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THE INFLUENCE OF TECHNOLOGICAL FACTORS ON THE CONTENT AND COMPOSITION OF SACCHARIDES IN LACTOSE-FREE MILK PRESERVED BY UHT METHOD®

Wpływ czynników technologicznych na zawartość i skład sacharydów w bezlaktozowym mleku utrwalanym metodą UHT®

The article presents the available knowledge about the influence of various factors on the composition and content of saccharides in lactose-free milk, i.e. milk with enzymatically degraded lactose. Various technological solutions applied in lactose-free milk production, in particular the sequence of lactose hydrolysis and heat treatment processes, were identified. The influence of lactose-hydrolyzed milk production procedure on saccharide transformations taking place, such as the process of sugar isomerization and Maillard reaction, was discussed. The relatively high variability in the quality of commercial lactose-free milk samples was pointed out. Special attention was paid to the importance of milk storage conditions on saccharide content changes. Moreover, the need to establish specific quality criteria for this type of milk was stressed.

Key words: lactose-free milk, saccharides, lactulose.

W artykule przedstawiono dostępną wiedzę o wpływie różnych czynników na skład i zawartość sacharydów w mleku bezlaktozowym, tj. z enzymatycznie rozłożoną laktozą. Wskazano na różne rozwiązania technologiczne stosowane w produkcji mleka bez laktozy, w szczególności sekwencyjność procesu hydrolizy laktozy i obróbki cieplnej. Omówiono wpływ procedury produkcji mleka z rozłożoną laktozą na zachodzące przemiany sacharydów, jak np. proces izomeryzacji cukrów oraz reakcje Maillarda. Wskazano na stosunkowo dużą zmienność jakości handlowych próbek mleka bezlaktozowego. Zwrócono uwagę na znaczenie warunków przechowywania mleka na zmiany zawartości sacharydów. Podkreślono konieczność ustalenia określonych kryteriów jakościowych dla tego rodzaju mleka.

Słowa kluczowe: mleko bezlaktozowe, sacharydy, laktuloza.

INTRODUCTION

At present, there is a significant increase in the production of lactose-free milk and milk products. The problem of consumer lactose intolerance is cited in the justification of these technological trends. This is not always justified on medical and health grounds [3].

The interpretation of the necessity to consume lactose-free milk and dairy products was presented in our earlier study [13].

In the evaluation of the quality of lactose-free milk, in particular milk preserved by the UHT method, attention is paid to the analysis of potential changes in the composition of proteins and saccharides in the preserved product, induced by the thermal process.

The composition and content of saccharides of lactose-free milk are determined by factors such as the type and biocatalytic properties of the β-galactosidase preparation used, its form (free or bound), as well as the conditions of lactose hydrolysis process, e.g. temperature, acidity and time [2, 7, 8]. The products of enzymatic reaction of lactose hydrolysis are mainly glucose and galactose. The β-galactosidase preparations used in the production of lactose-free milk also show transgalactosylation activity. This promotes the synthesis of galacto-oligosaccharides (GOS) with prebiotic properties. The scheme of transgalactosylation reactions is presented in Fig. 1 and Fig. 2 [14].

The efficiency of GOS synthesis depends on: type (source) of the enzyme and its activity, as well as on the reaction environment conditions, including mainly lactose concentration and reaction time [7]. The studies of Rodriguez-Colinas et al. [11] show that the kinetics of galacto-oligosaccharide synthesis reaction changes over time. After reaching a certain maximum concentration of galacto-oligosaccharides, there is

