EFFECT OF LACTOSE HYDROLYSIS ON MILK FERMENTATION AND SOME PROPERTIES OF CURD

József Csanádi^{1*}, Zsuzsanna Kárnyáczki², Ildikó Bajúsz¹, Ottilia Bara-Herzegh¹, József Fenvvessy¹

¹University of Szeged, Faculty of Engineering, 6724 Szeged Mars tér 7. Hungary; ²Körös-Maros Biofarm Ltd 5711 Gyula Külterület 2. Hungary

csanadi@mk.u-szeged.hu

ABSTRACT

Much of the World population suffers in lactose intolerance so these people mustn't eat milk and milk products without lactose pre-hydrolysis. Nowadays, dairy firms produce more and more lactose hydrolysed milk products for people suffered in lactose intolerance and enjoy the benefit of milk and milk products consumption. One of the most popular milk products are the fermented products such the yogurt. Well known, that lactose hydrolyzed milk has a sweeter taste and more prone to strong Maillard reaction. But the other technological properties and/or consequences of these are less investigated.

We observed a significant effect of the rate of lactose pre-hydrolysis on the acid clotting time, pH, and the gel firming. The pre-hydrolysis of raw milk (from 4.62% to 3.87%) accelerated the pH decrease so the clotting time was about 30 minutes shorter than in the control samples. The hydrolysis of raw milk accelerated the speed of pH decrease in lactase inactivated milk samples. Further difference was observed in the gel firming. The pasteurized than pre-hydrolysed and lactase inactivated milk samples showed better gel firmness at 4,6 pH. The biggest difference in gel firming was approximately four fold in hydrolysed raw milk samples versus control samples. The trend of pH and clotting changes was different in doubly pasteurized milk samples.

Keywords: lactose hydrolysed milk, fermentation, yogurt, acid gelation

1. INTRODUCTION

Lactose is the main carbohydrate in milk. The average content in cow's milk is usually between 4.5-5.0% (wt/vol) in cow, sheep and goat milk. During a normal digestion in a human lactose is hydrolyzed into glucose and galactose a process catalyzed by lactase normally in the intestinal mucosal cells and released to the intestinal juice (1). Low lactase activity is a relatively common abnormality of the small bowel in man. These lactose intolerant people don't able to hydrolyze lactose so lactose intake affects gastrointestinal symptoms in this people. Lactose intolerance is prevalent in most areas where there are few dairy animals or where adults use little or no milk. The proportion of lactose intolerance varies widely among region, from 10 to 90% (2).

Hydrolysis of lactose also will take place during milk processing, more exactly during fermentation and the monosaccharides produced are assumed to be utilized by the organisms. This fermentation reduces lactose in milk about to two thirds of the original level (2) so lactose intolerant to be given a chance to consume these products, but the partially hydrolysis don't guarantee the lack of symptoms fully. Lactose free milk products produced by lactase adding don't cause symptoms. Furthermore, the lactose maldigesters

should be able to tolerate foods small amount of lactose, not more than 2-6g per serving (3).

It is important to note, that the lactose derivatives lactulose, lactitol and galactooligosaccharides find applications in foods and pharmaceutical preparations as prebiotics to promote gut health. Similarly to non-digested lactose, these compounds enhance the intestinal absorption of calcium and magnesium. Other lactose-derived compounds (e.g., tagatose and lactobionic acid) have potential applications as bioactive ingredient in foods (4). So the lactose hydrolysed milk products have more benefit compared to the products from non-hydrolysed milk. It is found many lactase enzyme products in the market having different activity and properties, but problems can appear in milk processing inhibiting the fully hydrolysis. Further problems the excellent quality, heat stability, and the shelf life. It was observed a high reactivity of lactose-hydrolyzed milk to the Maillard reaction and the more limited chemical stability of products during storage (5, 6).

More inactivating and reactivating agents of lactase have already investigated. The enzyme can be inactivated by ethylenediaminetetraacetic acid tetrasodium salt or dialysis against distilled water, and activity can be recovered partially upon addition of magnesium ++ or manganese ++ salts at optimal concentrations. Heavy metals and p-chloromercuribenzoate can strongly inactivate the enzyme, indicating sulphydryl groups and their requirement for lactase activity. O-nitrophenyl-/3-D-galactopyranoside hydrolysis also can inhibit competitively by lactose, methyl-fl-D-galactoside, galactose, galacturonic acid, ribose, and galactitol; but neither glucose nor melibiose can affect enzyme activity (7).

