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RESEARCH ARTICLE

Effect of potential adjunct culture on physico-chemical and ripening parameters of goat milk white brined cheese

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Abstract: This study aimed at assessing the potential Lactobacillus strain which could survive conditions prevalent in goat milk white brined cheese (GM-WBC). Six single strains of Lactobacillus were screened for acidification, salt tolerance (3-9%), proteolytic activity, lipolytic activity and compatibility with starter cultures. *Lactiplantibacillus plantarum* was most tolerant to salt (7% NaCl) followed by *Lacticaseibacillus casei*. All strains exhibited good proteolytic activity with maximum being observed for *L. casei*. Two strains i.e. *L. casei* and *L. plantarum* were selected and added individually into GM-WBC to study their effect on physico-chemical and biochemical characteristics during ripening. Free fatty acids and water-soluble nitrogen increased significantly throughout ripening for 60 days in all samples with highest in case of GM-WBC with *L. casei*. SDS-PAGE of cheese extract showed unknown bands in GM-WBC with *L. casei* sample from 15th day of ripening depicting higher proteolytic activity of *L. casei compared to L. plantarum*. Thus, *L. casei* could be used as a potential adjunct culture for manufacture of GM-WBC.

Keywords: Adjunct cultures, White brined cheese, *Lactobacillus species*, Ripening period, Physico-chemical properties

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Introduction

Survivability of goats under harsh conditions entitles them as poor man's cow. The higher digestibility, alkalinity, high buffering capacity and therapeutic benefits are the key biological properties possessed by goat milk (de Almeida Júnior et al. 2015). Mediterranean and Balkan regions are known for their brined cheeses which often lack a rind and taste salty and mildly acidic due to ripening by lactic acid bacteria and preservation in a thick brine (12 to 18% NaCl). Salt and acid are the crucial factors for preserving these cheeses during ripening (Hayaloglu et al. 2008).

Starter lactic acid bacteria (SLAB) and non-starter lactic acid bacteria (NSLAB) rule the cheese microbiota. SLAB is responsible for acid development while NSLAB (adjunct cultures) helps in improving organoleptic properties (Blaya et al. 2018). NSLAB tend to grow after ripening due to their tolerance to heat or acid treatment which helps in bacterial autolysis during later stages. Such process facilitates release of enzymes (Lazzi et al. 2016). The potential ability of NSLABs is to survive extreme conditions like low pH, presence of salt, low moisture and depleted nutrition helps to increase their count after few months of ripening (Settanni and Moschetti, 2010). Major species in NSLAB family include facultative heterofermentative mesophilic *Lacticaseibacillus casei*, *Lacticaseibacillus paracasei*, *Lactiplantibacillus plantarum, Lacticaseibacillus rhamnosus* etc. Other genera are *Pediococcus*, *Micrococcus* and *Leuconostoc* (Bozoudi et al. 2016).

Proteolysis results in protein breakdown and causes changes in texture, water activity, pH, amino acid profile, etc. It also develops cheese flavour and sometimes off-flavour (bitterness) by production of peptides and free amino acids by different pathways (Sousa et al. 2001). Proteolysis causes changes in textural attributes like increased softening and reduction in water activity of cheese curd due to changes in water binding activity by formation of new carboxylic acid and amino groups (Fox et al. 2004).

To prevent the adventitious growth of NSLAB, off flavours and monitor the consistency of cheese prepared, it is important to select the potential NSLAB strains. In this study, six

Lactobacillus strains were screened based on their ability to survive conditions of white brined cheese (WBC). White brined cheese was prepared from goat milk using selected adjunct cultures and its effect on physico-chemical and biochemical attributes was also studied.

Materials and Methods

Materials

Pooled goat milk of Beetal breed was collected from Experimental Dairy Plant of College of Dairy Science & Technology, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana. Microbial rennet (FROMASE-2200 TL granulate) from *Rhizomucor miehi* was procured from Essdee Marketing, Pune, Maharashtra, India. Blend of thermophilic and mesophilic (*Streptococcus thermophilus*, *Lactococcus lactis* and *Lactococcus cremoris*) starter culture was procured from Chr. Hansen (India) for making GM-WBC. Six single strains (*Lactobacillus acidophilus* (NCDC-15), *Lacticaseibacillus casei* (NCDC-17), *Lactobacillus helveticus* (NCDC-288), *Lactobacillus delbrueckii* subsp. *bulgaricus, Lactiplantibacillus plantarum* and *Lacticaseibacillus rhamnosus*) were collected from National Collection on Dairy Cultures (NCDC) and Molecular Biology Laboratory of National Dairy Research Institute, Karnal, Haryana, respectively.

