THE INFLUENCE OF DIFFERENT FACTORS ON SYNERESIS OF RENNET CURD OBTAINED FROM EWE’S MILK

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ABSTRACT
Different factors affecting syneresis of rennet curd, made from ewe's milk, were examined. The experimental factors were: pasteurisation, temperature and pH of milk, curd firmness, curd temperature and rate of curd temperature rise, time (after renneting). Also the effect of above mentioned factors on dry matter content, yield and texture of fresh cheeses was established. It was concluded that syneresis of pasteurised milk curd was lower in comparison to syneresis of raw milk curd. Temperature and milk pH, curd temperature and rate of curd temperature rise, time of measurement influenced significantly syneresis from rennet curd. The pasteurisation and pH of milk influenced the yield, texture and dry matter content of fresh cheeses.

Key words: syneresis, rennet curd, ewe's milk
INTRODUCTION

The syneresis is a process of expelling of whey from curd with parallel curd shrinking. This process goes on with different intensity in time and it is difficult to establish its termination [15]. The meaning of syneresis is important in milk fermented drinks, and extremely meaningful in cheese production [8, 10, 15]. The volume of whey expelled influences the cheese and at the same time determines its quality. Production of different kind of cheese requires to keep large or small amounts of whey inside the cheese. Technologist achieve this by choosing different production parameters that decide syneresis process performance (whey drainage). Among factors influencing the syneresis are: milk composition, milk acidity, some functional additives (especially calcium); heating; homogenisation and ultrafiltration processes; amount of rennet added; curd firmness; magnitude and kind of area which expels whey (magnitude, and horizontal and vertical dimensions of curd grain); pressure during curd mixing; curd temperature (cooking temperature); curd temperature rise [4, 7, 9, 11, 15]. Some factors (size of curd granules, pressure, pH, gel temperature) have influenced syneresis whereas other have no influence [4, 9, 15]. The factors influencing syneresis determine also the final quality of cheeses[15]. A lot of papers has been already published concerning syneresis of rennet curd from cow’s milk. Far less evidence has been provided on the syneresis of rennet curd from ewe’s milk. Ewe’s milk is a very good material for cheese production because of very rich composition, higher dry matter, fat and protein (especially casein) contents than cow’s and goat’s milks [1]. The differences in chemical composition of ewe’s milk in comparison to cow’s milk can also influence the rennet curd syneresis and cheeses composition.

The aim of this research was to establish the influence of some chosen factors: milk pasteurisation process, milk temperature and initial pH at rennet addition, temperature and rate of curd heating on rennet curd syneresis. During the same experiments dependences between extent of curd syneresis (measured as amount of whey expelled during 60 minutes after curdling) and the cheeses quality, yield, texture and dry matter content were estimated.

MATERIALS AND METHODS

The material was ewe's milk obtained during spring from Polish long-fleece sheep breaded in sheepyard of University of Agriculture in Cracow. Milk was sampled at 5 l volume from milked batch and all analyses were performed during two subsequent hours.

The dry mass content by drying method [2], the fat content by Gerber method [6], the total nitrogen compounds content by Kjeldahl method, the casein content by Kjeldahl method (after precipitation with sodium acetate and acetic acid), the lactose content by Bertrand method were measured. Also the milk density was measured by lactodensimeter, the titratable acidity by Soxhlet – Henkel’s method and rennet coagulation time by Schern method were established [6].

Half of milk was pasteurised in 72°C during 15 sec, whereas half of milk was left in raw state. After pasteurisation the renneting time by Schern method and milk pH were measured.

The raw and pasteurised milks were subjected to 5 separate experiments. In each experiment raw milk (s) and pasteurised milk (p) in amount of 75 cm³ were poured to glass tubes (100 cm³ volume). The defined, constant temperature of milks was obtained by balanced heating. To all batches the same amount of rennet was added, with strength 1:1000 (0.25 cm³ per 75 cm³) to obtain the curd after 15 minutes. Next steps in each of 5 experiments are described below.

