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Some microbiological considerations applying to the conditioning, ageing and vacuum packaging of lamb

J. T. PATTERSON AND P. A. GIBBS

Summary

Laboratory investigations are described in which legs of lamb were conditioned at 18 and 10°C, followed by ageing at 3.3°C under controlled conditions of air movement and relative humidity and stored at 3, 7 and 15°C. It was concluded that the lower conditioning temperature was less likely to give problems associated with rapid microbial multiplication, though no potentially dangerous organisms were detected after conditioning and ageing at either temperature. The shelf-life of the meat conditioned at 18°C was shorter than at 10°C. In a subsequent investigation cuts of meat from a leg conditioned at 10°C and aged at 3.3°C were vacuum packaged and stored at 1–2 and 7°C for up to 6 weeks. The meat had spoiled in the vacuum-packages at 7°C after 6 weeks, but not after 3, while spoilage was not detected in the 6 week stored meat at 1–2°C, though faecal streptococci were present in all samples after 3 weeks. *Clostridium welchii* was found (26 per 100 g) in the 10°C conditioned meat stored at 1–2°C, after 6 weeks. Large numbers of Gram-negative organisms including psychrotrophic Enterobacteriaceae were found on the meat stored at 7°C.

Introduction

It is well known that the muscles of meat animals, and in particular lamb shorten when cooled too quickly. This is the so-called 'cold-shortening' phenomenon first observed by Locker & Hagyard (1963). The meat 'sets' in the shortened state as rigor sets in, and becomes extremely tough when it is subsequently cooked. Bendall (1974) has stated that 'cold-shortening' sets in during the chilling of the lamb, beef and chicken muscles (but not the white muscles of rabbit) if the conditions are such that the muscle temperature has fallen below 11°C before the pH has fallen below 6.2. Under easily attainable

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commercial rates of chilling cold-shortening may occur, while on the other hand optimal tenderness can be achieved in lamb or beef carcases if these are not chilled below 10°C until at least 10 hr after slaughter. Current chilling practice, e.g. as recommended by the US Department of Agriculture to reduce internal temperature of meat to 4.5°C within 16 hr of slaughter (Cutting, 1972a), or in the EEC Council Directives (Cutting, 1972b) which lay down that fresh meat intended for intra-Community trade must be chilled immediately after post-mortem inspection and kept permanently at an internal temperature not exceeding 7°C for carcases and parts thereof and 3°C for offal, may well cause meat to be tough after cooking. A considerable amount of work has been carried out in recent years, notably in New Zealand to find ways in which this shortening, and that due to thaw shortening caused if muscle is frozen pre-rigor and rapidly thawed, can be avoided. This work has been recently reviewed by Locker *et al.* (1975). The methods used have included conditioning of both lamb and beef carcases at relatively high temperatures (13–18°C) under standardized conditions of air velocity and humidity to avoid cold-shortening followed by a period of ageing (at 2–4°C) as whole carcases (lamb) or as vacuum packed cuts (beef) to develop tenderness. Bacteriological standards were also specified. Unconventional techniques were also described by these workers viz. altered posture during chilling and ageing, hot boning allied to conditioning at 10–15°C, high temperature conditioning (at 44–45°C) and electrical stimulation to speed the onset of rigor.

Some experiments have been carried out in this laboratory to examine the effects on the microbial flora developing on legs of lamb, of conditioning at 10 and 18°C for 24 hr followed by ageing for up to 7 days at 3.3°C under controlled conditions of humidity and air movement, and also whether conditioned and aged lamb was suitable for vacuum packaging. The published microbiological data have been based largely on total counts, and it is felt that more information was required as to the identity and biochemical activities of the organisms developing under these conditions.

Materials and methods

Conditioning and ageing procedures

The hind legs were removed from freshly butchered hogget carcases and immediately transported to the laboratory some 20 miles distant. In the first investigation, each leg was weighed, leg A aged for 7 days at 3.3 ± 1°C at a relative humidity of 88–91%. Leg B was conditioned at 18 ± 1.5°C for 24 hr at a relative humidity of 80–85% weighed and then aged for 72 hr at 3.3°C and a relative humidity of 88–91%, and reweighed. Leg B was then frozen to −20°C and held for 8 days before defrosting at 3.3°C for 39 hr. The air flow in the conditioning and ageing cabinets (Patra, Laboratory and Thermal Equipment Ltd, Oldham) was regulated to 90–100 ft/min. Temperature measurements were made by inserting thermocouples just under the surfaces and into the

deep muscles and near the bones of the legs and recorded every 20 min on a flat bed recorder (Kipp and Zonen, Model BD8), using an electronic thermometer and automatic selector unit (Comark Electronics Ltd, Littlehampton). In the second investigation the procedure was modified somewhat in that the conditioned leg was held at 10°C for 24 hr at a relative humidity of 82% and then aged at 3.3°C for 6 days at a relative humidity of 90%. The cut muscle surfaces of each leg was sealed by dipping in molten paraffin wax to reduce evaporation before transfer to the controlled environment cabinets. The procedure was modified still further in the third investigation, in that conditioning of leg B at 10°C for 24 hr at a relative humidity of 84% was followed by ageing for 1 day at 3.3°C at a relative humidity of 90%. Leg A was held at 3.3°C for 2 days at a relative humidity of 90%. In the fourth investigation a direct comparison was made between conditioning at 10°C (two legs) and at 18°C (two legs) for 20 hr at a relative humidity of 84% in both cases, prior to ageing at 3.3°C for 3 days. The relative humidities in the cabinets were checked frequently using a wet and dry bulb thermometer.

Storage of the meat after conditioning and ageing

Three cuts from the conditioned and aged meat from the first, second and fourth investigations, were taken by cutting across the bone thus exposing fresh surfaces, placed between two new polystyrene trays and overwrapped with a clear plastic film (WVTR 700 $g/m^2/24$ hr; O_2 transmission rate 8500 $ml/m^2/24$ hr; CO_2 transmission rate 70 000 $ml/m^2/24$ hr). One piece from each leg was stored for 1 day at 15°C; one piece for 2 days at 7°C and the third for 2 days at 3.3°C. After sampling the pieces were returned to storage until off odours were detected. Five cuts were similarly taken from each of the legs in the third investigation, vacuum packaged as described by Sutherland, Patterson & Murray (1975), and stored at 1–2°C (two cuts) and 7°C (two cuts) for up to 6 weeks. The remaining cut was used for initial sampling.

Microbiological examination

Initial counts on the surface of the legs were obtained from triplicate swabs of 10 cm^2 areas outlined by sterile metal templates. Each swab was moistened in sterile 0.5% (w/v) peptone water, and applied for 15 sec to the surface; the swabs were broken into 30 ml sterile 0.5% (w/v) peptone water and shaken for 10 min on a flask shaker to release the organisms into the diluent. After conditioning and ageing, this sampling was repeated on adjacent sites.

In the second investigation, after taking cuts across the bone for storage experiments, the remainder of the legs were sterilized by flaming followed by painting with a saturated alcoholic solution of crystal violet and brilliant green. The bone was then exposed aseptically, swab sampled and the swab and pieces of meat immediately surrounding the bone combined in sterile 0.5% peptone water diluent. Subsequent to storage at the three temperatures, 10 cm^2 areas

on other adjacent sites on the outside of the pieces, and on the cut surfaces were similarly sampled. In addition the bone was cut from each piece, rinsed in sterile diluent, the rinsings combined with small pieces of meat cut from around the bone, and blended for 1.5 min in a sterile Atomix cup (MSE Scientific Instruments, Crawley, Sussex). No special precautions were taken to avoid carrying contamination into the bone from the exterior when cutting pieces for storage in the first and second investigations, but in the third and subsequent investigation the surfaces were sterilized by direct flaming before cutting.

In the third investigation, after opening the vacuum packs the bone was removed, trimmed and shaken in 100 ml sterile 0.5% peptone water. The rinsings and the remainder of each cut were transferred to 100 ml sterile peptone water and mixed using the Colworth Stomacher for 2.5 min (Seward Ltd, 6 Stamford St, London). Decimal dilutions were prepared in 0.1% sterile peptone water by the method of Murray (1956) and 0.1 ml quantities from suitable dilutions spread on the surface of previously prepared plates. Total counts were obtained by plating on nutrient or plate count agar, (Oxoid CM 3 or CM 325). The colonies were counted after 3 days at 22°C. The rinsings from the bone were examined for total and sporing anaerobes by the method of Gibbs (1973), for faecal streptococci using the medium of Barnes (1956), and for total count of organisms capable of growing on plate count agar after 3 days at 30°C under reduced oxygen conditions, achieved by burning a candle to extinction in the incubation jar. In the third investigation, Enterobacteriaceae were isolated on violet red bile agar (Oxoid CM 107) containing 1% glucose as recommended by Mossel, Mengerink & Scholts (1962) and enumerated after 5 days at 15°C.

Representative isolates from the first, second and third investigations were picked off the total count plates using the method of Harrison (1938). These were purified by streaking and subjected to the following tests: colony characteristics on nutrient agar incubated for 3 days at 22°C in air; Gram reaction; catalase; oxidase; morphology and motility. Gram-negative rods were tested in the medium of Hugh & Leifson (1953) for ability to dissimilate glucose. If this ability was oxidative and the oxidase test was positive, the isolates were tested for the production of fluorescent pigments on the media of King, Ward & Raney (1954). Gram-positive cocci were checked for the production of catalase on a nutrient agar containing 1% (w/v) of added glucose. If catalase-positive on this medium, the ability to dissimilate glucose oxidatively or fermentatively was tested in the medium of Baird-Parker (1963). If fermentative the isolates were tested for the production of coagulase. Gram-positive, catalase-positive non-sporing rods (presumptive *M. thermosphactum*) were tested for the ability to grow on the medium of Gardner (1966), and that of Rogosa, Mitchell & Wiseman (1951).

Further identification of the Gram-negative isolates was made using the tests described by Hendrie, Hodgkiss & Shewan (1964), Cowan & Steel (1974) and the identification keys described in Bergey's Manual (Buchanan & Gibbons,

1974), while a selection of the oxidase positive Gram-negative isolates were stained for the presence of flagella by the method described by Rhodes (1958).

Extracellular enzymes of the isolates

The abilities of the isolates to hydrolyse casein, tributyrin and gelatin were tested by streaking each isolate on plates of casein agar prepared by adding 10% (v/v) of reconstituted skim milk powder to a nutrient agar medium, pH 7.4; tributyrin agar (Cowan & Steel, 1974) and by stabbing into a tube of gelatin medium prepared from Oxoid nutrient gelatin (CM 135 a), with certain additions (the CMG medium of Sutherland, Patterson, Gibbs & Murray, 1975). The plates and tubes were incubated at 25°C for up to 14 days.

Results and discussion

The results of the analysis of the microbial flora developing on the conditioned and aged meat are shown in Table 1. In the first investigation where conditioning at 18°C for 24 hr was followed by ageing for 72 hr at 3.3°C, freezing and thawing, the $\log_{10}$ total numbers of organisms recovered/cm^2 increased from 4.11 to 5.71, while the type of flora continued to be dominated by *Micrococcus* spp. Some of this increase in numbers may have occurred during the thawing of the meat prior to sampling. Comparable numbers on the conventionally aged meat were 4.47 and 3.95, and there were only small changes in the composition of the microflora after conventional ageing or conditioning and ageing.

In the second investigation, conditioning at 10°C for 1 day followed by ageing at 3.3°C for 6 days (twice as long as the maximum suggested by Locker *et al.*, 1975) also resulted in an increase in total count, although not as marked as at 18°C. The different periods of ageing for the 18 and 10°C conditioned meat make comparisons between the two results difficult in these investigations. The composition of the microflora also showed differences in that although still dominated by *Micrococcus* spp., there was a marked increase in the proportion of *Acinetobacter*-like organisms. Decreasing the duration of ageing from 6 days to 1 day (investigation III, Table 1) resulted in a slight decrease in total count. In the later investigation (IV, Table 1) the initial levels of contamination were generally lower and remained lower after conditioning and ageing than in comparable earlier investigations. The results indicated however that whereas conditioning at 10°C followed by ageing at 3.3°C resulted in a decrease in total count, conditioning at 18°C followed by ageing at 3.3°C resulted in a small increase in total count. Under the conditions in the conditioning and ageing cabinets the surface became quite dry, thus restricting microbial multiplication.

In the first investigation weight loss was 6.2% when aged conventionally and 5.1% when conditioned at 18°C and aged at 3.3°C. The deep muscle

Table 1. Microbiological condition of the meat after conditioning and ageing

				Composition of the flora (%)*													
Investigation	Treatment of meat	Log_{10} total no. of organisms cm^2	No. of isolates	*Ps.* III	*Flavo.*	*Morax.*	*Acineto.*	*Alc.*	*M. thermos.*	Coryneform	*Lact.*	*Bac.*	*Microc.*	*Staph.*	*Aerococc./Ped.*	Yeasts	Unidentified
I	Before ageing	4.47	61	2	3	18	7	0	0	8	2	2	39	6	8	0	5
II	Before ageing	3.85	61	0	0	2	2	0	0	0	2	0	80	10	0	0	4
III	Before ageing	3.67						Not done									
I	After ageing 7 days @ 3.3°C	3.95	60	0	0	15	5	0	5	7	0	2	48	10	0	1	7
II	After ageing 7 days @ 3.3°C	3.55	86	0	0	5	2	0	0	0	1	0	77	6	0	1	8
III	After ageing 2 days @ 3.3°C	3.87						Not done									
I	Before conditioning	4.11	60	0	2	3	2	0	0	8	0	0	80	3	0	0	2
II	Before conditioning	3.60	67	0	0	2	0	1	0	0	0	0	87	7	0	0	3
III	Before conditioning	4.43						Not done									
IV	Before conditioning	2.98						Not done									
	Before conditioning	4.04						Not done									
	Before conditioning	2.92						Not done									
	Before conditioning	2.95						Not done									
I	Conditioned at 18°C for 1 day, aged at 3.3°C for 3 days, held at −20°C for 8 days	5.71	60	0	2	0	0	0	0	0	0	5	92	1	0	0	0
II	Conditioned at 10°C for 1 day aged at 3.3°C for 3 days	4.04	60	0	2	2	17	0	3	0	0	0	60	3	0	0	13
III	Conditioned at 10°C for 1 day, aged at 3.3°C for 1 day	3.96						Not done									
	Conditioned at 10°C for 1 day, aged at 3.3°C for 3 days	2.36						Not done									
IV	Conditioned at 10°C for 1 day, aged at 3.3°C for 3 days	3.18						Not done									
	Conditioned at 18°C for 1 day, aged at 3.3°C for 3 days	3.57						Not done									
	Conditioned at 18°C for 1 day, aged at 3.3°C for 3 days	4.93						Not done									

* *Ps.* III, *Pseudomonas*, Group III; *Flavo.*, *Flavobacterium*; *Morax.*, *Moraxella*-like organisms; *Acineto.*, *Acinetobacter*-like organisms; *Alc.*, *Alcaligenes*; *M. thermos.*, *Microbacterium thermosphactum*; *Lact.*, *Lactobaccilus*; *Bac.*, *Bacillus*; *Microc.*, *Micrococcus*; *Staph.*, *Staphylococcus*, coagulase negative; *Aerococc./Ped.*, *Aerococcus/Pediococcus*.

Table 2. Microbiological condition of the meat subsequent to storage in air given after conditioning and ageing

Investigation	Conditioning and ageing	Log_{10} total count cm^{-2}	Log_{10} total count after storage (cm^{-2} or g^{-1})		
			2 days/3°C	2 days/7°C	1 day/15°C
I	7 days at 3.3°C	O 3.95	O 4.39	4.81	5.29
			M 2.80	4.14	4.18
			B 3.47	3.49	4.83
	1 day at 18°C, 3 days at 3.3°C frozen 8 days, thawed (39 hr at 3.3°C)	O 5.71	O 6.62	4.52	7.26
			M 3.68	5.00	4.63
			B 7.37	7.77	6.10
II	7 days at 3.3°C	O 3.55	O 2.18	2.43	3.76
			M<1.48	1.48	<1.48
		B<1.88	B<1.18	1.78	2.84
	1 day at 10°C, 6 days at 3.3°C	O 4.04	O 5.85	6.66	4.28
			M<1.48	<1.48	2.73
		B<1.88	B<1.18	2.59	2.25
IV	1 day at 10°C, 3 days at 3.3°C	O 2.36	M 1.0	2.45	2.79
			B 1.34	2.10	4.10
	1 day at 10°C, 3 days at 3.3°C	O 3.18	M<1.0	1.0	<1.0
			B 1.15	3.69	<1.48
	1 day at 18°C, 3 days at 3.3°C	O 3.57	M 2.15	2.78	2.92
			B 3.31	3.36	3.60
	1 day at 18°C, 3 days at 3.3°C	O 4.93	M<1.0	<1.0	4.31
			B<1.0	2.48	3.76

O = outer, uncut surface of meat; M = cut surface of meat; B = bone samples.

temperature in the legs reached that of the conditioning and ageing chambers overnight, and the conditioned leg at 18°C, decreased to the temperature of the ageing chamber (3.3°C) within another 8 hr. Shallow muscles and the regions just under the surfaces attained these temperatures much more rapidly, so that with the rapid air movement across the surfaces, conditions were not favourable for microbial multiplication.

After storage in air (Table 2), the microbiological condition of pieces of meat cut from legs conditioned at 18 or 10°C followed by ageing at 3.3°C, was generally poorer than pieces cut from conventionally aged legs. In the first investigation the counts of the bone samples after storage suggested that bone taint might be a problem with high temperature conditioned meat, although the later investigation (IV) did not confirm this, probably as a result of taking care not to carry surface contamination through to the bone when cutting pieces for storage. There was some indication however that bone samples from meat conditioned at 18°C were slightly more heavily contaminated than those from meat conditioned at 10°C after storage at different temperatures. In large scale investigations in New Zealand (Locker *et al.*, 1975) using conditioning temperatures of 13, 16 or 18°C (for 16–24 hr) followed by ageing at 3°C for up to 96 hr post mortem, there was no evidence of bone taint. Analysis of the flora recovered from the stored pieces of meat indicated that the flora on the outer uncut surface was still dominated by Gram-positive cocci, although the proportion had generally decreased. The flora on the cut surfaces however consist largely of Gram-positive and negative rods after storage. The organisms from around the bone were for the most part oxidase positive when tested on the plates and were probably Gram-negative rods; none or only low numbers of faecal streptococci were present on the Barnes plates and no sporing anaerobes were detected in the DRCM medium.

In the first and second investigations the meat remaining after removing the bone from the stored meat samples was returned to storage and off odours in meat conditioned at 18°C were detected after (in total) 2 days at 15°C, 5 days at 7°C, and 7 days at 3°C, and in meat conditioned at 10°C after 3 days at 15°C, 6 days at 7°C, while no off odours were detected during storage at 3.3°C for 32 days. Conventionally aged meat (7 days at 3.3°C) when cut and stored, failed to spoil in 32 days at 3.3°C but had a shelf life of 12 days at 7°C and 6 days at 15°C.

