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Denaturation of β-lactoglobulin and native enzymes in the plate exchanger and holding tube section during continuous flow pasteurization of milk

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The denaturation of β-lactoglobulin, alkaline phosphatase and lactoperoxidase and changes in the proteolytic activity, were studied at the heating and holding sections of a plate heat-exchanger, during treatment of milk under different conditions of temperature and flow rate. The heating rate contributed considerably to the heat-induced changes in the milk components during continuous flow pasteurization, whereas these indicators were less susceptible to differences in the holding times. Information gained in such studies on the denaturation effects occurring in the different sections could be useful in the design of heat exchangers and holding tubes. Copyright © 1996 Elsevier Science Ltd

INTRODUCTION

Characterization of heat-induced chemical changes in milk has been the subject of many papers aimed at finding indicators of the heat treatment conditions to which milk has been subjected. Denaturation of whey proteins occurs when milk is heated above 60°C and includes molecular unfolding, loss of globular configuration and association with the caseins and precipitation with them when milk pH is lowered to 4.6. The kinetics of the denaturation of β-lactoglobulin has been studied by several authors over a wide time/temperature range, which allowed an assessment of any heat treatment with respect to its effect on denaturation (Lyster, 1970; Hillier & Lyster, 1979; Manji & Kakuda, 1986; Dannenberg & Kessler, 1988; Resmini *et al.*, 1989*b*). Thus, the quantitative determination of undenatured β-lactoglobulin has been proposed for distinguishing different categories of heat-treated milk. A minimun content of 2600 mg of undenatured β-lactoglobulin per litre of pasteurized milk is within the limits proposed by the International Dairy Federation (Schlimme *et al.*, 1993). In addition, denaturation of native enzymes has been used in the retrospective determination of the heat treatment undergone by milk products. The negative alkaline phosphatase test is used as an index of adequate pasteurization and recovery of lactoperoxidase activity is taken as proof that milk has not been heated over 80°C (Griffiths, 1986).

The continuous development of heat-treatment processes and their application in the dairy industry has given rise to a number of commercial milks. Names such as 'high-pasteurized', 'ultra-pasteurized' and 'super-pasteurized' are being proposed for milks processed at high pasteurization temperatures. A knowledge of the denaturation effects occurring in the different sections of the pasteurizer, might allow one to choose the most appropriate technology and to manipulate the thermal regime for a particular pasteurizer, so that the final product possesses the optimal desirable properties. In this paper we report the effect of different pasteurization conditions on the degree of β-lactoglobulin denaturation and the activity of selected enzymes during the heating and holding phases in a plate heat-exchanger.

MATERIALS AND METHODS

Plate heat exchanger pasteurization system

Bulk raw cow's milk was obtained from a local farm and was kept refrigerated for up to 4 h until it was processed. Milk, preequilibrated to 20°C, was heated in a laboratory scale plate heat-exchanger designed to accurately reproduce the industrial HTST process

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(Armfield, Hampshire, UK). The system consisted of four sections: preheater (regenerative section), heater, holder and cooler. The temperature of the milk was measured at the entrance and exit of the heating section and at the end of the holding section. Heating was accomplished using water 6–8°C hotter than the pasteurization temperature (72.5, 80 and 85°C) as governed by a temperature control unit. Once the pasteurization temperature was reached, milk was passed through the holding section, followed by the regenerative and cooling plates, or diverted directly to the regenerative and cooling sections. Whenever holding times were applied, they were either 15 or 25 s, as given by flow rates of 300 and 180 ml/min, respectively. Come up times were also varied by controlling the flow rate of the peristaltic pump (Table 1). In a typical experiment, once the system was equilibrated, 1 l of heated milk was collected for analysis. All heat-treatment experiments were performed in duplicate.

