*Effect of adjunct cultures on some properties of white-brined cheese*
Proceedings of European Congress of Chemical Engineering (ECCE-6)
Copenhagen, 16-20 September 2007

# The effect of adjunct cultures on some chemical and biochemical properties of white-brined cheese

**Zekai Tarakci[1], Yusuf Tuncturk[2]**

[1]*Department of Food Science and Technology, Agriculture Faculty, Ordu University, 52200, Ordu, Turkey*
[2]*Food Engineering, Faculty of Agriculture, University of Yuzuncu Yil, 65080 Van, Turkey*

## Abstract

The influence of different adjunct cultures on the some chemical and biochemical properties of White-brined cheese was investigated. Four batches of cheese were produced: C, with no added adjuncts, containing only cheese culture (*Lactococcus lactis* ssp. *lactis plus Lc. lactis* ssp. *cremoris*); CY, containing C culture plus yoghurt culture (Streptococcus *thermophilus* + *Lb. delbrueckii* ssp. *bulgaricus*); CH, containing C culture plus Lactobacillus helveticus and CYH, containing all of them. Analyses were carried out on days of 2, 30, 60 and 90 of ripening. It was found that the addition of different adjunct starters to the cheese influenced ($P<0.01$) the chemical composition significantly, except salt contents ($P>0.05$). Total solids, protein, fat and pH values were higher in C cheese than the others. On the other hand, titratable acidity of C and CY cheeses was lower than that of CH and CYH cheeses. Although the lipolysis (acid degree value, ADV), water-soluble nitrogen (WSN) and trichloro acetic acid-soluble nitrogen (TCA-SN) values of cheeses were affected significantly ($P<0.01$) from these treatments, no significant differences exist ($P>0.05$) among phosphotungstic acid-soluble nitrogen (PTA-SN) values. Cheeses made with CYH culture had the highest lipolysis, WSN and TCA-SN. Degradation rates of $\alpha s_1$- and β-casein were higher in CYH cheese followed by CY, CH and C cheeses. Also, breakdown products of casein were higher in CY, CH and CYH cheeses compared to control cheese. The amounts of $\alpha s_1$- and β-caseins of all cheese samples decreased continuously during the ripening period, while the amounts of $\alpha_{S1}$-I casein and other breakdown products increased.

Keywords: White cheese, adjunct cultures, lipolysis, proteolysis, urea-PAGE electrophoresis

## 1. Introduction

Starter cultures contribute to cheese sensory quality with their peptidase activity. In artisanal cheese production, the cheese is made without the deliberate addition of a starter culture relying on the indigenous flora of the milk for ripening. Recently, mesophilic and/or thermophilic starter cultures were used in the manufacture of Turkish White-brined cheese. Little information on using adjunct culture in White

cheese has been reported and may provide information to improve the process and ripening by enhancing the quality of the final product while preserving its typical nature. Adjunct cultures can accelerate flavor development or contribute additional flavor to cheese as it ages, and control bitterness in aged cheeses depending on the fermentation process and adjunct type. They can also accelerate cheese ripening, which may allow substantial cost savings for the cheese industry. Adjunct cultures can be defined as selected strains of cheese related microorganisms that are added to the cheese milk to improve development of cheese sensory quality (El Soda *et al.* 2000). Recently, starter manufacturers are offering to cheese makers the adjunct cultures, some of which seem to have positive effects on the quality of conventional full-fat cheese, while others are more appropriate for enhancement of the quality of low-fat cheese. In most cases, the information offered by the culture manufacturer is limited to the levels of aminopeptidase activity of the culture (El Soda 1997). As known, it is desirable to have starters with low proteolytic but high peptidolytic activities (Mistry 2001). However, if they do not contribute through peptidase and esterase activities, some adjunct cultures may contribute little to flavour development in cheese (El Soda et al. 2000). Thus, the selection of appropriate adjunct cultures is becoming the important aspect of successful cheese production (Katsiari et al. 2002). For this purpose, *Lactococcus lactis* ssp*., Streptococcus thermophilus, Lactobacillus helveticus and Lactobacillus delbrueckii* ssp. *bulgaricus* can be used either individually or in various combinations depending on the cheese variety, for acid development and ripening of cheese (El Soda et al. 2000).

