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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.

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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 $\surd\ (a^2 + b^2)$, 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

Prep Inc., Elysian, MN, USA) was used to identify each FA. The identification of the conjugated linoleic acid (CLA) isomers was performed using a commercial mixture of cis- and trans-9,11- and 10,12-ocdecadienoic acid methyl esters (Sigma-Aldrich, Milano, Italy) and published isomeric profile (Kramer et al., 2004; Luna, de la Fuente, & Juárez, 2005).

2.6. Sensory analysis

VdB cheeses produced at the first cheese-making (28) were subject to panel test that was carried out following the ISO (2003a) indications. Sensory quality of VdB cheeses at different time of storage was evaluated by sensory analysis by a group of 6 panelists that had prior experience with descriptive analysis (3 man and 3 women, 25–50 years old). For all panel session, approximately 10 g of each sample were placed in coded white small plastic plates in a randomized order. All samples were left at ambient temperature (about 20 °C) for 30 min before administration.

The panelists evaluated fourteen descriptors regarding the aspect (color and uniformity of structure), the smell (strength of odor, milk, butter and unpleasant smell), the taste (salty, sweet, acid, spicy and bitter taste) and the consistency (soft/hard, solubility and grittiness following mastication). After tasting, each panelist rated the overall acceptability of the product. Quality was scored using a line scale anchored on the left (visual analogue scale) with dislike/low quality and on the right with like/high quality. The hedonic scale results were converted as distance (cm) of mark from the left end of the line.

2.7. Statistical analysis

Microbiological, chemical and physical parameters were analysed with the ANOVA linear model (GLM procedure of SAS 9.1.2 software, 2004) which include the fixed effects of storage (7 levels, from 0 to 180 days) and processing week (from 1 to 4). Cheese fatty acids (FA) composition, surface color and sensorial analysis were analysed with the ANOVA linear model which include the only fixed factor storage. On the another hand, chemico-physical parameters, FA and sensorial parameters were analysed by simple regressions, too (REG procedure of SAS 9.1.2 software, 2004). Moreover, sensorial parameters were analysed according to a multivariate approach, using the discriminant canonical analysis (CANDISC procedure of SAS 9.1.2 software, 2004).

3. Results and discussions

3.1. Microbiological analysis

The temporal evolution of the microbiological parameters of VdB cheeses during storage is reported in Fig. 1. Mesophilic and thermophilic LAB dominated the microbial community during the first 60 days of storage (Fig. 1a). Mesophilic LAB rods overcame the other groups from the day 90, while thermophilic LAB rods were always detected at subdominant levels among LAB population. TMC showed an increasing trend, but their levels were not superimposable to those of the LAB counts (Fig. 1b). The levels of enterococci increased of about 1.5 order of magnitude during time (Fig. 1b), reaching the highest level (almost 5.5 Log CFU*g^{-1}) at the end of the monitoring period. Coliforms and *E. coli*, CPS, SRA and pseudomonads were below the detection limit during the entire period of storage, while *Salmonella* spp. and *L. monocytogenes* were absent in all samples analysed. LAB evolution during the first days of investigation are almost in agreement with those produced in controlled conditions at pilot plant scale by Gaglio, Scatassa et al. (2014). The levels detected confirmed the common trend observed

