Available online at www.sciencedirect.com
ScienceDirect
Food Chemistry 104 (2007) 1192–1199
Food Chemistry
www.elsevier.com/locate/foodchem

# Evaluation of isolated starter lactic acid bacteria in Ras cheese ripening and flavour development

Sameh Awad *, Nour Ahmed, Morsi El Soda

*Department of Dairy Science and Technology, Faculty of Agriculture, University of Alexandria, Egypt*

Received 10 July 2006; received in revised form 6 January 2007; accepted 22 January 2007

**Abstract**

Eighteen cultures of starter lactic acid bacteria with or without added adjunct cultures, isolated from Egyptian dairy products, were evaluated in experimental Ras cheese for flavour development. Chemical composition of experimental cheeses was within the legal limit for Ras cheese in Egypt. All cultures used in this study had no effect on chemical composition of Ras cheese. Very significant variations in free amino acids, free fatty acids and sensory evaluation have been found among the cultures used in Ras cheesemaking. The levels of free amino acids and free fatty acids were correlated well with flavour development in Ras cheese. Seven of the tested cultures produced acceptable flavour and texture of Ras cheese. The highest overall score of flavour intensity, flavour and texture acceptability were in cheese made using YY47 lactic culture in addition to adjunct culture of *Lactobacillus helveticus*, *Lactobacillus paracasei* subsp. *paracasei*, *Lactobacillus delbrueckii* subsp. *lactis and Enterococcus faecium*. This culture can be recommended for Ras cheese manufacture using pasteurized milk.


*Keywords:* Ras cheese; Lactic acid bacteria; Adjunct culture; Flavour development

## 1. Introduction

Taste and aroma are very important features of cheese. Consumers make their choice of cheeses primarily on the basis of flavour characteristics. The cheese flavour results from lipolysis and proteolysis by starter cultures and non-starter lactic acid bacteria. Proteolysis is a major determinant of the intact casein which has a large impact on the texture of Cheddar cheese. For the development of an acceptable cheese flavour, a well-balanced breakdown of the curd protein (that is, casein) into small peptides and amino acids is necessary (Singh, Drake, & Cadwallader, 2003). Ras cheese had been made from raw milk for a long time, for that reason there are different groups of microorganisms present in the cheese, some of them participate in the flavour and texture development and some other may be pathogenic or cause defects in cheese (Abou-Donia, 2002; Awad, El Attar, Ayad, & El-Soda, 2003; Sabbour, 1966). However, the Egyptian Organization for Standardization and Quality Control (Egyptian standards, 2001) recommended the pasteurization of milk before cheese making to improve the hygienic quality of cheese. Pasteurised of milk will have a negative impact on the natural flora present in raw milk.

The microorganisms involved in cheese making and cheese ripening can be divided into two major groups: (1) microorganisms that are added to the cheese milk after being carefully selected by the starter manufacturer or the cheese-making company, and (2) non-starter lactic acid bacteria (Fox, McSweeney, & Lynch, 1998; Johnson, 1998). For the cheese industry to offer to the consumers safe and consistent cheeses with high organoleptic properties in a reasonable ripening time, they began to look for new technologies such as "adjunct cultures". Adjunct cultures can be defined as selected strains of cheese related microorganisms that are added to the cheese milk to

* Corresponding author. Tel./fax: +20 3 5903915.
*E-mail address:* sameh11eg@yahoo.com (S. Awad).

0308-8146/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2007.01.043

improve development of cheese sensory quality. In contrast to naturally occurring non starter lactic acid bacteria, adjuncts are specifically selected and intentionally added to supplement the microflora of cheese milk to improve overall quality of finished cheese (El Soda, Madkor, & Tong, 2000).

Starter and non-starter bacteria are important not only for the acid development which hastens the milk coagulation and assists in expulsion of whey, but also from the standpoint of its influence on flavour, body and texture of finished cheese (El Soda, 1993; Law, 2001). Enterococci strains are often isolated from Egyptian dairy products (El-Soda et al., 2003), it is believed that they may play a useful role during cheese ripening (Bahay-El-Din, El-Soda, & Ezzat, 2002). The beneficial effect of enterococci in cheesemaking has been attributed to hydrolysis of milk fat and protein (Bahay-El-Din et al., 2002). The enterococci were suggested for use as starter in the production of Cebreiro cheese and Feta cheese (Centeno, Menendez, & Rodriguez-Otero, 1996; Litopoulou-Tzanetaki, Tzanetakis, & Vafopoulou-Mastrojiannaki, 1993). A relatively high number of enterococci strains isolated from Egyptian dairy products were evaluated and the results indicated good technological performance for many strains of *Enterococcus faecium* (Ayad, Nashat, El-Sadek, & El-Soda, 2004b).

Today's Egyptian market clearly shows a growing demand for diversification. This makes it worthwhile to the cheese industry to develop products with improved flavour profiles and hygiene quality. In our previous work (Awad et al., 2003; Ayad, Awad, El-Attar, de-Jong, & El-Soda, 2004a), commercial Ras cheese was characterized for sensory evaluation, physico-chemical properties and flavour formation to establish the characterization of typical Ras cheese made from raw milk. The isolation and characterization of LAB from high quality traditional Egyptian dairy products in order to extend the number of available cultures has been considered in the search for new industrially important cultures (El-Soda et al., 2003). Starter cultures are not established yet for Ras cheesemaking. No detailed comparison has been made on proteolysis, lipolysis and flavour development in Ras cheese manufactured with individual starters. This work was planned to evaluate some starter and adjunct cultures isolated from Egyptian dairy products in Ras cheese ripening and flavour development.