White cheese is a pickled variety similar to Iranian white cheese **(**Khosrowshahi et al. 2006**),** Feta and Teleme cheeses. It is the most commonly produced and consumed cheese in Turkey (Cinbas and Kilic 2006). Starter cultures are not used for many cheeses made in Turkey including Turkish White-brined cheese. In artisanal cheese production, the cheese is made without the deliberate addition of a starter culture; the indigenous floras of the milk contribute to ripening. Recently, the use of mixed-strain, mesophilic or/and thermophilic starter cultures commenced in the manufacture of Turkish White-brined cheese (Hayaloglu et al. 2005). Among them, a mixture of *Lactococcus (Lc.) lactis* ssp. *lactis* and *Lc. lactis* ssp. *cremoris* bacteria in different ratios are used frequently in the production of high quality White cheese (Dagdemir et al. 2003). The aim of this study was to evaluate the influence of different commercial adjunct starter cultures on the chemical composition and biochemical properties of White-brined cheese and to determine an appropriate adjunct culture combination for White-brined cheese.

## 2. Materials and Methods

Cow milk was obtained from a local dairy plant. Totally, 260 kg of milk was used for experimental cheese production in each trial. Cheese culture (*Lactococcus lactis* ssp. *lactis* and *Lactococcus lactis* ssp. *cremoris*; C), yoghurt culture (*Streptococcus thermophilus* and *Lactobacillus delbrueckii* ssp. *bulgaricus; Y*), and *Lactobacillus helveticus* (H) obtained from Rhodia Food, (Ezal®, Dangé Saint-Roman, France) in lyophilized form. Commercial rennet was obtained from Mayasan Company®, (Istanbul, Turkey).

## Cheese manufacture

Turkish White-brined cheese was made in duplicate. In each trial, pasteurized milk (65°C/30 min) was cooled to 33°C, divided into four groups and inoculated with direct vat set starter cultures at the level of 0.1 g/L, totally. Samples of pasteurized milk groups were inoculated with *cheese culture* (0.1 g/L) (C, also control cheese); *cheese* (0.05 g/L) plus *yoghurt culture* (0.05 g/L) (CY); *cheese* (0.05 g/L) plus *Lb. helveticus cultures* (0.05 g/L) (CH) and *cheese* (0.033 g/L) plus *yoghurt* (0.033 g/L) plus *helveticus cultures* (0.033 g/L) (CYH) then they held for 30 min. All milk samples were coagulated with liquid calf rennet (strength 1: 12 000) for 90 min. The curd was cut into cubes of 1-2 $cm^3$ after coagulation with a wire knife and transferred to a cheese-cloth to drain; pressure (80-90 $kg/m^2$ for 130-150 min.) was then applied. After the removal of the whey, the curd was portioned into blocks of 7×7×7 $cm^3$, and the fresh cheeses were soaked in brine (140 g NaCl/L) at room temperature for 12 h. At the end of this time, cheese samples were packed in separate plastic boxes (15x24x16 $cm^3$) and filled with the same brine at 7±1°C. The ratio of brine: cheese was 1:4. After that, packed cheeses were stored at 5±1°C for ripening and were ripened during 90 day.

## Chemical and Biochemical Analysis in cheeses

The total solids were determined by oven-drying method (AOAC 1990); total nitrogen was determined by micro-Kjeldahl procedure (Case et al. 1985). The other compositional analyses were also carried out with the methods described by Case et al. (1985). WSN, TCA-SN, and PTA-SN were determined according to the method given by Kamaly et al. (1989) and Butikofer et al. (1993). Lipolysis was done using the BDI method and measured as acid degree value (Case et al. 1985). The electrophoretical analysis of protein patterns was conducted with the method of Creamer (1991) with some modifications (Tarakci et al. 2004). Discontinuous gel system was used for electrophoresis. The samples were electrophoresed using Hoefer Scientific Instrument model SE-600. After staining and destaining, the gels were scanned with a PC scanner and pictures were transferred to the PC. In this study, the production and storage of White-brined cheese together with all due analyses were performed in duplicate. Statistical calculations were performed using SAS Statistical Software (SAS 2006).

## 3. Results and Discussion

The chemical and biochemical changes of the cheeses were given in Table 1. The mean total solids of C cheeses were significantly ($P<0.01$) greater than that of CY and these were followed by CYH and CH cheeses, respectively. Although the cheese made with C culture had the highest protein content, there was no significant difference in protein content of other cheese treatments ($P>0.05$) statistically. The mean fat content of the cheese samples showed a similar trend to protein contents. During ripening, the total solids, fat and salt contents increased significantly ($P<0.01$) for all of cheese treatments, possibly owing to salt diffusion into the cheese body and causing loss of water.