Analytical determinations

The effects of the heat treatments were estimated by determining the amount of undenatured whey proteins and the activities of alkaline phosphatase, lactoperoxidase and proteolytic activity. Undenatured β-lactoglobulin was determinated on the fraction soluble at pH 4.6 by RP-HPLC using a PLRP-S 8 μm column (300 Å, 150×4.6 mm) (Polymer Laboratories Ltd, Church Stretton, Shropshire, UK), with a linear binary mobile phase gradient (Resmini *et al.*, 1989*a*). The fraction soluble at pH 4.6 was obtained adjusting the pH with 2 N HCl, followed by centrifugation (2500 g, 20 min, 5°C) and filtration through Whatman 40 filter paper. A standard curve for β-lactoglobulin (Sigma Chemical Co., St. Louis, MI) was used for calibration.

Alkaline phosphatase activity was determined qualitatively by the method of Aschaffenburg & Mullen (1949). Lactoperoxidase activity was measured by the spectrophotometric method described by Shindler *et al.* (1976). Milk (25 μl) was added to 5 ml of 1 mM ABTS [2,2′-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid), purchased from Sigma] in 0.1 M acetate buffer pH 4.4. Lactoperoxidase activity is expressed as percentage of the activity of the raw milk to take into account small variations among different batches.

Proteolytic activity was estimated after incubation of raw and heat-treated milk samples for 48 h at 37°C. Milk was mixed with three preservatives: 10 mM NaN_3, 0.1 ml/ml $CHCl_3$ and 0.1 ml/ml of toluene (Andrews, 1983). The fraction soluble at pH 4.6 was obtained by adjusting the pH with 2N HCl, followed by centrifugation (2500 g, 15 min, 5°C) and filtration through Whatman 40 filter paper. The proteolytic activity was calculated as the difference in nitrogen content, as determined by the Kjeldahl method, before and after the incubation, and expressed as percentage of the activity of the raw milk.

RESULTS AND DISCUSSION

Table 1 shows the effect of different time/temperature combinations on the denaturation of β-lactoglobulin, alkaline phosphatase and lactoperoxidase during pasteurization of milk. The heating rate influences the denaturation of the three indicators considered. A change in the come up time from 7.1 to 16.6 s increases the denaturation of β-lactoglobulin, indicating the effect

Table 1. Effect of different flow rates, heating temperatures, come up times and holding times on the denaturation of β-lactoglobulin, lactoperoxidase and alkaline phosphatase during continuous heating of milk in a plate heat exchanger

Flow (ml/min)	Temperature (°C)		Come-up time (s)	Holding time(s)	Undenatured β-lactoglobulin[a]		Lactoperoxidase		Phosphatase
	In	Out			mg/100 ml	SE[b]	% Activity[c]	SE[b]	
420	51.4	72.5	7.1	0	367	1.2	13.9	0.12	+
300	51.6	72.5	10.0	0	360	1.6	18.3	0.7	+
300	51.6	72.5	10.0	15	346	7.0	58.0	1.1	−
180	51.8	72.5	16.6	0	349	1.9	34.5	1.1	−
180	51.9	72.5	16.6	25	334	11.8	92.0	1.1	−
420	57.2	80.0	7.1	0	353	1.9	85.7	1.5	−
300	55.4	80.0	10.0	0	338	0.9	99.0	0.0	−
300	55.4	80.0	10.0	15	266	1.8	100	0.0	−
180	55.0	80.0	16.6	0	331	5.8	100	0.0	−
180	55.1	80.0	16.6	25	256	3.2	100	0.0	−
420	60.4	85.0	7.1	0	332	2.1	100	0.0	−
300	60.4	85.0	10.0	0	323	5.8	100	0.0	−
300	60.3	85.0	10.0	15	203	1.9	100	0.0	−
180	59.3	85.0	16.6	0	312	3.9	100	0.0	−
180	59.3	85.0	16.6	25	176	10.7	100	0.0	−

[a] The content of β-lactoglobulin of the raw milk was 377 mg/100 ml (SE = 0.9).
[b] SE = standard error of mean.
[c] Expressed as percentage of the activity of the raw milk.

of extended times in contact with hot surfaces. Nevertheless, the use of high flow rates in the plate heat-exchanger forces the temperature controller to switch on the heating element in the hot water tank and to increase the flow of hot water. This probably enlarges the temperature difference between the milk in contact with the surface of the heating plate and the rest of the liquid and might account for the comparatively greater degree of β-lactoglobulin denaturation in the milk pumped at the highest flow rates, especially at the highest temperature (12 and 18% of β-lactoglobulin denaturation at residence times on the plate heater of 7.1 and 16.6 s, respectively).

