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The use of the bacteriocin, nisin, as a preservative in ricotta-type cheeses to control the food-borne pathogen *Listeria monocytogenes*

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E.A. DAVIES, H.E. BEVIS AND J. DELVES-BROUGHTON. 1997. The efficacy of nisin to control the food-borne pathogen *Listeria monocytogenes* in ricotta-type cheeses over long storage (70 d) at 6–8°C was determined. Cheeses were prepared from unpasteurized milk by direct acidification with acetic acid (final pH 5·9) and/or calcium chloride addition during heat treatment. Nisin was added in the commercial form of Nisaplin® pre-production to the milk. Each batch of cheese was inoculated with 10^2–10^3 cfu g^{-1} of a five-strain cocktail of *L. monocytogenes* before storage. Shelf-life analysis demonstrated that incorporation of nisin at a level of 2·5 mg l^{-1} could effectively inhibit the growth of *L. monocytogenes* for a period of 8 weeks or more (dependent on cheese type). Cheese made without the addition of nisin contained unsafe levels of the organism within 1–2 weeks of incubation. Measurement of initial and residual nisin indicated a high level of retention over the 10-week incubation period at 6–8°C, with only 10–32% nisin loss.

INTRODUCTION

Listeriosis outbreaks linked to the consumption of contaminated dairy products have been well-documented (Ryser and Marth 1991). Post-process contamination from environmental sources has been identified as a leading cause (WHO Working Group 1988), and with respect to cheese production this is most likely due to the open nature of processing.

Soft, white, fresh cheeses that are subjected to minimal processing before packaging are highly perishable and thus have a short shelf-life, even at refrigerated temperature. However, due to the fact that *Listeria monocytogenes* is psychrotrophic and can grow at refrigeration temperatures, contamination by this organism could lead to a high risk factor. In 1985 consumption of contaminated Jalisco brand Mexican-style cheese (queso blanco) manufactured in California was directly linked to more than 142 cases of listeriosis, including 48 deaths (Linnan *et al.* 1988).

The polypeptide, nisin, produced by certain strains of the lactic acid bacterium *Lactococcus lactis* subsp. *lactis*, has antimicrobial activity against a wide range of Gram-positive bacteria, including sporeformers. Nisin has been approved world-wide for use as a natural food preservative and its main commercial application, under the trade name Nisaplin® (Aplin & Barrett Ltd, Trowbridge, UK), is in processed cheese to inhibit the outgrowth of spores (Delves-Broughton 1990). Sensitivity of *L. monocytogenes* to nisin has been demonstrated extensively (Mohamed *et al.* 1984; Benkerroum and Sandine 1988; Carminati *et al.* 1989; Harris *et al.* 1989; Spelhaug and Harlander 1989) and its effective addition to cottage cheese proposed (Benkerroum and Sandine 1988; Ferreira and Lund 1996).

It is suggested that the addition of nisin to milk in the production of soft, white, fresh cheeses made without starter culture (normally acidified or coagulated with rennet), such as ricotta, panir and Latin-American cheeses queso blanco and queso fresco (for guide to cheese types see Campbell-Platt 1987), could effectively control contamination with the pathogen *L. monocytogenes* and extend the shelf-life of the product. Cheeses developed with the use of starter culture would be unsuitable for nisin addition due to its inhibitory properties. However, nisin-resistant starter cultures in combination with nisin or nisin-producing strains have a potential application in cheese production, as has been demonstrated with Gouda cheese (Hugenholtz and de Veer 1991).

The purpose of this study was to evaluate the effectiveness of nisin to control *L. monocytogenes* in ricotta-type cheeses at 6–8°C under laboratory conditions.

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MATERIALS AND METHODS

Cheese preparation

Two alternative methods of manufacture were used.

Method 1. Unpasteurized raw cow's milk (pH 6·8), obtained from a local farm, was used to make each batch of cheese. Calcium chloride (anhydrous, 2000 mg l^{-1}), potassium sorbate (500 mg l^{-1}) and Nisaplin® (Aplin & Barrett Ltd) were added initially to the milk before heat treatment. Nisaplin® addition levels, expressed as weight of pure nisin, were 0, 1·25 and 2·5 mg l^{-1} (equivalent to 0, 50 ppm and 100 ppm Nisaplin®, respectively). The mixture was heated in a waterbath at 90°C for *ca* 30 min, during which time the curd precipitated. After cooling at ambient temperature the curd and whey were separated aseptically by sieve and their weights recorded.

Method 2. As for method 1, with the following modifications: a lower addition level of calcium chloride (anhydrous, 100 mg l^{-1}); addition of acetic acid (Sarsons white vinegar) when the temperature of the milk approached 90°C until the pH reduced to 5·9 (for this study *ca* 23 ml l^{-1}).

