Comparison of Milk Enzyme Activity in Different Lactation Periods

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Abstract

The study mainly measured enzyme activity of alkaline phosphatase and amylase in the different periods of milk, and seeking the best conditions for the enzyme activity of the respective in different pH, different temperature. Results showed that the activity of amylase in bovine colostrum was higher than in mature milk and terminal milk. The highest amylase activity, 103.28 U/dL, was detected at the temperature 40°C and pH 6. The best condition for Bovine colostrum of alkaline phosphatase activity is higher than mature milk and terminal milk. Alkaline phosphatase activity is the highest temperature at 40°C and pH 10, about 96.38 U/mL.

1. Introduction

According to lactation period milk can be divided into: colostrum, mature milk and terminal milk, the composition also exist certain differences in different periods. Milk contains all the nutrients for the human body requirement, as the rich nutrition and easy to digest absorb "white blood", it is the most ideal natural

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food. There are many kinds of enzymes in milk, they are mainly secreted by epithelial cells of the blood, breast, breast itself synthesis and invasive bacteria. Enzymes are a class of proteins with biological catalysis which produced by cells, and play an important role in the process of the body's metabolism. During lactation enzymes in milk play an important role in regulating, and also have antioxidant and congenital immune function.

Alkaline phosphatase (ALP) in milk is a nature phosphatase, participates in the metabolism, especially calcium and phosphorus metabolism (Xia Liu et al., 1997). ALP as one of endogenous enzymes in milk mainly exists on milk fat ball (A. L. Kelly et al., 2006). ALP is dairy cattle cell metabolic product, plays an important role in glucose metabolism and it is the key enzyme in the digestion and metabolism, participates in the fat metabolism. ALP biochemical role of milk is that digest an organic phosphoric acid compound in the milk such as casein with phosphoserine group and phospholipid into phosphoric acid and phosphoric acid combining with the original organic monomers, which activity is influenced by many factors. ALP in raw milk heat resistance is stronger than no spores of pathogenic microorganism; ALP also can be used as indicator of milk pasteurization (Fangdong Fan et al., 2003; Junshi Shen et al., 2009).

Amylase (AMS) mainly comes from salivary glands and pancreatic tissue in the body, its effect is generally recognized as complete food starch digestion process of hydrolysis of starch. Amylase is the main enzyme digestion of carbohydrates, baby's own salivary amylase and pancreatic amylase yet to develop completely, the activity of amylase in the body is extremely low (Newton ER, 2004). Amylase can digest the polysaccharide which does not exist in milk, and decompose starch and glycogen through hydrolysis of α-1, 4 glycosidic bonds. Births has not yet in contact starchy food supplement,, highly active AMS in colostrum for births of exclusive breastfeeding role is still poorly understood. Reports have pointed out that it could by relative single-mindedly decompose bacteria cell walls and play antibacterial effect (Lonnerdal B, 2003).

At present, there are more than 60 inherent enzymes which are isolated in the breast milk (Tanker IA et al., 2001). Enzyme activity was affected by many factors, so the enzyme activity of different lactation milk can provide important theoretical basis for dairy products processing and preservation conditions, developing new baby foods. This experiment makes sure the optimal pH and temperature of the two kinds of the enzyme activity in milk, though comparing different lactation amylase and alkaline phosphatase in milk under different pH, temperature, enzyme activity.

2. Materials methods

2.1. Materials and instrument

2.1.1. Milk sample

The total of collecte 30 cow milk samples were collected from three cattle farms in Shenyang. Each lactation collected 10 samples, each sample was 20 ml.

2.1.2. Instrument

Digital temperature water bath HH-6 Guohua Electric Appliance Co., Ltd.; UV-Vis spectrophotometer UV-2000 Shanghai Guigo Industrial Co., Ltd.; Electronic balance Shenyang Dragon Electronic Weighing Instrument Co., Ltd.; High-speed refrigerated centrifuge Shanghai GuNing instrument co., Ltd.;

2.2. Method

2.2.1. Milk sample collection and processing

Milk samples centrifuged 20 min under 4°C, 10000 r/min, removed cream to make from skim milk, made after the whey using isoelectric point method to remove casein, in -20°C cryopreservation. In order to make the experiment more representative, 20ml of colostrum samples were mixed to prepare a mixture of milk.
before the experiment, matured milk, and late milk like above.

2.2.2. AMS activity determination
   Used iodine-starch colorimetric method (Jigui Wang, 1996).

2.2.3. APL activity determination
   Used benzene disodium phosphate method (Jigui Wang, 1996).

3. Results and Discussion

3.1. AMS activity determination

3.1.1. AMS activity determination under different pH
   When the temperature was 37 °C, pH 4, 5, 6, 7, 8, 9, measured the AMS activity value of milk as shown in table 1.