The effect of the holding time on the extent of β-lactoglobulin denaturation begins to be noticeable at 80°C and increases with the temperature and the time of exposure. Resmini *et al.* (1989*b*) carried out industrial HTST pasteurization experiments at temperatures from 72 to 90°C with holding times from 2 to 45 s and reported lower denaturation values. This could be attributed to differences in the heating rate, as the present results show that it contributes more than the holding time to the heat-induced changes in the milk components during continuous flow pasteurization.

When the intensity of the heat-treatment is low, lactoperoxidase inactivation may be more appropriate than β-lactoglobulin denaturation for assessing the thermal effect. At 72.5°C, the come up time and holding time affect considerably the activity of lactoperoxidase, with a greater decrease in activity being observed than for β-lactoglobulin denaturation. Among other milk enzymes, lactoperoxidase has been found to offer the most promising method for assessing HTST treatments of the order 76°C for 15 s; however, its thermostability depends on the heating method employed (Griffiths, 1986).

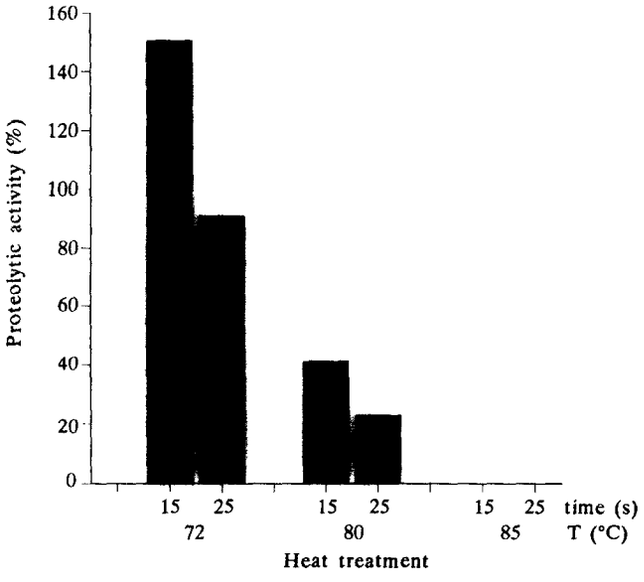

Fig. 1. Proteolytic activity of milks heated at 72.5, 80 and 85°C for 15 and 25 s, expressed as percentage of the activity of the raw milk.

Figure 1 compares the increase in pH 4.6-soluble nitrogen (as compared with raw milk) during 48 h at 37°C, of milks heated at 72.5, 80 and 85°C, with holding times of 15 and 25 s. Treatment of milk at 72.5°C for 15 s leads to an increase in the proteolytic activity over the raw milk, whereas more drastic treatments result in a reduction of activity. Richardson (1983) found a higher rate of activation of plasminogen in milk pasteurized at low temperatures over raw milk and attributed it to the denaturation of inhibitors of the plasminogen activator. Heat-treatment at 72.5°C for 15 s probably causes denaturation of the inhibitors of the conversion of plasminogen into plasmin whereas, under more severe conditions, denaturation of plasmin becomes noticeable.

Heat-induced changes occurring under severe processing conditions can have negative effects on the sensory properties, nutritive value and shelf life of milk. In addition, a high proteolytic activity is responsible for the development of off flavours, especially bitterness, upon storage (Baker, 1983). However, although the most severe heat-treatments applied considerably reduce the proteolytic activity in milk, it has been found that rigorous pasteurization conditions result in a shorter shelf life and a more rapid deterioration of the sensory quality of stored milk. This effect could be related to the destruction of antimicrobial systems and other antagonistic effects or to activation of spores (Cromie *et al.*, 1989).

These results show the relative contribution of the two main steps of the pasteurization process on heat-induced changes in the milk components. Short come up times considerably reduce the denaturation of proteins and enzymes, whereas these biochemical indicators are less affected by differences in the holding times. The determination of these parameters provides useful information for the design and operation of the heat exchanger and holding tube sections of pasteurization equipment.

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