The whey drainage was measured starting from 5 minutes after curding and then after each subsequent 15 minutes up to 60 minutes. The drainage was estimated by measuring the summed volume of expelled whey basing on Marshall method [12].

The curd was separated from cylinder walls and cross cut into 1 cm depth rows with long, slim knife, then after subsequent 15 minutes curd was gently poured out into the plastic sieve and whey was separated into measuring cylinder.
Plan of experiments.

Experiment 1

It was aimed to estimate the influence of curd firmness, which was measured as time from curding (after 5, 30 and 60 minutes), on syneresis. The experiment took place in 30°C in milk with normal pH. The volume of expelled whey was measured after coagulation during 5 minutes (1s and 1p), 30 minutes (2s and 2p) and 60 minutes (3s and p).

Simultaneously, the texture of curds, made from raw and pasteurised milks with different firmness, was measured. The texture measurements were done with TA-XT2 Micro System Texture Analyser with plastic conical penetrator, φ 20 mm, with penetrated curd into 20 mm depth with 1 cm per second speed. Basing on above measurements texture parameters were established with Texture Expert v. 1.05 program, algorithm “Fracture TPA”. The parameters measured were: hardness, adhesiveness, cohesiveness, springiness, and gumminess. Hardness was estimated as the end force needed for sample deformation (this is a point on curve of maximal deformation during first cycle of pressing); adhesiveness was calculated as minus peak area; cohesiveness as multiplication of first and second pressing peaks’ areas; springiness as the rate of time measured starting from beginning of second pressing cycle to reaching of maximal deformation during this cycle to time measured from beginning of first cycle of pressing to obtain of maximal deformation of this cycle; gumminess was calculated as multiplication of hardness and cohesiveness [13, 14].

Experiment 2

It was aimed to check the influence of milk temperature at the moment of rennet addition on syneresis process. The experiment was performed on milk with normal pH. The syneresis of curds obtained from milk heated to 25°C (1 s and 1 p), to 30°C (2s and 2p) and to 35°C (3s and 3p) was examined.

Experiment 3

It was aimed to check the influence of milk pH on curd syneresis at the moment of rennet addition. Milk temperature was 30°C. The measurements of amount of expelled whey from curds obtained from milk with normal pH (1s and 1p), with pH 6.3 (2s and 2p) and with pH 6.0 (3s and 3p) were performed. To lower pH of milk the 4,5 M lactic acid solution was used [12].

Experiment 4

It was aimed to check the influence of curd heating temperature on its syneresis. At the moment of rennet addition milk had normal pH and temperature of 30°C. The rate of temperature rising was 1°C/1 min. Curd heating was done by placing of glass pots with curd into aluminium water batch pots, which were heated on electrical plate. The measurements of syneresis from not heated (1s and 1p) and heated to 40°C (2s and 2p) and heated to 50°C (3s and 3p) were performed.

Experiment 5

It was aimed to check the influence of rate of temperature rise on curd syneresis. At the moment of rennet addition milk had normal pH and temperature of 30°C. The end heating temperature of curd was 40°C. The amount of whey expelled from not heated curd (1s and 1p), from curd heated at rate of 1°C/1 min (2s and 2p) and from curd heated at rate of 1°C/3 min (3s and 3p) was measured.

After finishing of all syneresis measurements (in experiments 1-5) the curds were gently put on weighed sieves and left for 12 hours. The yield of fresh cheese (12-hours drained curd) was calculated as percentage rate of cheeses’ weights to weights of milks used (for each cheese preparation). Also the dry mass content was established by drying method [AOAC, 1999] and their texture was measured with TA-XT2 as described above. The aluminium cylindrical penetrator, φ 50 mm, was used. The probe deformation was foreseen for 60% of cheese mass cut into 1,5 cm dimensions cube. The parameters measured were: hardness, adhesiveness, cohesiveness, springiness and chewiness. The way of hardness, adhesiveness, springiness and cohesiveness calculation was the same as described above, for curds texture measurements. The chewiness was defined as the multiplication of hardness, springiness and cohesiveness [13, 14].
All obtained results were estimated statistically with Statgraphic programme v. 5.2, which allowed for ANOVA performance and checking of statistical significance of pasteurisation and different experimental factors (experiments 1-5) influence on syneresis process performance during 60 minutes in curds and on yield, dry mass content and texture of 12-hour cheeses.