It can be seen from Table that, CH cheeses contained the highest mean titratable acidity followed by CYH, C and CY cheeses, respectively. The titratable acidity of C and CY were not statistically different. The differences in pH values between different cheeses were probably due to differences of starter culture used.

Table 1: Chemical and biochemical changes of White cheeses with adjunct cultures

| | Cheese types | | | |
|---|---|---|---|---|
| Properties | C | CY | CH | CYH |
| Total solids (%) | 40.78±0.53$^{a}$ | 38.64±0.87$^{b}$ | 37.54±0.78$^{c}$ | 38.35±0.55$^{bc}$ |
| Protein (%) | 15.83±0.39$^{a}$ | 14.90±0.43$^{b}$ | 14.48±0.62$^{b}$ | 14.58±0.34$^{b}$ |
| Fat (%) | 18.90±0.31$^{a}$ | 17.75±0.53$^{b}$ | 17.43±0.79$^{b}$ | 17.81±0.44$^{b}$ |
| Salt (%) | 3.95±0.15$^{a}$ | 4.06±0.14$^{a}$ | 4.06±0.13$^{a}$ | 3.94±0.15$^{a}$ |
| Acidity (%) | 0.641±0.02$^{c}$ | 0.618±0.04$^{c}$ | 0.841±0.05$^{a}$ | 0.716±0.04$^{b}$ |
| pH | 6.04±0.09$^{a}$ | 5.72±0.06$^{b}$ | 5.17±0.03$^{d}$ | 5.41±0.04$^{c}$ |
| Lipolysis (ADV) | 1.368±0.08$^{bc}$ | 1.336±0.21$^{c}$ | 1.536±0.07$^{ab}$ | 1.581±0.07$^{a}$ |
| WSN (%) | 13.50±0.29$^{b}$ | 13.49±0.65$^{b}$ | 13.87±0.58$^{b}$ | 14.85±0.77$^{a}$ |
| TCA-SN (%) | 6.50±0.53$^{c}$ | 6.81±0.64$^{bc}$ | 7.38±0.61$^{ab}$ | 7.95±0.76$^{a}$ |
| PTA SN (%) | 3.91±0.32$^{a}$ | 3.86±0.40$^{a}$ | 3.63±0.49$^{a}$ | 3.99±0.50$^{a}$ |

C; cheese with *cheese culture*, CY; cheese with *cheese* and *yoghurt culture*, CH; cheese with *cheese* and *helveticus culture*, CYH; cheese with *cheese + yoghurt culture + helveticus cultures.*
$^{abc}$ letters indicate differences ($P$<0.01) between mean values of cultures.

## Biochemical changes

While C cheeses had the lowest mean value of lipolysis in the first day of ripening, CYH cheeses had the highest level. As the ripening advanced, differences between the cheese samples became clearer in terms of fat acidity. The results indicate that the use of *Lb. helveticus* as adjunct culture caused an increase in the lipolysis of cheese. In the White-brined cheeses, the lipolysis values showed a linear significant (P<0.01) increase during ripening. While the mean WSN ratio of CYH cheese was higher and differed significantly (P<0.01) from the other cheeses there were no significant differences among the cheeses of C, CY and CH. These showed that the proteolytic activity of the cheese of trio mixed cultures (CYH) was slightly higher than those of the other culture combination cheeses.

The highest mean value for the TCA-SN was found in the cheeses containing CYH cultures, while the lowest mean level was obtained from the C culture cheese (P<0.01). The addition of *Lb. helveticus* to cheese increased significantly the TCA-SN values as it enhanced the peptidase activity of the microorganisms (Moatsou et al. 2004). Although, the highest PTA-SN values were obtained from the CYH cheese, no statistically significant differences were found in the mean PTA-SN values (P>0.05) of the cheeses with different adjunct cultures. On the contrary to our results, Drake et al. (1997) was found that the use of *Lb. helveticus* as an adjunct culture in reduced fat Cheddar cheese exhibited significantly greater rates of proteolysis than control cheeses.