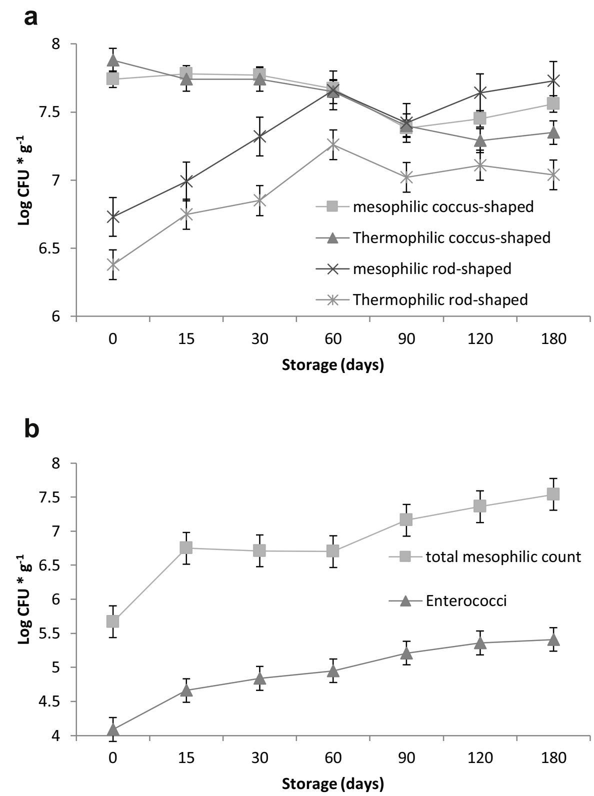

Fig. 1. a: Trend of LAB during the storage (LSM ± s.e.) b: Trend of TMC and Enterococci during the storage (LSM ± s.e.).

for VdB cheeses produced at farm level throughout the production area (Gaglio, Francesca et al., 2014). The absence of pathogenic species and spoilage/opportunistic pathogen bacteria could be strictly dependent on the quality of raw milk utilised (Oliver, Jayarao, & Almeida, 2005); moreover, the thermal treatment applied during stretching (Gaglio, Francesca et al., 2014) and the microbial biofilms of the wooden vats (Scatassa et al., 2015) also contributes to the safety of the resulting cheeses. On the whole, the VdB microbiological profile could be considered safety until 180 days of storage.

3.2. Chemico-physical analysis

The least square means (LSM) of chemical and physical parameters are reported in Table 1. Storage produced significant variation on proteolytic index, salt, pH and a_w parameters. Proteolytic index of VdB cheeses increase during storage ($b > 0$; $P < 0.001$) from 8.34% to 26.54% at 180 days. This reflects the natural metabolic evolution to which cheeses undergo during the storage period, since caseins are gradually hydrolyzed by proteolytic enzymes. These phenomena are also responsible for the increase of pH values over time, in fact pH determined in fresh VdB cheeses was 5.44 to increase up to 5.92 for cheeses stored for 180 days, showing a positive trend ($b > 0$; $P < 0.001$). This significant change is due to the formation of nitrogen compounds of alkaline character, and the products of catabolism of lactic acid that physiologically occur during cheese ripening (McSweeney & Fox, 1997; Park, 2001). On the another hand, Hassan, Johnson, and Lucey (2004) showed that during the first 3 months of storage, approximately 20% of the insoluble Ca in cheese becomes soluble and a corresponding amount of phosphate ions would also be released from the protein. At the pH of cheese, the phosphate ions will be mainly $H_2PO_4^-$, whereas they are generally unprotonated

Table 1
Trend of chemical-physical cheese parameters during storage.