From these limited investigations it would appear that lamb can be conditioned at a relatively high temperature for the purposes of avoiding toughness without any great microbiological problems, provided initial contamination is kept to a minimum. However a shorter shelf life might be expected than with conventionally chilled and aged meat though the ageing period we used was longer than that recommended by Locker *et al.* (1975). There was no evidence to suggest the rapid multiplication of potentially dangerous organisms at either conditioning temperature in our investigations.

The microbiological condition of the meat after vacuum-packaging (the third investigation) is shown in Table 3, where an interesting feature was the rapid

Table 3. Microbiological condition of conditioned and aged meat after vacuum-packaging

Investigation	Period in vacuum-package	Log_{10} total no. of organisms/g	No. of isolates	Composition of the flora (%)		
					Gram +ve rods	
				Gram −ve rods	Catalase +ve	Catalase −ve
III Leg A (aged 2 days at 3.3°C)	21 days at 1–2°C	6.79	45	29	71	Nil
	42 days at 1–2°C	7.76	28	18	75	7
	21 days at 7°C	6.80	31	61	39	Nil
	42 days at 7°C	8.06*	30	100	Nil	Nil
III Leg B (conditioned 1 day at 10°C aged 1 day at 3.3°C)	21 days at 1–2°C	6.45	29	69	31	Nil
	42 days at 1–2°C	7.60	33	18	70	12
	21 days at 7°C	6.81	43	98	2	Nil
	42 days at 7°C	8.23*	23	96	Nil	4

* Off-odours detected on opening package.

Table 4. Composition of the Gram-negative flora present on vacuum-packaged lamb after storage for up to 42 days at 2 and 7°C

Investigation	Period of storage	No. of Gram-negative isolates	Log_{10} no./g meat	Identified isolates (%): *Pseudomonas* spp. and *Alcaligenes*	*Moraxella*	*Vibrio*/*Aeromonas*	Psychrotrophic Enterobacteriaceae: Lactose +ve	Psychrotrophic Enterobacteriaceae: Lactose −ve
III Conventionally aged (leg A; 2 days at 3.3°C)	21 days at 1–2°C	13	6.25	38	62	Nil	Nil	Nil
	42 days at 1–2°C	5	6.92	100	Nil	Nil	Nil	Nil
	21 days at 7°C	19	6.59	26	5	Nil	21	47
	42 days at 7°C	30	8.06*	Nil	Nil	3	Nil	97
III Conditioned and aged (leg B; 1 day at 10°C, 1 day at 3.3°C)	21 days at 1–2°C	20	6.29	15	Nil	Nil	25	60
	42 days at 1–2°C	6	6.86	100	Nil	Nil	Nil	Nil
	21 days at 7°C	42	6.80	21	Nil	5	24	50
	42 days at 7°C	22	8.21*	5	Nil	Nil	14	82

* Off-odours detected on opening package.

Table 5. Extracellular enzymes produced by isolates from vacuum-packaged lamb

Isolate	No. tested	Incidence of enzymes (%)		
		Lipolytic	Caseolytic	Gelatinolytic
M. thermosphactum	95	Nil	Nil	Nil
Lactobacillus spp.	7	Nil	Nil	Nil
Other Gram +ve rods, +ve cocci	3	33	Nil	33
Pseudomonas gp I	9	100	78	100
Pseudomonas gp II	24	Nil	Nil	13
Moraxella-like organisms	9	Nil	Nil	11
Alcaligenes sp.	1	Nil	Nil	Nil
Vibrio/Aeromonas spp.	3	33	67	100
Psychrotrophic Enterobacteriaceae				
Lactose +ve	22	46	41	46
Lactose −ve	89	67	67	67
Totals	262	31	30	33

increase in the number of Gram negative isolates, particularly on the meat held at 7°C and the low numbers of catalase negative organisms, normally expected on vacuum packaged meat. Barlow & Kitchell (1966) found *M. thermosphactum*, which is Gram-positive and catalase positive to be the predominant organism on vacuum-packaged lamb chops held at 5°C for 6 days, a much shorter storage time than employed by us. Storage at this temperature would represent poor factory practice and should not occur, and the more likely holding temperature, if lamb was to be vacuum packaged on any scale would be <2°C. At this temperature only the meat stored for 21 days from the leg conditioned at 10°C had a high proportion of Gram-negative organisms which were potential spoilers. The catalase positive, Gram-positive rods were found to be *M. thermosphactum*, while the catalase negative rods were lactobacilli.

The composition of the Gram-negative flora present when the vacuum-packages were opened is given in Table 4. The isolates from the conventionally aged meat stored at 1–2°C were *Pseudomonas* gps I, II and III and *Moraxella*-like organisms together with one strain of *Alcaligenes*. The majority of isolates from conditioned meat stored at 1–2°C, and from both legs stored at 7°C were psychrotrophic Enterobacteriaceae although none of these organisms were detected in other aerobic storage investigations. Some of these were lactose negative and would not be detected on media normally used to isolate coliform bacteria such as violet-red-bile agar. Similar organisms have been described by Eddy & Kitchell (1959); Gardner, Carson & Patton (1967) and Hechelmann *et al.* (1974). Ingram & Dainty (1971) suggested that when fresh meats are vacuum-packaged in relatively impermeable films, the O_2 becomes largely replaced by CO_2 and the *Pseudomonas-Achromobacter* element in the flora is

suppressed by more tolerant strains of the *Enterobacter-Hafnia* group, *Kurthia*, *M. thermosphactum* and lactobacilli.

Spoilage had taken place in the vacuum-package after 6 weeks at 7°C, since strong off-odours were present on opening, particularly around the bone where the meat was greenish in appearance. Smears prepared from these sites showed large numbers of non-sporing Gram-positive rods, probably lactobacilli, and a few sporing rods. Low numbers of *Clostridium sporogenes* were found in the meat from leg A stored at 2°C for 3 weeks, and in all samples after 6 weeks. *Cl. welchii* was found (26 per 100 g) from leg B stored at 1–2°C for 6 weeks. Faecal streptococci were also present in all samples both after 3 and 6 weeks, with low numbers at 1–2°C and the highest recovery being $\log_{10}$ 6.48/g on meat from leg A stored at 7°C for 6 weeks. These results emphasize the need for strict temperature control, and the inadvisability of unduly extending the storage time of vacuum packaged lamb, even at temperatures near to 0°C. If these criteria are met conditioned lamb could be used for vacuum-packaging. Reagan *et al.* (1971) found that lamb loins could be successfully vacuum-packaged, provided low storage temperatures could be maintained and the storage interval short, otherwise shelf-life was poor.

One hundred and eleven isolates described as psychrotrophic Enterobacteriaceae in Table 4 were examined in detail. Few of the isolates exactly fitted the descriptions given by Cowan & Steel (1974) or in Bergey's Manual (Buchanan & Gibbons, 1974) but the majority could be described as strains resembling *Serratia liquefaciens* (70); others were identified as *Hafnia alvei* (21), *Enterobacter cloacae* (14), *Enterobacter aerogenes* (3), *Klebsiella aerogenes* (2), with one unidentified. The source of these organisms from further work appears to be from the animal gut, but whether they are of public health significance has not been determined. Further work on their growth characteristics has shown that representative strains had the ability to grow slowly at 0°C, with a doubling time of 30–60 hr, and that this shortened *c.* 7 hr at 7°C.

The extracellular enzymes produced by the isolates are shown in Table 5. *Pseudomonas* group I strains were particularly active as were the *Vibrio/Aeromonas* and *Hafnia* isolates. This is at variance with the definition of *H. alvei* given by Cowan & Steel (1974) where this organism was described as gelatin negative, though the test media used were different. Of all the groups, *Pseudomonas*, *Vibrio/Aeromonas* and the psychrotrophic Enterobacteriaceae would from these results be likely to spoil the meat under aerobic conditions if ability to cause spoilage is related to the degradation of these complex substrates.

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The effect of low temperature on the growth and survival of *Staphylococcus aureus* and *Salmonella typhimurium* when inoculated on to bacon

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Summary

Samples of bacon (10 g) were inoculated with *Staphylococcus aureus* and *Salmonella typhimurium* and the effect of storage at −22, 5 and 16°C was studied. The numbers of *S. aureus* increased significantly at 5 and 16°C, whilst at −22°C a significant decrease was recorded. The *S. typhimurium* counts increased at 16°C but decreased when the storage temperature was 5 and −22°C.

Introduction

A study was undertaken to investigate the growth and survival of *Staphylococcus aureus* and *Salmonella typhimurium* when inoculated on to bacon. These two organisms were selected because of their pathogenicity and their occurrence on meats. Jay (1962) noted the presence of *S. aureus* in spiced ham, while Eddy & Ingram (1962) stated that normal bacon may be regularly contaminated with food poisoning staphylococci. Thatcher, Robinson & Erdman (1962) showed that under certain conditions staphylococcal toxin production occurs on bacon and that the toxin can survive cooking at 205°C. Dempster, Reid & Cody (1973) reported that vacuum packed hams were contaminated with coagulase positive staphylococci, during the packaging operation, following cooking. These authors pointed out that if slicing and packing are not hygienically controlled early contamination can take place by human operators, who are a likely source of food poisoning bacteria.

Dempster & Kelly (1973) studied the growth of *S. aureus* in Wiltshire bacon and bacon produced hygienically. The two bacons were inoculated with a high (10^6/g) and a low (10^3/g) dosage of the organism and stored at 5 and 15°C. With the high inoculum the test organism grew in both bacons at 5°C, and survived at 5°C with the low inoculum. At 15°C the test organism grew, growth

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being more pronounced in the 'hygienic' than in Wiltshire bacon. The increasing importance of *Salmonella*, as a food pathogen, has been well documented, e.g. Weissman & Carpenter (1969), Hobbs (1974), McCoy (1975) and Tompkin (1976). Matches & Liston (1968) noted that the low temperature growth capability of *Salmonella* could be significant in foods stored for long periods of time at low temperatures (5°C).

The survival of any organism in a food is subject to a number of factors, e.g. temperature, salt concentration, pH and the presence of other organisms in the food. These factors are interdependent on each other. None of them can, therefore, be considered in isolation.

Variation in temperature affects the type of spoilage obtained, due to its influence on bacterial development (Cavett, 1962). The storage life of a product may be increased by retention at a low temperature. At these low temperatures organisms become more susceptible to other restrictive influences, (Ingram, 1960). The optimum temperature for growth of *S. aureus* and *S. typhimurium* is recorded in Bergey's Manual (Buchanan & Gibbons, 1974) as 37°C. The temperature at which food products may be stored fall into three broad ranges – room temperature, refrigeration temperature and freezer temperature. It was with this in mind that the experiments outlined in this paper were carried out at temperatures of 16, 5 and –22°C.

By using a consistent type and cut of bacon, throughout the experiments, it was possible to maintain the salt content at a constant level. Dempster (1976) pointed out that temperature has a controlling influence on the action of salt. The lethal action of salt on micro-organisms is less effective at low temperatures because a temperature rise almost invariably increases the velocity of a bactericidal reaction.

In the case of pH, changes are not of major importance in spoilage of meats due to their high buffering capacity, but if there is a high concentration of lactic acid, retardation of bacterial growth will occur (Ingram, 1960).

Competition between organisms is also of importance. As noted by Thatcher, Robinson & Erdman (1962) the competitive action of spoilage bacteria, under certain conditions, may repress multiplication of specific pathogens. By following the changes in the natural flora of the uninoculated samples, as reported here, it is possible to determine the influence of this factor on the test organism.

Materials and methods

Test organisms

Staphylococcus aureus, ATCC 25923; *Salmonella typhimurium*, ATCC 14028.

Media

In the enumeration of total viable organisms, Plate Count Agar (PCA, Oxoid) plus 3% sodium chloride (NaCl) was used. *S. aureus* counts were obtained on

Staphylococcus Medium No. 110 (S110, Oxoid). Brilliant Green Agar (BGA, Oxoid) was used in the enumeration of *S. typhimurium*. The standard inocula were grown in Nutrient Broth (Oxoid) and Ringer's solution was the diluent used in the preparation of all serial dilutions.

Bacon

The bacon was obtained at a retail outlet and all samples were produced by the same manufacturer. It was vacuum packaged, unsliced, smoked, contained little fat, no rind and all samples were from the back quarter, which normally had a salt content of 3.2% (w/w) and a nitrite content of 16.5 ppm. The same type and cut of meat was used in all experiments.

Sampling

The bacon packs were opened and 10 g portions were sliced aseptically and placed in sterile Petri dishes in which the samples were stored during the course of the experiments. The humidity of the samples was not controlled.

Sample inoculation

Serial dilutions were prepared of an 18 hr culture of the test organism. The optical densities of the suspensions were obtained using a Colman (295E) spectrophotometer at a wavelength of 600 nm. The number of viable bacteria, per ml of selected dilutions, was determined. From these results a standard graph was obtained. This was used for the standardization of the culture, which was inoculated on to the surface of the bacon samples and spread with a sterile glass spreader. The numbers present in the inoculum were confirmed by plate counts on S110, in the case of *S. aureus* and on BGA when the test organism was *S. typhimurium*. Uninoculated samples were set up at the beginning of each experiment.

Bacteriological analysis

Samples of bacon, 10 g, were placed in sterile plastic bags, to which 90 ml of Ringer's solution was added. The contents of the bags were then macerated using a Stomacher (Colworth 400). From this homogenate, serial dilutions were prepared, and 0.1 ml quantities were inoculated on to poured plates of the required medium and spread over the surface using a sterile glass spreader. All plates were incubated for 48 hr at 37°C. Counts were recorded on those plates yielding colonies in the range 30–300. In the examination of the samples, both uninoculated and inoculated, total counts were noted. The test organism was enumerated in the case of the inoculated samples. Sufficient bacon samples were set up initially to provide for the analysis of triplicate 10 g portions at each storage interval over a period of 30–32 days on 7 to 9 days.

pH determination

The pH values of the macerated samples, were determined electrometrically using a pH meter with a glass electrode (Corning meter, model 7).

Analysis of data

Analysis of variance was carried out on the data according to Snedecor (1956) and using Duncan's multiple range test to compare individual means.

Results and discussion

The effect of storage at −22°C on S. aureus

A significant decrease occurred in the *S. aureus* counts during the storage period (Fig. 1). The unfavourable temperature could account for this result. It should be noted, however, that 41.8% of the *S. aureus* organisms remained viable after 30 days storage. The total counts of the inoculated samples decreased significantly during the experiment. In the uninoculated samples a significant increase was noted between day 0 and 1, which may be accounted for by multiplication before the storage temperature was reached. The results of day 1 and day 30 showed no significant change. The pH trends of the uninoculated and inoculated samples did not change significantly during the experiment. This is in agreement with the reports of Georgala & Hurst (1963).

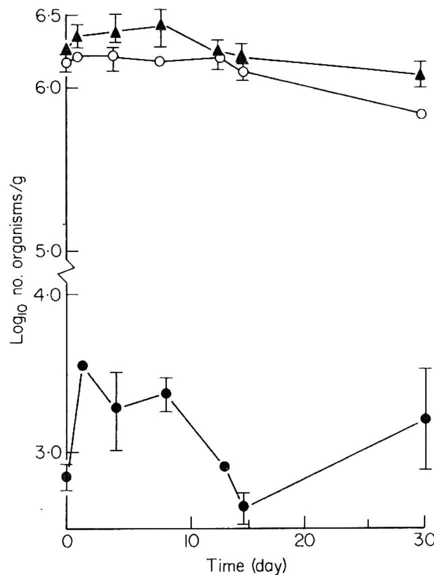

Figure 1. Changes in number of organisms when stored at −22°C in the experiment using *Staphylococcus aureus.* ●, total counts of uninoculated samples on PCA + 3% NaCl; ▲, total counts of samples inoculated with *Staphylococcus aureus* on PCA + 3% NaCl; ○, *Staphylococcus aureus* count on S110.

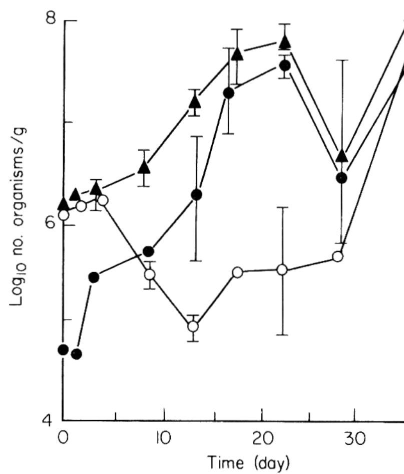

Figure 2. Changes in number of organisms when stored at 5°C in the experiment using *Staphylococcus aureus.* ●, total counts of the uninoculated samples on PCA + 3% NaCl; ▲, total counts of samples inoculated with *Staphylococcus aureus* on PCA + 3% NaCl; ○, *Staphylococcus aureus* count on S110.

The effect of storage at 5°C on S. aureus

The increase in *S. aureus* counts until day 3 was not significant, but in the interval from day 3 to 13 a significant decrease was recorded (Fig. 2). Since these decreases were accompanied by a drop in pH and a significant increase in the total viable count, it may indicate that it was due to the lactic acid group of bacteria. This agrees with the findings of Graves & Frazier (1963), who reported that species of *Lactobacillus* cause inhibition of *S. aureus*. From day 13 to 28 a gradual but significant increase in the *S. aureus* counts was recorded. This could be explained by the development of a less antagonistic flora and a more favourable pH (Table 1). In the interval from day 28 to 35, a significant

Table 1. Changes in pH means at 5 and 16°C when *Staphylococcus aureus* was the test organism

Day	Uninoculated samples at 5°C	Inoculated samples at 5°C	Uninoculated samples at 16°C	Inoculated samples at 16°C
0	5.9	5.9	6.0	6.2
1	5.8	5.9	5.7	5.8
3	6.1	6.2	—	—
4	—	—	5.1	5.7
8	6.0	5.9	5.9	8.0
13	5.9	5.6	8.0	9.5
17	5.3	5.3	—	—
20	—	—	9.7	10.2
22	5.4	5.6	—	—
25	—	—	10.4	10.7
28	5.6	6.7	10.6	10.7
35	6.5	7.75	10.5	10.7

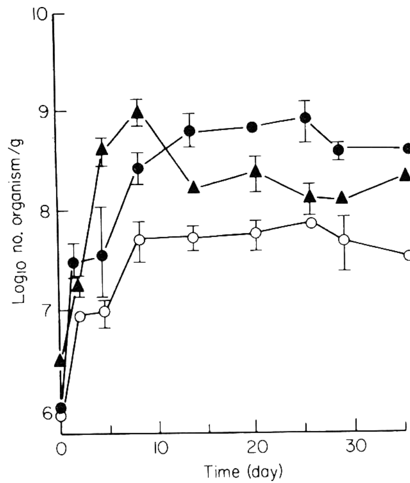

Figure 3. Changes in number of organisms when stored at 16°C in the experiment using *Staphylococcus aureus*. ●, total counts of the uninoculated samples on PCA + 3% NaCl; ▲, total counts of samples inoculated with *Staphylococcus aureus* on PCA + 3% NaCl; ○, *Staphylococcus aureus* counts on S110.

increase in all counts was recorded. This coincides with the continued increase in pH. The changes observed in the uninoculated samples were similar to those in the inoculated samples as were the pH trends.

