °C.

2.3. Physicochemical analyses

Lactose and organic acids were analyzed by high-performance liquid chromatography (HPLC). A Varian HPLC (Varian Associates Inc. 2010, CA, USA) equipped with an RI detector (GBC Scientific Equipment Pty Ltd., LC 1240, Vic., Australia) was used with a Bio-Rad Aminex HPX-87H column (300 mm × 7.8 mm, Hercules, CA, USA). Before HPLC analysis, proteins and fat of samples were removed according to the procedure of Standard 28A described by the International Dairy Federation (IDF, 1974). One microlitre of the filtrate was mixed with 50 μl 70% perchloric acid, for the precipitation of very small peptides, and centrifuged at 14,000 rpm and 4 °C for 30 min (Heraeus Sepatech, Biofuge 22R). The upper phase was passed through a filter (0.45 μm) and the filtrate was injected into the 20 μl loop for HPLC analysis. Analysis was performed isocratically at 35 °C with 5 mM H_2SO_4 and at a flow rate of 0.5 ml/min. Analytical grade organic acids were used as standards. Quantification was based on peak area measurements, using a Waters recorder and data module (model 746, Waters Corporation, Milford, USA).

The volatile compounds present in the headspace fraction of samples were isolated and identified using a balance pressure (static) Perkin–Elmer HS40 headspace system (Perkin–Elmer Analytical Instruments, Uberlingen, Germany) coupled to a GC/MS-Q 5050 (Shimadzu Co., Kyoto, Japan). Five grammes of each milk and Halloumi cheese sample were weighed and introduced into 22 ml vials, which were then sealed with aluminium–rubber septa. The vials containing the samples were held at 80 °C for 30 min, purged and pressurized with helium at a flow rate of 35 ml/min. The volatile compounds were driven through the transfer line that was held at 100 °C to the injector of the GC. The volatile compounds were separated on an HP Innowax capillary column (60 m length × 0.25 mm internal diameter, 0.25 μm film thickness) under the following conditions: injector temperature, 200 °C; carrier gas helium 0.6 ml/min; temperature programme: 35°C for 3 min, increasing to 80 °C at a rate of 4 °C/min, held for 12 min and then raised to 200 °C at a rate of 7 °C/min and held for 6 min. The GC column was directly connected without splitting to the ion source of a QP 5050 quadrupole mass spectrometric detector, operating in the scan mode within a mass range of m/z 35–300 at 2 scans/s. The interface line to the MS was set at 250 °C. The MS operated in an electron impact mode at electron energy of 70 eV and was calibrated by auto-tuning. Identification of the compounds was carried out by computer-matching of mass spectral data with those in the Shimadzu NIST62 mass spectral database and by comparing their retention times and mass spectra with those of some standard compounds (when available) using the same conditions The quantification of the volatile compounds was performed using the Shimadzu Class 500 software by integrating the peak areas of total ion chromatograms (TIC).

Free fatty acids (FFA) were extracted from cheese according to the method of Nieuwenhof and Hup (1971) and determined by gas chromatography. A Shimadzu model GC-17A gas chromatograph, equipped with an on-column injector and a flame ionization detector (FID) was used with a Nucol fused silica capillary column (length 30 m, internal diameter 0.25 mm) coated with free fatty acid (FFA) phase OV-351 (bonded polyglycol- nitroterephthalic, film thickness 0.25 μm). Direct on-column injection took place at 110 °C. The injector temperature was raised from 110 to 220 °C at a rate of 8 °C/min and then held at 220 °C for 55 min. Oven temperature was initially held at 60 °C for 2 min. Afterwards, it was raised to 70 °C at a rate of 1 °C/min and then immediately raised to 220 °C at a rate of 10 °C/min and held at 220 °C for 18 min. The FID temperature was 230 °C. The carrier gas (helium) flow rate was 2.5 ml/min. The identification of the individual fatty acids of the cheese samples was based on a comparison of the retention times of the unknown FFA with those obtained from known FFA standards (Sigma, Steinheim, Germany) under identical conditions. The quantification of the FFA of cheese samples was performed using the internal standardization technique with C7:0 and C17:0 as internal standards and processing the chromatograms with the Chromeleon™ version 6.2 Software System (Shimadzu Scientific Instruments Inc., Dionex Corp., P.D., Sunnyvale).

2.4. Sensory evaluation

Following storage for 1, 15, 30 and 45 days, cheese was subjected to sensory evaluation by a 10-member panel of the Dairy Laboratory of the Agricultural University of Athens. Panel members, who were familiar with Halloumi cheese, evaluated each cheese for appearance, texture and flavour (odour and taste) using a 10-point scale, scoring 1 for the worst and 10 for the best quality. The attributes of flavour and texture were given priority over appearance, as advised by the IDF (1987), by multiplying their scores by 5 and 4, respectively. The total score was obtained by adding the scores for the three attributes. An excellent cheese would receive a total score of 100. The results are expressed as a mean score by the whole panel for each cheese.

2.5. Statistical analysis

The results for cheese at each sampling age, were subjected to analysis of variance (ANOVA) using the software Statgraphics (Statistical Graphics Corp., Rockville, MD, USA). A randomized complete block design was used and paired comparisons of means were made using the Duncan test ($P \leqslant 0.05$).