items	Storage								P value		Regression model		
	0	15 d	30 d	60 d	90 d	120 d	180 d	SEM	Storage	Processing week	b	P value	r
n.	16	16	16	16	16	16	16						
Dry Matter (%)	56.68	57.16	57.37	57.37	57.23	57.69	58.08	0.41	ns	***	0.006	*	0.91
Fat (% DM)	46.61	45.93	46.04	46.52	46.05	45.65	45.99	0.49	ns	*	−0.003	ns	−0.27
Total Protein (% DM)	47.10	45.73	47.65	45.67	45.55	45.89	46.57	0.45	ns	***	0.000	ns	0.46
Proteolytic index (%)	8.34	10.59	11.76	18.59	24.48	25.69	26.54	1.92	***	***	0.112	***	0.71
NaCl (% DM)	2.08	2.00	2.04	2.11	2.16	2.22	2.40	0.07	***	***	0.002	***	0.82
Ash (% DM)	6.18	5.98	6.19	5.92	5.78	5.96	5.81	0.12	ns	**	−0.002	*	−0.43
pH	5.44	5.52	5.73	5.85	5.74	5.94	5.92	0.04	***	***	0.003	***	0.74
aw	0.984	0.989	0.984	0.988	0.987	0.976	0.971	0.002	***	***	−0.000	***	−0.56
n.	4	4	4	4	4	4	4						
L*	83.93	80.10	79.84	80.62	79.78	77.72	80.25	0.599	***	–	−0.015	**	−0.45
a*	−4.49	−5.44	−6.48	−5.87	−5.69	−5.71	−5.74	0.135	***	–	0.003	ns	0.28
b*	14.41	15.77	19.63	18.39	16.56	17.02	17.83	0.410	***	–	0.009	ns	0.29
Chroma	15.09	16.68	20.67	19.30	17.51	17.95	18.73	0.427	***	–	0.009	ns	0.29
Hue angle	−0.31	−0.34	−0.33	−0.32	−0.34	−0.34	−0.32	0.004	***	–	0.000	ns	0.04

P value: *P < 0.05; **P < 0.01; ***P < 0.001; ns = not significant.

when present as insoluble calcium phosphate. Thus the pH of cheese increases as the concentration of H^+ ions decreases since protons are absorbed by the phosphate ions that are dissolved into the cheese serum.

Water activity and pH are the most important factors that affect cheese stability (Di Marzo et al., 2006). In our study, a_w was not constant during storage and decreased until cheese surface was in equilibrium with the surrounding space. Along the storage, a_w was similar for cheeses until 90 days, while decreased successively, showing an overall downward trend ($b < 0$; $P < 0.001$) in accordance with McMahon et al. (2014) and Pappa, Samelis, Kondyli, and Pappas (2016). This fact is considered positively, because an increase of a_w values during the storage encourage the development of undesirable microbial populations (Robertson, 1993).

Salt (NaCl) determined on VdB cheeses was lower than other studies (Todaro, Bonanno, & Scatassa, 2014), but its trend ($b > 0$; $P < 0.001$) showed an increase during storage specially after 60 days. Also Gaucheron, Le Graët, Michel, Briard, and Piot (1999) found a slightly increase during 14 days of storage; moreover, these authors established a migration of sodium, potassium and chloride ions from the outer layer versus the core of cheese which ended after 5 days. Other authors, on Minas cheese (Felicio et al., 2016),

Table 2
Trend of cheese fatty acids (g/100 g of FAME).

