Volume 9 Number 2 June 1974

Journal of Food Technology

Published for the Institute of Food Science and Technology (U.K.) by Blackwell Scientific Publications Oxford London Edinburgh Melbourne

JOURNAL OF FOOD TECHNOLOGY

Institute of Food Science and Technology (U.K.)

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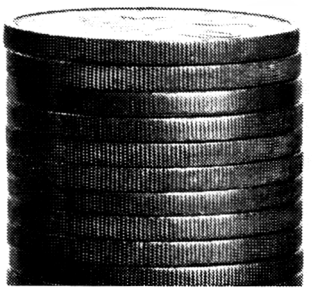

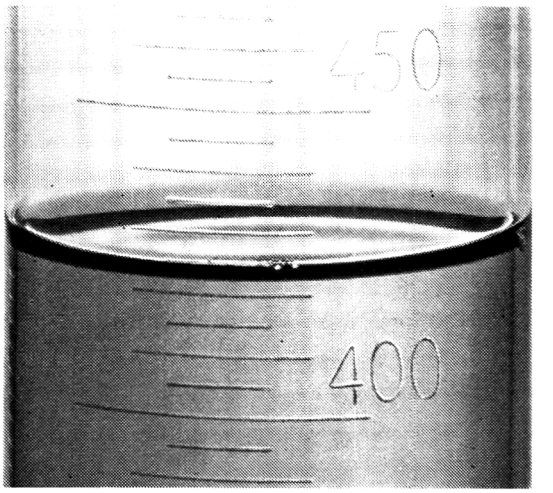

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Meat tenderness and the sliding-filament hypothesis

B. B. MARSH* AND W. A. CARSE

Summary

A detailed study was made of the relationship between the tenderness of beef and the extent of its early post mortem length change, observations covering the range from more than twice to less than one half the initial excised length. The 'peak' of toughness previously shown to occur at about 35–40% shortening was more accurately defined, and a secondary, much smaller peak was detected in muscles which had been held in a 25–30% *extended* state during rigor onset. These results strongly support the view that ultimate actomyosin configuration is a major determinant of meat tenderness, since the complex pattern of tenderness variation with length change can be explained entirely in terms of the varying degree of overlap of actin and myosin filaments.

Introduction

The tenderness of meat, cooked after rigor onset, is determined largely by the extent of cold shortening (Locker & Hagyard, 1963) undergone by the muscle during the first few hours post mortem. Meat is relatively tender if shortening during this period was either relatively small or very considerable; at intermediate values, however, a marked toughening is observed (Marsh & Leet, 1966a). The results are compatible with the view that the toughening is related directly to the interaction between actin and myosin during rigor onset (Marsh & Leet, 1966b), but this interpretation has been questioned (Bendall & Voyle, 1967; Voyle, 1969).

The shortening/toughening relationship has now been reinvestigated. Its extension to a wider range of muscle lengths has provided further evidence that meat tenderness is strongly influenced by a sliding-filament mechanism, and that actomyosin is a major contributor to toughness.

Materials and methods

Strips of sternomandibularis muscle, taken from ox or cow carcasses within a half hour of slaughter, were laid horizontally on a smooth surface and held without restraint at 15°C. At intervals during the first 24 hr post mortem they were transferred to an

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incubator at −0·5°C; this cold treatment induced shortening which ranged from more than 50% in the samples chilled very early after slaughter to less than 10% (usually below 5%) in those held at 15°C for 24 hr.

Other muscle strips were clamped in adjustable metal frames, their fibres parallel to the direction of restraint. The frames were then extended to hold the strips firmly at varying lengths up to 2·1 times their initial excised lengths. The clamped strips were held at 15°C until 24 hr post mortem before transfer to −0·5°C, still in their restraining frames.

All samples were plastic-wrapped 48 hr post mortem after removing the clamped strips from their frames, and were cooked in a water bath to an internal temperature of 80°C. The cooled samples were cut to a uniform size and their tenderness was assessed with a MacFarlane-Marer (1966) tenderometer. In all, 662 samples were examined within the one strictly standardized experimental routine.

Results and discussion

To facilitate presentation and interpretation, the data relating tenderness and pre-cooking length changes have been grouped at shortening intervals of 5% and lengthening intervals of 10%. The mean tenderness values (± standard error) for each group are shown in Fig. 1. Results from the earlier investigation have not been included because of slight differences in experimental procedure and an intentional exclusion of older animals and bulls from the present work.

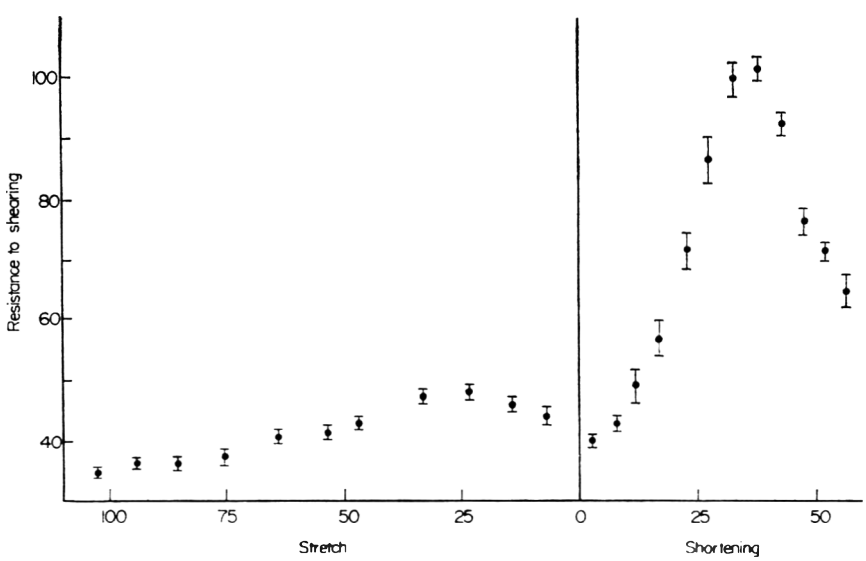

FIG. 1. The effect of extension or shortening before rigor completion on the toughness of meat cooked in rigor. Length change as per cent initial excised length. Vertical bars: mean shearing force ± s.e.

The shortening data (right-hand side of the origin in the figure) agree well with those of the previous study. The clearly defined peak of toughness, somewhat lower in this investigation because of the choice of younger beasts, occurs at about 35% shortening. It separates a pronounced upward curve (0–30%) from a steep downward trend (40–60%); a few other points in the >60% region, too few in number for statistical validity, lend support to the approximate linearity of the latter phase. This tenderizing with higher shortening, the subject of the following paper (Marsh, Leet & Dickson, 1974), is shown here only to locate the position of the peak; we believe it is due to an effect quite unrelated to that operating in the stretched and lower-shortening ranges.

Results from the stretched samples (to the left of the origin) reveal a most interesting minor peak at a muscle extension of about 25–30% of the initial excised length. This feature was quite unexpected for two reasons: first, the downward sweep of the curve as shortening declined from 30% toward 0% might reasonably be expected to continue as moderate stretching was imposed, and second, a slight tenderizing with stretch has in fact been reported (Herring *et al.*, 1967).

Statistical treatment indicates that the small peak is indeed genuine. The two mean force values in the 20–29% and 30–39% ranges of stretch are each significantly greater than that required to cleave samples in the range of 0–9% stretch ($P < 0{\cdot}05$) or of 0–5% shortening ($P < 0{\cdot}001$); in the other direction they are also significantly greater than the mean force value in the 40–49% stretch-range ($P < 0{\cdot}005$) and in all more highly extended ranges ($P < 0{\cdot}001$). The mean force value in the 10–19% stretch range does not differ significantly from those in the 20–29% and 30–39% ranges of extension, but is significantly greater ($P < 0{\cdot}005$) than the value in the 0–5% shortening range. For twenty of the individual points in the range of 20–39% stretch, paired comparison was possible, for control samples from the same individual muscle had been left unclamped under identical conditions of time and temperature. Following clamp removal and cooking, the stretched strips (mean extension 30% of the initial excised length) were found to be tougher than their controls (mean shortening 7%) in every one of the twenty comparisons, the mean difference being $7{\cdot}3 \pm 0{\cdot}9$ (s.e.) force units.

The relationship between shearing-force requirement and relative muscle length is thus an even more complex one than was envisaged when the study was re-opened. A plausible explanation of the tenderness pattern can be given, however, if certain parameters of bovine muscle are applied to the sliding-filament concept of muscular contraction (Hanson & Huxley, 1955). The values to be used in later discussion are as follows.

(i) Length of the bovine myosin filament: 1·5 μm (Bendall & Voyle, 1967).

(ii) Length of the bovine actin filament in each half sarcomere, from tip to Z-line mid-point: 1·3 μm* (unpublished result of Bendall & Page, quoted by Bendall & Voyle, 1967).

* See addendum.

(iii) Thickness of the bovine Z-line: 0·1 μm. (This value is an approximate one only, obtained by measurement of published electron micrographs from several sources. Its more exact assessment would not materially affect the validity of our argument.)

(iv) Mean sarcomere length of bovine sternomandibularis muscle in rigor, after approximate correction for the shortening which almost invariably accompanies rigor onset: 2·1 μm. (This value, the basis of all shortening measurements in the present study and corresponding to 0% in Fig. 1, was obtained for us by M. R. Dickson and N. G. Leet. Using methods described in the following paper, they first determined the sarcomere lengths of muscles which had passed into rigor at 15°C, and then applied corrections for the small amounts of shortening (based on the distance between two marks near the ends of each strip) which had occurred during rigor onset. Their value of about 2·1 is less than the 2·29 μm found by Voyle (1969), whose treatment of the muscles (vertical suspension under their own weight during rigor onset) would have encouraged some degree of stretching.)

(v) Length of the bridge-free pseudo H-zone in the central segment of the myosin filament: 0·2 μm (Huxley, 1963).

Applying the specific values (i)–(v) to the general sliding-filament hypothesis, we may calculate several 'critical values'. The reasoning parallels that used by Bendall (1969) in describing the length-tension relationship in stimulated muscle fibres.

(a) At 95% stretch (sarcomere length 4·10 μm) the tips of the actin and myosin filaments are only just in contact (Fig. 2a). The slightest additional extension will separate them entirely; conversely, shortening from this position will be accompanied by cross-bridge formation between the two sets of filaments.

(b) At 33% stretch (S.L. 2·80 μm) the actin tips reach the edges of the unreactive central region of the myosin filament (Fig. 2b). Additional shortening from this configuration will not increase the number of myosin-actin bridges, the bare pseudo H-zone being devoid of projections capable of interaction with the active sites of actin.

(c) At 24% stretch (S.L. 2·60 μm) the tips of the actin filaments within each sarcomere are just in contact (Fig. 2c). Further shortening from this position causes a double overlapping of actins, and we may suppose that the overlap interferes to some extent with the formation of myosin-actin links once the actin tips have traversed the unreactive bare region of the myosin filament.

(d) At 24% shortening (S.L. 1·60 μm) the tips of the myosin filaments reach the Z-lines (Fig. 2d), and shortening beyond this point must result in either Z-line penetration (Fig. 2e, i) or myosin crumpling (Fig. 2e, ii). Stromer & Goll (1967) and Voyle (1969) have presented evidence that both effects can occur in highly shortened bovine muscle, and observations in this laboratory confirm that myosin filaments can and do pierce the Z-line; Plate 1 (kindly provided by Mr N. G. Leet) shows several examples of penetration in a muscle which had cold-shortened by 33% (S.L. 1·41 μm between Z-line mid-points). It would thus be expected that, if myosin filaments (1·5 μm) could penetrate the Z-line, they would be on the point of finally breaking through, their

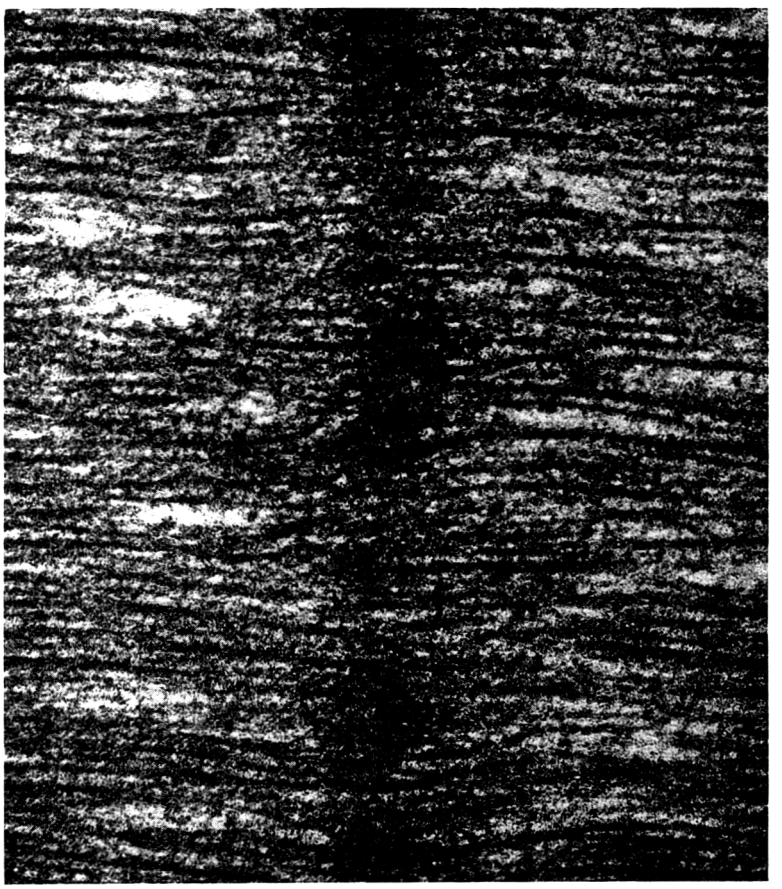

PLATE 1. Z-line of a bovine sternomandibularis muscle in rigor, cold-shortened by 33% of its initial excised length during rigor onset. Note several thick-filament intrusions into or through the Z-line. × 102,000. (Photograph kindly provided by N. G. Leet.)

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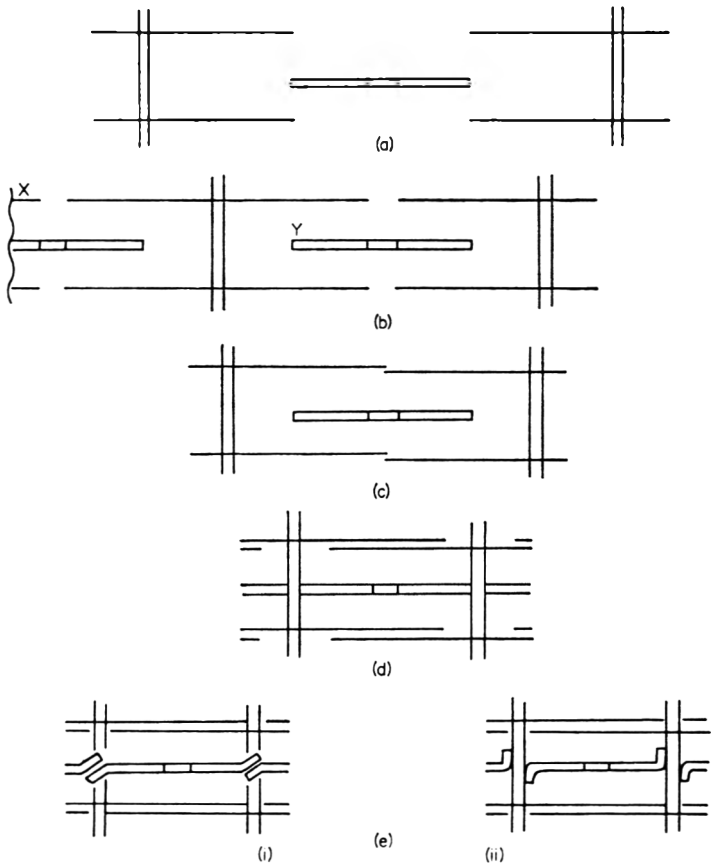

FIG. 2. Configuration of actin and myosin filaments and Z-lines at (a) 95% stretch, (b) 33% stretch, (c) 24% stretch, (d) 24% shortening, (e) 35% shortening. Only the longitudinal dimensions are to scale. Bent thick filaments in (e) are diagrammatic only, to indicate (i) penetration of Z-lines, (ii) rejection by Z-lines.

tips starting to appear on the other side and a number of the filaments do indeed appear to have reached this stage.

(e) At 35% shortening (S.L. 1·37 μm) actin tips reach the ends of those myosin filaments which have successfully penetrated the Z-lines from *neighbouring* sarcomeres (Fig. 2e, i). If we assume that polarity considerations apply to cross-linkage formation in rigor as they appear to in contraction (Huxley, 1963) then the two filament species (their ends on the point of overlap and their structures correctly polarized for interaction) can now form rigor linkages. The entire structure thus becomes firmly cross-bridged throughout its length.

With these critical values defined, we are in a position to attempt an elementary

mathematical treatment of the relationship between meat tenderness and muscle length at rigor onset.

It is generally accepted that the shearing resistance of cooked meat is due to the presence of both connective tissue and actomyosin (Marsh & Leet, 1966b). With some risk of over-simplification, it will now be assumed that the toughness of the former ('background toughness') is unaffected by the degree of stretch or shortening; without this assumption, further speculation would be impossible on the basis of present knowledge. It is further supposed that actin and myosin filaments, when not interbridged (either because one component is locally absent or because polarity considerations prevent interaction), contribute negligibly to shearing resistance. It follows that length-dependent differences in toughness are due to length-related changes in the actomyosin component.

Stretch exceeding 95%

In very highly stretched muscle, where actin and myosin filaments do not overlap at all, the toughness corresponds only to that of connective tissue present. Because bovine sternomandibularis muscle cannot be extended much beyond twice its initial length without tearing, it has been impossible to confirm the constancy of shearing resistance (F) with increasing length beyond about 105% stretch. The results (Fig. 1), however, are compatible with the view that a constant base-line toughness of about 35 shearing units is reached with extreme extension; in this length range, therefore, $F = 35$.