**Electrophoretogram changes**

The extent of degradation of major caseins and their hydrolysis products was determined by the urea-PAGE. Figure 1 shows the electrophoretogram of the $\alpha_{s1}$-, β-, $\alpha_{s1}$-I casein fractions and other breakdown products of experimental cheeses. $\alpha_{S1}$-casein was observed with a high intensity at the beginning of ripening, then its intensities decreased during ripening as a result of proteolysis by milk plasmin, microbial enzymes and the clotting enzyme used (Carmona et al. 1999). As ripening progressed, hydrolysis of the caseins became clearer. At the end of ripening, $\alpha_{s1}$-casein hydrolysis was observed as lower in C and CH cheeses while CY and CYH cheeses were characterized by the extensive hydrolysis of $\alpha_{s1}$-casein, Proteolysis may be mainly attributed to chymosin activity in the model cheeses, which manufactured without cooking the curd (Cagno et al. 2006) since the activity of plasmin was weak at the lower pH degrees and proteinase activity of starter cultures was insufficient. The highest $\alpha_{s1}$-casein degradation was observed in cheese made using CYH mixed culture, followed by CY and CH cheeses. The highest density of $\alpha_{s1}$-casein was however found in cheese produced with C culture. Another reason of higher degradation rate of $\alpha s_1$-casein in CYH, CY and CH cheeses may be enhanced activity of starter and non-starter bacteria depending on decrease of pH in these cheeses.

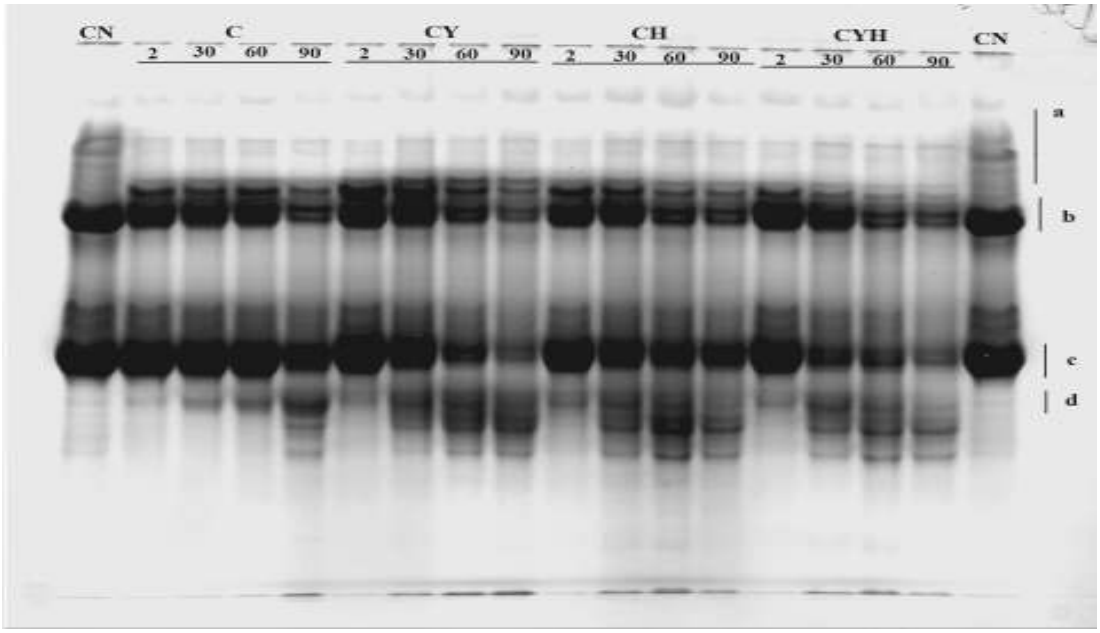


Figure 1*: Urea-PAGE electrophoretogram of white cheeses. CN: Casein; C: Cheese culture (control) cheese, CY: Cheese + Yoghurt culture cheese, CH: Cheese + *Lb. helveticus* culture cheese, CYH: Cheese + Yoghurt and *Lb. helveticus* culture cheese. a: γ-caseins, b: β-casein, c: $\alpha_{S1}$-casein, d: Casein degradation products.

It has been established that β-casein is more resistant to proteolysis, especially in cheese matrix, either by calf rennet or starter enzymes, owing to its structure and particularly, their tendency to associate. Breakdown of β-casein in cheeses inoculated with CY and CYH cultures was denser than the other starter's cheeses at the end of

the ripening period as in the case of $\alpha_{s1}$-casein degradation of CYH cheese. The β-casein band intensities decreased continuously in the experimental cheeses during ripening (Figure 1). Some of γ-casein patterns can be observed from Figure 1, however, their density is low and bands are not clearly separated. Casein break-down products were seen more in the cheeses made with CY, CH and CYH culture than C cheese. This trend was in good agreement with the indices of proteolysis reported previously.

