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# Texture and flavour development in Ras cheese made from raw and pasteurised milk

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**Abstract**

Texture, proteolysis and flavour development in Ras cheeses made from raw or pasteurised milk with two different thermophilic lactic cultures were monitored during ripening. Results showed that at day 1 of manufacture, the moisture content and pH were lower in raw milk cheese than in pasteurised milk cheeses. Levels of water-soluble nitrogen, casein breakdown, free amino groups and free fatty acids were higher in cheese made from raw milk than in that made from pasteurised milk. Textural characteristics, such as hardness, cohesiveness and chewines, increased in all treatments during the first 60 days of ripening due to the reduction in the moisture level during the second stage of salting (dry salting during the first 60 days of ripening). Cheese made from raw milk received the highest texture and flavour scores by panellists.


*Keywords:* Ras cheese; Proteolysis; Texture; Raw milk; Pasteurised milk

## 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 typical cheese flavour results from lipolysis, proteolysis and further degradation of amino acids 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 (Creamer & Olson, 1982). For the development of an acceptable Cheddar 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). These products of proteolysis either contribute directly to flavour (Visser, 1993) or act as precursors of flavour compounds. Major textural changes occur during curing. The texture of cheese depends upon the cheese composition and the extent of biochemical changes during ripening (De Jong, 1976; Fox, Guinee, Cogan, & McSweeney, 2000; Lawrence, Creamer, & Gilles, 1987).

Ras cheese is the most popular hard cheese in Egypt. It is similar to the Greek variety "Kefalotyri" (Phelan, Renaud, & Fox, 1993). This cheese is manufactured in small factories located in the Delta region. It is made from raw cow's milk or a mixture of cow's and buffalo's milks without using starter cultures (Awad, El-Attar, Ayad, & El-Soda, 2003). The fermentation always occurs by the native microflora from the raw milk and the environment. Moreover, Ras cheese is usually stored in moist and uncontrolled hygienic conditions which support the growth of moulds and yeasts. Consequently, the final flavour and texture will be influenced by the actions of all these factors (Ayad, Awad, El-Attar, de-Jong, & El-Soda, 2004).

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Recently, the Egyptian Organisation for Standardisation and Quality Control published new standards indicating that all cheese varieties must be made from pasteurised milk. Milk pasteurisation influences both the extent and characteristics of proteolysis during cheese ripening (Singh et al., 2003). It causes very limited heat-induced interactions of whey protein with casein which result in the retention of additional whey proteins in cheese. The presence of heat-denatured whey protein in cheese influences the accessibility of casein to proteinases during ripening (Lau, Barbano, & Rasmussen, 1991).

Applying the new Egyptian standards to the Ras cheese making will require using starter cultures. Therefore, maintaining the typical Ras cheese flavour would be a challenge. The high cooking temperature (45 °C) used in making Ras cheese allows only thermophilic cultures to grow. This work was planned to investigate the changes in flavour, proteolysis and texture during ripening of Ras cheese made from raw and pasteurised milk using two different thermophilic lactic cultures.

## 2. Materials and methods

### 2.1. Milk and cultures

Raw cow's and buffalo's milks were obtained from the farm of Faculty of Agriculture, Alexandria University. Recombinant chymosin (Chy-max) and commercial "direct to vat set" thermophilic starter cultures (FRC60 and YY47) were obtained from Chr. Hansen, Denmark.

### 2.2. Ras cheese making

A mixture of raw cow's and buffalo's milks (80:20, 4.6% fat and 0.18% acidity as lactic acid) was divided into two portions: the first portion was used without pasteurisation for making raw milk cheese, while the second one was heated at 63 °C/30-min, and then cooled to 30 °C. Calcium chloride (0.02%, w/w) was added to the pasteurised milk which was divided into 2 portions: portion A to which the FRC60 culture (0.01%, w/w) was added and portion B to which the YY47 culture (0.01%, w/w) was added. After 1 h of milk ripening at 30 °C, the appropriate amount of Chymosin was added to clot the milk in 40 min. The coagulum was cut and cooked to 45 °C over 45 min and held at this temperature for 15 more minutes. The curd was salted (3%, w/w, NaCl), hooped and pressed as described by Hofi, Youssef, Ghoneim, and Tawab (1970). Dry salt was applied to the surfaces of cheese during the first 60 days of ripening (Hofi et al., 1970). The raw milk cheese was made using the above procedure without pasteurisation or addition of $CaCl_2$ or starter culture. Cheeses were stored without waxing at 13 ± 2 °C and 80 ± 5% relative humidity for 180 days.

