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ORIGINAL ARTICLE

Growth and survival of *Listeria monocytogenes* in two traditional foods from the United Arab Emirates

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The survival of Listeria monocytogenes *in two foods of Arabic origin, namely labneh and houmos, was studied. Counts of* L. monocytogenes *in excess of 10 000 cfu* g^{-1} *from labneh (pH 3·8) were reduced to zero within 72 h at 4 and 10°C, but increased survival was noted at pH 4·5, especially in unsalted labneh; at 30°C, no organisms could be detected 24 h after inoculation. From a starting count of 27 000 cfu* g^{-1}, L. monocytogenes *was able to survive on houmos throughout the anticipated shelf life (3 days); when houmos was stored at 4 and 10°C under olive oil, the numbers of* Listeria *declined slowly over 3 days. It was concluded that care should be taken to avoid the contamination of houmos with* Listeria *during preparation in the kitchen.*

Introduction

Although a recent survey of retail foods from the United Arab Emirates (UAE) revealed that the incidence of *Listeria* was low (Gohil et al. 1995), the range of products did not include base materials that might be further modified in the kitchen. Thus, uncooked foods into which fresh herbs, spices or other ingredients are blended manually in the home are very popular among the national and expatriate populations of the UAE, and careless handling could present an obvious risk. The high ambient temperatures of the Gulf also fuel concerns about the possibility that a home-made dish could become suspect following cross-contamination with *Listeria*; the often fatal consequences of infection for unborn or neonatal infants has given listeriosis a higher profile than is, perhaps, merited by the number of recorded incidents.

Consequently, the survival of *L. monocytogenes* in two foods, chosen on the basis of their widespread popularity, was followed over their anticipated shelf lives. The selected products were labneh, a milk-based fermented food made by concentrating yoghurt, and houmos, a thick gruel made from chick peas and tahina (sesame) paste. In both cases, the products are often subject to extensive manipulation before serving.

Nature of the products

Labneh is prepared both at home, as well as commercially in the UAE, and the basic yoghurt is produced with a starter culture consisting of *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus*; after incubation at 42°C to the desired acidity, some manufacturers blend in a quantity of salt. This yoghurt is then poured into

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muslin bags and hung for 48 h at 1–4°C. Gentle mixing to obtain a smooth consistency follows, and the finished labneh is placed in suitable containers. The composition of a typical commercial brand from the UAE: 27% total solids; 10% fat; 9·0% protein; 1·0% salt and pH 3·8 is about average for the product in question (Robinson and Tamime 1993); the anticipated shelf life is 15 days. Obviously the low pH of commercial labneh should be bacteriocidal to *Listeria* but, as batches made in the home are often less acidic, it was felt that the examination was justified.

Houmos is a popular choice as a cold starter for a meal, and is included almost daily in the Arabic diet. It is made from mashed chick peas and sesame paste, to which salt, garlic, lemon and chopped green pepper are added, along with a little olive oil on the top. Different ratios of mashed chick peas and sesame paste are employed to suit individual tastes, but a typical composition might be: 29·4% total solids; 5·0% fat; 5·2% protein; 1·7% ash and pH 5·5. Unlike labneh, the shelf life for fresh houmos is only 1 day, and it is generally not used after 24 h from the time of preparation; houmos stored under olive oil may be held for 2–3 days before consumption.

Materials and Methods

Preparation of the inoculum

L. monocytogenes NCTC 10888 was cultured for 24 h at 35°C on slants of trypticase soya yeast extract agar (TS-YEA) (Anon. 1994) and, after making an initial suspension, 10-fold serial dilutions to 10^{-8} were prepared in peptone water (9 ml). Duplicate aliquots (1 ml) from each of the three highest dilutions (10^{-6}, 10^{-7}, 10^{-8}) were transferred to sterile Petri dishes, and TS-YEA (12–15 ml) tempered at 45–48°C was added to each plate and mixed with the inoculum. When the agar had solidified, the plates were inverted and incubated for 24 h at 35°C. The original dilutions were stored at 4°C. After incubation, plates with 25–250 colonies were counted, and an estimated count per millilitre of the relevant dilution was established.

Although it was anticipated that limited growth of the culture would occur at 4°C, it was assumed that the figures obtained from the plate counts would be sufficiently accurate to calculate the numbers of colony-forming units (cfu) in inocula prepared from the stored dilutions.

Preparation of the samples: labneh

Bulk samples of freshly-prepared labneh (one with 1·0% salt and one without) were collected from a local dairy on the day of production in sterile containers and delivered to the laboratory in a refrigerated van.