## 2. Materials and methods

### 2.1. Starter cultures

*Lactococcus lactis* subsp. *lactis* 56 L was isolated from Domiati cheese, while the *Lactococcus lactis* subsp. *lactis* 104 L and 102 L were isolated from traditional Ras cheese. These strains were identified and characterised as described earlier (El-Soda et al., 2003). Commercial lactic culture (DVS YY47) was obtained from Chr. Hansen's Laboratory, Denmark. The YY47 culture contained *Lactococcus lactis* subsp. *lactis*, *Lactococcus lactis* subsp. *cremoris, Sterptococcus thermophilus and Lactobacillus helveticus*.

All lactococci strains were grown in M17 broth supplemented with 5 g/L lactose. Each strain was subculture (1% vol/vol) 3 times and then transferred to reconstitute skim milk for overnight incubation at 32 °C to produce the cheese starter culture.

### 2.2. Adjunct culture

Adjunct cultures are nonstarter lactic acid bacteria, consisting mainly of *Lactobacillus* sp., which are used in addition to a standard starter to improve and to enhance the flavour of cheese (Madkor, Tong, & El Soda, 2000). *Lactobacillus delbrueckii* subsp. *lactis* 119TH, *Lactobacillus acidophilus* 83ST, *Lactobacillus paracasei* subsp. *paracasei* 27ST, *Lactobacillus helveticus* 120B, *E. faecium* 102E, 136E and 241E were obtained from the collection of Faculty of Agriculture, Alexandria University (El-Soda et al., 2003). Enterococcal strains were examined for haemolytic activity before using in cheese making.

Lactobacilli strains were grown in MRS broth at 30 °C (mesophilic strains) or 42 °C (thermophilic strains), while entercocci strains were grown in M17 broth at 37 °C. Cells growth phase were monitored by measuring the absorbance at 650 nm using a LKB (Pharmacia Nova Spectrophotometer II, Cambridge, England). After 8–12 h, early stationary phase cells were harvested by centrifugation at 2800*g* for 20 min at 4 °C. The pellet was then washed twice with 0.01 M potassium phosphate buffer pH 7.0. The pellet was then suspend in phosphate buffer and adjusted to 1 OD and stored at −20 °C overnight. All the starters and adjunct cultures used in this study are summarized in Table 1.

### 2.3. Cheese making procedure

Raw whole cow and buffalo milk were obtained from the dairy barn at the Alexandria University. A mixture of 70:30 of raw cow and buffalo milk (fat: 45 g $kg^{-1}$ and acidity: 0.16–0.17% as lactic acid) was pasteurized at 74 °C for 15 s. Three replicates of experimental cheeses for each starter mixture were processed using computer-controlled cheese equipment (INRA, Poligny, France) equipped with four 11 L vats. Starter cultures (*Lactococcus lactis* subsp. *lactis*, 15 mL $kg^{-1}$, or DVS culture, 0.15 g $kg^{-1}$), freeze-shocked suspension of adjunct cultures (1 mL $kg^{-1}$, for cheeses made with adjunct culture) and $CaCl_2$ (0.12 g $kg^{-1}$) were added individually to milk at 32 °C. The inoculated milk is held for 60–75 min at 32 °C and then 9 ml of 2% liquid rennet (Chymax-II 500: Chr. Hansen's Lab., Denmark) was added to each vat to coagulate the milk in 30 min. The coagulum was cut into cubes (~2 cm) and the curds were allowed to rest in the whey for 5–10 min. The curds were cooked to 45 °C over 45 min and held at this temperature for 15 more minutes. The whey was drained when its acidity reached to 0.14% (as lactic acid

Table 1
Starter/adjunt cultures combination used for Ras cheesemaking

| Culture number | Starter culture | Adjunct culture |
|---|---|---|
| 1 | *Lc .lactis* subsp. *lactis* 56 L[a] | – |
| 2 | *Lc .lactis* subsp. *lactis* 56 L | *Lb. helveticus* |
| 3 | *Lc .lactis* subsp. *lactis* 56 L | *Lb. acidophilus* |
| 4 | *Lc. lactis* subsp. *lactis* 56 L | *Lb. acidophilus* and *Lb .helveticus* |
| 5 | *Lc .lactis* subsp. *lactis* 104 L | – |
| 6 | *Lc. lactis* subsp. *lactis* 104 L | *Lb. helveticus* |
| 7 | *Lc. lactis* subsp. *lactis* 104 L | *Lb. acidophilus* |
| 8 | *Lc .lactis* subsp. *lactis* 104 L | *Lb. paracasei* subsp. *paracasei* |
| 9 | *Lc .lactis* subsp. *lactis* 104 L | *Lb. delbrueckii* subsp. *lactis* |
| 10 | *Lc. lactis* subsp. *lactis* 102 L | – |
| 11 | *Lc .lactis* subsp. *lactis* 102 L | *Lb. helveticus* |
| 12 | *Lc .lactis* subsp. *lactis* 102 L | *Lb. paracasei* subsp. *paracasei* |
| 13 | Thermophilic lactic cultures YY47 [b] | – |
| 14 | Thermophilic lactic cultures YY47 | *Lb. helveticus* |
| 15 | Thermophilic lactic cultures YY47 | *Lb. paracasei* subsp. *paracasei* |
| 16 | Thermophilic lactic cultures YY47 | *Lb. paracasei* subsp. *paracasei* and *Lb. helveticus* |
| 17 | Thermophilic lactic cultures YY47 | *Lb. helveticus*, *Lb. paracasei* subsp. *paracasei and Lb. delbrueckii* subsp. *lactis* |
| 18 | Thermophilic lactic cultures YY47 | *Lb. helveticus*, *Lb. paracasei* subsp. *paracasei*, *Lb. delbrueckii* subsp. *lactis and En. faecium* 102E,. *faecium* 136E and. *faecium* 241E |