Heat treatment also affect the enzyme activation. It was observed that heat treatment of milk significantly increases lactase activity, due to the liberation of free SH groups. Authors suggested, that this enzyme activation can be reversed by oxidizing the reactive sulfhydryl groups, proving that the observed effect may be related to the release of free SH to the medium, rather than to the denaturation of a thermo labile protein inhibitor (8)

The heat sensitivity of enzymes extracting from different microorganisms is different. The lactase from Bacillus stearothermophilus is quite heat resistant (9) so this enzyme can used in hot milk (70°C) so we can make lactose hydrolysis and microbe killing simultaneously. These mentioned observes confirms, that the lactose hydrolysis has significant effects on the processing and quality of these milk products.

Our aim was to observe the effect of lactose hydrolysis on the fermentation speed and the coagulation properties (coagulation time, viscosity of gel) using yogurt as a test product.

2. MATERIAL AND METHODS

2.1. Making of samples



Figure 1. The coagulation measurer system

Raw com milk was homogenized (20 MPa), pasteurized (90°C with 30 sec. holding) and cooled (at 40°C) cow milk for lactose hydrolysis adding 1.00 ml Maxilact 2000 lactase enzyme (Gijst Brocades) to 3.0 litres milk. Hydrolysis was performed at 40°C in a thermostat (Memmert UNB 200, Schwabach, Germany) for 15, 30 and 45 minutes in raw milk samples and for 60 minutes in pasteurized milk samples than the samples were heated up to 80 °C (with 1 min. holding) to inactivate the enzyme. The samples were cooled to 45 °C for inoculation and fermentation. So we had samples which were heat treated once and twice.

For fermentation Chr. Hansens Yo Fast 88 starter was used inoculating into the pre-treated samples. First, solution was made from 0.20 g lyophilized culture and from 9.80g distilled water, than 30 minutes conditioning was used before inoculation with 0.500 ml into 100 ml milk. Each measuring was repeated fivefold.

2.2. PH and gelation measure

PH was measured with Thermo Orion-3 Star pH meter (Thermo Fisher Scientific Inc).

Coagulation properties were investigated with SV 10 oscillatory viscometer (A&D Company, Japan). The sensors of viscometer cut the protein net partly, but this time (point) is affected by the clotting ability of milk. Therefore it able the observing to show the differences in the clotting ability of the milk samples resulted different pre-treatment (hydrolysis). The constant temperature of samples was kept with water bath (Memmert WNB 14 Schwabach, Germany)

3. RESULTS AND DISCUSSION

We observed unexpected differences in the pH and gel properties between once and double pasteurized milk samples. The development of fermentation was faster in hydrolyzed but only once heated milk samples than in non hydrolyzed samples (Fig 2.). This phenomenon corresponds with the information of the producer.



Figure 2. PH changing in milk samples during the fermentation

The clotting occurred sooner (after 210 min) in hydrolysed milk samples than in non hydrolysed samples. The clotting time of non hydrolysed samples was 240 min. So the clotting time of hydrolysed milk samples were 30 min. shorter than in non hydrolysed milk. The explanation of this remarkable difference of clotting time and pH can be the faster microbe growing in milk is rich in monosaccharides This difference is markedly affect the processing time of lactose hydrolysed yogurt but this time gain in the fermentation only compensates the time of hydrolysis.

The trend of lactose decrease was similar both in hydrolysed and control milk samples. The lactose content of control samples was 3.30% at the clotting (30% decrease). The degree of lactose decrease was better in hydrolysed milk samples (40%) as compared to the initial 3.83% and the lactose content at the clotting was 2.32% (Fig 3.).

This markedly lower lactose content can decrease further during the cooling and storage probably, but most likely doesn't reach the wanted low level, so this is not a lactose free milk product. Even so, this low lactose level in yogurt doesn't causes symptoms likely in many lactose intolerant people or these people can eat more yogurt without symptoms (10).



Figure 3. Lactose changing in milk samples during the fermentation

Fig 4. demonstrates the changes in the clotting time and gel viscosity among the control and hydrolysed samples with different hydrolysation time. As it can be seen, longer lactose hydrolysis time caused shorter clotting time (the peak of curves).



Figure 4. Clotting curves of control and different scale hydrolysed milk samples (represented time values are the time of hydrolysation)

The first 15 min. hydrolysis decreased the clotting time about a half compared to the control. This finding agree the result of (11) observed the clotting time decreasing effect in Twarog production. Further hydrolysis did not cause remarkable changes in clotting time but it caused big changes in viscosity of gels. The viscosity of hydrolysed (for 60 min) milk gel was about four fold than in the control samples, 4.1mPas versus 22.56mPas. Now, we have to note that this viscosity is not typical for a common set type yogurt gel has 50-120 mPas viscosity. These observed low values occurred due to the conditions of the experiments presumably.