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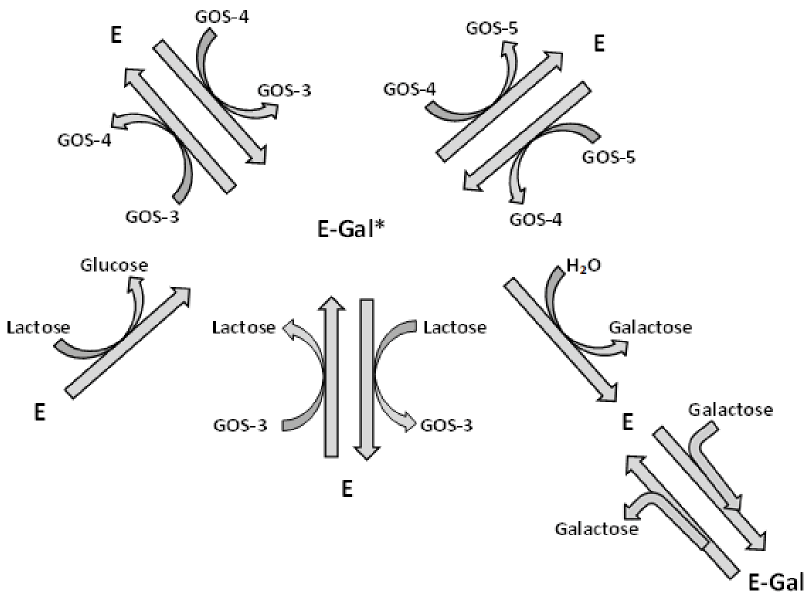

Fig. 1. Mechanism of synthesis of GOS with β-galactosidase from A. oryzae.
Rys. 1. Mechanizm syntezy GOS z udziałem β-galaktozydazy z A. oryzae.

Explanation of abbreviations: E – free enzyme concentration (mmol/kg or mM), E-Gal* – galactosyl-enzyme transition complex concentration (mmol/kg or mM), E-Gal – galactose-enzyme complex concentration (mmol/kg or mM), GOS-3, GOS-4, GOS-5 – galactooligosaccharides composed of three, four or five sugar residues.

Objaśnienie skrótów: E – stężenie wolnego enzymu (mmol/kg or mM), E-Gal* – stężenie kompleksu przejściowego galaktozyl-enzym (mmol/kg or mM), E-Gal – stężenie kompleksu galaktoza-enzym (mmol/kg or mM), GOS-3, GOS-4, GOS-5 – galaktooligosacharydy zbudowane z trzech, czterech lub pięciu reszt cukrowych.

Source: Own study based on [14]

Źródło: Opracowanie własne na podstawie [14]

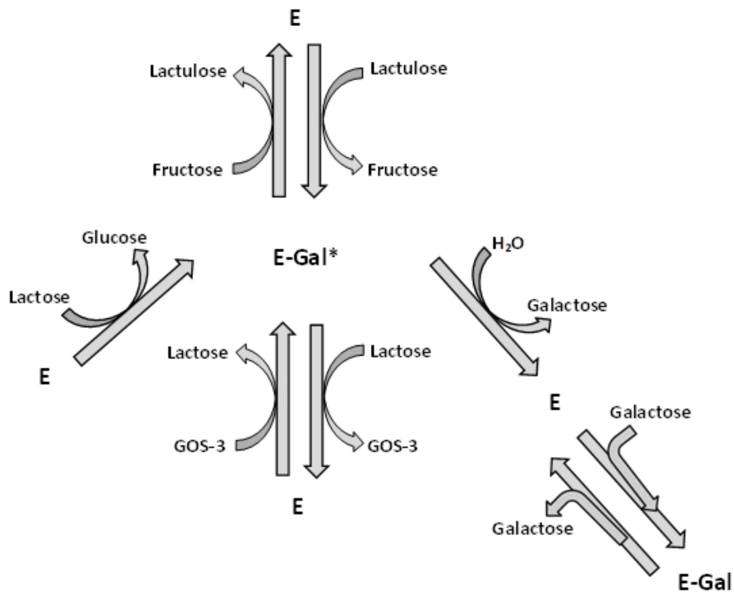

Fig. 2. Mechanism of synthesis of lactulose with β-galactosidase from A. oryzae.
Rys. 2. Mechanizm syntezy laktulozy z udziałem β-galaktozydazy z A. oryzae.

