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Influence of Starter Cultures on the Accumulation of Histamine and Tyramine in Vacuum-Packaged Otlu (Herby) Cheese During Ripening

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Effect of cheese starters (*Lactococcus lactis* ssp. *lactis* and *Lactococcus lactis* ssp. *cremoris*; CC), yoghurt starters (*Streptococcus thermophilus* and *Lactobacillus delbrueckii* ssp. *bulgaricus*; YC) and helveticus starter (*Lactobacillus helveticus*; HC) on histamine, tyramine contents and some microbiologic properties of Otlu (herby) cheese were investigated in this study. The moisture and salt contents of cheese were similar for difference cultures but pH of CC cheese was higher than those of other cultures at the beginning of the ripening. Histamine and tyramine contents of CC cheese, on the other hand, were lower than other cheese samples and increased significantly during ripening. Coliform counts were determined as under < 1 at the end of the storage period in all cheeses while *Enterobactericeae* counts were decreased approximately to a value of 1 cfu. Lactic acid bacteria, yeast and mould counts of helveticus culture cheeses at 60 days was lower than that of other ($p < 0.05$) samples. Results indicated that addition of the different starter cultures on Otlu cheeses had no significant ($p > 0.05$) effect on compositional (moisture, salt contents and pH value) and microbiological (total aerobic mesophilic bacteria, coliform and *Enterobactericeae* counts) properties when compared with the other cheese samples.

Key Words: Otlu (herby) cheese, Starter cultures, Histamine, Tyramine.

INTRODUCTION

Herby cheese ("Otlu Peynir" in Turkish) has been produced and consumed in Eastern and Southern cities of Turkey for a long time. Although there are many traditional cheese varieties in Turkey, their production is largely based on small-scale dairies and family farms. The industrial production of Otlu cheese (cheese added different herbs) has been introduced recently. Otlu cheese, a semi-hard, salty and herb added cheese, is manufactured in small family businesses for their needs and commercial purposes from raw sheep's and cow's milk, between May and June, in the Eastern and South-Eastern of Turkey. For the production; Otlu cheese milk is filtered through a coot-cloth and heated up to 30-35 ºC. Then, it is coagulated by using rennet. Following the coagulation, curd is cut into small cubes. The herbs

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were added on the curd weight. The herbs commonly used in cheese are as follows: *Allium* sp., *Chaerophyllum macropodum*, *Antriscus nemorosa*, *Silene vulgaris*, *Ferula* sp., *Prangos* sp., *Tymus* sp. and *Mentha* sp. and they are added to cheese at different ratios[1]. These herbs have odour, flavour and bio preservative characteristics in the dairy products and they are added to cheese at different ratios. Nevertheless, the traditional production method is still more desirable among the consumers. Starter cultures (*lactococci* and *lactobacilli*) used for cheese fermentation degrade milk proteins by means of proteases and peptidases to peptides and free amino acids. These breakdowns of the products support the growth of starter cultures and are also important for the achievement of the characteristic texture and aroma of the cheese. On the other hand, they may increase the potential for amine formation during cheese fermentation, which exhibit a decarboxylase activity, when microorganisms are present[2]. Utilization of starter cultures enables producers to make food products with a standard quality in a shorter time.

Histamine accumulates, especially during the ripening in cheese, due to the presence of lactic acid bacteria coming from milk and other sources. The formation of free amino acids and biogenic amines is in fact directly influenced by the bacterial activity, pH and salt concentration. Water activity, storage temperature and ripening time all influence amine formation indirectly[3]. Different biogenic amines are predominant in different kind of cheeses. Putrescine, cadaverine and tyramine were predominant biogenic amines in Beyaz cheeses manufactured with and without starter culture. Tyramine was also reported as predominant biogenic amine in Feta and Domiati cheeses. The content of histamine, phenylethylamine and cadaverine is correlated with the number of *lactobacilli*, tyramine, with number of *enterococci*, putrescine and with the number of Enterobacteria, respectively[4]. The predominant amines in Turkey are putrescine, cadaverine, histamine, tyramine and spermidine in fresh Kasar, Mihalic, Otlu (herby) and Orgu cheeses. Phenylethylamine, cadaverine, tyramine and spermidine are predominant in Urfa cheese while the predominance of putrescine, cadaverine, histamine and tyramine is observed in Civil cheese. Putrescine and tyramine are also predominat in Beyaz cheese; which is commonly consumed in Turkey. There is a lack of information regarding the effects of starter cultures on the accumulation of histamine and tyramine in Otlu (herby) cheese during ripening which is the aim of this study.

