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Influence of Adjunct Cultures of *Lactobacillus* spp. on Chemical, Microbiological and Sensory Properties of White Pickled Cheese

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This paper reports a comparison of changes in the microflora and chemical parameters related with *Lactococcus* and *Lactobacillus* cultures during 90 days ripening in Turkish white pickled cheese. One control (K) and four experimental cheeses were manufactured by the addition of *Lactobacillus casei* (A) 1 % and (B) 10^6 cfu mL^{-1} and *Lactobacillus plantarum* (E) 1 % and (F) 10^6 cfu mL^{-1} plus commercial starter culture comprising *Lactococcus lactis* subsp. lactis and *Lactococcus lactis* subsp. cremoris. As a result of the ripening process, all the sensorial, microbiological and chemical characteristics were significantly affected and some *Lactobacillus* ssp. might be considered suitable as adjunct cultures in the Turkish white pickled cheese production process because of their positive contribution to cheese flavour.

Key Words: Cheese ripening, Chemical properties, White pickled cheese.

INTRODUCTION

White pickled cheese is widely consumed in Balkan Countries. The average annual rate of this cheese is *ca.* 67 % of total cheese production in Turkey. It is manufactured as a semi soft cheese from cow's, sheep's, or a mixture of cow's and sheep's and goat's milk and ripened in brine (14-15 % salt). Initially, this cheese was manufactured from non-pasteurized milk, but in the last decades, it has been produced from pasteurized milk using commercial lactic cultures. In the manufacturing process, *Lactococcus* spp. strains of starter cultures are used and ripened for 90 d. However, it has been determined that the microflora, represented by the *Lactobacillus casei*/*paracasei* (*Lb. casei/paracasei*) and *Lactobacillus plantarum* (*Lb. plantarum*) strains, predominate during the greater ripening period. These strains could predominate during the last stages of ripening period when the starter culture components are no longer present. These microorganisms are known to dominate over the microflora of many other cheeses. These species were also found in Domiati cheese[1], Bulgarian white pickled cheeses made from cow's and ewe's milk during the first month of ripening[2] and Greek white pickled cheese[3]. Facultatively heterofermentative *Lactobacilli* (FHL) are one of the principal groups of non-starter microflora isolated from similar white pickled cheeses. The species *Lb. casei/paracasei* and *Lb. plantarum* are the

main components of the non-starter microflora, with the first of these being the most common component in a number of different cheeses such as Serra da Estrella[4], Cheddar[5], Emmental[6] and Fiore Sardo[7]. Therefore, FHL has the potential to be used as adjunct cultures in the cheese milk to improve the flavour of the cheese[8]. According to Mannu *et al.*[7], the microorganisms *Lb. casei/paracasei* and *Lb. plantarum* are the only ones that continue to multiply during cheese ripening, reaching the level of 10^7 colony-forming unit (cfu) g^{-1}. To reach these levels, the FHL must have a suitable energy source other than milk lactose, which is not available in the cheese due to the activity of the starter culture. Therefore non-starter mesophilic *Lactobacilli* have the ability to utilize sugars as an energy source where the lactose level is very low, such as in the cheese environment[9]. Citrate is also a potential source of carbon and strains of *Lb. casei* and *Lb. plantarum* are capable of metabolizing this compound derived from the fat globule membrane glycoproteins[10]. Nevertheless, the available citrate concentration is usually insufficient for these bacteria to reach such high levels and the other sources of energy from proteolysis and lipolysis[11] or from the autolysis of other bacteria may be used. During this process, aromatic amines and volatile components are produced and could contribute to the aroma development in cheese. In addition, this mechanism may control undesired detrimental microbial activities *e.g.* of clostridia and gas-forming *Lactobacilli*[12]. In this study, we aimed to examine the effects of different adjunct cultures *Lb. casei* and *Lb. plantarum* (FHL) on the development of chemical, microbiological and sensory characteristics of white pickled cheeses during ripening.