Explanation of abbreviations: E – free enzyme concentration (mmol/kg or mM), E-Gal* – galactosyl-enzyme transition complex concentration (mmol/kg or mM), E-Gal – galactose-enzyme complex concentration (mmol/kg or mM), GOS-3 – galactooligosaccharides composed of three sugar residues.

Objaśnienie skrótów: E – stężenie wolnego enzymu (mmol/kg or mM), E-Gal* – stężenie kompleksu przejściowego galaktozyl-enzym (mmol/kg or mM), E-Gal – stężenie kompleksu galaktoza-enzym (mmol/kg or mM), GOS-3 – galaktooligosacharydy zbudowane z trzech reszt cukrowych.

Source: Own study based on [14]

Źródło: Opracowanie własne na podstawie [14] a gradual decrease in the amount of GOS due to competition between hydrolysis and transgalactosylation reactions [11].

The biocatalytic specificity of β-galactosidase used and the process management conditions also determine the content and composition of the resulting galacto-oligosaccharides. The transgalactosylation reaction may produce tri-, tetra- or pentasaccharides. Their proportion changes over the course of the process. For example, it is known that the concentration of tri- and tetra-saccharides reaches its maximum after 30 minutes of reaction and then gradually decreases. It follows that in lactose-free milk the composition of galacto-oligosaccharides may be variable [2].

In the production of lactose-free milk preserved by the UHT method, lactulose, i.e. disaccharide composed of fructose and galactose combined with a β-1,4-glycosidic bond, may also be formed. It is synthesized in the reaction of internal transformation of glucose in the lactose molecule to fructose by conversion of the aldose form to the ketose form [2, 4, 9].

Lactulose is not a natural component of milk. However, its presence is found in milk after heat treatment. The heating of milk promotes thermal isomerization of lactose. The presence of lactulose in milk is often assumed as an indicator (marker) of changes in milk caused by the thermal process [10].

Interestingly, lactulose is a saccharide with beneficial functional, including prebiotic, properties [2, 9].

According to the available literature information, monosaccharides, disaccharides and galacto-oligosaccharides may be present in lactose-free milk preserved by the UHT process. Their composition and content largely depends on the technological process conditions [8, 11, 12].

In this article, according to the available literature, a decision was made to confirm the above dependencies.

THE INFLUENCE OF TECHNOLOGICAL PROCESS CONDITIONS ON SACCHARIDE CONTENT AND COMPOSITION

It is known that lactose-free milk has a higher content of reducing sugars compared to conventional UHT-preserved milk. As a result, lactose-free milk is more susceptible to ingredient changes. This is because, inter alia, the monosaccharides, glucose and galactose produced after lactose hydrolysis are more active than lactose in the formation of the Maillard reaction products during thermal preservation as well as during milk storage [6, 12].

There are a number of lactose-free UHT milk production procedures known. The differences concern mainly the sequence of the lactose hydrolysis process and the thermal preservation process. Both pre- and post-UHT lactose hydrolysis is possible. Currently, the production of lactose-free milk is predominantly based on two processes, referred to as batch (lactose hydrolysis prior to thermal treatment) and aseptic (lactose hydrolysis following thermal treatment), both of which use a free-form enzyme. Processes with an immobilized enzyme offer an alternative solution. However, their application in the industrial production of lactose free milk is more difficult to popularize due to problems with final product microbiological stability. Furthermore, the recycling of the immobilized enzyme is limited, which reduces the profitability of this procedure; the product is more vulnerable to quality defects when compared to the product obtained in the process using the free, soluble form of the enzyme [5, 8].

Depending on the procedure used (pre- or post-UHT lactose hydrolysis), lactulose and furosine, which are markers of the intensity of changes in sugars and proteins during the technological process, may appear in various quantities in the finished product. According to the research by Messia et al. [8], the use of UHT treatment prior to lactose hydrolysis induces minor changes in milk, e.g. less loss of lysine, the main substrate of the Maillard reaction [8].

Performance of lactose hydrolysis in heat-treated milk (post-UHT) requires preservation of conditions hindering microbial proliferation. This concerns mainly the technique of aseptic enzyme dosing into milk [12].