Compositional analysis of milk

Raw goat milk was analysed for fat, solid-not-fat and protein content using Milkoscreen (IndiFoss, Ahmedabad, India). Acidity, ash and moisture content were determined according to the method described in AOAC (2005). pH was recorded using handheld pH meter (Cole Parmer, Mumbai, India). Casein content was determined using Pyne's method (Pyne, 1932).

In-vitro screening of adjunct cultures

Six single strains (*L. acidophilus* (NCDC-15), *L. casei* (NCDC-17), *L. helveticus* (NCDC-288), *L. delbrueckii* subsp. *bulgaricus, L. plantarum* and *L. rhamnosus*) were screened based on their ability to survive conditions of white brined cheese.

The compatibility of *Lactobacillus* strain with starter culture was determined by spotting 10 µl of each adjunct culture alongside the starter culture on nutrient agar (pH 6) followed by incubation at 37°C for 48 hrs. Zone of inhibition depicted incompatibility of adjunct culture with starter culture.

Acidification activity was measured for their ability to reduce pH with reference to Meng et al. (2018). Adjunct cultures were sub-cultured in 25 ml sterile reconstituted skim milk (10% w/v) aseptically and pH was recorded at 0, 3, 6, 18 and 24 h interval of incubation at 37°C.

Salt tolerance was determined by the method of da Silva Ferrari et al. (2016) at different salt concentration (3, 5, 7 and 9% w/w) via their growth in the MRS media containing 0.017% bromocresol purple solution. A change in colour from purple to yellow was observed which was the evidence of cell growth and positive result for salt tolerance.

Proteolytic activity was determined as per de Almeida Júnior et al. (2015) on skim milk agar (1% skim milk powder to nutrient agar) for formation of transparent halo zone around the spots was considered positive for proteolytic activity.

Lipolytic activity was determined as per Albayrak and Duran (2021) method using nutrient agar with 0.01% (w/v) $CaCl_2$ and 1% (v/v) of Tween 80 and observed for visual precipitation zones around colonies due to precipitation of $CaCl_2$ and fatty acids on lipolytic agar which indicated the ability of strains to lipolyze lipids.

Production of white brined cheese (WBC) using adjunct cultures

White brined cheese (WBC) was prepared from goat milk standardized to casein to fat ratio of 0.69-0.72 as per the method described by Zaravela et al. (2021) with slight modifications. Goat milk was batch pasteurized at 63°C/30 min followed by cooling at 35°C. Starter culture (calculated quantity mentioned on the DVS sachets, blend of thermophilic and mesophilic culture) was added along with 0.5% (v/v) of individual adjunct culture to cheese milk. Milk was left undisturbed for period of 60 min to allow culture to activate and hydrate itself in cheese milk. Later, 0.01% (w/v) of $CaCl_2$ and 13 mg/L of rennet was added at 35°C. Milk was allowed to set into firm curd for 50-60 min followed by cutting the curd into 2 cm^3 cubes. Cubes were heated at 35°C for 20 min with gentle stirring for every 5 min to avoid shattering of curd and further prevent lump formation and then transferred to perforated moulds to promote whey drainage. Once the curd was moulded, it was then pressed under its own weight. Curd mass was inverted every 1 hr interval for first 3-4 hrs followed by overnight whey drainage at 16-18°C in stability chamber till pH reached in the range of 4.6 to 4.8. The next day, cheese block was dry salted with 2.5% (w/w) of NaCl and left at 16-18°C in stability chamber (Macro Scientific Works, Delhi, India) for 24 h. The cheese blocks were then dipped in 7% brine solution containing 0.2% $CaCl_2$ and 5% citric acid until the pH reached to 4.6 and kept for ripening under brine at 4°C for 60 days. Control GM-WBC sample was prepared without addition of adjunct cultures while experimental samples were prepared using selected adjunct cultures such as *L. plantarum* (GM-WBC(P)) and *L. casei* (GM-WBC(C)) based on salt tolerance, acidification, proteolytic and lipolytic activity. The control and experimental samples were analysed for biochemical and physico-chemical characteristics at 15 days interval from 0 to 60 days.