items	Storage								Anova	Regression model		
	0	15 d	30 d	60 d	90 d	120 d	180 d	SEM	P value	b	P value	r
n.	4	4	4	4	4	4	4					
C4	2.98	3.07	2.90	2.63	2.95	3.08	3.00	0.09	*	0.0003	ns	0.10
C6	2.71	2.86	2.70	2.57	2.76	2.84	2.79	0.06	*	0.0004	ns	0.17
C8	2.48	2.62	2.48	2.46	2.53	2.60	2.58	0.04	*	0.0004	ns	0.26
C10	6.82	7.16	6.84	6.87	6.96	7.10	7.07	0.08	*	0.0009	ns	0.30
C12	3.55	3.67	3.58	3.59	3.60	3.64	3.64	0.03	*	0.0003	ns	0.29
C14	10.09	10.22	10.15	10.20	10.14	10.22	10.24	0.04	ns	0.0005	*	0.39
C16	24.15	23.97	24.22	24.35	24.03	24.11	24.11	0.10	ns	−0.0001	ns	−0.04
C18	9.94	9.75	9.91	9.97	9.85	9.86	9.84	0.06	ns	−0.0002	ns	−0.10
C18:1 *t11*, VA[1]	2.98	2.93	2.98	2.99	2.96	2.94	2.95	0.02	ns	−0.0001	ns	−0.21
C18:1 *c9*	15.27	14.97	15.22	15.27	14.93	14.91	14.97	0.08	**	−0.0016	**	−0.45
C18:2 n-6 *c9 c12*	1.98	1.93	1.98	1.99	1.95	1.94	1.93	0.01	*	−0.0002	*	−0.37
C18:3 n-3 α-linolenic	1.46	1.42	1.45	1.46	1.43	1.45	1.45	0.01	ns	0.0000	ns	0.00
CLA C18:2 *c9 t11*, RA[2]	1.24	1.21	1.23	1.24	1.22	1.22	1.23	0.01	*	0.0000	ns	0.17
CLA isomers	0.42	0.42	0.40	0.39	0.42	0.38	0.36	0.02	ns	−0.0002	*	−0.34
Saturated FA	68.22	68.86	68.30	68.22	68.80	68.85	68.55	0.19	*	0.0016	ns	0.22
Monounsaturated FA	24.36	23.88	24.32	24.34	23.95	23.88	24.20	0.14	*	−0.0008	ns	−0.16
Polyunsaturated FA	7.41	7.25	7.38	7.44	7.25	7.27	7.25	0.06	ns	−0.0007	*	−0.35
Unsaturated FA	31.78	31.13	31.70	31.78	31.20	31.15	31.45	0.19	*	−0.0016	ns	−0.22
Saturated/Unsaturated	2.15	2.21	2.15	2.15	2.21	2.21	2.18	0.02	*	0.0002	ns	0.23
Σ omega-6	0.92	0.92	0.94	0.96	0.88	0.92	0.92	0.02	*	−0.0001	ns	−0.14
Σ omega-3	3.99	3.88	3.96	3.99	3.91	3.92	3.91	0.03	*	−0.0002	ns	−0.26
omega-6/omega-3	0.23	0.24	0.24	0.24	0.23	0.23	0.24	0.01	ns	0.0000	ns	0.05
BCFA[3]	2.26	2.20	2.25	2.26	2.15	2.13	2.00	0.06	*	−0.0013	**	−0.57
Σ C4-C11	15.32	16.10	15.27	14.86	15.54	15.99	15.80	0.24	*	0.0022	ns	0.22
Σ C12-C16	42.70	42.74	42.83	42.99	44.66	42.80	43.04	0.16	ns	0.0013	ns	0.25
Σ C17-C24	41.99	41.16	41.90	42.14	41.80	41.26	41.16	0.22	*	−0.0034	*	−0.36

[1]VA = vaccenic acid; [2]RA = rumenic acid; [3]BCFA = Branched chain fatty acids; P value: *P < 0.05; **P < 0.01; ns = not significant.

reported a significant decrease in sodium during the first 14 days of storage, while no significant variation was reported from 25 to 360 days of storage by Pappa et al. (2016) for Urda whey cheese.

3.3. Surface colour

LSM and trend of colour parameters was reported in Table 1. Lightness (L*) decreased during the storage time ($b < 0$; $P < 0.01$) causing a slight browning of the VdB cheese. As the cheeses were kept in the dark, this fact is probably due to positively correlation between browning and proteolysis in cheeses (Mukherjee & Hutkins, 1994), as observed in our cheeses (Table 1).

The a* (red–green), b* (yellow–blue) and chroma values underwent small changes up to 30 days, then remained stable up to 180 days, but no significant trend were observed. Similar trend was found by Kristensen, Hansen, Arndal, Trinderup, and Skibsted (2001) for cheeses storage with similar experimental conditions (stored at 5 °C in the dark). In fact is well known as temperature and light influence the colour and the oxidative stability of processed cheese (Kristensen et al., 2001; Mortensen, Sørensen, & Stapelfeldt, 2003).