EXPERIMENTAL

Full fat cow milk was obtained from a local dairy plant. Cheese cultures (*Lactococcus lactis* ssp. *lactis* and *Lactococcus lactis* ssp. *cremoris*), yoghurt cultures (*Streptococcus thermophilus* and *Lactobacillus delbrueckii* ssp. *bulgaricus*) and helveticus culture (*Lactobacillus helveticus*) obtained from Rhodia Food, Ezal®, Dange Saint-Roman and France were used as starter cultures. Commercial rennet was obtained from Mayasan Company®, Istanbul. Herbs known as "sirmo" (*Allium* sp.) in eastern region were obtained from dairy products market in Van, Turkey.

Cheese manufacturing: The raw cow's milk was pasteurized at 65 °C for 0.5 h, cooled to 32 °C and then divided into three groups. Starter cultures, *Lactococcus lactis* ssp. *lactis* and *Lactococcus lactis* ssp. *cremoris* (cheese starters, CC), *Streptococcus thermophilus* and *Lactobacillus delbrueckii* ssp. *bulgaricus* (yoghurt starters, YC) and *Lactobacillus helveticus* (helveticus culture, HC) were then added at the ratio of 1 % in each group and incubated for 0.5 h. Milks were coagulated with rennet for 1.5 h. Curds were cut into 8-10 mm cubes with a wire knife. The herb was added to the all groups with the ratio of 1 % milk weight following the coagulation. The herbs were then thoroughly mixed into the curds. Curds were pressed and all cheeses were then cut into blocks with dimensions of 7 cm × 7 cm × 5 cm. These cheese blocks were salted with dry salt (5 %) and kept for 2 days. After the 2 days period, vacuum-packed cheeses were stored in the 7 ± 1 °C to ripe the experiment cheese for 3 months.

Chemical analyses: The moisture content was analyzed by oven-drying (Nuve, Ankara) in a laboratory oven at 105 ºC until a constant weight was obtained. Salt content was determined according to the Mohr method and the pH value of each sample was recorded using a digital pH meter (pH 211, Hanna Instruments, Centralino, Italy) equipped with a glass electrode that was inserted directly into the cheese samples as described by Case *et al.*[5]. All analyses were performed with duplicates.

Histamine and tyramine analyses: Sample preparation and biogenic amines (histamine and tyramine) analyses were carried out as described by Hurst[6]. The fluorescence of the compound was measured by fluorometery (Perkin-Elmer LS 50 B Luminescence spectrometer, Wellesley, USA). The developed fluorescence intensity was recorded using an excitation wavelength of 350 nm and emission wavelength of 450 nm for histamine and an excitation wavelength of 464 nm and emission wavelength of 520 nm for tyramine[7]. The standard curves were automatically obtained by fluorometery using standard solutions. The results were expressed as mg/100 g wet weight.

Microbiological analyses: Representative 10 g cheese samples were homogenized with 90 mL of sterile 2 % (w/v) sodium citrate solution at 45 °C in a stomacher blender (2300/400, Barcelona, Spain) for 2 min. Decimal dilutions were prepared in 0.1 % sterile peptone water and plated in duplicate. Total aerobic mesophilic bacteria (TAMB) counts were realized on Plate Count Agar (Oxoid) at 30 °C for 48 h. Coliforms and *Enterobacteriaceae* were determined on Violet Red Bile Lactose (Merck) and Violet Red Bile Glucose Agar (Merck), respectively and then incubated at 30 °C for 24-48 h. Lactic acid bacteria were grown anaerobically on M17 agar (oxoid) with its pH reduced to 5.7 at 30 °C for 5 days and cycloheximide (Sigma) was added (100 mg/L) to prevent the growth of yeasts. Yeasts and moulds were created on Potato Dextrose Agar (Oxoid) adjusted to pH 3.5 with 10 % lactic acid (Merck) at 25 °C for 3 days. After the incubation, colonies were counted and results were expressed as log cfu/g of the cheese[8].