The effect of storage at 16°C on S. aureus

During the first 8 days of storage all counts increased significantly (Fig. 3). Following day 8 no significant changes occurred in the staphylococci counts. In the case of the total counts of the inoculated samples, the decrease recorded on day 13 was significant but following this there were no significant changes. The latter may have resulted from unfavourable pH or lack of nutrients as noted by Daly, Sandine & Elliker (1972). The uninoculated samples showed a significant increase between day 0 and 25, followed by a significant decrease. The pH trends are illustrated in Table 1. The pattern for both types of samples was the same, but the increases for the inoculated samples were greater.

The effect of storage at −22°C on S. typhimurium

The marked decrease in *S. typhimurium* counts during storage was significant (Fig. 4). At the conclusion of the experiment on day 32, 10.4% of the initial number of organisms inoculated on, remained viable. The trend obtained for the total counts was similar. The total counts of the uninoculated samples had values less than 3×10^3/g throughout the experiment. These results are in agreement with the findings of Woodburn & Strong (1960) and of Enkiri & Alford (1971). The latter recorded the survival of salmonellae when inoculated on to the surface of meat and stored at −18°C over a period of 10 weeks,

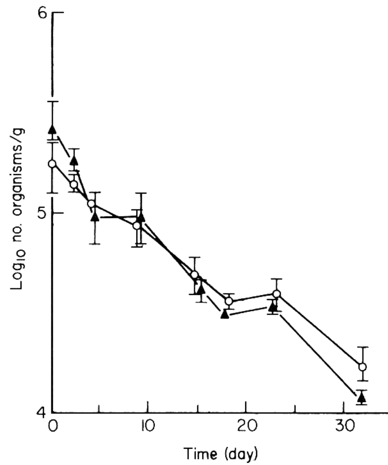

Figure 4. Changes in number of organisms when stored at −22°C in the experiment using *Salmonella typhimurium*. ▲, total counts on PCA + 3% NaCl; ○, *Salmonella typhimurium* counts on BGA.

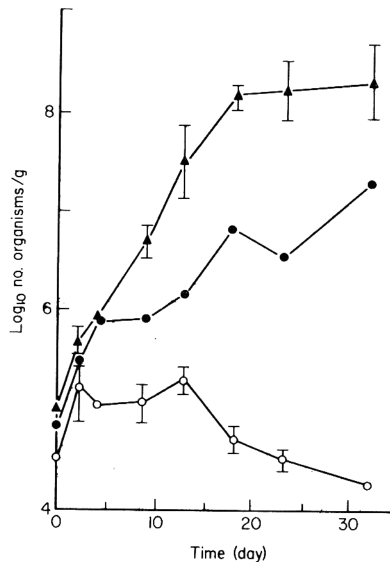

Figure 5. Changes in number of organisms when stored at 5°C in the experiment using *Salmonella typhimurium*. ●, total counts of the uninoculated samples on PCA + 3% NaCl. ▲, total counts of the inoculated samples on PCA + 3% NaCl. ○, *Salmonella typhimurium* counts on BGA.

The effect of storage at 5°C on S. typhimurium

A significant increase in the *S. typhimurium* counts occurred during the first 2 days of storage, while in the interval day 2 to 13 the changes did not differ significantly (Fig. 5). *Salmonella* counts yielded a significant decrease during the remainder of the storage period. The percentage survival of *S. typhimurium* at this temperature was greater than at −22°C. The results are in contrast to

those obtained for *S. aureus* when stored at 5°C. These results are borne out by the work of Goepfert & Chung (1970) who noted the ability of *Salmonella* to survive protracted storage at 5°C when inoculated on to the surface of luncheon meats but grew when incubated at room temperature. Angelotti, Foter & Lewis (1961) observed no growth of salmonellae in custard or ham salad between 4.4 and 10° C; however, in chicken à la king growth of salmonellae occurred at temperatures of 6.7°C and above. These authors concluded that growth of salmonellae in perishable foods was prevented when the internal temperature was at or below 5.6°C.

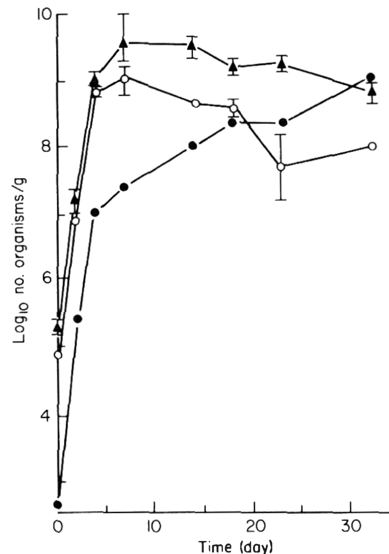

Figure 6. Changes in number of organisms when stored at 16°C in the experiment using *Salmonella typhimurium*. ●, total counts of the uninoculated samples on PCA + 3% NaCl; ▲, total counts of the inoculated samples on PCA + 3% NaCl. ○, *Salmonella typhimurium* counts on BGA.

The effect of storage at 16°C on S. typhimurium

The increase recorded in *Salmonella* counts until day 7 was siginficant, while in the interval day 7 to 18 the numbers did not differ significantly (Fig. 6). Following day 18 a significant decrease was noted. This latter result may have arisen from competition for nutrients. The overall increase in *S. typhimurium* numbers is to be expected as the temperature becomes more favourable for growth. Matches & Liston (1972) noted growth of *Salmonella* at 12°C, when the salt content was 3.5%. The total counts of the inoculated samples increased significantly to a maximum on day 7, followed by a significant decrease during the remainder of the experiment. The uninoculated samples showed a continued increase during the storage period.

Conclusions

From the work reported here it can be concluded that both *S. aureus* and *S. typhimurium* can survive storage at −22°C, over a period of 30 days. If a sample of bacon is contaminated with sufficient of these organisms (greater than 1×10^6 /g; Frazier, 1967) prior to storage at −22°C, there is the possibility that food poisoning may occur. At 5°C staphylococci show a marked increase in numbers. If a sample is contaminated with this organism, sufficient growth may occur to cause illness. In the case of *Salmonella* stored at 5°C, unless present in sufficient numbers to cause infection, prior to storage, there is no hazard. At 16°C, if either organism is present initially, sufficient growth could occur to cause infection or poisoning.

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Pasteurization of dried egg white by high temperature storage

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Summary

Inactivation of *Salmonella oranienburg* and some other members of the Enterobacteriaceae during high temperature storage of egg white flakes and powder was studied. During the manufacture of egg white flakes, microbially fermented egg white was inoculated with the test organisms, blended with citric acid or ammonia, and pan dried. Similarly enzyme fermented egg white was inoculated with test organisms and spray dried. Storage for 2 weeks at 55 or 49°C gave a 6–7 decimal reduction of the test organisms in flakes and powder respectively. The heat resistance of *S. oranienburg* was greater than that of the other Enterobacteriaceae tested.

Introduction

Raw egg products are frequently contaminated with Salmonella, therefore they are pasteurized in order to protect the consumer. Pasteurization is important particularly for egg white used in nougat creams, marshmallow whip and in other confectioneries, because these products are cooked insufficiently to kill Salmonella. Of the various methods of pasteurization a preferred method is the storage of the dried product in a 'hotroom' for a number of days at about 50°C (Ayres & Slosberg, 1949). This method is not detrimental to the functional properties of the dried product, and when the product is heat treated in the package, recontamination is impossible (Banwart & Ayres, 1956). Hotroom pasteurization is now a common practice in the production of pan dried egg white and spray dried egg white, but insufficient data are available on the microbiological aspects of the treatment and the bacteriocidal effects of the commercial process preceding the hotroom pasteurization.

The present study was conducted in order to determine the minimal hotroom temperature and storage time necessary to give a 10^6 reduction of *S. oranienburg*

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and other members of the Enterobacteriaceae when these were inoculated into fermented egg white during the manufacture of egg white to flakes and powder.

Materials and methods

Test organisms

Salmonella oranienburg was chosen as a test organism because this serotype was reported by Banwart & Ayres (1956) to be more resistant than other Salmonella serotypes to hotroom drying. Moreover this serotype is frequently isolated from egg products. Other test organisms were *Enterobacter cloacae*, isolated from spray dried egg white, *Klebsiella pneumoniae* and *Proteus vulgaris*, isolated from meat and bone meal respectively, *Enterobacter aerogenes* and a *Citrobacter* sp., strains of both showing a greater heat resistance than Salmonella during the manufacture of feed pellets (O. Pietzsch, personal communication).

Pan dried egg white experiments

Deep frozen, fermented egg white was obtained from a commercial egg processing plant using a fermentation method with an unspecified mixture of bacteria and yeasts. After thawing, 12 litre amounts of egg white were inoculated with 18-hr old cultures of the test organisms in brain heart infusion broth resulting in an initial number of 10^7 to 10^8 test organisms per ml egg white. Four hours after inoculation the pH of the egg white was adjusted with 25% ammonia or with 10% citric acid. The pH adjustment was according to the practice of the manufacturer. After a holding period of 24 hr at 20°C the egg white was poured onto trays to a depth of 2 cm. The egg white was dried in a forced air oven for 48 hr. Dry bulb temperature and relative humidity in the oven were 40°C and 40 to 45% R.H. respectively. The dried flake was broken up, mixed and placed in 250 ml screw capped jars. After holding the jars for 24 hr at 40°C (in order to simulate the increase of temperature in the commercial package) the incubation temperature was adjusted to the required hotroom temperature of 49, 51, 53 or 55°C.

Spray dried egg white

Deep frozen egg white, treated with the enzyme glucose oxidase in order to remove the glucose, was obtained from a commercial egg processing plant. After thawing, 120 litre of egg white was inoculated with test organisms as described for the pan dried egg white experiments, however, Salmonella was not used as a test organism, in order to avoid contamination of the commercial scale spray dryer. The liquid egg was spray dried according to procedures considered by the egg processing industry to give a product having acceptable functional properties. Products with various moisture contents were obtained by adjusting both the inlet temperature (157 to 184°C) and the feed rate. After drying, the powder was stored at 49, 51, 53 or 55°C.

Bacteriological methods

The level of organisms present after inoculation was determined by plating on violet red bile agar (Difco B12) with 10 g glucose per litre (VRBG); the plates were incubated for 24 hr at 37°C. To estimate the number or organisms present after the addition of citric acid or ammonia, after drying, and during the hot-room treatment, the Most Probable Number technique including a pre-enrichment stage and using five tubes or flasks for each dilution was applied. Depending on the number of test organisms expected, 100, 10 and 1 g of the product were transferred to a ten-fold larger volume of buffered peptone water. Further serial dilutions were also made in buffered peptone water. To estimate the surviving population of Salmonella, 1 or 10 ml of the dilution in buffered peptone water incubated at 37°C for 16–20 hr was transferred to 10 or 100 ml respectively of tetrathionate bile brilliant-green medium (ISO 3565). After incubation for 24 and 48 hr at 37°C, loops of broth were streaked onto brilliant-green phenol red agar (Oxoid CM 329). Plates were examined for typical colonies after 24 hr incubation at 37°C. To estimate the number of the other Enterobacteriaceae 1 or 10 ml of the dilution in buffered peptone water was transferred to 10 or 100 ml respectively of Enterobacteriaceae enrichment medium (Difco 0566). After incubation in this broth for 24 hr at 37°C broth was streaked out onto VRBG; plates were incubated for 24 hr at 37°C and examined for typical colonies.

Moisture content

Moisture content of the dried product was determined by the loss in weight of a 2 g sample upon drying at 105°C in a forced air oven for 48 hr.

Results

Results obtained for the inactivation of *S. oranienburg* in pan dried egg white of 10.5 to 10.7% moisture during the two process methods are presented in Table 1. Addition of citric acid or ammonia had a direct effect on the survival of *S. oranienburg* and an indirect effect later on during drying and hotroom treatment. During the holding time of 24 hr after addition of citric acid the direct effect was slight; the inactivation was proportional to the amount of citric acid added. During drying also an inactivation proportional to the amount of citric acid added was noticed. On the other hand a large amount of citric acid had a slightly indirect effect on the inactivation of *S. oranienburg* during hotroom treatment. The ammonia process method showed a marked inactivation of *S. oranienburg*, directly after addition of ammonia, and during hotroom treatment. However, the inactivation during spray drying was slight and not proportional to the amount of ammonia added. Figure 1 shows that during drying and hotroom treatment *S. oranienburg* was more resistant than *K.*

Table 1. Decimal reduction of *Salmonella oranienburg* during processing of pan dried egg white (10.5–10.7% moisture)

Process step	With citric acid adjusted pH			With ammonia adjusted pH			
	4.7	4.9	5.1	9.6	9.7	9.8	10.0
During 24 hr after pH adjustment	0.7	0.4	0.2	0.4	1.0	2.3	>2.5
Pan drying	1.5*	1.4	0.9	2.0	1.0	0.4	0.5
Hotroom treatment of 14 days							
49°C	—†	1.8	—	—	—	5.4	—
51°C	—	3.3	—	—	—	4.4	—
53°C	1.9	2.8	3.6	1.9	4.0	4.0	4.0
55°C	—	4.8	—	—	—	7.0	—

* Per gram solids, before and after drying.
† Not tested.

pneumoniae, Citrobacter and *P. vulgaris*. In the hotroom, the inactivation of Citrobacter and other Enterobacteriaceae was more than a hundred times greater than that of *S. oranienburg*. In other experiments with pan dried egg white with a lower moisture level (8.7 and 9.2%) we found that the inactivation of *S. oranienburg* was similar to the results presented. The results of Salmonella

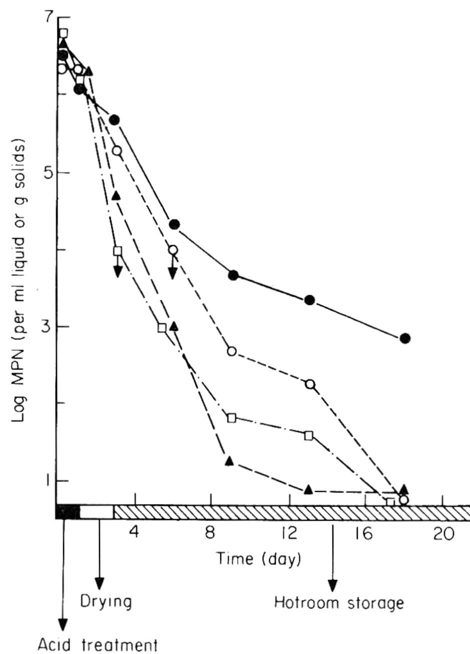

Figure 1. Reduction of test organism during acid treatment at pH 4.9, pan drying, and storage at 53°C. ●, *Salmonella oranienburg;* ○, *Klebsiella pneumoniae;* ▲, Citrobacter; □, *Proteus vulgaris.*

Table 2. Decimal reduction of three test organisms during processing of spray dried egg white

Process step	*Citrobacter* sp.				*Klebsiella pneumoniae*			*Enterobacter cloacae*		
	Dry product, moisture (% w/w)									
	5.9	6.8	7.9	8.8	5.2	6.0	6.8	5.8	6.9	8.8
Spray drying	4.0*	4.0	2.9	2.1	>4.5	>4.5	>4.5	2.7	2.5	1.8
Hotroom treatment of 14 days										
49°C	4.9	4.5	—†	—	5.4	5.7	5.7	—	—	—
51°C	5.2	—	6.2	—	—	—	—	—	—	—
53°C	5.5	6.0	—	7.3	—	—	—	6.4	6.7	>8
55°C	6.2	6.5	7.1	—	6.6	6.0	6.6	—	—	—

* Per gram solids, before and after drying.
† Not tested.

shown in Fig. 1 were obtained in a test, which differed from that used for Table 1. The results in the tests agree after adding 1 to the total reduction in Fig. 1 necessary to compensate the change of MPN per ml to MPN per g after drying.

The results for spray dried egg white are shown in Table 2. The inactivation of the test organisms was greater during spraying of a low moisture egg white powder. However, the inactivation in a low moisture powder during hotroom treatment was less than that in a high moisture powder. The inactivation of *E. aerogenes*, not shown in Table 2, was similar to that of *K. pneumoniae*. At a hotroom temperature of 53°C the inactivation of Citrobacter in spray dried egg white with a low moisture content was about 2 log greater than the inactivation of *S. oranienburg* in pan dried egg white.

Discussion and conclusion

The elimination of salmonellae from egg products by pasteurization is often checked by the relatively simple presence/absence test for Enterobacteriaceae. In order to meet a specification requiring absence of Enterobacteriaceae in 0.1 g of product, a reduction of 10^6 is needed, because the number of Enterobacteriaceae in the fermented egg white frequently amounts 10^6 per ml of liquid egg white, or 10^7 per g of dry product. Although the number of salmonellae might be very small compared with the total number of Enterobacteriaceae, a reduction of 10^6 is required to achieve absence of Salmonella in a large amount of product. In pan dried egg white, this degree of inactivation of salmonellae is achieved by the citric acid process with a hotroom temperature of 55°C. In the ammonia process an effective inactivation of salmonellae is achieved after adjusting the pH to 9.8 in combination with a hotroom temperature of 49°C.

In order to evaluate the results of spray dried egg white the inactivation of Citrobacter has to be compared with that of *S. oranienburg*. In pan dried egg white the inactivation of Citrobacter was 2 log greater than that of *S. oranienburg* (Fig. 1). Banwart & Ayres (1956) demonstrated with *S. oranienburg* a higher inactivation in spray dried product than in pan dried product. Results of McBee & Cotterill (1971) showed a more than 10^8 inactivation of *S. oranienburg* after spray drying and hotroom storage. Therefore, the conclusion can be drawn that an 10^8 inactivation of Citrobacter guarantees effective reduction of *S. oranienburg*. In the spray dried product this was achieved at a hotroom temperature of 49°C.

The difference in inactivation of *S. oranienburg* and other Enterobacteriaceae tested in our experiments was surprising. However, the results explain the data of van Schothorst (1977), who isolated Salmonella from a 25 g sample of pan dried egg white although Enterobacteriaceae were absent in 1 g. This means that after processes causing different inactivation rates for salmonellae and other members of the Enterobacteriaceae, the presence/absence test for Enterobacteriaceae, although of value as a check for recontamination, is not useful to check for the absence of salmonellae. Salmonellas should be tested for directly in dried egg white pasteurized by the hotroom treatment.

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Evaluation of spices and oleoresin-VI-pungency of ginger components, gingerols and shogoals and quality

SHANTHI NARASIMHAN AND V. S. GOVINDARAJAN

Summary

The pungent compounds of ginger had earlier been shown to be homologous gingerols, the dehydration product shogaols and the degradation product zingerone. The changes in these compounds are reported to affect quality with reduction in pungency and formation of off-flavour. Contrary to earlier assumptions, careful threshold tests for pungency of different oleoresins and purified pungent isolates, gingerols and shogaols established that shogaols are twice as pungent as gingerols. With this ratio of pungency of gingerols and shogaols, the calculated values agreed well with the estimated values of pungency in different ginger oleoresin samples. Occurrence of other non-pungent phenolics with close R_f values to the pungent compounds have been confirmed.

