enzyme supplies

Product manufacturing information sheet

Lactase 5000Lx

An *Enzyme Supplies* beta-galactosidase enzyme preparation for the dairy & dietetics industry

Introduction

Lactase 5000Lx is a purified lactase preparation, isolated from a special strain of the dairy yeast *Saccharomyce (Kluyveromyces) marxianus* var. lactis. This yeast, firstly described by Beijerinck in 1889, is a well-known dairy organism, which is used in the production of certain types of yoghurt (kefir). The International Enzyme Number is EC 3.2.1.23. The product is non-GM & also contains glycerol (50%), & potassium chloride (2%).

Lactase 5000Lx is suitable for the following main application areas:

- Milk hydrolysis
- Yoghurt-milk hydrolysis
- Cream hydrolysis
- Whey hydrolysis
- Animal feed applications (whey, cat milk)
- Dulce de leche

In general, a sweeter product will be obtained due to the more sweetness of glucose and galactose.

Technical Characteristics

Lactase 5000Lx, hydrolyses the milk sugar, lactose, into the two monosaccharides, glucose and galactose. During the reaction, one molecule of water is used and binds to the sugar molecule. The reaction conditions, i.e. temperature, acidity, processing time, lactose- and enzyme concentration, determine the speed of the reaction.

1. Inhibitors and activators

The most important conditions for a continuing enzyme activity are the pH and the temperature. Heavy metals such as zinc (>5x10^{-4}M) and copper (>5x10^{-4}M) have a strong inhibiting effect on the enzyme.

Free calcium in higher concentrations (>10^{-4}M) also inhibits the enzyme. In contrast, lactase activity and/or stability are improved by magnesium (10^{-4}M), manganese (10^{-4}M) and potassium (10^{-4}M). Phosphate concentrations up to 10^{-2}M will influence stability positively through calcium binding. In practice, both activating and inactivating minerals are present and the actual influence of metallic ions would have to be determined for each substrate. In normal milk or sweet whey however there are no

special requirements if hydrolysis takes place in stainless steel equipment. If a pH correction is required (usually to increase the pH) it is advisable to use potassium hydroxide, as potassium has a strong boosting effect on the activity.

2. Effect of temperature and pH

Because Lactase 5000Lx is derived from a dairy yeast, the optimal conditions are close to the natural pH and temperature of milk, i.e. a pH 6.6-6.8 and a temperature of 35-40°C.

Typical reaction conditions:

pH: 6.6
Time: 4 hours
Enzyme amount: 1000 NLU/l

During the hydrolysis the solution should be stirred.
pH correction can be made, if necessary with either 4N KOH or 4N H_2SO_4.

Lactase 5000Lx is also active at lower temperatures, even down to 5°C. This property is especially important because growth of spoilage bacteria at these low temperatures is very moderate. Sufficient activity is maintained to treat milk (or whey) during an overnight storage period.

3. Applications of Lactase 5000Lx in milk products

1. Milk hydrolysis and lactose intolerance

It is known that, due to a low intestinal lactase activity, not all humans are able to digest the lactose in milk with the same ease. The percentage of lactose malabsorption in the Caucasian human race ranges from 2% (Danish) to approx. 19% (White American). However, the indigenous populations of Eastern Europe, the Mediterranean, Africa, Asia, Latin America and Australia show a much higher incidence ranging from 60% (e.g. Indian, Israeli, Latin- and North-American Indians) to values of 80% (e.g. Chinese, Japanese, Nigerian, Australian aboriginal.

Lactase intolerance also occurs in the following cases:

- Due to a very rare congenital disease some people lack lactase activity from the very moment of birth. This results in severe gastrointestinal disorders, which can be fatal.
- Premature birth may cause an abnormally low lactase activity. Lactase activity is however almost fully restored within one or two months. Heavily undernourished children that have a protein calorie malabsorption. Lactase activity can be completely absent on a temporary basis.

Due to intestinal tract operations the lactase activity may be lost temporarily. Pre-treatment of the milk with Lactase 2000L will prevent these intestinal problems. Depending on the level of hydrolysis, the treated product will have a slightly sweeter taste. This is caused by the higher sweetness of glucose and galactose in comparison to lactose. The balance between milk intake, level of lactose malabsorption and hydrolysis percentage will determine if symptoms of lactose intolerance will occur or not. In the market milk products with different degrees of lactose hydrolyses are sold.

2. Production of hydrolysed milk

- Pasteurise the fresh milk and adjust the temperature to fit the chosen time-temperature-enzyme concentration relationship. To prevent un-desirable microbial growth, we advise to bactofuge the milk prior to hydrolysis and treat the milk below 10°C (50°F). If shorter incubation times are essential, higher temperatures up to 37°C (98.6°F) can be used but only for a short time. In this case the microbiological quality of the milk must be excellent.

