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Effect of refrigerated storage on microbiological, chemical and sensory characteristics of a ewes' raw milk stretched cheese

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ABSTRACT

This study aimed to describe the effects of refrigerated storage up to 180 days on microbiological, chemical, physical, and sensory characteristics of a PDO ewes' raw milk stretched cheese. To this aim, a total of 224 cheeses were manufactured in four consecutive production weeks, and series of 32 of them were examined before packaging and after 15, 30, 60, 90, 120, and 180 d of storage at 4 ± 2 °C in the dark, respectively. Lactic acid bacteria cocci displayed the highest levels (7.8 Log CFU*g^{-1}) during early storage and decreased progressively over time (7.4 Log CFU*g^{-1}), while the opposite trend was observed for lactic acid bacteria rods (from 6.5 to 7.3 Log CFU*g^{-1}). TMC and enterocci significantly increased during the storage. Chemical parameters showed a natural increase of proteolytic index during storage, an increase of pH (from 5.44 to 5.92), salt (from 2.08 to 2.40% of DM) and a decrease of a_w (from 0.984 to 0.971). Storage modified the color of the cheeses, provoked a slight browning, while a* value (red–green) and b* value (yellow–blue) increased until 30 days and then remained unchanged. Cheese fatty acids composition didn't show particular trend during the storage, while several panel test parameters changed. Cheeses after 180 days of storage showed higher solubility, greater odor of butter and less odor of milk than fresh cheeses, that determined an high overall satisfaction of the panelists at the end of storage.

1. Introduction

In the last years, there has been a growing attention towards food quality and food safety, as well as an increasing demand for "natural" products, especially those enjoying a 'recognition of quality' status and recognition of 'geographical indications and traditional specialties' (PDO, PGI and TSG) conferred by the European Community to promote and protect the names of quality agricultural products and foodstuffs.

Recently, the food policies undertaken by many government institutions to protect small-scale producers, as well as those promoting nutrition guidelines, made consumers more sensitive to food culture. In particular, great attention is given to the presence of chemical preservatives for food conservation (Settanni & Moschetti, 2010) and for this reason there has been a re-discovery of traditional food products, such as raw milk cheeses. Once the peculiar characteristics of a given food are defined, a key role in preserving the organoleptic, sensory, quality and safety profile is played by the conditions applied during storage.

"Vastedda della valle del Belice" (VdB) is a typical cheese of the homonymous valley of Sicily (Italy) that, recently, gained the PDO status (OJC no. C 42/16 19.2.2010). This cheese is a stretched (*pasta filata*) cheese made with raw milk of Valle del Belice sheep without the addition of starter cultures (Mucchetti et al., 2008). Although VdB is made with raw milk, it undergoes the stretching process that is a strong thermal treatment of the acidified curd. This technology reduces greatly the microbiological risks associated with the final stretched cheese products (Mucchetti, Carminati, & Addeo, 1997).

In order to ensure the safety/quality of cheese, the post-processing contaminations might be strongly limited through an appropriate storage condition. Actually VdB is stored under vacuum, refrigerated and consumed within three months after production, this is the shelf life utilized by all dairy farmers belonging to the Consortium of protection. The choice of the shelf life (90 days) was made on the basis of only organoleptic analysis by Consortium, but missing the study on the evolution of

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microbial, chemical, physical and organoleptic parameters during a long time storage. The aim of this study is to evaluate the variation of microbiological, chemical and physical parameters during the storage and provide evidences on the extension of shelf life of VdB cheeses for more than three months.

2. Material and methods

2.1. Cheese production, packaging and sample collection

The cheeses were produced according to the disciplinary of production (OJC no. C 42/16 19.2.2010) in a typical small dairy factory belonging to the consortium for the protection of VdB cheese during May and June 2014. The experimental plan included four experimental cheese-making trials, performed in four consecutive weeks. Fifty-six VdB cheeses were made in each production week, eight of which were sampled and analysed before packaging (T_0) and storage at 4 ± 2 °C. The other cheeses were transferred into pouches made of polyamide bioriented (OPA) and polypropylene (PP) (15 μm OPA/75 μm PP) characterized by an oxygen permeability of 30 $cm^3 m^{-2}$ 24 h^{-1} at 25 °C (Alpaksrl, Taurisano, Italy). The pouches were evacuated, flushed and sealed using a Lavezzini device (Fiorenzuola d'Arda, Piacenza, Italy). The packaged cheeses were collected at 15, 30, 60, 90, 120 and 180 days of storage for all trials.