[a] FAAU; *Lactococcus lactis* subsp. *lactis* 56 L , 104 L, 102 L and all adjunct cultures were isolated by El-Soda et al. (2003) and stored at Faculty of Agriculture, Alexandria University, (FAAU).
[b] Thermophilic lactic culture YY47 was obtained from Chr. Hansen's Laboratory, Denmark.

w/v). The obtained curd was hooped and pressed at 66 Psi for 4 h, and then the cheeses were turned and repressed at 120 Psi for 12 more hours. The resultant cheeses were salted in brine solution (230 g $kg^{-1}$) at 13 °C for 3 days. During the first ripening month, the second salting was performed. The cheeses were daily turned upside down and manually salted in order to obtain 3.5–4% salt in cheese (Hofi, Youssef, Ghoneim, & Tawab, 1970). When the salt in cheese reached to 3.5–4%, the cheese surface was well washed with warm water using a smooth brush, and then was dried. Cheeses were waxed by quick immersion in the molten wax (Chr. Hansen's Lab., Denmark) and lifted up to cool for 2 h before removal to the ripening room (12 ± 2 °C and 80 ± 5% relative humidity).

### 2.4. Cheese composition analysis

Total protein was measured by Kjeldahl (AOAC, 2000), Fat content by Gerber method (AOAC, 2000). A Corning flat surface combination electrode was used to measure the pH on the well-mixed ground cheese samples. Titratable acidity (TA) was determined by the method developed by Lau, Barbano, and Rasmussen (1991). TA was expressed as percentage lactic acid content of cheese by weight. The moisture content was determined using the moisture analyser (Mettler Toledo Model HR73). Salt content was determined using chloride meter (Jenway, England, UK).

### 2.5. Assessment of proteolysis and lipolysis

The water-soluble extract (WSE) was prepared by the method development by Kuchroo and Fox (1982) and the free amino acids (FAA) were determined in WSE by to the Cd-ninhydrin method of Folkertsma and Fox (1992) and expressed as mM leucine equivalent in water-soluble extracts, using a standard curve. Free fatty acids (FFA) were determined by the method of Deeth, Fitz-Gerald, and Wood (1975) and expressed as mmol equivalent $g^{-1}$ cheese fat.

### 2.6. Sensory evaluation

A panel consisting of 10–15 cheese graders, including staff members, cheese producers and consumers, carried out the sensory evaluation. Each individual was given 4 blocks (8 × 2 × 2 cm) of cheese per sample. Samples were presented in identical plastic sample cups sealed with plastic lids and identified by a random 3-digit number. The coded samples were randomly presented. The graders were asked to give the cheese an overall grade out of (100) and to whether each sample was typical Ras cheese or not and any additional comments. Cheeses were graded at 4 months of age and the following scales were used: 0–25, unacceptable; 26–50, poor; 51–75, acceptable; 76–100, good.

### 2.7. Statistical analysis

Data reported are the average of three measurements. The SAS statistical analysis software package (SAS, 1999) was used for analysis of variance. Differences were considered significant at $P < 0.05$.

## 3. Results and discussion

### 3.1. Cheese composition

All cultures used in this study had little or no impact on the gross chemical composition (moisture, fat, total protein and salt) of Ras cheese. The average moisture at one day of manufacture was $455.0 \pm 14.6$ g kg$^{-1}$ (Table 2). The moisture content significantly decreased in Ras cheese during ripening, most of the moisture loss occurred during the first month of ripening, the reason for this loss during this period may be attributed to second stage of salting and before cheeses waxing. There is a gradual increase in salt content in the first months of ripening, and then it slightly increased until the end of the ripening period. This could be attributed to the second stage of salting that took place during this period (Hofi et al., 1970). Decreasing the moisture content and increasing the salt level during ripening of Ras cheese were also reported in others studies (Awad, 2006; Hofi et al., 1970).

The fat and protein content in cheeses were found to be related to the moisture content in cheeses during ripening. The protein and fat content on dry basis were not significantly different ($P < 0.05$) in all cheeses. There were no significant differences in gross chemical composition among all treatments. The gross chemical composition of aged Ras cheese are in agreement with that reviewed by Abou-Donia (2002), and were within the legal limit for Ras cheese in Egypt (Egyptian standards for Ras cheese, 2001). The corresponding values for 1st grade Ras cheeses are pH 5.2–5.4; salt 36–39 g kg$^{-1}$; moisture: 330–340 g kg$^{-1}$; fat in dry matter (FDM): 520–550 g kg$^{-1}$ (Awad et al., 2003).

### 3.2. pH and titratable acidity

The pH values and titratable acidity of experimental cheeses determined during ripening are presented in Table 3. Using mesophilic or thermophilic lactic cultures did not appear to have significant effect on cheese pH values on day 1 of manufacture. The pH of all cheeses decreased ($P < 0.05$) gradually throughout the first 60 days followed by increasing thereafter. At 1 and 60 days of ripening, cheeses made using mesophilic or thermophilic lactic cultures with added lactobacilli showed significantly ($P < 0.05$) lower pH values compared to the one made without added lactobacilli, except cheeses made with *Lactococcus lactis* subsp. *lactis* 102 L (cultures 10–12), as there were no differences between cheese made with or without added lactobacilli. The pH value of cheeses made with *Lactococcus lactis* subsp. *lactis* 56 L (cultures 2–4) was much lower than all other cheeses at 60 days of ripening.