RESULTS AND DISCUSSION

Ewe’s milk used for experiments contained 16.44 % ± 2.11 of dry mass where 5.12 % ± 0.24 was fat, 5.24 % ± 0.11 total nitrogen compounds, 4.15 % ± 0.15 casein and 4.56 % ± 0.09 was lactose. The density of milk was 1.0342 ± 0.0002 g/cm³. Its pH before pasteurisation was 6.66 ± 0.01 and renneting time 107 ± 10 sec. After pasteurisation the pH of milk was lower (6.56 ± 0.010) and renneting time was longer (122 ± 0.15 sec). The chemical composition and density were in agreement with literature data for ewe’s milk. Milk’s pH was normal typical for fresh ewe’s milk [1]. According to Alichanidis & Polychroniadou [1] renneting time for ewe’s milk is shorter than for cow’s milk because of casein fractions composition. Observed longer milk renneting time after pasteurisation and its pH lowering were in agreement with results obtained by Balcones [3].

The process of rennet curd syneresis estimated as the volume of whey drainage measured during the first hour after renneting in five experiments is presented in Figures 1-5, with differentiation for pasteurised and raw milks.

There are presented the three factor MANOVA results of rennet curd syneresis in 5 experiments in dependence of: 1) milk pasteurisation, 2) three experimental parameters called in each experiment as A, B, C and 3) time of measurement of expelled whey (i.e. 15, 30, 45, and 60 minutes after coagulation).

In experiment 1, as it is shown on Figure 1, the process of whey drainage from raw milk curd and pasteurised milk curd was quite similar after 5, 30 and 60 minutes after coagulation. The highest drainage was in raw milk curd examined after 30 minutes and the lowest was in pasteurised milk curd examined after 5 minutes (less firm curd). The MANOVA results did not indicate the significant influence of curd firmness, measured as time for its occurrence, on amount of whey expelled (Table 1).

Figure 1. The whey drainage depending on curd firmness

1s – 3s – raw milk curds, 1p – 3p – pasteurized milk curds, 1s and 1p – the curds 5 min, 2s – 2p – 30 min, 3s – 3p – 60 min after curd formation
Table 1. Lowest squared means for volume of whey expelled from curd (as % of the original milk volume) depending on different factors

<table>
<thead>
<tr>
<th>Number and kind of experiments</th>
<th>Total means</th>
<th>Standard error</th>
<th>Pasteurisation</th>
<th>Factors in experiments 1-5</th>
<th>Time of syneresis measuring (minutes)</th>
<th>significance of differences</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>None</td>
<td>72°C/15 sec</td>
<td>A</td>
<td>B</td>
</tr>
<tr>
<td>1. The influence on curd firmness</td>
<td>9.87</td>
<td>0.46</td>
<td>10.85</td>
<td>8.89</td>
<td>-</td>
<td>9.83</td>
</tr>
<tr>
<td>2. The influence on milk temperature</td>
<td>8.56</td>
<td>0.45</td>
<td>9.78</td>
<td>7.35</td>
<td>1-2**</td>
<td>4.61</td>
</tr>
<tr>
<td>3. The influence on milk pH</td>
<td>14.97</td>
<td>0.45</td>
<td>15.73</td>
<td>14.20</td>
<td>-</td>
<td>9.38</td>
</tr>
<tr>
<td>4. The influence on curd temperature</td>
<td>21.18</td>
<td>0.68</td>
<td>23.21</td>
<td>19.15</td>
<td>1-2*</td>
<td>11.18</td>
</tr>
<tr>
<td>5. The influence on rate of curd temperature rise</td>
<td>17.68</td>
<td>0.87</td>
<td>20.66</td>
<td>14.70</td>
<td>1-2**</td>
<td>11.18</td>
</tr>
</tbody>
</table>

Denotes a statistically significant difference between means (p ≤ 0.05); and **(p ≤ 0.01).

