Research of lactose hydrolysis depending on the type of the enzyme

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Abstract. In order to produce low-lactose dairy products the process of lactose hydrolysis is studied and optimal process parameters depending on the type of enzyme samples are determined. By the example of two types of enzymatic samples of lactase (β-galactosidase): “Lacta-free” TM “Lactoferm ECO”, obtained from mushrooms Aspergillus oryzae, is produced by Biochem srl. (Italy) and “Maxilact-2000”, obtained from Kluyveromyces lactis yeast, is produced by "DSM Food Specialties" (Netherlands) and the most effective samples has been tested. Optimal parameters for "Lacta-free": at temperature (40±2)°C, duration of hydrolysis process (5.0±0.5) hours; for "Maxilact-2000": at temperature (37±2)°C, duration of hydrolysis process (4.0±0.5) hours.

1. Introduction

In the modern day, a great attention is paid to the products have medical and functional purpose, which include low-lactose and lactose-free products. These products for people who, because of genetic or health reasons, cannot digest milk sugar and therefore cannot consume milk. Both children and adults are found to be lactose intolerant. The decrease in digestive enzymes, including the enzyme lactase (β-galactosidase), is observed with age. Lactose intolerance is found in different ethnic groups, but it varies. Lactose intolerance is more common in populations in Africa and Southeast Asia, also a rare disease in European populations [1,2]. The modern market for functional foodstuffs, including low-lactose and non-lactose ones, is expanding in assortment also increasing in sales segment. In order to reduce the mass fraction of lactose in dairy raw materials, various industrial methods and biotechnological techniques are have been used: enzymatic hydrolysis of lactose; baromembrane methods – water treatment methods; bioconversion of lactose by lactic acid bacteria (sour-milk squashing); production of dairy low-lactose and lactose-free composite products by mixing various micro- and macro-components with milk protein isolated by ultrafiltration and diafiltration [3]. The most common way to reduce lactose mass fraction in industry is lactose hydrolysis with enzyme samples of lactase (β-galactosidase).

The enzyme β-galactosidase is selected considering the conditions of the hydrolysis process. In this regard, the study and determination of optimal parameters of lactose hydrolysis process is an urgent task.
The purpose this work - to study and determine optimal parameters of the process of lactose milk hydrolysis by different enzymes of β-galactosidase for the development of low lactase product.

To achieve the above objectives, the following tasks need to be accomplished:

- pick out enzymatic samples for lactose milk hydrolysis;
- determine the dependence of lactose hydrolysis degree on temperature, concentration of the enzyme samples put in and duration of the process;
- develop recommendations for further use of selected enzyme samples in production of low lactose dairy products;
- drinking milk in accordance with GOST 31450-2013;
- enzymatic samples of β-galactozidases: "Lacta-free" TM "Lactoferm ECO", obtained from mushrooms Aspergillus oryzae, produced by "Biochem srl" (Italy); "М "Maxilact-2000", obtained from Kluyveromyces lactis yeast, produced by "DSM Food Specialties" (Netherlands). In milk it was brought from 0,04 to 0,20 % from mass of raw materials and from that enzyme samples with each sample increase by 0,04 %.

2. Materials and methods

The research was conducted in two stages. At the first stage, study the influence of enzymes mass fraction (%) and temperature of hydrolysis (°C) on the degree of lactose hydrolysis at the interval of temperature variation from 10 to 50°C and duration of the process is 4 hours. At the second stage, study the influence of enzymes mass fraction (%) and duration of hydrolysis (h) on the degree of lactose hydrolysis at the range from 2 to 10 hours, at temperature 40°C.

The mass fraction of lactose was determined by refractometric method using RL-1 refractometer, the degree of hydrolysis was calculated [5].

3. Results and discussion

The main results of the study of the first stage are presented in the form of graphical, dependencies of hydrolysis degree on the mass fraction of enzymes and process temperature (figure 1).

Figure 1. Dependence of lactose hydrolysis degree in milk on temperature and mass fraction of enzyme samples: a) "Lacta-free"; b) "Maxilact-2000".

The regression equations describing the process of enzymatic hydrolysis and reliability of the obtained data are given in table 1.
Table 1. Regression analysis of lactose hydrolysis dependence in milk on temperature and enzyme mass fraction.