In the discussed technological variant, the use of the immobilized form of the enzyme seems to be more advantageous, despite the aforementioned reservations, as by conducting the hydrolysis of lactose with the free form of the enzyme, it may be expected that the enzyme will be active in the finished product, in which it may, for example, hydrolyze the lactulose produced after thermal lactose isomerization. Lactulose hydrolysis may be confirmed by the presence of fructose in lactose-free milk samples [15].

However, fructose and tagatose are sometimes present in lactose-free milk as products of thermal isomerization of glucose and galactose, respectively [12].

The diversity of composition and saccharide content in commercial samples of lactose-free milk is confirmed by the results of the research by Ruiz-Matute et al. [12] presented in Table 1.

Table 1. Saccharide content in seven samples of commercial lactose-free UHT milk

Tabela 1. Zawartość sacharydów w siedmiu próbkach handlowego bezlaktozowego mleka UHT

Saccharides	Content [g/l]	Average content [g/l]
Galactose	17,3 23,5	21,0
Glucose	19,9 25,1	22,8
Fructose	0,076 0,185	0,117
Tagatose	0,036 0,098	0,076
Lactose	0,033 0,291	0,096
Allolactose	0,139 0,841	0,348
β-1-6-Galactobiose	0,166 0,747	0,348
6'-galactosyl-lactose	0,271 1,703	0,825
4'-galactosyl-lactose	0,028 0,254	0,065
Unidentified galactooligosaccharides	0,349 1,020	0,580
Total galactooligosaccharides	0,956 4,350	2,134

Source: Own study based on [12]

Źródło: Opracowanie własne na podstawie [12]

The content of individual saccharides varied in lactose-free milk samples analyzed by the aforementioned authors. Glucose content fluctuated from 19.9 g/l to 25.1 g/l, and galactose content fluctuated from 17.3 g/l to 23.5 g/l. In the tested milk samples, lactose content was between 0.033 and 0.291 g/l. Five out of the seven tested milk samples met the criterion of lactose content in lactose-free milk (below 0.01%) (Table 1). The above-presented glucose and galactose content in the compared samples of lactose-free milk indicates that probably no other lactose removal technique was used apart from its hydrolysis. Glucose and galactose content differences in the tested milk samples indicate that part of the galactose was used in the enzymatic synthesis of galacto-oligosaccharides. The content of galacto-oligosaccharides was significantly different in the analyzed milk samples. This concerns both the identified ones, e.g. 6'-galactosyl-lactose: from 0.271 to 1.703 g/l and 4'-galactosyl-lactose: from 0.028 to 0.254 g/l, and the unidentified ones: from 0.349 to 1.020 g/l (Table 1) [12].

Significant influence on the composition and content of galacto-oligosaccharides (including those given in Table 1) is exerted by factors such as the source and properties of β-galactosidase used and the temperature and time of hydrolysis reaction. This was confirmed by the research by Rodriguez-Colinas et al. [11], which compared the effects of transgalactosylation, including the composition and content of galacto-oligosaccharides after reaction with various β-galactosidase preparations: Biolactase NTL-CONC (β-galactosidase from *Bacillus circulans*), Lactose pure 6500L (β-galactosidase from *Kluyveromyces lactis*) and Lactase F (β-galactosidase from *Aspergillus oryzae*) [11].

The presence of fructose and tagatose in the evaluated UHT lactose-free milk samples confirms that they were formed in the process as products of thermal isomerization of glucose and galactose, respectively. Fructose content oscillated between 0.076 and 0.185 g/l and tagatose content oscillated between 0.036 and 0.098 g/l. The presence of fructose in milk does not depend on the sequence of lactose hydrolysis process (pre- or post-UHT). It may be formed, as mentioned above, after glucose isomerization if the hydrolysis process occurs prior to thermal treatment. If the UHT process precedes enzymatic hydrolysis, part of lactose is converted to lactulose, which is then broken down to fructose and galactose by enzymatic hydrolysis [12].