Compositional and biochemical analysis of WBC

Fat, moisture, ash and acidity of GM-WBC samples were analysed as per AOAC (2005) method. pH of grated cheese (2 g mixed with 2 ml of distilled water) was measured using handheld pH meter (Cole Parmer, Mumbai, India). Salt content was analysed using Mohr's method. Total protein was calculated by Kjeldahl method (AOAC, 2005).

Free fatty acid (FFA) content was determined by extraction-titration method given by Deeth and Fitz Gerald (1976). Procedure of Kuchroo and Fox (1982) was adopted for determining water soluble nitrogen (WSN) content.

Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) was performed by the method given by Jin and Park (1996) to separate the protein fractions at different stages of ripening. Cheese samples for electrophoresis were prepared by the method given by Mbye et al. (2021). The protein in prepared sample was quantified in the sample using Nanodrop One (Thermo Fisher Scientific, Madison, USA). Broad range molecular weight markers of the range 3.5 to 205 KDa (GeNei, Bangalore, India) were used.

Statistical analysis

Statistical Software Package IBM SPSS version 26 was used to compare the results using two-way ANOVA and significant differences between groups was evaluated by Post hoc Tukey's test with a significance level P<0.05 throughout the study. The data was presented as mean ± standard deviation in Microsoft excel.

Results and Discussion

The average compositional analysis of goat milk used for preparing white brined cheese is given in Table 1.

***In vitro* screening of Lactobacillus adjunct cultures**

All *Lactobacillus* strains were compatible with blend starter culture since no zone of inhibition was observed (Fig. 1). Our results agreed with Kandola (2018) who demonstrated that cell free extracts of yoghurt culture *Lactobacillus delbrueckii* subsp. *bulgaricus* (Lb-09 and Lb-305) and *Streptococcus thermophilus* (ST-311 and ST-74) were compatible with *L. acidophilus* (LA-13).

Result for acidification activity is shown in Figure 2. Fastest reduction in pH was observed in case of *L. acidophilus* (pH

Fig 1. Spot assay of *Lactobacillus* species and blend of thermophilic and mesophilic starter culture (RST: Blend of thermophilic and mesophilic starter culture; P: *L. plantarum*; C: *L. casei*; R: L. *rhamnosus*; B: *L. delbruekii* subsp. *bulgaricus*; A: *L. acidophilus*; H: *L. helveticus*)

3.55) while *L. delbreukii* subsp. *bulgaricus* showed the slowest reduction in the pH value (5.84) from an initial pH of 6.55 after a period of 24 h. Nieto-Arribas et al. (2009) reported similar results with *L. plantarum* strains that showed low acidifying capacity than *L. paracasei* subsp. *paracasei* strains due to its heterofermentative nature. Herreros et al. (2003) also found that *L. plantarum* and *L. brevis* showed significantly lower acidification than most strains of *L. casei* subsp. *casei*. Strains of *L. casei* (TAUL 1522 and TAUL 1580) developed acidification at similar rates as of *Lactococci* after 24 h.

The growth of all six *Lactobacillus* species was inhibited at 9% salt concentration. Only *L. plantarum* and *L. casei* were able to tolerate till 7% salt concentration by showing growth which indicated by colour change while *L. bulgaricus* and *L. acidophilus* were unable to tolerate even 3% salt concentration (Fig. 3). *L. rhamnosus* and *L. helveticus* started to show inhibition at 5% salt concentration. Yao et al. (2020) suggested that two strains of *L. plantarum* isolated from fermented Chinese Dongbei kimchi showed no growth above 8% NaCl concentration. While strain of *L. plantarum* D_{31} was able to survive at 8% salt concentration. Nath et al. (2020) also reported that *L. plantarum*

Table 1. Composition of goat milk

Fat (%)	Solids-not-fat (%)	Protein (%)	Lactose (%)	Ash (%)	pH	Acidity (% lactic acid)
4.0 ± 0.2	8.7 ± 0.05	3.7 ± 0.08	4.0 ± 0.04	0.84±0.02	6.45±0.06	0.17 ± 0.01

strain GCC_19M1 isolated from fermented milk sample was able to survive till 7.5% salt concentration beyond which viability decreased.