The factors in experiments 1: A - 5 minutes after curd formation, B - 30 minutes and C - 60 minutes; 2: A - temperature of milk 25°C at the moment of rennet addition, B - 30°C and C - 35°C; 3: A - pH of milk 6.61 at the moment of rennet addition, B - pH 6.3 and C - pH 6.0; 4: A - curd without heating (temperature 30°C), B - curd heated to 40°C and C - to 50°C; 5: A - curd without heating (temperature 30°C), B - the rate of curd temperature rising 1°C/1 minute and C - 1°C/3 minutes.
In experiment 1 the texture measurement of curd after 5, 30 and 60 minutes after coagulation was performed. The results for texture are shown in Table 2. The MANOVA results showed that milk pasteurisation did not influenced significantly the texture of curd whereas the curd hardness and cohesiveness of curd were significantly growing with time, after renneting. The adhesiveness of curd was also growing when gumminess falling down, but not significantly. It could be observed that starting from coagulation moment of curd its texture had been changed with time (5, 30 and 60 min. after renneting) what confirmed the experiment thesis that curd cohesiveness could be estimated as time which lapsed from curd formation moment.

Table 2. Lowest squared means for texture parameters of curd made from ewe’s raw and pasteurised milks depending on the time measured after curd formation

<table>
<thead>
<tr>
<th>The curd texture parameters</th>
<th>Means total</th>
<th>Standard errors</th>
<th>Pasteurisation</th>
<th>Time after curd formation (min)</th>
<th>significance of differences</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>none</td>
<td>72°C/15 sec</td>
<td>5</td>
</tr>
<tr>
<td>Hardness TPA [G]</td>
<td>49.82</td>
<td>3.64</td>
<td>55.5</td>
<td>42.9</td>
<td>-</td>
</tr>
<tr>
<td>Adhesiveness TPA [G.s]</td>
<td>-118.1</td>
<td>15.97</td>
<td>-123.7</td>
<td>-109.3</td>
<td>-</td>
</tr>
<tr>
<td>Springiness TPA</td>
<td>0.95</td>
<td>0.03</td>
<td>0.95</td>
<td>0.95</td>
<td>-</td>
</tr>
<tr>
<td>Cohesiveness TPA</td>
<td>0.61</td>
<td>0.01</td>
<td>0.60</td>
<td>0.62</td>
<td>-</td>
</tr>
<tr>
<td>Gumminess [G]</td>
<td>29.28</td>
<td>1.61</td>
<td>31.6</td>
<td>26.3</td>
<td>-</td>
</tr>
</tbody>
</table>

Denotes a statistically significant difference between means (p ≤ 0.05).

In experiment 2, where the rennet curd was obtained from milk with different temperature, the highest syneresis showed the raw milk curd with temperature of 35°C, and the lowest the pasteurised milk curd with temperature 25°C (Fig. 2).

MANOVA results confirmed the significant influence of pasteurisation on rennet curd syneresis (Table 1). Marshall [12] also reported that rise in milk temperature from 25°C to 35°C caused whey drainage growth. Calvo and Espinoza [7] reported that raw milk had been characterised by greater syneresis coefficient in comparison to the pasteurised milk.

Figure 2. The whey drainage depending on rate of curd temperature rise

The results for the syneresis process from curds obtained from milks with different pH (at the moment of rennet addition) are shown on Figure 3. The biggest whey drainage was observed from curds made of raw milks with starting pH 6.0 whereas the smallest drainage was in curds obtained from pasteurised milk with normal starting pH. The data in Table 1 showed that lowering of milk pH to 6.3 and to 6.0 influenced significantly the growth of curd syneresis. According to Kiermeie et all. [11], Marshall[12] and Walstra [15] cow milk pH lowering had influenced significantly curd syneresis growth.

The changes of rennet curd syneresis with heating temperature are presented on Figure 4. As it is shown the curd obtained from raw milk heated to 50°C had the biggest whey drainage whereas the curd obtained from pasteurised, not heated (30°C) milk had the smallest whey drainage.
The differentiation of syneresis process in this experiment in dependence of experimental factors was the biggest among all experiments. MANOVA has showed the significant influence of temperature of curd heating and of milk pasteurisation on the amount of expelled whey (Table 1).

