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The use of the bacteriocin, nisin, as a preservative in pasteurized liquid whole egg

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Nisin used at a level of 5 mg/l resulted in a significant increase in refrigerated shelf life of pasteurized liquid whole egg from between 6–11 d to 17–20 d. In the first of two trials, nisin also protected the liquid egg from growth of *Bacillus cereus*. *Bacillus cereus* was not presented in the egg in the second trial. Effective residual levels of nisin were detected in the liquid egg post-pasteurization.

It is a statutory requirement in the UK that liquid whole egg is pasteurized at a temperature of not lower than 64·4°C for at least 2·5 min to ensure the destruction of *Salmonella*. This heat process will result in approximately 99% of the bacteria present in raw liquid whole egg being killed. Surviving organisms may include both Gram-negative and Gram-positive species such as the spore-forming *Bacillus* (Cunningham 1977; Schafi *et al.* 1970; Payne *et al.* 1979; Wood and Waites 1988). Many of these surviving bacteria are capable of growth at refrigeration temperatures and pasteurized liquid whole egg has generally a shelf life of 10–11 d maximum when held at these temperatures. The use of a preservative in the product that results in a significant increase in this shelf life would have a considerable benefit to the egg processing industry. Also the food poisoning spore-forming species, *Bacillus cereus*, is a common contaminant of liquid whole egg (Wood and Waites 1988), and inhibition of this organism would result in increased consumer safety.

This work describes two trials on the use of nisin as a preservative in commercially pasteurized liquid whole egg held at refrigeration temperatures. Nisin is a bacteriocin produced by *Lactococcus lactis*, and has been widely used as a food preservative for many years. It is accepted as safe for food use by JECFA and national/community food additive legislating bodies including US FDA, UK and EEC. It is active against Gram-positive bacteria but is thought to have little action against Gram-negative bacteria, yeasts and moulds (Delves-Broughton 1990; Fowler and Gasson 1991). The site of action against susceptible vegetative cells is the cytoplasmic membrane. Recent studies have shown that the disruption of the outer membrane of Gram-negative bacteria by chelating agents can make these bacteria susceptible to the bactericidal effect of nisin (Stevens *et al.* 1991).

Materials and Methods

The nisin used in this study was the commercial preparation, Nisaplin. This preparation contains 25 mg nisin/g. Processing of the liquid whole eggs was carried out in a UK egg processing factory and the pasteurization regime employed was 64·4°C for 2·5 min. Nisin (5 mg/l) was added to the raw egg prior to pasteurization and multiple 20 ml samples in sterile screw-capped jars were aseptically collected post-pasteurization for shelf life determination.

Trial 1: Pasteurized samples were incubated at 6°C and individual jars were removed for

Table 1. Residual nisin levels as measured by bioassay in pasteurized and raw liquid whole egg

	Trial 1	Trial 2
Raw egg with no nisin added	—	2·69 mg/l*
Pasteurized egg with no nisin added	0·5 mg/l*	0·96 mg/l*
Raw egg with nisin (5 mg/l) added	4·3 mg/l	5·38 mg/l
Pasteurized egg with nisin (5 mg/l) added	3·65 mg/l	4·65 mg/l

* Inhibition not due to nisin.

examination at appropriate intervals (see Table 2) for both aerobic bacteria/ml on Plate Count Agar (Oxoid) and total anaerobes on Reinforced Clostridial Agar (Oxoid). The aerobic count plates were incubated at 30°C for 3 d, and the anaerobic plates at 37°C for 2 d. Gram stains of predominant isolates were examined microscopically.

Trial 2: As for trial 1, except that samples were not analysed for anaerobic bacteria but were analysed for *Bacillus cereus*/g using *Bacillus cereus* Selective Agar (Oxoid) with supplements.

In both trials samples were also assessed for visual appearance, off-odours and pH.

Nisin assays. The retention of added nisin was determined by extraction and bioassay using the horizontal agar plate diffusion assay (*Micrococcus luteus* indicator organism) as described by Fowler *et al.* (1975). Assays were carried out on the raw and pasteurized whole egg samples, both with and without added nisin.