Introduction

Ginger, a spice known from very early times is valued for its characteristic aroma and pungency. Though in use for many thousands of years the nature of components contributing to these two sensory qualities have been studied only in the last hundred years. Connell (1970) reviewed the early work on the aroma and pungent compounds and summarized recent work of his studies on Australian ginger. He showed that the major pungent constituents of ginger are a mixture of *o*-methoxy phenyl alkanones, the gingerols, with varying lengths of side chains and shogaols the related dehydration product. Based on the aldehyde released from the side chain on alkaline degradation, he proposed naming them (6)–, (8)–, and (10)– gingerols. The three gingerols were found in the ratio 56:13:31 (Connell & Sutherland, 1969). By analysis of ginger oleoresin from fresh green ginger, sliced and quickly dried ginger, whole dried ginger and oleoresin stored over 18 months from the same batch of ginger, Connell (1969) showed the decrease of gingerols and increase of shogaols, both due to drying conditions and storage of oleoresin. These progressive storage changes in the

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pungent constituents have been confirmed by the recent quantitative studies by Ananthakrishna & Govindarajan (1974). In another analysis of a number of oleoresins of varying quality, Connell (1969) demonstrated that high quality oleoresin had predominantly gingerol and some shogaol while the oleoresins of lower quality showed the reverse proportions of the two constituents. An oleoresin considered very poor in quality with off flavour had neither gingerol not shogaol but only a small quantity of zingerone, a degradation product. Thus the proportion of gingerol homologues as also the proportion of gingerol, shogaol and zingerone can vary depending on the source, processing conditions and length and conditions of storage.

Reviewing pungency of synthetic analogous compounds, Dyson (1950) concluded that generally unsaturated compounds were more pungent while Pravatoroff (1967) generalized that unsaturated chemical combinations are less pungent than their partially reduced products. However, in these early experiments the determination of sensory pungency may not have been evaluated rigorously.

Though no experimental evidence is given it has been stated by Kulka (1967) and quoted by Connell (1969) that shogaol is less pungent than gingerol. Gingerols and shogaols are essentially pungent compounds and the significance of their relative proportion in different gingers and conversion due to processing and storage to total flavour quality would appear to require examination.

In this communication we have examined the pungency of isolated gingerols and shogaols and fresh and stored oleoresins with varying gingerols and shogaols content objectively estimated and their sensory quality.

Materials and method

Commercially dried whole ginger and sliced and quickly dried ginger were used for the preparation of oleoresins in the laboratory by column percolation using ethylene dichloride. Along with these oleoresins, samples obtained from commercial sources, stored at ambient temperature for 12 to 36 months and one alkali treated sample were also used in the experiments.

Method

Quantitative estimation of gingerols and shogaols content was done by the TLC-taste testing procedure developed by Ananthakrishna & Govindarajan (1974).

Gingerols and shogaols were isolated from several preparative TLC plates using the same solvent system and procedure for quantitative estimation and purified by repetition of the TLC separation.

Sensory pungency of oleoresins and isolated gingerols and shogaols were determined by the procedure standardized in our laboratory (Govindarajan,

Table 1. Pungent compounds (%) and pungency (SHU*) of ginger oleoresins

Sample	Gingerols	Shogaols	Pungency (SHU*)		
			Estimated	Calculated	
				A†	B‡
Sliced, dried fresh	26.8	1.3	25.7	18.0	22.1
Commercial dry ginger, fresh	20.3	4.1	21.3	18.3	21.4
Commercial, old	8.8	12.9	25.4	24.6	25.9
Laboratory dried, fresh	22.2	1.8	17.0	16.0	19.3
Laboratory dried, alkali treated	2.5	15.5	24.6	24.7	25.1

* SHU, Scoville heat units in thousands.
† Calculated using pungency values from Table 2.
‡ Calculated using pungency value of shogaol as 150×10^3 and gingerol as half of shogaol (75×10^3).

Shanthi Narasimhan & Dhanaraj, 1977) and expressed as Scoville Heat Units (SHU). These tests were done as threshold tests. The standardized procedure involves screening panelists for homogeneous sensitivity and training for avoiding bias, use of dilution series of test samples in 3% sugar solution, with a concentration difference with one 'just noticeable difference' levels. The results are expressed as mean $\pm\sigma$. The panel sensitivity is defined in terms of threshold value for pure piperine which helps in comparing the values from panels of different sensitivity.

Results and discussion

The gingerols and shogaols content in some samples of oleoresin and their pungency in SHU is given in Table 1. The oleoresins varying widely in their gingerol and shogaol contents, showing similar sensory pungency set us on checking the earlier observation on the pungency of gingerols and shogaols.

The isolates of gingerols and shogaols from the preparative TLC plates were estimated by analytical TLC and were confirmed to be free from each other but still contained traces of the adjacent higher R_f non-pungent components. The individual pungency of these purified pungent compounds by the standardized threshold method are given in Table 2.

The results clearly establish that shogaols contrary to earlier observations, exhibit more than twice the pungency of gingerols. The quantitation of the pungent compounds are based on the colour values determined by reaction with Folin-Ciocalteau reagent, and we have no reason to believe that gingerols and shogaols will react in different ways or to different degrees that would explain the difference in pungency now found.

Gingerols and shogaols are very well separated by TLC (R_f 0.3 and 0.7 respectively) and there is no possibility of any contamination of each other.

Table 2. Pungency (SHU*) of purified gingerols and shogaols

Component	Range (SHU*)	Mean $\pm\sigma$ (SHU*)
Gingerols	54.50 – 63.50	60.57 ± 2.43
Shogaols	136.00 – 160.50	150.50 ± 8.38

* SHU, Scoville heat units in thousands.

However it has been well established in our earlier paper that there are some phenolic compounds having no pungency which occupy adjacent areas on the TLC plates and it is likely there is some contamination with these, in the separated gingerols and shogaols (Ananthakrishna & Govindarajan, 1974). These non-pungent compounds have similar ultra-violet and infrared spectra as gingerols and shogaols (unpublished observations). These may be the homologues with longer side chains (10)–, (12)– reported to have higher R_f in TLC (Connell & McLachlan, 1972) or the gingediols and gingediacetate which have recently been reported by the Japanese group (Murata, Shinohara & Miyamoto, 1972; Masada *et al.*, 1974). Connell (1970) or Masada *et al.* (1974) do not record if the gingerols having shorter or longer side chains differ in intensity of pungency or have any pungency. However in line with the observations on synthetic analogs of capsaicin (Nelson, 1919), and the paradols (Locksley, Rainey & Rohan, 1972), it could be expected that highest pungency is noted in the dominant natural product, the (6)–gingerol and (6)–shogaol and other analogs with shorter and longer side chains have lower or no pungency. Murata *et al.* (1972) record that the gingediol tastes more bitter than pungent. Our observation is that these adjacent spots do not show any pungency even at as low a dilution level of 2 000, a thirtieth of the dilution for gingerol.

The value of twice the pungency for shogaol than gingerol would explain the high pungency obtained for the old sample of ginger oleoresin which analyses to lower gingerols and higher shogaols (Table 2). A fresh ginger oleoresin sample treated with alkali to convert part of gingerol to shogaol according to Connell & Sutherland (1969) showed that with a decrease in total pungent compounds and individually a large decrease in gingerol and an increase in shogaol as determined by TLC estimation, pungency increased in the treated sample over the untreated sample. This confirmed the results obtained with pure gingerols and shogaols. While the actual value in SHU for the pungency of gingerols and shogaols could vary with the sensitivity of the panel used in the subjective testing, the ratio of values for gingerols to shogaols would remain the same (Govindarajan *et al.*, 1977). The sensitivity of the panel used in this study is defined as 100 000 SHU for pure piperine.

In view of the difficulties of preparing and checking the purity of gingerols and shogaols, we have attempted to relate the estimated pungency (in SHU) of

five samples of fresh and stored oleoresins by calculating the pungency from objectively estimated values of gingerols and shogaols (Table 1) using the mean threshold values given in Table 2 for pure gingerols and shogaols. Data in column 5 of Table 1 show that in the case of stored or treated oleoresins which have higher shogaol contents the calculated pungencies agree closely with the estimated ones, whereas in the case of fresh oleoresins which have a greater proportion of gingerols to shogaols the agreement is less close. However, the values calculated assuming the pungency of gingerol as one half of shogaol given in the last column of Table 1 have shown the best agreement between the estimated and calculated values. This would indicate that the threshold value for shogaols is truer and hence the purity of this component is better than gingerols. Thus a more accurate threshold value of the pungent homologues of gingerols and shogaols would be 75×10^3 and 150×10^3 respectively. This is being checked with further purification and evaluation of individual homologues.

In these and related studies we have found that the conversion of gingerols to shogaols *per se* does not have any relation to aroma quality and any observation of lowering of flavour quality by Connell should only be coincidal and not causative. However in oleoresins where both shogaols and gingerols are low and zingerone is found, the off flavour is clear due to the degradative formation of the aldehydes which are well known to contribute to off-flavours. Our experience has been that even in oleoresins stored for about 36 months at room temperature (27–30°C) the total pungent compounds do not change substantially but the pungency increases. This sample was however rated poor in aroma, possibly more because of loss of desirable ginger aroma attributes by oxidative and polymerization reactions affecting the terpenic components than degradative formation of zingerone and aldehydes. The decrease in shogaols content also does not appear to happen very easily. The conditions under which the formation and polymerization of shogaols occur as demonstrated by Connell (1969) are rather drastic, 120°C for 12 h, and not likely to be met with the normal production or storage conditions. The possibilities of degradative conversion of gingerol to zingerone also appears not to be easily occurring under normal conditions of processing and reasonable storage, though the β-hydroxy ketone group in gingerols is very susceptible.

In view of the higher pungency of shogaols established in this paper and since shogaols are found in most of the commercial samples, it is necessary to estimate both the pungent gingerols and shogaols in ginger oleoresin samples to validly estimate its pungency. Any method which estimates total phenolics or gingerols and related compounds alone grossly (Nambudiri *et al.*, 1975) will therefore be an unsatisfactory estimate of pungency. Improvements to the TLC taste testing method of estimating the two pungent components of ginger have been worked and will be published in another communication. This improved method is being used in the determination of pungent components of gingerols and shogaols in a number of oleoresins to develop a correlation and predictive multiple regression.

ห้องสมุด กรมวิทยาศาสตร์

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Determination of ammonia in dressed thornback ray (*Raja clavata* L.) as a quality test

W. VYNCKE

Summary

The sensory determination of ammonia in raw, steamed and boiled ray wings using a scoring system appeared to be a valuable method of quality assessment. The borderline of acceptability was reached after 10 (±1) days in ice. A combination of the steaming and boiling tests (with addition of acetic acid and salt) was useful to confirm definite spoilage or to indicate the approach to the borderline of acceptability.

The chemical determination of ammonia appeared to be a useful objective method in addition to the organoleptic tests.

Thornback ray was borderline at a concentration of 60–70 mg N. From the other related parameters determined (urea, pH, redox potential, total bacterial count, α-amino nitrogen) only pH appeared to be of value.

Introduction

The occurrence of a high level (1 to 2.5%) of urea in the muscle, blood and organs is a characteristic of the Elasmobranchs (sharks, rays, skates). During spoilage, this urea breaks down with formation of ammonia due to the activity of bacterial urease (Simidu & Oisi, 1951). The often rapid development of ammonia in cartilaginous fish causes problems to the fish trade of countries such as Belgium where those species are popular.

In a previous paper (Vyncke, 1970) the determination of the ammonia content as an objective quality assessment method for several fish species and crustaceans was evaluated and appeared to be useful for spurdog (*Squalus acanthias* L.) and spotted dogfish (*Scylliorhinus canicula* O.). This work was continued on ray. Thornback ray (*Raja clavata* L.) which together with the common skate (*Raja batis* L.) is the most commonly landed species in Western Europe was chosen for the present experiments. Besides the chemical determination, special attention was paid to the sensory assessment of ammonia.

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Materials and methods

Fish

Thornback rays from the Southern North Sea weighing 2.5 to 3.5 kg and 2 (±1) days old at landing were prepared on the premises of a wholesale trader by the method usually adopted for this fish species, i.e. severing the wings from the trunk and skinning them. They were washed by dipping them for 30 sec in tap water.

Laboratory methods

Ammonia: with the accelerated microdiffusion method described previously (Vyncke, 1968a).

Urea: with urease according to Conway (1962) but using the modified ammonia determination.

pH: measured directly in minced fish with glass electrode.

Redox potential: measured in expressed fish fluid (Vyncke, 1968b) with a combined platinum-calomel electrode after 5 min stirring under a stream of nitrogen.

α-Amino nitrogen: with 2, 4, 6-trinitrobenzene-1-sulfonic acid according to Satake *et al.* (1960) on a 7.5% trichloracetic acid extract (4 g of fish per 200 ml).

Total bacterial count: *c.* 5 g of muscle aseptically cut from the central part of five ray wings and blended for 1 min in a sterile Waring blendor beaker after addition of a tenfold of sterile water. An appropriate dilution series was made and the resulting suspension inoculated on Petri dishes containing Plate Count Agar (Difco); incubation time was 72 hr at 20°C.

Differentiation of organisms on the basis of urease activity: fifty colonies taken at random from the plates used for the total bacterial count were first cultured on nutrient agar (Difco) slants in test tubes at 20°C for at least 4 days. The bacteria were then inoculated on urea agar base (BBL Cockeysville, Maryland, U.S.A.) slants in test tubes, according to Christensen (1946) but incubating for 1 day at 20°C.

Organoleptic tests: the degree of intensity of the odour of ammonia was graded on raw, steamed and boiled rays by a taste panel consisting of 3–4 members of laboratory staff experience on quality research on Elasmobranchs. The following scale was used:

5 – not present
4 – just recognizable
3 – slight
2 – moderate
1 – strong.

The raw fish was allowed to warm up to room temperature before the test.

Steaming was carried out in Pyrex dishes with loose lids over a boiling water bath for 30 min; about 200 g of boneless fish taken from five wings were used. The dishes were then placed in a thermostatic water bath kept at 60°C. Ammonia was assessed immediately upon removing this lid.

Boiling was performed in a litre beaker containing a solution of 0.15% acetic acid and 1% sodium chloride in water. The beaker was covered with a watch glass. The fish (200 g) was introduced when the liquid was boiling and cooked for 10 min. The sample was then removed and put in a Pyrex dish with loose lid and further assessed as in the steaming test.

General appearance, taste and texture were also examined.

Procedure

The ray wings were divided into two batches. A first batch was iced immediately and stored at 0°C. In order to enhance spoilage and for the sake of comparison, a second batch was kept for 15 hr in a room at 15°C before being iced and stored at 0°C.

At five time intervals after catching (Figs 1 and 2) five wings from each batch were taken for objective and organoleptic tests, which were carried out on pooled samples.

Besides the determination of ammonia and its precursor, urea, pH and redox potential were also measured as these parameters are linked to the bacterial ammonia production; α-amino nitrogen was also determined as ammonia can also be formed by deamination of amino acids. Ammonia being freed by bacterial action, total viable count and urease-producing organisms were also assessed.

For the organoleptic tests, the bacteriological analyses and the determination of redox potential, whole pieces of muscle were cut from the wings. The rest of the flesh was minced in an electric meat grinder and thoroughly mixed. The mince was used for the chemical determinations.

The experiments were repeated five times during the period January–May.

Results and discussion

The average results of the five experiments are reported graphically in Figs 1 and 2. The range of experimental data is also indicated.

Exposing the fish to a temperature of 15°C for 15 hr markedly changed the spoilage pattern as measured by the different subjective and objective methods. This treatment decreased shelf life of the rays by 2 to 3 days. Using the mentioned grading system for raw and steamed fish the taste panel judged the rays to be of acceptable commercial quality up to a score of 3. The panel however agreed that in actual commercial practice this score could be 0.5 units higher or lower according to consumer acceptance. Score 3 was reached after

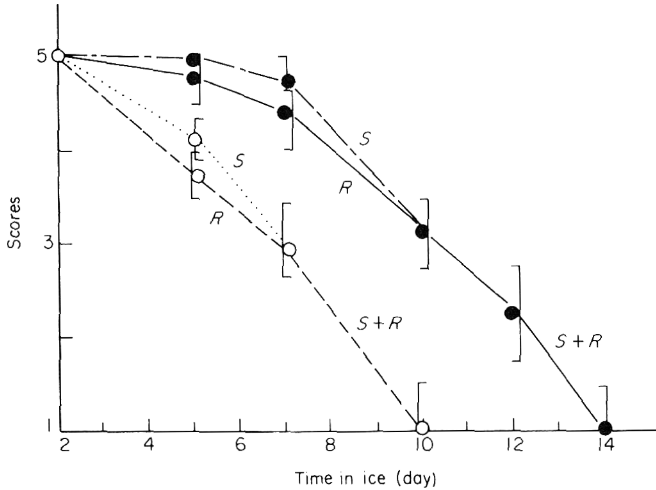

Figure 1. Evolution of organoleptic scores during storage of thornback ray in ice ●, immediately iced; ○, iced after 15 hr exposure to 15°C; *R*, raw odour score; *S*, steamed odour score.

9 to 11 days (average 10) for the immediately iced fish and after 6 to 8 days (average 7) for the 15°C fish.

When comparing the scores of the raw and steamed fish (Fig. 1), the latter ones appeared to be higher during the first 6 or 7 days of storage. One could conclude this test to be less sensitive. However, it should be emphasized that a certain amount of blood is present at the surface of skinned rays. As Elasmobranch blood usually has a higher urea content than the muscle (Simidu, 1961) and is readily attacked by bacteria present at the surface of the fish, the ammonia thus formed in an early stage of storage is in fact not a good indicator of spoilage and can easily be removed by washing; the fish flesh itself is still unchanged.

From a theoretical view-point, the steaming test could suffice. However, as assessing the raw odour score does not require special preparations and is rapid, it should also be carried out, increasing the reliability of the whole organoleptic judgment. On the other hand, when no ammonia is detected on the raw rays, the steaming test can be omitted.

After a series of screening experiments it was decided to add a boiling test to the procedure. This test is nearer to actual consumer practice, where a certain amount of vinegar is usually added to the cooking water to bind small amounts of ammonia. The boiling test was performed daily as soon as the score (raw and steamed) had reached about 3. Ammonia could not be detected in the boiled ray until the product was really unacceptable. This occurred 1 or 2 days after reaching score 3 on the raw or steamed rays. For quality control (e.g. in cases of dispute) a combination of steaming and boiling tests could be very useful, the latter indicating either that the borderline of acceptability has approached (when negative, the raw and steaming scores being around 3) or had been passed (when positive).