Statistical analyses: Statistical analyses of data for determining the effects of starter cultures and ripening times on some chemical, histamine and tyramine contents and microbiological properties on Otlu cheese were performed by ANOVA procedures using SAS[9]. The differences between means were evaluated by implementing the Duncan's multiple range tests.

RESULTS AND DISCUSSION

The values of moisture, salt, pH values and biogenic amine contents of cheeses at different ripening times are presented in Table-1. The moisture contents of HC culture added cheeses were slightly higher than cheeses containing CC and YC. It was found that starter cultures and ripening periods did not significantly affect the moisture content ($p > 0.05$). The salt contents of cheeses were varied between 3.57 and 4.09 %. The salt content of all cheese samples did not show a change during ripening. The type of starter culture types was not found to be important on the salt content ($p > 0.05$) of Otlu cheese. The rate of salt absorption was very high at the first month due to the movement of NaCl molecules, as a result of the osmotic pressure and difference in the moisture contents of cheeses[10]. Salting of the cheese can influence the pH value of the cheese due to its effect on microbial activity. Low levels of salt can stimulate bacterial activity whereas concentrations that are higher than 2.5 % display a negative effect[11]. The pH values of cheeses were simply statistically ($p < 0.05$) affected by starter cultures types in 2 days. The pH value significantly ($p < 0.05$) decreased until the 30 days of ripening and then displayed a change slowly. The decrease in the pH value is a result of the production of organic acids (primarily lactic acid) in which *Lactobacillus* is responsible for most of the sugar fermentation[10].

The histamine and tyramine values of HC culture added cheeses were higher than cheese samples containing CC and YC, as can be seen in Table-1. The histamine and tyramine values showed a significant ($p < 0.05$) increase during the ripening of all cheese samples. These values are much lower than those reported for Swiss cheese and Cheddar cheese by Taylor *et al.*[11] and Voight and Eitenmiller[12], respectively but almost similar with the findings of Tarakci *et al.*[13] and Ekici *et al.*[14] for Tulum cheese with black cumin and pasteurized and non-pasteurized Otlu cheeses, respectively. Since the main factor for the production of biogenic amines in cheese is the presence of microorganism with high decarboxylation activity, several studies have been published on this concept matter[15]. This microorganism presence is in fact even desirable in most types of cheese because proteolysis and acidification are very important factors for the quality of the final product. Therefore, minimizing tyramine accumulation by changing the production conditions would seem not to be the solution. The solution, rather, is to prevent the appearance of tyramine producing bacteria. In this respect, the use of pasteurized milk acquires special importance[16].

Some bacteria growing on the surface of Munster cheeses, on the other hand, degrade histamine. Strains of *B. linens* reduced histamine and tyramine contents, in course of deamination, by 55-70 % during a 4-week ripening period[2]. Yetismeyen[17],