3.4. Cheese fatty acids

Table 2 reports the effect of storage on cheese fatty acid composition. Although several cheese FAs resulted significantly influenced by storage, a specific positive ($b > 0$) trend was found only for C14, while significant negative ($b < 0$) trends were found for oleic acid (C18:1 c9) ($P < 0.01$), linoleic acid (C18:2 n-6 c9 c12) ($P < 0.05$), CLA isomers ($P < 0.05$), BCFA ($P < 0.01$), PUFA ($P < 0.05$) and, consequently, long chain FAs ($P < 0.05$). The sum of saturated and unsaturated FAs or the sum of short and medium FAs didn't show significant variations. Accordingly, it is possible to conclude that the FA composition of the VdB cheese remains quite stable up to 180 days of storage. While the slight decreasing trend observed for some polyunsaturated long chain FA could be attributed to a slow microbial catabolic activity on free FAs released as a result of lipolysis, leading to the production of aroma compounds (Collins, McSweeney, & Wilkinson, 2004), and occurring presumably during storage. In literature the variation of FA composition in fresh cheeses during the storage is little studied, while the variation during ripening has been more investigated. Only two studies were found on effect of storage on FA composition and in particular on CLA concentration, but these papers report conflicting results. Shantha, Ram, O'leary, Hicks, and Decker (1995) showed that for several cheeses, such as Mozzarella, Gouda and Cheddar, storage did not affect CLA concentration, suggesting that CLA is a stable component. While Rodríguez-Alcalá and Fontecha (2007) reported a decrease of total CLA in fresh CLA-fortified cheese samples detected after 10 weeks of refrigerated storage, possibly related to an increase in microbiota growth.

Overall, the VdB cheese fatty acids composition is similar with those reported by Todaro et al. (2014) for VdB cheeses produced in spring when ewes are fed with green forage, so these cheeses maintains some beneficial properties for human health, which a good level of PUFA among which rumenic acid (C18:2 *c9 t11*), and its precursor vaccenic acid (C18:1 *t11*). In this regard, the levels of rumenic acid, the more abundant among the CLA isomers, known for its anti-cancer and anti-atherogenic effects (Parodi, 2009), were comparable to those reported by Bonanno et al. (2016) and Nudda, McGuire, Battacone, and Pulina (2005) for sheep cheeses produced by milk from grazing ewes in the same seasonal period.

3.5. Sensory findings

Table 3 reports the changes in sensory scores attributed to the cheese descriptors registered over time. From the beginning of the experiment until the end of storage, uniformity of structure, odor of butter, solubility, grittiness and overall satisfaction descriptors scores increased, while salty, acid, bitter and chewiness significantly decreased, showing that the acceptability and palatability of the VdB cheeses improved during storage. Similar results were found by Abdalla and Mohamed (2009) for a white soft cheese of the Sudan, stored under vacuum for 45 days at 5 °C.

Multivariate analysis confirmed the results found with univariate analysis. The Mahalanobis distances between the centromeres of the point clouds were statistically significant ($P < 0.001$) for all

Table 4
Canonical discriminant analysis: Mahalanobis quadratic distances for different storage.

Storage	0 (A)	15 d (B)	30 d (C)	60 d (D)	90 d (E)	120 d (F)	180 d (G)
0 (A)	0	18.77	10.88	6.46	15.95	17.34	22.41
15 d (B)		0	21.46	13.18	12.28	14.68	17.53
30 d (C)			0	5.98	7.04	10.23	14.80
60 d (D)				0	4.27	6.68	10.70
90 d (E)					0	2.48*	5.21
120 d (F)						0	1.78*
180 d (G)							0

*not significant; other distances are significant at $P < 0.001$.

Table 3
Effect of storage on sensorial parameters.