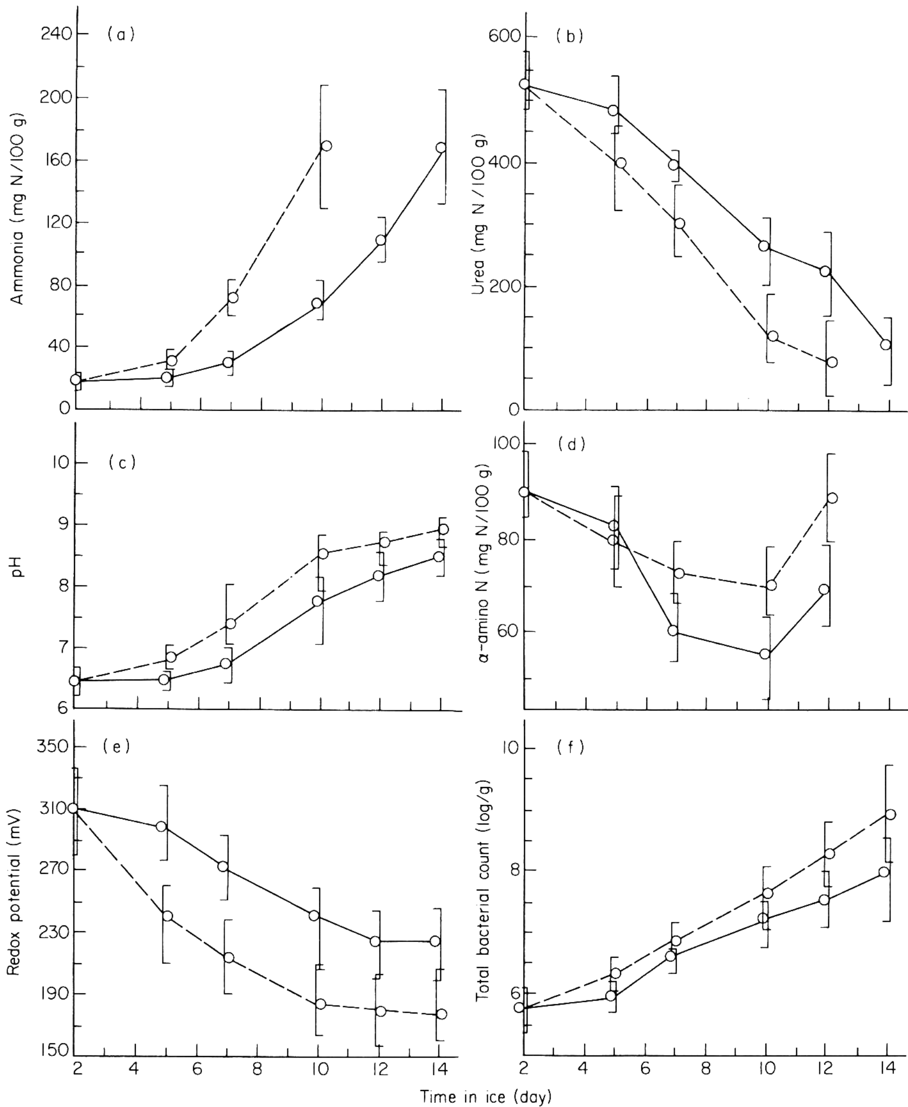

Figure 2. Evolution of (a) ammonia, (b) urea, (c) pH, (d) α-amino nitrogen, (e) redox potential and (f) total bacterial count, during storage of thornback ray in ice. ——, Immediately iced (0°C); – – –, iced after 15 hr exposure to 15°C.

The other organoleptic characteristic appeared to be less valuable with skinned ray wings. Other odours and flavours normally associated with fish spoilage could also be detected but were dominated by the development of ammonia and hence of less importance. No important discolorations were observed except at the end of the shelf life when the wings became yellowish. Texture became softer both in the raw and cooked rays but only when approaching the limit of acceptability.

When comparing the graphs giving the evolution of the concentration of

ammonia and the organoleptic scores (Figs 1 and 2), there appeared to be a good relationship between both parameters. A slight odour (score 4–4.5) was detected at a concentration of 30–40 mg of ammonia–N.

The borderline of acceptability could be set at 60–70 mg which is higher than the values noted earlier for dogfish, i.e. 55–60 mg (Vyncke, 1970) and also above the limit of 30 mg quoted by James & Olley (1971) for shark.

The concentration of the main precursor of ammonia, urea, decreased sharply during storage. When comparing the figures for urea and ammonia however it can be concluded that the leaching effect is much stronger than the breakdown to ammonia. This is further confirmed by the fact that the 15°C curve runs parallel to the 0°C-curve when it would be expected to diverge.

Owing to the development of ammonia, pH also increased significantly. From a value of about 8 onwards however the progress was distinctly slowed down. As the levelling-off effect occurred only when the ray was practically unacceptable, pH appeared to be a good complementary quality assessment method. The limit of acceptability could be set between 7.2 and 7.8, values superior to 8 indicating definite spoilage.

The determination of α-amino nitrogen did not allow to conclude that ammonia is also formed in significant amounts by deamination. The concentration in fact depends upon the rate of formation of new amino acids by the breakdown of proteins and peptides, their deamination (and other reactions) and leaching by the melting ice. Nevertheless, this test showed the activity of the bacterial exopeptidases to be greater than that of the deaminases when spoilage was enhanced either by natural causes (growth of bacteria during storage in ice) or artificially (temperature influence). This could be assessed by the fact that at the end of the storage period, when spoilage was pronounced, the content of α-amino nitrogen increased significantly. Moreover, the 15°C samples showed a higher value after a few days' storage. As many amino acids show an activating effect on urease by binding heavy metals, thereby protecting the urease sulphydrylgroups (Pinter, Tashovski & Karas, 1954), the relative increase of α-amino nitrogen probably also furthered urea breakdown.

Bacterial activity was clearly reflected by the evolution of the redox potential making the medium more reducing with progressing spoilage. Exposing the fish to a temperature of 15°C markedly decreased the potential. Values of 230–250 mV indicated the onset of spoilage, but some discrepancy was observed between 0 and 15°C experiments in this respect, rendering redox potential measurements a doubtful objective quality method.

Total bacterial count increased regularly during spoilage. It can be noticed that the initial load was already rather high, ranging from log 5.3 to 6.2 (average 5.7). It should be stressed that rays have a large amount of very viscous mucus on the surface and that contamination of skinned ray wings with bacteria from this slime is difficult to avoid. For the same reason the total viable count was high at the borderline of acceptability, ranging from log 6.5 to 7.8

From investigations by Liston (1957) the bacterial population of skin and gills of skate (*Raja* spp.) appeared to be composed principally of Gram-negative rods of the *Pseudomonas* and *Achromobacter* genera and was in fact similar to the population of other fish species of the North Sea. No further differentiation of the microbial flora was carried out except for the organisms showing urease activity.

Table 1. Differentiation of bacteria on the basis of urease activity (in % of total count)

Temperature	Storage time (day)				
	0	5	7	10	12
0°C	20	25	29	36	44
15°C	20	31	42	45	62

Although the culture medium used probably did not react on all urease producing bacteria present, it was considered to give a good estimate of the urease activity. The results reported in Table 1 indicated the percentage of micro-organisms showing urease activity to increase significantly during spoilage. This was also confirmed by the higher numbers of the 15°C experiment, where spoilage was enhanced from the beginning of the storage period. The changes in pH and redox potential are at least partially responsible for the changes in microbial flora.

It should further be stressed that urease is known to occur in over two hundred species of bacteria (Sumner & Somers, 1953) emphasizing the high risks of ammonia development in Elasmobranchs.

The present experiments showed the chemical determination of ammonia to be a useful objective test in addition to the organoleptic assessment of this compound in ray. From the other related parameters studied, especially pH appeared to be of value. An ammonia content exceeding 60 mg and a pH higher than 7.2 should be regarded as indicating rays of suspect quality.

Acknowledgment

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Changes in the heat stability of milk protein during the manufacture of dried skim-milk

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Summary

The effects on the heat stability of milk protein, when heated to 140°C, of variations in milk composition and in processing conditions during the manufacture of skim-milk powder have been investigated. The heat stability of the skim milk powder was largely determined by the stability of the original milk and by the severity of the forewarming treatment applied during processing. It was also shown that the heat stability of milk powder reconstituted to 10% total solids could be optimized by adjustment of milk pH, by supplementing the natural urea level and by applying a minimal forewarming treatment.

Introduction

The advent of sophisticated technology has enabled the modern milk processor to tailor the physical properties of dried skim milk to satisfy a wide range of consumers (Sanderson, 1977). The factors controlling solubility, dispersibility and wettability of powders are also well known and high standards of quality are routinely acheived (American Dry Milk Institute, 1971). However, much less information is available concerning the chemical stability of protein in dried milk although in some end uses, such as custard formulations and as whiteners in hot beverages, the heat stability of milk protein assumes considerable importance.

Sweetsur (1976) examined the stability of 'instant' dried skim-milk when added to hot coffee and showed that the formation of insoluble material was inversely related to the maximum coagulation time (CT) of the reconstituted powder measured at 140°C and directly related to the casein number of milk protein (i.e. the percentage of milk protein precipitated at pH 4.6). As the history of the milk from which the powders were made was unknown, it was not possible to differentiate between natural variations in heat stability of the raw milk supply and subsequent changes brought about by

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denaturation of milk protein during processing (leading to increases in casein number).

The object of the investigations reported here was to establish the relation between the heat stability (measured at 140°C) of the protein in skim-milk and that of the milk protein after processing to milk powder. In addition, the experiments were designed to simulate the relative effects of seasonal variation in heat stability and the changes in heat stability which could be induced by variation of processing treatment.

Recent studies have demonstrated that the naturally occurring level of urea in milk is a major determinant of heat stability (Muir & Sweetsur, 1976, 1977) and in addition that, with the exception of a 6 week period in late May and early June, the heat stability of the milk supply to manufacturing creameries in south-west Scotland is highly correlated with the urea level (C. Holt, Muir & Sweetsur, unpublished results). Therefore by manipulation of the urea level in milk it is possible to simulate seasonal variations in heat stability.

The manufacturing process for milk powder consists of three distinct stages. (a) Heat treatment of skim-milk – technically called forewarming – which reduces microbial contamination but which, in special cases, is also used to improve powder characteristics (for example, Greenbank *et al.*, 1927 showed that the properties of powders used in bread-making are improved by severe heat treatment). (b) Concentration of the forewarmed milk to a level of 40–50% total solids by evaporation of water under reduced pressure and, (c) spray drying the concentrate to remove all but 3–4% of the remaining water.

In laboratory studies, the forewarming treatment has been shown to influence heat stability (Sweetsur & White, 1974) probably as a result of whey protein denaturation (Sweetsur, 1976). Therefore in this work, the extremes of forewarming treatment likely to occur in commercial practice were simulated by two contrasting procedures. The minimum heat treatment required to pasteurize milk was employed as the first treatment and in the second case the milk was heated in conditions which promoted almost complete denaturation of the whey proteins (Davies & White, 1959).

This paper details the changes in heat stability which occurred during the manufacture of dried skim-milk in experimental situations designed to represent the extremes of variation both in the nature of the raw-milk supply and in the processing conditions used.

Materials and methods

Materials

Bulk milk (90 litre) was collected from the Hannah Research Institute Farm bulk tank (during December 1975), warmed to approximately 40°C and the fat then separated using a continuous-flow centrifugal separator. After cooling to around 20°C, urea (Analar Grade) was added to one portion of the milk (40 litre) at a level of 0.26 g per litre and another 40 litre sample of the milk was used as the control.

The control and urea supplemented milks were each then sub-divided and subjected to a heat treatment. For each urea level, one sample of milk was pasteurized by holding the milk at 64°C for 30 min and another sample of each milk was heated at 85°C for 30 min.

Preparation of powders

Immediately after heat treatment the four sub-samples of milk were introduced into a small-scale single pan *Scott* evaporator and concentrated to about 38% total solids (TS). The temperature of the milk during concentration was maintained around 52°C.

After concentration, the milk samples were dried in a *Scott* spray drier equipped with a disc atomizer. Average drying conditions were: air inlet temperature 135°C and air outlet temperature 85°C, chamber temperature 105°C. The moisture contents of the powders were less than 4.0%. The powders were stored until required for testing in air tight containers.

Analyses

Total solids content of skim milk and concentrate was estimated by the appropriate British Standard Method (1963). Moisture content of the powders was measured by drying to constant weight in a fan-assisted oven at 102°C.

The urea levels in milk, concentrates and re-dispersed powder were measured by the method of Fawcett & Scott (1960) as adapted by Muir & Sweetsur (1976).

The amount of whey protein denaturation was estimated by measurement of the casein number as described in Sweetsur (1976).

Heat stability

The heat stability of the milks was determined by observing the time taken for visible clots to appear when the milk was heated at 140°C. The milk pH was adjusted by the addition of HCl or NaOH, and the coagulation time (CT)/pH profiles were measured by the subjective method of Sweetsur & White (1974). The CT/pH profiles of concentrate and powder were measured after dilution of the material with distilled water to the TS concentration corresponding to the original skim-milk.

Results

Whey protein denaturation during processing

Heat treatment of milk may promote significant denaturation of the whey protein if the temperature of processing exceeds 60°C (Davies & White, 1959).

Table 1. Changes in the level of whey protein denaturation during the production of skim-milk powders

Treatment	Casein number*			
	Control milk		Urea-supplemented milk	
	'low heat'	'high heat'	'low heat'	'high heat'
Skim-milk	79.2	79.2	78.0	78.0
After pasteurization†	81.3	n.a.	79.4	n.a.
After forewarming‡	n.a.	91.4	n.a.	92.9 §
After spray-drying	80.3	91.2	78.5	89.0

n.a. = not applicable.
* Casein number = percentage of total nitrogen precipitated at pH 4.6.
† Milk heated at 64°C for 30 min.
‡ Milk heated at 85°C for 30 min.
§ Approximate value found in a small-scale experiment, large-scale sample was lost.

Since most of the denatured whey protein co-precipitates with casein at pH 4.6, measurement of the casein number of the milk or milk product provides a useful indication of the level of denaturation.

The casein numbers of the skim-milks and powders were determined during processing and the results are shown in Table 1. For the 'low heat' powders the casein number showed little change during processing indicating that only slight denaturation of whey protein occurred during pasteurization and that the subsequent operations of concentration and drying had little further effect. The results of forewarming at 85°C on the control and urea-supplemented milks were in contrast to those for the low heat treatment: in both cases heating at 85°C denatured over 75% of the whey protein. These findings parallel those of O'Connor, McKenna & O'Sullivan (1969) who also found that during the manufacture of skim-milk powder most of the whey protein denaturation occurred during the heat treatment of milk prior to concentration.

Changes in heat stability during processing

The effect of forewarming treatment

In this work, the heat stability of the milks was measured over a large range of milk pH values because small changes in milk pH can cause large changes in CT (Rose, 1961a). Therefore, measurement of CT of milk or re-constituted milk at a single pH value may give a misleading impression of overall changes in heat stability. Manipulation of the pH of concentrated milk and milk powder is possible within the current food legislation and addition of suitable salts to milk powder is included in the new proposals for 'Regulations on condensed milk and dried milk products' (Ministry of Agriculture, Fisheries and Food, 1976).

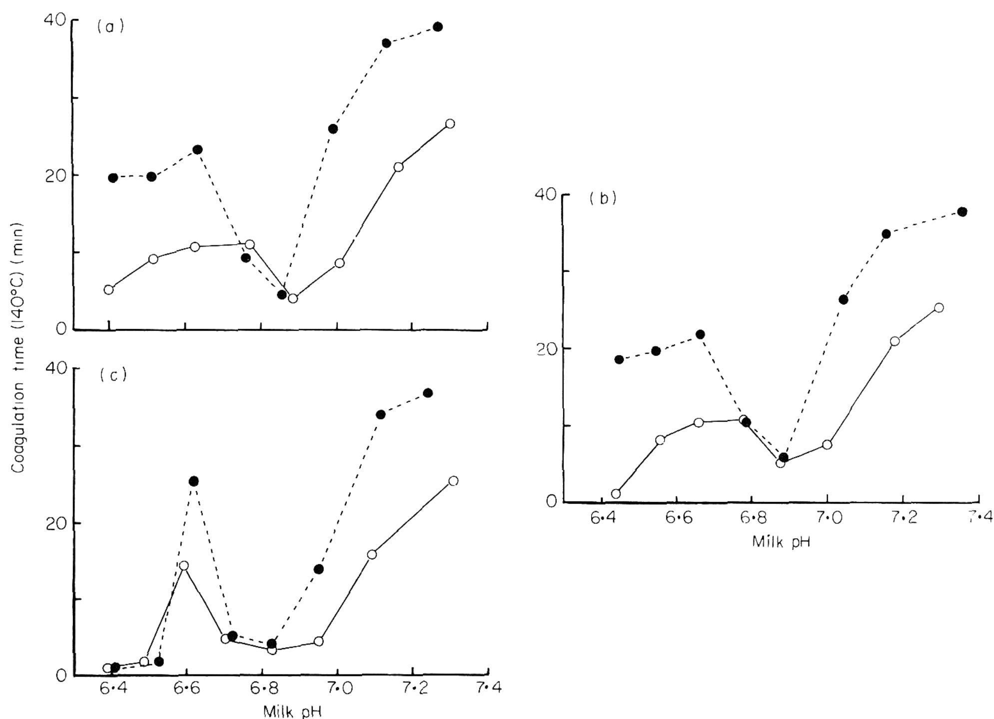

Figure 1. Effect of preheat treatment and addition of urea (0.26 g/litre milk) on the coagulation time (CT)/pH profile of milk. ○, milk; ●, milk + urea; (a), no preheat treatment; (b), low preheat treatment (64°C, 30 min); (c), high preheat treatment (85°C, 30 min).

The CT/pH profiles for the control and urea-supplemented milks are shown in Fig. 1(a). The effect of high urea concentration was marked over a wide range of milk pH values but, as reported previously (Muir & Sweetsur, 1976, 1977), urea had less influence on CT within the 'minimum' of the CT/pH profile (6.65 < milk pH < 7.10). Nevertheless, the CT/pH profiles were representative of the extremes of seasonal variation observed in Hannah Research Institute bulk milk over a 2 year period.

Heat treatment of the skim-milk for 30 min at 64°C did not cause a significant change in the CT/pH profile (Fig. 1(b)), but forewarming at 85°C for the same time induced marked differences (Fig. 1(c)). The extent of the CT/pH 'minimum' was increased although the maximum CT (*c.* milk pH of 6.6) was largely unaffected by forewarming. Notwithstanding, these changes induced by high heat treatment, the effect of urea supplementation on the general level of heat stability was retained.

The irreversible effect of concentration

Concentration effects a marked reduction in the heat stability of skim-milk (e.g. Hunziker, 1935; Rose 1961b; Schmidt & Koops, 1965) but it is not known

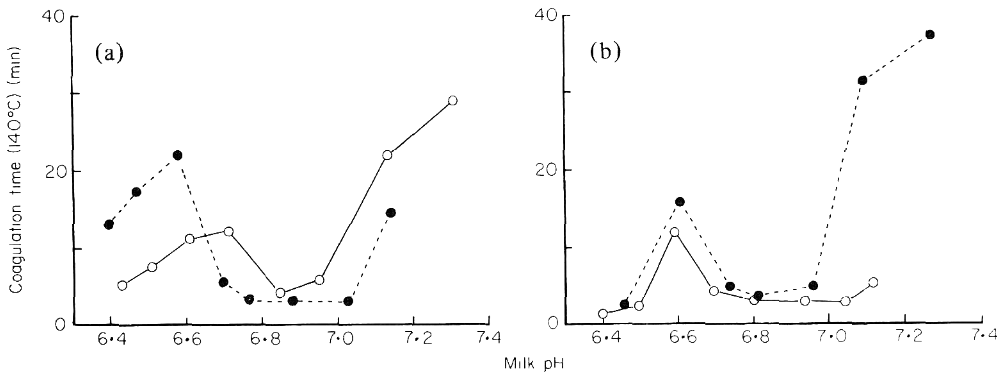

Figure 2. Coagulation time (CT)/pH profiles for concentrated milks, rediluted to their original total solids level. ○, milk; ●, milk + urea; (a), low preheat treatment (64°C, 30 min); (b), high preheat treatment (85°C, 30 min).

if the effect is reversible. For comparison with the skim-milks, the concentrates in this study were diluted with distilled water to the original level of TS in the skim-milks, prior to the measurement of the CT/pH profiles. By this means, only the permanent effect of concentration on heat stability was considered.

