ORIGINAL RESEARCH

Use of hydrogen peroxide detection strips to determine the extent of pasteurization in whole milk

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Commercially available hydrogen peroxide (H_2O_2) detection strips were shown to be effective in determining known levels of H_2O_2 in milks where the lactoperoxidase system (LPS) had been inactivated. In addition, the strips were evaluated for determination of residual H_2O_2 in LPS-activated milks, following a range of heat treatments. The detection of residual H_2O_2 corresponded with results of zero lactoperoxidase activity. Hence, the detection strips offer a simple and accurate method for detecting the absence of lactoperoxidase, and therefore can be used to determine whether the milk has been subjected to overpasteurization. The technique provides a more convenient method than the standard procedure based on oxidation of 1,4-phenylenediamine.

Keywords Detection, Heat treatment, Hydrogen peroxide strips, Inactivation, Lactoperoxidase, Rapid method pasteurization.

INTRODUCTION

It is now a requirement that pasteurized milk should show a positive activity for lactoperoxidase, to ensure that it has not been over-processed. Lactoperoxidase is also the major component of the lactoperoxidase system (LPS), which is a naturally occurring antimicrobial system found in milk. The other two components of the system are thiocyanate (SCN^-) and hydrogen peroxide (H_2O_2). Lactoperoxidase catalyses the oxidation of SCN^-, by H_2O_2, to intermediate antimicrobial compounds,[1] the main one being hypothiocyanite ($OCNS^-$). It may play a role in helping to extend the shelf-life of pasteurized milk, so it is important to ensure that milk is not overprocessed.

There is always sufficient lactoperoxidase in raw milk for the LPS to operate, so when H_2O_2 and thiocyanate are added to raw milk, the LPS is activated. The H_2O_2 is utilized immediately and cannot be detected as long as the lactoperoxidase remains active.[2] If the lactoperoxidase has been inactivated by heat,when H_2O_2 and thiocyanate are added, the H_2O_2 content can be estimated by means of enzyme-impregnated detection strips. The strips are able to detect small levels of H_2O_2 and are commercially available (Merck, Dorset, UK), being used primarily for water testing. Using these assumptions, it can be hypothesized that the degree of inactivation of the LPS by thermal processing could be quantified by these detection strips. The strips will detect between 1 and 25 ppm H_2O_2 with excellent reproducibility and speed. They are far simpler to use than the standard method for peroxide determination, which requires the addition of H_2O_2 and the hazardous chemical 1,4-phenylenediamine ($C_6H_8N_2$) to a milk sample. The latter method is based on the principle that if lactoperoxidase is present it can decompose the H_2O_2, and the liberated oxygen can oxidize the colourless $C_6H_8N_2$ to the purple indophenol (Storch test). Although this test can be performed almost as quickly as the detection strip method, the reagent ($C_6H_8N_2$) is only stable for 1 or 2 days and needs to be made up regularly, whereas H_2O_2 is stable for 1 month.

The objectives of this study were to test the validity of the strips for detecting the presence of active lactoperoxidase in milk. Detection strips were tested with LPS-inactivated milk (boiled) containing known quantities of added H_2O_2. In addition, the strips were used to estimate the concentrations of H_2O_2 remaining in LPS-activated milks, following their heat treatment over a range of time and temperature combinations in the pasteurization region.

MATERIALS AND METHODS

To determine the H_2O_2 level, a strip was immersed in the sample for 10 s and the excess shaken off gently. Colour formation was allowed to proceed for 30 s before comparing against a colour chart. The H_2O_2 concentration could then be read directly from this chart.

Lactoperoxidase activity was determined using an adaptation of the International Dairy Federation

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(IDF) method[3] described by Barrett *et al*.,[4] where the rate of oxidation of 2,2 azinobis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) was measured spectrophotometrically at 412 nm.

In the first experiment, the sensitivity and accuracy of the strips for detecting known concentrations of H_2O_2 in milk were assessed. The strips were first calibrated by immersing them in accurately prepared dilutions of H_2O_2 (1, 5, 10, 15, 20 and 25 ppm) and comparing the observed colour with that on the chart. It was confirmed that the strips had a high sensitivity and the colour chart was accurate. Raw whole milk was subsampled; one sample was unheated whilst the other was boiled for 1 h to inactivate the enzyme completely. Three levels of H_2O_2 addition to milk were investigated (1, 10 and 100 ppm) as well as a control. The LPS was activated once the samples had been cooled to 8°C by adding 10 ppm thiocyanate and the appropriate level of H_2O_2 to both unheated and boiled milks. Thiocyanate was added as sodium thiocyanate and H_2O_2 was added in the form of sodium percarbonate, which liberates H_2O_2 when in contact with water. The level of H_2O_2 was determined after 0, 60 and 120 min using the detection strips and colour chart. Lactoperoxidase activity was measured in all samples.