Stretch of 95–33%

As the degree of extension declines from 95% toward 33% of the initial excised length (phase a–b of Fig. 2), the tips of the actin filaments within each sarcomere approach each other and increasingly overlap the myosin unit. The formation at rigor onset of cross-bridged actomyosin creates an additional cleavage resistance directly proportional to the length of the linked actin-myosin overlap, i.e. to $95 + S$ (the per cent shortening, S, being negative in the stretched range).

The progressive cross-linking of thick and thin filaments, however, is not the only toughening effect of length reduction in this range. Shearing resistance is also dependent on the size of the *un*bridged areas, for cleavage will obviously occur preferentially in those zones where actomyosin formation does not take place with rigor onset. The H-zone is one such region, the myosin there being unaccompanied by actin, but it is insignificant in comparison with the much greater distance between the myosin of one sarcomere and the actin advancing toward it from the farther Z-line of the adjacent sarcomere. This 'gap' (X–Y) is illustrated in Fig. 2b. Until this weak zone closes at 35% shortening (see (e) above, and Fig. 2e, i), it will remain an area of fragility, since only at or after complete closure will the cleavage-resistant cross-linked actomyosin extend through and beyond Z-lines and into the adjacent sarcomeres. Because the

X–Y gap is greater at all muscle lengths than the H-zone, we may ignore the latter entirely; cleavage will always occur at the point of greatest vulnerability, regardless of the presence of other zones of almost (but not quite) the same weakness.

The effect of this gap on toughness will be inversely proportional to its span, i.e. to $35-S$, since at 35% shortening the weak zone disappears entirely. This proposed inverse dependence of strength on length parallels the reciprocal relationship between the safe loading of a wooden beam or the breaking weight of a metal girder and the distance it spans (Thurston, 1951).

In the 95–33% stretch range, therefore, the combined toughening effects of connective tissue, length of cross-linked filaments, and size of weak gap may be expressed as

$$F=35+\frac{p(95+S)}{35-S},$$

p being a constant and S the per cent shortening.

Stretch of 33–24%

With further decline of extension (phase b–c of Fig. 2) the approaching tips of the thin filaments traverse the 0·2 μm central segment of the thick filament. Actin-myosin interaction cannot occur here because of the absence of the necessary bridge-forming projections, so the number of cross-links does not increase with shortening during this phase; the numerator of the second term thus remains constant, with $S=-33$. The denominator, on the other hand continues to decrease with shortening (or reduced stretch), since the 'gap' is still closing. The appropriate expression relating toughness and length in this range is thus

$$F=35+\frac{p(95-33)}{35-S}=35+\frac{62p}{35-S},$$

p being a constant.

24% *stretch*–24% *shortening*

The H-zone closes as stretch declines to 24% and with further shortening actin filaments from opposite ends of the sarcomere commence to overlap each other (Fig. 2, phase c–d). The intruding actin filaments would be expected to weaken normal actin-myosin cross-bridge formation, just as they appear to do in reducing tension development in shortened muscles by 'interfering mechanically and chemically with the interaction of the correctly oriented actin and myosin molecules' (Huxley, 1965). To recognize this interference, we therefore introduce a *tenderizing* effect proportional to the extent of actin-actin overlap, i.e. to $24+S$.

Apart from the insertion of this new term, the equation remains unaltered from that applying in the previous length range. The advancing actin tips, even though now beyond the bare central segment of the thick filament, are unable to interact with the

distal part of the myosin because of their antiparallel orientation, while the weak (X–Y) gap continues to diminish in size with reduced stretch. The expression for this range thus becomes

$$F = 35 + \frac{62p}{35 - S} - q(24 + S),$$

q being a constant.

For the length ranges considered so far (> 95% stretch to 24% shortening), a single general equation relates length to toughness:

$$F = 35 + \frac{p(95 + S)}{35 - S} - q(24 + S).$$

Depending on the range under scrutiny, logical modifications must be made to this equation to compensate for range-specific features. Thus the terms $95 - S$ and $24 + S$ are measures of filament overlap (actin-myosin and actin-actin respectively); negative values of these terms are clearly to be read as zero since they would merely indicate an absence of overlap. Similarly, the term $95 + S$ is to be equated to 62 for all values of S exceeding -33, for cross-bridges cannot increase in number once the advancing actin tip has reached the edge of the projection-free central segment of myosin.

Specified values for the constants p and q have been derived empirically from the experimental data, and found to be 13·5 and 0·70 respectively for all ranges where they apply. The resulting equation

$$F = 35 + \frac{13{\cdot}5(95 + S)}{35 - S} - 0{\cdot}7(S + 24)$$

is plotted in Fig. 3, together with the experimental points (already shown in Fig. 1) and the critical values (a)–(e). In the span of shortening means from -102% to $+23\%$, not a single mean value differs significantly from the theoretical relationship deduced above. We regard as particularly important the ability of the equation to explain the minor peak at moderate stretch, for it gives the most direct evidence so far of the involvement of actomyosin in meat texture; no theory reckoning connective tissue as the sole determinant of toughness could account on present knowledge for the elevation in shearing resistance with muscle extension.

Shortening exceeding 24%

Problems arise when shortening is greater than 24%, for from this point onward the theoretical curve indicates a continuing increase in the rate of toughening with shortening while the experimental results show clearly that the rate of toughening decreases and eventually becomes negative. The first difficulty—an obviously false prediction of infinite toughness at 35% shortening—is readily explained as a consequence of over-simplified mathematical treatment; it arises because, having assumed that cross-linked

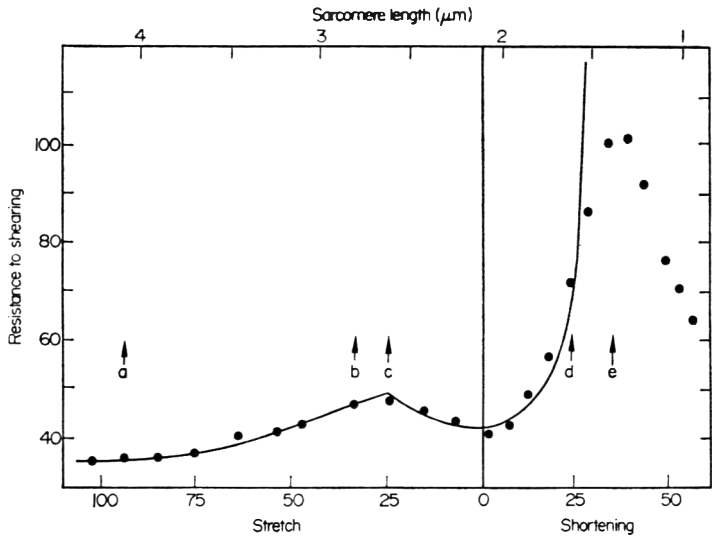

FIG. 3. The effect of muscle length on toughness. Line: theoretical relationship based on actin-myosin interaction. Points: experimental mean values. a–e: critical values (see text).

actomyosin is resistant to cleavage, we have neglected to place a finite maximum on the resistance. Perhaps the best interpretation of the equation is that a shortening of the weak area toward zero causes the shearing requirement to tend *toward that of the continuously cross-linked structure*, rather than toward infinity. In this connection it is of interest that the muscle illustrated in Plate 1 had one of the highest shearing resistances after cooking (136 units) encountered in the entire series, an observation to be associated with the nearness of its shortening (33%) to the critical 35% value.

The second problem—a declining rate of toughness increase with shortening—is accounted for in terms of critical value (d), the 24% shortening at which the ends of the thick filaments reach the Z-lines. With slight additional shortening, some of them penetrate this barrier (Fig. 2e, i) and are able to continue their approach toward the actins of the adjacent sarcomere; others, however, are crumpled, bent, or folded back (Fig. 2e, ii) and in consequence can no longer contribute to gap-closing activity. Phase d–e therefore represents a continued toughening with shortening, but at a decreasing rate brought about by the Z-line rejection of many of the myosin filaments.

The phase of massive toughening eventually comes to a halt at critical value (e), 35% shortening, when those thick filaments successfully penetrating the barrier have encountered the advancing thin filaments in the next sarcomere; the weak zone of ready cleavage has closed, and toughness has attained its maximum. The apparently paradoxical tenderizing which accompanies further shortening involves a quite different mechanism, and will be dealt with in the following communication.

Since the methods used in the present study follow those employed in earlier work

(Marsh & Leet, 1966b), the conclusions are still open to the criticism (Bendall & Voyle, 1967) that our argument is based on a relationship between the shear value of the *cooked* meat and the extent of shortening of the *raw* material. However, Dickson & Leet (unpublished observations) have found that, once the actin-myosin overlap is 'locked' by rigor onset, the application of heat causes about the same relative changes in A- and I-band lengths as it does in sarcomere length (about 20%, for instance, in samples retained at their initial excised lengths until rigor completion). This evidence indicates that heat coagulation is without effect on the *proportionate* size of the areas of weakness. We are confident that the same type of general relationship would apply if total shortening (including that due to cooking) had been substituted for pre-cook shortening, though with very different constants in the equation; by adopting this course, however, we could not have utilized the established filament-length data on which the present hypothesis depends.

Finally, we may speculate briefly on the condition of the primary filaments at and beyond the critical point (e). At 35% shortening the ends of those myosins which have successfully penetrated the Z-line have just reached the free tips of the actins in the neighbouring sarcomere, and the structural polarities of the two sets of filaments are correctly orientated for interaction. We propose that this configuration is the starting-point for a further shortening phase of renewed vigour, with cross-bridge formation now taking place between actins and myosins originally in adjacent sarcomeres. A mechanism of this sort could perhaps account for the magnitude of the supraphysiological length changes observed in cold shortening (Locker & Hagyard, 1963), thaw shortening (Perry, 1950), and the delta state (Ramsey & Street, 1940).

Acknowledgments

The authors are grateful to Dr M. R. Dickson and Mr N. G. Leet for providing data on sarcomere lengths and on the proportionate size reduction which accompanies heat coagulation. They also appreciate the constructive criticisms made by Dr J. R. Bendall (Meat Research Institute, Bristol) in the course of this work.

References

Bendall, J.R. (1969) *Muscles, Molecules and Movement.* Heinemann, London.
Bendall, J.R. & Voyle, C.A. (1967) *J. Fd Technol.* **2,** 259.
Hanson, J. & Huxley, H.E. (1955) *Symp. Soc. exp. Biol.* **9,** 228.
Herring, H.K., Cassens, R.G., Suess, G.G., Brungardt, V.H. & Briskey, E.J. (1967) *J. Fd Sci.* **32,** 317.
Huxley, H.E. (1963) *J. molec. Biol.* **7,** 281.
Huxley, H.E. (1965) *Sci. Amer.* **213,** 18.
Locker, R.H. & Hagyard, C.J. (1963) *J. Sci. Fd Agric.* **14,** 787.
Macfarlane, P.G. & Marer, J.M. (1966) *Fd Technol., Champaign,* **20,** 838.
Marsh, B.B. & Leet, N.G. (1966a) *Nature, Lond.* **211,** 635.
Marsh, B.B. & Leet, N.G. (1966b) *J. Fd Sci.* **31,** 450.

MARSH, B.B., LEET, N.G. & DICKSON, M.R. (1974) *J. Fd Technol.* **9,** 141.
PERRY, S.V. (1950) *J. gen. Physiol.* **33,** 563.
RAMSEY, R.W. & STREET, S.F. (1940) *J. Cell Comp. Physiol.* **15,** 11.
STROMER, M.H. & GOLL, D.E. (1967) *J. Fd Sci.* **32,** 386.
THURSTON, A.P. (1951) *Molesworth's Handbook of Engineering Formulae and Data,* 34th edn, pp. 668, 678. E. and F. N. Spon Ltd, London.
VOYLE, C.A. (1969) *J. Fd Technol.* **4,** 275.

(*Received* 10 *November* 1973)

Addendum

The 2·6 μm over-all length of the thin filament is the latest available published value for bovine muscle. Dr A. F. Huxley has informed us that it should now be reduced by about 5%, S.G. Page (1968, *J. Physiol.* **197,** 709) having introduced a correction to her earlier results. Substituting the lowered figure in our calculations alters four of the five critical values slightly, the greatest changes being in (b) and (c), with the result that the theoretical minor peak should be moved 6% to the right. Agreement with the experimentally determined values then becomes slightly less satisfactory, though the fitting of new constants leaves all but one point (that at 24% stretch) still insignificantly different from the theoretical curve. In view of the approximations made in this work (in particular the initial excised length of 2·1 μm sarcomere length, which itself could be up to 5% in error), and also of the primitive assumption that cleavage of connective tissue is unaffected by stretch or shortening, we believe further refinements to the general equation should await more precise determinations of several of the basic values and confirmation of our suppositions.

We are grateful to Dr Huxley for drawing our attention to Page's more recent work.

J. Fd Technol. (1974) **9**, 141–147.

The ultrastructure and tenderness of highly cold-shortened muscle

B. B. MARSH,* N. G. LEET AND M. R. DICKSON†

Summary

In an attempt to explain the paradoxical tenderizing, observed when muscle shortening exceeds about 40% of the initial excised length, the fine structure of highly cold-shortened muscle has been studied by light and electron microscopy. Tissue at the 'peak' of toughness is uniformly shortened and shows no sign of structural weakness or damage, but with further shortening supercontraction and node formation take place in an increasing proportion of the sarcomeres. Widespread fibre fracturing is seen in the internodal zones when the length change has exceeded about 50%. This disruption appears sufficient to account for the declining toughness which accompanies supraphysiological shortening.

Introduction

Cold shortening (Locker & Hagyard, 1963) is now recognized as a major determinant of tenderness in both beef (Locker, 1960; Marsh, 1964; Herring, Cassens & Briskey, 1965) and lamb (Marsh, Woodhams & Leet, 1968; McCrae *et al.*, 1971). The relationship between shortening and tenderness is complex. Toughness increases with the extent of the length change which has preceded or accompanied rigor onset, attaining a maximum value at about 35–40% shortening; it then declines towards its initial relatively low value as the change in length approaches 60% (Marsh & Leet, 1966a, b).

This latter phase of decreasing toughness is of no real practical significance. It is nevertheless of considerable theoretical concern, for until it is explained any hypothesis concerning the shortening/toughening relationship (e.g. Marsh & Carse, 1974) must remain incomplete and perhaps suspect. The ultrastructural studies of highly cold-shortened bovine muscle undertaken by Weidemann, Kaess & Carruthers (1967) and Stromer & Goll (1967a, b) shed no light on the paradoxical tenderizing at high shortening values. Voyle (1969) observed myofibril rupture which he suggested might be

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related both to the exudation of free fluid and to the reversal of toughness development, these latter features having been associated earlier by Marsh & Leet (1966a, b).

The present investigation is concerned solely with this cold-induced supraphysiological shortening range. It deals with the structure of maximally cold-shortened muscle and with the changes in structure as maximum shortening is approached, and attempts to relate these changes to the observed decline in toughness. The presumably related phenomenon of 'thaw shortening' (Perry, 1950), in which even more extreme muscle shortening can be produced (Marsh & Thompson, 1958), is not examined in this study, in order to avoid possible complicating structural changes due solely to ice-crystal formation and consequent cell-rupture effects.

Material and methods

Ox sternomandibularis (neck) muscles, excised within 20 min of slaughter, were cut into strips at least 7 cm long parallel to the fibre axis and about 12 cm^2 cross-section. A small nail was inserted near each end of every strip to facilitate the measurement of later length changes. The samples were laid horizontally and unrestrained on polyethylene sheets at 15°C, and remained there for varying periods of up to 12 hr before being transferred to a cold room (0°C). The length changes provoked by this treatment ranged from 50–58% of the initial excised length (if chilled within 4 hr post mortem) to 35–40% (if chilled 10–12 hr post mortem). Storage at 0°C was continued until 48 hr post mortem, when final length measurements were made. A longitudinal internal strip was cut from each sample and fixed in 5% glutaraldehyde buffered with 0·1 M Sorenson's phosphate (pH 7·4). The remainder of each sample was cooked as described by Marsh, Woodhams & Leet (1966), and its toughness was measured in arbitrary units with a tenderometer (Macfarlane & Marer, 1966).

The fixed strip was reduced in size over a 4 hr period to pieces of about 1 mm^3, with two changes of phosphate-buffered glutaraldehyde during this time. The tissue was washed overnight in 0·1 M phosphate buffer, then fixed with osmium tetroxide and uranyl acetate (schedule 3 of Terzakis, 1968), dehydrated with ethanol, impregnated with Fluka Durcapan/epoxy propane mixtures, and embedded the following day. Polymerization took place at 60°C.

Thick sections (0·5–1 μm) were cut from the epoxy-embedded specimens, dried on to glass slides, stained with 5% toluidine blue and borax, and mounted beneath a coverslip with unpolymerized resin. A Leitz Orthoplan microscope with high-power dry plano objective and Orthomat camera were used for photomicrography.

The tip of the conical end of a BEEM capsule was cut off, and the truncated cone so formed was placed over the section following cover-slip removal. It was first polymerized in position with a small amount of resin, and later was filled completely with resin and polymerized.

Thin sections were cut with a diamond knife, the fibres being aligned parallel to the

knife edge to reduce compression effects during sectioning. The sections were stained with uranyl acetate and lead citrate and examined with a Phillips EM 200 microscope at 80 kV.

Results

In the course of this investigation, thirty-two samples from thirteen animals were examined, the cold-shortening values ranging from 35% to 58% of the initial excised length. To eliminate possible inter-animal variability and facilitate direct comparison of structural features, we have selected three samples from a single neck muscle of one ox for detailed presentation and discussion. It was verified that the conclusions drawn from these applied equally to material of comparable shortening from other animals.

In each plate a single sample is presented at three magnifications: (a) ×550 (light microscope), (b) ×2960 (low-power electron microscope) and (c) ×23,200 (higher-power electron microscope), the boxed area in each of the first two pictures indicating the full area of the next higher magnification. Distinguishing features (unique banding patterns, fibre boundaries and slight irregularities) serve to relate the photographs to one another within the same plate.