Results and Discussion

Residual nisin levels for raw and pasteurized liquid whole egg are shown in Table 1. It is apparent from these results that other antimicrobial factors present in both the raw and pasteurized product, apart from nisin, were able

Table 2. Trial 1: Bacteriological analysis, pH, appearance and presence of off-odours in pasteurized liquid whole egg, with and without nisin, at 6°C storage

Days incubation	Total bacteria/ml	Anaerobes/ml	pH	Appearance	Off-odour
1. With nisin (5 mg/l)					
1	3	3	7·67	Good	None
4	10*	<10	7·55	Good	None
7	$4{\cdot}0 \times 10^{2}$*	<10	7·46	Good	None
10	$2{\cdot}0 \times 10^{1}$*	50	7·72	Good	None
14	$7{\cdot}0 \times 10^{4}$*	<10	7·68	Good	None
17	$2{\cdot}0 \times 10^{2}$	<10	7·67	Good	None
21	—	—	7·74	Slight loss of colour	None
22	$>10^{7}$*	<10	7·46	Slight loss of colour	None
23 (1)	$>10^{7}$*	<10	7·59	Slight loss of colour	None
23 (2)	$>10^{7}$*	<10	7·56	Slight loss of colour	None
23 (3)	$>10^{7}$*	<10	7·59	Slight loss of colour	None
2. Control (no nisin)					
1	90†	$2{\cdot}7 \times 10^{2}$‡	7·59	Good	None
4	$2{\cdot}4 \times 10^{4}$†	$3{\cdot}0 \times 10^{2}$‡	7·55	Good	None
7	$5{\cdot}3 \times 10^{6}$†	$5{\cdot}0 \times 10^{3}$	6·88	Loss of colour	Slight
10	$7{\cdot}3 \times 10^{7}$†	$3{\cdot}0 \times 10^{2}$	6·23	Complete loss of colour/ separation/congealed	Strong

* Gram-negative rods (Pseudomonads).
† *Bacillus*, subsequently identified as *Bacillus cereus*.
‡ Gram-positive cocci.

to produce significant zones of inhibition in the agar plate diffusion assay. This problem could be overcome by use of a more specific assay for nisin such as the ELISA method (Falahee *et al.* 1990). However, good residual nisin levels were detected in the pasturized samples (3·65 and 4·65 mg/l for trials 1 and 2 respectively).

Results of bacteriological analysis, pH, appearance and presence of off-odour are shown in Tables 2 and 3.

In trial 1, the product without nisin had a shelf life at 6°C of 4–6 d. Spoilage was attributed to the growth of *Bacillus* species, which was subsequently identified as *Bacillus cereus* by streaking isolates on to *Bacillus cereus* Selective Agar and using the confirmatory method described by Holbrook and Anderson (1980). Pasteurized product containing added nisin had a shelf life of between 17 and 20 d and spoilt due to the growth of Gram-negative rod-shaped bacteria (Pseudomonads).

In trial 2, the control samples had a shelf life of 11 d whereas samples with added nisin lasted for 20 d. The spoilage flora in the control samples were predominantly *Pseudomonas* species. Spoilage flora in the treated samples were tentatively identified as *Bacillus* species (mainly 3–8 μm in length), catalase positive, forming mucoid colonies. With no isolates was the presence of spores demonstrated.

