The Influence of Papain on the Content of Amino Acids from Holland Cheeses

Mirela JIMBOREAN¹, Cornel LASLO¹, Dorin ȚIBULCĂ¹, Aurora ȚIBULCĂ², Adriana PĂUCEAN¹

¹University of Agricultural Sciences and Veterinary Medicine, Faculty of Agriculture, 3-5 Mănăștur Street, 400372 Cluj-Napoca, Romania, mirelajimborean2004@yahoo.com
²S.C RAAL S.A., Bistrița

Abstract. The aim of the present paper is to study the variation of amino acid content during the ripening of semi-hard cheese (type Holland), after adding in the milk, used for the coagulation, of a proteolytic enzyme - papain - and the comparison with the data obtained from the analysis of a witness cheese (without enzymes added) obtained and ripen in the same technological conditions. For investigations were collected and analyzed samples at different times during ripening.

Keywords: physical-chemical indicators, protein, protein content, amino acids, cheese maturation, proteolytic enzymes.

INTRODUCTION

The cheeses ripening starts in the processing tank, so the lactic fermentation phase is rapidly taking place during the preparation for coagulation and during milk coagulation. By adding in the pasteurized milk the selected cultures of lactic bacteria, the ripening process can be directed in order to obtain final products with constant and uniform characteristics, thus preventing the influence of microbiological daily variations of collected milk on the quality of cheeses. After the obtaining of curd, the aim of all further operations is to bring the clot in a compact mass. In this way, the optimal conditions for specific lactic bacteria growth are assured, in order to have the required enzymatic activity for the transformation of cheeses main compounds.

MATERIALS AND METHODS

This study took into consideration two types of semi-hard cheese:

✓ A witness sample – the cheese was obtained using the classic production technology for Holland cheese assortment;
✓ A cheese with added proteolytic enzymes; proteolytic enzymes (papain) were also added in milk before coagulation.

The samples were analyzed at a certain time intervals, from production (at 24 hours from production, 25 and 50 days of ripening):

M₁ – the witness sample at 24 hours from production;
M₂ – the witness sample at 25 days of ripening;
Bp₂ – the cheese sample with proteolytic enzymes at 25 days of ripening;
M₃ – the witness sample at 50 days of ripening;
Bp₃ – the cheese sample with proteolytic enzymes at 50 days of ripening.

The sampling was made according to STAS 9535/1-87.
Using the gas chromatography we were able to appreciate the proteolytic changes from Holland cheeses, by determining their amino acids content. For the analysis of amino acids, the next steps were:

a. Extraction and hydrolysis procedures;
b. Two-step amino acids derivatization: esterification followed by trifluoroacetilation;
c. Quantitative gas-chromatographic analysis.

RESULTS AND DISCUSSIONS

The conditions of temperature and humidity were monitored, throughout the ripening of the experimental samples of cheese, so that the temperature ranged between 13.5 and 15°C and humidity between 83 and 86%.

The obtained results, regarding the content of amino acids during semi-hard cheese ripening, type Holland, are presented in Tab. 1.

<table>
<thead>
<tr>
<th>Nr.</th>
<th>Amino acid</th>
<th>Milk</th>
<th>M₁</th>
<th>M₂</th>
<th>M₃</th>
<th>Bp₂</th>
<th>P₃</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Ala</td>
<td>4.37</td>
<td>5.21</td>
<td>4.39</td>
<td>2.81</td>
<td>3.48</td>
<td>2.89</td>
</tr>
<tr>
<td>2.</td>
<td>Gly</td>
<td>13.02</td>
<td>0.11</td>
<td>1.20</td>
<td>0.65</td>
<td>1.38</td>
<td>-</td>
</tr>
<tr>
<td>3.</td>
<td>Thr</td>
<td>3.28</td>
<td>3.45</td>
<td>2.19</td>
<td>2.86</td>
<td>3.62</td>
<td>4.47</td>
</tr>
<tr>
<td>4.</td>
<td>Ser</td>
<td>14.73</td>
<td>15.24</td>
<td>2.22</td>
<td>8.72</td>
<td>25.64</td>
<td>22.94</td>
</tr>
<tr>
<td>5.</td>
<td>Val</td>
<td>1.86</td>
<td>9.91</td>
<td>7.51</td>
<td>7.75</td>
<td>8.96</td>
<td>6.26</td>
</tr>
<tr>
<td>7.</td>
<td>15N-Ile</td>
<td>6.69</td>
<td>0.44</td>
<td>0.21</td>
<td>0.61</td>
<td>0.45</td>
<td>0.26</td>
</tr>
<tr>
<td>8.</td>
<td>Ile</td>
<td>2.56</td>
<td>1.10</td>
<td>0.98</td>
<td>1.44</td>
<td>1.16</td>
<td>0.91</td>
</tr>
<tr>
<td>11.</td>
<td>Phe</td>
<td>8.47</td>
<td>18.14</td>
<td>21.48</td>
<td>5.71</td>
<td>12.80</td>
<td>2.15</td>
</tr>
<tr>
<td>12.</td>
<td>Glu</td>
<td>3.70</td>
<td>5.80</td>
<td>8.37</td>
<td>15.54</td>
<td>1.45</td>
<td>7.89</td>
</tr>
<tr>
<td>13.</td>
<td>Lys</td>
<td>0.76</td>
<td>0.36</td>
<td>0.42</td>
<td>28.11</td>
<td>1.59</td>
<td>10.94</td>
</tr>
<tr>
<td>14.</td>
<td>Tyr</td>
<td>5.33</td>
<td>11.64</td>
<td>17.56</td>
<td>3.35</td>
<td>3.63</td>
<td>26.00</td>
</tr>
<tr>
<td>15.</td>
<td>Met</td>
<td>-</td>
<td>-</td>
<td>1.59</td>
<td>2.73</td>
<td>-</td>
<td>3.20</td>
</tr>
<tr>
<td>16.</td>
<td>Orn</td>
<td>-</td>
<td>-</td>
<td>7.15</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