Plate 1: 43% *shortening*, 110 *toughness units*

Apart from having a rather shorter sarcomere length, this sample did not differ significantly in appearance from 36% shortened material from the same muscle, and showed essentially the same features as samples (from other animals) which had shortened from 35% to 43%. The structure depicted is thus characteristic of muscle at the peak of shortening-induced toughness or very slightly beyond it.

Plate 1a shows a general patchiness suggesting at first sight a developing unevenness or irregularity within the fibre. That this is not so, however, is shown in Plate 1b; considering the internal strains which we assume must develop in muscle shortened to this extent, the Z-lines have remained quite remarkably in register across the fibre and the sarcomeres are very uniform in length.

Since at this shortening the sarcomere length (1·15–1·20 μm) is appreciably less than the normal length of the myosin filament (1·5 μm), we would expect to find either buckled, bent and folded myosin units (if the Z-line resisted penetration), or direct evidence that thick filaments had pierced the Z-line. Plate 1c offers no sign of the former, showing instead the presence of reasonably straight and uncrumpled filaments. By contrast, several muscles used in this and the preceding study yielded positive evidence of Z-line penetration when shortening exceeded about 30%; an example was shown in Fig. 3 of the previous paper (Marsh & Carse, 1974).

Plate 2: 48½% *shortening*, 87 *toughness units*

Although the material illustrated in this plate has cold-shortened only 5½% more than that shown previously, the samples differ very appreciably in microscopic appear-

ance. The irregular patchiness has been replaced by a much more definite and less random pattern of dark and light zones, which mostly extend across the full width of the fibre (Plate 2a). The dark areas, although by no means precisely spaced or of uniform width even within the same fibre, nevertheless resemble each other closely in appearance and density and in their fairly strict transverse direction.

Plate 2b indicates that the cause of the banding pattern is the tight longitudinal packing of sarcomeres in some regions, these high-density areas alternating with light zones in which no further shortening, beyond that seen in Plate 1, has taken place. Sarcomere lengths in the dark areas frequently indicate a shortening of about 60–80% of the initial excised length over short sequences of three to four sarcomeres. No significant disruption of fibres has taken place, and little or no stretching of sarcomeres has occurred; the change is essentially one of super-contraction in limited areas without compensating extension elsewhere. The additional over-all shortening of the sample is thus determined by the number of sarcomeres undergoing super-contraction rather than by the extent of contraction of individual sarcomeres, and this number varies widely both among fibres and among sites within a single fibre.

Plate 2c, selected to include both dark and light areas, shows that, as in the previous plate, very little buckling or bending of the myosin filaments has occurred, the Z-line apparently still offering negligible resistance to penetration. In the light areas, Z-lines are less precisely defined than before, probably because the myosin units have pushed a little further into the adjoining sarcomeres to give a double-myosin 'fringe' to each boundary. Between these fringes in the typical light-zone sarcomere, the structure is relatively open, for the myosin filaments are here only singly arrayed; but in the dark zones the fringes have advanced inwards to the point of contact, their continuous overlap giving a uniformly high density. The transition from relatively long sarcomeres (with only small fringes of double myosins) to super-contracted sarcomeres (with fringes in contact) is seen in the sarcomere sequence A, B, C.

Plate 3: 55% *shortening*, 63 *toughness units*

Plate 3a, showing maximally cold-shortened tissue, reveals a more extreme form of the dark–light alternation than seen in the previous sample. The banding is now less regular; Plate 3b shows that the dark areas are due, as before, to tight longitudinal packing of sarcomeres, but the number of these highly shortened units within each band varies from three to twenty or more. Although the bands are still generally latitudinal in orientation, they are clearly less well ordered than in the previous sample.

The degree of sarcomere contraction achieved in the highly shortened areas is no greater than in the material in Plate 2, but a much higher proportion of each fibre is in the super-contracted state. The resulting greatly increased packing density does not cause a proportionally large increase in over-all shortening, however, because a new effect has appeared; complete cleavage has occurred in many of the light areas, the breaks almost compensating for the greatly increased number of super-contracted

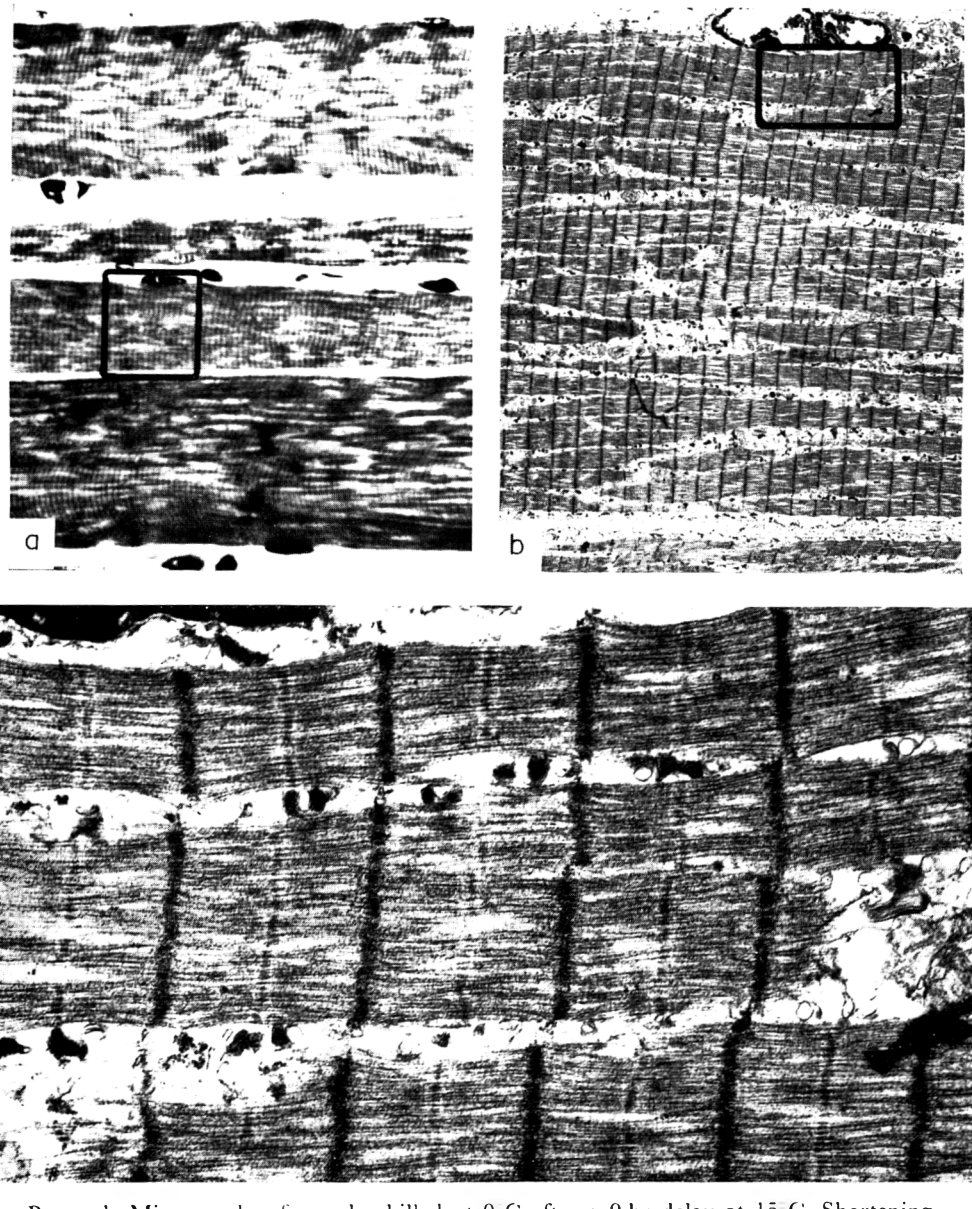

PLATE 1. Micrographs of muscle chilled at 0 C after a 9 hr delay at 15 C. Shortening 43% of initial excised length; toughness 110 arbitrary units. (a) × 550 (light microscopy), (b) × 2960 (EM), and (c) × 23,200 (EM).

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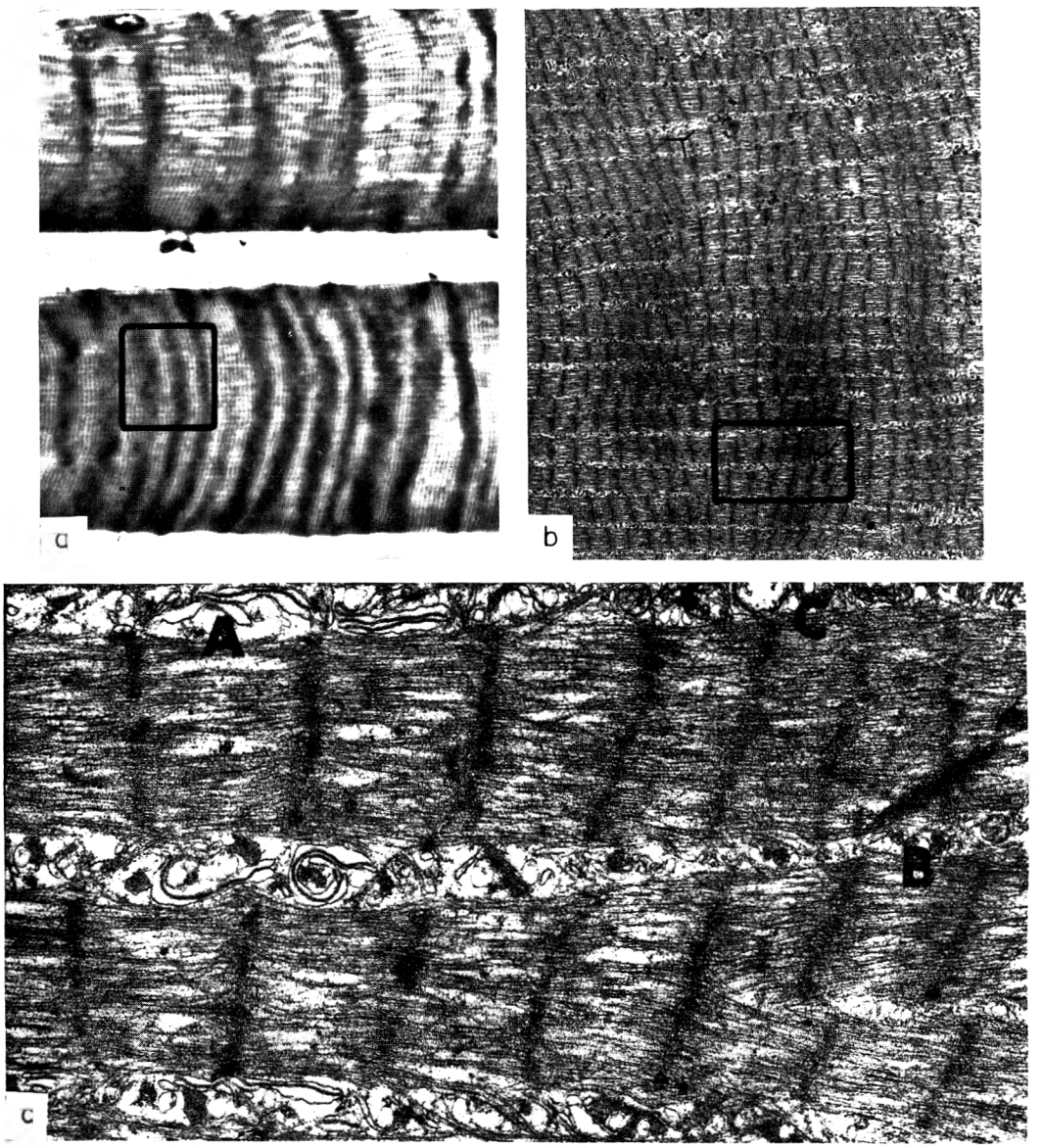

PLATE 2. Micrographs of muscle chilled at 0 C after a 6 hr delay at 15 C. Shortening $48\frac{1}{2}\%$; toughness 87 units. (a) × 550 (light microscopy), (b) × 2960 (EM) and (c) × 23,200 (EM).

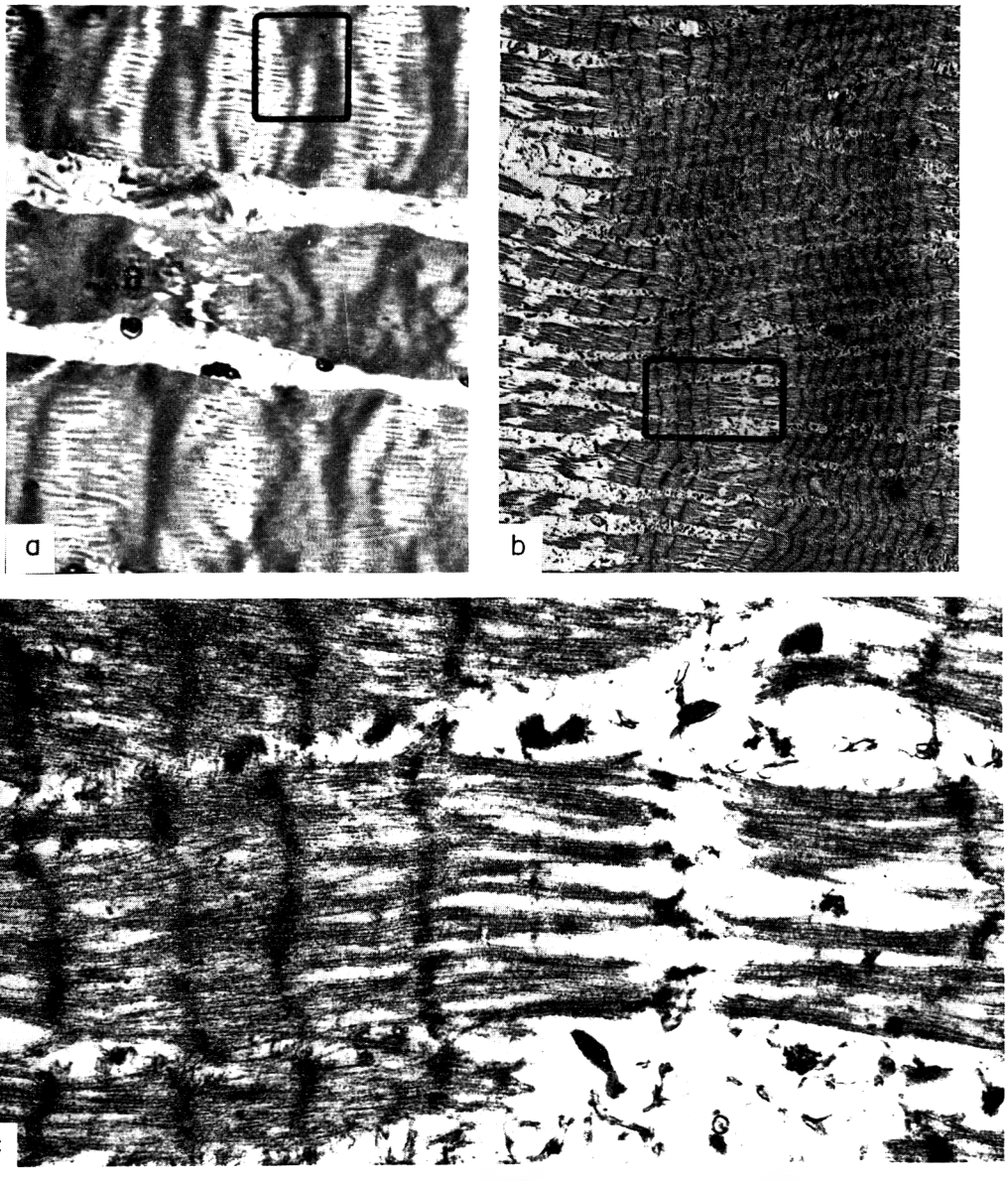

PLATE 3. Micrographs of muscle chilled at 0 °C after a 3 hr delay at 15 °C. Shortening 55%; toughness 63 units. (a) ×550 (light microscopy), (b) ×2960 (EM) and (c) ×23,200 (EM).

sarcomeres in the dark zones. In some places the cleavages run only part way through the fibre, as in the central vertical break in Plate 3b; in others they appear to continue through the full width, as seen in the upper fibre of Plate 3a and along the left edge of Plate 3b. Irregular though it is, the pattern of fibre fracture is obviously very effective in shattering the longitudinal structural elements, only a few of the highly shortened segments exceeding 20 μm in length.

Plate 3c shows an area in which sarcomere length varies almost three-fold within a three-sarcomere segment. Some sarcomeres are longer than any seen in the earlier plates of less shortened muscle, but on present evidence it is not possible to say if this is a genuine length increase or merely the early effects of fibre cleavage. Even if it could be established that some parts of the structure had lengthened from a more shortened configuration, however, the high-shortening tenderness could not be explained in terms of a return to a low or zero-shortened state; the longest of the observed sarcomeres still correspond to a shortening of about 30%, and consequently should display almost maximal toughness. Such an explanation, in any case, is now quite unnecessary, for the massive rupturing which accompanies high shortening appears to offer an entirely adequate reason for the toughness decline.

Discussion

Evidence presented here and in the preceding paper indicates that the thick filament can and does penetrate the Z-line in the shortening range associated with maximum toughness. As described in the previous study (Marsh & Carse, 1974), the resulting overlap of myosin from one sarcomere with actin from the next would produce, with the later formation of rigor cross-bridges, a continuously linked actomyosin extending for considerable lengths along the fibre. Furthermore, if it is accepted that actomyosin is the constituent responsible for shortening-induced toughness (Marsh & Leet, 1966a), then the observed strict regularity (Plate 1) must indicate that shearing resistance would be uniform, with no weak or broken areas to facilitate cleavage. Muscle which has shortened to this extent, therefore, is very tough simply because the cross-bridged primary filaments run homogeneously and without interruption through the tissue.

When shortening approaches 50% of the initial excised length (Plate 2), supercontraction occurs in some sarcomeres, resulting in localized shortenings of as much as 80%. The pattern is semi-regular; while there is little dimensional uniformity among the dark zones along the fibre, there is a very pronounced regularity of pattern *across* the fibre, the stimuli to super-contract appearing to have spread inwards at right angles to the long axis. The result is a reasonably well-disciplined banding, rather than the random patchwork which might have been expected had there been no transverse tubular network of interfibrillar communication.