Demonstration of tagatose in samples of lactose-free UHT milk may indicate that lactose was hydrolyzed prior to the thermal process, as free galactose is present in small amounts (about 0.01%) and tagatose is not present in conventional UHT milk [1, 12].

It is interesting that no lactulose (below 0.01 g/l) was found in the analyzed milk samples, which, as described earlier, is a marker of the range of thermal changes, mainly lactose isomerization. Notably, the analyzed milk samples came from different producers and there is no information about the technological process procedure. From the analysis of saccharide content, e.g. lactulose, it is difficult to indicate unequivocally whether lactose hydrolysis was carried out prior to or after milk heat treatment. This is due to the fact that the absence of lactulose in lactose-free milk is independent of whether the UHT treatment is carried out prior to or after enzymatic hydrolysis. Lactulose is produced by isomerization of lactose during heat treatment of milk, so if UHT treatment is applied after lactose hydrolysis, there is not enough substrate (lactose) to produce lactulose. By means of an alternative procedure, i.e. post-UHT lactose hydrolysis, the lactulose formed from lactose is also hydrolyzed under the influence of β-galactosidase remaining in milk [12].

The results of the research carried out by Messia et al. [8] indicate that the UHT treatment of milk with partially degraded lactose promotes lactulose formation. The experimental technological process of milk production included the following stages: pasteurization (72°C/15 s), lactose hydrolysis in milk at 4°C for 36 h and then UHT treatment with infusion system. After enzymatic hydrolysis, the milk contained lactose and lactulose in the quantities of 6.0 g/l and 0.014 g/l, respectively. After the UHT process, a lactulose content of 0.078 g/l, which was five times higher than in the pre-UHT milk sample, was present in the analyzed milk sample. Such a large increase in lactulose concentration in the examined post-UHT milk sample is probably due to the fact that milk contained a comparatively high concentration of lactose (6.0 g/l), thus a substrate for the lactulose formation reaction [8].

The subject of the research by Messia et al. [8] was also saccharide and furosine analysis in 14 different samples of commercial UHT, lactose-hydrolyzed semi-skimmed milk. The lactose, lactulose and fructose content of the samples of lactose-hydrolyzed UHT milk was within 3.0÷12.0 g/l (mean: 8.0 g/l), 0.0÷0.401 g/l (mean: 0.191 g/l), and 0.047÷0.655 g/l (mean: 0.168 g/l), respectively. The presence of lactulose was demonstrated in almost all of the UHT milk samples. The lowest content of lactose (3.0÷4.0 g/l) and the highest content of galactose (22.0÷25.0 g/l) and glucose (23.0÷25.0 g/l) reported for milk samples no. 11, 12 and 13 may have resulted from the applied technology consisting in aseptic addition of β-galactosidase to milk after UHT treatment, as indicated by producers on the packaging. In the indicated samples, lactulose, fructose and furosine content was in the range of 0.265÷0.401 g/l, 0.052÷0.088 g/l and 0.484÷0.603 g/100 g of protein, respectively. In comparison with other milk samples, the relatively high lactulose and furosine content may indicate that indirect UHT treatment (membrane heat transfer) was applied. Fructose present in the listed samples may have originated from lactulose degradation under the influence of β-galactosidase during enzymatic hydrolysis [8].

In the characteristics of other tested milk samples, the applied technology impact on the composition of saccharides is not readily determinable, as no details of their production are specified. However, based on the content of saccharides and furosine, the authors evaluated the tested samples with an indication of the possible production process. The low content of lactulose (0.018÷0.139 g/l) and furosine (0.162÷0.188 g/100 g protein) and the significant quantity of fructose (0.056÷0.110 g/l) in milk samples nos. 5, 7 and 10 may indicate that a mild UHT treatment (infusion) was performed after hydrolysis. The absence of lactulose and increased levels of fructose (0.391 g/l) and furosine (0.319 g/100 g protein) in milk sample no. 8 may indicate that indirect (membrane) UHT treatment was performed after lactose hydrolysis. The high content of furosine (0.397 g/100 g of protein), lactulose (0.382 g/l) and fructose (0.655 g/l) in milk sample no. 3 may indicate that thermal treatment was performed both prior to and after lactose hydrolysis [8].