In Fig. 1 is presented the separation of free amino acids chromatogram (FAA) from a sample of milk raw material. In Fig. 2 is presented the amino acid content from milk raw material, used in the obtaining of experimental cheeses.

In milk, a quantity over 20 µg/ml was found for the next amino acids: Gly – 48.9 µg/ml, Ser – 55.3 µg/ml, Pro – 59.7 µg/ml, Asp – 37.5 µg/ml, Phe – 31.8 µg/ml, Lys – 28.4 µg/ml and Tyr – 20 µg/ml. Met was not identified, and for Val, Leu, and Ile a quantity smaller than 10 µg/ml was detected.
Fig. 1. Separation of free amino acids (FAA) by chromatography for milk sample

Fig. 2. Variation of amino acids content from raw milk

In Fig. 3 is shown the variation of FAA content at semi-hard cheese witness sample. In the case of witness cheese, the amount of amino acids increases in the first 25 days of ripening, and then decreases to 50 days of ripening, except the following amino acids:

- **Ser**, which at the obtaining is found in relatively large amount: 537.3 mg/g, at 25 days of ripening decreases to 157.1 mg/g and at 50 days of ripening increases slightly, being found in a quantity of 183.49 mg/g;

- **Asp** is found in amount of 418.2 mg/g at the obtaining of the cheese, it is increasing by 2.8 times in 25 days, reaching to 1181.7 mg/g, but at 50 days of ripening it is not detected;

- **Lys** is increasing throughout ripening, from 12.8 mg/g to 29.7 mg/g at 25 days, and reaches to 591.6 mg/g at 50 days of ripening;

- **Tyr** is increasing and then decreasing during ripening: in the first 25 days is increasing from 410.2 mg/g to 1245.4 mg/g, and than at 50 days of ripening is decreasing to 70.5 mg/g;
- **Met** is not identified at the obtaining, but at 25 days of ripening is found in proportion of 113 mg/g and is decreasing in 50 days of ripening at 57.5 mg/g;
- At the end of ripening, **Orn** is detected in a proportion of 7.15% of total amino acids. The chains of amino acids may be modified in the cheese. Hydrolysates may release the ammonia from Asn and Gin, or by partial hydrolysis of the guanidine group from Arg, forming citrulina or by degradation, ornitina.

![Graph showing FAA content variation during ripening](image)

**Fig. 3. Variation of the content of FAA during ripening at the witness sample**

In a small proportion (less than 2%) were found, at the end of ripening the amino acids: Gly = 0.65% and Ile = 1.44%, and in a bigger proportion of 10% the: Glu = 15.54% and Lys = 28.11%.

In Fig. 4 is presented the FAA content variation of the type Holland cheese assortment with proteolytic enzymes:

The characteristics of the amino acids dynamics is that during ripening, the concentration of some amino acids grows progressively, while for others, the concentration reaches a maximum point and than decreases.
In the case of experimental sample with proteolytic enzymes, after 25 days of ripening the quantity of amino acids varies as follows:

- **Ala** decreases from 183 mg/g (at the obtaining) to 91.5 mg/g cheese (at 25 days of ripening) and then increases to 131.27 mg/g at 50 days of ripening; **Thr** decreases from 121.6 mg/g to 95.3 mg/g in the first stage, then increases to 272.57 mg/g at 50 days; **Ser** increases during ripening from 537.3 mg/g at the obtaining, to 674 mg/g in 25 days and than reaches to 1399.93 mg/g at 50 days of ripening; **Val** decreases in the first 25 days from 349.5 mg/g to 235.6 mg/g and then increases to 382.08 mg/g at 50 days of ripening; **Leu** decreases from 321.8 mg/g (at the obtaining) to 248.6 mg/g at 25 days and then increases at least to 266.57 mg/g at 50 days, the **Ile** and **Pro** is increasing a little after 25 days from 30.6 mg/g to 55.58 mg/g, respectively from 16.56 mg/g to 435.3 mg/g to 471.97 mg/g at 50 days of ripening, the **Glu** decreases from 204.5 mg/g to 38.1 mg/g at 25 days of ripening, and increases to 481.2 mg/g at 50 days; **Lys** increases throughout ripening from 12.8 mg/g at 41.8 mg/g at 25 days and at 667.45 mg/g at 50 days, and **Tyr** decreases in the first phase from 410 mg/g to 95.5 mg/g at 25 days and increases to 1587.31 mg/g at 50 days;