Table 3. Trial 2: Bacteriological analysis, pH, appearance and presence of off-odours in pasteurized liquid whole egg, with and without nisin, at 6°C storage

Days incubation	Total bacteria/ml	*Bacillus cereus*/ml	pH	Appearance	Off-odour
1. With nisin (5 mg/l)					
1	<10	<10	7·72	Good	None
4	<10	<10	7·71	Good	None
5	<10	<10	7·67	Good	None
6	10	<10	7·71	Good	None
7	<10	<10	7·66	Good	None
8	10	<10	7·68	Good	None
9	10	<10	7·70	Good	None
10	10	<10	7·72	Good	None
11	50	<10	7·69	Good	None
12	10	<10	7·71	Good	None
13	15	<10	7·74	Good	None
14	100*	<10	7·70	Good	None
15	25*	<10	7·72	Good	None
16	2×10^3*	<10	7·72	Good	None
17	$4{\cdot}8 \times 10^3$*	<10	7·72	Good	None
18	$1{\cdot}5 \times 10^4$*	<10	7·74	Good	None
19	$1{\cdot}0 \times 10^4$*	<10	7·67	Good	None
20	$5{\cdot}0 \times 10^3$*	<10	7·71	Good	None
21	$3{\cdot}3 \times 10^6$*	<10	7·71	Good	None
2. Control (no nisin)					
1	$8{\cdot}3 \times 10^2$†	<10	7·67	Good	None
4	$1{\cdot}2 \times 10^3$†	<10	7·64	Good	None
5	$1{\cdot}1 \times 10^3$†	<10	7·68	Good	None
6	$8{\cdot}0 \times 10^2$†‡	<10	7·64	Good	None
7	$1{\cdot}3 \times 10^3$†	<10	7·65	Good	None
8	$1{\cdot}9 \times 10^3$†‡	<10	7·67	Good	None
9	$1{\cdot}5 \times 10^3$†	<10	7·61	Good	None
10	$1{\cdot}5 \times 10^3$†	<10	7·66	Good	None
11	$1{\cdot}6 \times 10^3$*†	<10	7·68	Good	None
12	$1{\cdot}7 \times 10^8$§	10	7·57	Good	Very slight fruit odour
13	$1{\cdot}9 \times 10^8$§	<10	7·58	Good	Slight fruit odour

* Mucoid colony. Gram-positive aerobic rod (mainly 3–4 μm up to 7–8 in length). No spores visible. Catalase positive. *Bacillus*.

† Predominantly yellow pigmented colonies. Gram-variable small rods, catalase positive. Coryneforms.

‡ *Bacillus* colony. Few in number.

§ Gram-negative, oxidase positive Pseudomonads.

Spoilage of controls in trial 1 was associated with a strong odour, loss of colour, the product congealing, and a significant drop in pH. Nisin treated samples, when eventually spoilt, showed only a slight loss of colour and a small drop in pH.

Spoilage of controls in trial 2 was associated with a fruity off-odour product becoming more viscous, and a small drop in pH. Treated samples showed no visible spoilage characteristics of odour, or drop in pH, and were judged to be spoilt by having a high bacterial content.

The use of nisin at 5 mg/l resulted in a significant increase in shelf life of pasteurized liquid whole egg held at refrigerated temperatures. Increased consumer safety may also occur due to the inhibition, if present, of *Bacillus cereus*. The spoilage flora varied between the two trials and most probably reflects changes in the type of contamination present at the plant on the dates of the two trials. The first trial was carried out in October and the second in February.

It is noted that in trial 2 the controls spoilt due to the growth of predominantly Gram-negative *Pseudomonas* species. Thus nisin would appear to be controlling the growth of a Gram-negative bacterium whereas current knowledge on its antimicrobial spectrum indicates that little or no action of nisin against Gram-negative bacteria can be expected. It is possible that the pasteurization process employed (64·4°C for 2·5 min) is sufficient to cause some disruption and an increase in the permeability of the outer membrane of the Gram-negative cell wall. This may then allow access of the nisin to the cytoplasmic membrane where it can act in a bactericidal fashion by interaction with the phospholipids in the cell membrane (Henning *et al.* 1986). The phenomenon of non-lethal heat causing increased permeability of the outer membranes of Gram-negative bacteria has been reported by Tsuchido *et al.* (1985). Another possibility is that natural antimicrobial factors present in egg, e.g. lysozyme (see Board 1969), act synergistically with nisin in a similar way to chelating agents, such as EDTA, to increase permeability of the outer membrane thus allowing nisin access to the cytoplasmic membrane.

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