Nisin bioassay

Before inoculation of the cheese with *L. monocytogenes*, samples were removed for initial and residual nisin bioassay. Initial samples were frozen (−20°C) and bioassayed within the week, whereas samples determining residual nisin were incubated along with the inoculated samples at 6–8°C and bioassayed at the end of the shelf-life analysis.

The bioassay procedure was conducted as described by Fowler *et al.* (1975). Mean results from duplicate samples were used.

Inoculation of the cheese with *L. monocytogenes*

The cheese was inoculated with a cocktail of five *L. monocytogenes* strains: Scott A (clinical isolate) and four obtained from the Campden Food Research Association culture collection, 4184 (cheese isolate), 3930 (dairy isolate), 4722 (dairy isolate) and 4753 (cheese isolate). The initial combined inoculum level was targeted at *ca* 10^2–10^3 cfu g^{-1} as follows. The *L. monocytogenes* strains were grown overnight in brain–heart infusion broth (BHI; Oxoid) at 30°C to *ca* 10^9 cfu ml^{-1}. Samples (100 μl) of each strain were added together in maximum recovery diluent (MRD; Oxoid; 9·5 ml) and this cocktail was further diluted to 10^{-3} in MRD to give *ca* 5×10^4 cfu ml^{-1}. A 1-ml volume of this was then inoculated into *ca* 200 g cheese to give *ca* 250 cfu g^{-1}. Mixing was conducted in a stomacher (Model 400; Seward, London, UK) for 30 s.

Shelf-life analysis

After inoculation, the cheese was distributed in roughly 15-g quantities to sterile containers (50 ml; Sterilin) and incubated at 6–8°C. Shelf-life analysis was conducted on the samples, at first twice a week and later at weekly intervals. This involved homogenizing 10 g of sample in 90 ml of MRD in a stomacher for 40 s and conducting further decimal serial dilutions in MRD before enumerating by the direct spread plating procedure. Listeria selective agar (Oxford formulation; Oxoid CM856 + SR140) was used to select for *L. monocytogenes*. Plates were incubated at 30°C for at least 48 h.

RESULTS AND DISCUSSION

Nisin was added pre-production (to the milk) rather than post-production (to the cheese curd) because when added to the curd, even though good mixing is achieved (results not shown), the consistency of the cheese is altered completely into a paste.

Product yield and nisin distribution are shown in Table 1. A higher yield of cheese was obtained using method 1 than method 2. On average method 1 produced 302 g l^{-1} while method 2 produced 222 g l^{-1}. Between 46 and 56·4% of the nisin added was distributed in the cheese curd, more than double the percentage of that distributed in the whey by-product. Percentage values were slightly higher in the cheese prepared by method 1 than method 2.

Both control samples (no nisin) contained an initial inoculum level of *ca* 5×10^2 cfu g^{-1} *L. monocytogenes* (Figs 1 and 2). In contrast, three out of four samples containing nisin were below the detectable limit, indicating the immediate listericidal effect of nisin. *Listeria monocytogenes* grew well in both control samples, although its growth rate was slower in the cheese prepared by method 2, most probably due to the antimicrobial action of acetic acid. At 7 d incubation, levels

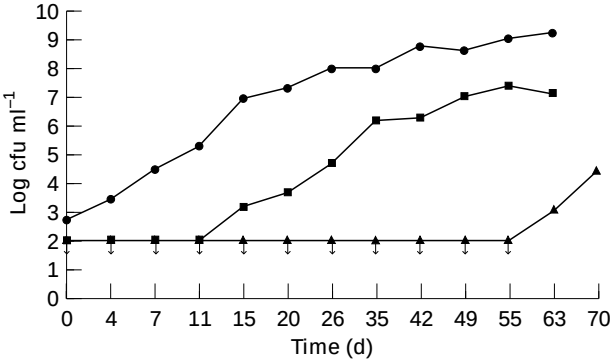

Fig. 1 The effect of nisin in ricotta-type cheese to control the food-borne pathogen *Listeria monocytogenes* at 6–8°C. Cheese prepared using method 1. ●, 0 mg l^{-1} nisin (control); ■, 1·25 mg l^{-1} nisin; ▲, 2·5 mg l^{-1} nisin; ↓, less than 2 log cfu ml^{-1}

Table 1 Product yield and nisin distribution

Method of cheese preparation*	Level of nisin added to milk (mg l^{-1})	Product yield (g l^{-1})		Recovered nisin levels (mg l^{-1})		Nisin distribution† (%)	
		Curd	Whey	Curd	Whey	Curd	Whey
1 (pH 6·1)	0	264·3	690·1	<0·25	<0·25	—	—
	1·25	320·4	652·4	2·2	0·4	56·4	22·2
	2·50	321·7	658·5	4·0	0·9	51·2	23·1
2 (pH 5·8)	0	217·8	776·9	<0·25	<0·25	—	—
	1·25	219·2	779·1	2·7	0·3	47·4	20·3
	2·50	228·8	764·4	5·0	0·7	46·0	21·4

* Refer to text.
† Nisin distribution values (%) are corrected for product yield.