The inhomogeneity created by this intermittent shortening is clearly a state intermediate between the regularity of the first plate and the disruption of the third. By

the time over-all shortening has attained 55% of the initial excised length (Plate 3), the increasing number of super-contracted sarcomeres has fractured the internodal zones. It is of interest that, in this highly shortened muscle, sarcomeres showing more than about 35% length change are almost never observed to be broken; conversely, fracture is seen in every sarcomere which has shortened by less than this amount (or alternatively has been re-extended to it from a more highly shortened state). This observation supports the concepts developed by Marsh & Carse (1974), in that small zones of weakness would still be present in muscle which had shortened by a little less than 35%, whereas no such fragile areas would remain when shortening exceeded this value. Furthermore, we would predict that these more readily cleaved points would be immediately adjacent to the Z-line, since it is here that the weak zone is finally closed at 35% shortening (see Fig. 2e, (i) of Marsh & Carse, 1974); it is precisely in this position that fracture of highly shortened muscle is found to take place (Plate 3c).

Whatever the molecular explanation for the massive disruption, there is no doubt that it is just this fracturing process which accounts for the paradoxical increasing tenderness at very high shortening values. Progressive rupturing was in fact suggested earlier as an explanation of the effect (Marsh & Leet, 1966a) but at that time it was envisaged that the shattering process might exert its influence on tenderness by permitting a decrease in internal strain, with a consequent realignment of previously distorted cleavage planes. No such return to a pre-existing, easily cleaved pattern is now required; the rupturing is itself sufficiently extensive and devastating in the highly shortened tissue to account entirely for the observed decline in toughness.

Acknowledgments

The authors are grateful to Drs J. P. Chalcroft, C. L. Davey and R. H. Locker for their helpful advice and constructive criticism, and to the Auckland Farmers' Freezing Co-operative Limited for supplying muscle in a pre-rigor condition.

References

Herring, H.K., Cassens, R.G. & Briskey, E.J. (1965) *J. Fd Sci.* **30,** 1049.
Locker, R.H. (1960) *Fd Res.* **25,** 304.
Locker, R.H. & Hagyard, C.J. (1963) *J. Sci. Fd Agric.* **14,** 787.
McCrae, S.E., Seccombe, C.G., Marsh, B.B. & Carse, W.A. (1971) *J. Fd Sci.* **36,** 566.
Macfarlane, P.G. & Marer, J.M. (1966) *Fd Technol., Champaign,* **20,** 838.
Marsh, B.B. (1964) In: *Carcass Composition and Appraisal of Meat Animals* (Ed. by D.E. Tribe), Paper 12. C.S.I.R.O., Australia.
Marsh, B.B. & Carse, W.A. (1974) *J. Fd Technol.* **9,** 129.
Marsh, B.B. & Leet, N.G. (1966a) *J. Fd Sci.* **31,** 450.
Marsh, B.B. & Leet, N.G. (1966b) *Nature, Lond.* **211,** 635.
Marsh, B.B. & Thompson, J.F. (1958) *J. Sci. Fd Agric.* **9,** 417.

MARSH, B.B., WOODHAMS, P.R. & LEET, N.G. (1966) *J. Fd Sci.* **31,** 262.
MARSH, B.B., WOODHAMS, P.R. & LEET, N.G. (1968) *J. Fd Sci.* **33,** 12.
PERRY, S.V. (1950) *J. gen. Physiol.* **33,** 563.
STROMER, M.H. & GOLL, D.E. (1967a) *J. Fd Sci.* **32,** 329.
STROMER, M.H. & GOLL, D.E. (1967b) *J. Fd Sci.* **32,** 386.
TERZAKIS, J.A. (1968) *J. Ultrastruct. Res.* **22,** 168.
VOYLE, C.A. (1969) *J. Fd Technol.* **4,** 275.
WEIDEMANN, J.F., KAESS, G. & CARRUTHERS, L.D. (1967) *J. Fd Sci.* **32,** 7.

(*Received* 10 *November* 1973)

J. Fd Technol. (1974) **9**, 149–156.

Measurement of the heat production in beef muscle during rigor mortis

M. J. MORLEY

Summary

The post mortem heat production in beef at body temperature has been measured by observing the rise in temperature of the muscles M. semitendinosus and M. extensor carpi radialis when enclosed in an insulated container submerged in a water bath at 37°C. The variation with time of the muscle's radial temperature profile was measured using a multi-junction thermocouple probe and a data logger, and this enabled the heat loss to be calculated. The mean values obtained for the total heat production during rigor mortis were 6·4 kJ/kg for M. semitendinosus and 7·9 kJ/kg for M. extensor carpi radialis, these being equivalent to temperature rises of 1·8°C and 2·2°C respectively, in perfectly insulated muscles. The heat production per unit decrease in pH averaged 5·4 kJ/kg for M. semitendinosus and 6·8 kJ/kg for M. extensor carpi radialis. The rate of heat production reached a mean maximum level of 1·5 kJ/kg hr, which was maintained until 4–5 hr post mortem before decreasing to zero. These results are in general conformity with calculations based on the known relations of glycogen, phosphocreatine and ATP.

Introduction

It is well known that mammalian temperature rises during the rigor mortis process and values as high as 43°C or more have been occasionally registered in carcasses from normal healthy animals (Sybesma & Logtestijn, 1966). However, such measurements provide only a rough guide to the heat evolved because heat is being continuously lost from the carcass. There is therefore some uncertainty about the precise quantity of heat liberated. It is important to know this, not only because of its contribution to the total heat that must be removed in refrigeration, but also since the heat liberated deep inside the carcass can result in internal putrefaction. The calorimetric experiments on rabbit muscle by Smith (1930) and the theoretical approach by Bendall (1973) disagree with reported observations of post mortem increases in temperature of up to 8°C (Golovkin *et al.*, 1958). Bendall (1973) has calculated that the total heat produced

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during post mortem biochemical changes could theoretically raise meat temperature by 2·1–2·7°C for beef and 3·0–3·5°C for pork. In view of the uncertainty that exists, and especially as post mortem heat production has only actually been measured on rabbit muscle, a further investigation was considered necessary.

Methods

Animals were slaughtered in the Institute's licensed abattoir and the muscles M. extensor carpi radialis and M. semitendinosus, which are fairly large and roughly cylindrical, were dissected as soon as possible after death. A multi-junction thermocouple probe was then inserted radially into the muscle at its mid point. The thermocouple probe consisted of nine individually calibrated copper-constantan thermocouples (36 SWG), spaced 1 cm apart along the probe shaft, made of 5 mm o.d. 'Tufnol' tubing. The muscle was then enclosed in a polyethylene bag and placed in an insulated container submerged in a water bath at 37°C (Fig. 1). By 1 hr post mortem the muscle had sufficiently stabilized to enclosure temperature to allow measurements to commence. The variation with time of the muscle's radial temperature profile was followed at intervals of 1 min until completion of rigor mortis, using a data logger reading to ±0·01°C. The temperature of the water bath was varied throughout the period of measurement so as to give as small and symmetrical a temperature profile as possible, in order to minimize the cooling rate and increase the accuracy of its determination.

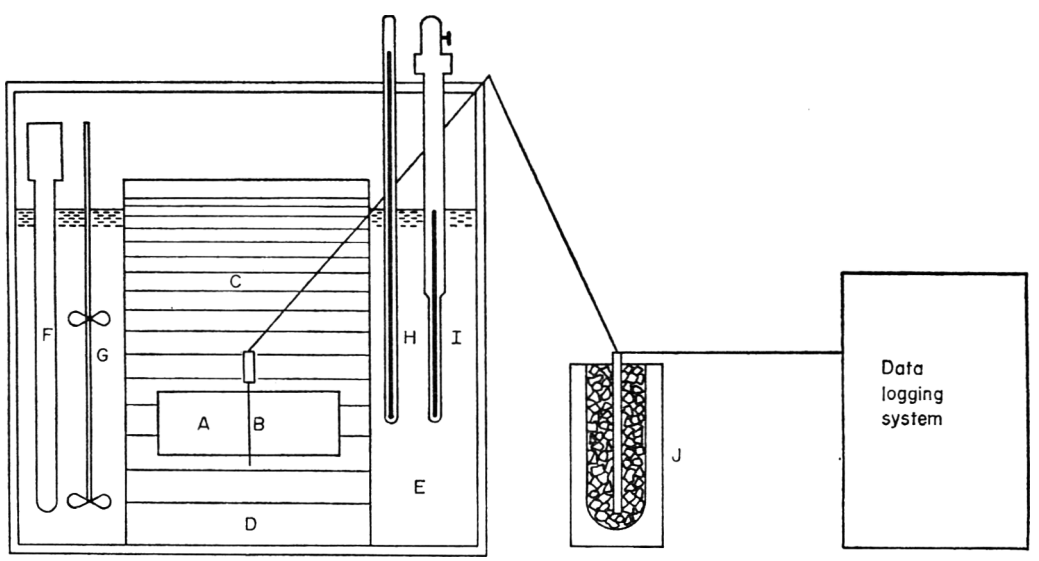

FIG. 1. The apparatus. A, Muscle sample; B, thermocouple probe; C, vermiculite insulation; D, sinker weight; E, controlled temperature water bath; F, heater; G, stirrer; H, thermometer; I, control thermometer; J, ice junction.

Measurement of pH were taken at intervals, on the end portions of the muscle, which were cut off initially and kept in an incubator at the same temperature as the remainder of the muscle. The pH was measured in a homogeneous mixture of muscle and iodoacetate–KCl solution, using a glass and a calomel electrode.

The adiabatic temperature rise during any period of time is given by the sum of the observed temperature rise and the cooling correction. The latter can be evaluated from the heat conduction equation, in cylindrical coordinates (r, ϕ, z), for a homogeneous isotropic solid in which heat is generated at a rate Q per unit volume (Carslaw & Jaegar, 1959):

$$\frac{Q}{c\rho} = \frac{\partial\theta}{\partial t} - \kappa\left[\frac{\partial^2\theta}{\partial r^2} + \frac{1}{r}\frac{\partial\theta}{\partial r} + \frac{1}{r^2}\frac{\partial^2\theta}{\partial\phi^2} + \frac{\partial^2\theta}{\partial z^2}\right]$$

where t is the time, c the specific heat, ρ the density, κ the thermal diffusitivity, and θ the temperature at radial distance r.

For a symmetrical profile $\partial^2\theta/\partial\phi^2 = 0$, and if the muscle's length is much greater than its diameter, the axial conduction term $\partial^2\theta/\partial z^2$ can be neglected. The equation then becomes:

$$\frac{Q}{c\rho} = \frac{\partial\theta}{\partial t} - \kappa\left[\frac{\partial^2\theta}{\partial r^2} + \frac{1}{r}\frac{\partial\theta}{\partial r}\right]$$

i.e. the rate of adiabatic temperature rise is given by the sum of the observed rate of temperature increase and the rate of cooling,

$$-\kappa\left[\frac{\partial^2\theta}{\partial r^2} + \frac{1}{r}\frac{\partial\theta}{\partial r}\right].$$

The terms $\partial^2\theta/\partial r^2$ and $1/r\ \partial\theta/\partial r$ were determined by numerical differentiation of the observed temperature profile, using Newton's interpolation formula. Cooling rates were calculated at the nearest points on either side of the radial centre. The total cooling during any period of time was obtained by numerical integration of the corresponding cooling rates.

An estimation of the accuracy of the cooling determined by this method was obtained by comparing, in each experiment, the cooling as observed after the heat production had ceased with the cooling as calculated from the temperature profiles during the same period of time. It was thereby found that the calculated cooling underestimated the true cooling by on average 0·03°C/hr and since the cooling rate was adjusted to be similar to that during heat production, it enabled this correction to be used to modify the calculated cooling during the heat production period. This underestimation of the cooling might arise from the fact that in the above theory, axial conduction is assumed negligible, i.e. $\partial^2\theta/\partial z^2 = 0$, and temperature profiles are assumed to be symmetrical i.e. $\partial^2\theta/\partial\phi^2 = 0$, whereas in fact they are somewhat asymmetric.

Results

A typical variation of muscle centre temperature with time is shown in Fig. 2a. It can be seen that after an initial period of stabilization (region A), the temperature then increases (region B), levelling off to a plateau (region C) before finally decreasing (region D). Figure 2b shows the variation with time of the muscle centre temperature corrected for heat loss, for the muscle sample in Fig. 2a. Tables 1 and 2 show, for each experiment, the adiabatic increase in temperature, the heat production, the corresponding decrease in pH, and the variation with time of the rate of heat production. In calculating heat production from adiabatic increase in temperature, a specific heat of 3·6 kJ/kg °C was used.

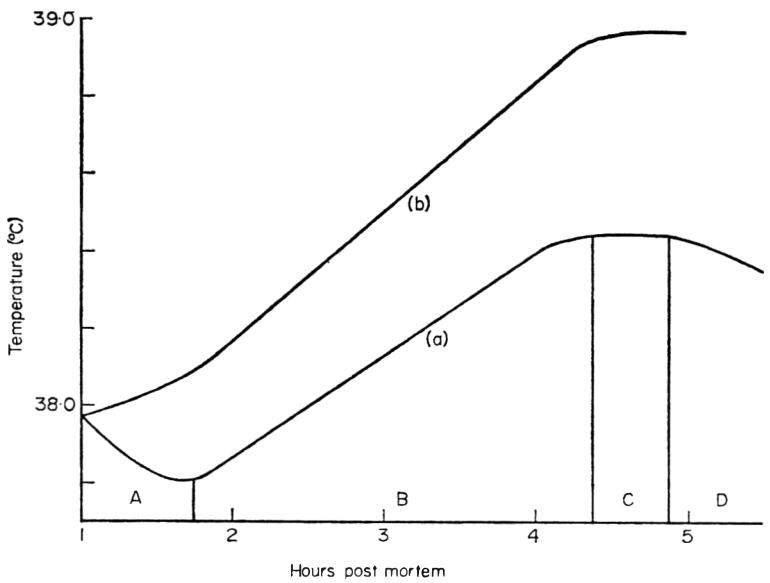

FIG. 2. Typical variation of the temperature of the muscle centre with time: (a) as observed; (b) if perfectly insulated.

The means and standard deviations of the heat productions for the M. semitendinosus and M. extensor carpi radialis samples are $4{\cdot}50 \pm 0{\cdot}9$ kJ/kg and $4{\cdot}14 \pm 0{\cdot}7$ kJ/kg respectively. The tables show that the rate of heat production reaches a maximum level between 1 and 2 hr post mortem (mean = 1·5 kJ/kg hr), this maximum rate being maintained for a further 2 or 3 hr before finally decreasing to zero.

On calculating the heat production per unit decrease in pH, the following mean values are obtained in the pH ranges specified (Table 3).

Since the differences between these means are not statistically significant, an average value will be taken for the heat production per unit decrease in pH, of 5·4 kJ/kg for M. semitendinosus and 6·8 kJ/kg for M. extensor carpi radialis.

TABLE 1. Heat production in beef M. semitendinosus

Expt no.	Period post mortem (min)	Adiabatic increase in temperature (°C)	Heat production (kJ/kg)	Rate of heat production (kJ/kg.hr)	pH change	Ultimate pH	Total heat production (kJ/kg)
1	60–110	0·15	0·54	0·65	6·75–6·6		
	110–180	0·42	1·51	1·29	6·6–6·35		
	180–240	0·37	1·33	1·33	6·35–6·15	5 65	3·92
	240–255	0·10	0·36	1·44	6·15–5·95		
	255–280	0·05	0·18	0·43			
2	60–135	0·31	1·12	0·90	6·55–6·2		
	135–205	0·54	1·94	1·66	6·2–6·2	5 6	4·29
	205–245	0·28	1·01	1·51	6·1–5·75		
	245–275	0·06	0·22	0·44			
3	60–125	0·73	2·63	2·43	6·55–6·15		
	125–205	0·75	2·70	2·03	6·15–5·65	5·65	6·48
	205–250	0·27	0·97	1·29	5·65–5·65		
	250–275	0·05	0·18	0·43			
4	60–120	0·38	1·37	1·37	6·7–6·5		
	120–185	0·37	1·33	1·23	6·5–6·1	5·65	3·92
	185–240	0·32	1·15	1·25	6·1–5·95		
	240–250	0·02	0·07	0·42			
5	60–110	0·12	0·43	0·52	6·5–6·3		
	110–160	0·33	1·19	1·43	6·3–6·2		
	160–210	0·26	0·94	1·13	6·2–6·0	5·6	3·25
	210–230	0·16	0·58	1·74	6·0–5·9		
	230–240	0·03	0·11	0·66			
6	60–105	0·15	0·54	0·72	6·4–6·35		
	105–155	0·30	1·08	1·30	6·35–6·15		
	155–210	0·24	0·86	0·94	6·15–5·9	5·6	3·92
	210–265	0·35	1·26	1·37	5·9–5·65		
	265–290	0·05	0·18	0·43			
7	60–115	0·10	0·36	0·39	6·55–6·55		
	115–180	0·32	1·15	1·06	6·55–6·3		
	180–305	0·81	2·92	1·40	6·3–5·65	5·65	4·97
	305–325	0·11	0·40	1·20	5·65–5·65		
	325–335	0·04	0·14	0·84			
8	60–100	0·12	0·43	0·65	6·55–6·4		
	100–155	0·33	1·19	1·30	6·4–6·4		
	155–200	0·31	1·12	1·49	6·4–6·1	5·6	4·51
	200–250	0·41	1·48	1·78	6·1–5·75		
	250–270	0·08	0·29	0·87			

TABLE 1 (*continued*)