Two batches of salted and two batches of unsalted labneh—each of *c.* 3·2 kg—were weighed into sterile, stainless-steel buckets (10 l). One batch of salted labneh was then subjected to continuous blending with a slow-speed, electric egg-beater, and the acidity adjusted to pH 4·5 by the stepwise addition of 10% NaOH solution. After each addition of alkali, the labneh was mixed gently for 5 min, and the pH was then recorded with a Kent EIL 7020 Meter and combination electrode; the process was repeated until the desired end-point had been achieved. The second batch of salted labneh was used at its original pH of 3·8. Two similar batches of unsalted labneh were prepared at the same time.

From the original dilution of *L. monocytogenes* that gave an estimate count of $3{\cdot}8\times10^{7}$ cfu ml^{-1}, 1 ml was added to 9 ml peptone water and, after agitation on whirl-mix, the entire culture (10 ml) was added to one of the four batches of labneh; this volume gave an estimated count of 11 800 cfu g^{-1} labneh. The container of labneh was then placed in a laminar-flow cabinet, and the inoculum carefully mixed throughout the product with the electric egg beater. The same procedure was repeated with the three remaining batches to give a final pattern of inoculated products (3·2 kg) of: unsalted pH 3·8, unsalted pH 4·5, salted pH 3·8 and salted pH 4·5.

Because the removal of samples from the bulk material could have lead to 'puddles' of whey forming in the labneh, sub-samples (150–200 g) of each batch were distributed into 18 sterile, screw-cap containers. This allocation allowed for pairs of containers to

be examined for *L. monocytogenes* for 3 successive days after inoculation, and then on alternate days for a typical shelf life (15 days). All the containers were placed in a refrigerator maintained at 4°C until analysed.

On the following 3 days, the entire procedure from collecting the labneh through to setting-up the duplicate sub-samples was repeated, but with the sets of 18 containers being stored at 10, 20 and 30°C.

Enumeration of L. monocytogenes

On each occasion, two screw-cap containers (150–200 g) were selected at random from each temperature regimen, and duplicate samples (25 g) of labneh were weighed from each container into sterile Stomacher bags. Sterile peptone water (225 ml, 0·1%) was added to prepare the first dilution (10^{-1}), and the second dilution (10^{-2}) was prepared by adding 1 ml of the first dilution to 99 ml sterile diluent. Aliquots (0·1 ml) of each dilution were spread onto each of two plates of listeria selective agar (LSA) (Anon. 1994). Typical colonies of *L. monocytogenes*, i.e. colonies with a sunken centre and black colouration of the medium, were counted after 48 h incubation at 35°C. The number of *L. monocytogenes* per gram of labneh was calculated from the average of the four counts (duplicate plates for two samples).

Preparation of the samples: houmos

It emerged during some initial trials that houmos is an excellent growth medium for bacteria, for a survey of freshly-prepared houmos from restaurants included an examination for total bacterial load (aerobic plate count on plate count agar at 35°C for 48 h); coliform count (violet red bile agar at 35°C for 24 h, followed by confirmation in brilliant green bile broth at 35°C for 24–48 h); presence of faecal coliforms (MacConkey broth at 35°C for 24–48 h, followed by confirmation in brilliant green bile broth at 44·5°C in a water-bath for 24–48 h); *Staphylococcus aureus* (Baird–Parker medium at 35°C for 48 h, followed by confirmation of black colonies with or without an opaque zone by the coagulase test); and *Salmonella* (lactose broth at 35°C for 18–24 h followed by selenite broth at 35°C for 24 h; loopfuls of the latter broth were streaked onto Hektoen enteric agar for preliminary identification of genus (Anon. 1994, Richardson 1985).

A specific examination of any naturally-occurring *Listeria* involved weighing 25 g houmos into a sterile Stomacher bag, and adding 225 ml listeria enrichment broth (LEB) (Lovett et al. 1987). The contents of the bag were blended in a Stomacher (Type 400) for 30 s, and incubated at 35°C for 48 h. A loopful was then streaked onto LSA and incubated at 35°C for 48 h (Curtis et al. 1989).

Faecal coliforms were isolated from all 22 samples examined, *S. aureus* from 13 (50–1400 cfu g^{-1}) and *Salmonella* sp. from one sample. The aerobic plate counts (17 000–3·4 $\times 10^6$ cfu g^{-1}) and coliform counts (95–22 000 cfu g^{-1}) were also unacceptable; no *Listeria* spp. were isolated during the survey. The pHs of the samples ranged between 4·7–5·6 and, although no direct correlation emerged, it is likely that the higher values had encouraged microbial development.