<table>
<thead>
<tr>
<th>Milk samples enzyme activity (U/dL)</th>
<th>pH=4</th>
<th>pH=5</th>
<th>pH=6</th>
<th>pH=7</th>
<th>pH=8</th>
<th>pH=9</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colostrum</td>
<td>10.30</td>
<td>63.18</td>
<td>101.06</td>
<td>58.72</td>
<td>34.74</td>
<td>8.09</td>
</tr>
<tr>
<td>Mature milk</td>
<td>7.62</td>
<td>49.58</td>
<td>77.11</td>
<td>42.91</td>
<td>19.61</td>
<td>2.25</td>
</tr>
<tr>
<td>Terminal milk</td>
<td>5.39</td>
<td>35.88</td>
<td>49.01</td>
<td>27.21</td>
<td>12.65</td>
<td>1.66</td>
</tr>
</tbody>
</table>

Table 1 listed in when the temperature is 37 °C, three lactations: colostrum, mature milk and terminal milk sample in the pH of 4, 5, 6, 7, 8, and 9 determining amylase activity values. By comparing the milk could be drawn from different lactation amylase activity as follows: colostrum, mature milk and terminal milk. And it could obtain the best pH of the amylase activity in the milk around 6. Colostrum enzyme activity at pH=6 maximum, about 101.06 U/dL. Thus, amylase content and activity were higher in bovine colostrum; the milk of the enzyme activity curve was shown in figure 1 at different pH value.

3.1.2. AMS activity determination under different temperature
   When pH=6, temperature 10 °C, 20 °C and 30 °C, 40 °C, 50 °C, 60 °C determining amylase activity values are shown in table 2.

<table>
<thead>
<tr>
<th>Milk samples enzyme activity (U/dL)</th>
<th>10°C</th>
<th>20°C</th>
<th>30°C</th>
<th>40°C</th>
<th>50°C</th>
<th>60°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colostrum</td>
<td>14.11</td>
<td>37.23</td>
<td>62.59</td>
<td>103.28</td>
<td>42.79</td>
<td>9.59</td>
</tr>
<tr>
<td>Mature milk</td>
<td>11.19</td>
<td>23.07</td>
<td>45.66</td>
<td>88.70</td>
<td>37.02</td>
<td>6.45</td>
</tr>
<tr>
<td>Terminal milk</td>
<td>7.64</td>
<td>16.86</td>
<td>29.07</td>
<td>56.55</td>
<td>28.32</td>
<td>2.24</td>
</tr>
</tbody>
</table>

Table 2 listed in three lactations: colostrum, mature milk and terminal milk sample in the temperature 10 °C, 20 °C and 30 °C, 40 °C, 50 °C, 60 °C determining amylase activity values. By comparing the milk could be drawn from different lactation amylase activity as follows: colostrum, mature milk and terminal milk. And it could obtain the best temperature of the amylase activity in the milk around 40°C. Colostrum enzyme activity at temperature 40°C maximum is about 103.28 U/dL. Thus, amylase content and activity were higher in bovine colostrum, different temperature; the milk of the enzyme activity curve was shown in figure 2.
It could be concluded that milk amylase enzyme activity was the highest in colostrum. When the pH value was 6, the temperature was about 40 °C; the best amylase enzyme activity was 103.28 U/dL. AMS as a kind of protein antibody protection, could improve the neonatal anti-infection ability, and could help new born digest starch and had antimicrobial effect (Zengqiang Chen et al., 2003), so people should recognize and improve the utilization value of bovine colostrum.

3.2. ALP activity determination

3.2.1. ALP activity determination under different pH

According to the alkaline phosphatase is measured at 510 nm absorbance values, establish standard curve, the regression equation can be obtained as follows:

\[ y = 0.0277x + 0.0227 \quad (R^2=0.9988) \]

\( y \) is the absorbance value in this formula, \( x \) is alkaline phosphatase activity in milk samples (U/mL), with good linear relationship(Ge Zhang,2013).

When the temperature was 37 °C, pH of 4, 5, 6, 7, 8, 9, 10, 11, measured milk samples in wavelength of 510 nm absorbance value, and was calculated from the regression equation of colostrum, mature milk, and terminal milk of the alkaline phosphatase activity, as shown in table 3.