Table 2
Gross composition of experimental Ras cheese made with different starter and adjunct cultures

| Culture | Ripening time (days) | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Moisture (g kg$^{-1}$) | | | Fat in dry matter (g kg$^{-1}$) | | | Protein (g kg$^{-1}$) | | | Salt (g kg$^{-1}$) | | |
| | 1 | 60 | 120 | 1 | 60 | 120 | 1 | 60 | 120 | 1 | 60 | 120 |
| 1 | 468 | 361 | 352 | 489 | 485 | 509 | 210 | 235 | 240 | 17 | 33 | 40 |
| 2 | 451 | 391 | 338 | 455 | 526 | 529 | 209 | 232 | 250 | 23* | 42* | 47* |
| 3 | 442 | 395 | 337.7 | 475 | 529 | 536 | 216 | 234 | 250 | 24* | 43* | 49* |
| 4 | 431 | 351 | 323 | 439 | 524 | 546 | 214 | 250 | 262 | 17 | 32 | 40 |
| 5 | 447 | 355 | 332 | 470 | 481 | 479 | 215 | 235 | 239 | 17 | 33 | 40 |
| 6 | 480 | 379 | 346 | 461 | 515 | 512 | 204 | 250 | 261 | 18 | 34 | 41 |
| 7 | 465 | 364 | 331 | 505 | 503 | 508 | 201 | 241 | 250 | 18 | 35 | 43 |
| 8 | 474 | 381 | 364 | 513 | 501 | 535 | 202 | 242 | 253 | 17 | 33 | 40 |
| 9 | 452 | 380 | 346 | 456 | 516 | 535 | 245 | 242 | 252 | 17 | 32 | 41 |
| 10 | 446 | 374 | 351 | 487 | 495 | 516 | 200 | 232 | 240 | 18 | 35 | 41 |
| 11 | 452 | 379 | 349 | 477 | 513 | 524 | 212 | 243 | 251 | 19 | 37 | 42 |
| 12 | 440 | 382 | 357 | 465 | 510 | 513 | 221 | 250 | 262 | 18 | 34 | 41 |
| 13 | 463 | 351 | 312 | 437 | 509 | 498 | 226 | 254 | 263 | 17 | 34 | 39 |
| 14 | 456 | 374 | 357 | 441 | 527 | 536 | 214 | 245 | 251 | 18 | 35 | 41 |
| 15 | 480 | 390 | 350 | 500 | 508 | 523 | 238 | 254 | 263 | 17 | 33 | 40 |
| 16 | 469 | 390 | 352 | 471 | 500 | 494 | 218 | 230 | 266 | 17 | 34 | 40 |
| 17 | 442 | 371 | 352 | 448 | 493 | 509 | 231 | 261 | 270 | 17 | 35 | 40 |
| 18 | 440 | 359.2 | 351 | 441 | 505 | 509 | 227 | 264 | 268 | 17 | 36 | 41 |
| Av | 455 | 374 | 344 | 468 | 508 | 517 | 217 | 244 | 255 | 18 | 35 | 41 |
| SD | 14.6 | 14.1 | 13.1 | 23.4 | 14.0 | 17.3 | 12.7 | 10.2 | 9.7 | 2.0 | 3.0 | 2.6 |

Av, Average of all treatments.
SD, Standard deviations among all treatments.
* Statistical analysis showed that there were no significant different ($P < 0.05$) among all treatments, except the salt content was higher in cheeses made with cultures 2 and 3.

Reducing the pH value in cheeses during the first 60 days of ripening could be attributed to the continued production of lactic acid by live cells of lactobacilli that could survive much longer in cheese than lactococci, and/or the liberation of certain amino acids, such as aspartic and glutamic acids that could influence cheese pH (Sallami, Kheadr, Fliss, & Vuillemard, 2004; Trepanier, El Aboudoi, Lee, & Simard, 1992).

The pH values significantly increased in all cheeses at 4 months of ripening. However, most of cheeses made with added lactobacilli showed significantly ($P < 0.05$) higher pH values compared with the cheeses made without added lactobacilli. The increase in pH at the end of ripening has been attributed to the combined effects of the utilisation of lactic acid, formation of non acidic decomposition products and weaker or less highly dissociated amino acids and liberation of alkaline products of protein decomposition (Webb, Johnson, & Alford, 1983).

At day 1 of manufacture, cheeses made with added lactobacilli had significantly ($P < 0.05$) higher titratable acidity value as compared to the other cheeses made with same mesophilic or thermophilic lactic culture without added lactobacilli. As the cheese ages, the titratable acidity value increased (Table 3). Within the first 10 days of cheese making, the lactose present in the curd is degraded. Therefore, lactic acid might have contributed directly to the increase in titratable acidity (Lau et al., 1991). However, as cheese ages, more caseins and high molecular weight peptides are hydrolysed into low molecular weight peptides that increase their carboxyl groups, which could interfere with the titration process.

### 3.3. Proteolysis

Amino acid release, expressed as mM leucine equivalent, in water-soluble extracts of experimental cheeses at different ripening stages is shown in Table 3. FAA concentration increased significantly ($P < 0.05$) as ripening progressed. In general, the free amino acids value was higher in cheeses made with added adjunct culture than in cheeses made without adjunct culture. The FAA concentration was higher in cheeses made with thermophilic starter than in cheeses made with mesophilic starter. The adjunct single culture of *Lactobacillus paracasei* subsp. *paracasei* (culture 15) produced higher level of free amino acids than any other adjunct single culture in the current study. Awad, Bahay-El Din, and El-Soda (2001) observed that free amino acids concentration in pseudo curd and cheese slurries containing lactobacilli were significantly higher than those from pseudo curd and cheeses slurries containing lactococci or enterococci.