It was concluded from this pattern of contamination that: (1) pathogenic bacteria, including perhaps *Listeria* spp., could grow and survive in houmos; and (2) the direct enumeration of *L. monocytogenes* from 'spiked' samples onto LSA could result in contaminants outnumbering the *Listeria*. Consequently, it was decided to use canned houmos as the test material, even though it contained citric acid along with the usual basic ingredients (mashed chick peas and sesame paste). The inclusion of citric acid in the formulation could have rendered the product marginally bacteriocidal *vis-a-vis* fresh houmos, but it was decided that: (1) the risk of low rates of survival of *Listeria* in the canned houmos could be moderated, for experimental purposes, by raising the inoculation rate from 11 000 cfu g^{-1} (labneh) to around 30 000 cfu g^{-1}; and (2) if the organism did survive well in the canned houmos, then it could be assumed that its longevity in freshly-prepared houmos would be extended even more.

One brand was selected on the basis of the ingredient specification and, after wiping the lids of the cans with cotton wool soaked with

70% alcohol and flaming, the cans were opened under sterile conditions; the contents were then transferred to a sterile, stainless steel vessel. The experimental houmos was prepared as directed on the labels of the cans, and this stage involved: (1) washing green chillies, peeled garlic and lemon thoroughly in tap water; (2) grinding these ingredients and a teaspoon of salt into a paste; and (3) mixing the paste into the houmos; a portion was collected for chemical analysis.

Inoculation of the houmos

The preparation of the inoculum was similar to the procedure described for labneh. The APC of the selected dilution was $8{\cdot}28\times10^{7}$ cfu ml^{-1}, so that when 1·0 ml in peptone water (9 ml) was added to 3·0 kg houmos, the anticipated count of *L. monocytogenes* was 27 600 cfu g^{-1} houmos. The inoculated material was blended with the slow-speed egg beater to ensure uniform distribution, and three further batches of houmos was prepared in the same manner. The four batches of 'spiked' houmos were then distributed (150–200 g) into 40 sterile, screw-cap containers. A layer of olive oil (2·5 cm) was poured onto the houmos in 20 of the containers, while the others were left with the surfaces exposed to the air. Five containers from each treatment were then placed in a refrigerator maintained at 4°C, while similar groups were allocated to incubators at 10, 20 and 30°C. This allocation allowed for one container of each treatment to be examined for *L. monocytogenes* at 8, 16, 24, 48 and 72 h after distribution, and the enumeration procedure was the same as that described for labneh.

The number of *L. monocytogenes* per gram of any given sample of houmos was calculated from the average of the four counts (duplicate plates for two samples from each container).

The data were analysed using the General Linear Models procedure of SAS (1987), and differences between treatments were tested using Duncan's multiple range test.

Results and Discussion

After allowing for the slight variations in weight of the labneh, the estimated counts were: 11 800 cfu g^{-1} of salted labneh (pH 3·8 and 4·5); 11 700 cfu g^{-1} of unsalted labneh (pH 3·8) and 11 600 cfu g^{-1} of unsalted labneh (pH 4·5).

After holding the individual containers overnight to reach the required temperatures, analyses of the different samples showed that the numbers of *L. monocytogenes* had been reduced by *c.* 75% (see Table 1). In most cases, this dramatic rate of cell loss continued and, irrespective of the salt content, *L. monocytogenes* did not survive more than 1 day at 4°C or 10°C at pH 3·8. At pH 4·5, some cells survived for 7 days at 4°C and for 5 days at 10°C in unsalted labneh but, in the salted variety, the organism only survived until the fifth day of incubation at 4°C.

Clearly, survival was influenced principally by pH ($P<0{\cdot}05$), with salt ($P<0{\cdot}05$) and temperature applying additional stresses. In particular, one of the reasons for the poor survival of *L. monocytogenes* at the higher temperatures may have been an increasing level of lactic acid generated by the starter bacteria (Tamime 1990). In addition, as it is the undissociated form of lactic acid that is inhibitory to *Listeria* (Hunter and Seigel 1973), the higher temperatures may have encouraged more rapid diffusion into the cells.

It may be relevant also that *L. monocytogenes* is known to be inhibited by the lactic acid bacteria found in starter cultures (Schaak and Marth 1988). In particular, the species of bacterium used in the preparation of labneh, namely *L. delbrueckii* sub-sp. *bulgaricus* and *S. thermophilus* were shown to be inhibitory to *L. monocytogenes* in skim-milk and yoghurt mix. The same workers found that *L. delbrueckii* sub-sp. *bulgaricus* was more inhibitory than *S. thermophilus*, and that the degree of inhibition was affected by both the level of starter addition and the temperature of incubation. It is possible, therefore, that the destruction of *L. monocytogenes* in the present study was the result of the combined action of pH, salt and an inhibitory effect from the starter culture.