The obtained results indicate that the technology of lactose-hydrolyzed milk production (thermal treatment and enzymatic hydrolysis intensity and sequence) has an effect on the composition of saccharides and the intensity of Maillard reaction. The analysis of furosine content and the composition of saccharides, in particular lactulose and fructose, may be helpful in the evaluation of lactose-hydrolyzed milk quality, and indicate the sequence of enzymatic hydrolysis and thermal treatment [8].

The scope of the research by Messia et al. [8] also included the assessment of the influence of storage conditions, i.e. its time (from 1 to 4 months) and temperature (4°C and 20°C), on changes in lactulose and fructose content in lactose-hydrolyzed milk samples differing in technological procedure – lactose hydrolysis sequence (pre- or post-UHT), as well as UHT treatment type (direct and indirect). Milk that was subjected to lactose hydrolysis prior to the thermal process (abbreviated as "A-UHT-HD") was preserved by direct UHT treatment (infusion). Whereas milk that was subjected to lactose hydrolysis after the thermal process (abbreviated as "B-UHT-HD") was preserved by indirect UHT treatment. The content of the above-mentioned saccharides in the control samples, which was traditional UHT milk produced without lactose hydrolysis (marked as "A-UHT" and "B-UHT" respectively), was also analyzed for two variants of UHT treatment, analogous to the production of lactose-hydrolyzed milk. There were significant differences in the content of the aforementioned saccharides in the compared samples. The initial lactulose content in lactose-hydrolyzed milk, in which post-UHT lactose hydrolysis was carried out (B-UHT-HD), was 0.117 g/l, while it was 0.340 g/l in the traditional milk control sample (B-UHT). This confirms that lactulose was hydrolyzed by the continuously active β-galactosidase present in milk. Lower lactulose concentrations were reported for A-UHT-HD lactose-hydrolyzed milk (pre-UHT lactose hydrolysis) and the control sample (A-UHT); 0.0445 g/l and 0.110 g/l, respectively. The lower lactulose content in lactose-hydrolyzed milk (A-UHT-HD) vs. the control sample (A-UHT) may result from the reduced amount of lactose in heat-treated milk [8].

For each of the variants, the initial concentration of fructose in lactose-hydrolyzed milk (0.0912 g/l and 0.0892 g/l) was several times higher than in the control samples (0.0236 g/l and 0.0242 g/l). Depending on the sequence of lactose hydrolysis and UHT treatment processes, this results from the release of fructose by lactulose hydrolysis (for post-UTH lactose hydrolysis milk) or glucose isomerization (for pre-UTH lactose hydrolysis milk) [8].

The results of the aforementioned studies also indicate the significance of the storage temperature of milk samples for changes in lactulose and fructose content. Those changes were more intense in samples stored at 20°C than in samples stored at 4°C. It should be noted that under these conditions (20°C), lactulose content in traditional UHT milk (A-UHT) and lactose-hydrolyzed milk (A-UHT-HD, pre-UHT lactose hydrolysis) increased by about 50% after 4 months of storage. By comparison, the lactulose content of traditional UHT milk (B-UHT) increased by about 10%, and that of lactose-hydrolyzed milk (B-UHT-HD, post-UHT lactose hydrolysis) decreased by about 20% of the initial value after 4 months of storage. The decrease in lactulose concentration in lactose-hydrolyzed milk, in which lactose hydrolysis was carried out after UHT treatment, was caused by β-galactosidase activity in milk during storage. In all milk samples stored at 20°C, a fructose concentration increase was detected, with the highest increase being observed for B-UHT-HD milk (lactose hydrolysis after UHT treatment), which was also marked by a significant lactulose concentration decrease. This confirms the activity of β-galactosidase present in milk even for several months of storage [8].

It is worth noting that the authors of the cited studies also demonstrated the unfavorable effect of higher storage temperature and longer storage time of milk on the increase in the content of furosine, an important marker of thermal changes in proteins [8].