TABLE-1
CHEMICAL AND BIOCHEMICAL PROPERTIES OF
CHEESE SAMPLES DURING RIPENING

Property	Cheese	Ripening time (day)			
		2	30	60	90
Moisture (%)	CC	54.34 ± 1.06	53.09 ± 0.65	54.32 ± 1.56	53.57 ± 1.56
	YC	55.12 ± 0.79	54.92 ± 2.65	55.08 ± 0.29	55.61 ± 1.60
	HC	56.18 ± 1.25	56.74 ± 0.44	56.13 ± 2.07	55.87 ± 0.54
pH	CC	$5.59 \pm 0.05^{a,A}$	5.37 ± 0.04^{B}	5.28 ± 0.06^{B}	5.34 ± 0.05^{B}
	YC	$5.54 \pm 0.01^{a,A}$	5.36 ± 0.02^{B}	5.31 ± 0.04^{B}	5.28 ± 0.04^{B}
	HC	$5.43 \pm 0.04^{b,A}$	5.29 ± 0.04^{B}	5.26 ± 0.03^{B}	5.27 ± 0.06^{B}
Salt (%)	CC	3.71 ± 0.52	3.60 ± 0.18	3.59 ± 0.05	3.80 ± 0.35
	YC	3.89 ± 0.33	3.84 ± 0.53	3.65 ± 0.16	3.45 ± 0.15
	HC	3.57 ± 0.26	3.62 ± 0.27	3.86 ± 0.13	4.09 ± 0.22
Histamine (mg/100 g)	CC	3.015 ± 0.05^{C}	13.068 ± 3.23^{B}	19.670 ± 0.13^{B}	$37.596 \pm 5.88^{b,A}$
	YC	3.252 ± 0.11^{C}	14.495 ± 1.59^{B}	18.179 ± 2.35^{B}	$43.159 \pm 0.65^{ab,A}$
	HC	3.169 ± 0.09^{D}	9.622 ± 1.10^{C}	43.875 ± 0.71^{B}	$51.627 \pm 0.83^{a,A}$
Tyramine (mg/100 g)	CC	20.462 ± 0.80^{C}	$27.085 \pm 4.63^{ab,BC}$	$44.069 \pm 3.55^{b,B}$	$89.529 \pm 15.38^{b,A}$
	YC	16.885 ± 4.29^{C}	$30.981 \pm 3.06^{a,B}$	$34.269 \pm 0.05^{c,B}$	$107.198 \pm 2.21^{ab,A}$
	HC	16.424 ± 4.93^{C}	$17.657 \pm 3.11^{b,C}$	$77.642 \pm 3.05^{a,B}$	$122.591 \pm 2.81^{a,A}$

CC; cheese added *Lc. lactis* ssp. *lactis* and *Lc. lactis* ssp. *cremoris*, YC; cheese added *St. thermophilus* and *Lb. delbrueckii* ssp. *bulgaricus*, HC; cheese added *Lb. helveticus*. ^{abc}Letters indicate differences ($p < 0.05$) between culture groups. ^{ABC}Letters indicate differences ($p < 0.05$) between ripening times.

who investigated[18] samples of Otlu cheese, found a positive relationship between the formation of biogenic amines and the levels of titratable acidity, protein, water-soluble nitrogen, non-protein nitrogen and tyrosine[19]. Durlu-Ozkaya[18] reported that the concentrations of amines in Otlu cheese were much lower than the toxic levels. Histamine levels in brined herbs used in the manufacturing of Otlu cheese were determined by Ekici *et al.*[14] and 23.8, 23.0, 24.9 and 24.2 mg/kg histamine were found in brined herbs including *Allium*, *Ferula*, *Anthriscus* and *Silene*, respectively. These values can be considered negligible for Otlu cheese to which the herbs are added at a level of 1 %.

Changes in some microbiological properties of the cheese samples are shown in Table-2. Total aerobic mesophilic bacteria (TAMB) counts decreased until 30 d, then increased and again decreased during ripening and these changes were significant for YC and HC cheese samples ($p < 0.05$). Otlu cheeses, which the starters cultures were added, were not statistically ($p > 0.05$) significant regarding TAMB counts. Coliform bacteria counts, on the other hand, decreased statistically ($p < 0.05$) in 60 days of ripening and all cheeses were not treated for 90 days. The results found were similar to the results reported by other researchers. The coliform counts of HC culture added cheeses were higher than cheeses containing CC and YC. This difference might be high for biogenic amines in the same cheese samples. Statistical differences between *Enterobactericeae* counts of the cheese samples were not significant ($p >$

0.05) and *Enterobactericeae* counts decreased continuously during the ripening. The lactic acid bacteria (LAB) was the dominant bacteria during the storage time and may contribute to the production of cheese. The general trend for lactic acid bacteria values was to increase up during 60 days of storage period and then the values decreased. The lactic acid bacteria and yeast-mould counts of HC culture added cheeses in 60 days was merely higher, statistically, than cheeses containing CC and YC cultures ($p < 0.05$). Yeast and mould levels in the samples decreased continuously during ripening and they were lowest at the end of ripening. Yeast and moulds may grow in the higher acidity values. Microorganisms possessing the enzyme histidine decarboxylase, which converts histidine to histamine, are responsible for the formation of histamine in foods. One organism among them, *Lactobacillus buchneri*, may be vital to the dairy industry due to its involvement in cheese-related outbreaks of histamine-poisoning. The toxicity of histamine appears to be enhanced by the presence of other biogenic amines found in foods that can inhibit histamine metabolizing enzymes in the small intestine[20].