### 2.3. Cheese analyses

Cheese was analysed for fat by Gerber method (AOAC, 2000) and total protein by macro-Kjeldahl (AOAC, 2000). The moisture content was determined using the moisture analyser (Mettler Toledo Model HR73, Switzerland). Salt content was determined using the chloride analyser (Jenway, England, UK). The pH was measured in slurry prepared by macerating 20 g of grated cheese in 20 ml of deionised water.

### 2.4. Assessment of proteolysis

#### 2.4.1. Water-soluble nitrogen

The water-soluble extracts (WSE) were prepared according to the method of Kuchroo and Fox (1982) and their nitrogen content was determined using the macro-Kjeldahl method (IDF, 1993).

#### 2.4.2. Electrophoresis

Cheese samples and their water-soluble extracts were analysed by urea–polyacrylamide gel electrophoresis (PAGE) using a protean II vertical slab gel unit (Bio-Rad Laboratories, USA) as described by Andrews (1983). The gels were stained directly with Commassie brilliant blue G250.

#### 2.4.3. Free amino groups

The concentrations of free amino groups in cheeses were determined by the Cd–ninhydrin method of Folkertsma and Fox (1992). A standard curve based on leucine was used to convert $A_{507}$ to its leucine equivalent (mg $g^{-1}$ cheese).

### 2.5. Assessment of lipolysis

Free fatty acids (FFA) were determined by the method of Deeth, Fitz-Gerald, and Wood (1975) and expressed as mg oleic acid $g^{-1}$ cheese fat.

### 2.6. Texture profile analysis

Samples for texture profile analysis (TPA) were obtained from the middle of the whole cheese block rather than from the surface to avoid surface effects. Cheese cubes ($20 \times 20 \times 20$ mm) were placed in plastic cups, sealed (to prevent dehydration) and tempered to 12 ± 0.5 °C prior to analysis. A two-bite penetration test was performed using the Texture Analyser (TA1000, CNS-Farnell, England) with the TA 17 probe (30° and 25 mm diameter) and operated at a crosshead speed of 1 mm $s^{-1}$ and penetration distance of 10 mm. Hardness, cohesiveness, springiness and chewiness were evaluated

in triplicate according to the definitions given by IDF (1991).

### 2.7. Sensory evaluation

The sensory evaluation was carried out at Department of Dairy Science and Technology, Alexandria University by a panel consisting of 10–15 cheese graders, including staff members and assistants, cheese producers and consumers. Cheeses were graded at 60, 120 and 180 days of age for flavour intensity and flavour and texture acceptability on a 0–100 point scale, where 0–25 = unacceptable, 26–50 = poor, 51–75 = acceptable, and 76–100 = good.

### 2.8. Statistical analysis

The reported data are the average of three measurements per replicate. Cheeses were made three times. 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

The composition of experimental Ras cheeses is summarised in Table 1. Pasteurised milk cheeses retained more moisture ($P < 0.05$) than raw milk cheeses at day one of manufacture. The moisture in all cheeses decreased during ripening. Most of the moisture losses occurred during the first 30 days of ripening. This could be attributed to the second stage of salting that took place during this period (Hofi et al., 1970). The fat and protein contents on dry basis were not different ($P > 0.05$) among cheeses. Ras cheese was salted in two stages: before and after pressing. There was a constant increase in the salt content during the first 60 days of ripening after which, only a slight increase was observed throughout the ripening. There were no significant differences ($P > 0.05$) in salt content among all cheeses. No significant differences were seen in composition between the two types of pasteurised milk cheeses. The aged cheeses were within the reported limits for salt, moisture, fat and protein in market Ras cheese (Abou-Donia, 2002; Awad et al., 2003).