	Storage								Anova	Regression model		
	0 d	15 d	30 d	60 d	90 d	120 d	180 d	SEM	P value	b	P value	r
n.	4	4	4	4	4	4	4					
Color	45.92	35.00	63.73	47.31	42.94	45.22	44.67	2.37	***	−0.018	ns	−0.07
Uniformity of structure	87.59	61.06	84.92	83.28	84.83	90.33	90.33	2.75	***	0.080	***	0.30
Strength of odor	48.52	56.11	63.69	54.53	63.06	52.94	51.89	3.51	**	−0.009	ns	−0.03
Odor of butter	32.96	44.12	58.41	43.19	52.89	53.94	52.50	3.06	***	0.071	***	0.26
Odor of milk	48.52	30.09	51.15	47.10	39.72	37.61	37.70	3.63	***	−0.040	ns	−0.13
Unpleasant odor	3.15	1.90	3.29	1.90	2.03	1.83	1.83	0.66	ns	−0.006	ns	−0.13
Salty	25.56	20.74	23.97	21.54	19.00	11.06	12.72	2.41	***	−0.008	***	−0.38
Sweet	42.96	33.06	39.37	47.81	41.67	34.94	33.94	3.65	*	−0.028	ns	−0.10
Acid	29.72	18.64	15.80	18.49	9.44	10.06	10.06	2.54	***	−0.090	***	−0.40
Bitter	13.70	10.74	7.06	4.14	2.37	2.77	3.44	1.44	***	−0.056	***	−0.43
Spicy	6.29	2.31	2.74	3.12	1.56	3.11	3.45	0.80	***	0.006	ns	0.10
Chewiness	39.81	27.08	19.53	13.32	4.49	9.55	7.47	3.13	***	−0.161	***	−0.52
Solubility	65.18	59.21	80.99	73.37	90.61	89.78	89.78	3.35	***	0.162	***	0.50
Grittiness	8.33	12.57	5.52	6.61	6.83	12.72	30.06	3.24	***	0.107	***	0.38
Overall satisfaction	40.93	57.41	62.74	58.70	69.44	64.61	64.06	4.40	***	0.093	**	0.25

P value: *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; ns = not significant.

Table 5
Standardized canonical discriminant function coefficients.

Variable	1st canonical variable	2nd canonical variable
Color	0.66	**0.78**
Uniformity of structure	0.37	**0.55**
Strength of odor	0.05	−0.31
Odor of butter	**−0.73**	0.15
Odor of milk	**0.70**	0.29
Unpleasant odor	0.02	0.30
Salty	**0.50**	0.32
Sweet	0.36	−0.16
Acid	0.15	−0.03
Bitter	−0.04	−0.25
Spicy	−0.41	0.20
Chewiness	**0.65**	−0.47
Solubility	−0.12	**0.73**
Grittiness	**0.56**	0.03
Overall satisfaction	−0.10	−0.27
Explained variance (%)	43.2	35.1

Bold values signifies high loading values.

cheeses analyzed during the different time points of storage, except between 90 days and 120 days and between 120 days and 180 days (Table 4). The standardized canonical discriminant function coefficients (Table 5) showed that this distinction was caused by both the canonical variables, which together explained 78.3% of total variance. In particular the variables odor of butter, odor of milk, salty, chewiness and grittiness were most correlated with canonical 1, while color, uniformity of structure and solubility were more correlated with canonical 2.

Plot of Canonical 1 × Canonical 2 is reported in Fig. 2. All cheeses could be grouped into three areas. The area 1, which included the cheeses stored for 15 d (B), showed the characteristics of the fresh VdB cheeses, with lower colour (yellow), uniformity of structure and solubility versus cheeses stored for longer periods (areas 2 and 3). The area 3 has grouped the cheeses stored for 90, 120, and 180 days (E, F and G), showed significant differences along the canonical 1, if compared to the cheeses grouped in the area 2, stored for shorter periods (C and D). In particular, the cheeses in the area 3, respect those of area 2, presented less chewiness and grittiness, greater odor of butter and less odor of milk and lower perception of salty.

The good persistence of the odor of butter, the low perception of salt, acid and bitter flavors until the end of the observation period, together with the high solubility and low grittiness of VdB cheese stored up to 180 days, is a positive result for this cheese typology. In fact, despite the enzymatic processes that occurred in all cheeses during storage, the characteristics of freshness were nevertheless maintained in the cheeses stored for longer periods, so that an increasing of the overall satisfaction was observed too (Table 4).