Expt no.	Period post mortem (min)	Adiabatic increase in temperature (°C)	Heat production (kJ/kg)	Rate of heat production (kJ/kg.hr)	pH change	Ultimate pH	Total heat production (kJ/kg)
9	60–100	0·22	0·79	1·19	6·6–6·3		
	100–150	0·30	1·08	1·30	6–3–6·2		
	150–210	0·47	1·69	1·69	6·2–5·6	5·6	6·01
	210–285	0·66	2·38	1·90	} 5·6–5·6		
	285–310	0·02	0·07	0·17			
10	60–95	0·10	0·36	0·62	6·55–6·35		
	95–150	0·40	1·44	1·57	6·35–6·25		
	150–215	0·48	1·73	1·60	6·25–5·7	5·65	4·53
	215–250	0·24	0·86	1·47	} 5·7–5·65		
	250–265	0·04	0·14	0·56			
11	60–105	0·10	0·36	0·48			
	105–185	0·52	1·87	1·40	6·65–6·45		
	185–260	0·37	1·33	1·06	6·45–6·0	5·7	3·70
	260–275	0·04	0·14	0·56	} 6·0–5·75		

Discussion

Tables 1 and 2 show that, in general, the heat production apparently ceases before the ultimate pH is reached. However, since it is known that the biochemical reaction responsible for pH fall, namely the breakdown of glycogen to lactate, also produces heat, the heat production would be expected to continue until the ultimate pH is reached. The reason for it apparently not doing so is thought to be that the heat production rate reaches so low a value that it becomes undetectable, this corresponding to the slow rate of fall in pH as the ultimate pH is approached. This undetectable heat production can be estimated from the mean heat production per pH unit and the difference in pH between the ultimate pH and the pH at which the heat production just becomes undetectable. Similarly an estimate of the heat produced before the experiments commenced, can be obtained from the difference between the initial pH and the pH at 1 hr post mortem. This was taken to be 0·25 pH unit for M. semitendinosus and 0·1 pH unit for M. extensor carpi radialis, being twice the mean observed decrease in pH between $\frac{1}{2}$ and 1 hr post mortem. The heat production became undetectable at an average 0·1 pH unit above the ultimate pH for M. semitendinosus and at 0·45 pH unit for M. extensor carpi radialis. Thus the total 'undetected' heat production was estimated to be 1·89 kJ/kg for M. semitendinosus and 3·74 kJ/kg for M. extensor carpi radialis. Hence the mean total heat production during the whole of the rigor mortis process is 6·4 kJ/kg for the M. semitendinosus samples and 7·9 kJ/kg for the

TABLE 2. Heat production in beef M. extensor carpi radialis

Expt no.	Period post mortem (min)	Adiabatic increase in temperature (°C)	Heat production (kJ/kg)	Rate of heat production (kJ/kg.hr)	pH change	Ultimate pH	Total heat production (kJ/kg)
12	60–110	0·45	1·62	1·94	6·95–6·8		
	110–185	0·67	2·41	1·93	6·8–6·6	6·05	4·68
	185–215	0·15	0·54	1·08	6·6–6·45		
	215–240	0·03	0·11	0·26			
13	60–140	0·39	1·40	1·05	7·05–6·8		
	140–200	0·41	1·48	1·48	6·8–6·6		
	200–275	0·52	1·87	1·50	6·6–6·4	5·8	5·14
	275–300	0·09	0·32	0·77	6·4–6·35		
	300–310	0·02	0·07	0·42			
14	60–110	0·18	0·65	0·78	6·9–6·8		
	110–145	0·19	0·68	1·17	6·8–6·65		
	145–200	0·32	1·15	1·25	6·65–6·45	5·9	3·34
	200–280	0·22	0·79	0·59	6·45–6·3		
	280–295	0·02	0·07	0·28			
15	60–115	0·24	0·86	0·94	6·75–6·6		
	115–185	0·47	1·69	1·45	6·6–6·4	5·75	3·99
	185–245	0·35	1·26	1·26	6·4–6·3		
	245–270	0·05	0·18	0·43	6·3–6·25		
16	60–140	0·15	0·54	0·41	7·0–6·75		
	140–200	0·30	1·08	1·08	6·75–6·45		
	200–275	0·42	1·51	1·21	6·45–6·4	5·9	3·53
	275–295	0·08	0·29	0·87	6·4–6·25		
	295–310	0·03	0·11	0·44			

M. extensor carpi radialis samples, these values being equivalent to temperature rises, under adiabatic conditions of 1·8°C and 2·2°C respectively.

Smith (1930) measured the heat of rigor in the leg of rabbit by observing the rise in temperature of muscles kept in a Dewar flask immersed in a constant temperature

TABLE 3. Heat production per unit pH decrease

	M. semitendinosus				M. extensor carpi radialis		
pH range	6·7–6·4	6·4–6·2	6·2–5·9	5·9–5·6	7·0–6·7	6·7–6·5	6·5–6·2
kJ/kg per pH unit	4·5	5·5	4·9	7·8	6·7	6·3	8·4
Standard error	1·0	1·0	0·6	3·1	1·5	0·8	2·8

water bath at 21°C. The muscle temperature was initially about 0·5°C below the bath temperature and rose to about 0·5°C above the bath temperature during the experiment. The cooling coefficient of the calorimeter was determined by observing the rate or fall in temperature of a similar quantity of autoclaved muscle at a lower and then at a higher initial temperature than that of the water bath. He obtained an average heat production, in four experiments, of 4·58 kJ/kg. This is the heat production from 1 hr post mortem onwards, and is close to the corresponding value obtained in this investigation.

Bendall (1973) considered the enthalpies of the main biochemical reactions yielding heat, namely the anerobic breakdown of glycogen to lactate and, in the early post mortem stages, the breakdown of phosphocreatine to creatine and inorganic phosphate. He obtained a total heat production during rigor mortis of between 7·5 and 9·6 kJ/kg for beef. The heat production for M. semitendinosus between pH 6·6 and 5·75 (the mean pH range in Table 1) would be 4·55 kJ/kg, i.e. an average of 97 kJ/mol of lactate formed, since 0·055 mol of lactate per kg is formed per pH unit (Bendall, personal communication). These values agree with those obtained for M. semitendinosus in this investigation, namely 4·5 kJ/kg and 5·4 kJ/kg per unit decrease in pH i.e. 98 kJ/kg/mol of lactate. Smith (1930) obtained an average enthalpy per mole of lactate of 106 kJ for rabbit at 21°C.

Conclusions

The mean values obtained for the total heat production in beef during rigor mortis were 6·4 kJ/kg for M. semitendinosus and 7·9 kJ/kg for M. extensor carpi radialis, the corresponding average heat productions per unit decrease in pH being 5·4 kJ/kg and 6·8 kJ/kg respectively. There appears to be reasonable agreement between the results of this investigation and those of Bendall and Smith and hence the weight of evidence is against the much larger increases in temperature sometimes reported in the literature.

References

Bendall, J.R. (1973) A hypothesis to account for the heat production in muscles during rigor mortis. *Memorandum No.* **10.** Meat Research Institute, Langford.

Carslaw, H.S. & Jaeger, J.C. (1959) *Conduction of Heat in Solids*, 2nd edn. Clarendon Press, Oxford.

Golovkin, N. A., Alyamovsky, I.G., Pershina, L.I. & Shagan, O.S. (1958) *Bull. Int. Inst. Refrig.* Annexe **1958–2,** 441.

Smith, E.C. (1930) *Proc. R. Soc. B* **107,** 214.

Sybesma, W. & Logtestijn, J. G. van (1966) *XIIth European Meeting of Meat Research Workers*, Sandefjord. Paper E13.

(*Received* 25 *August* 1973)

J. Fd Technol. (1974) **9**, 157–169.

Chilling of poultry: the effects of process parameters on the level of bacteria in spin-chiller waters

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Summary

A survey has been made of the effects of process parameters on the microbiological contamination of spin-chiller waters at five poultry process plants in the UK. Water samples, taken at hourly intervals, from both the first and second chillers were examined bacteriologically and for the levels of residual chlorine pH value and turbidity. Water usage was also monitored. In one process line comparison was made of the effect of substantial modification to the process plant resulting in changes in both water usage and chlorination levels.

The colony counts of presumptive coliform bacteria and other bacteria were reduced generally to < 1 l and < 32 ml^{-1} respectively when high water usage (> 4 l/kg carcass weight) was combined with high total residual chlorine (30–50 ppm). Higher numbers of bacteria (normally $< 10^5$ ml^{-1}) were recovered when high water usage (> 4 l/kg) was combined with low residual total chlorine (< 5 ppm) or when low water usage (< 1 l/kg) was combined with moderate levels of residual chlorine (7–16 ppm). The signifiance of the results is discussed with reference to the hygienic operation of spin-chillers.

Introduction

Rapid chilling of poultry immediately after evisceration is an important step in the prevention of spoilage and in the control of potential food poisoning organisms which may be present on the carcass. Spin chilling is the most frequently used commercial method in the USA, UK, Denmark, Holland and many other countries. However, a recent directive of the EEC (Council Directive, 1971) prohibits the use of spin chilling of poultry on hygienic grounds from 1 January 1977.

The major argument against the use of spin chilling is that of possible cross-contamination of previously uninfected carcasses with pathogenic organisms (especially salmonellae) derived from infected carcasses and transmitted through the water. In spite of

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evidence that the major opportunity for cross-contamination occurs prior to chilling, i.e. during defeathering, evisceration and head removal (Galton *et al.*, 1955; Wilder & MacCready, 1966; Woodburn & Stadelman, 1968; Surkiewicz *et al.*, 1969; v. Schothorst, Notermans & Kampelmacher, 1972) attention has been drawn to the possibility of cross-contamination by bacteria washed from the carcasses by the action of the spin chiller plant.

Berner & Scholtyssek (1968) and Peric, Rossmanith & Leistner (1971) have suggested that the numbers of organisms on the carcasses increased during the period of spin chilling, but their results differ from those of other workers (Buchli, v. Schothorst & Kampelmacher, 1966; Surkiewicz *et al.*, 1969; Mead & Thomas, 1973b) who have demonstrated that the numbers of faecal organisms and staphylococci on the carcasses decrease during passage through the chiller. Indeed, even the work of Peric *et al.* (1971) suggested that initially there was a decrease in the bacterial load of spin-chilled carcasses. Evidence that these chillers can be operated hygienically comes from the work of Surkiewicz *et al.* (1969) who showed that the number of carcasses contaminated with salmonellae neither increased nor decreased during spin chilling and that the numbers of coliforms on the carcasses decreased. Buchli *et al.* (1966) surveyed a number of process plants and demonstrated that the level of bacterial contamination of spin chilled carcasses was less than that of air-chilled carcasses.

The importance of maintaining an adequate water throughput and the desirability of maintaining an adequate free chlorine residual in the water has been shown by Barnes & Mead (1971) and by Mead & Thomas (1973a). In a commercial poultry process plant Mead & Thomas (1973a) demonstrated that bacteria washed from the carcasses are destroyed by chlorine. They showed also that the build up of suspended organic matter in the water can be controlled by the use of suitable water flow rates. The present investigation was undertaken to compare the effects of water usage and chlorination levels on the hygiene of spin-chillers operated commercially under widely varying conditions.

Materials and methods

Five process lines from within the British Poultry Industry were selected for study. The survey was organized such that each process line was studied on each of two occasions over a full working day of 8–9 hr. In one process line the investigation was repeated following modification to the plant. Details of the process lines are summarized in Table 1 and the methods of chlorination of the process waters are summarized in Table 2.

The preparation of bacteriological media and the sampling and analysis was undertaken by the staff of the collaborating companies working under the direction of one of the authors (RMB). Water samples (see below) collected at hourly or 2-hourly intervals from the chiller tanks were examined for bacterial colony counts at 1°, 20°

TABLE 1. Data on the spin-chilling plant used in the various process lines

		Process line					
		A (1 & 2)	A (3)	B	C	D	E
Type of chiller	(1)†	1 RK/4*	1 RK/4*	2 RK/4*	2 RK/4*	ZEBARTH	1 RK/4*
	(2)	2 RK/4*	3 RK/4*	3 RK/4*	'Drag Chain'	ZEBARTH	3 RK*
Capacity of chiller	(1)	1200	1200	2400	2400	3300	1500
(gal)	(2)	2000	3600	3600	5200	6600	3500
Direction of water flow		Parallel	Parallel	Parallel	Parallel	Counter	Parallel

* Gordon Johnson-Stevens Ltd, Model No.

† Refers to chiller (1) or chiller (2). Chiller (1) received water only and chiller (2) received both water and ice.

TABLE 2. Mode of addition and type of chlorination used on the various process lines

Process line	Chlorination	
	In-plant	Additional to chillers
A (1, 2 and 3)	None	Automatic, chemical*†
B	Automatic, gas, $\geq$ 15 ppm	Manual, chemical to Chillers 1 and 2‡
C	Automatic, gas, $\geq$ 10 ppm to supply for Chiller 1 only	Manual, chemical to Chiller 2 only‡
D, E	Automatic gas, $\geq$ 10 ppm	None

* Alkaline based hypochlorite

† Added to input water.

‡ Added directly to the chillers.

and 37° and for presumptive coliforms. Estimations of turbidity, pH values, temperature, residual total and free chlorine concentrations, water flow rate and carcass throughput were also made. The data was collated and analysed by the authors.

Bird throughput

The throughput of birds was determined by counting the carcasses entering the first chiller during timed periods of 15 or 30 min at hourly or 2-hourly intervals. The mean weights of the eviscerated carcasses were obtained for each investigation from the factory data.

Water flow rate

Normally the rate of flow of the water entering the chillers was determined by recording the time to collect 2 gal of input water at hourly or 2-hourly intervals.

In two plants direct readings were taken at hourly or 2-hourly intervals from water flow meters. The quantity of ice added to the second chiller on each line was recorded separately.

Water usage

The data for throughput of birds, the total weight of eviscerated carcasses processed, the water flow rate and the water capacity of the chiller was used to compute the water usage in terms of volume of water (litres) per kg carcass weight processed.

Water temperature

The temperature of the water in the chillers was measured using a Multirange Temperature Unit (In-Line Process Control, Food R.A., Leatherhead, Surrey) or a Dependatherm Electronic Thermometer (Kane May Ltd, Swallowfields, Welwyn Garden City, Herts.).

Collection of water samples

Samples were collected in jugs previously sterilized with alcohol from one site in each section of each chiller tank. The by-pass of the Gordon-Johnson Stevens chillers was sampled at 4-hourly intervals. Samples for bacteriological analysis were transferred immediately to 6 oz (150 ml) bottles containing 10 ml 3% (w/v) sodium thiosulphate solution to neutralize the chlorine. Exceptionally, samples were transferred to 8 oz (200 ml) bottles containing 10 ml 4% (w/v) sodium thiosulphate. The bottles were filled to the neck, capped and mixed several times by inversion. An appropriate dilution factor was applied in calculation of the bacterial colony counts.

pH value of the water

The pH values of chiller water samples were measured electrometrically using a Model 29 pH Meter (Radiometer, Copenhagen, Denmark) with a combined glass-calomel electrode (Ref. GK2311C).

Turbidity of the water

The turbidity of the samples was measured with an EEL Nephelometer Head and a Unigalvo Type 200 (Evans Electroselenium Ltd, Halstead, Essex) using $\frac{3}{4}$ in diameter optically clean test tubes. The instrument was calibrated using a tube of distilled water and a standard opacity tube (EEL Ref. 05399004).

Chlorine levels

Residual chlorine concentrations were determined colorimetrically using a Lovibond Comparator 1000 (BDH Ltd, Poole, Dorset) with moulded 13·5 mm cuvettes calibrated

3–12 ml. A 'White Light Meter' (BDH Ltd) was used for all tests. Residual total chlorine was measured using potassium iodide tablets and free chlorine using diethyl-p-phenylenediamine tablets (BDH Ltd).

Bacteriological analyses

Serial decimal dilutions of each neutralized sample were prepared in one quarter strength Ringers Solution (Oxoid) containing 0·1% (w/v) Peptone (Oxoid). Total colony counts were made on Plate Count Agar (Oxoid) using the one-quarter plate surface spread technique of Farrell & Barnes (1964). Plates were air dried at 50–55° for $1\frac{3}{4}$ hr before use. Exceptionally, when a severe contamination problem was encountered the drop count method of Miles & Misra (1938) was used. Replicate sets of plates were incubated at 37°, 20° and 1° and counted after 2, 5 and 14 days respectively. Prior to incubation at 1° all Petri dishes were incised in four places to permit a more liberal supply of air within the dish. MacConkey Agar No 3 (Oxoid) was used to enumerate presumptive coliform organisms in 0·1 ml aliquots of the undiluted neutralized sample or in dilutions prepared as above. The aliquots were spread over the surface of the agar and plates were incubated at 37° and counted after 1 and 2 days. When low levels of coliforms were anticipated, 1 ml aliquots of the neutralized undiluted sample were inoculated into each of three bottles of MacConkey Purple Broth (Oxoid). The broths were incubated at 37° and examined for the presence of acid and gas after 1 and 2 days.

The presence of *Escherichia coli* type 1 was confirmed by subculture from positive MacConkey broths; colonies selected randomly from MacConkey Agar plates were also tested. Tests were carried out at the process plants in Brilliant Green Bile Broth (Oxoid) or MacConkey Purple Broth (Oxoid) incubated at $44 \pm 0{\cdot}5°$ and in Tryptone or Peptone water (Oxoid). Confirmatory tests undertaken by the authors were made in Brilliant Green Bile Broth and Tryptone Water. The ability to ferment lactose with the production of gas at 44° in the presence of bile salts and to produce indole from tryptophan was considered as evidence for *E. coli* type I.