### 3.2. pH changes

The pH values of experimental cheeses determined during ripening are presented in Fig. 1. At day 1 of manufacture, raw milk cheese showed lower ($P < 0.05$) pH values than did pasteurised milk cheeses. The pH of all cheeses decreased ($P < 0.05$) gradually during the first 60 days, after which a significant increase was observed. The lowest pH values in the raw milk cheese and pasteurised milk cheeses were found after 30 and 60 days of ripening, respectively. The pH of cheese is influenced by the growth of both starter and non-starter lactic acid bacteria in raw and pasteurised milk cheeses. The increase in pH at the end of ripening has been attributed to the utilisation of lactic acid, formation of non acidic decomposition products and liberation of alkaline products of protein decomposition (McSweeney & Fox, 1993).

Table 1
Chemical composition (% w/w) of Ras cheese made from raw and pasteurised milk

| Cheese code[1] | Ripening time (days) | Moisture | Fat | FDM[2] | Protein | Salt | SM[3] |
|---|---|---|---|---|---|---|---|
| Raw milk | 1 | 40.63[b] | 29.75 | 50.11 | 22.85 | 1.70 | 4.18 |
| | 30 | 36.10[d] | 32.40 | 50.71 | 24.23 | 2.12 | 5.88 |
| | 60 | 33.20[g] | 33.75 | 50.52 | 25.75 | 3.45 | 10.39 |
| | 120 | 32.66[h] | 34.00 | 50.49 | 26.65 | 3.75 | 11.48 |
| | 180 | 32.35[h] | 34.00 | 50.27 | 26.89 | 3.95 | 12.21 |
| FRC60 | 1 | 42.22[a] | 29.40 | 50.88 | 21.6 | 1.75 | 4.14 |
| | 30 | 38.19[c] | 31.07 | 50.27 | 22.72 | 2.01 | 5.26 |
| | 60 | 35.43[e] | 32.50 | 50.33 | 23.75 | 3.45 | 9.74 |
| | 120 | 34.27[f] | 33.25 | 50.58 | 25.85 | 3.87 | 11.29 |
| | 180 | 34.02[f] | 33.50 | 50.77 | 26.12 | 4.05 | 11.90 |
| YY47 | 1 | 42.33[a] | 29.20 | 50.63 | 22.95 | 1.77 | 4.19 |
| | 30 | 38.60[c] | 31.15 | 50.73 | 23.34 | 2.24 | 5.80 |
| | 60 | 35.33[e] | 32.50 | 50.25 | 23.2 | 3.35 | 9.48 |
| | 120 | 34.39[f] | 33.25 | 50.67 | 25.25 | 3.79 | 11.02 |
| | 180 | 34.17[f] | 33.50 | 50.88 | 26.31 | 4.05 | 11.85 |

[a,b,c,d,e,f,g,h] means within a column with no common subscript differ $P < 0.05$.

[1] Raw milk, Ras cheese made from raw milk. FRC60, Ras cheese made from pasteurised milk with FRC60 culture. YY47, Ras cheese made from pasteurised milk with YY47 culture.

[2] FDM, fat in dry matter.

[3] SM, salt in moisture.

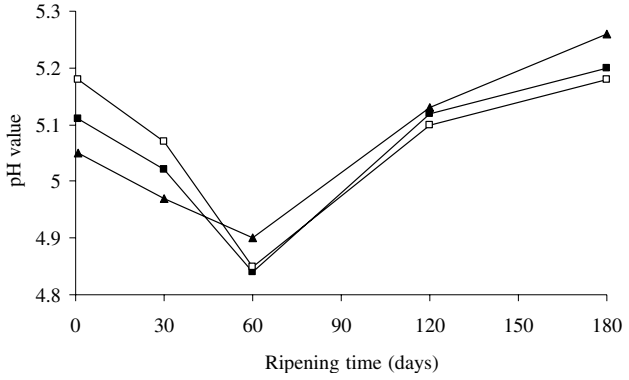


Fig. 1. Changes in pH during ripening of Ras cheese made from raw (▲) and pasteurised milk with (■, FRC60 culture; □, YY47 culture).