EXPERIMENTAL

Whole cow's milk was supplied from a dairy plant (Tekirdag, Turkey). Commercial calf rennet was obtained from Mayasan Company, Istanbul, Turkey. Commercial starter culture comprising *Lactococcus lactis* subsp. *lactis* and *Lactococcus lactis* subsp. *cremoris* (Lactoprox MT 1001-1 20398 Lot 313223-50 UA) was supplied from Chr. Hansen's (Kopenhagen, Denmark). Adjunct cultures (*Lb. casei* and *Lb. plantarum*) were obtained from Microbiology Culture Collection of Ankara University, Department of Food Engineering (Ankara, Turkey). All the chemicals were supplied by Merck (Darmstadt, Germany).

For cheese making, five cheese batches were manufactured: A control batch was made from pasteurized cow's milk (K); second (A) and third (B) batches were made with the addition of different rates of Lb casei in order of 1 % and 10^6 cfu mL^{-1}. Fourth (E) and fifth (F) batches were made with the addition of *Lb plantarum* at two different rates, 1 % and 10^6 cfu mL^{-1}, in order of. A commercial starter comprising *Lactococcus lactis* subsp. *lactis* and *Lactococcus lactis* subsp. *cremoris* was also added to all vats at different rates (1.5 % for A and E groups; 2.25 for % B and F groups). The FHL strains of the two species used as the adjunct cultures were selected strains isolated and identified from white pickled cheeses in an earlier work. In each trial, pasteurized (72 °C for 20 min) cow's milk was cooled to 32 °C, divided

into 5 equal batches and inoculated with determined levels of the mentioned cultures. Calcium chloride was also added to the milk at a level of 0.2 g L^{-1}. The inoculated milk (32 °C) was held for *ca.* 0.5 h until the pH reached 6.30 and liquid (1/10.000) calf rennet was added at a concentration of 0.1g L^{-1} of cheese milk (sufficient to coagulate the milk in 1.5 h). Following coagulation, the coagulum was cut into cubes (2 to 3 cm^3 sides) and allowed to rest for 10 min for whey releasing. The curds were carefully transferred from the cheese vat into the molds. After 1 h of draining (without pressing), pressure was applied at room temperature (21 °C) for 3 h or until whey drainage had stopped and the block of cheese was cut into cubes of about 7 cm × 7 cm × 7 cm with a knife. The pieces, weighing 350 to 400 g each, were placed in brine (14 % NaCl) for *ca.* 12 h at 21 °C. After salting, the cheese blocks were packed in cans (16 cm × 8 cm × 8 cm) and covered with brine. The cans, which contained about 1 kg of cheese were sealed hermetically and the cheese samples ripened at 6 to 8 °C for 90 d. Samples obtained from pasteurized milk were analyzed at 1st, 30th, 60th and 90th days of ripening period. Trials were made in duplicate.

The following groups of microorganism were examined: Lipolytic bacteria and proteolytic bacteria on modified nutrient agar (MNA)[13], total bacteria were determined with plate count agar (PCA)[13], mould and yeast counts were determined with pH adjusted potato dextrose agar (PDA) and *Lactobacillus* spp. on MRS agar[14].

The percentage of dry matter (DM) and fat were determined according to IDF methods[15,16]. A combined electrode digital pH meter (WTW pH 330/SET-1) was used for pH determinations. The Kjeldahl method was used for total nitrogen (TN) and water soluble nitrogen (WSN) determinations[17,18]. Salt concentration was determined by titration with $AgNO_3$[19]. The total concentration of acid degree value (ADV) in the cheeses was determined according to the method described by Nunez *et al.*[20].

Cheese samples were evaluated organoleptically in one month intervals by a 7 member non-professional tasting panel. This panel graded the surface appearance (1-5), inter appearance (1-5) structure (1-5), odour (1-5) and taste (1-5) according to the Turkish Standards for Turkish White pickled cheese[21].

The SPSS program (version 11.0, SPSS Inc. Chicago, IL, USA.) was used for statistical analysis. For evaluation of the obtained values, variance analysis was done and differences among the means were compared with Duncan's Multiple Range Test.