The appropriate CT/pH profiles for the diluted concentrates are shown in Fig. 2. For the low heat treatment, the CT/pH profile of the control milk was not significantly altered but, in the case of the urea-supplemented milk the 'minimum' in the CT/pH profile was extended over a wide pH range (Fig. 2(a)). This phenomenon was accentuated for both control and urea-supplemented milks which had received high heat treatments (Fig. 2(b)). Furthermore, with high heat treatments much of the difference between the high and low urea levels had disappeared: even at the CT 'maximum' (pH 6.6) the difference in CT was 3.5 min compared to 10.5 min for the same skim-milks before concentration.

It was not possible to simulate in a batch evaporator the conditions prevailing in a modern multiple effect evaporator where the milk temperature in the first stage of evaporation may be as high as 80°C and thereafter fall by stages to around 40°C in the fourth effect. However, additional experiments were carried out with skim-milk concentrate (43% TS) from a commercial four-effect, falling-film evaporator and the irreversible changes in the CT/pH profiles of the milk were very similar to those shown in Fig. 2(b).

The effect of spray-drying

The heat stability of the spray dried powders was measured after reconstitution of the powders with distilled water to the same TS concentrations as those of the original skim-milks and diluted concentrates. The CT/pH profiles are shown in Fig. 3.

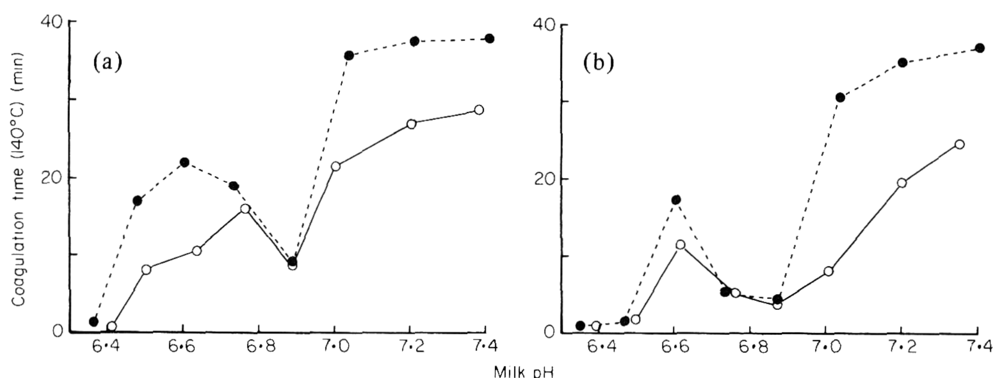

Figure 3. Coagulation time (CT)/pH profiles for milk powders, reconstituted to their original total solids level. ○, milk; ●, milk + urea; (a), low preheat treatment (64°C, 30 min); (b), high preheat treatment (85°C, 30 min).

When the milk received a low heat treatment during processing (Fig. 3(a)), not only were the changes observed after concentration reversed, but the extent of the CT/pH minima were also reduced in comparison to the skim-milks from which the powders were made (Fig. 1(b)). A parallel effect occurred for the high heat powders (Fig. 3(b)), for the CT/pH profiles were more stable than those of the corresponding concentrates (Fig. 2(b)). However in this case, the CT/pH profiles were slightly less stable than for the corresponding skim milks (Fig. 1(c)). Nevertheless, for both mild and severe heat treatments, the differences between low and high urea levels – which had been reduced after concentration – were restored by spray drying to values similar to those observed in the original milks.

Since duplication of conditions in a commercial spray dryer was not feasible in the laboratory size dryer, confirmatory experiments were carried out using a pilot-scale, (evaporative capacity of 35 kg water per hr) tall-form, co-current spray-dryer with pressure atomization and air inlet temperatures to 190°C. Using this spray-dryer, the changes in heat stability after concentration and spray drying were indistinguishable from the results obtained in the laboratory-scale dryer.

Discussion and conclusions

The results of this work demonstrate that the heat stability characteristics of a reconstituted skim-milk powder are determined by the heat stability of the original milk and by the nature of the processing treatment. The effect of the urea level on the CT/pH profile was maintained, albeit to varying extents, throughout the processing sequences, irrespective of the type of forewarming treatment. Consequently, it is expected that seasonal variations in the heat stability of milk, associated with natural variations in urea level, will be reflected by parallel changes in the heat stability of reconstituted milk powder.

The severity of the forewarming treatment of milk prior to concentration had an equally marked effect on heat stability and where high heat treatments were used substantial losses in heat stability were recorded.

It was also noted that, although the heat stability characteristics of skim-milk powder resemble those of the corresponding substrate, concentration and spray drying had opposite and approximately equal effects on the CT/pH profiles. Further investigations of these effects are under way since there appears to be potential for improvement of heat stability of milk powder by increasing the benefits conveyed by spray-drying at the expense of the deleterious effects of concentration.

From the results of this paper, several criteria appear to be important for optimization of the heat stability of reconstituted skim-milk powder.

First, the natural stability of the skim-milk from which the powder is to be made must be satisfactory, for inherent instability of the starting milk will be reflected in the properties of the powder. Natural instability (not associated with bacterial degradation of milk protein) is most likely to occur in the winter period when the milk urea levels are low (Muir & Sweetsur, 1976) and for short periods in late May and early June when other compositional changes in the milk occur (Holt, Muir & Sweetsur, in preparation). Although supplementation of the natural urea level may overcome natural instability associated with winter milk such addition is not permitted under current food legislation.

The second criterion to achieve high levels of heat stability is to limit the severity of heat treatment of the milk. This requirement must, however, be balanced against the need to maintain low levels of bacterial contamination in the final powder.

Finally, the pH of the milk powder on reconstitution to the original level of TS should be outside the CT/pH 'minimum' for, if this condition is not fulfilled, the potential heat stability of the milk may not be realized. For example, a shift in milk pH of 0.1 units may lead to a five-fold decrease in heat stability (Fig. 3). Further investigation will be required to establish how the use of currently permitted additives may regulate milk pH.

Acknowledgments

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A model system for the formation of N-nitrosopyrrolidine in grilled or fried bacon

M. H. COLEMAN

Summary

A simple model system has been developed which mimics the formation of the principal nitrosamine, N-nitrosopyrrolidine, in grilled or fried bacon. The yield of the nitrosamine in the model system may be estimated by a simple GC method. This has permitted the rapid screening of a variety of compounds which may influence nitrosamine in bacon. The antioxidant ethoxyquin inhibits nitrosamine formation in the model system.

Introduction

The present work describes the development of a simple model system, designed to mimic the formation of the principal volatile nitrosamine N-nitrosopyrrolidine (NNP), found in bacon subjected to high-temperature cooking, i.e. grilling or frying.

In preliminary experiments with bacon, the formation of NNP was shown to be virtually confined to the fatty tissue, in agreement with the observation of Fiddler *et al.* (1974). In confirmation of the suggestion of Scanlan (1975) it was shown that the fatty tissue reached much higher temperatures during grilling, than the lean. It was also found that NNP was formed when the fat, (rendered from bacon at a low temperature), was heated alone, at 170°C; whereas when rendered pork fat was so heated, no NNP formation occurred, but the pyrrolidine content was greatly increased. The amount of pyrrolidine found in heated pork fat was more than sufficient to account for the NNP found in cooked bacon.

These experiments suggest that pyrrolidine may be an intermediate in the formation of NNP in cooked bacon, but is not the initial precursor. Further *in vitro* experiments showed that NNP was readily formed when pyrrolidine was heated with nitrite; and of the possible precursors of pyrrolidine, likely to be present in bacon, proline gave the highest yield of pyrrolidine when

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heated at high temperatures. Subsequent experiments on the addition of proline to bacon established a linear relationship between the level of free proline present at cooking, with the level of NNP found in the cook-out fat.

Various techniques have been employed for the estimation of volatile nitrosamines in cooked cured meats, including i.r. and u.v. spectroscopy (Ender *et al.*, 1964); TLC, Sakshang *et al.*, 1965; TLC and GC, Sen *et al.*, 1969 and DuPlesis, Nunn & Roach, 1969), and GC with special detectors (Howard, Fazio & Watts, 1970; Sen, 1970; Althorpe *et al.*, 1970; Fiddler *et al.*, 1971): but the combination of GC with high-resolution mass-spectrometry (Telling, Bryce & Althorpe, 1971; Fazio *et al.*, 1971) would seem to be the method of choice for the unambiguous quantitative estimation of volatile nitrosamines. However the GC-MS procedure is costly and time-consuming in execution. It could obviously be advantageous if a simple model system could be devised to mimic the formation of NNP in cooked bacon, which would avoid both the complex clean-up procedure and the time-consuming GC-MS, as well as the variability of results associated with the analysis of meat samples.

The preliminary experiments on bacon indicated that proline was the likely precursor of NNP, and pyrrolidine a possible intermediate in its formation. However no pyrrolidine could be detected when proline was heated at 170°C in aqueous solution; although significant amounts were formed when it was heated in an inert solvent such as tetralin, or the more polar solvent, methanol. As sodium nitrite is appreciably soluble in methanol, it was found that a homogeneous reaction mixture yielding NNP in sufficient amounts to be estimated by GC alone, could be prepared by heating a methanolic solution of proline and sodium nitrite at 170°C in a sealed tube. This has provided the model system in which the effects of various additives on the formation of NNP have been studied.

Thus the effects of halide and thiocyanate ions, which have been reported as catalysing the formation of nitrosamines (Ridd, 1961; Boyland, Nice & Williams, 1971); and also the similar effects of phenolic compounds (Challis & Bartlett, 1975) have been tested.

The effects of ascorbic acid (Mirvish *et al.*, 1972) and other antioxidants (Sen *et al.*, 1974), which have been reported to inhibit nitrosamine formation, have also been examined. In this system the antioxidant ethoxyquin has been found to reduce the formation of NNP substantially, (and its effect is even more marked in bacon itself). Each of the experiments reported here has been performed several times, and the same results have been obtained in each case.

Materials and methods

Bacon used in these experiments was either normal commercial back bacon, or similar material prepared in the laboratory by an in-pack curing procedure (Coleman, Hannan & Osborne, 1974) using salt and sodium nitrite only, to final levels of 5% w/w and 200 ppm respectively.

Reagents used were of analytical grade, or of the purest grade commercially available.

Nitrosoproline was prepared essentially by the method of Lijinsky, Keefer & Loo (1970) except that the initial product was extracted from the reaction mixture with ethyl acetate which, after drying over anhydrous sodium sulphate, was removed under reduced pressure.

Bacon was cooked by grilling in a 'Baby Belling' electric cooker,* at approximately 70 mm from the grill element, operating at its maximum setting. The rashers were held flat during cooking by being clipped between the two halves of a specially made grid, centrally located in a grill-pan which filled the inside of the oven space. Rashers were grilled for 4 min on one side followed immediately by 2 min on the other; the resulting bacon being moderately well-cooked, the fat slightly crisp, but the lean still succulent. To follow the temperatures attained during grilling, 30-gauge copper-constantan thermocouples were inserted into the rasher at various points in both the lean and adipose tissues. Temperature measurements were made at approximately 10-sec intervals, using a 'Comark' thermocouple meter (type 160c). Both the cooked rasher, and the cook-out fat, which collected in the grill-pan, were analysed for nitrosamines by the method of Telling *et al.* (1971) using the GC-MS procedure.

To prepare rendered pork or bacon fat, the tissue was macerated in a beaker, warmed gently on a steam-bath, and the fat released filtered through glass-wool. Pyrrolidine was estimated by gas-chromatography on an 18 ft column containing 10% w/w of 'Carbowax 20M' with 5% w/w of potassium hydroxide as a stationary phase, on 80–100 mesh acid-washed Celite as support. The column was run isothermally at 63°C, and the identity of the pyrrolidine confirmed by mass-spectrometry.

For experiments with the model system a reaction mixture containing 800 ppm of proline and 200 ppm of sodium nitrite in methanol was normally used. In some experiments the reaction mixtures were heated in sealed tubes made from 10 mm diameter tubing, containing 3–4 ml, and heated in an oil bath for an hour after reaching 170°C. In other experiments much smaller volumes of the reaction mixtures were sealed in m.p. tubes, and dropped into the oil bath at 170°C for the required length of time. From such reaction mixtures, the NNP yields were measured by gas-chromatography by applying 5 μl aliquots to a 5 ft polythene glycol column in a Pye 104 chromatograph fitted with a FID detector, running isothermally at 175°C.

Nitrite was estimated by a method developed from that of Shin (1941).

Results

Preliminary experiments with bacon

Temperatures attained during grilling. Figure 1 illustrates temperatures achieved during grilling: it will be seen that the lean hardly exceeds 100°C, whilst the fat approaches 200°C at the end of the cooking period.

* Belling & Co. Southbury Rd, Enfield, Middlesex.

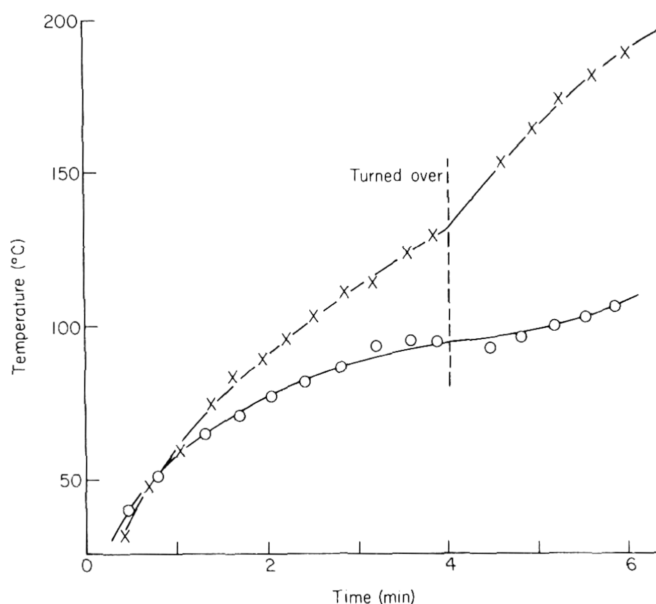

Figure 1. Temperatures attained during grilling of bacon rashers: ○, lean; X, fat.

Distribution of nitrosamines. No NNP could be detected in raw commercial bacon when analysed by the GC-MS method; and no volatile nitrosamines could be detected in boiled ham. Both DMN and NNP could be detected in grilled or fried bacon. In view of the difference in temperature achieved by the lean and fat during cooking, the distribution of nitrosamines between the lean and fat was investigated in some 1600 g of laboratory-cured bacon, having a nitrite content of 207 ppm immediately before cooking. This material was divided into two equal portions, one of which was cooked as intact rashers; the other being dissected into lean and fat, and the two tissues then being cooked separately.

Table 1 gives the results of this experiment.

Table 1. Distribution of nitrosamines between lean and fat of cooked bacon

Row	Bacon cooked as	Bacon fraction	Fraction weights (g)		Nitrosamines (ppb)	
			Raw	Cooked	DMN	NNP
(a)	Lean and	Lean	410	228	3.4	0.3
(b)	fat separately	Fat	276	53	8.4	74.3
(c)		Cook-out fat		169	8.3	22.7
(a) + (b)	Totals		686	281	4.3	14.2
(d)	Intact rashers	Rasher	658	326	3.8	10.4
(e)		Cook-out fat		131	9.9	21.6

Compare (a) + (b) with (d); and (c) with (e).

Organic precursors of N-nitrosopyrrolidine. A 150 g sample of rendered pork fat was heated at 170°C for 1.5 hr, in a slow stream of nitrogen, which was subsequently passed through 7 ml of M HCl in a Pettenkofer tube. The fat was then cooled, and extracted twice with 100 ml portions of M HCl, the extract evaporated to dryness under reduced pressure, and 2 ml of ether added to the residue. The free base was liberated with 20% w/v NaOH, and dried over sodium sulphate. A 10 μl aliquot was examined for pyrrolidine by gas-chromatography. The HCl from the Pettenkofer tube was similarly examined.

Other samples of pork fat, both heated and unheated were similarly examined, and the results are given in Table 2.

Two 125 g samples of rendered bacon fat from the same batch of laboratory-cured bacon were heated, under reflux, with stirring for 10 min after reaching 170°C. To one of the samples 0.1 g of proline dissolved in 0.5 ml of water, was

Table 2. The pyrrolidine content of heated and unheated pork fat

Pork sample	Pyrrolidine (ppb)			
	Unheated	Heated		
		Fat	Volatiles	Total
A	37	—	—	—
B	111	—	—	—
C	148	630	445	1075
D	—	370	104	474
E	—	185	222	407
F	—	148	74	222
Mean	98.7			544.5

Table 3. Effect of added proline on nitrosamine yields

Expt. no.	Pork sample	Nitrite added (ppm)		Proline added (ppm)		Nitrosamines (ppb) Rendered fats	
						DMN	NNP
1	A	200		—		0.2	0.2
	A	200		800*		1.0	2550
2(a)	B	200		—		9.0	0.3
	B	200		333†		16.6	17.7
2(b)	C	200		—		13.6	4.5
	C	200		333†		13.2	62.0
		Added	Measured	Added	Measured	Cook-out fats	
3	D	800	298	—	82	2.7	17.5
	D	800	292	200†	168	2.9	62.0
	D	800	285	400†	332	2.9	135.5

*Added to rendered fat. † Added to curing brine.

added before heating commenced. The fat was then cooled and analysed for nitrosamines by the GC-MS method. In a second experiment, proline was added to the curing brine of half the packs of a sample of laboratory-cured bacon, before rendering and heating the fat as before. This was repeated on a second sample of bacon, and the results of these experiments are given in Table 3. A third experiment was performed in which a batch of laboratory-cured bacon was divided into three portions, and proline was added at the equivalent of 200 and 400 ppm to two of them. All three portions of bacon were subsequently cooked, and the cook-out fats analysed for nitrosamines by the GC-MS procedure. For this third experiment the nitrite was increased to 800 ppm added, so that it should not be limiting to the yield of nitrosamines. Both the nitrite (Shin, 1941) and the proline contents (Chinard, 1952) were measured in the bacon, immediately before cooking. The results are also given in Table 3.