When *E. faecium* was added with *Lb. helveticus* and *Lb. paracasei* subsp. *paracasei* (culture 18), higher concentration of free amino acids were obtained compared to Ras cheese made without added *E. faecium* (culture 17). The production of high level of free amino acids in cheese made

Table 3
Biochemical and organoleptic properties of experimental Ras cheese made with different starter and adjunct cultures

| Culture | Ripening time (days) | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | pH value | | | Acidity[A] | | | FAA[B] | | | FFA[C] | | | Sens[D] |
| | 1 | 60 | 120 | 1 | 60 | 120 | 1 | 60 | 120 | 1 | 60 | 120 | 120 |
| 1 | 5.10$^{c}$ | 5.03$^{a}$ | 4.97$^{d}$ | 1.55$^{cd}$ | 1.80$^{d}$ | 2.20$^{e}$ | 0.10 | 0.25 | 0.61$^{i}$ | 1.20 | 3.52 | 8.32$^{h}$ | 25$^{h}$ |
| 2 | 4.96$^{d}$ | 4.76$^{c}$ | 5.12$^{c}$ | 1.70$^{b}$ | 2.20$^{b}$ | 2.40$^{c}$ | 0.20 | 0.69 | 1.51$^{f}$ | 1.82 | 5.44 | 11.52$^{f}$ | 35$^{g}$ |
| 3 | 4.94$^{de}$ | 4.59$^{e}$ | 5.11$^{c}$ | 1.85$^{ab}$ | 2.12$^{bc}$ | 2.50$^{b}$ | 0.14 | 0.39 | 0.76$^{i}$ | 2.33 | 8.32 | 14.72$^{e}$ | 45$^{e}$ |
| 4 | 4.97$^{d}$ | 4.69$^{d}$ | 5.21$^{b}$ | 1.98$^{a}$ | 2.20$^{b}$ | 2.45$^{bc}$ | 0.27 | 1.43 | 1.65$^{f}$ | 3.05 | 14.27 | 17.92$^{d}$ | 54$^{d}$ |
| 5 | 5.16$^{b}$ | 4.97$^{b}$ | 4.95$^{d}$ | 1.55$^{cd}$ | 1.70$^{e}$ | 2.10$^{f}$ | 0.09 | 0.25 | 0.87$^{g}$ | 0.90 | 1.70 | 2.98$^{j}$ | 40$^{f}$ |
| 6 | 5.12$^{c}$ | 4.93$^{b}$ | 5.30$^{a}$ | 1.49$^{d}$ | 2.15$^{bc}$ | 2.35$^{c}$ | 0.12 | 0.46 | 2.00$^{e}$ | 1.30 | 3.52 | 5.98$^{g}$ | 43$^{e}$ |
| 7 | 5.11$^{c}$ | 4.96$^{b}$ | 5.20$^{b}$ | 1.55$^{cd}$ | 1.90$^{d}$ | 1.98$^{h}$ | 0.07 | 0.12 | 1.65$^{f}$ | 1.16 | 2.94 | 6.59$^{h}$ | 44$^{e}$ |
| 8 | 5.02$^{d}$ | 4.86$^{c}$ | 5.10$^{c}$ | 1.60$^{c}$ | 1.86$^{d}$ | 2.10$^{f}$ | 0.11 | 0.52 | 1.55$^{f}$ | 1.12 | 2.78 | 4.13$^{i}$ | 64$^{c}$ |
| 9 | 5.11$^{c}$ | 4.95$^{b}$ | 5.30$^{a}$ | 1.60$^{c}$ | 1.92$^{d}$ | 2.20$^{e}$ | 0.25 | 1.09 | 2.14$^{e}$ | 1.24 | 3.84 | 7.68$^{h}$ | 62$^{c}$ |
| 10 | 5.13$^{b}$ | 5.04$^{a}$ | 5.22$^{b}$ | 1.30$^{e}$ | 1.65$^{e}$ | 1.80$^{g}$ | 0.07 | 0.25 | 1.13$^{h}$ | 1.03 | 2.37 | 5.60$^{g}$ | 38$^{g}$ |
| 11 | 5.27$^{a}$ | 5.04$^{a}$ | 5.20$^{b}$ | 1.35$^{e}$ | 1.90$^{d}$ | 2.10$^{f}$ | 0.14 | 0.75 | 2.12$^{e}$ | 1.07 | 2.56 | 8.32$^{h}$ | 43$^{e}$ |
| 12 | 5.27$^{a}$ | 5.04$^{a}$ | 5.20$^{b}$ | 1.35$^{e}$ | 1.90$^{d}$ | 2.10$^{f}$ | 0.17 | 0.85 | 2.60$^{d}$ | 1.22 | 3.52 | 9.92$^{f}$ | 63$^{c}$ |
| 13 | 5.24$^{a}$ | 4.99$^{ab}$ | 5.06$^{c}$ | 1.50$^{d}$ | 2.30$^{b}$ | 2.40$^{c}$ | 0.23 | 1.51 | 2.46$^{d}$ | 0.06 | 0.96 | 14.72$^{e}$ | 45$^{f}$ |
| 14 | 5.17$^{b}$ | 4.75$^{cd}$ | 5.21$^{b}$ | 1.60$^{c}$ | 2.50$^{a}$ | 2.60$^{a}$ | 0.28 | 2.86 | 5.16$^{c}$ | 2.11 | 8.54 | 17.28$^{d}$ | 62$^{c}$ |
| 15 | 5.10$^{c}$ | 4.66$^{d}$ | 5.11$^{c}$ | 1.65$^{c}$ | 2.23$^{b}$ | 2.60$^{a}$ | 0.35 | 3.22 | 6.12$^{ab}$ | 3.26 | 16.32 | 23.04$^{b}$ | 82$^{b}$ |
| 16 | 5.09$^{c}$ | 4.93b | 5.20b | 1.65$^{c}$ | 1.90$^{d}$ | 2.30$^{d}$ | 0.32 | 2.90 | 5.45$^{bc}$ | 2.20 | 11.20 | 21.44$^{c}$ | 65$^{c}$ |
| 17 | 5.06$^{cd}$ | 4.82$^{c}$ | 5.10$^{c}$ | 1.70$^{bc}$ | 2.10$^{c}$ | 2.40$^{c}$ | 0.34 | 3.10 | 6.40$^{ab}$ | 3.85 | 18.56 | 25.92$^{b}$ | 84$^{b}$ |
| 18 | 5.10$^{c}$ | 4.75$^{c}$ | 4.92$^{d}$ | 1.80$^{b}$ | 2.10$^{c}$ | 2.30$^{d}$ | 0.38 | 3.35 | 6.78$^{a}$ | 3.88 | 19.87 | 27.69$^{a}$ | 90$^{a}$ |