- **Gly**, **Phe** and **Asp** decreases during ripening, at 25 days, Gly was found in amount of 36.2 mg/g and at 50 days it has not been identified; Asp decreases from 418.2 mg/g to 258, 4 mg/g at 25 days and it is not identified at 50 days, Phe decreases from 639.5 mg/g to 336.4 mg/g at 25 days and further to 131.27 mg/g at 50 days aging;

- **Met** is identified only in the harvested sample at 50 days of ripening, in amount of 195.35 mg/g, representing 3.2% of total amino acids;

In Fig. 5 is shown the variation of free total amino acid content for the analyzed samples:
Fig. 8. Variation of free amino acids (FAA) content during ripening for Holland cheeses

For the cheese sample with proteolytic enzymes added, it decreases until 2629 µg/g and than it significantly increases at 50 days of ripening reaching to 6103.45 µg/g of cheese; in the case of witness sample, the quantity of amino acids decreases at 50 days, remaining at a higher level than at the beginning: 2104.55 µg/g.

CONCLUSIONS

➢ The ripening of the experimental samples took place in air-conditioned warehouse, where it was monitored the temperature and the relative humidity of air. The average temperature was between 13.5 and 15°C and the values of the relative humidity of air were between 83 and 86%.

➢ In the case of experimental sample with added proteolytic enzymes, the amino acids with bitter taste, has the following evolution:
  - Leu: 248.6 µg/g cheese at 25 days of ripening and 266.57 µg/g cheese at 50 days of ripening;
  - Phe: 336.4 µg/g cheese at 25 days and 131.27 µg/g cheese at 50 days of ripening;
  - Lys: 41.8 µg/g cheese at 25 days and 667.45 µg/g cheese at 50 days of ripening.

➢ In the case of experimental witness sample (without added enzymes) these bitter taste amino acids had the following evolution during ripening:
  - Leu: 321.8 µg/g cheese at obtaining, 629.4 µg/g cheese at 25 days of ripening and 182 µg/g cheese at 50 days of ripening;
  - Phe: 639.5 µg/g cheese at obtaining, 1523 µg/g cheese at 25 days of ripening and 120.17 µg/g cheese at 50 days of ripening;
  - Lys: 12.8 µg/g cheese at obtaining, 29.7 µg/g cheese at 25 days of ripening and 591.6 µg/g cheese at 50 days of ripening.

➢ Amino acids with sweet taste (Ala, Gly, Thr, Ser and Pro) had the following evolution during ripening of analyzed experimental cheeses sample:

✓ Wetness sample:
  - Ala: 183.7 µg/g cheese at obtaining, 311 µg/g cheese at 25 days of ripening and 59.06 µg/g cheese at 50 days of ripening;
  - Gly: 38.4 µg/g cheese at obtaining, 85.4 µg/g cheese at 25 days of ripening and 13.77 µg/g cheese at 50 days of ripening;
- **Thr:** \(121.6 \, \mu g/\, g\) cheese at obtaining, \(155.5 \, \mu g/\, g\) cheese at 25 days of ripening and \(60.13 \, \mu g/\, g\) cheese at 50 days of ripening;
- **Ser:** \(537.3 \, \mu g/\, g\) cheese at obtaining, \(157.1 \, \mu g/\, g\) cheese at 25 days of ripening and \(183.49 \, \mu g/\, g\) cheese at 50 days of ripening;
- **Pro:** \(233.5 \, \mu g/\, g\) cheese at obtaining, \(449.1 \, \mu g/\, g\) cheese at 25 days of ripening and \(80.1 \, \mu g/\, g\) cheese at 50 days of ripening.

✓ **Cheese sample with proteolytic enzymes added:**
- **Ala:** \(91.5 \, \mu g/\, g\) cheese at 25 days of ripening and \(176.59 \, \mu g/\, g\) cheese at 50 days of ripening;
- **Gly:** \(36.2 \, \mu g/\, g\) cheese at 25 days of ripening and \(0 \, \mu g/\, g\) cheese at 50 days of ripening;
- **Thr:** \(95.3 \, \mu g/\, g\) cheese at 25 days and \(272.57 \, \mu g/\, g\) cheese at 50 days of ripening;
- **Ser:** \(674 \, \mu g/\, g\) cheese at 25 days and \(1399.93 \, \mu g/\, g\) cheese at 50 days of ripening;
- **Pro:** \(435.3 \, \mu g/\, g\) cheese at 25 days and \(471.97 \, \mu g/\, g\) cheese at 50 days of ripening.

✓ After the analyses of experimental samples, it was noticed that the used proteolytic enzymes (papain) intervenes with success in the degradation of proteins and lipids, leading to reaction products which offers superior qualitative properties.

**REFERENCES**