Similar studies (Conner et al. 1986, Sorrels et al. 1989) concluded that the inhibition of growth and/or survival of *L. monocytogenes* is a function of acidity and incubation tem-

Table 1. Recovery of *L. monocytogenes* (mean cfu g^{-1}) on *Listeria* selective agar from different samples of labneh inoculated with estimated counts (see text) in excess of 11 000 cfu g^{-1} and held at the temperatures indicated

Temperature		Time from inoculation (days)				
		1	2	3	5	7
4°C	Salted/pH 3·8	1550	125	0	0	0
	Salted/pH 4·5	3000	1875	675	275	0
	Unsalted/pH 3·8	3250	200	0	0	0
	Unsalted/pH 4·5	3150	2800	1325	275	25
10°C	Salted/pH 3·8	1500	150	0	0	0
	Salted/pH 4·5	3000	475	0	0	0
	Unsalted/pH 3·8	3250	275	0	0	0
	Unsalted/pH 4·5	3150	2100	475	250	0
20°C	Salted/pH 3·8	1550	0	0	0	0
	Salted/pH 4·5	3000	25	0	0	0
	Unsalted/pH 3·8	3250	25	0	0	0
	Unsalted/pH 4·5	3150	550	0	0	0
30°C	Salted/pH 3·8	1550	0	0	0	0
	Salted/pH 4·5	3000	0	0	0	0
	Unsalted/pH 3·8	3250	0	0	0	0
	Unsalted/pH 4·5	3150	0	0	0	0

Table 2. Recovery of *L. monocytogenes* (mean cfu g^{-1}) on *Listeria* selective agar from samples of houmos inoculated with estimated counts of 27 000 cfu g^{-1} and held at the temperatures indicated; 50% of the samples were covered with olive oil immediately after inoculation

Temperature		Time (h)					
		0	8	16	24	48	72
4°C	With oil	27 000^{a}	23 000^{bc}	24 000^{b}	19 400^{d}	21 500^{c}	19 100^{d}
	Without oil	27 000^{c}	29 000^{b}	22 000^{d}	27 000^{c}	27 500^{bc}	32 500^{a}
10°C	With oil	27 000^{a}	23 500^{b}	24 000^{b}	13 900^{c}	12 100^{d}	12 100^{d}
	Without oil	27 000^{ab}	25 500^{b}	25 000^{b}	26 000^{b}	28 000^{ab}	30 000^{a}
20°C	With oil	27 000^{a}	26 000^{a}	22 500^{b}	15 500^{c}	10 000^{d}	9000^{d}
	Without oil	27 000^{a}	27 500^{a}	28 000^{a}	22 000^{b}	8000^{c}	9000^{c}
30°C	With oil	27 000^{b}	30 000^{a}	21 500^{c}	9850^{d}	7000^{e}	8000de
	Without oil	27 000^{b}	30 000^{a}	21 000^{c}	12 000^{d}	8500^{e}	8000^{e}

[a]Means within a row lacking a common superscript differ, significance $P<0·05$.

perature, and Parish and Higgins (1989) showed that there was a decline in numbers of *L. monocytogenes* at pH<4·5. Consequently, it is not surprising that *Listeria* cannot survive long in labneh, or that the product has a good record with respect to consumer safety. Nevertheless, more than 1000 cfu g^{-1} did survive for 3 days in unsalted labneh at pH 4·5, and hence gross contamination in the home could pose a marginal risk to susceptible individuals.

The results of the analyses of the houmos

are shown in Table 2, and it is noticeable that, in houmos stored without olive oil, the cell counts showed a slight increase in number at 4°C over the 72 h period of incubation. At 10°C, the counts remained stable, whereas at 20°C and 30°C, the counts had declined significantly ($P<0{\cdot}05$) after 16 h. Once again, survival of the cells was best at low temperature, so that chilled fresh houmos contaminated with *Listeria* would remain a risk within its expected shelf life of 24 h. At higher temperatures, it could be that the citric acid in houmos is exercising a bacteriocidal effect but, unless the product is subject to severe temperature abuse, the inhibition will be of little benefit to the retailer and consumer.

When the houmos was covered with oil, the counts decreased significantly ($P<0{\cdot}05$) over 72 h at all temperatures, although the decline was sharpest at ambient temperatures. The significant difference ($P<0{\cdot}05$) between the overall counts with or without olive oil may have been a result of an antimicrobial effect of olive oil *per se* (Raina 1993) but, although *Listeria* spp. are facultative anaerobes, the exclusion of oxygen could have affected survival of the strain in question.

Overall, it was evident that, although an acidic product like labneh can be regarded safe, with respect to *Listeria*, high counts of *L. monocytogenes* are able to survive in houmos for up to 3 days—the maximum expected shelf life of the product. Obviously the initial counts employed in this trial were much higher than should be reached by casual handling in a kitchen. Nevertheless, the analysis of normal retail products suggested that houmos is prone to contamination during preparation, so that steps to avoid any contact with *Listeria* should be taken seriously by producers.

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