Presence of precursors in pork fat. The polar fraction was obtained by extracting a solution of 100 g of rendered pork fat in 300 ml of petroleum ether with three portions of 95% methanol, saturated with petroleum ether (Nichols, 1964). The combined methanolic extracts were washed three times with petroleum ether (saturated with 95% methanol), and evaporated to dryness. The residue was refluxed for 2.5 hr with ethanolic HCl, and the hydrolysate analysed for amino-acids. Table 4 gives the M % of possible precursors of pyrrolidine.

Development of the model system

Yield of pyrrolidine from precursors. To compare yields, 0.1 g samples of the various amino acids were heated in 20 ml portions of tetralin (Chatelus, 1964) for 1 hr at 170°C. After cooling, the mixtures were extracted with 20 ml portions of M HCl, and the extracts washed several times with ether. The washed extracts were evaporated to dryness under reduced pressure, 10 ml portions of ether added, and the solutions made alkaline with 20% NaOH. After drying over sodium sulphate, the solutions were analysed for pyrrolidine by gas-chromatography. Table 5 gives the yields.

Table 4. Pyrrolidine precursors in pork polar lipids

Amino acids	Mole (%)
Ornithine	Trace
Hydroxyproline	0.7
Citrulline	0.7
Arginine	7.4
Proline	2.1

Table 5. Pyrrolidine yields from various precursors after heating in tetralin

Precursor (100 mg)	Pyrrolidine (μg)
Ornithine	2
Hydroxyproline	7
Citrulline	18
Arginine	18
Proline	9722

In addition to pyrrolidine, pyrroline is also formed. Thus in another experiment when 100 mg of proline was heated in 20 ml of tetralin for 1 hr at 170°C, 8.4 mg of pyrrolidine was obtained, and 0.8 mg of pyrroline.

When proline was heated in water at 170°C for 1 hr, in a sealed tube, no pyrrolidine was detected. But when proline was heated in mixtures of tetralin and methanol, or in pure methanol, pyrrolidine was found in significant amounts (see Table 6): only in mixtures containing methanol was the proline in solution.

Formation of N-nitrosopyrrolidine in a model system. A series of reaction mixtures were prepared in methanol containing 800 ppm of proline, and nitrite ranging from 32 to 3200 ppm. These were heated in 10 mm diameter sealed tubes for 1 hr after reaching 170°C; and on cooling the contents were analysed for NNP by gas-chromatography. The results are given in Table 7.

Table 6. Pyrrolidine yield from proline heated in tetralin-methanol mixtures

Solvent mixture		Pyrrolidine yield
Methanol (ml)	Tetralin (ml)	(mg)
0	3	0.24
1	2	30.2
2	1	3.9
3	0	3.9

Table 7. Effect of nitrite concentration on NNP yield from heating proline in methanol

Nitrite (ppm)	Yield of NNP (ppm)
32	1.5
200	9.0
320	10.7
400	15.7
800	22.7
1600	29.4
3200	31.1

The effect of varying the proline concentration in methanolic reaction mixtures containing a fixed level of 200 ppm of nitrite was investigated using MP tubes heated for 1 hr at 170°C (see Table 8).

The effect of heating time on the NNP yield for reaction mixtures containing 800 ppm of proline and 200 or 400 ppm of nitrite in methanol was similarly investigated (see Table 9).

Table 8. Effect of proline concentration on NNP yield when heated with nitrite in methanol

Proline (ppm)	Yield of NNP (ppm)
10	0.65
50	1.3
100	1.4
250	2.0
500	2.8
750	7.4
1000	9.1

Table 9. Effect of heating time on NNP yield for mixtures of proline and nitrite in methanol

200 ppm nitrite		400 ppm nitrite	
Time (min)	Yield on NNP (ppm)	Time (min)	Yield of NNP (ppm)
0	0	10	5.1
2	0.2	20	6.1
4	0.45	30	8.1
8	0.9	40	10.8
12	1.3	50	14.3
24	1.9	60	17.0
48	3.6		
60	6.5		

Table 10. Effect of temperature on NNP yield from heating proline/nitrite mixtures in methanol

Temperature (°C)	Yield of NNP (ppm)
140	0.08
150	0.16
160	0.5
170	0.9
180	2.0
190	2.7
200	3.4

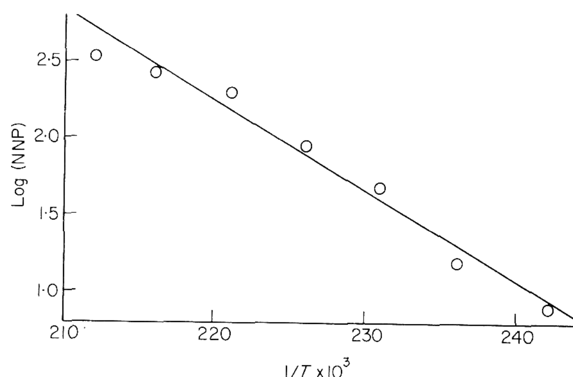

Figure 2. Arrhenius plot for the formation of N-nitrosopyrrolidine in the model system.

The effect of temperature was investigated using a reaction mixture containing 800 ppm proline and 200 ppm of nitrite in methanol which was heated in MP tubes at the required temperature for 10 min (see Table 10).

A plot of log(NNP) against the reciprocal of the absolute temperature is illustrated in Fig. 2, and from this energy of activation may be calculated as 27.2 kcal.

The effect of the addition of water is illustrated in Table 11 for a reaction mixture containing 800 ppm or proline, 400 ppm of nitrite heated for 30 min at 170°C in MP tubes.

Formation of NNP from various precursors. A series of reaction mixtures were prepared, each containing a 10 mg aliquot of one of the various precursors of pyrrolidine previously examined, and 10 ml of a methanolic solution of 1000 ppm of sodium nitrite. These were sealed in 10 mm tubes, and heated at 170°C in an oil bath for 1 hr. The contents were then analysed for NNP by GLC. Table 12 gives the results.

Decomposition of nitrosoproline. Solutions containing 100 or 1000 ppm of nitrosoproline were heated in aqueous or methanolic solution in 10 mm diameter sealed tubes for 10 min after reaching 170°C. After cooling the contents were analysed for free proline colorimetrically (Chinard, 1952) and for pyrrolidine and NNP, by gas-chromatography. The results are given in Table 13.

Table 11. Effect of water on NNP yield from heating proline/nitrite mixtures in methanol

Water (% v/v)	Yield of NNP (ppm)
1	3.0
2	2.5
4	1.9
10	1.45
20	0.2
100	0.0

Table 12. Yield of NNP from various precursors heated with nitrite in methanol

Precursor (10 mg)	Yield of NNP (ppm)
Pyrrolidine	62.2
Agmatine	30.4
Putrescine	42.6
Proline	12.4
Ornithine	6.1
Arginine	3.3
Citrulline	1.5

Table 13. Products of heating nitrosoproline in solution

Nitrosoproline (ppm)	Proline (ppm)	Pyrrolidine (ppm)	NNP (ppm)
Heated in methanol			
1000	39	2.44	0.8
100	—	0	0.4
Heated in water			
1000	96	3.4	0.16
100	18	1.56	—

Samples of nitrosoproline solutions in methanol containing 1000 or 10000 ppm were heated for an hour at 170°C in sealed 10 mm diameter tubes. Aliquots of the reaction products were applied to an 18 ft polyethylene glycol column coupled to a mass-spectrometer. Products other than pyrrolidine and NNP included pyridazine and methyl pyridazine. Tentative identification was obtained for pyridine, pyrrole and acetic acid, but fifteen other components have not yet been identified.

Use of model system for testing additives

Halide and thiocyanate ions. Table 14 illustrates the effects of the addition of potassium chloride, bromide and iodide and ammonium thiocyanate at a final concentration of 3.3 mM to the standard reaction mixture containing 400 ppm of sodium nitrite, and 800 ppm of proline, in methanol.

Lipid hydroperoxide. Table 15 illustrates the effect of the hydroperoxide of 2–oleodistearin at 2000 ppm on the NNP yield (with a control containing 2–oleodistearin), in a methanolic reaction mixture containing 400 ppm of sodium nitrite and 800 ppm of proline.

Phenolic compounds. Reaction mixtures containing 400 ppm of sodium nitrite and 800 ppm of proline in methanol were heated with the addition of gallic acid, chlorogenic acid or caffeic acid in equimolar amounts to the proline

Table 14. Effects of halide and thiocyanate ions on NNP yield from heating proline/nitrite mixtures in methanol

Additive (at 3 mM)	NNP yield (ppm)
KCl	9.75
KBr	9.5
KI	9.25
NH_4CNS	22.5
None	8.5

Table 15. Effect of lipid hydroperoxide on NNP yield from heating proline/nitrite mixtures in methanol

Additive (at 2000 ppm)	NNP yield (ppm)
None	11.9
2–oleodistearin	11.5
2–oleodistearin hydroperoxide	18.3

Table 16. Effect of phenolic compounds on NNP yield from heating proline/nitrite mixtures in methanol

Expt no.	Additive (7 mM)	Residual nitrite (ppm)	NNP yield (ppm)
1	None		11.8
	Gallic acid	—	6.1
	Chlorogenic acid	—	40.4
	Caffeic acid	—	27.2
2	None	211	11.2
	Gallic acid	0	4.6

present. Table 16 gives the results for these experiments. Since gallic acid was the only compound showing a reduction in NNP, this was repeated, with the additional step of measuring the residual nitrite after heating. This is also illustrated in Table 16.

Ascorbate. Reaction mixtures containing 800 ppm of proline and 400 ppm of nitrite in methanol were sealed in 10 mm tubes with varying levels of ascorbic acid. The tubes were heated in a 'Techne Driblock' DB 3H adjusted to 170°C; but on insertion of the tubes the block fell to 161°C, and did not subsequently rise above 166°C. The heating was prolonged for 90 min, but the yield in the control tube was low. The results are given in Table 17.

Table 17. Effect of ascorbate on NNP yield from heating proline/nitrate mixtures in methanol

Ascorbic acid added (ppm)	Residual nitrite (ppm)	NNP yield (ppm)
0	330.1	2.7
100	265.0	32.8
250	219.4	37.5
500	145.0	26.0
750	101.0	22.3
1000	62.3	14.9
2000	0.0	1.35

Table 18. Effect of antioxidants on NNP yields from heating proline/nitrite mixtures in methanol

Antioxidant (1% w/v)	NNP yield (ppm)
None	5.2
BHT	4.0
BHA	3.0
Ethoxyquin	1.6

Table 19. Effect of ethoxyquin concentration on NNP yield from heating proline/nitrite mixtures in methanol

Ethoxyquin added (% w/v)	NNP yield (ppm)
None	12.9
0.25	9.3
0.5	4.5
0.75	4.8
1.0	3.9
1.25	3.3

Antioxidants. Reaction mixtures were prepared containing 800 ppm of proline and 200 ppm of nitrite with 1% w/v of three antioxidants. These were sealed in capillary tubes and heated for 1 hr at 170°C: Table 18 gives the respective yields of NNP in the mixtures containing butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and ethoxyquin.

The effect of the varying level of ethoxyquin was tested in a reaction mixture containing 500 ppm of proline and a large excess of nitrite, of 2500 ppm, heated at 170°C for 45 min in MP tubes. Table 19 gives the results.

Table 20. Effect of ethoxyquin on residual nitrite level after heating proline/nitrite mixtures in methanol

Proline added (ppm)	Ethoxyquin added (% w/v)	Nitrite (ppm)		NNP yield (ppm)
		Added	Residual	
—	—	400	399	—
—	0.33	400	379	—
800	—	400	252	12.4
800	0.33	400	254	8.2

Table 21. Effect of ethoxyquin at various heating times of proline/nitrite mixtures in methanol

Heating time (min)	NNP yield (ppm)	
	Control	0.33% w/v ethoxyquin
15	9.0	9.8
30	13.2	7.8
45	15.2	8.4
60	25.2	8.4

The effect of ethoxyquin at 0.33% on the level of residual nitrite in reaction mixtures containing 400 ppm of sodium nitrite and 800 ppm of proline is illustrated in Table 20 and its effect at the same concentration for various heating times on similar reaction mixtures is illustrated in Table 21.

Discussion

The results given in Table 1 show that more than 92% of the total nitrosamines, and more than 99% of the NNP is found in the fatty portion of grilled bacon: this observation is in agreement with that of Fiddler *et al.* (1974).

As Scanlan (1975) has previously suggested, and as the present work has confirmed experimentally, the adipose tissue reaches much higher temperatures during grilling than the lean. This indicates that either the formation of NNP itself, or of its precursor, requires a high temperature. If, as proposed by Ender & Ceh (1971) and as the present work suggests, proline is the organic precursor of NNP, the high temperature reaction would presumably be a decarboxylation. This might be the direct decarboxylation of proline with the formation of pyrrolidine, which is then nitrosated: or the nitrosation of proline which then decarboxylates to yield NNP (Lijinsky & Epstein, 1970). However, the present results show that on heating in solution, the yield of NNP from nitrosoproline is small; the molecule appears to lose both the carboxyl group and the nitroso group to yield pyrrolidine; of course this could then nitrosate readily in the presence of nitrite.

However, the presence of more than sufficient proline in pork fat, (Wasserman & Spinelli, 1972; Gray *et al.*, 1976) to account for the observed levels of NNP in bacon fat; the substantial increase in pyrrolidine levels when pork fat is heated; and the ready nitrosation of pyrrolidine in the presence of nitrite, reported in the present work, all indicate that proline is the likely precursor of NNP. The direct proportionality between the yield of NNP in the cook-out fat, with the level of free proline at the time of cooking, provides additional evidence for this concept. It may be noted that proline decarboxylates at a lower temperature than most amino acids (Wendlandt, 1960).

The inhibitory effects of water on nitrosamine formation reported here, suggest certain conclusions in relation to nitrosamine formation in bacon. First in bacon, the evaporative cooling effect of water in the lean tissue prevents the attainment of temperatures necessary for decarboxylation. Secondly, the reduction of NNP yield with increasing water content of the model system suggests a more direct chemical effect, and this is confirmed by the absence of proline decarboxylation in water, even when heated to 170°C. This could be a second factor in restricting nitrosamine formation to the fatty tissue, since only this portion can provide a non-aqueous environment during cooking. This also suggests that the conditions for the nitrosation reaction in aqueous solution may be irrelevant to the situation in cooking bacon.

The requirement for a high temperature, the inhibitory effects of water and antioxidants, and the catalytic effect of a lipid hydroperoxide, are consistent with the involvement of a free-radical in the formation of NNP. This might also explain (see Table 19) the diminishing effect of the inhibitor ethoxyquin with increasing concentration. It is of interest to consider what the effective nitrosating species might be. From the results with ascorbic acid in the model system, it would appear to be a product of the reaction between ascorbate and nitrite; since although the residual nitrite level may be greatly reduced, the NNP yield is greatly increased until a very large excess of ascorbate is added. The initial product of the nitrite-ascorbate reaction is nitric oxide; and to test this as a nitrosating agent, 4 ml of an 800 ppm solution of proline in methanol was heated with 2.5 ml of the gas, in a sealed tube for 1 hr at 170°C. This gave a yield of 59 ppm of NNP, approximately twice that expected for an equimolar amount of sodium nitrite. The reaction tube was flushed with nitric oxide for 10 min before sealing to remove oxygen.

The phenolic substances which increase the yield of nitrosamines during nitrosation in aqueous solution (Challis & Bartlett, 1975) also do so in the model system; the exception, gallic acid, which actually reduces the yield of NNP, apparently does so by removing the nitrite present.

Clearly ethoxyquin does not act in this way, since it does not significantly affect the level of residual nitrite. Even at high levels of ethoxyquin, some NNP is formed; and its effect at various heating times reveals an initial formation of NNP (during the first 15 min of heating), which is not inhibited by ethoxyquin. Further formation of NNP is thereafter inhibited; which suggests that NNP may be formed in the model system by two routes, only one of which is susceptible

to ethoxyquin inhibition. Ethoxyquin is very effective in bacon, as an inhibitor of nitrosamine formation (Coleman, 1976) and its effects in this context will be described elsewhere.

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Technical note: Lead and copper uptake by food prepared in tinned-copper utensils

C. REILLY

Introduction

Tinned metal utensils have been in use for food preparation since Roman times. The relatively low melting point of tin (232°C) and the ease with which a layer can be applied to other metals by dipping or wiping permitted its use as a protective plate on other less-resistant metals even in technologically unsophisticated ages. Today we recognize that the electrochemical relations between tin and other metals, as well as its surface activity and non-toxicity, make it an ideal material for surfacing food handling and processing equipment fabricated from stronger metals.

While the main use of tin in the food industry is as tinplate in canning, a certain amount is also used as a coating on copper cooking utensils. Because of the toxic nature of some salts of copper as well as its catalytic effect on fat oxidation, it is desirable to place a barrier between the metal and food. Thus it is normal practice to cover the surface of copper utensils and food processing equipment with a layer of tin. This metal is non-toxic, but pure tin is not normally used for plating. In fact, 'Standard tin' sold on the London Metal Exchange may contain up to 0.1% lead. While it is accepted that 'the tin used for tin plate and for hot-tinning fabricated articles should be of high quality, low in lead' (Hedges, 1960), a Cooking Utensils (Safety) Regulation issued by the Home Secretary in December 1972 under the Consumer Protection Act of 1971, allows a maximum of 0.2% lead in the tin plate. This Regulation superseded a 1964 British Standards Specification which had allowed a higher level of 0.25% lead. There is evidence that tinned cooking utensils which do not conform to this regulation, or even to the earlier British Standards Specification, are sometimes sold and used at the present time. In 1966 the Consumer Association found tinned frying pans which contained between 40 and 60% lead in the tin layer on sale to the public (Consumer Association, 1973). We have shown that some tinned copper saucepans still in use in a restaurant were capable of contributing a high level of lead to food (Reilly, 1976).

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The present report is a further contribution to this investigation. A random sample of food cooked under normal restaurant conditions was tested for lead and copper. In addition, a selection of dishes of a more acid and less usual nature, though still standard restaurant fare, was made. Identical portions were prepared in aluminium and tinned copper utensils. The cooked food, as well as the raw materials, were analysed for the two metals. The results show that lead uptake is related to the type of food and conditions of cooking as well as to the state of wear and length of use of the utensils.

Materials and methods

Food and food preparation

Food was obtained from commercial restaurant suppliers and was prepared according to widely used catering procedures (Cracknell & Kaufmann, 1972). Samples analysed were of two types:

Normal restaurant fare. A complete luncheon as served to customers and consisting of soup (tomato) and bread rolls, main course (lamb chop, boiled new potatoes and peas) and dessert (apple pie), was taken. Total edible portions of the main course, as well as the whole serving of apple pie, were homogenized separately and 10 g (wet weight) samples of the homogenates, in triplicate, as well as similar weights of the liquid soup and the bread rolls, used for analyses as described below.

Selected foods. The individual dishes examined were: *Whiting à la Portuguese* (recipe number 190, Cracknell & Kaufmann) in which the fish is simmered for 30 min in oil; *Poulet sauté bourguignonne* (number 829), chicken cooked for 1 hr in a red wine sauce; *Chou rouge flamande* (number 960), red cabbage cooked for 2 hr in vinegar followed by a further 45 min heating with cooking apples; *Pommes Delmonico* (number 1052), potatoes gently heated in milk for 1 hr; *Sorbet au Citron* (number 1364), prepared by boiling sugar and lemon juice in water to a thick syrup and then freezing.