## 4. Conclusion

The results of this study showed that the use of adjunct culture in White-brined cheese somewhat developed the ripening characteristics. Particularly, *Lb. helveticus* had positive contribution on the ripening of cheese. Lipolysis, WSN and TCA-SN values increased with *Lb. helveticus* as adjunct culture. On contrary to expected, no significant differences exist in terms of PTA-SN values between cheeses made with adjunct culture and control cheese. Activity of Y culture was found insufficient on lipolysis and nitrogen fractions. Degradation of $\alpha_{S1}$ and β-caseins was slightly higher in the adjunct culture containing cheeses than the control. Additionally, high amounts of casein degradation products were observed in the experimental cheeses than the control cheese produced with standard culture.

## 5. References

AOAC, (1990). Association of Official Analytical Chemistry. Official methods of analysis. Vol II. (15th edition). Arlington, USA.

Butikofer, U., Ruegg, M. and Ardo, U., (1993). Determination of nitrogen fractions in cheese: Evaluation of a collaborative study. *Lebensm Wiss Technology*, 26, 271-275.

Cagno, R. D., Quinto, M., Corsetti, A., Minervini, F. and Gobetti, M., (2006). Assessing the proteolytic and lipolytic activities of single strains of mesophilic lactobacilli as adjunct cultures using a Caciotta cheese model system. *International Dairy Journal*, 16, 119-130.

Carmona, M. A., Sanjuan, E., Gomez, R. and Fernanandez-Salguero, J., (1999). Effect of starter cultures on the physico-chemical and biochemical features in ewe cheese made with extracts from flowers of *Cynara cardunculus*. *Journal of the Science of Food and Agriculture*, 79, 737-744.

Case, R. A., Bradley, R. L. and Williams, R. R., (1985). Chemical and Physical Methods. In: Standard Methods for the Examination of Dairy Products, Ed. Richardson, G.H., 15th Ed., 327-404, American Public Health Association, Baltimore, U.S.A.

Cinbas, T. and Kilic, M., (2006). Proteolysis and lipolysis in White cheeses manufactured by two different production methods. International Journal of Food Science and Technology, 41, 530-537.

Creamer, L. K., (1991). Electrophoresis of Cheese. Bulletin of the IDF No:261/1991, 14-28.

Dagdemir, E., Celik, S. and Ozdemir, S., (2003). The effects of some starter cultures on the properties of Turkish white cheese. *International Journal of Dairy Technology*,

56, 215-218.
Drake, M. A., Boylston, T. D., Spence, K. D. and Swanson, B. G., (1997). Improvement of sensory quality of reduced fat Cheddar cheese by a *Lactobacillus* adjunct. *Food Research International*, 30, 35-40.
El Soda, M., (1997). Control and enhancement of flavour in cheese. In B. A. Law (Ed.), Microbiology and biochemistry of cheese and fermented milk. Pp. 219–252). London: Chapman & Hall.
El Soda, M., Madkor, S. A. and Tonh, P.S., (2000). Adjunct cultures: Recent development and potential significance to the cheese industry. *Journal of Dairy Science*. 83, 609-619.
Hayaloglu, A. A., Guven, M., Fox, P. F. and McSweeney, P. L. H., (2005). Influence of starters on chemical, biochemical, and sensory changes in Turkish white-brined cheese during ripening. *Journal of Dairy Science*, 88, 3460-3474.
Kamaly, K. M., Johnson, M. E. and Marth, E. H., (1989). Characteristics of Cheddar cheese made with mutant strains of lactic streptococci as adjunct sources of enzymes. *Milchwissenschaft*, 44, 343-346.
Katsiari, M. C., Voutsinas, L. P. and Kondly, E., (2002). Improvement of sensory quality of low-fat Kefalograviera-type cheese with commercial adjunct cultures. International Dairy Journal, 12, 757-764.
Khosrowshahi, A., Madadlou, A., Ebrahimzadeh Mousavi, M. and Emam-Djomeh, Z., (2006). Monitoring the chemical and textural changes during ripening of Iranian White cheese made with different concentrations of starter. *Journal of Dairy Science*, 89, 3318-3325
Mistry, V. V., (2001). Low fat cheese technology. International Dairy Journal, 11, 413–422.
Moatsou, G., Moschopoulou, E. and Anifantakis, E., (2004). Effect of different manufacturing parameters on the characteristics of Graviera Kritis cheese. *International Journal of Dairy Technology,* 57**,** 215-220.
SAS/STAT Software, 2006. Statistics (version 9.1.13), SAS Institute Inc., Cary, N.C., U.S.A.
Tarakci, Z., Coskun, H. and Tuncturk, Y., (2004). Some properties of fresh and ripened herby cheese, a traditional variety produced in Turkey. *Food Technology and Biotechnology*, 42, 47-50.