- Agitate the milk to assure proper mixing of the enzyme and to keep the solution homogeneous.

- Take two samples of the milk for use during the preparation of the standard hydrolysis curve

- Add the required amount of Lactase 5000Lx to the milk according to the chosen incubation time and temperature.

- During the hydrolysis, regular pH or titrate-able acidity determinations are effective tools for the detection of bacterial growth.

- At the expected end of the hydrolysis one should again determine the degree of hydrolysis. When the desired degree of hydrolysis is achieved, the milk is ready for further processing.

- The milk can now be pasteurised or preferably UHT-treated

3. Milk hydrolysis and the prevention of crystallization of lactose

Due to the higher solubility of the single sugars, highly concentrated or frozen milk products can be obtained without the phenomenon of "sandiness". The latter is an advantage in the production of products like ice cream and *dulce de leche.* The level of hydrolysis needed to prevent crystallisation of lactose in dulce de leche is about 30%. Higher levels can be used if increased sweetness is also wanted.

4. Milk hydrolysis and its use in fermented dairy products (yoghurt, quark)

Although most strains of starter organisms for yoghurt production, i.e. *Streptococcus thermophilus* and *Lactobacillus bulgaricus,* have been selected for their ability to ferment lactose, the actual splitting of lactose appears to be the rate limiting step. The hydrolysis of the lactose therefore often further stimulates the growth of these cultures. In traditional fruit yoghurts or other yoghurt based desserts the lactose does not contribute to the sweetness. Hydrolysed lactose, being 3 times sweeter, substantially contributes to sweetness, thereby reducing the need for added sugar.

Example: production of yoghurt. There are 3 possible methods:
I. Pre-hydrolysis of pasteurised yoghurt milk at low temperatures.
II. Pre-hydrolysis of pasteurised yoghurt milk at higher temperatures.
III. Simultaneous hydrolysis and acidification of the yoghurt milk.
All three can be used for stirred- and set yoghurt. The target for hydrolysis is 85%.

Method I:
A. The temperature of the pasteurised milk is set at 6-10°C (42-50°F), the pH of the milk is about pH 6.6.
B. Per litre of milk (4.6% lactose) add 2000 of Neutral Lactase Units (NLU). Mix well.
C. Keep the milk for 15 hours while gently stirring.
Give the milk its normal heat treatment and add starter culture.

Method II:
A. The milk is pasteurised and cooled to 37°C (98.6°F), pH of the milk is about pH 6.6.
B. Per litre of milk (4.6% lactose) about 2000 Neutral Lactase Units are added. Mix well.
C. Keep the milk for 2 hours while gently stirring.

Give the milk its normal heat treatment and add starter culture.

Method III:
A pre-condition for this third method is that the incubation temperature does not exceed 40°C (104°F). The decrease in pH is measured during a normal acidification process to determine how much time elapses before reaching a pH below 5.7. At this pH the lactase will be inactivated, however, the elapsed time before reaching pH 5.7 can be used for hydrolysis. The quantity of Lactase 2000L to be used must be enough to give 85% hydrolysis in this period. The lactase dosage can be reduced by a short pre-hydrolysis, e.g. add Lactase 2000L 1-2 hours before the culture.

Applications of Lactase 5000Lx in whey

1. Whey, whey protein concentrate, and whey protein for human consumption

With a partial hydrolysis of the lactose, the sweetness of the end product and solubility of the sugars will be increased. The glucose/ galactose mixture has, depending on the concentration, a sweetness of 65-80% relative to sucrose. Microbiologically stable syrups up to 75% total solids can be prepared and can be used in the different application areas.

1.1 Bakery Products: Bread, Biscuits and Cakes

Since whey syrup primarily consists of a mixture of un-denatured proteins and sugars, both chicken egg white and sucrose can be replaced. Reports indicate that up to 30% of the egg-white can be substituted, while the single sugars in the syrup contribute greatly to the improvement of the colour of the baked product.

1.2 Candies and Confectionary Products

Hydrolysed whey syrups can replace large percentages of the sweetened condensed milk used in the manufacture of toffee, fudge, candy bars and dulce de leche. No granulation or sandiness caused by lactose crystals will appear and the caramelisation is improved.

Enzyme Supplies Limited
John Eccles House
Robert Robinson Avenue
The Oxford Science Park
Oxford OX4 4GP
UK

Telephone: +44 (01865) 578557
Telefax: +44 (01865) 338100
E-mail: info@enzymesupplies.com