[A] Acidity as lactic acid % in cheese.
[B] Free amino acids mmol leucine equivalent.
[C] Free fatty acids mmol equivalent $g^{-1}$ cheese fat.
[D] The sensory evaluation of flavour intensity, flavour and texture acceptability on a 0–100 scale, where 0–25, unacceptable; 26–50, poor; 51–75, acceptable; 76–100, good.
[a–j] Means within the same columns with different subscriptions are significantly different ($P < 0.05$). Statistical analysis showed that there were significantly different ($P < 0.05$) among values in function of ripening time whiten all treatments.

with added adjunct culture containing *E. faecium* can be attributed to high tolerance of entrococci to salt and acid during cheese ripening (Litopoulou-Tzanetaki, 1990; Wessels, Jooste, & Mostert, 1990) and production of proteolytic enzymes involved in casein degradation (Bahay-El-Din et al., 2002).

The major contributors to the production of small peptides and FAA are probably the starter and non-starter bacteria enzymes (El Soda et al., 2000; Lane & Fox, 1996). Differences were observed among the cheeses made using different cultures, indicating that the starter and non-starter bacteria (NSLAB) seems to be responsible for the production of the free amino acids in Ras cheese during ripening. The role of the starter culture is to ensure consistent acid development during cheese making. This group is also involved in the degradation of protein and fat during ripening. Adjunct cultures were also developed to accelerate cheese ripening, which may allow substantial cost savings to the cheese industry (El Soda et al., 2000).

### 3.4. Lipolysis

The lipolysis in Ras cheese during ripening was measured in terms of total FFA and expressed as mmol equivalent $g^{-1}$ cheese fat. FFA increased gradually with increasing ripening time (Table 3). Cheeses with added lactobacilli showed higher acid values during ripening. However, cheeses containing *Lb. paracasei* exhibited a higher acid value than did those with other lactobacilli. Moreover, lactobacilli with YY47 culture produced even highest values during ripening. High acid values in cheeses containing lactobacilli has been previously reported by El Soda, Hantira, Ezzat, and El-Shafei (1992), Ezzat and El-Shafei (1991) and Madkor et al. (2000), and attributed to the release of intracellular esterases and lipases.

Commonly, acid values follow the same trend as soluble nitrogen, suggesting that factors affecting proteolysis may have similar impact on lipolysis (Kebary, Khader, Zedan, & Mahmoud, 1996). The highest values of free amino and free fatty acids were recorded in cheese made using YY47 culture and adjunct culture of *Lb. helveticus*, *Lb. paracasei* subsp. *paracasei*, *Lb. delbrueckii* subsp. *lactis* and *E. faecium*. These results indicate that adjunct cultures contribute to lipolysis in cheese and different adjunct strains have different lipolytic activity. Increasing the rate of lipolysis with added adjunct during cheese ripening has been observed by other authors (El Soda et al., 1992; Ezzat & El-Shafei, 1991).

### 3.5. Organoleptic quality of cheese

The main objective for this study is to establish a promising culture for Ras cheese-making using pasteurised milk. The acceptability of cheese depends on its appearance and sensory properties (flavour, texture, and appearance), among these, flavour is the most important attribute for the consumer. Hence, the most important for selecting culture is to produce typical Ras cheese flavour. Therefore, experimental Ras cheeses were made from pasteurized milk using different mixtures of selected strains as shown in Table 1. The averages of scores for sensory evaluation of experimental Ras cheeses are summarized in Table 3. The grading at 4 months of ripening shows that each cheese obtained a special grade score. Among all cheeses, the experimental Ras cheeses made with single *Lactococcus* spp. strain (culture number 1, 5 and 10) received low scores and unacceptable flavour. Addition of lactobacilli adjunct cultures with *Lactococcus* spp. improved the cheese flavour, but most of flavour acceptability of these cheeses was in the poor range, and some received Cheddar or Gouda flavour. Only 3 lactobacilli adjunct cultures (cultures 8, 9 and 12) out of 8 used with *Lactococcus spp.* produced acceptable cheese flavour.

Cheeses made with YY47 lactic culture with added adjunct culture (cultures 14–18) received high scores with acceptable Ras cheese flavour. Interesting, the flavour density and acceptability in all cheeses made with added *Lactobacilli paracasei* subsp. *paracasei* with either mesophilc or YY47 cultures were in the acceptable range. The grade awarded to cheeses made with YY47 lactic culture and adjunct culture of *Lb. helveticus*, *Lb. paracasei* subsp. *paracasei*, *Lb. delbrueckii* subsp. *lactis and E. faecium* remained the highest among all experimental cheeses.