### 3.3. Proteolysis

#### 3.3.1. Development of water-soluble nitrogen

The level of WSN%TN in cheeses increased from ~4.2–4.9% at day one to ~21.5–26.5% after 180 days of ripening. The increase in water-soluble nitrogen (WSN) was at a higher rate in raw milk cheese than in both pasteurised milk cheeses throughout ripening (Fig. 2). The rate of the increase in WSN was similar in both cheeses made from pasteurised milk.

#### 3.3.2. Urea–polyacrylamide gel-electrophoresis (PAGE)

Electrophoretograms of the experimental cheeses at various stages of ripening are shown in Fig. 3. The overall degradation pattern of the control cheese was similar to that previously reported for Ras cheese (Awad et al., 2003). In general, the trend observed with urea–PAGE was consistent with that of WSN, with the overall level of proteolysis in raw milk cheese being higher than that in pasteurised milk cheeses. Generally, the $\alpha_{s1}$-casein was more extensively hydrolysed than β-casein. The $\alpha_{s1}$-casein was hydrolysed initially to $\alpha_{s1}$-1-casein (f24-199) and subsequently to other peptides with electrophoretic mobilities faster than that of $\alpha_{s1}$-I-casein. After 180 days of ripening, $\alpha_{s1}$-casein was almost completely degraded in raw milk cheese and decreased to low concentration in pasteurised milk cheeses.

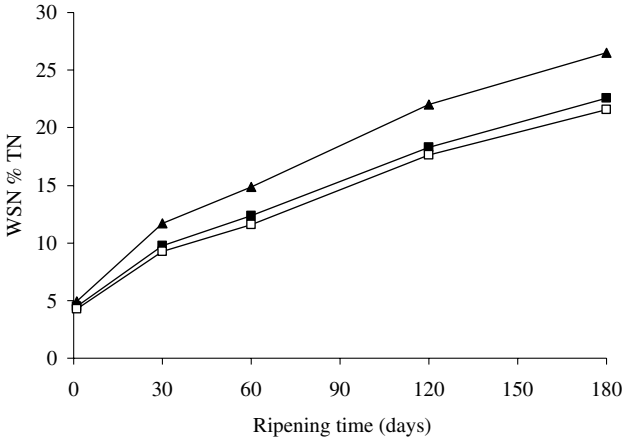


Fig. 2. Evolution of water-soluble nitrogen (WSN) as percent of total nitrogen (TN) in Ras cheese made from raw (▲) and pasteurised milk with (■, FRC60 culture; □, YY47 culture).

The intensity of the band corresponding to β-casein decreased slightly throughout ripening with a concomitant increase in the bands corresponding to the γ-caseins, suggesting that β-casein was degraded primarily by the indigenous milk enzymes, mainly plasmin, which have a high specificity for β-CN (Fox, Law, McSweeney, & Wallace, 1993). Slight quantitative differences were evident between the electrophoretograms of both pasteurised milk cheeses with regard to $\alpha_{s1}$, β-casein, and their degradation products.

Differences in the level of WSN and casein breakdown between the raw milk and pasteurised milk cheeses could be due to the action of non-starter peptidases present in raw milk, the greater retention and activity of the coagulant in raw milk cheese resulting from their lower pH at day one of manufacture (Lane & Fox, 1997), and the presence of heat-denatured whey proteins in pasteurised milk cheese which influences the accessibility of casein to proteinases (Lau et al., 1991),

Electrophoretic patterns of the water-soluble extract (WSE) of the cheeses are present in (Fig. 3). Both qualitative and quantitative differences were observed among WSE from all treatments during ripening. After 180 days of ripening, high relative densities of bands were present in WSE of pasteurised milk cheeses compared to those of the raw milk cheeses. Some minor quantitative differences were also observed between the WSE of the two cheeses made from pasteurised milk. These results confirm the finding of Lane and Fox (1997) that proteinases of lactic acid bacteria present in raw milk can hydrolyse water-soluble peptides.

#### 3.3.3. Liberatiion of free amino groups

The level of free amino groups was higher in the raw milk cheese than in pasteurised milk cheeses at all stages of ripening (Fig. 4). In addition, the concentration of free amino groups was higher in pasteurised milk cheese made with FRC60 than in cheese made with the YY47 culture. Visser (1977) and Lane and Fox (1996, 1997) reported that the starter and non-starter enzymes were the major contributors to the production of small peptides and the accumulation of amino acids in cheese during ripening.