2.2. Microbiological analysis

Twenty-five grams of each cheese sample were weighed into sterile stomacher bags and homogenized in 225 mL of sodium citrate (2% w/v solution) by means of a stomacher (Type 400; Seward London, UK) for 6 min at 260 rpm. Decimal dilutions of cell suspensions were prepared in Ringer's solution (Sigma-Aldrich, Milan, Italy) and subjected to the analysis of the following microbial groups: total mesophilic count (TMC) on plate count agar (PCA) incubated aerobically at 30 °C for 72 h (ISO 4833-1:2003); coliforms on violet red bile agar (VRBA) incubated aerobically at 37 °C for 24 h (ISO 4832:2006); *Enterobacteriaceae* on violet red bile glucose agar (VRBGA) incubated aerobically at 37 °C for 24 h according to ISO 21528 (2004b); mesophilic and thermophilic rod-shaped lactic acid bacteria (LAB) on de Man-Rogosa-Sharpe (MRS) agar, acidified to pH 5.4 with lactic acid (5 mol*L^{-1}) and incubated anaerobically in hermetically sealed jar added with the AnaeroGen AN25 system (Oxoid, Milan, Italy) at 30 and 44 °C for 72 h respectively, followed by Gram stain, catalase and oxidase tests; mesophilic and thermophilic coccus-shaped LAB on M17 agar incubated aerobically at 30 and 44 °C for 48 h respectively, followed by Gram stain, catalase and oxidase tests; enterococci on rapid *Enterococcus* agar (REA) incubated aerobically at 44 °C for 48 h followed by catalase and esculin hydrolysis test on the suspected colonies (BioradHercules, CA, USA); pseudomonads on *Pseudomonas* agar base (PAB) supplemented with 10 mg/ml cetrimide fucidin, incubated aerobically at 25 °C for 48 h; *Escherichia coli* β-glucuronidase positive on tryptone bile glucuronide Agar (TBX) at 44 °C for 24 h (ISO 16649–2:2010); sulphite-reducing anaerobic organisms (SRA) incubated anaerobically on iron sulphite agar at 37 °C for 24 h (ISO 15213: 2003); coagulase positive staphylococci (CPS) on Baird Parker RPF Agar at 37 °C for 24–48 h according to ISO 6888 (1999).

Detection of *Salmonella* spp. and *Listeria monocytogenes* were carried out on 25 g of each sample by an enzyme linked fluorescentassay (ELFA) in an automatic system VIDAS (bioMerieux, Marcy-l'Etoile, France): the AFNOR BIO 12/23-05/07 method including a pre-enrichment step in Buffered Peptone Water at 37 °C for 16–20 h and a subsequent step performed by VIDAS Immuno-Concentration Salmonella II (ICS2) was used for *Salmonella* spp.; the AFNOR BIO 12/11-03/04 method was performed with Half Fraser broth at 30 °C for 24–26 h and then Fraser Broth (FB) at 37 °C for 24–26 h for *L. monocytogenes*. Furthermore, for the last pathogen, one portion of the FB culture was then used for the *L. monocytogenes* VIDAS test (LMO2). All culture media were purchased from Oxoid except otherwise stated. Microbiological count were carried out in duplicate.

2.3. Chemico-physical analysis

Samples of cheese were analysed for moisture, fat, protein, salt and total solid by indirect near infrared transmittance employing the FoodScan analyser (Foss, Hillerød, Denmark). For each cheese, a representative sample was homogenized by grinding; approximately 180 g of ground sample was placed in a 140 mm round sample dish, and the dish was placed in the FoodScan.

Total and soluble nitrogen were assessed by Kjeldahl method (IDF, 1964) and results were displayed for percent (g*100 g^{-1}). Proteolytic index has been calculated as percentage ratio (%) between soluble and total nitrogen. The salt content was determined by the Volhard method (AOAC, 2000). pH was assessed using a pH-meter (DocuMeter Sartorius; Data Weighing Systems, Inc., Elk Grove, IL, USA). Water activity (a_w) was determined according the ISO 21807 (2004a) using HygroPalm water activity indicator (Rotronic, Bassersdrof, Germany).

2.4. Surface colour

VdB cheeses produced at the first cheese-making (28) were analysed for surface color of the top slice in the cheese package, measured by a Minolta tristimulus Chromometer CR-300 (Minolta, Osaka, Japan) using CIELAB L*a*b* values (Hunter, 1975). The measure of lightness (L* values, range 0–100) represents black to white, the redness measurement (a* values) describes green to red, and the yellowness measurement (b* values) represents blue to yellow. Beside these attributes, the (a*, b*) combination also determines the parameters hue angle and chroma: the hue angle (a*/b*) gives the predominant wavelength composing the color; chroma or saturation, equal to $\sqrt{(a^2 + b^2)}$, accounts for the vividness or the color purity. The chromometer was standardized using a white standard plate. The results reported are averages of five measurements on the same cheese slice.

2.5. Analysis of cheese fatty acids

VdB cheeses produced at the first cheese-making (28) were analysed for fatty acids composition. Fatty acids in lyophilized cheese samples (100 mg) were directly methylated with 2 mL of 0.5 M $NaOCH_3$ at 50 °C for 15 min, followed by 1 mL of 5% HCl in methanol at 50 °C for 15 min (Lee & Tweed, 2008). Fatty acid methyl esters (FAME) were recovered in hexane (1.5 mL). One microliter of each sample was injected by auto-sampler into an HP 6890 gas chromatography system equipped with a flame-ionization detector (Agilent Technologies Inc., Santa Clara, CA).

Fatty acid methyl esters from all samples were separated using a 100-m length, 0.25-mm i.d., 0.25-μm capillary column (cp-sil 88; Chrompack, Middelburg, the Netherlands). The injector temperature was kept at 255 °C and the detector temperature was kept at 250 °C, with an H_2 flow of 40 mL/min, air flow of 400 mL/min, and a constant He flow of 45 mL/min. The initial oven temperature was held at 70 °C for 1 min, increased at 5 °C/min to 100 °C, held for 2 min, increased at 10 °C/min to 175 °C, held for 40 min, and then finally increased at 5 °C/min to a final temperature of 225 °C and held for 45 min. Helium, with a head pressure of 158.6 kPa and a flow rate of 0.7 mL/min (linear velocity of 14 cm/s), was used as the carrier gas. Fatty acid methyl ester hexane mix solution (Nu-Chek