All *Lactobacillus* strains exhibited proteolytic activity by forming halo zone around them while *L. casei* showed the maximum activity (Fig. 4). Meng et al. (2018) reported proteolytic activities in case of 9 *L. paracasei* and 6 *L. rhamnosus* strains isolated from goat milk semihard cheeses. Rahmati (2017) reported that 6 strains belonging to *L. casei*, *L. plantarum* and *S. cerevisiae* exhibited proteolytic activity with zone diameter ranging between 15-19 mm on agar plates. Ma et al. (2012) also stated that 36 strains of *Lactobacilli*, isolated from Chinese fermented milks exhibited proteolytic activity in the range 17-48 mg Gly/L milk. Suresh and Nampoothiri (2022) observed that two strains, *L. plantarum* FCW2 and *L. plantarum* FCW4, showed clear zones on MRS agar plates added with skim milk.

For lipolytic activity, precipitation was only observed around colonies of *L. rhamnosus*, *L. bulgaricus* and *L. casei* on lipolytic agar (Fig. 5). The results were in agreement with Rahmati (2017) who found only 5 strains out of 16 isolated from Iranian traditional dairy products (*L. casei* GYL1, *L. casei* AKL2, *L. casei* DDL2, *L. plantarum* ACL4 and *S. cerevisiae* DDy2) which could hydrolyse fat where *L. plantarum* possessed poor lipolytic activity. The results of Meng et al. (2018) are contradictory with ours' as they reported no lipolytic activity in *L. paracasei* and *L. rhamnosus* on tributyrin agar.

Based on these results, *L. plantarum* and *L. casei* were selected and used as adjunct culture in WBC from goat milk.

Compositional analysis of GM-WBC

Addition of adjunct cultures retains more moisture during ripening when compared to cheese made without adjunct culture. The moisture content of all samples increased significantly ($P<0.05$) during cheese ripening (Table 2). This might be due to the diffusion of brine water into the cheese matrix until an equilibrium was reached. Kocak et al. (2020) reported a significant ($P<0.05$) increase in moisture content with increased ripening period. Though, no significant ($P>0.05$) effect was reported by use of adjunct cultures. The increase in moisture content of white brined cheese was reported to be due to an increase in the water uptake at cold storage at low salt concentration (<20%) where migration of water occurred from brine to cheese. An increase in moisture is an indicative of formation of ionic bonds which have a water binding capacity by breaking of peptide bonds (Creamer and Olson, 1982). No significant difference ($P>0.05$) was observed in fat content between the samples (Table