### 3.4. Lipolysis

The level of free fatty acids (FFA) increased markedly throughout ripening (Fig. 5). Raw milk cheese consistently showed higher FFA levels throughout ripening. The release of FFA in cheese contributes to the

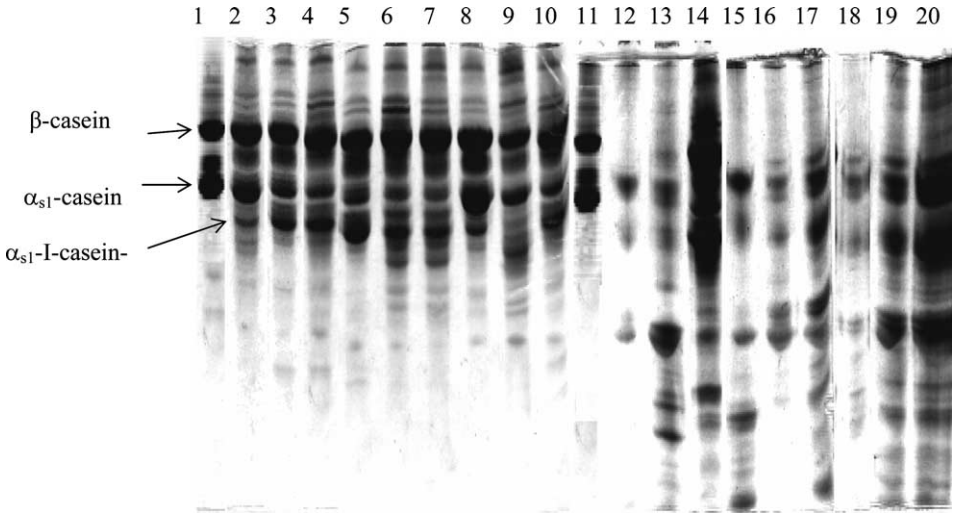


Fig. 3. Urea–polyacrylamide gel electrophoretograms of sodium caseinate (lanes 1 and 11), cheese made from pasteurised milk with FRC60 culture after ripening for 30, 120 and 180 days (lanes 2, 3 and 4), cheese made from raw milk after ripening for 30, 120 and 180 days (lanes 5, 6 and 7), and cheese made from pasteurised milk with YY47 culture after ripening for 30, 120 and 180 days (lanes 8, 9 and 10). Water-soluble extract (WSE) of cheese made from pasteurised milk with FRC60 culture after ripening for 30, 120 and 180 days (lanes 12, 13 and 14), WSE of cheese made from raw milk after ripening for 30, 120 and 180 days (lanes 15, 16 and 17), and WSE of cheese made from pasteurised milk with YY47 culture after ripening for 30, 120 and 180 days (lanes 18, 19 and 20).

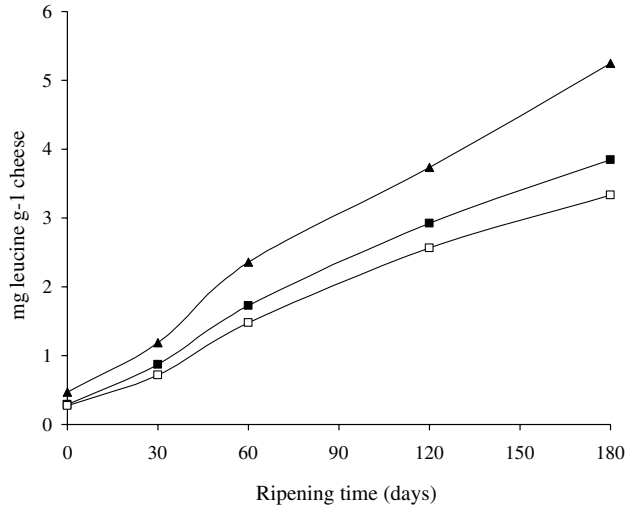


Fig. 4. Liberation of free amino groups in Ras cheese made from raw (▲) and pasteurised milk with (■, FRC60 culture; □, YY47 culture).