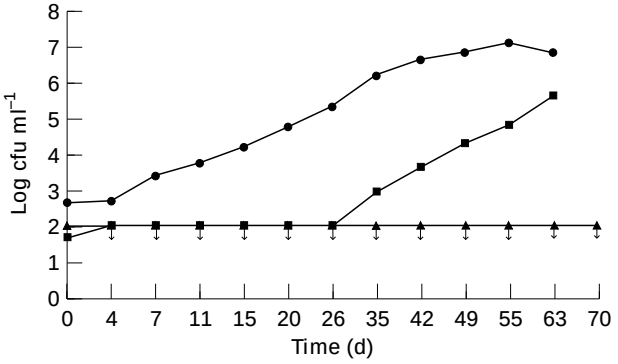

Fig. 2 The effect of nisin in ricotta-type cheese to control the food-borne pathogen *Listeria monocytogenes* at 6–8°C. Cheese prepared using method 2. ●, 0 mg l^{-1} nisin (control); ■, 1·25 mg l^{-1} nisin; ▲, 2·5 mg l^{-1} nisin; ↓, less than 2 log cfu ml^{-1}

had reached 10^4 cfu g^{-1} (method 1) and 10^3 cfu g^{-1} (method 2). In contrast, *L. monocytogenes* was not detectable ($<10^2$ cfu g^{-1}) in any of the samples containing nisin (1·25 and 2·5 mg l^{-1}) at this time. As the shelf-life analysis progressed, *L. monocytogenes* continued to grow to high numbers in the control samples, e.g. *ca* 10^7 cfu g^{-1} (method 1) and 10^4 cfu g^{-1} (method 2) at 15 d incubation. A nisin concentration of 1·25 mg l^{-1} delayed the onset of growth until after 11 d incubation for method 1 and after 26 d incubation for method 2. However, a nisin concentration of 2·5 mg l^{-1} was so effective that it inhibited growth of *L. monocytogenes* until after 55 d for method 1, and with method 2 *L. monocytogenes* was not detected ($<10^2$ cfu g^{-1}) by the end of the trial (70 d). The presence of the mould inhibitor potassium sorbate may have contributed to nisin's bactericidal action, as it has been shown in BHI broth (pH 5·5) that potassium sorbate and nisin acting in combination produce an enhanced listericidal effect compared with nisin alone (Buncic *et al.* 1995). The addition of potassium sorbate was considered necessary due to extensive mould contamination in a previous study. However, the amount added (500 mg l^{-1}) is typical of that used by cheese manufacturers.

The residual levels of nisin in the cheese after 10 weeks of incubation at 6–8°C are shown in Table 2. The results indicate that a relatively high percentage of the initial nisin level

Table 2 A comparison of initial and residual nisin levels in ricotta-type cheese after 10 weeks of incubation at 6–8°C

Method of cheese preparation*	Level of nisin added to milk (mg l^{-1})	Initial nisin levels (mg l^{-1})	Residual nisin levels (mg l^{-1})	% Nisin loss after 10 weeks
1 (pH 6·1)	0	<0·25	<0·25	—
	1·25	2·2	1·5	32
	2·50	4·0	3·6	10
2 (pH 5·8)	0	<0·25	<0·25	—
	1·25	2·7	2·1	22
	2·50	5·0	3·5	30

* Refer to text.

is retained in the cheese for at least 10 weeks at 6–8°C. This is applicable to both methods of preparation, with around 10–32% loss.

It is suggested that the use of nisin, at a concentration of 2·5 mg l^{-1}, in ricotta-type cheese production may extensively increase the shelf-life of the product when stored at 6–8°C. This has been demonstrated with the food-borne pathogen *L. monocytogenes*, which was effectively controlled, when present in low numbers, for a period of 8 weeks or even longer with acetic acid addition. The increased effectiveness of nisin in the presence of acetic acid and sorbate is a good example of the use of nisin in the 'hurdle' concept of food safety (Muriana 1996). Ricotta-type cheese made without the addition of nisin contained unsafe levels of *L. monocytogenes* within 1–2 weeks of incubation.

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