The results confirmed the influence of the milk production procedure on milk saccharide content, as well as on the intensity of the components transformations, e.g. Maillard reaction.

The UHT-induced changes may lead to the emergence of unfavorable taste and smell, and sometimes to the formation of sediment during milk storage. It turns out that these changes may be inhibited by introducing polyphenols into milk, e.g. in the form of green tea extract containing catechins [6].

CONCLUSION

The technology used in the production of lactose-free and lactose-reduced milk, as well as the type of β-galactosidase preparation influence the transformations of milk components (mainly proteins) and saccharide composition. Maillard reaction and saccharide isomerization intensified by heat treatment show different rates by milk composition (the content of the substrates for these reactions) and milk production process, in particular the lactose hydrolysis and heat treatment sequence, heat treatment type, as well as lactose hydrolysis time and degree. Therefore, traditional milk and lactose-hydrolyzed milk are characterized by a different content of saccharides as well as compounds which are markers of changes caused by the thermal process, e.g. furosine and lactulose. Milk with partially hydrolyzed lactose was found to contain about 2-3 times less lactulose compared to the traditional milk control samples. Moreover, no lactulose was found in lactose-free milk (lactose content <0.01%). The content of the aforementioned compounds also shows high variability depending on the technology of lactose-hydrolyzed milk production. The presence of tagatose may, for instance, indicate that lactose hydrolysis was carried out prior to thermal treatment. The analysis of the results of various commercial samples of lactose-hydrolyzed milk indicates a high variability of their quality, which in turn highlights the need to establish specific quality criteria for this type of milk so as to ensure product quality. Detailed analysis of the content of saccharides (e.g. lactose, lactulose, fructose, tagatose) as well as furosine may be instrumental in assessing the effect of the technological process on changes in the composition of saccharides and on the nutritional value of lactose-free milk and milk with partially hydrolyzed lactose preserved by the UHT method.

PODSUMOWANIE

Technologia stosowana w produkcji mleka bezlaktozowego i o obniżonej zawartości laktozy, jak również rodzaj preparatu β-galaktozydazy wpływają na przemiany składników mleka (głównie białek) i skład sacharydów. Reakcje Maillarda i izomeryzacja sacharydów intensyfikowane obróbką cieplną wykazują różne tempo w zależności od składu mleka (zawartości substratów tych reakcji) oraz procesu produkcji mleka, a w szczególności sekwencyjności hydrolizy laktozy i obróbki termicznej, rodzaju obróbki termicznej, a także czasu i stopnia hydrolizy laktozy. W związku z powyższym, mleko tradycyjne oraz z rozłożoną laktozą charakteryzują się różną zawartością sacharydów, a także związków, stanowiących markery zmian wywołanych procesem cieplnym, np. furozyny i laktulozy. Mleko z częściowo rozłożoną laktozą charakteryzowało się około 2-3-krotnie mniejszą zawartością laktulozy w porównaniu do próbek kontrolnych mleka tradycyjnego. Ponadto nie odnotowano obecności laktulozy w mleku bezlaktozowym (zawartość laktozy <0,01%). Zawartość w/w związków wykazuje również dużą zmienność w zależności od technologii produkcji mleka z rozłożoną laktozą. Obecność tagatozy może np. wskazywać, że hydroliza laktozy była przeprowadzona przed obróbką termiczną. Analiza wyników badań różnych handlowych próbek mleka z rozłożoną laktozą wskazuje na dużą zmienność ich jakości, co z kolei podkreśla konieczność ustalenia określonych kryteriów jakościowych dla tego rodzaju mleka w celu zapewnienia jakości produktu. Kompleksowa analiza zawartości sacharydów (np. laktozy, laktulozy, fruktozy, tagatozy), a także furozyny może być pomocna w ocenie wpływu procesu technologicznego na zmiany składu sacharydów oraz na wartość żywieniową mleka bezlaktozowego i z częściowo rozłożoną laktozą utrwalanego metodą UHT.

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