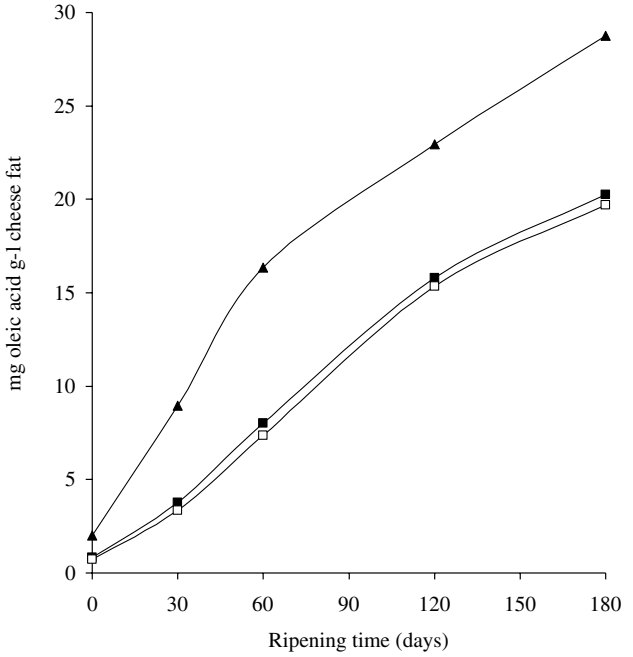


Fig. 5. Liberation of free fatty acids in Ras cheese made from raw (▲) and pasteurised milk with (■, FRC60 culture; □, YY47 culture).

development of flavour and is due to the lipolysis of fat by different enzymatic systems in cheese. High FFA levels in cheeses containing adjunct culture have been previously reported by El-Soda, Hantira, Ezzat, and El-Shafei (1992); Madkor, Tong, and El-Soda (1999), and were attributed to the release of intracellular esterases and lipases. Commonly, the levels of FFA follow the same trend as soluble nitrogen, suggesting that same factors affect both proteolysis and lipolysis (Kebary, Khader, Zedan, & Mahmoud, 1996).There were no significant differences between FFA in the two cheeses made from pasteurised milk. Most of lactic cultures used in the manufacture of fermented milk products have weak intracellular lipase and esterase activities (Paulsen, Kowalewska, Hammond, & Glatz, 1980).

### 3.5. Texture profile analysis

The changes in texture profile analysis parameters (hardness, cohesiveness, springiness and chewiness) during ripening of experimental cheeses are shown in

Table 2. Generally, values for these parameters tended to increase gradually during first 60 days of ripening at rates that depended on the moisture loss. At day one of manufacture, raw milk cheese was significantly harder than pasteurised milk cheeses. The hardness increased consistently during first 60 days of ripening, and then remained almost constant until the end of the ripening. The increase in hardness during the first 60 days of ripening is related to decreasing moisture which acts as a plasticiser in the protein matrix, thereby making it less elastic and more susceptible to fracture upon compression (Fox et al., 2000). In general, pasteurisation of milk reduces the hardness of fresh cheese which might due to the increased moisture content. At the end of ripening, a decrease in the hardness of raw milk cheese was observed (Table 2). This could be due mainly to the higher proteolysis in raw milk cheese than in pasteurised milk cheese. Hardness has been reported to have a good correlation with proteolysis (Fedrick, 1987).

There were no differences ($P > 0.05$) in cohesiveness among all aged cheeses. Cohesiveness increased gradually during the first 120 days of ripening, and then slightly decreased. The springiness slowly increased as the ripening period progressed up to 120 days, and then levelled off. Chewiness was higher ($P < 0.05$) in fresh raw milk cheese than in pasteurised milk cheese. After 180 days of ripening the raw milk cheese became softer and smoother and hence, the chewiness values decreased. The chewiness of aged raw milk cheese was lower than that of the pasteurised milk cheese, due to the high proteolysis in the former type.