Table 3 Milk samples ALP enzyme activity under different pH

<table>
<thead>
<tr>
<th>Milk samples enzyme activity (U/dL)</th>
<th>pH=4</th>
<th>pH=5</th>
<th>pH=6</th>
<th>pH=7</th>
<th>pH=8</th>
<th>pH=9</th>
<th>pH=10</th>
<th>pH=11</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colostrum</td>
<td>9.23</td>
<td>16.69</td>
<td>25.37</td>
<td>32.51</td>
<td>52.16</td>
<td>89.39</td>
<td>90.72</td>
<td>50.96</td>
</tr>
<tr>
<td>Mature milk</td>
<td>6.33</td>
<td>14.16</td>
<td>20.20</td>
<td>30.73</td>
<td>48.65</td>
<td>70.66</td>
<td>76.38</td>
<td>51.31</td>
</tr>
<tr>
<td>Terminal milk</td>
<td>4.28</td>
<td>13.84</td>
<td>18.54</td>
<td>26.96</td>
<td>30.89</td>
<td>45.44</td>
<td>57.21</td>
<td>30.22</td>
</tr>
</tbody>
</table>

Table 3 listed the three lactation milk colostrum, mature milk; milk sample in pH 4, 5, 6, 7, 8, 9, 10, 11, the determination of the ALP activity value. By comparing the milk could be drawn from different lactation ALP activity as follows: colostrum, mature milk and terminal milk. The optimum pH of ALP in milk was about 10, this time in the colostrum ALP activity was 89.39 U/dL, mature milk was 70.66 U/dL, and terminal milk was 45.44 U/dL. From figure 3, it could be intuitively Seen in the pH = 10, with a strong alkaline phosphatase activity.

3.2.2. ALP activity determination under different temperature

When the temperature was pH 10, the temperature was 10°C, 20°C and 30°C, 40°C, 50°C, 60°C, measured the milk samples in wavelength of 510 nm absorbance value, and was calculated from the regression equation of colostrum, mature milk, terminal milk the alkaline phosphatase activity, as shown in table 4.
Table 4. Milk samples ALP enzyme activity under different temperature

<table>
<thead>
<tr>
<th>Milk samples enzyme activity (U/mL)</th>
<th>10°C</th>
<th>20°C</th>
<th>30°C</th>
<th>40°C</th>
<th>50°C</th>
<th>60°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colostrum</td>
<td>16.10</td>
<td>40.26</td>
<td>75.39</td>
<td>96.38</td>
<td>70.63</td>
<td>39.08</td>
</tr>
<tr>
<td>Mature milk</td>
<td>13.03</td>
<td>35.28</td>
<td>64.69</td>
<td>78.16</td>
<td>57.37</td>
<td>30.12</td>
</tr>
<tr>
<td>Terminal milk</td>
<td>9.87</td>
<td>24.94</td>
<td>48.95</td>
<td>50.32</td>
<td>30.09</td>
<td>15.13</td>
</tr>
</tbody>
</table>

Table 4 listed in three latications: colostrum, mature milk and terminal milk sample in the temperature 10 °C, 20 °C and 30 °C, 40 °C, 50 °C, 60 °C determining ALP activity values. By comparing the milk could be drawn from different latication amylase activity as follows: colostrum, mature milk and terminal milk. And it could obtain the best temperature of the ALP activity in the milk around 40°C. Colostrum enzyme activity at temperature 40°C maximum is about 96.38 U/dL. Thus, ALP content and activity were higher in bovine colostrum, different temperature; the milk of the enzyme activity curve was shown in figure 4.

![Fig.3. Milk samples ALP enzyme activity under different pH](image)

![Fig.4. AMS enzyme activity under different temperature](image)

It could be concluded that milk ALP enzyme activity was highest in colostrum. When the pH value was 10, the temperature was about 40 °C, the best ALP enzyme activity was 96.38 U/mL.

4. Conclusions

With the development of the society and the improvement of living quality, people pay more attention to nutrition, health and health care. Milk occupies very important status in our daily life, because most people recognize the milk containing essential nutrients. And it is the necessary nutrients for infant growth and development process. In addition to the familiar nutrition function, the milk also contains many important enzymes; in the formation of milk, antioxidant and antibacterial system formation is great significance (Weizheng Li, 2010).

The study mainly measured enzyme activity of alkaline phosphatase and amylase in the different periods of milk, and seeking the best conditions for the enzyme activity of the respective in different pH, different temperature. Results showed that the activity of amylase in bovine colostrum was higher than in mature milk and terminal milk. The highest amylase activity, 103.28 U/dL, was detected at the temperature 40°C and pH 6. The best condition for Bovine colostrum of alkaline phosphatase activity is higher than mature milk and terminal milk. Alkaline phosphatase activity is the highest temperature at 40°C and pH 10, about 96.38 U/mL. Enzyme in milk can play an important role in breast tissue or milk. Most enzyme activity of colostrum is higher than mature milk and terminal milk; make timely births to eat colostrum that will be good for the health and growth. In addition, because milk contains abundant enzymes, should be considered in the development of new generation emulsion compound to add this kind of active substance, and the influence of
pH and temperature on the enzyme activity.

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References