

# Studies on some low-lactose milk products

Wafaa Sadie El-Sabie supervisor by A. H. Dawood, Hayam El-Gazzar, M. Osman

The importance of milk and its products are very well considered for infants and adults from the nutritional and health point of view. However, low-lactose milk and/or its products is of great importance for sensitive and intolerant consumers to the ordinary milk and its products. This study was planned to produce purified  $\beta$ -galactosidase enzyme from *K. lactis* yeast which immobilized through cellulose fabric strips, and used in making low lactose milk and some other low lactose dairy products. Moreover, low lactose ultrafiltered milk was used in preparing low lactose ice cream, yoghurt and white soft cheese. The investigation was carried out in three parts. Part I : Production of  $\beta$ -galactosidase enzyme from *K. lactis* and its properties: Section A This section deals with the suitable and favourable conditions for biosynthesis and cell free extraction of  $\beta$ -galactosidase enzyme. The results can be summarized as follows : 1- The obtained supernatant  $\beta$ -galactosidase activity, protein content as well as specific activity increased continuously as the incubation period increased from where the highest enzyme activity was achieved. Afterwards, there was slight continuous decrease. 2- The highest activity was attained at 30°C, pH 4.8 and inoculum size of 1.0% (v/v). 3- The sonication period to give the optimum activity of the enzyme was 10 min. Section B and C: Purification and characterization of *K. lactis*  $\beta$ -galactosidase. Results could be summarized as follows : 1. Crude  $\beta$ -galactosidase enzyme performed from sonication yeast was partially purified by acetone precipitation. The best ratio was found to be 1 : 1 (v/v) at 4°C as it gave the highest volume of activity, specific activity and purification fold of 2.6. The final purification of the enzyme was achieved using Gel filtration on Sephadex G.2DD and sodium phosphate buffer for elution at pH 6.5. Studying the properties of crude and purified enzyme indicated that : \* Assay time of the enzyme was 5 min for both of crude and purified enzyme. optimum temperature was 40°C, while the optimum pH was 6.5 for both crude and purified enzyme. The enzyme was activated by  $\text{Ca}^{++}$ ,  $\text{Mg}^{++}$ ,  $\text{Mn}^{++}$  and  $\text{K}^{+}$ , while  $\text{Zn}^{++}$ ,  $\text{Fe}^{+++}$ , and  $\text{Cu}^{++}$  cations were considered as inhibitors for the enzyme activity. \* The crude and purified enzyme were stable at -10°C for 14 weeks. The kinetic parameters of the purified *K. lactis*  $\beta$ -galactosidase showed that the Michaelis Menten constant ( $K_m$ ) was 1.2 UM and the  $V_{max}$  was 8.4 mole unit/mg protein/ minute. Part II : Immobilization of *K. lactis*  $\beta$ -galactosidase enzyme on cellulose fabric strips and its efficiency in hydrolysing lactose. Pilot experiments were conducted to evaluate the efficiency and capability of cellulose fabric strips for immobilization of  $\beta$ -galactosidase enzyme. The results can be summarized as follows: -1- Some experiments were carried out to achieve the optimum hydrolysis for 6 runs. Lactose hydrolysis was estimated quantitatively in each run through the Osazone fraction. 2- Evaluating the validity of reusing the immobilized  $\beta$ -galactosidase cellulose fabric strips indicates that a reasonable percentage of lactose hydrolysis could be achieved by using such strips for two or three times. 3- The efficiency of lactose hydrolysis by immobilized  $\beta$ -galactosidase cellulose fabric strips was evaluated by comparing with soluble enzyme. This was applied on lactose (5%) aqueous solution and pasteurized skim milk for 6 h or runs. Therefore the results concluded that : \* Lactose hydrolysis in 5% lactose solution was almost similar when using soluble or immobilized strips for the first 3 h or runs. However, using soluble enzyme gave a slight increase in hydrolysis through the last 2 h or runs. \* Immobilized enzyme was more efficient in hydrolysing lactose of skim milk than soluble enzyme. Part III : Manufacturing of low lactose Dairy products: Section A: Low lactose Ice Cream Low lactose Ice

cream was prepared from UF whole milk retentate (diluted with water with ratio (1: 1) and immobilized milk and they compared with that made from whole buffalo milk. The composition and properties of the mixes as well as the resultant ice cream were studied and the data obtained can be summarized as follows:

1. Fat content of mixes was standardized to be 6%~ lactose content was 5.0, 2.1 and 2.3% for the control, immobilized and UF-retentate mixes respectively. The respective protein content was 4.0~ 4.2 and 5.7%, while the T.S. was 32.28, 32.5 and 32.10%.
2. Hydrolysis of lactose increased the acidity and decreased the pH of the mix.
3. Specific gravity and related weight /gallon as well as viscosity of the UF-treated mix were higher than the immobilized treatment which in turn higher than the control.
4. Hydrolysis of lactose by the immobilized enzyme lowered the freezing point of the mix, while decreasing the lactose by UF treatment caused high freezing point than the control mix.
5. Specific gravity and its corresponding weight/gallon of the ice cream were the highest in UF treatment, followed by immobilized milk treatment and at last comes the control treatment.
6. The overrun was the highest in control (65.94%) followed by the immobilized treatment (52.35/0), Then the UF treatment (47.91 %).
7. Milting resistance of ice cream made from UF retentate was the highest, then control, at last was the ice cream made from immobilized milk.
8. Organoleptic tests of ice cream showed that the increase of protein and low lactose contents in UF - treatment recipe improved body and texture of the resultant ice cream. While the hydrolysis of lactose by the J3-galactosidase enzyme increased its sweetness. So, the results proved the validity of using immobilized milk and UF -retentate to increase the milk SNF without sandiness and to give a good quality ice cream.

**Section B: Low Lactose Yog.**

Three treatments of yoghurt were prepared from buffaloes' milk as a control, diluted UF- retentate ( 1 :1 w/w) and partly hydrolysed lactose milk by immobilized fl-galactosidase enzyme. The chemical composition of buffaloes' milk, diluted UF retentate and immobilized milk showed that the UF retentate was low in T.S., S.N.F, lactose, fat, acidity content, but it was high in protein content, while the immobilized-milk resembled the composition of buffaloes milk except the reduction in lactose content. Concerning yoghurt, results revealed that:

1. The hydrolysis of lactose in the immobilized treatment caused an acceleration in the developed acidity during processing and storage, while the UF treatment showed a decrease in acidity development. The pH took the trend of acidity but in an opposite direction.
2. Curd tension of yoghurt was almost affected with acidity and TS. Thus, it was low in the UF-treatment than the other treatments.
3. Continuous decrease in lactose content during processing and storage was observed in all treatments.
4. Yoghurt of immobilized milk had the highest acetaldehyde content, followed by that of buffalo milk and at last yoghurt of 50% retentate. There was a decrease in acetaldehyde content during storage.
5. Lactic acid bacterial count increased in all treatments during incubation and storage up to the 3rd day, after which it began to decrease gradually. Almost the total bacterial count was the highest in immobilized treatment and the lowest in UF treatment.
6. The organoleptic evaluation proved the possibility of making a good quality low lactose yoghurt from immobilized milk, while decreasing lactose content by diluting UF retentate caused some defects in the resultant yoghurt compared with control.

**Section C: Low Lactose white soft cheese:**

Lowering lactose content of cheese milk of white soft cheese and its effect on the resultant product was the ultimate target of this study. Therefore, white soft cheese was made from retentate diluted with water at concentration of 80% and 50% and compared with that from buffaloes milk. The result indicated that:

1. The chemical composition of cheese milk revealed that 80% retentate was of the highest percentages of all constituents except lactose content. The 50% retentate was almost similar to control except lactose content.
2. Lactic acid bacterial count as well as total count were the highest in the control, followed by the 80% retentate and at the last came the 50% retentate when fresh. Substantial continuous reduction was noticed for the three treatments as storage duration period proceeded.
3. Curd tension widely varied among the three treatments of cheese. The highest curd tension was for the control followed by the 80% retentate cheese, then the 50% retentate cheese. This result matches well with the behaviour of acidity content.
4. Results revealed differences in the gross components (T. S., fat, lactose, T.N.) of the various treatments due to the variations in the cheese milk composition.
5. Fresh cheese made from 80% retentate contained the highest amount of SN, tryptophan and tyrosine, followed by that made from 50% retentate after which was the control cheese. During storage, all these parameters increased due to the protein breakdown with different

rates.6. Organoleptic scoring of fresh cheese indicates the superiority of control compared with the other two treatments. However, the scoring for the consequent last three weeks showed an improvement in the quality of the control and 80% retentate cheese as they approached in scoring points, while the cheese made from 50°A. retentate imparted some defects.