RESULTS AND DISCUSSION

Microbiological properties: Table-1 summarizes the changes that took place in populations of the total bacteria, *Lactobacilli*, coliform, proteolytic, lipolytic bacteria and mould and yeast. Starting out at similar levels (108 cfu g^{-1}), the total bacteria content declined in all cheese batches over the ripening period. Significant differences were observed during the ripening period ($p < 0.05$). The decrease in total bacteria was most marked in all the cheeses examined. The decline in total

TABLE-1
MICROBIOLOGICAL PROPERTIES OF WHITE PICKLED CHEESE DURING RIPENING (log cfu g^{-1})

Trials	Days	Total bacteria	Lipolytic bacteria	Proteolytic bacteria	Lactobacillus	Mould/yeast	Coliform
K	0	8.301^{abB}	5.602^{abC}	4.146^{aA}	4.732^{aA}	1.778^{cC}	3.88^{aB}
	30	8.579^{abC}	5.176^{abB}	4.681^{aC}	5.079^{aAB}	1.763^{cC}	1.908^{aA}
	60	7.763^{abA}	4.819^{abA}	4.462^{aBC}	8.792^{aB}	1.732^{cB}	-
	90	7.672^{abA}	4.505^{abA}	3.477^{aAB}	5.204^{aAB}	1.544^{cA}	-
A	0	8.392^{bB}	6.176^{bC}	4.643^{bA}	7.770^{bA}	1.748^{aC}	3.838^{aB}
	30	8.698^{bC}	5.380^{bB}	5.523^{bC}	7.707^{bAB}	1.755^{aC}	2.792^{aA}
	60	7.929^{bA}	4.845^{bA}	5.146^{bBC}	7.672^{bB}	1.431^{aB}	-
	90	7.792^{bA}	4.591^{bA}	4.982^{bAC}	7.278^{bAB}	1.380^{aA}	-
B	0	8.301^{aB}	5.491^{aC}	4.880^{bA}	6.113^{bA}	1.792^{abC}	3.643^{aB}
	30	8.342^{aC}	4.924^{aB}	5.324^{bC}	6.863^{bAB}	1.732^{abC}	2.643^{aA}
	60	7.079^{aA}	4.633^{aA}	5.322^{bBC}	6.913^{bB}	1.505^{abB}	-
	90	7.623^{aA}	4.397^{aA}	5.176^{bAB}	6.826^{bAB}	1.361^{abA}	-
E	0	8.530^{bB}	5.863^{abC}	4.982^{bA}	7.633^{bA}	1.763^{bcC}	3.301^{aB}
	30	8.643^{bC}	5.602^{abB}	6.146^{bC}	7.255^{bAB}	1.792^{bcC}	2.146^{aA}
	60	7.662^{bA}	4.301^{abA}	5.322^{bBC}	7.880^{bB}	1.633^{bcA}	-
	90	7.579^{bA}	4.414^{abA}	5.079^{bAB}	7.176^{bAB}	1.518^{bcA}	-
F	0	8.301^{abB}	5.419^{aC}	4.342^{bA}	6.255^{bA}	1.799^{cC}	3.633^{aB}
	30	8.707^{abC}	5.477^{aB}	5.792^{bC}	6.707^{bAB}	1.819^{cC}	2.301^{aA}
	60	7.477^{abA}	4.301^{aA}	5.518^{bBC}	9.991^{bB}	1.662^{cB}	-
	90	7.643^{abA}	4.176^{aA}	5.204^{bAB}	6.707^{bAB}	1.579^{cA}	-

a, b, c indicate differences among the trials ($p < 0.05$).
A, B, C, D indicate differences among the ripening periods ($p<0.05$)
(K) Control, (A) *Lactobacillus casei* 1 %, (B) *Lactobacillus casei* 10^6 cfu mL^{-1},
(E) *Lactobacillus plantarum* 1 %, (F) *Lactobacillus plantarum* 10^6 cfu mL^{-1}.

bacteria were reported to be more gradual in cheddar cheese[22] and Swiss-type cheese[23] made from raw milk compared to cheeses made from pasteurized or microfiltered milk.