The levels of free amino acids were significantly ($P < 0.05$) higher in the cheese made with YY47 lactic culture and adjunct culture of *Lb. paracasei* subsp. *paracasei* and *Lb. helveticus* throughout ripening than in all other cheeses except for the cheese with added *E. faecium*. Adding the *E. faecium* strain with the lactobacilli culture increased the free amino and fatty acids levels and enhanced the Ras cheese flavour. These results indicated that the proteinase and peptidases of starter and non-starter bacteria is very important for the production of small peptides and the accumulation of amino acids in Ras cheese during ripening; Lane and Fox (1997) reported similar results in Cheddar cheese. There has been considerable interest in using defined strains of nonstarter lactic acid bacteria as adjunct cultures to accelerate and improve flavor and texture development during cheese ripening (Drake, Boylston, Spence, & Swanson, 1996; El Soda et al., 1992).

There were a positive relationship between the free amino acids and free fatty acids content and flavour development in Ras cheese. The idea that free amino acids and their decomposition products could be important in cheese flavour formation was put forward many years ago (Kosikowski & Mocquot, 1958). Later reporter also repeatedly emphasized that a relationship exists between the amino nitrogen content and the flavour intensity of a cheese (Singh et al., 2003).

Giraffa (1995) reported that strains of enterococci isolated from dairy products do not produce haemolysin, and it was suggested that absence of haemolytic activity should be a selection criterion for starter strains for dairy

use. In our laboratory, enterococcal strains were examined for haemolytic activity before using in cheese making. The results of this study indicated that addition of *E. faecium* to adjunct culture of *Lb. paracasei* subsp. *paracasei* and *Lb. helveticus* produced a typical Ras cheese flavour. It has been reported that the dominance or persistence of enterococci in some cheeses during ripening can be attributed to their wide range of growth temperature, their high tolerance to salt and acid (Litopoulou-Tzanetaki, 1990; Wessels et al., 1990).

## 4. Conclusions

The differences between individual strains were not reflected in their performance during cheese manufacture or the composition of the cheeses. The use of adjunct cultures in Ras cheese had no effect on the gross chemical composition. The most interesting effect of YY47 lactic acid starter and adjunct cultures of lactobacilli were the accumulation of significantly higher amounts of free amino acids and free fatty acids. Free amino acids and free fatty acids contribute to Ras cheese flavour directly or indirectly, by acting as precursors for the formation of cheese flavour compounds. Indeed, the sensory evaluation showed that the cheeses made with YY47 starter culture received significantly ($P < 0.5$) higher scores for flavour intensity and texture than the cheeses made with misophilic starter cultures. YY47 starter culture and adjunct culture of *L. helveticus*, *L. paracasei* subsp. *paracasei*, *L. delbrueckii* subsp. *lactis* and *E. faecium* produced the typical Ras cheese flavour.

The beneficial role of enterococci in the development of cheese aroma led to inclusion of selected enterococcal strains in certain starter cultures for Ras cheese. Further work is in progress to establish the possible use of *E. faecium* in commercial starter preparation.

## References


Abou-Donia, S. A. (2002). Recent development in Ras cheese research: a review. *Egyptian Journal of Dairy Science, 30*, 155–166.

AOAC (2000). Official methods of analysis. Association of Official Analytical Chemists, International, Arlington, VA.

Awad, S. (2006). Texture and Flavour development in Ras cheese made from raw and pasteurised milk. *Food Chemistry, 97*, 394–400.

Awad, S., Bahay-El Din, B., & El-Soda, M. (2001). Proteolytic activity of *Lactobacillus*, *Lactococcus* and *Enterococcus* strains in pseudo-curd, casein hydrolysate and cheese slurry. *Egyptian Journal of Dairy Science, 29*, 87–97.

Awad, S., El Attar, A., Ayad, A. H., & El-Soda, M. (2003). Characteristic of Egyptian market Ras cheese; 1-sensory evaluation, rheological, physico-chemical properties and microbiological analysis. *Egyptian Journal of Dairy Science, 31*, 289–303.

Ayad, E. H. E., Awad, S., El-Attar, A., de-Jong, C., & El-Soda, M. (2004a). Characterisation of Egyptian Ras cheese. 2. Flavour formation. *Food Chemistry, 86*, 553–561.

Ayad, E. H. E., Nashat, S., El-Sadek, N., & El-Soda, M. (2004b). Selection of wild lactic acid bacteria isolated from traditional Egyptian dairy products according to production and technological criteria. *Food Microbiology, 21*, 715–725.

Bahay-El-Din, B. B., El-Soda, M., & Ezzat, N. (2002). Proteolytic, lipolytic and autolytic activities of enterococci strains isolated from Egyptian dairy products. *Lait, 82*, 289–304.

Centeno, J. A., Menendez, S., & Rodriguez-Otero, J. L. (1996). Main microbial flora present as natural starters in Cebreiro raw cow's milk cheese (Northwest Spain). *International Journal of Food Microbiology, 33*, 307–313.

Deeth, C. H., Fitz-Gerald, C. H., & Wood, A. F. (1975). A convenient method for determining the extent of lipolysis in milk. *Australian Journal of Dairy Technology, 30*, 109–111.

Drake, M. A., Boylston, T. D., Spence, K. D., & Swanson, B. G. (1996). Chemical and sensory effects of a *Lactobacilus* adjunct in Cheddar cheese. *Food Research International, 29*, 381–387.

Egyptian standards (2001). Ras cheese Part 5, ES: 1007/2001. *Ministry of Industry and Technological Development*, Egypt.

El Soda, M. (1993). Control and enhancement of flavour in cheeses in microbiology of flavour in cheese. In B. Law (Ed.), *Microbiology and Biochemistry of cheese and fermented milk* (pp. 219–246). London: Blackie Academic professional.