4. Conclusions

The VdB cheeses stored for a long time, up to 180 days at 4 °C in the dark, showed a normal evolution of LAB microorganisms, while, despite the increase of pH, the low levels of undesirable bacteria and the absence of pathogenic species and spoilage/opportunistic pathogen bacteria, make healthy these cheeses. Storage caused a slight browning of VdB cheeses after 90 days, but this fact has not caused a decrease of acceptance by the tasters, that

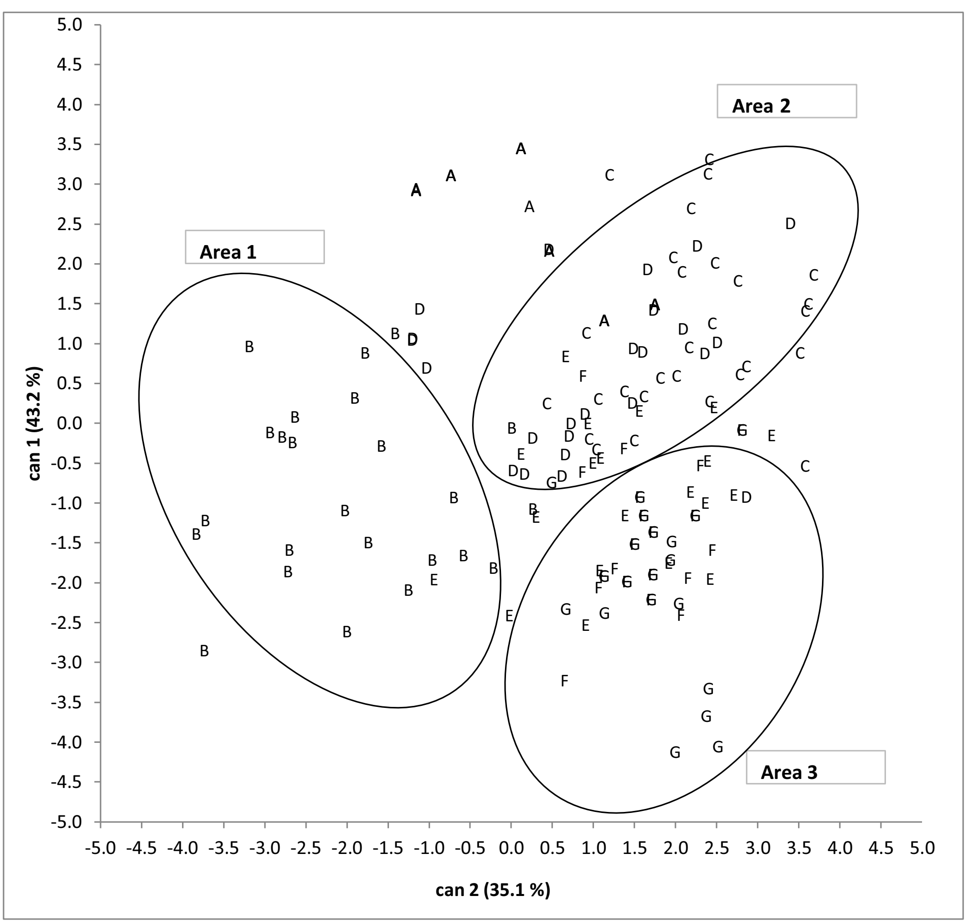

Fig. 2. Plot of Canonical 1 × Canonical 2 – variable: Storage.

found the cheeses after 180 days of storage more homogeneous in their structure, with higher solubility and with greater odor of butter. The stability of the cheese composition, including the FA composition and the high overall satisfaction at the panel test, provided evidences on the extension of shelf life of VdB cheeses for more than three months, indicated by the Consortium of protection.

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