Table 2
Texture profile analysis of Ras cheese made from raw and pasteurised milk

| Parameter | Ripening time (days) | Cheese code[1] | | |
|---|---|---|---|---|
| | | Raw | FRC60 | YY47 |
| Hardness (g) | 1 | 485[a] | 455[b] | 448[b] |
| | 60 | 660[a] | 624[b] | 607[b] |
| | 120 | 660[a] | 631[b] | 628[b] |
| | 180 | 642[a] | 640[a] | 636[a] |
| Cohesiveness | 1 | 0.39[a] | 0.38[a] | 0.37[a] |
| | 60 | 0.54[a] | 0.51[ab] | 0.49[b] |
| | 120 | 0.57[a] | 0.57[a] | 0.56[a] |
| | 180 | 0.53[a] | 0.56[a] | 0.55[a] |
| Springiness (mm) | 1 | 6.70[a] | 6.80[a] | 6.75[a] |
| | 60 | 7.10[a] | 7.12[a] | 7.13[a] |
| | 120 | 7.20[a] | 7.13[b] | 7.15[b] |
| | 180 | 7.15[a] | 7.15[a] | 7.16[a] |
| Chewiness (g $s^{-1}$) | 1 | 1267.31[a] | 1175.72[b] | 1118.88[c] |
| | 60 | 2530.44[a] | 2265.87[b] | 2120.68[c] |
| | 120 | 2708.64[a] | 2564.45[b] | 2514.51[b] |
| | 180 | 2432.86[c] | 2562.56[a] | 2504.57[b] |

[a,b,c] means within a row with no common subscript differ $P < 0.05$.
[1] Raw milk, Ras cheese made from raw milk. FRC60, Ras cheese made from pasteurised milk with FRC60 culture. YY47, Ras cheese made from pasteurised milk with YY47 culture.

Table 3
Sensory evaluation of Ras cheese made from raw and pasteurised milk

| Parameter | Ripening time (days) | Cheese code[1] | | |
|---|---|---|---|---|
| | | Raw | FRC60 | YY47 |
| Flavour intensity[2] | 60 | 52.36[a] | 42.15[b] | 39.45[b] |
| | 120 | 64.89[a] | 44.45[b] | 42.67[b] |
| | 180 | 78.68[a] | 50.25[b] | 49.58[b] |
| Flavour acceptability[2] | 60 | 61.98[a] | 44.23[b] | 45.65[b] |
| | 120 | 75.67[a] | 56.01[b] | 54.65[b] |
| | 180 | 83.74[a] | 62.58[b] | 61.24[b] |
| Texture acceptability[2] | 60 | 65.74[a] | 65.47[a] | 62.58[a] |
| | 120 | 73.75[a] | 70.67[a] | 71.25[a] |
| | 180 | 82.45[a] | 74.58[b] | 75.65[b] |

[a,b] means within a row with no common subscript differ $P < 0.05$.
[1] Raw milk, Ras cheese made from raw milk. FRC60, Ras cheese made from pasteurised milk with FRC60 culture. YY47, Ras cheese made from pasteurised milk with YY47 culture.
[2] Flavour intensity, flavour and texture acceptability on a 0–100 scale. 0–25 = unacceptable, 26–50 = poor, 51–75 = acceptable, 76–100 = good.

### 3.6. Sensory analysis

The mean grades for flavour intensity and flavour and texture acceptability of cheeses at 60, 120 and 180 days of ripening are shown in Table 3. Generally, the raw milk cheese received higher scores than pasteurised milk cheeses, but both aged cheeses were considered acceptable. The flavour intensity increased as the ripening period progressed. The typical Ras cheese flavour was not noticed in aged cheeses made from pasteurised milk. This might due to their relatively lower levels of free amino groups and free fatty acids. The concentrations of low molecular weight peptides and free amino acids have considerable influence on the cheese flavour (Lowrie & Lawrence, 1972). In addition, the concentration of free fatty acids, especially the short chain ones, is responsible for the characteristic cheese flavour (Kanawjia, Rajesh, Latha, & Singh, 1995).

## 4. Conclusion

The results of this study confirm the general view that cheese made from raw milk develops the characteristic flavour more rapidly than that made from pasteurised milk. Lactic acid bacteria in raw milk are important not only for the acid development which hastens the milk coagulation and assists in expulsion of whey, but also because of their influence on flavour, body and texture of the final cheese. The flavour and texture were acceptable in pasteurised milk cheese made using thermophilic lactic starter cultures. Consumers might prefer safer pasteurised milk cheese which may not develop the typical Ras cheese flavour found in that made from raw milk.

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