The curd heated to 50°C in comparison to curds heated to 40°C and to not heated (30°C) had the highest syneresis. Kammerlehner [9] and Kiermeier et all.[11] had established that with the rose of curd temperature its syneresis also had risen.

In experiment 5 (Fig. 5) the highest whey drainage was observed for curd from raw milk heated with temperature increase of 1°C for 3 min, followed by raw milk curd heated with 1°C/1 min whereas the smallest drainage was for pasteurised milk curd from not heated milk.
The slower rate of curd temperature rise (1°/3min) caused the faster growth in whey drainage in comparison to curd temperature rise 1°/1min and in comparison to not heated curd (Table 1). Kammerlehner [9] had established that the shorter time of the temperature of cow milk curd rise the smaller the syneresis had been.

In experiments 1 and 2, where the influence of curd firmness and milk temperature on curd syneresis were measured the amount of whey expelled after 15 minutes was a few percent and after 60 minutes it had grown to more than 15%. In experiment 3 (the milk pH influence) that growth was bigger from more than 5% to more than 24%. In experiments 4 and 5 (the curd temperature and rate of curd temperature rise influence) after 15 minutes of measurements the amount of whey drainage was larger than in the rest of experiments. After 60 minutes of measurements the largest amount of whey drainage was in experiment 4, where the influence of temperature of curd heating (40°C and 50°C) was examined. The smallest volume of whey drainage, after 60 minutes of measurements, was found in experiments 2 and 1, where the influence of milk temperature and curd firmness were examined. In the rest of experiments the volume of whey drained was high, the highest in experiment 4, where the influence of curd temperature was examined (Table 1). Many authors had reported that whey drainage had grown steeply - mostly during the fist hour after renneting and intensity of syneresis depended on different factors [9, 11, 12, 15].

All obtained curds were left in sieves until the next day to obtain fresh cheeses. The analyses of fresh cheeses were performed for confirmation of further syneresis process development.

The results are presented in Table 3, with pasteurisation factor influence and A, B, C – experimental factors influence on cheeses yields, their dry matter content and their texture. As it is shown the milk pasteurisation influenced cheeses yield significantly in experiments 2, 3, and 4. We could also observe that cheeses from pasteurised milk in comparison to raw milk cheeses contained less dry matter, what was statistically significant in experiment 3. Dry matter content lowering in pasteurised milk cheeses and their yields growth confirmed that bigger amount of water had been captured and that the syneresis was smaller at the same time. Milk pasteurisation also influenced the lowering in hardness and chewiness of cheeses and growth of their adhesiveness. Statistics showed only the significant influence of pasteurisation on cheeses adhesiveness in experiments 1 and 5.

In our previous research [5] we had found that milk pasteurisation had caused the growth of cheeses yield, lowering of dry matter content, lowering of hardness, chewiness and cohesiveness and small growth of adhesiveness.

Experimental factors in experiments 1-5 did not influence significantly cheeses yield (Table 3). The dry matter content was significantly influenced by milk pH and it was growing with pH lowering (Table 3). In other experiments (1, 2, 4 and 5) experimental factors did not influence significantly the dry matter content in cheeses. Only milk pH influenced significantly growth in cheeses hardness. Parameters A, B and C did not influence significantly adhesiveness, springiness, cohesiveness and chewiness of cheeses, but there could be observed some differences in cheeses hardness and chewiness in experiments 4 and 5. In experiment 5 there was observed the statistically significant growth in cohesiveness of curd heated with the slower rate (1°/3min).]
Table 3. Lowest squared means for fresh cheeses parameters depending on the influence of different factors