Coliform group bacteria were no longer detectable in the cheeses after only 30 days of ripening. It is thought that the bacteriocins produced by contaminant non-starter *Lactobacilli* and the adjunct cultures of these microorganisms may affect bacterial survival. Different studies have shown that strains of FHL have antibacterial substances active against a wide range of bacteria[24,25]. *Lactobacilli* were not detected in the pasteurized milk, as reported in other works[26,27]. In present study, *Lactobacilli* were also determined in the cheeses made from the pasteurized milk without the addition of adjunct culture (K). But, Lactobacillus counts were low and may be ascribed to contamination during cheese manufacture[28]. The counts of *Lactobacilli* found in the cheeses could also be due to recovery of heat-shocked cells. *Lactobacilli* levels displayed significant differences ($p < 0.05$) among the four cheese batches over the course of ripening. On day 1, Lactobacillus counts were highest in the cheeses in which the experimental adjunct cultures had been added (batches A and E).

Lactobacillus counts remained nearly constant in all the batches between 30-90 days of ripening and then remained constant in the cheeses in batch B and F at around 10^6 cfu g^{-1} and in batches A and E at around 10^7 cfu g^{-1}. These findings indicate that the *Lactobacilli* selected as adjunct cultures for this experiment survived well in all cheese groups and indeed grew rapidly. Yeast and mould counts in all cheese groups were similar at the beginning of the ripening process. Similar results were observed in many soft and semi-soft cheese varieties, probably originating from the processing equipment and the environment[29]. A significant source of yeast contamination is the brine solution[30]. During the ripening process, the yeast-mould counts were gradually decreased.

Physico-chemical properties: Table-2 shows the physico-chemical properties of the cheeses. As a result of the ripening process, dry matter content of the cheeses increased ($p < 0.05$). Nevertheless, during the first 30 days, dry matter values attained the same levels in all experimental samples. The fat contents of K, A, B, E and F group cheeses were significantly different from each other ($p < 0.05$) during the ripening period, with the highest levels being recorded for the cheeses containing adjunct culture of *Lb. plantarum* (10^6 cfu mL^{-1}). The NaCl content of all cheeses ranged from 4.65 to 5.02 % on day 30 and increased in the ripened cheese ranging from 5.02 to 5.27 %. Adjunct culture addition and the ripening process caused significant differences among the four cheese batches ($p < 0.05$). During the ripening period, the pH in all cheese groups declined due to acid production by cheese micro-organisms. The decrease in pH in these batches during cheese making was lower than the values reported by Olarte *et al.*[31], probably as a consequence of the action of the *Lactobacilli* added as adjunct cultures. The results showed that there were no differences among the control and experimental samples. The slight increases in pH until the end of ripening period can be attributed to the consumption of lactic acid by yeasts and to the release of ammonia[32]. As a result of the evolution of proteolysis in the cheeses, were no statistically significant differences between the water soluble nitrogen contents of the experimental and control cheeses. On the other hand, the intentional addition of *Lb casei* increased the proteolysis more significantly than *Lb plantarum* in terms of water soluble nitrogen content. The water soluble nitrogen contents of the cheeses were found to be similar to those reported by Tzanetakis and Litopoulous[3]. Giori *et al.*[33] and Peterson and Marshall[28] reported that *Lb. casei* has a higher proteolytic activity than *Lb plantarum*. The extent of lipolysis of cheeses expressed as acid degree value increased continuously during the ripening period, but cheese A had significantly higher ($p < 0.05$) acid degree values than the other cheese groups (K, B, E and F). At day 90th, the acid degree value's were 9.850 meq-KOH 100 g^{-1} fat and 8.575 meq-KOH 100 g^{-1} fat for cheese A and cheese B, respectively. The results indicate that each adjunct starter culture group and the amounts in which they were used resulted in a different level of acid degree value in the cheese samples. Similar acid degree value's have been reported by Georgala *et al.*[34].