Results

Water usage, residual chlorine levels, pH value and turbidity of the water

The calculated water usage per kg carcass weight processed, together with the mean residual chlorine levels and pH values are presented in Table 3. The data in Table 3 demonstrates the marked differences in water usage and chlorination levels in the different process lines. Water usage ranged from about 1 to about 14 l per kg carcass weight and the mean residual chlorine levels ranged from < 5 to 50 ppm total chlorine and from 0·1 to 30 ppm free chlorine. The data for the third investigation on process line A was obtained after substantial modification to the plant involving an increase

TABLE 3. Water usage, pH values and mean residual chlorine levels of the water in spin-chillers on the various process lines

Process line	Investigation	Carcass throughput	Total carcass wt (kg) processed	Water in tank* (litres)	Water throughput	Water usage litres/kg carcass wt processed*: (a) in relation to flow of water	Water usage litres/kg carcass wt processed*: (b) in relation to total water	Mean residual chlorine (ppm): Total*	Mean residual chlorine (ppm): Free*	Mean water pH value*
A	1	22,000	31,500	(1) 5460 (2) 9100	(1) 13,982 (2) 2711†	(1) 0·44 (2) 0·09 } 0·53	(1) 0·62 (2) 0·37 } 0·99	(1) 13·5 (2) 15·8	(1) 11·5 (2) 8·7	(1) 7·3 (2) 7·3
	2	17,054	28,300	(1) 5460 (2) 9100	(1) 11,466 (2) 3438†	(1) 0·40 (2) 0·09 } 0·49	(1) 0·60 (2) 0·41 } 1·01	(1) 7·3 (2) 6·7	(1) 4·8 (2) 1·7	(1) 7·1 (2) 7·0
	3§	26,433	31,300	(1) 5460 (2) 15,925	(1) 79,500 (2) 46,300‡	(1) 2·54 (2) 1·48 } 4·02	(1) 2·71 (2) 1·99 } 4·70	(1) 34 (2) 38	(1) 30 (2) 35	(1) 8·45 (2) 8·54
B	1	30,500	41,300	(1) 10,920 (2) 16,380	(1) 62,553 (2) 83,447‡	(1) 1·52 (2) 2·02 } 3·54	(1) 1·78 (2) 2·42 } 4·20	(1) 46·0 (2) 23·8	(1) 2·7 (2) 1·9	(1) 7·9 (2) 7·6
	2	23,500	40,000	(1) 10,920 (2) 16,380	(1) 72,072 (2) 92,046‡	(1) 1·80 (2) 2·30 } 4·10	(1) 2·07 (2) 3·71 } 4·78	(1) 31·1 (2) 12·4	(1) 6·7 (2) 0·7	(1) 7·3 (2) 7·0
C	1	31,198	42,500	(1) 10,920 (2) 23,660	(1) 61,243 (2) 68,015‡	(1) 1·44 (2) 1·60 } 3·04	(1) 1·70 (2) 2·16 } 3·86	(1) 7·2 (2) 30·3	(1) 1·7 (2) 12·6	(1) 7·7 (2) 7·6
	2	32,664	44,550	(1) 10,920 (2) 23,660	(1) 72,072‡ (2) 74,529	(1) 1·62 (2) 1·67 } 3·29	(1) 1·86 (2) 2·20 } 4·06	(1) 2·5 (2) 35·4	(1) 1·3 (2) 6·4	(1) 7·6 (2) 8·0
D	1¶	1,180	17,730	(1) 29,120 (2) 58,240	(1) 32,760 (2) 32,305‡	(1) 1·84 (2) 1·82 } 3·66	(1) 3·48 (2) 5·10 } 8·58	(1) < 5 (2) < 5	(1) 0·4 (2) 0·1	(1) 7·2 (2) 7·3
	2	10,115	14,390	(1) 29,120 (2) 58,240	(1) 52,657 (2) 58,458‡	(1) 3·66 (2) 4·06 } 7·72	(1) 5·68 (2) 8·10 } 13·78	(1) < 5 (2) < 5	(1) 0·6 (2) 0·2	(1) 7·2 (2) 7·3
E	1	20,687	28,500	(1) 6825 (2) 15,925	(1) 65,520 (2) 14,765‡	(1) 2·30 (2) 0·52 } 2·82	(1) 2·54 (2) 1·08 } 3·62	(1) 2·5 (2) < 5	(1) 0·5 (2) 0·2	(1) 7·1 (2) 7·5
	2	21,241	25,220	(1) 6825 (2) 15,925	(1) 65,520 (2) 32,760‡	(1) 2·60 (2) 1·30 } 3·90	(1) 2·86 (2) 1·93 } 4·79	(1) 3·5 (2) 8·4	(1) 0·3 (2) 0·1	(1) 7·3 (2) 7·4

* Figures in parentheses relate to chiller tank nos (1) and (2).

† Ice only.

‡ Ice and water.

§ Plant modified, see text.

¶ 4 hr run only.

in the capacity of the second chiller by the incorporation of an extra section of chiller tank, and increases in the rate of water throughput and the level of chlorination.

The turbidity of the water was used as an indirect measure of the suspended organic matter. In general, the turbidity increased initially with time and with carcass throughput. At high water usage (e.g. $>4\,l\,kg^{-1}$) the turbidity increased to a lesser extent and an equilibrium was established after a period of 2–4 hr.

Chiller water temperature

The primary objective of chillers for poultry is the rapid reduction of carcass temperature prior to chill or deep freeze storage and the conditions necessary for adequate chilling have been discussed elsewhere (Ranken, 1973). The water temperatures measured varied considerably from one process line to another but were generally within the limits recommended by the American Poultry Inspectors Handbook (USDA, 1972). The overall mean temperatures in the first and second chillers were 13·4 and 5·2°C respectively.

Bacterial counts

The range and mean logarithmic colony count per ml water at 1°, 20° and 37° and the presumptive coliform count, for all sites within any one chiller is presented in Table 4 for the individual investigations on each process line. The mean and range of counts for different sampling sites, including the water by-pass, within any one chiller were comparable. The differences observed for the two investigations on any one process line are within the range of variation which might be expected for normal production conditions where the source, numbers and carcass weights of the poultry will vary. Whilst most investigations extended over a full working day of 8–9 hr the first investigation on process line D was restricted to 4 hr.

Changes with time in the counts of bacteria and in the residual chlorine levels are illustrated in Figs 1 and 2 for the second investigation on each of three process lines (lines A, B and C). The changes observed in colony counts for water samples from lines D and E were similar to those in the two initial investigations on line A. Comparisons of the colony counts at 1° and of the presumptive coliform counts before and after modifications to the plant in process line A are presented in Fig. 3.

In the case of both chillers in process line B and the second chiller in process line C the levels of surviving coliform bacteria ($<11\,ml^{-1}$) were below the level of detection of the counting method used. Similar results were obtained on the modified plant in process line A (Fig. 3). The 'presence or absence of coliforms' test indicated a level of less than one presumptive coliform ml^{-1} water in most samples tested from these process lines. Of the presumptive coliforms tested, between 67 and 95% were identified as *Escherichia coli* type I, the number varying according to the actual process line.

TABLE 4. Range and (mean) $\log_{10}$ colony counts per ml chiller water

Process line	Investigation	Range and (mean) $\log_{10}$ colony counts per ml chiller water							
		Chiller 1				Chiller 2			
		Presumptive coliform	Total 37°C	Total 20°C	Total 1°C	Presumptive coliform	Total 37°C	Total 20°C	Total 1°C
A	1	< 1·04–3·80 (2·86)	NT	NT	< 1·50–3·40 (1·85)	< 1·04–3·31 (2·05)	NT	NT	< 1·50–2·28 (1·55)
	2	< 1·04–4·40 (3·31)	< 1·50–4·50 (3·51)	< 1·50–4·93 (3·93)	< 1·50–3·31 (2·51)	< 1·04–4·33 (2·86)	< 1·50–4·90 (3·39)	< 2·50–4·35 (3·25)	< 1·50–3·37 (2·52)
	3	< 1·04–1·73 (< 1·04)	NT	NT	< 1·50	< 1·04–2·12 (< 1·04)	NT	NT	< 1·50–1·90 (< 1·50)
B	1	< 1·04–2·07 (1·04)	< 1·50–3·32 (2·43)	1·80–2·76 (2·39)	< 1·50	< 1·04	< 1·50–3·62 (2·11)	< 1·50–2·82 (2·05)	< 1·50
	2	< 1·04–1·95 (1·04)	< 1·50–2·35 (1·50)	< 1·50–3·72 (2·47)	< 1·50	< 1·04–1·77 (1·04)	< 1·50–2·12 (1·50)	< 1·50–2·72 (2·11)	< 1·50
C	1	2·75–4·14 (3·65)	4·47–5·24 (4·85)	3·78–5·15 (5·04)	3·02–4·06 (3·41)	< 1·04–1·90 (1·04)	< 1·50–3·71 (2·04)	< 1·50–3·91 (3·13)	< 1·50–3·83 (1·94)
	2	< 1·04–4·35 (3·54)	< 1·50–5·03 (4·22)	< 1·50–5·10 (4·34)	< 1·50–4·76 (3·89)	< 1·04–2·54 (1·40)	< 1·50–2·89 (2·02)	< 1·50–3·75 (2·91)	< 1·50–3·44 (2·48)
D	1	< 1·04–3·12 (2·11)	< 2·50–4·26 (2·99)	< 2·50–3·90 (3·31)	2·19–3·60 (2·98)	< 1·04–3·00 (2·20)	< 2·50–4·03 (3·10)	< 2·50–4·34 (3·72)	< 2·50–3·96 (3·18)
	2	< 1·04–3·83 (2·79)	2·50–4·34 (3·51)	< 2·50–4·13 (3·42)	2·19–3·76 (3·00)	< 1·04–3·54 (3·68)	2·50–4·29 (3·38)	2·50–4·35 (3·70)	< 2·50–3·72 (3·17)
E	1	< 1·04–3·33 (2·16)	< 2·50–4·26 (3·29)	< 2·50–5·36 (3·94)	< 2·50–4·74 (3·13)	< 1·04–3·99 (3·00)	< 2·50–4·97 (4·04)	2·50–4·35 (4·82)	< 2·50–4·94 (4·26)
	2	< 1·04–3·47 (2·19)	< 2·50–4·71 (3·33)	2·74–5·74 (3·97)	< 2·50–3·47 (2·84)	< 1·04–3·47 (3·00)	< 2·50–4·77 (3·92)	3·49–5·73 (4·66)	3·33–4·64 (4·19)

NT = Not tested.

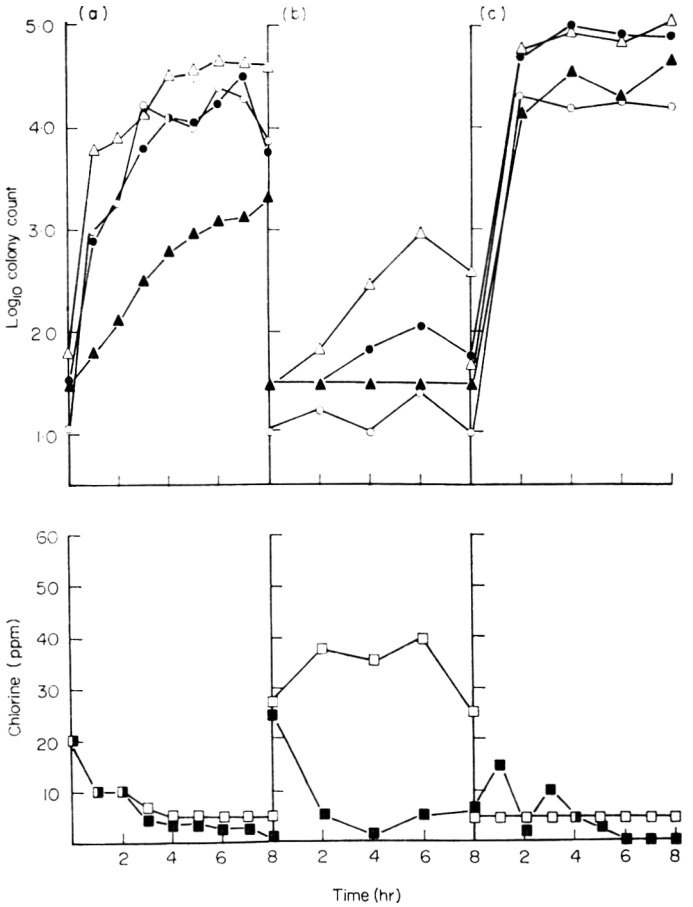

FIG. 1. Changes in the presumptive coliform count (○) and the colony count at 37° (●), 20° (△) and 1° (▲) and in the residual total (□) and free (■) chlorine levels in water samples from the first chiller taken during the second investigation on (a) line A, (b) line B and (c) line C.

Discussion

A significant relationship between water usage, chlorination level and the number of viable bacteria in spin-chiller waters has recently been demonstrated for a three stage chiller system in a single poultry process plant (Mead & Thomas, 1973a). In the present study similar observations have been made in process lines operating under a variety of commercial conditions. Quantitative differences associated with different types of plant were small in comparison with the differences resulting from variation in water usage and chlorination levels. However, direct comparison of the bacteriological results with those of Mead & Thomas (1973a) suggests that a three stage

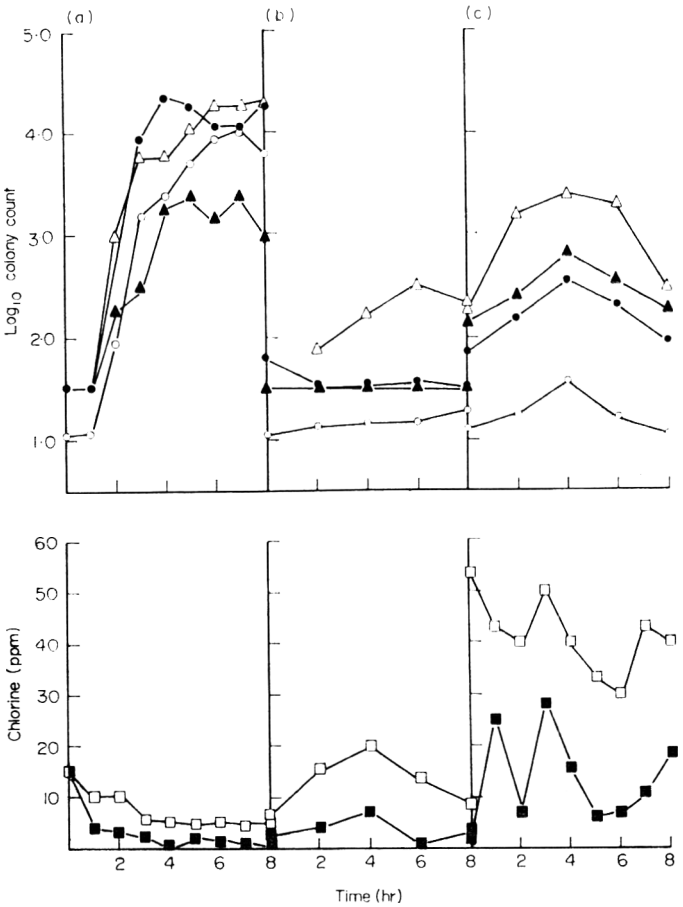

FIG. 2. As Fig. 1 but samples taken from the second chiller on lines A, B and C.

chiller system may be more efficient than the two stage systems studied in the present investigation, since in the third chiller low bacterial counts were obtained even in the absence of chlorination.

The results obtained in the present study are summarized in Table 5. When low water usage (*c.* 1 litre kg^{-1} carcass weight) was combined with moderate chlorination (6–16 ppm total chlorine), or when moderate (3·6–4·8 l kg^{-1}) or high (>8 l kg^{-1}) water usage was combined with low level chlorination (<5–8 ppm), the maximum bacterial count in the chiller waters approached a level of 10^5 organisms ml^{-1} and the maximum coliform count ranged from 10^3 to 10^5 organisms ml^{-1}. Moderate water usage combined with moderate to high levels of chlorination (12–46 ppm total residual chlorine) resulted in colony counts which only rarely exceeded 10^2 organisms ml^{-1} and presumptive coliform counts were near or below the limit of the method of detection

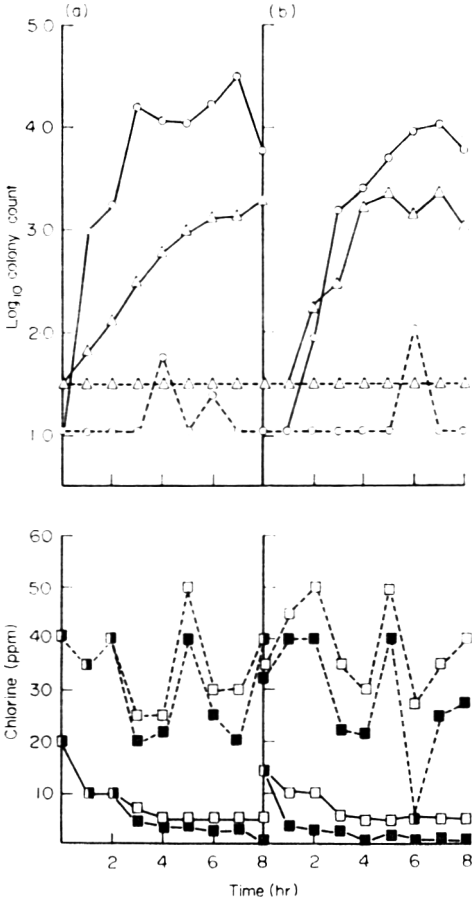

FIG. 3. Changes in the presumptive coliform count (○), the colony count at 1° (△) and the residual total (□) and free (■) chlorine levels for water samples from (a) the first chiller and (b) the second chiller on process line A before (——) and after (– – – –) modifications to the process plant. For further details see text.

(eleven organisms ml^{-1}). This is illustrated by the results obtained on process line C where the first chiller received only 'in plant' chlorination and the second chiller was chlorinated manually to a level in excess of 30 ppm total residual chlorine. In both chillers the water usage was almost identical (cf. Table 3).

Data of this type is of considerable value in ensuring the maintenance of good hygienic standards in poultry process plants. The modifications to process line A were made after the initial results were obtained. The increased levels of water usage and chlorination resulted in significant reductions in the numbers of viable bacteria in the chiller waters throughout the working day.

No attempt has been made to determine the possible presence of salmonellae in the

TABLE 5. Comparison of the highest bacterial counts observed in the water of spin-chillers operated under various conditions

Total water usage (l kg^{-1} carcass)	Mean residual total chlorine (ppm)	Highest colony count ml^{-1} in water from			Reference
		Chiller 1	Chiller 2	Chiller 3	
>8	<5	22,000	22,000	—	This work
3–6	<5–8	550,000	540,000	—	
3–6	12–46	5200	8100	—	
<1	6–16	85,000	80,000	—	
8	Nil	87,000	36,000	3300	Mead & Thomas (1973a)
5	Nil	18,000	19,000	2500	
2·5	Nil	410,000	150,000	21,000	
4·5	Nil	N.T.	10,300,000	—	Peric *et al.* (1971)
1·5	Nil	N.T.	37,000,000	—	

chiller waters. Other workers (see Lee & Riemann, 1971) have shown that chlorine has the same antibacterial effect on salmonellae as on *E. coli* type I. The results for coliform bacteria may therefore be taken as indicative of the trends for any salmonellae which may have been present in the chiller waters. At the observed temperatures of the chiller waters it is unlikely that organisms of faecal origin would have grown although slow growth of psychrophilic spoilage organisms could have occurred (cf. Farrell & Barnes, 1964).