| Mass fraction of the enzyme, % | Regression equations | Approximation confidence value ($R^2$) |
|-------------------------------|----------------------|---------------------------------------|
| «Lacta-free»                  | «Maxilact»           |                                       |
| 0.04                          | $y = -0.025x^2 + 2.2386x$ + 0.2857 $y = -0.0236x^2 + 1.9814x$ – 1.4286 |
| 0.08                          | $y = -0.04x^2 + 3.1343x$ + 2.1429 $y = -0.0304x^2 + 2.7664x$ – 2.5 |
| 0.12                          | $y = -0.0496x^2 + 3.765x$ + 4.2143 $y = -0.0368x^2 + 3.5221x$ – 2.5 |
| 0.16                          | $y = -0.0586x^2 + 4.36x$ + 5.8571 $y = -0.0482x^2 + 4.0993x$ + 0.2143 |
| 0.2                           | $y = -0.0689x^2 + 4.8893x$ + 7.7857 $y = -0.0593x^2 + 4.6443x$ + 1.5714 |
| «Lacta-free»                  | $R^2 = 0.995$        | $R^2 = 0.9896$                        |
| «Maxilact»                    | $R^2 = 0.9896$       | $R^2 = 0.9876$                        |

According to the obtained data, shown in figure 1, the following dependence is observed: with increasing temperature from 10°C to 40°C, the degree of lactose hydrolysis increases, and when reached 50°C it begins to decrease, which can be explained by the thermolability of the enzyme. The study showed that the optimal hydrolysis temperature for the enzyme Lacta-free is in the range of (40±2) °C, and for the enzyme Maxilact-2000 is (37±2) °C. The increase in the concentration of enzymes put in, leads to an acceleration of lactose hydrolysis. The rational concentration of the enzyme is (0.18±0.02)% for both studied samples. According to the results of the first stage of the study, the next stage was planned and carried out, which consisted in determining the fermentation period at the optimal temperature for cultivation of the research samples "Lacta-free" and "Maxilact-2000".

The main results of the study in the second stage are presented in the form of graphical, it shows the dependencies of lactose hydrolysis degree on the duration of hydrolysis process and mass fraction of the research samples.

**Figure 2.** Dependence of lactose hydrolysis degree in milk on process duration and mass fraction of enzyme samples: a) "Lacta-free"; b) "Maxilact-2000".
The regression equations describing the process of enzymatic hydrolysis and reliability of the obtained data are given in Table 2.

**Table 2.** Regression analysis of lactose hydrolysis dependence in milk on hydrolysis duration and enzyme mass fraction.

| Mass fraction of the enzyme, % | Regression equations | Approximation confidence value (R²) |
|--------------------------------|----------------------|-----------------------------------|
| <br>«Lacta-free» <br> «Maxilact» | <br>«Lacta-free» <br> «Maxilact» | <br>«Lacta-free» <br> «Maxilact» |
| 0.04  <br>y = -1.0893x² + 16.064x + 3.2857  <br>y = -0.7366x² + 13.18x - 0.3929 | R² = 0.9734  <br>R² = 0.9989 |  |
| 0.08  <br>y = -1.3259x² + 19.302x + 4.6071  <br>y = -1.1964x² + 18.907x - 1 | R² = 0.9644  <br>R² = 0.9874 |  |
| 0.12  <br>y = -1.5089x² + 23.032x + 6.5  <br>y = -1.6339x² + 25.111x - 0.6429 | R² = 0.9606  <br>R² = 0.9807 |  |
| 0.16  <br>y = -1.7545x² + 25.459x + 8.0357  <br>y = -1.7143x² + 26.129x - 0.2857 | R² = 0.9426  <br>R² = 0.9755 |  |
| 0.2   <br>y = -1.8839x² + 26.796x + 8.9286  <br>y = -1.7812x² + 26.913x + 0.25 | R² = 0.9346  <br>R² = 0.9782 |  |

According to the presented graphs, the increasing of lactose hydrolysis degree is observed, it increases by proportion of enzyme samples input. As to the data have showed, it was noted that in order to obtain the degree of lactose hydrolysis up to 90% the duration of the hydrolysis process in enzyme "Lacta-free" is (5.0 ± 0.5) hours and in enzyme "Maxilact-2000" is (4.0 ± 0.5) hours. At the same time, the enzyme samples "Maxilact-2000" has a higher enzymatic activity of (0.16±0.02)%, which is 12.5% higher than the enzyme "Lacta-free".

4. Conclusions

From the presented experiment, the data show that the degree of lactose hydrolysis depends on temperature, concentration of enzyme samples and hydrolysis duration. It was found that the effect of different commercial lactase samples on the process of lactose hydrolysis has different optimal parameters. For "Lacta-free" samples they were: at temperature (40 ± 2)°C, duration of hydrolysis process (5.0 ± 0.5) hours, concentration (0.18 ± 0.02)% and for "Maxilact-2000" samples: temperature (37 ± 2)°C, duration of hydrolysis process (4.0 ± 0.5) hours, concentration (0.16 ± 0.02)%.

Thus, the comparative analysis of optimal parameters of the hydrolysis process has shown a high efficiency of the enzyme samples β-galactosidase "Maxilact-2000" made of Kluyveromyces lactis yeast, produced by "DSM Food Specialties" (Netherlands).

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