<table>
<thead>
<tr>
<th>Measured parameters of cheeses</th>
<th>Number of experiments</th>
<th>Total means</th>
<th>Standard error</th>
<th>Pasteurisation</th>
<th>The factors in experiments 1-5*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>None 72°C/15 sec</td>
<td>A 3 4 5 significance differences</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1 2</td>
<td>A 3</td>
</tr>
<tr>
<td>Yield [%]</td>
<td>1  2  3  4  5</td>
<td>26.99</td>
<td>1.28</td>
<td>24.85</td>
<td>29.19 -</td>
</tr>
<tr>
<td>Dry mass [%]</td>
<td>1  2  3  4  5</td>
<td>34.03</td>
<td>0.75</td>
<td>35.42</td>
<td>32.64 -</td>
</tr>
<tr>
<td>Hardness TPA [G]</td>
<td>1  2  3  4  5</td>
<td>695.2</td>
<td>67.7</td>
<td>813.2</td>
<td>577.2 -</td>
</tr>
<tr>
<td>Adhesivness TPA [G-s]</td>
<td>1  2  3  4  5</td>
<td>-53.87</td>
<td>1.15</td>
<td>-5.73</td>
<td>-11.04 1-2*</td>
</tr>
<tr>
<td>Springiness TPA</td>
<td>1  2  3  4  5</td>
<td>0.98</td>
<td>0.94</td>
<td>1.02</td>
<td>0.94</td>
</tr>
<tr>
<td>Cohesivness TPA</td>
<td>1  2  3  4  5</td>
<td>0.40</td>
<td>0.01</td>
<td>0.41</td>
<td>0.40</td>
</tr>
<tr>
<td>Chewiness</td>
<td>1  2  3  4  5</td>
<td>289.6</td>
<td>39.9</td>
<td>354.4</td>
<td>224.9 -</td>
</tr>
<tr>
<td>TPA [G]</td>
<td>1  2  3  4  5</td>
<td>277.0</td>
<td>40.4</td>
<td>326.8</td>
<td>229.2</td>
</tr>
</tbody>
</table>

- denotes a statistically significant difference between means – p ≤ 0.05.

The factors in experiments 1: A - 5 minutes after curd formation, B - 30 minutes and C - 60 minutes; 2: A - temperature of milk 25°C at the moment of rennet addition, B - 30°C and C - 35°C; 3: A - pH of milk 6.61 at the moment of rennet addition, B - pH 6.3 and C - pH 6.0; 4: A - curd without heating (temperature 30°C), B - curd heated to 40°C and C - to 50°C; 5: A - curd without heating (temperature 30°C), B - the rate of curd temperature rising 1°C/1 minute and C - 1°C/3 minutes.
To estimate the relationship between the amount of whey expelled from rennet curds and the amount of dry matter content in fresh cheeses obtained from above mentioned curds the correlation coefficients between amount of whey expelled (after 60 minutes of syneresis measurements) and dry matter content in fresh cheeses were calculated. It was concluded that correlation coefficients were not significant and equal for curds and cheeses made of raw milk $r = +0.29$ and for products made of pasteurised milk $r = +0.07$.

It can be concluded that observed different rate of syneresis from rennet curds, in experiments 1-5, during 60 minutes of measurements, depending from different experimental parameters was equalized after 12 hours. Milk pasteurisation and experimental factors in experiments: pH and temperature of milk, curd firmness, curd temperature and rate of curd temperature rise had bigger influence on syneresis in at the very beginning after curds formation than on total amount of expelled whey after 12 hours of cheeses formation, and on cheeses properties.

**CONCLUSIONS**

1. It was concluded that the changes of texture of rennet curd with time have had place and its hardness, adhesiveness, cohesiveness and gumminess have grown.
2. The syneresis of curd made from pasteurised milk is lower than syneresis of curd made of raw milk.
3. The temperature and pH of milk, curd temperature and rate of curd temperature rise and the time of measurements influence significantly the syneresis of rennet curd produced from ewe’s milk what is in agreement with results found in reports published on curd renneted from cow’s milk.
4. Among experimental factors the pasteurisation and pH of milk influence mostly the yield, texture and dry matter content in fresh cheeses.

**REFERENCES**

Responses to this article, comments are invited and should be submitted within three months of the publication of the article. If accepted for publication, they will be published in the chapter headed ‘Discussions’ in each series and hyperlinked to the article.