TABLE-2
MICROBIAL PROPERTIES OF CHEESE SAMPLES DURING RIPENING

Property	Cheese	Ripening time (day)			
		2	30	60	90
Total aerobic mesophilic bacteria	CC	9.26 ± 0.51	8.57 ± 0.59	9.27 ± 0.52	8.04 ± 0.06
	YC	8.45 ± 0.23^{B}	8.77 ± 0.45^{B}	9.66 ± 0.03^{A}	8.14 ± 0.08^{B}
	HC	8.55 ± 0.29^{BC}	8.83 ± 0.03^{B}	9.48 ± 0.20^{A}	8.05 ± 0.21^{C}
Coliform	CC	$3.69 \pm 0.12^{b,A}$	$3.25 \pm 0.13^{b,B}$	< 1	< 1
	YC	$4.66 \pm 0.13^{a,A}$	$4.51 \pm 0.12^{a,A}$	3.08 ± 0.00^{B}	< 1
	HC	4.35 ± 0.10^{a}	4.64 ± 0.11^{a}	3.23 ± 1.31	< 1
Enterobacteriaceae	CC	4.26 ± 0.67^{A}	3.06 ± 0.40^{AB}	3.15 ± 0.78^{AB}	1.15 ± 1.63^{B}
	YC	3.71 ± 1.00	3.97 ± 0.26	3.40 ± 0.28	< 1
	HC	4.17 ± 0.55^{A}	4.04 ± 1.05^{A}	2.89 ± 0.16^{B}	1.95 ± 2.75^{B}
Lactic acid bacteria	CC	8.41 ± 0.10^{C}	8.72 ± 0.05^{B}	$9.56 \pm 0.11^{a,A}$	8.18 ± 0.04^{D}
	YC	8.64 ± 0.37^{B}	8.69 ± 0.10^{B}	$9.45 \pm 0.04^{a,A}$	8.28 ± 0.03^{B}
	HC	8.58 ± 0.33	8.36 ± 0.18	8.62 ± 0.40^{b}	8.28 ± 0.28
Mould and yeast	CC	7.23 ± 0.83^{A}	7.42 ± 0.16^{A}	$7.53 \pm 0.11^{ab,A}$	5.94 ± 0.91^{B}
	YC	6.92 ± 0.48^{B}	7.37 ± 0.86^{AB}	$8.69 \pm 0.42^{a,A}$	6.86 ± 0.31^{C}
	HC	6.90 ± 0.23^{A}	7.25 ± 0.27^{A}	$6.93 \pm 0.47^{b,A}$	6.04 ± 1.05^{B}

CC; cheese added *Lc. lactis* ssp. *lactis* and *Lc. lactis* ssp. *cremoris*, YC; cheese added *St. thermophilus* and *Lb. delbrueckii* ssp. *bulgaricus*, HC; cheese added *Lb. helveticus*. ^{ab}Letters indicate differences ($p < 0.05$) between culture groups. ^{ABCD}Letters indicate differences ($p < 0.05$) between ripening times.

However, the selection of the starter culture should be realized by only considering not only the lactic acid production of the strains but also their activity in biogenic amine synthesis[21]. Biogenic amines are organic bases with an aliphatic, aromatic or heterocyclic structure, which have been found in many foods, such as fishery products, cheese, wine, beer and other fermented foods[21]. Utilization of raw or pasteurized milk in cheese making, higher ripening temperature, excessive proteolysis, high pH

and low salt concentration may contribute to the ability of an organism to produce histamine[22]. The increases in the amine content of cheese, however, may be attributed to various microorganisms. These microorganisms may form the flora, associated with the milk used to make the cheese, by the contamination during cheese making and storage. Thus, they may be added to the cheese deliberately as a part of the starter cultures[23].