Cooking utensils: (i) Aluminium pans, regular kitchen stock; (ii) copper pans also in regular use in kitchen with, in most cases, evidence of wear on tin surface; (iii) copper pans of similar vintage to (ii) but which had been in store and unused since purchase. In the case of uncooked food, all the raw materials used in preparation of a particular dish were homogenized together and samples taken for analysis.

Preparation and analysis of samples: Triplicate 10 g samples (approx. wet weight) were taken, weighed, dried in hot air oven at 70°C to constant weight, which was recorded, and ashed overnight at 500°C in a muffle furnace. The ash was dissolved in 2 cm^3 N HCl and centrifuged. This solution was used directly, without further treatment, for analysis by atomic absorption spectrophotometry.

Solutions were analysed according to the standard procedures using EEL 240 Model Spectrophotometer and results expressed as the mean of three samples.

Table 1. Lead and copper contents of dishes served at luncheon

Food	Metal content (mg/kg, wet weight mean of three samples)	
	Lead	Copper
Soup (tomato)	0.08	0.35
Bread rolls	0.58	0.40
Main course (lamb chops, potato, peas)	0.38	1.71
Apple pie	0.21	2.73

Standard solutions of copper and lead were prepared from stock standards supplied by BDH Chemicals.

When necessary, analytical solutions were diluted using distilled, ion-free water.

Results and discussion

Lead and copper contents of typical luncheon fare

Table 1 shows the average lead and copper content of the food eaten in a meal served to a customer in a traditional restaurant. The food was prepared using a selection of different cooking utensils, including aluminium and stainless steel as well as tinned and untinned copper vessels. A tinned steel mixing bowl was used in making the bread rolls. The lead contents of all samples were well below the present statutory limit of 2 mg/kg for all but certain specified foods (MAFF 1975a). Except for the soup, levels were, however, higher than the average of 0.09 mg/kg for foods in general reported by the Working Party on the Monitoring of Foodstuffs for Heavy Metals (MAFF 1975b). There is no general permitted maximum levels for copper but the Food Standards Committee recommends a limit of 20 mg/kg in most foods and 2 mg/kg in ready-to-drink beverages. In addition, there are specific restrictions in the case of gelatin to a maximum of 30 mg/kg and for tomato ketchup 20 mg/kg under Food Standards Orders (Pearson 1976). The foods served in the restaurant luncheon were well below all these limits.

Selected foods

Table 2 summarizes the results of analyses of the different food dishes, prepared as described. Results are given both in relation to wet and dry weight. The former is used since statutory limits are expressed in this manner and also is easier to interpret in relation to food portion sizes in catering. Dry weights, however, give a clearer idea of actual increases or decreases of metal content of food following different methods of preparation. With regard to lead, it is clear that cooking of food in tinned utensils results in an increase in content of

Table 2. Changes in copper and lead contents of food (mg/kg) after preparation in aluminium and tinned-copper utensils

	Fish		Chicken		Cabbage		Potato		Sorbet		
	Pb	Cu	Pb	Cu	Pb	Cu	Pb	Cu	Pb	Cu	
Uncooked	0.31 (1.87)	0.82 (6.38)	0.14 (1.05)	2.21 (17.15)	0.15 (0.55)	1.36 (7.36)	0.19 (0.67)	3.10 (10.89)	0.27 (0.41)	1.46 (2.04)	wet wt. (dry wt.)
Aluminium utensils	0.36 (1.91)	1.37 (7.23)	0.21 (1.04)	2.52 (6.67)	0.18 (0.53)	1.04 (5.88)	0.16 (0.72)	1.87 (8.40)	0.19 (0.36)	1.28 (2.26)	wet wt. (dry wt.)
Tinned-copper (old)	0.42 (2.00)	5.70 (27.01)	0.25 (1.62)	6.36 (15.35)	0.29 (0.66)	2.07 (8.01)	0.22 (0.84)	2.39 (9.07)	0.58 (0.70)	2.00 (4.43)	wet wt. (dry wt.)
Tinned-copper (unused)	1.09 (4.22)	2.24 (11.47)	0.94 (3.14)	4.05 (13.56)	0.79 (3.29)	1.93 (5.74)	0.26 (1.90)	1.88 (7.32)	0.88 (1.10)	2.96 (3.68)	wet wt. (dry wt.)

the metal. The state of wear of the tinning is reflected in the level of lead uptake, with only low level accumulation in food cooked in well-used pans. Cooking in pans with intact tinning gives lead values in most cases close to the recently recommended general maximum of 1 mg/kg (MAFF 1975a). Nevertheless, though all the dishes were deliberately chosen for their acidity and are, presumably, more plumbosolvent than the ordinary lunch menu, lead uptake was considerably less than was the case in a previous study (Reilly, 1976) in which tomato soup was found to contain 0.80 mg/kg (wet weight) when prepared in worn saucepans and 3.17 mg/kg when previously unused tinned copper saucepans were used.

In the earlier study it was also found that the tinning on some unused saucepans contained 0.28% lead (0.08% above the legally permitted maximum), while worn tin plate had as little as 0.072% of the metal.

The results for copper show, as might be expected, a higher level in food cooked in saucepans with worn tinning than in copper pans with intact plating or in aluminium utensils. In no case does the level of copper exceed the recommended limit of 20 mg/kg wet weight. On the contrary, it should be noted that several of the foods, even when cooked in copper vessels, suffered a marked loss of copper. This type of loss of essential trace elements during food processing may be of some significance if the availability of the metals in fresh foods is limited.

The Inter-Departmental Working Group on Heavy Metals in its paper on 'Lead in the Environment . . .' (Department of the Environment, 1974) noted that tin-coated utensils can contaminate food with lead. The results given here show the levels of lead contamination which may be expected in meals served in a restaurant where traditional tinned-copper utensils are used. When referring to the corresponding problem of lead pick-up by food from some poorly made glazes on ceramic ware, the Working Group recommended that 'amateur potters, including those working at schools or colleges, should restrict the use of their glazed ware to decorative purposes only unless evidence of its suitability for cooking or food storage is available'. While not suggesting the same restriction on the tinned saucepans, it would seem that, at the least, care should be taken to see that those in use conform to the present regulations which limit the amount of lead which may be present. Perhaps even this level should be kept under review and, if possible, the permitted amount of lead reduced or at least methods of making the metal less easily extracted by food, investigated. However, according to the professional tinners, the figure of 0.2% lead is the finest limit at which it is practical to tin anything on a production basis (Barrett, 1977). Almost 40 years ago when the situation was far worse and as much as 20% lead was to be found in some tin plate, Monier-Williams (Ministry of Health, 1938) pointed out that lead alloys used in plating could contaminate food. His appeal for a reduction of the amount of lead entering food in this way to the lowest possible limit was finally answered by the 1964 British Standard. But, when appealing for a lower limit, Monier-Williams wondered whether 'any limit, however small, for a cumulative poison can be regarded

as safe'. Others today echo his doubts and would like to see lead prohibited as a constituent of any container in which food is stored or cooked (Bryce-Smith & Waldron, 1974). This view is not shared by the Food Additives and Contaminants Committee which points out that it can be misleading to concentrate attention on extreme cases of lead contamination. Most people eat a mixed diet and receive a lead intake of less than half the FAO/WHO 'provisional tolerable weekly intake' of 3.0 mg for adults. While it is true that a constant diet of *Whiting à la Portuguese* or *Poulet sauté bourguignonne*, cooked in the manner described here, might result in a build up of lead in the body, the consumption of the type of food served in the restaurant for normal luncheon, even if this were consumed at each meal of the day, would, on the assumption that the total weekly intake of food is 10.5 kg and that the main course only is eaten, contribute approximately 4 mg of lead to the body of an adult. It is, of course, highly improbable that anyone would eat a diet entirely composed of such food, though the situation could arise with hotel staff who take all their meals in the hotel or in families where tinned kitchen utensils are used.

Acknowledgment

The technical assistance of A. Hirst and N. Maynard is gratefully acknowledged.

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Book reviews

Energy Saving Techniques for the Food Industry. Ed. by M. E. Casper. New Jersey: Noyes Data Corporation, 1977. Pp. xiv + 657. U.S. $39.00.

The book is based on two reports entitled *Energy Efficiency Improvement Targets, Food and Kindred Products Industry* and *Industrial Energy Study of Selected Food Industries* prepared for the U.S.A.'s Federal Energy Administration in order to develop energy improvement targets for 1980. The first chapter is concerned with a general description of the food industries in the U.S.A. with particular reference to the structure and operations, energy-use patterns and energy conservation achievement and programme. The methodology used to establish an energy efficiency improvement target is discussed in Chapter 2. The third chapter is concerned with a general description of energy efficiency improvement measures – tables show the energy conservation techniques and procedures which were considered during the survey. The conversion from the use of scarce energy forms is discussed in Chapter 4. Government regulations can directly affect the energy use requirements of industry and this aspect is reviewed in the next chapter. The energy efficiency improvement target which should be achievable by 1980 is defined in Chapter 6. The remaining forty-seven chapters of the book give for each industry a description of the structure, energy consumption patterns, energy conservation achievement and programmes, energy efficiency improvement analysis, and the technological, economic and legal considerations affecting the industry. Industries described include sugar and sugar confectionery products, corn milling and baker products, pasta products, cereal breakfast foods, meat packing and meat products, fish and seafoods, oils and fats, milk and dairy products, poultry and egg processing, pet food, fruit and vegetables, soft drinks, wines and spirits, beverages, chocolate and cocoa products, and pickles, sauces and salad dressings.

The data presented will be of interest to food manufacturers in the United Kingdom, and some engineers will wish to ascertain the practicability of applying suggested savings measures in their factories.

Two, perhaps minor, criticisms of the book are the lack of an index and the quality of the printing. Fortunately each industry chapter has a similar format and therefore the required information can be found by reference to the table of contents. In order to keep the price of the book to a reasonable level the publishers have reproduced by photo offset directly from the original typewritten report and unfortunately the quality of printing leaves much to be desired.

This reference book can be recommended as desirable reading by factory management responsible for the efficient utilization of energy resources.

D. Blackburn

Tea and Soluble Tea Products Manufacture. N. D. Pintauro.
New Jersey: Noyes Data Corporation, 1977. Pp. xii + 261. U.S. $36.

The author has surveyed the U.S. and British patent literature of the last twenty years or so, which he describes as 'the largest and most comprehensive collection of technical information in the world'. This is the first of many errors, since patents by and large are not intended to impart technical data, or even define a process closely; they are designed to protect inventions from competition, even if they only exist in the imagination of the inventor. This misunderstanding runs throughout the book, and sets its tone. As a result, while the coverage is quite good, the standard of reviewing is very variable. There is a good deal of intricate detail describing individual processes, often including results that are quite unrepeatable. A good example of this is a graph on p. 17 which is supposed to illustrate the 'course of fermentation', something which will in fact vary with the source and variety of tea as well as with differences in processing. Another result of the almost verbatim account of patent claims is the unwarranted implication of precision and reproducibility.

A concise summary of the principles and mechanics of each process, followed by a few generalized and critical descriptions from patents, would have been more suitable in such a review, but in fact no criticism is made of the patentee's claims. Moreover it is unfortunate that the author has failed to achieve his intention of eliminating 'legal jargon and juristic phraseology' from the patents; instead they are served up in a largely undigested form, full of such phrases as 'preferably', 'between – and – ', and 'substantially'. The last is particularly objectionable when it appears as 'substantially completely eliminated' (p. 207).

Individual patents have been reproduced more or less intact, and are arranged sequentially so that little attempt has been made to correlate and integrate the data contained in different patents. Because of this some sections contain material which would have been placed better elsewhere.

The author's failure to be critical has led him into factual errors in several places. It is no valid excuse that these errors are originally the fault of the patentees. This book should have filled a gap in the literature, for we badly need a good modern treatise on tea technology. Regrettably in our opinion, Mr Pintauro has failed to provide this.

G. V. Stagg

Cooling Technology in the Food Industry. A. Ciobanu, G. Lascu, V. Bercescu and L. Niculescu.
Tunbridge Wells: Abacus Press, 1976. Pp. 500. £16.50.

This substantial book is a revision by the senior author of *Frigul Artificial în Fabricarea şi conserverea Produselor Alimentare*, first published by Editura Technică, Bucharest, in 1971, translated into English by Mr Ciobanu and Mr C. Sturza, with Dr John Hammel acting as translation editor. The text is, by and large, clear and readable, though one does find a minor asperity in language on, perhaps, every other page.

The book is divided into three main sections: Scientific Background, General Systems Applied in Food Refrigeration and Applications. In the first and shortest section, in two chapters, the authors discuss the biochemical, biological, nutritional and physical changes occurring in stored foods and the effect of temperature thereon. In the second section there are chapters devoted in turn to chilling, freezing, air conditioning and freeze drying – together with a particularly short chapter (barely eight pages of text) covering treatments which might be combined with refrigeration. Half of this chapter is devoted to modified atmosphere storage, while in the rest, vacuum packaging, dehydrofreezing, blanching, chemical and antibiotic treatments and irradiation are dismissed.

The final section (which comprises half the book) is written on a commodity basis and includes chapters on: Meat and Meat Products, Poultry, Fish and Fish Products, Milk and Dairy Products, Eggs, Fruits and Vegetables, Ice Cream, Prepared Foods and Fermented Beverages, together with a chapter on various products not included in the above categories and another on The Cold Chain.

The approach is that of the food technologist, rather than the refrigeration engineer. For instance, in the chapter on fruits and vegetables there are sections on the selection of raw materials, cleaning, peeling, blanching, protective additives and quality control – sections in which cooling, as such, is hardly mentioned. In writing a wide-ranging book such as this, one must decide in advance what it is to be assumed that the reader already knows. The authors, particularly in their introductory section, do not really come to grips with this problem. The terms 'solute' and 'solvent' are given formal definitions, yet a few pages later 'colligative', 'dendrite' and 'evanescent spherulite' are introduced without any explanation as to their meaning. However, the reader with even an elementary knowledge of food technology should have no problem with the text.

Rather unusually, for a technical book published in England today, C.G.S. units are employed throughout. An appendix lists conversion factors between C.G.S., S.I. and Imperial measures. Other appendices give a table of the enthalpies of a variety of foods over the temperature range +30 to –30°C together with Mollier and Carrier Psychrometric Charts (this last being in Imperial units). Symbols used in mathematical sections of the work are

variously defined in the text or listed at the *end* of the appropriate section – which can cause some confusion at first reading.

Copious references are given throughout to published sources – both to general texts and specialist original papers. In the earlier sections some of the references to general texts are, unfortunately, to works in Romanian. One would have thought that, in preparing a work for English-speaking readers, equivalent texts in English could have been cited. On the other hand, the research citations are largely to works in English, French and German. Surprisingly few references to Eastern European publications are given.

In revising the text, the citations have been brought up to the year 1975. Taking, at random, the chapter on freezing, almost 40% of the 197 references are from the period 1972–75. Yet, even with this extensive revision, the book has rather a traditional air. For instance, the section on freezing times discusses Planck's equation for four pages, yet dismisses computer methods of freezing time estimation in two lines and three references. There is little discussion of the limitation of Planck's equation nor is it appreciated that the formulation given in equation 4.5 embraces those given in equations 4.3 and 4.6. Indeed, throughout the book the smell of the paste and the snip of the scissors is more in evidence than an independant re-assessment of the published literature. Nevertheless, even the experienced reader should find this a useful book for general reference.

The volume itself, which has been produced in Romania, is well printed and bound but, alas, is innocent of an index.

N. D. Cowell

Books received

Sweeteners and Enhancers. Food Technology Review No. 40. N. D. Pintauro.
New Jersey: Noyes Data Corporation, 1977. Pp. xi + 392. US$39.

The book reviews the American and some of the British patent literature on compounds proposed and used as sweeteners and sweetness enhancers.

Radiation Chemistry of Major Food Components. Ed. by P. S. Elias and A. J. Cohen.
Amsterdam: Elsevier Publishing Company, 1977. Pp. xii + 220. £17.00.

A collection of monographs on the radiation chemistry of lipids, proteins, carbohydrates and vitamins.

Developments in Food Carbohydrates. Ed. by G. G. Birch and R. S. Shallenberger.
London: Applied Science Publishers, 1977. Pp. x + 199. £12.50.

Five of the ten papers in this collection deal with specific problems of food technology.

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Standard usage. The *Concise Oxford English Dictionary* is used as a reference for all spelling and hyphenation. Verbs which contain the suffix ize (ise) and their derivatives should be spelt with the z. Statistics and measurements should always be given in figures, i.e. 10 min, 20 hr, 5 ml, except where the number begins the sentence. When the number does *not* refer to a unit of measurement, it is spelt out except where the number is greater than one hundred.

Abbreviations. Abbreviations for some commoner units are given below. The abbreviation for the plural of a unit is the same as that for the singular. Wherever possible the metric SI units should be used unless they conflict with generally accepted current practice. Conversion factors to SI units are shown where appropriate.

SI UNITS

gram	g	Joule	J
kilogram	kg = 10^3 g	Newton	N
milligram	mg = 10^{-3} g	Watt	W
metre	m	Centigrade	°C
millimetre	mm = 10^{-3} m	hour	hr
micrometre	μm = 10^{-6} m	minute	min
nanometre	nm = 10^{-9} m	second	sec
litre	l = 10^{-3} m^3		

NON SI UNITS

inch	in	= 25·4 mm
foot	ft	= 0·3048 m
square inch	in^2	= 645·16 mm^2
square foot	ft^2	= 0·092903 m^2
cubic inch	in^3	= 1·63871 × 10^4 mm^3
cubic foot	ft^3	= 0·028317 m^3
gallon	gal	= 4·5461 l
pound	lb	= 0·453592 kg
pound/cubic inch	lb in^{-3}	= 2·76799 × 10^4 kg m^{-3}
dyne		= 10^{-5} N
Calorie (15°C)	cal	= 4·1855 J
British Thermal Unit	BTU	= 1055·06 J
Horsepower	HP	= 745·700 W
Fahrenheit	°F	= 9/5 T°C + 32

Figures. In the text these should be given Arabic numbers, e.g. Fig. 3. They should be marked on the backs with the name(s) of the author(s) and the title of the paper. Where there is any possible doubt as to the orientation of a figure the top should be marked with an arrow. Each figure must bear a reference number corresponding to a similar number in the text. Photographs and photomicrographs should be unmounted glossy prints and should not be retouched. Line diagrams should be on separate sheets; they should be drawn with black Indian ink on white paper and should be about four times the area of the final reproduction. Lines and lettering should be of sufficient thickness and size to stand reduction to one-half or one-third. Whenever possible, the originals of line diagrams, prepared as described above, should be submitted and not photographs. The legends of all the figures should be typed together on a single sheet of paper headed 'Legends to figures'.

Tables. There should be as few tables as possible and these should include only essential data; the data should not be crowded together. The main heading should be in bold with an Arabic number, e.g. **Table 2**. Each table must have a caption in small letters. Vertical lines should not be used.

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