Table 2. Composition of goat milk white brined cheese (GM-WBC) during ripening

Parameters	Days	Cheese samples		
		GM-WBC	GM-WBC(C)	GM-WBC(P)
Moisture (%)	0	61.70 ± 0.05^{dA}	62.15 ± 0.07^{dA}	62.33 ± 0.07^{cA}
	15	63.84 ± 0.03^{cB}	64.23 ± 0.09^{cA}	64.24 ± 0.01^{bA}
	30	64.09 ± 0.06^{bB}	64.32 ± 0.03^{bcA}	64.22 ± 0.01^{bA}
	45	64.23 ± 0.02^{aB}	64.44 ± 0.03^{abA}	64.25 ± 0.01^{bB}
	60	64.33 ± 0.04^{aC}	64.51 ± 0.02^{aA}	64.42 ± 0.02^{aB}
Fat (%)	0	15.62 ± 0.19^{aA}	15.64 ± 0.06^{aA}	15.63 ± 0.12^{aA}
	15	15.60 ± 0.11^{abA}	15.61 ± 0.15^{bA}	15.62 ± 0.08^{aA}
	30	15.58 ± 0.03^{bcA}	15.59 ± 0.14^{bcA}	15.58 ± 0.15^{bA}
	45	15.57 ± 0.07^{bcA}	15.58 ± 0.09^{cA}	15.58 ± 0.13^{bA}
	60	15.56 ± 0.02^{cA}	15.58 ± 0.07^{cA}	15.58 ± 0.11^{bA}
Protein (%)	0	14.63 ± 0.05^{aA}	14.64 ± 0.11^{aA}	14.63 ± 0.19^{aA}
	15	14.36 ± 0.06^{bA}	14.22 ± 0.16^{bB}	14.33 ± 0.13^{bA}
	30	14.24 ± 0.20^{cA}	14.05 ± 0.05^{cB}	14.24 ± 0.10^{cA}
	45	13.76 ± 0.18^{dA}	13.58 ± 0.05^{dB}	13.74 ± 0.12^{dA}
	60	13.34 ± 0.05^{eA}	13.24 ± 0.16^{eB}	13.32 ± 0.13^{eA}
Salt (%)	0	4.06 ± 0.08^{cA}	4.07 ± 0.10^{cA}	4.09 ± 0.05^{bA}
	15	4.02 ± 0.05^{dA}	4.02 ± 0.07^{dA}	4.02 ± 0.06^{cA}
	30	4.27 ± 0.04^{bA}	4.26 ± 0.05^{bA}	4.28 ± 0.08^{aA}
	45	4.28 ± 0.06^{abA}	4.29 ± 0.09^{aA}	4.28 ± 0.08^{aA}
	60	4.30 ± 0.05^{aA}	4.31 ± 0.02^{aA}	4.32 ± 0.07^{aA}

Values (mean ± SD, n = 3) with different superscript 'abcde' within column indicates the days of ripening, has significant ($P<0.05$) effect on dependent variables.
Values (mean ± SD, n = 3) with different superscript 'ABC' among row indicates the type of adjunct culture, has significant ($P<0.05$) effect on dependent variables.

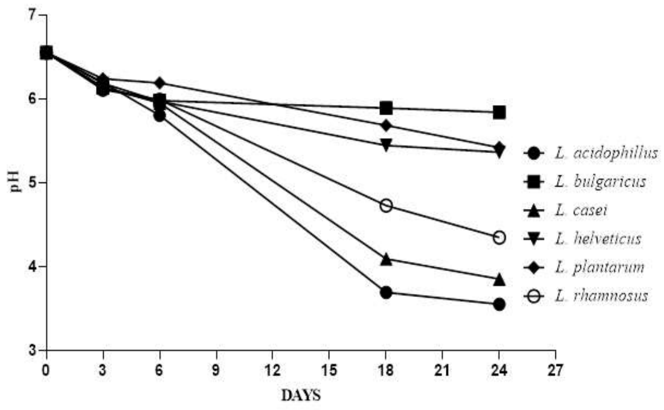


Fig 2. Acidification activity of different *Lactobacillus* species

Fig 3. Proteolytic activity of different Lactobacillus species (P: *L. plantarum*; C: *L. casei*; R: L. *rhamnosus*; B: *L. delbruekii* subsp. *bulgaricus*; A: *L. acidophilus*; H: *L. helveticus*)

Fig 4. Lipolytic activity of *Lactobacillus* species (a) *L. rhamnosus* (b) *L. delbruekii* subsp. *bulgaricus* (c) *L. casei*

2). While decrease in fat content ($P<0.05$) was observed during ripening period in all samples due to increase in moisture content which affected the overall composition of WBC. Lipolysis might also contribute a decreased fat content of cheese. The protein content of all the samples decreased significantly ($P<0.05$) during the ripening due to protein degradation (Table 2). Smiljanić et al. (2014) reported higher levels of protein loss in white brined cheese from goat or ovine milk than from cow milk due to compact structure of cow milk cheese. However, Protein content of control and GM-WBC(P) showed no significant difference ($P>0.05$). Salt content showed a significant ($P<0.05$) increase in all samples due to diffusion of salt inside the curd to balance the osmotic pressure of cheese and brined water. However, no significant difference was observed between treatments throughout storage period. Kaminarides et al. (2019) also reported a significant ($P<0.05$) increase in salt content of sample stored under brine (7% NaCl) at 4°C due to diffusion of water from brine to cheese to attain osmotic pressure equilibrium.