In conclusion, it would be appropriate to say that the amount of histamine and tyramine in Otlu cheese increases during the ripening period. On the other hand, it is clear that no general deduction may be derived from this fact since there is no standardization in Otlu cheese production and these types of cheese may be kept ripening for longer periods than the ripening period in this study. The histamine and tyramine contents of cheeses with CC starters (*Lactococcus lactis* ssp. *lactis* and *Lactococcus lactis* ssp. *cremoris*) were higher than the values of yoghurt cultures (*Streptococcus thermophilus* and *Lactobacillus delbrueckii* ssp. *bulgaricus*) and helveticus culture (*Lactobacillus helveticus*). Nonetheless, it must be further investigated whether high maturing temperatures are significant in terms of histamine and tyramine formation in herb cheese or not through detailed researches in the future.

REFERENCES

1. Z. Tarakci and N. Akyuz, *Int. J. Food Prop*., **12**, 496 (2009).
2. R.G.K. Leuschnert and W.P. Hammes, *J. Food Protect*., **61**, 874 (1998).
3. S. Vale and M.B.A. Gloria, *J. AOAC Int*., **60**, 651 (1997).
4. F. Durlu-Ozkaya, E. Alichanidis, E. Litopolou-Tzanetaki and N. Tunail, *Milchwissenschaft*, **54**, 680 (1999).
5. R.A. Case, R.L. Bradley and R.R.Williams, In: Standard Methods for the Examination of Dairy Products, Am. Public Health Assoc., edn. 15, Baltimore, 327 (1985).
6. W.J.J. Hurst, *J. Liq. Chromatogr*., **13**, 1 (1990).
7. S.L. Rice, R.R. Eitenmiller and P.E. Koehler, *J. Milk Food Tech*., **38**, 256 (1975).
8. Apha, Compendium of Methods for the Microbiological Examination of Foods, Washington, (1992).
9. Sas/Stat Software, Changes and Enhancements Through Release 6.12., Sas Inst., Inc., Cary, N.C. (1998).
10. T.P. Guinee and P.F. Fox, In: Cheese Chemistry, Physics and Microbiology, London 257 (1993).
11. S.L. Taylor, J.T. Kefe, S.E. Windham and J.F. Howell, *J. Food Protect*., **45**, 455 (1982).
12. M.N. Voigt and R.R. Eitenmiller, *J. Food Protect*., **41**, 182 (1978).
13. Z. Tarakci, K. Ekici, O. Sagdic and E. Kucukoner, *Arch Lebensmittelhyg*, **56**, 135 (2005).
14. K. Ekici, H. Coskun, Z. Tarakci, E. Ondol and R. Sekeroglu, *J. Food Biochem*., **30**, 362 (2006).
15. Z. Oner, O. Sagdic and B. Simsek, *Eur. Food Res. Tech*., **219**, 455 (2004).
16. M. Fernandez, D.M. Linares, A. Rodriguez and M.A. Alvarez, *Appl. Microbiol. Biotech*., **73**, 1400 (2007).
17. A. Yetismeyen, Ankara University Scientific Research Project Report, Turkey (2005).
18. F. Durlu-Ozkaya, *J. Food Process. Preservat*., **26**, 259 (2002).
19. A.A. Hayaloglu and P.F. Fox, *Dairy Sci. Tech*., **88**, 245 (2008).
20. J.E. Stratton, R.W. Hutkins and S.L. Taylor, *J. Food Protect*., **54**, 460 (1991).
21. A. Halasz, A. Barath and W.H. Holzapfel, *Z. Lebensm. Unters. Forsch. A*, **208**, 434 (1999).
22. F. Gardini, M. Martuscelli, M.C. Caruso, F. Galgano, M.A. Crudele, F. Favati, M.E. Guerzoni and G. Suzzi, *Int. J. Food Microbiol*., **64**, 105 (2001).
23. M. Marino, M. Maifreni, S. Moret and G. Rondinini, *Lett. Appl. Microbiol*., **31**, 169 (2000).

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