El Soda, M., Hantira, A. A., Ezzat, N., & El-Shafei, H. K. (1992). Accelerated ripening of Ras cheese using freeze-shocked mutant strains of *Lactobacillus casei*. *Food Chemistry, 44*, 179–184.

El Soda, M., Madkor, S. A., & Tong, P. S. (2000). Adjuncts cultures: recent developments and potential significance to cheese industry. A review. *Journal of Dairy Science, 83*, 609–619.

El-Soda, M., El-Ziney, M., Awad, S., Osman, G., Omran, N., Gamal, G., et al. (2003). A culture collection of lactic acid bacteria isolated from raw milk and traditional Egyptian dairy products. *Egyptian Journal of Dairy Science, 31*, 23–41.

Ezzat, N., & El-Shafei, H. (1991). Acceleration ripening of Ras cheese using freeze and heat-shocked *Lactobacillus helveticus*. *Egyptian Journal of dairy Science, 19*, 347–358.

Folkertsma, B., & Fox, P. F. (1992). Use of the cadmium-ninhydrin reagent to assess proteolysis in cheese during ripening. *Journal of Dairy Research, 59*, 217–224.

Fox, P. F., McSweeney, P. L. H., & Lynch, C. M. (1998). Significance on non starter lactic acid bacteria in Cheddar cheese. *Australian Journal of Dairy Technology, 53*, 83–89.

Giraffa, G. (1995). Entercoccal bacteriocins: potential as antilisteria factors in dairy technology. *Food Microbiology, 12*, 291–299.

Hofi, A. A., Youssef, E. H., Ghoneim, M. A., & Tawab, G. A. (1970). Ripening changes in Cephalotyre "Ras" cheese manufactured from raw and pasteurised milk with special reference to flavour. *Journal of Dairy Science, 53*, 1207–1212.

Johnson, M. E. (1998). Cheese products. In E. H. Marth & J. L. Steele (Eds.), *Applied dairy microbiology* (pp. 213–249). New York: Marcel Dekker Inc.

Kebary, K. M. K., Khader, A. E., Zedan, A. N., & Mahmoud, S. F. (1996). Accelerated ripening of low fat Ras cheese by attenuated lactobacilli cells. *Food Research International, 29*, 705–713.

Kosikowski, F.V., and Mocquot, G. (1958). Advances in cheese technology. FAO Agric Stud Nr 38. Rome, Italy: *Food and Agriculture Organization* (FAO). 15 p.

Kuchroo, C. N., & Fox, P. F. (1982). Soluble nitrogen in Cheddar cheese. Comparison of extraction procedures. *Milchwissenschaft, 37*, 331–335.

Lane, C. N., & Fox, P. F. (1997). Role of starter enzymes during ripening of Cheddar cheese made from pasteurized milk under controlled microbiological conditions. *International Dairy Journal, 7*, 55–63.

Lane, C. N., & Fox, P. F. (1996). Contribution of starter and adjunct lactobacilli to proteolysis in Cheddar cheese during ripening. *International Dairy Journal, 6*(7), 715–728.

Lau, K. Y., Barbano, D. M., & Rasmussen, R. R. (1991). Influence of pasteurization on protein breakdown in Cheddar cheese during ripening. *Journal of Dairy Science, 74*, 727–740.

Law, B. A. (2001). Controlled and accelerated cheese ripening: the research base for new technology. *International Dairy Journal, 11*, 283–396.

Litopoulou-Tzanetaki, E. (1990). Changes in numbers and kinds of lactic acid bacteria during ripening of Kefalotyri cheese. *Journal of Food Science, 55*, 111–113.

Litopoulou-Tzanetaki, E., Tzanetakis, N., & Vafopoulou-Mastrojiannaki, A. (1993). Effect of type of lactic starter on microbiological, chemical and sensory characteristics of Feta cheese. *Food Microbiology, 10*, 31–41.

Madkor, S. A., Tong, P. S., & El Soda, M. (2000). Ripening of Cheddar cheese with added attenuated adjunct cultures of lactobacilli. *Journal of Dairy Science, 83*, 1684–1691.

Sabbour, M.M. (1966). Bacteriological studies on dye reduct on and keeping quality tests of milk. M.Sc. Thesis, *Faculty of Agriculture, Ain Shams University*, Cairo.

Sallami, L., Kheadr, E. E., Fliss, I., & Vuillemard, C. J. (2004). Impact of autolytic, proteolytic, and nisin-producing adjunct cultures on biochemical and textural properties of Cheddar cheese. *Journal of Dairy Science, 87*, 1585–1594.

SAS® (1999). *User's guide: statistics* (8 ed.). Cary, NC: SAS Inst.

Singh, T. K., Drake, M. A., & Cadwallader, K. R. (2003). Flavour of Cheddar cheese: a chemical and sensory perspectives. *Comprehensive Reviews in Food Science and Food Safety, 2*, 139–162.

Trepanier, G., El Aboudoi, M., Lee, B. H., & Simard, R. E. (1992). Accelerated maturation of cheddar cheese: Microbiology of cheese supplemented with *Lactobacillus casei* subsp. *casei*. *Journal of Food Science, 57*, 345–347.

Webb, B. H., Johnson, A. H., & Alford, J. A. (1983). *Fundamentals of dairy chemistry* (2nd ed.). Westport, CT: AVI Publishing Co. Inc.

Wessels, D., Jooste, P. J., & Mostert, J. F. (1990). Technologically important characteristics of *Enterococcus* isolates from milk and dairy products. *International Food Microbiology, 10*, 349–352.