Even under the worst conditions of water usage and the lowest levels of chlorination, few of the 443 samples of water tested from five process lines gave counts higher than 10^5 organisms ml^{-1} and none exceeded $5{\cdot}5 \times 10^5$ organisms ml^{-1}. Mead & Thomas (1973a) also rarely observed counts greater than 10^5 organisms ml^{-1} even under conditions of low water usage in the absence of chlorination (see Table 5). These results are at variance with the data of Berner & Scholtyssek (1968) and Peric *et al.* (1971). Bacterial counts in excess of 10^7 organisms ml^{-1} were reported by Peric *et al.* (1971) after operating a spin-chiller for periods of $2\frac{1}{2}$–6 hr. The water usage data quoted by these workers as 1·5, 3·0 and 4·5 l per carcass for their three experiments are misleading since the majority of water went into the first chiller which was not tested bacteriologically. Counts were made only on the second chiller in their system which received 0·1, 0·2 and 0·5 l per carcass respectively in the different experiments. In these circumstances, particularly where only 0·1 and 0·2 l per carcass were used, the second chiller would be acting largely as a spin-chiller with no significant water flow but with mechanical agitation to wash bacteria from the carcasses. In these conditions continuous build up of organisms would be inevitable. The equilibration achieved in the build up of turbidity in the present investigations shows that the flow of water through the chiller dilutes the detritus washed from the carcasses. A similar effect will undoubtedly occur

with bacteria suspended in the water provided that sufficient water flow is maintained (Jarvis & Blood, 1973). Further control of bacterial numbers can be achieved by chlorination of the water.

The investigations of Surkiewicz *et al.* (1969) demonstrated that the number of faecal organisms on carcasses decrease during passage through a spin-chiller when it is operated under carefully controlled conditions. By controlling the build up of bacteria in the water by adequate water usage and by chlorination, the possibilities for cross-contamination of carcasses will be reduced to very low levels.

Acknowledgments

The authors are indebted to the British Poultry Meat Association Limited for the facilities made available by their members and to the management and staff of the companies which participated for their interest and their active assistance. The authors wish to thank Dr Ella M. Barnes for helpful discussion during the course of this work and Dr G. C. Mead and Mr N. L. Thomas for a pre-publication copy of their paper.

References

Barnes, E.M. & Mead, G.C. (1971) Clostridia and salmonellae in poultry processing. In: *Poultry Disease and World Economy* (Ed. by R.F. Gordon and B.M. Freeman), pp. 47–63. British Poultry Science, Edinburgh.

Berner, H. & Scholtyssek, S. (1968) *Fleischwirtschaft*, **48,** 422.

Buchli, H., van Schothorst, M. & Kampelmacher, R.H. (1966) *Archiv Lebensmittelhyg.* **17,** 97.

Council Directive (1971) of 15th February 1971 relating to the health problems involved in the trade of fresh poultry meat (71/118/EEC), Article 14.

Farrell, A.J. & Barnes, E.M. (1964) *Br. Poult. Sci.* **5,** 89.

Galton, M.N., Mackel, D.C., Lewis, A.L., Haire, W.C. & Hardy, A.V. (1955) *Am. J. vet. Res.* **16,** 132.

Jarvis, B. & Blood, R.M. (1973) The relationship between water usage and the level of bacterial contamination of water in spin chillers. *Proc. Symp. Wld Poult. Ass.* Roskilde, Denmark, August 1973.

Lee, W.H.R. & Riemann, H.P. (1971) The inhibition and destruction of *Enterobacteriaciae* of pathogenic and public health significance. In: *Inhibition and Destruction of the Microbial Cell* (Ed. by W.B. Hugo), pp. 399–418. Academic Press, New York.

Mead, G.C. & Thomas, N.L. (1973a) *Br. Poult. Sci.* **14,** 99.

Mead, G.C. & Thomas, N.L. (1973b) *Br. Poult. Sci.* **14,** 413.

Miles, A.A. & Misra, S.S. (1938) *J. Hyg., Camb.* **38,** 732.

Peric, M., Rossmanith, E. & Leistner, L. (1971) *Fleischwirtschaft*, **51,** 216.

Ranken, M.D. (1973) Chlorination and the hygienic operation of spin chillers. In: *Proceedings of the Fourth Europe Poultry Conference*, London, pp. 353–358. British Poultry Science, Edinburgh.

v. Schothorst, M., Notermans, S. & Kampelmacher, E.H. (1972) *Fleischwirtschaft*, **52,** 749.

Surkiewicz, B.F., Johnston, R.W., Moran, R.W. & Krumm, G.W. (1969) *Fd Technol., Champaign*, **23,** 1066.

U.S.D.A. (1972) *American Poultry Inspectors Handbook.* U.S.D.A. Consumer and Marketing Service, Poultry Division, Washington D.C.

Wilder, A.N. & MacCready, R.A. (1966) *New Engl. J. Med.* **274,** 1453.

Woodburn, M. & Stadelman, W.J. (1968) *Poultry Sci.* **47,** 777.

(*Received* 10 *June* 1973)

J. Fd Technol. (1974) **9**, 171–177.

Utilization of edible protein from meat industry by-products and waste

II. The spinning of blood plasma proteins

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Summary

Spun proteins which may be acceptable as meat analogues have been fabricated from blood plasma. It was found that plasma protein, initially concentrated by partial freeze drying, could be converted into a suitable form for spinning by denaturation with alkali followed by the addition of a weak acid to effect stabilization of the resulting high viscosity solution. A method for producing textured plasma protein on a laboratory scale is described, together with some of the properties of the resultant products.

Introduction

The recovery of protein from blood represents an area of the meat by-products industry as yet not exploited to its fullest extent. More efficient processing of animal blood would provide a valuable source of protein whilst concomitantly alleviating a pollution problem.

The potential of blood as a human food source was recognized some years ago in the development of spray dried plasma which could be used as a substitute for dried egg albumin (Brooks & Ratcliff, 1959). Attempts have also been made to produce dried protein concentrates from whole blood (Vickery, 1968).

More recently, it has been suggested that blood plasma could be included in meat products and meat analogues derived from soya protein to improve binding quality and nutritional value (Gordon, 1971). However, the fabrication of blood protein into palatable foods has not so far been described. It seems highly desirable therefore to assess the possibility of texturizing blood protein into organoleptically attractive forms.

Since the protein of blood plasma is in solution at high concentration, it is in a convenient state for conversion into fibres using the spinning process so that the resultant product should resemble meat. The generation of texture in vegetable proteins by this technique has been described elsewhere (Boyer, 1954; Ashton, Burke & Holmes, 1970). In this study, the modification of plasma protein into a denatured form suitable for spinning and its subsequent fabrication into a spun product has been investigated.

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Materials and methods

Blood collection and separation of plasma

Bovine blood was collected directly from the jugular vein. A concentration of 50 mg of heparin in 4 ml 0·9% saline was used as anticoagulant for 1 litre of blood. The collected blood was centrifuged twice at 1000 ***g*** for 20 min to separate the plasma.

Concentration of plasma

The desired concentration of protein for spinning was achieved by employing a partial freeze drying method. A calibration curve of water loss against time was initially constructed for 150 ml volumes of plasma, blast frozen at −25°C for 24 hr and freeze dried for 2, 4, 8, 16 and 24 hr respectively. Freeze drying proceeded at an internal pressure of 0·05 mm Hg and a plate temperature of 40°C. The samples were then thawed and filtered. Final protein contents were measured using the microkjeldahl method so that losses of soluble protein due to denaturation could be estimated.

Preparation of spinning dope

About 1 l bovine blood plasma was collected and divided into two samples. The samples were concentrated to protein contents of 98 mg/ml and 85 mg/ml respectively by partial freeze drying. The effect of various amounts of NaOH, ranging between 0·8% and 1·0% (w/v) of total volume, on the viscosity of the plasma was determined. The desired concentration of alkali was achieved by the addition of 40% (w/v) NaOH with thorough mixing.

Measurements of the viscosity of 120 ml volumes of plasma were made using a modification of the falling sphere technique. Spinning dope (alkali-treated plasma) was supported vertically in a glass tube of 2 cm internal diameter. The time taken for a 1/8 in diameter steel ball to sink 20 cm after reaching terminal velocity was taken as a measure of viscosity. All measurements were made at room temperature (21°C). An estimate of the absolute viscosity in poises was derived by applying Stoke's Law.

Stabilization of dope viscosity

Although several workers describe the attainment of a stable viscosity suitable for spinning merely by treating the protein solutions with alkali (Naismith, 1954; Kelley & Pressey, 1966), this procedure was found to cause gel formation when applied to plasma in the present work. A similar effect has also been reported for groundnut protein solutions (Green *et al.*, 1960). It was shown that the viscosities of these solutions could be effectively stabilized after the addition of alkali by reducing the pH to 10·5–11·0. This approach was investigated by preliminary treatment of plasma with NaOH followed by addition of acetic acid to effect pH reduction.

Heparinized bovine plasma was concentrated to 112 mg/ml protein by partial freeze drying. It was found convenient to add NaOH to the plasma so that the ratio

NaOH : protein was 0·1 which initiated a viscosity increase to about 50 P in 15 min. This was accomplished by adding 2·8 ml of 40% NaOH to 100 ml volumes of concentrated plasma, the final pH being 12·4.

After the initial viscosity rise, 2 N acetic acid was added in volumes within the range of 6·0–8·0 ml with increments of 0·2 ml. Solutions were thoroughly mixed to dissolve local coagulation of protein due to the acid. The variation of viscosity with time was again measured by the falling sphere method.

The spinning of protein fibres

Fibres were spun from bovine plasma converted to a protein dope of suitable stable viscosity. This was found to be 250 P. The procedures used to obtain this high viscosity dope were derived from the preceding experiments.

Pilot scale spinning apparatus similar to that described by Wormell (1954) was used. The protein dope was poured into a reservoir and a pressure of 20–30 lb in^{-2} applied with a manual pump. The dope was thus forced through to a gear pump which provided a steady flow to the spinneret. The pumping pressure could be varied to give varying flow velocities. Any extraneous matter present in the dope was removed by double filtration. The stainless steel spinneret was immersed in the coagulating bath and contained forty holes each of 0·008 cm diameter. After precipitation, the fibres were drawn from the bath using a rotating godet wheel which wound the fibres in bundles or tows. The rotational speed of the wheel could be varied which in turn altered the degree of stretching of the fibres. The spinneret was immersed as deeply as possible in the bath to provide maximum dwell time of the precipitated fibres in the coagulating solution. This solution was generally 11% Na_2SO_4 or 20% NaCl in 1 N acetic acid. Samples were also obtained using a H_2SO_4/salt coagulant.

The fibres were removed from the godet wheel in tows and left to drain of excess coagulating solution. They were then sliced into chunks and stored at 0°C. Protein, moisture and ash contents of the samples were analysed.

Results

Partial freeze drying proved to be an efficient method of concentrating blood plasma. It was found that plasma (originally containing 75 mg/ml protein) could be concentrated to 220 mg/ml protein over a period of 24 hr freeze drying. Using a calibration curve, protein concentrations could be predicted with reasonable accuracy, although it was necessary to filter the plasma after freeze drying to remove some protein coagulates. Comparison of actual protein concentrations after partial freeze drying with theoretical values, calculated on the basis of water loss alone, gave errors of $\pm$ 0·6 mg/ml to 12·0 mg/ml protein from low to high concentration. These errors indicate the low extent of protein loss due to coagulation.

Figure 1 illustrates the effect of NaOH on the viscosity of concentrated plasma. The viscosity remained low after the addition of alkali in all cases for up to 20 min but increased rapidly after this initial period. An interesting feature was that the viscosity rise did not level off with time, gelation occurring fairly quickly after the viscosity increase commenced. Reducing the protein or alkali concentration appeared merely to delay the onset of the viscosity rise. The suitable range of viscosity for spinning has been established at 25–380 P (Green *et al.*, 1960). It can be seen from Fig. 1 that with alkali treatment the rapidly-increasing plasma viscosity would fall within this range for only a few minutes. Under these conditions, the dope would not be suitable for spinning since a much greater degree of stability is required for the process.

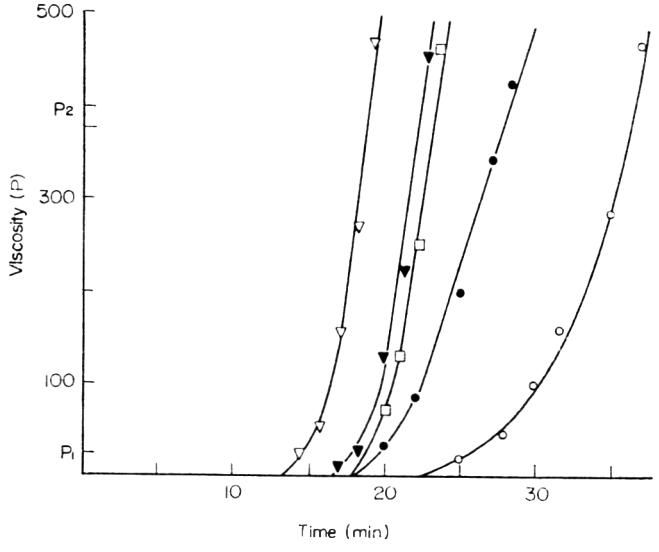

FIG. 1. The alteration of viscosity of concentrated plasma with time as affected by concentration of alkali. ▽ 1% NaOH (protein 98 mg/ml); ▼, 0·9% NaOH (protein 98 mg/ml); □, 0·85% NaOH (protein 98 mg/ml); ●, 0·85% NaOH (protein 85 mg/ml); ○, 0·8% NaOH (protein 85 mg/ml). The values P_1 and P_2 are the limits of suitable viscosity for spinning.

It was found that the viscosity of plasma previously treated with NaOH could be stabilized indefinitely within the range 25–380 P by the addition of acetic acid such that the pH of the solution was reduced from 12·4 to 11·1–11·3. However, the range of concentration of acetic acid required to achieve this effect was narrow. Additions of 2 N acetic acid greater than 7·2 ml or less than 6·8 ml to 100 ml plasma treated with NaOH produced stabilized viscosities which were outside the desirable limits for spinning. Thus, the amount of acetic acid required, considered in terms of acid to protein ratio appeared to be 0·073–0·077. Figure 2 shows the stabilization of dope viscosity by acetic acid. An important observation was that the time of addition of acetic acid

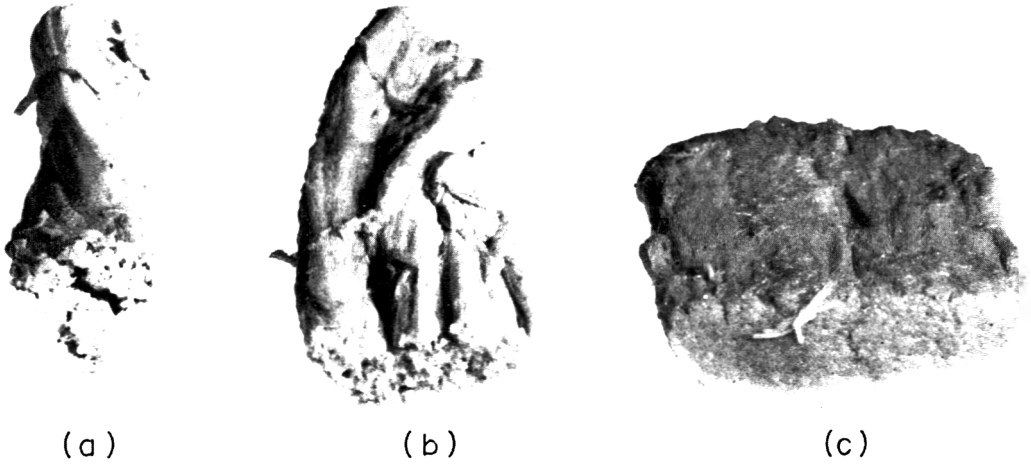

PLATE 1. Spun products fabricated from blood plasma. Note the differences in appearance due to the variation in the constituents of the coagulating solution: (a) 14% NaCl/0·7 N acetic acid/0·3 N H_2SO_4; (b) 20% NaCl/1 N H_2SO_4; (c) 11% Na_2SO_4/1 N acetic acid.

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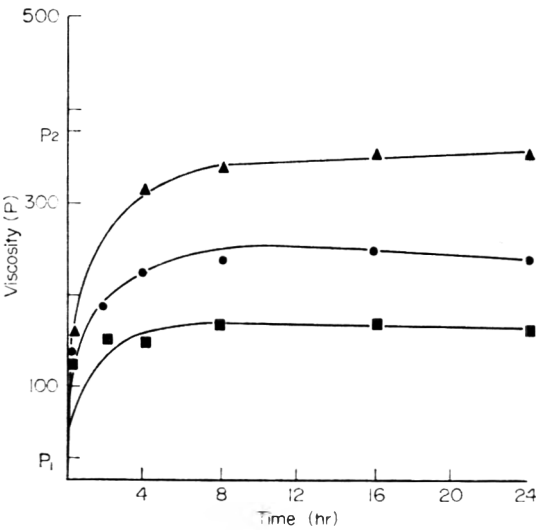

FIG. 2. The stabilization of viscosity of alkali-treated plasma by acetic acid. The plasma protein was initially concentrated to 112 mg/ml and treated with 11·2 mg/ml NaOH in each case. The curves indicate the effect of acid concentration on the final value of viscosity obtained. ▲, 8·16 mg/ml acetic acid (acid : protein 0·073); ●, 8·40 mg/ml acetic acid (acid : protein 0·075); ■, 8·64 mg/ml acetic acid (acid : protein 0·077).

was critical. It proved necessary to wait until the viscosity had reached about 50 P before adding the acid. If the acid was included before this value was reached, the increase in viscosity was completely inhibited.