The second experiment involved estimating the concentrations of H_2O_2 and lactoperoxidase activity remaining in LPS-activated milks following heating over a range of time and temperature combinations. Milks were heated at a range of conditions within the pasteurization region. Temperatures ranged from 68 to 80°C at 2°C intervals and holding times of 15, 40, 60 and 80 s were used. Heat treatment was carried out using a small high temperature, short time (HTST) plate-type pasteurizer (JHE301, APV Baker, Peterborough, UK) and samples were cooled to below 6°C and stored immediately at 4°C. Six 1-litre samples were collected for each time/temperature combination. Three samples of each were LPS activated by adding 10 ppm thiocyanate and 10 ppm H_2O_2 (as directed by the IDF[5]), the remainder being used as controls. Each sample was analysed in triplicate. The H_2O_2 was measured 2 min after LPS activation.

Table 1 Residual hydrogen peroxide (H_2O_2) levels in raw and boiled milks as determined by peroxide detection strips at 0, 60 and 120 min after H_2O_2 addition

		Residual H_2O_2 level as read by detection papers (ppm)		
	Added H_2O_2 (ppm)	*0 min*	*60 min*	*120 min*
Raw milk (LPS active)	0	0	0	0
	1	0	0	0
	10	0	0	0
	100	2	0	0
Boiled milk (LPS inactive)	0	0	0	0
	1	0	0	0
	10	10	7.5	7.5
	100	>25	>25	>25

LPS = lactoperoxidase system.

Table 2 Results of hydrogen peroxide (H_2O_2) detection strips (ppm) in milks processed over a range of heat treatments*

	H_2O_2 concentration at specified holding time			
Heat treatment	*15 s*	*40 s*	*60 s*	*80 s*
Raw	0 (100)	0 (100)	0 (100)	0 (100)
68°C	0 (91.3)	0 (87.4)	0 (85.4)	0 (65.8)
70°C	0 (78.4)	0 (73.1)	0 (69.2)	0 (58.8)
72°C	0 (69.4)	0 (57.8)	0 (48.0)	0 (32.1)
74°C	0 (29.5)	0 (24.5)	0 (2.0)	0 (1.6)
76°C	0 (1.8)	0 (0.8)	10 (0)	10 (0)
78°C	0 (0.9)	10 (0)	10 (0)	10 (0)
80°C	10 (0)	10 (0)	10 (0)	10 (0)

*The lactoperoxidase system is activated after heating by addition of 10 ppm thiocyanate and 10 ppm H_2O_2 (% residual lactoperoxidase activities are shown in parentheses).

RESULTS AND DISCUSSION

The first experiment (Table 1) demonstrated the efficiency of the lactoperoxidase enzyme in H_2O_2 removal. Lactoperoxidase assays confirmed that the raw milk samples contained appreciable enzyme activity, while no activity was detectable in any of the boiled milks. In the raw milks, there was no detectable H_2O_2 after any level of addition after 60 or 120 min, and only 2 ppm was detected immediately after addition of 100 ppm H_2O_2. However, in the LPS-inactivated boiled milks, appreciable levels of H_2O_2 were detected at all times after addition of 10 and 100 ppm H_2O_2. No H_2O_2 could be detected after addition of 1 ppm, which is probably below the detection limit for the strips, while addition of 100 ppm H_2O_2 gave residual levels above the scale range. Thus, 10 ppm was chosen for H_2O_2 addition in subsequent experiments. After LPS activation with 10 ppm of both thiocyanate and H_2O_2, the peroxide strips provided a simple visual indication of the extent of heat treatment, without the need for lengthy analysis.

The second experiment involved heating milk between 68 and 80°C for 15–80 s; half these samples had their LPS activated and half were left untreated as controls. No H_2O_2 was detected in any of the control samples, as expected, since none had been added. For the activated samples (Table 2), no H_2O_2 could be detected where the LPS system was operational. This is because all of the added H_2O_2 was fully consumed during the reaction.[2] In contrast, when the enzyme had been completely inactivated by heating (shown as 0% residual

lactoperoxidase activity in Table 2), the lactoperoxidase system could not operate and these samples gave readings of 10 ppm H_2O_2 when tested exactly 2 min after LPS activation. For the time/temperature combinations chosen, overpasteurization (shown by the presence of H_2O_2) was seen in all milks heated at 80°C, those at 78°C for 40 s or more and those at 76°C for 60 s or more. The detection strips clearly offer a simple and accurate method of detecting overpasteurization. It has been observed that HTST conditions of 80°C for 15 s result in a poorer keeping quality than 72°C for 15 s.[4,6,7] This higher temperature corresponds to zero detectable levels of lactoperoxidase in milk, suggesting that the LPS may be contributing to the overall keeping quality of standard pasteurized milk.

CONCLUSIONS

This detection strip method is rapid and inexpensive, and does not require the preparation and use of hazardous reagents and so reduces operator error whilst increasing safety. The strip test is as rapid as the European Commission (EC) method[8] but is much cheaper, since it only requires one detection paper and nominal quantities of chemicals. Both methods rely on inactivation of the enzyme to give an overpasteurization result and presumably have the same theoretical sensitivity. The detection strip test always gives an overpasteurization result when no enzyme activity can be detected, whereas the peroxidase test sometimes indicates that there is no activity when, in fact, some can be detected. For example, if the same sample is tested several times, it may give peroxidase-negative and -positive results, as opposed to the detection strips which consistently give the same result for the same sample.

The peroxide detection strip method could provide an excellent alternative to the peroxidase test[8] for detecting overpasteurization.

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