The trends clotting time and viscosity changed in double heated milk samples (Fig 5.). Control samples clot sooner than hydrolysed samples and the gels of hydrolysed samples had higher viscosity, but the differences were not too big. The difference in clotting time was 12 min. but in viscosity was only 0.8 mPas. So the use of HTST (High Temperature

Short Time) to hydrolyse and and lactase inactivation at 80 °C for 1 min. gave different results compared to the use of raw milk for hydrolysis.

The different heat treatments of the samples may have caused this different tendency. Heat treatment significantly increases the enzyme activity (8) and affects the microbe activity so the speed of hydrolysis and the exact changes in milk during the fermentation also can be different.

The different lactose content and some side product (galacto-oligosaccharides and other prebiotics) of hydrolysis at the lactase inactivation by heating also can cause differences in the gelation.



Figure 5. Clotting curves of control and hydrolysed HTST milk samples (60 min.: 60 min. pre-hydrolysis of HTST milk)

Further, we observed difference in the sensory properties of yogurt made from hydrolysed and non-hydrolysed milk. Yogurt from hydrolysed milk had lower flavour intensity mainly lower acid flavour. This result agrees with results investigated the sensory properties of cheeses from low, high and lactose free milk (12).

CONCLUSION

We observed contrary phenomena's during the fermentation of non-hydrolysed and different scale hydrolysed milk samples. The clotting time was shorter and the viscosity was higher using raw milk for hydrolysis versus using HTST milk for hydrolysis. Even so, these differences between the properties of acid gelation of different milk samples were remarkable. Other important observe was the lower flavour intensity in yogurt from lactose hydrolysed milk. Important problem was the lack of expected sour flavour in yogurt from pre-hydrolysed milk. We can not give an exact explanation for these contrary results but the pre-treatment of milk, the side products of lactose hydrolysis and also the second heating of milk (lactase inactivation) can play a role separately or jointly in the appearance of gelation of these samples. We have to continue these preliminary experiments due to the mismatching data to the interest of obvious results, and to clear the trend in the acid gelation of lactose hydrolysed milk. The finally aim our future work is to give data and methods for producing of lactose free yogurt with the expected texture and sensory properties.

ACKNOWLEDGEMENT

This research was supported by the Hungarian and EU Governments in a Research Program Jedlik Ányos, NKFP 06 A2.

REFERENCES

- Orten, J.M., and Neuhaus, O.W. (1975): Human Biochemistry. C. V. Mosby Co., St. Louis
- Walstra, P., Wouters, J.T.M., Geurts, T.J. (2006): Dairy Science and Technology, Taylor and Francis group, London
- Hertzler, S., Bao-Chau Huynh, L., Savaianom, D.A. (1998): How much lactose is low lactose? Journal of the American Dietetic Asociation, 96, 243-246 p.
- Schaafsma, G. (2008): Lactose and lactose derivatives as bioactive ingredients in human nutrition International Dairy Journal 18, 458-465 p.
- Pellegrino, L., De Noni, I., Resmini, P. (1995): Coupling of lactulose and furosine indices for quality evaluation of sterilized milk. International Dairy Journal, 5, 647–659 p.
- Messia, M.C., Candigliota, T., Marconi, E. (2007): Assessment of quality and technological characterization of lactose-hydrolyzed milk. Food Chemistry 104, 910– 917 p.
- Castillo, F.J., Moreno, B. (1983): Properties of Lactase Produced by Candida pseudotropica Journal of Dairy Science 66, 1616-1621 p.
- Jimenez-Guzman, J., Cruz-Guerrero, A.E., Rodriguez-Serrano, G., Lopez-Munguia, A., Gomez-Ruiz, L., Garcia-Garibay, M. (2002): Enhancement of Lactase Activity in Milk by Reactive Sulfhydryl Groups Induced by Heat Treatment. Journal of Dairy Science 85, 2497–2502 p.
- Chen, W., Chen, H., Xia, Y., Zhao, J., Tian, F., Zhang, H. (2008): Production, Purification, and Characterization of a Potential Thermostable Galactosidase for Milk Lactose Hydrolysis from Bacillus stearothermophilus. Journal of Dairy Science 91, 1751–1758 p.
- Alm, L., (1982): Effect of Fermentation on Lactose, Glucose, and Galactose Content in Milk and Suitability of Fermented Milk Products for Lactose Intolerant Individuals. Journal of Dairy Science 65, 346-352 p.
- Omar, M.M., Hosaja, M. (1986): Microstructure and chemical changes in Twarog cheese made from ultrafiltrated milk and from lactose-hydrolysed milk. Food Chemistry 22, 147-163 p.
- Rehman, S.U., Waldron, D., Fox, P-F. (2004): Effect of modifying lactose concentration in cheese curd on proteolysis and in quality of Cheddar cheese. International Dairy Journal, 14, 591-597 p.