TABLE-2
PHYSICO-CHEMICAL PROPERTIES OF WHITE PICKLED CHEESE DURING RIPENING

Trials	Days	DM (%)	Fat (%)	Salt (%)	pH	TN (%)	WSN (%)	WSN/TN (%)	ADV (meq KOH 100 g^{-1} fat)
K	0	41.42^{bA}	17.00^{aA}	4.43^{aA}	4.75^{aC}	2.608^{aA}	0.252^{bA}	9.662^{bA}	4.050^{aA}
	30	41.12^{bA}	16.50^{aAB}	4.65^{aB}	4.60^{aB}	2.684^{aB}	0.677^{bC}	25.22^{bC}	5.630^{aB}
	60	41.82^{bAB}	17.00^{aBC}	4.88^{aC}	4.54^{aA}	2.761^{aC}	0.618^{bB}	22.38^{bB}	6.750^{aC}
	90	$42,24^{bB}$	17.50^{aC}	5.08^{aD}	4.52^{aA}	2.780^{aBC}	0.761^{bD}	27.37^{bD}	8.225^{aD}
A	0	40.85^{aA}	16.00^{aA}	4.51^{aA}	4.81^{bC}	2.783^{bA}	0.307^{bA}	11.03^{bA}	6.175^{bA}
	30	40.86^{aA}	16.00^{aAB}	4.70^{aB}	4.75^{bB}	2.845^{bB}	0.693^{bC}	24.35^{bC}	6.850^{bB}
	60	41.28^{aAB}	17.25^{aBC}	4.68^{aC}	4.66^{bA}	2.905^{bC}	0.641^{bB}	22.06^{bB}	7.315^{bC}
	90	41.12^{aB}	17.00^{aC}	5.02^{aD}	4.62^{bA}	2.854^{bBC}	0.799^{bD}	27.99^{bD}	9.850^{bD}
B	0	41.11^{abA}	16.00^{aA}	4.20^{aA}	4.76^{aC}	2.814^{cA}	0.281^{bA}	9.98^{abA}	4.975^{aA}
	30	41.37^{abA}	16.50^{aAB}	4.66^{aB}	4.63^{aB}	2.945^{cB}	0.669^{bC}	22.71^{abC}	5.760^{aB}
	60	41.43^{abAB}	16.25^{aAC}	4.76^{aC}	4.56^{aA}	2.959^{cC}	0.577^{bB}	19.49^{abB}	6.915^{aC}
	90	41.61^{abB}	17.00^{aC}	5.02^{aD}	4.55^{aA}	2.918^{cBC}	0.786^{bD}	26.93^{abD}	8.575^{aD}
E	0	40.44^{abA}	16.00^{aA}	4.64^{bA}	4.70^{bC}	2.738^{bA}	0.348^{abA}	12.71^{abA}	4.855^{aA}
	30	41.17^{abA}	16.25^{aAB}	5.02^{bB}	4.77^{bB}	2.871^{bB}	0.651^{abC}	22.67^{abC}	5.850^{aB}
	60	41.47^{abAB}	17.25^{aBC}	5.22^{bC}	4.73^{bA}	2.909^{bB}	0.562^{abB}	19.25^{abB}	6.225^{aC}
	90	42.46^{abB}	18.00^{aC}	5.27^{bD}	4.69^{bA}	2.894^{bBC}	0.678^{abD}	23.42^{abD}	7.680^{aD}
F	0	42.08^{bA}	17.00^{bA}	4.43^{bA}	4.77^{aC}	2.767^{bcA}	0.253^{aA}	9.14^{aA}	4.750^{aA}
	30	41.52^{bA}	17.75^{bAB}	4.81^{bB}	4.62^{aB}	2.907^{bcB}	0.621^{aC}	21.36^{aC}	5.300^{aB}
	60	41.72^{bAB}	17.75^{bBC}	5.35^{bC}	4.54^{aA}	2.908^{bcC}	0.528^{aB}	18.15^{aB}	7.015^{aC}
	90	42.21^{bB}	18.50^{bC}	5.37^{bD}	4.54^{aA}	2.919^{bcBC}	0.641^{aD}	21.95^{aD}	7.350^{aD}

a, b, c indicate differences among the trials ($p < 0.05$).
A, B, C, D indicate differences among the ripening periods ($p < 0.05$).
(K) Control, (A) *Lactobacillus casei* 1 %, (B) *Lactobacillus casei* 10^6 cfu mL^{-1}
(E) *Lactobacillus plantarum* 1 %, (F) *Lactobacillus plantarum* 10^6 cfu mL^{-1}

Sensory properties: The cheese samples prepared by supplementation with *Lb. plantarum* and *Lb. casei* and ripened for 2 months were most preferred by the panelists. As a result of the ripening process, all the sensorial characteristics were significantly affected and the total flavour scores of all the groups increased in the first two months of the ripening period (Table-3). *Lactobacilli* cultures substantially improved the taste of experimental cheeses relative to the control (K). The cheeses manufactured with *Lactobacilli* had the highest scores compared to the control group cheese.