Physicochemical analysis

A sudden increase in the pH value for all the samples during initial days of ripening (at 15th day) could be attributed to the onset of proteolysis which are basic in nature (Poullet et al. 1991). Thereafter, pH decreased significantly ($P<0.05$) due to adaptation of lactic acid bacteria and production of lactic acid (Table 3). The results of change in pH during ripening were in accordance with Mahmoudi et al. (2012), who claimed significant ($P<0.05$) increase and then decrease in pH of the of Iranian white brined cheese containing starter culture (*L. lactis* subsp. *lactis*, *L. lactis subsp. cremoris*, *L. delbrueckii* subsp. *bulgaricus* and *S. thermophilus*) and in sample prepared using *Bifidobacterium animalis* during 60 days of ripening. GM-WBC(C) and GM-WBC(P) samples had

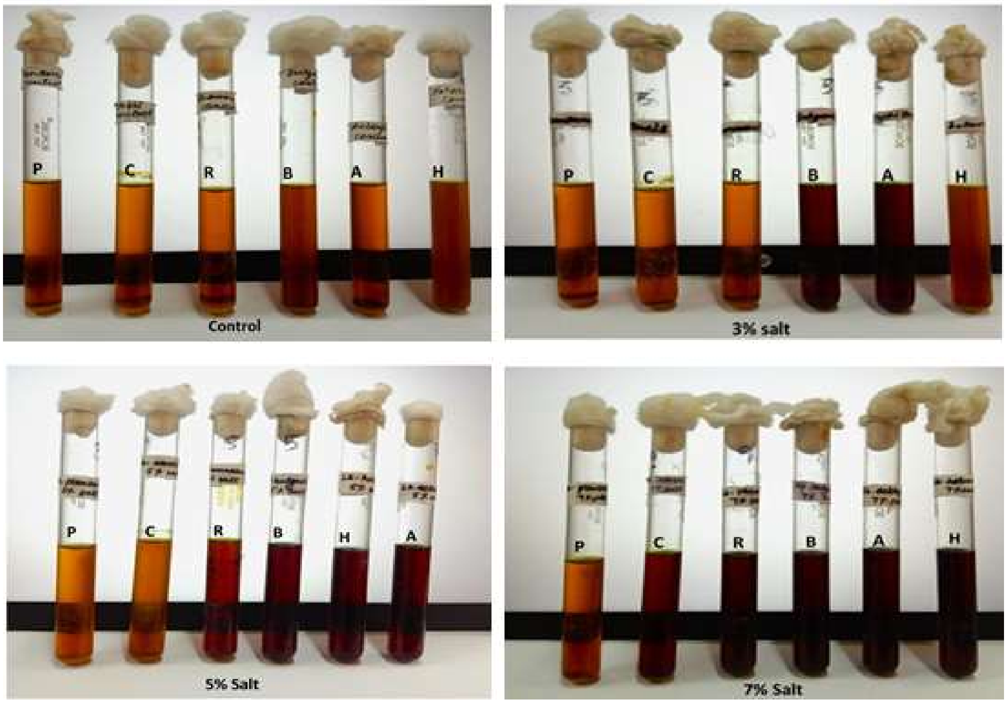


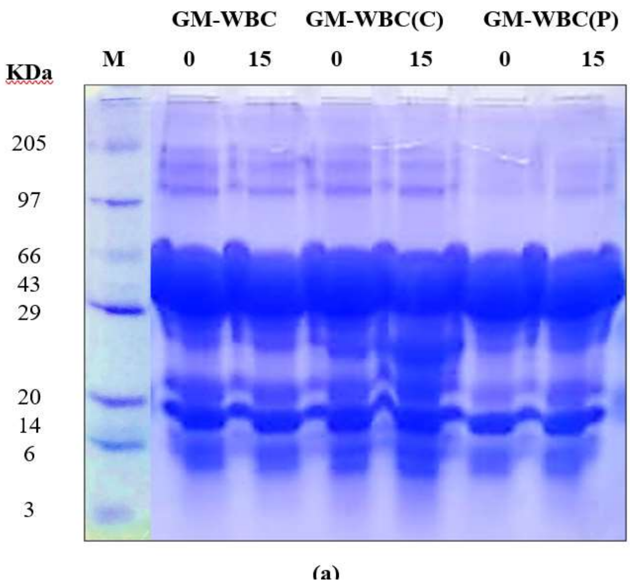
Fig 5. Salt tolerance of Lactobacillus species at different salt concentrations (control (0), 3, 5 and 7%) (P: *L. plantarum*; C: *L. casei*; R: *L. rhamnosus*; B: *L. delbruekii* subsp. *bulgaricus*; A: *L. acidophilus*; H: *L. helveticus*)