In conclusion, addition of different amounts of the adjunct cultures of *Lb. plantarum* and *Lb. casei* significantly affected most of the physicochemical properties of cheeses made from pasteurized cow's milk over the course of the ripening period ($p < 0.05$). Chemical and sensory evaluation data indicated that some *Lactobacillus* ssp. might be considered suitable as adjunct cultures in the white pickled cheese production process because of their contribution to cheese flavour. The two different adjunct cultures (*Lb. casei* and *Lb. plantarum*) used in this research had good implementation

TABLE-3
SENSORY PROPERTIES OF WHITE PICKLED CHEESE DURING RIPENING

Trials	Days	Surface appear	Inter appear	Structure	Odour	Taste	Total score
K	30	4.3^{cA}	4.6^{aA}	4.2^{aA}	4.2^{aA}	3.8^{aA}	21.1
	60	4.5^{cB}	4.7^{aA}	4.6^{aB}	4.5^{aB}	4.0^{a}	18.7
	90	4.7^{cC}	4.8^{aA}	4.7^{aC}	4.4^{aA}	4.0^{aA}	19.0
A	30	4.3^{bcA}	4.6^{aA}	4.2^{aA}	4.2^{abA}	4.0^{aA}	17.7
	60	4.5^{bcB}	4.6^{aA}	4.6^{aB}	4.3^{abB}	4.1^{aA}	22.1
	90	4.6^{bcC}	4.4^{aA}	4.6^{aC}	4.3^{abA}	4.0^{aA}	21.9
B	30	4.1^{aA}	4.5^{aA}	4.2^{aA}	4.3^{abA}	3.9^{aA}	21.0
	60	4.3^{aB}	4.5^{aA}	4.5^{aB}	4.5^{abB}	4.0^{aA}	21.8
	90	4.6^{aC}	4.5^{aA}	4.7^{aC}	4.3^{abA}	4.1^{aA}	22.2
E	30	4.1^{abA}	4.4^{aA}	4.2^{aA}	4.1^{aA}	4.2^{aA}	21.0
	60	4.4^{abB}	4.7^{aA}	4.5^{aB}	4.3^{aB}	4.2^{aA}	22.1
	90	4.6^{abC}	4.7^{aA}	4.7^{aC}	4.3^{aA}	4.0^{aA}	22.3
F	30	4.2^{bcA}	4.4^{aA}	4.2^{aA}	4.4^{bA}	3.7^{aA}	20.9
	60	4.5^{bcB}	4.5^{aA}	4.7^{aB}	4.5^{bB}	4.1^{aA}	22.3
	90	4.7^{bcC}	4.6^{aA}	4.7^{aC}	4.3^{bA}	4.2^{aA}	22.5

a, b, c indicate differences among the trials ($p < 0.05$).
A, B, C, D indicate differences among the ripening periods ($p < 0.05$)
(K) Control, (A) *Lactobacillus casei* 1 %, (B) *Lactobacillus casei* 10^6 cfu mL^{-1}
(E) *Lactobacillus plantarum* 1 %, (F) *Lactobacillus plantarum* 10^6 cfu mL^{-1}

in the cheese and both of them had a synergic effect on the *Lactococci*. Addition of the adjunct cultures in pasteurized cheeses rapidly decreased the levels of contaminant microorganisms, possibly due to the release of antimicrobial substances. In general, the cheeses with adjunct cultures showed similar microbiological changes with the control group during the ripening period. Therefore, the adjunct culture constituted by *Lb. casei* and *Lb. plantarum* can be considered to be suitable for making white pickled cheese from pasteurized cow's milk due to the characteristics of accelerated cheese ripening and improved sensorial properties.

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