Table 3. Physicochemical parameters of goat milk white brined cheese (GM-WBC) during ripening

Parameters	Days	Cheese samples		
		GM-WBC	GM-WBC(C)	GM-WBC(P)
pH	0	4.75 ± 0.01^{cA}	4.60 ± 0.01^{dC}	4.65 ± 0.01^{bB}
	15	4.81 ± 0.01^{aB}	4.84 ± 0.01^{aA}	4.81 ± 0.01^{aB}
	30	4.74 ± 0.01^{cA}	4.72 ± 0.01^{bB}	4.70 ± 0.01^{bC}
	45	4.72 ± 0.01^{dA}	4.67 ± 0.01^{cB}	4.70 ± 0.01^{bA}
	60	4.77 ± 0.01^{bA}	4.62 ± 0.01^{dB}	4.40 ± 0.01^{dC}
Acidity (% lactic acid)	0	0.535 ± 0.04^{cB}	0.765 ± 0.05^{bA}	0.734 ± 0.02^{cA}
	15	0.556 ± 0.03^{bcC}	0.813 ± 0.05^{abA}	0.746 ± 0.05^{abB}
	30	0.565 ± 0.02^{bcC}	0.834 ± 0.06^{abA}	0.746 ± 0.09^{abB}
	45	0.587 ± 0.01^{bC}	0.860 ± 0.04^{aA}	0.797 ± 0.05^{bB}
	60	0.627 ± 0.02^{aC}	0.898 ± 0.08^{aB}	1.308 ± 0.02^{aA}
Water soluble nitrogen (%)	0	0.21 ± 0.10^{dA}	0.23 ± 0.06^{dA}	0.20 ± 0.10^{cA}
	15	0.22 ± 0.09^{dB}	0.29 ± 0.07^{cA}	0.24 ± 0.06^{bcB}
	30	0.26 ± 0.08^{cB}	0.35 ± 0.08^{bA}	0.26 ± 0.08^{bB}
	45	0.32 ± 0.07^{bC}	0.44 ± 0.10^{aA}	0.36 ± 0.04^{aB}
	60	0.38 ± 0.07^{aB}	0.46 ± 0.05^{aA}	0.39 ± 0.09^{aB}
Free fatty acids (% oleic acid)	0	0.441 ± 0.14^{dB}	0.569 ± 0.07^{dA}	0.551 ± 0.11^{dA}
	15	0.445 ± 0.11^{dC}	0.663 ± 0.14^{cB}	0.767 ± 0.14^{cA}
	30	0.792 ± 0.14^{cB}	1.180 ± 0.07^{bA}	0.781 ± 0.12^{cB}
	45	0.845 ± 0.14^{bC}	1.185 ± 0.07^{abA}	0.871 ± 0.11^{bB}
	60	0.981 ± 0.13^{aB}	1.208 ± 0.06^{aA}	0.992 ± 0.09^{aB}

Values (mean ± SD, n = 3) with different superscript 'abcd' within column indicates the days of ripening, has significant (P<0.05) effect on dependent variables.
Values (mean ± SD, n = 3) with different superscript 'ABC' among row indicates the type of adjunct culture, has significant (P<0.05) effect on dependent variables.

lower pH values than control due to high acidification activity of adjunct cultures. A significant (P<0.05) increase in titratable acidity of cheese added with adjunct cultures compared to control with ripening period was observed. GM-WBC(P) showed a sudden increase in the acidity after 60th day of ripening due to the better adaptability of *L. plantarum* to salt at later stages (Table 3).



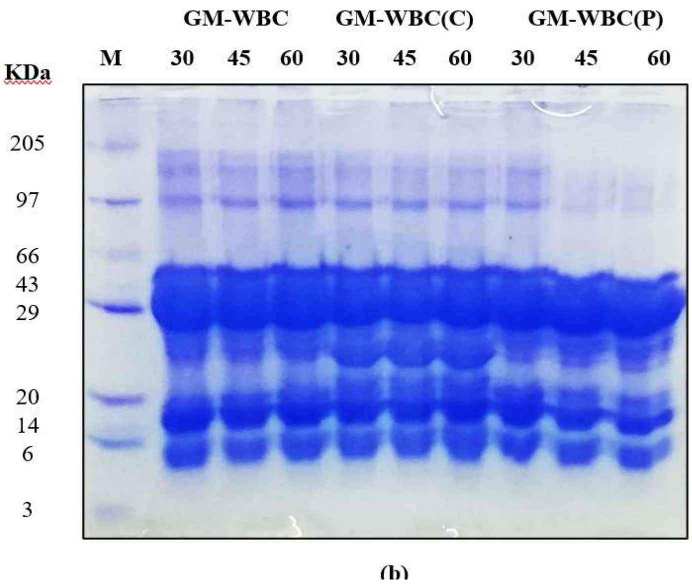


Fig 6. Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) of proteins of goat milk white brined cheese (GM-WBC) with or without adjunct culture ripened for (a) 0 and 15 days (b) 30, 45 and 60 days (M - Molecular weight marker ranging from 3 to 205 KDa).

Dabevska-Kostoska et al. (2015) reported a significant increase ($P<0.05$) in the titratable acidity due to addition of *L. casei* as adjunct culture in white brined cheese compared to control sample.

Water soluble nitrogen (WSN) is used as an index for maturation or cheese ripening. WSN consists of high, medium and low molecular weight peptides along with free amino acids (Fox et al. 2004). Significant increase ($P<0.05$) in the levels of WSN was observed in all samples during ripening. Highest WSN content was observed in GM-WBC(C) due to more proteolytic activity of *L. casei* (Table 3). Kocak et al. (2020) reported similar results with significant ($P<0.05$) increase in WSN content from 0.12 to 0.67% at 90th day of ripening of WBC prepared using *L. casei* as adjunct culture. Addition of *L. plantarum* MU12 and *L. plantarum* S6-4 increased WSN-total nitrogen values from 4 to 21% and 4.8 to 32%, respectively from 30 to 90 days in Cheddar cheese (Duan et al. 2019). The Free fatty acids (FFA) content of cheese samples showed a significant ($P<0.05$) difference among control and treated samples throughout ripening period. FFA content was highest for GM-WBC(C) than the GM-WBC(P) and control which might be due to lipolytic nature of *L. casei* (Table 3). Kumar et al. (2015) reported a significant ($P<0.05$) increase in the levels FFA content during ripening. Highest FFA (4.23μg/g) was reported at 60th day in case of feta cheese made with *L. helveticus*. A consistent increase in FFA with ripening period was reported due to increase in intracellular lipases produced by *Lactobacilli species*.

Electrophoresis

Figure 6 represents the SDS-PAGE results of the GM-WBC samples at 15 days interval during ripening for 60 days under brine. The results of electrophoresis suggests that there had been some degradation of α and β fractions of caseins especially in GM-WBC(C) sample after 15th day ripening which was shown by formation of extra bands (Fig. 6). However, no such extra bands were observed in control as well as GM-WBC(P) sample after 15 days of ripening which represented minimal degradation of casein. This could be due to inhibition of hydrolysis of casein especially β-casein by rennin or pepsin due to presence of salt. Plasmin activity is minimal due to pH and salt conditions in white brined cheese (Hashemi et al. 2009). Our results were in agreement with Tarakci and Tuncturk (2008) who observed degradation of α_s-casein fractions more in the case of cheese prepared using *L. helveticus* due to enhanced activity by starter and non-starter lactic acid bacteria on decreasing pH.

Conclusions

Among the six strains of *Lactobacillus* screened for adjunct cultures, *L. casei* and *L. plantarum* were selected for preparation of GM-WBC based on salt tolerance, acidification, proteolytic and lipolytic activity. The study suggests the use of *L. casei* as potential adjunct culture in GM-WBC that not only improves the physico-chemical properties but also initiates ripening at early stages. The results are suggestive of formation of degradation products due to onset of ripening which might have some bioactivity.

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