Protein fibres spun from modified plasma are shown in the photograph. The mean analysis of these samples was as follows.

Protein	17·3%
Moisture	73·3%
Ash	8·6%

The protein concentration of the spun product was found to increase up to 20% after a few days storage due to further drainage of excess solution. Variation in the type of salt used in the coagulating solution had no apparent effect on the fibres. But when sulphuric acid was included in the bath in place of acetic acid a marked effect on the appearance of the precipitating fibres was observed, the fibrous coagulate being more dense and white than that previously obtained. Thus, the final product was a tow consisting of fibres far more coarse than those precipitated in an acid bath. This feature can be seen clearly in the photograph (Plate 1). However, the finer coagulation of protein in the acetic acid/salt mixture resulted in a tow more closely resembling a meat product. The fibres produced by coagulation in sulphuric acid were noticeably more brittle, possessing less binding capacity and elasticity than those coagulated in acetic acid.

Discussion

The elaboration of procedures for modifying protein solutions into forms of suitable protein concentration and viscosity is of great importance in the spinning process. These procedures vary with the type of protein utilized. From this study, it appears that protein dopes can be formed from blood plasma (concentrated to 100–120 mg/ml protein) by the following steps:

(i) the addition of NaOH to the plasma (NaOH: protein = 0·1);
(ii) a period of rest for about 15 min to allow a viscosity increase to 50 P;
(iii) the addition of acetic acid with thorough mixing to reduce the pH of the solution to 11·1–11·3 (acid: protein = 0·075).

By maintaining these ratios of alkali and acid, protein dopes of suitable viscosity for spinning may be obtained from plasma irrespective of volume or protein concentration. It is essential that the high viscosities required remain stable throughout the period of the spinning process. Clearly the results indicate that this can be achieved.

Although these spun products were not treated with edible binders, the protein fibres appear to possess reasonable binding capacity and elasticity. It is also interesting to note that the protein and moisture contents of the final product are similar to those for raw meat. Thus, the work has shown that it is possible to convert blood plasma protein into a fibrous form which appears analogous to meat and that the properties of the fibres can be varied by changing the composition of the coagulating solution. Since there is no heat involved in this process, the high quality protein of the plasma is unlikely to suffer any degree of damage, and the spun product should be of high nutritional merit. This is an important factor with the increasing awareness of the nutritional value of foods. With further development, such a process would provide a valuable outlet for those animal proteins which are at present largely wasted.

Acknowledgments

This work was supported by a grant from the Meat and Livestock Commission which is gratefully acknowledged. The authors are also indebted to Professor C. S. Whewell, Dr G. C. East and Mr D. Storer of the Department of Textile Industries at the University of Leeds for their helpful advice and assistance with the spinning apparatus.

References

Ashton, M.R., Burke, C.S. & Holmes, A.W. (1970) *Textured Vegetable in Proteins.* BFMIRA Scientific and Technical Surveys, No. 62.

Boyer, R.A. (1954) *U.S. Patent* 2,682,466.

Brooks, J. & Ratcliff, P.W. (1959) *J. Sci. Fd Agric.* **9**, 486.

GORDON, A. (1971) *Fd Trade Rev.* **4,** 29.
GREEN, E., GRAHAM, R.W., GARDNER, H.S. & HOLMES, A.W. (1960) *U.S. Patent* 2,958,606.
KELLEY, J.J. & PRESSEY, R. (1966) *Cereal Chem.* **43,** 195.
NAISMITH, W.E.F. (1954) *J. appl. Chem.* **4,** 188.
WORMELL, R.L. (1954) *New Fibres from Proteins.* Butterworths, London.
VICKERY, J.R. (1968) *Meat Industry Bulletin* (*Aust.*), January, p. 31.

(*Received* 1 *July* 1973)

J. Fd Technol. (1974) **9**, 179–184.

Further experiments on the hot-cutting of lamb

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Summary

If joints of lamb, prepared by hot-cutting, are conditioned in a carton for 24 hr in air at 1°C and subsequently frozen, all the meat is tender when roasted from the frozen state, and bacterial counts are acceptable. Legs or shoulders may also be frozen in a carton without conditioning, since toughening does not occur, due to skeletal restrain of muscles. However, it is necessary to condition double loins and double racks to maintain acceptable tenderness.

Distortion due to packing in a carton is not a problem with shoulders, double loins and double racks stacked on end, but occurs to a degree in legs. Experiments on cutting after accelerated rigor produced by electrical stimulation showed an improvement in the handling of legs and shoulders, but not loins. Brief blast chilling of a carcass while suspended from the pelvis allowed easy cutting, including long loins, but the rounded conformation characteristic of hot-cut legs and shoulders is lost.

Introduction

This report explores further the practical processing possibilities of hot cutting, raised by a previous paper (McLeod *et al.*, 1973), in which hot-cut joints of lamb and mutton were 'conditioned' (i.e. given sufficient time to go into rigor at a temperature which avoids cold shortening) and then frozen separately.

As it would be an advantage if subsequent processing could be carried out in cartons, we have attempted the conditioning and freezing of joints packed into cartons immediately after hot-cutting. The results are satisfactory in most respects. Preliminary study of cutting techniques has also been made.

Experimental

Lambs were processed, cut into legs, double loins, double racks and shoulders, and shrink wrapped, as previously described (McLeod *et al.*, 1973).

In the conditioning experiment, the legs from five lambs were packed on the medial

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surface into one carton, six as a bottom layer and four on top. Remaining cuts were stacked as mixed packs in two other cartons—shoulders, two deep lying on the rib surface, and double racks and double loins two to three deep, lying horizontally on the ventral surface. Thermocouples were inserted deep into each type of joint (seven sites), at contact points between joints (five sites), between joints and the box (six sites), and in the air space inside (two sites) and outside (three sites) the carton.

The cartons of meat were chilled in an air stream of velocity 500 ft/min at 10°C. After 24 hr, freezing was commenced, in air of velocity 850 ft/min and temperature falling to −18°C.

Bacteriological sampling was done after freezing at a total of ten sites (four on legs and two each on shoulders, loins and racks). Incubations were carried out at 25°C and 37°C in plate count agar (Difco).

For the immediate freezing experiment, a further ten legs were packed into one carton as a single layer, all lying on the pin-bone with the shanks inwards, crossed in herring-bone array. Ten shoulders were packed as a single layer in another carton, all standing neck up on the cut ribs. The two cartons were then blast frozen immediately. The double racks and double loins from these animals were not frozen immediately, but were packed in a separate carton, conditioned as previously, then frozen. These cuts were packed vertically, half on the neck end, half on the leg end.

Joints were roasted from the frozen state and assessed as previously described (McLeod *et al.*, 1973). In statistically analysing the results, the 'Student's *t*-test' was used.

Results and discussion

(a) *Conditioning and freezing in a carton*

A uniform and steady conditioning temperature of 10°C, as found suitable for cuts, could not be reproduced in a carton. An ambient air temperature had to be chosen which would provide cooling rapid enough to prevent excessive bacterial growth, yet slow enough to avoid cold shortening and allow adequate conditioning. From existing data on cooling rates in cartons, 1°C was chosen.

Table 1 shows the temperatures recorded at various sites during the conditioning period and subsequent freezing. The figures suggest that only after 12 hr has the meat reached temperatures where cold shortening is likely, but at this time the meat is too near rigor to respond. Also, by this time the temperatures were not conducive to rapid growth of micro-organisms.

The mean bacterial counts per cm^2 from the frozen cuts were $8{\cdot}7 \times 10^4$ (25°C) and $7{\cdot}1 \times 10^4$ (37°C). The counts ranged from $3{\cdot}2 \times 10^3$ to $3{\cdot}4 \times 10^5$. Freezing would have killed some organisms, but even so the counts were satisfactory. No special precautions were taken and the degree of handling was greater than normal.

Tenderness results are shown in Table 2. There was no significant difference in tenderness between legs and shoulders that had been conditioned individually in the

TABLE 1. Carton conditioning and freezing. Thermocouple readings in °C

Site	No. of thermo-couples	Hours of cooling						
		2	6	12	18	24*	30	48
Outside air	3	2	0	2	0	2	−15	−18
Inside air	2	17	12	7	5	3	−7	−12
Contact between joints and box	6	18–21	8–14	6–10	3–6	3–4	−9 to −4	−13 to −6
Deep joints and contacts between joints†	12	22–29	17–22	10–16	7–10	4–6	−2 to 1	−6 to −3

* Change to freezing at 24 hr.

† Includes the contact between one leg and the bottom of the box.

TABLE 2. Tenderness* of hot-cut lambs

Treatment†		Shoulder				Leg			
		TB‡	IS	SS	LD	SM	BF	GM	ST
1 Conditioned singly 24 hr, 10°C, frozen§	±	14·8 2·8	11·6 2·1	17·9 3·3	19·8 5·8	16·6 3·6	13·8 3·6	17·9 4·1	14·8 3·5
2 Conditioned, frozen in carton	±	19·8 3·1	11·6 2·9	17·0 2·9	15·4 2·1	15·8 1·3	14·6 3·1	15·0 4·1	13·4 2·3
3 Blast frozen in carton	±	21·3 10·7	14·0 4·2	20·5 5·6	23·6 9·9	17·3 3·0	12·4 2·6	23·3 9·6	14·5 1·9

		Double racks			Double loins		
		Neck up	Neck down	Horizontal	Neck up	Neck down	Horizontal
2 Conditioned, frozen in carton	±	17·1 4·4	27·8 6·8	23·5 2·8	18·9 6·0	16·4 3·4	22·4 3·2

* Mean shear force and standard deviation for five animals in each treatment.

† All cuts roasted from the frozen state.

‡ Muscle code: TB, triceps brachii; IS, infraspinatus; SS, supraspinatus; LD, longissimus dorsi; SM, semimembranosus; BF, biceps femoris; GM, gluteus medius; ST, semitendinosus.

§ After McLeod *et al.* (1973).

earlier study, and those that had been conditioned in the carton. The tenderness of all cuts was highly acceptable. Meat of shear force value below 20 on the MIRINZ tenderometer (Macfarlane & Marer, 1966) is regarded as very tender.

An interesting effect was observed with the double racks. The longissimus in those packed with the neck end upwards was significantly more tender ($P < 0{\cdot}01$) than in

those in the reverse position. Packing position had no effect on tenderness of double loins.

For conditioning in a carton, ambient air at 1°C is satisfactory in terms of microbial control and tenderness.

(b) *Immediate freezing in a carton*

Mean shear values for legs and shoulders, listed in Table 2, show that immediate freezing has no detrimental effect on tenderness of the cartoned cuts. Shear force values were a little higher but not significantly so.

The only toughening observed in legs was in a single muscle of two cuts which had their lateral face against the box. In these two, the gluteus medius scores were 34 and 42 (no other leg muscle score exceeded 21). The highest score recorded for a shoulder muscle was 46 in the triceps brachii of a shoulder packed with the lateral face against the box. A score of 42 was also registered for the longissimus of a shoulder packed on the inside. No other shoulder muscle scored above 30. Thus the position of cuts in the carton has no practical significance for tenderness.

The double loins and double racks from the above animals were used in the conditioning experiment (a). A few have been subsequently blast-frozen in a carton. A double loin gave a score of 41, and three double racks 52, 46 and 28.

Results clearly indicate that hot-cut lamb legs and shoulders (but not double loins and double racks) can be cartoned and frozen immediately without detriment to the tenderness of the meat. The reason for this is that the hot-cut leg is a virtual 'natural' posture, which has been shown to confer immunity to toughening by cold shortening or thaw shortening. A balance of tension between adductor and flexor groups results in a fairly uniform skeletal restraint on the muscles (Davey & Gilbert, 1973; Quarrier, Carpenter & Smith, 1972). The shoulder is little affected for the same reason, irrespective of hanging or cutting methods. The good results of the carton conditioning experiment are therefore (for legs and shoulders) not a reflection of adequate conditioning time, but rather the lack of a need to condition. However, the higher shear forces for early-frozen loins and racks show that conditioning is necessary. It should be pointed out that leg steaks cut from the early-frozen material, after brief frozen storage, and cooked from the frozen state, would be quite vulnerable to thaw shortening (Davey & Gilbert, 1973).

(c) *Distortion of cuts*

The carton conditioning experiment showed clearly that joints cannot be stacked more than one deep. The packing of shoulders, double loins and double racks on end, as in the early freezing experiment, led to negligible distortion. In the case of legs, the herring-bone array produced a dent in the lower leg and some puckering of the wraps. Thus the problems of distortion, while not severe, are only partly resolved. Brief chilling of the carcass before cutting, as described in the next section, may help.

Freezing in a carton is efficient for handling but not in terms of heat transfer, and other systems, more efficient from the latter point of view, might also dispose of distortion problems.

(d) *Cutting techniques*

The softness of meat and fat and the flexible state of the carcass make hot cutting on a normal band-saw a difficult and dangerous operation. Special blades, sliding cradles or other devices might help to overcome these problems. These have yet to be investigated, but some attempt has been made to modify the meat rather than the equipment.

It has recently been shown in the laboratory that electrical stimulation of lambs immediately after slaughter accelerates the onset of rigor mortis dramatically, the carcasses becoming quite stiff in 40 min (Chrystall & Hagyard, 1973). Two animals so treated were cut at 1 hr post mortem on a band saw. The stiff carcasses were much easier to handle. Two transverse cuts were made without difficulty, and the leg and shoulder pairs were then easily separated. However, the loins were still floppy and the flaps tended to catch in the saw. Also, the table became coated with fat. In spite of the stiffness of the limbs, the meat appeared as soft as that of unstimulated carcasses. The shrink wraps produced the same attractive rounded conformation as before in leg and shoulder. The loins, however, distorted badly, exactly as described in the previous paper.

Since the cutting of chilled carcasses on band-saws is a standard trade practice, it seemed that a brief pre-chill might assist cutting. A lamb, hung from the pelvis to avoid cold shortening, was blast-chilled for 80 min in a blast-freezer running at $-12°C$. The deep loin had then reached 6°C. The carcass, although pre-rigor, was quite stiff due to hardening of the fat, and sawed as easily as if frozen or chilled in rigor. One side was returned to the freezer, and the other was cut immediately into joints. The square edges of the cuts were preserved on shrink wrapping, and the conventional long loin did not distort. The cuts were then frozen immediately in a carton. They were barely distinguishable from those from the other side, cut and wrapped after freezing. It appears that hardness of the fat is more important than a rigor condition of the muscle in promoting ease of sawing. Thus brief chilling is a big advantage in this respect, but the rounded configuration of leg and shoulder is lost.

It should be noted that the early-cut loin from this experiment was tough when roasted from the frozen state after storage for 4 weeks at $-12°C$ (shear force 41), whereas the loin frozen before cutting was tender (shear force 18). This difference can only be due to cold shortening, which must have occurred inside the undistorted shell of fat during freezing in the carton. The conclusion from the earlier results, that loins need to be conditioned before freezing, still applies with the pre-chill technique. A combination of the electrical stimulation with the pre-chill should produce undeformed loins which could be frozen immediately, since the pH is already below the lower limit for cold shortening (Chrystall & Hagyard, 1973).

(e) *Shelf life*

Two legs and two shoulders were roasted after 12 months storage in a carton at *c.* −12°C. These joints were pairs with those conditioned and frozen in the carton and cooked soon after. The shear force values were unchanged by storage and there was no sign of off-odours in the oven or off-flavours on eating. It is concluded that the frozen shelf life of this material is at least a year at normal store temperatures.

Conclusion

While the technique of hot cutting is still far from application as an on-line process, the experiments described extend the possibilities and make clearer some of the problems.

Whether processing in cartons is the best direction for development is a matter for further study, but in terms of tenderness and control of bacteria, early packing is acceptable if either conditioning or immediate freezing is intended. Conditioning is needed only for the double loins and double racks. However, some problems in distortion of cuts remain.

Early cutting of the carcasses is clearly possible, but needs further work. The methods adopted are likely to vary according to the cuts desired and the stage at which packing occurs.

Acknowledgments

The authors wish to acknowledge the co-operation of the staff of the Auckland Farmers' Freezing Co-operative Ltd in processing, the services of Mrs R. Wyborn in bacteriological counts, the assistance of Dr B. B. Chrystall in the electrical stimulation experiment, and discussions with Dr C. L. Davey.

References

Chrystall, B.B. & Hagyard, C.J. (1973) In preparation.

Davey, C.L. & Gilbert, K.V. (1973) Submitted for publication.

Macfarlane, P.G. & Marer, J.M. (1966) *Fd Technol., Champaign*, **20**, 134.

McLeod, K., Gilbert, K.V., Wyborn, R., Wenham, L.M., Davey, C.L. & Locker, R.H. (1973) *J. Fd Technol.* **8**, 71.

Quarrier, E., Carpenter, Z.L. & Smith, G.C. (1972) *J. Fd Sci.* **37**, 130.

(*Received* 16 *August* 1973)

J. Fd Technol. (1974) **9**, 185–193.

Measurement of the stickiness and other physical properties of bread crumb

E. E. McDERMOTT

Summary

A simple apparatus for measuring the stickiness, compressibility and resilience of fresh bread crumb is described. Results are discussed in relation to the α-amylase activity of the flour, and to the early stages of bread staling.

Introduction

The stickiness of crumb is an important quality characteristic of bread. Stickiness can be adversely affected by faults during baking, and, more commonly, by the use of flour with excessive α-amylase activity, which results in an increased attack on the starch by the enzyme. Thus, not only is the amount of water that can be held by the starch decreased in both the dough and the bread as it is baked, but also excessive amounts of starch degradation products are formed. These products (dextrins and sugars) contribute to a degree of crumb stickiness that is unacceptable by the consumer, and can also create serious technical problems at the bread-slicing stage, for there may be a build up of gummy substances on the cutting blades that can lead to crumb tearing, loaf deformation and eventually to blockage at the slicing machine.

A practical investigation of the problem of crumb stickiness should include, therefore, a study of other crumb characteristics such as its strength, softness, and springiness. The study should also be made on very fresh bread 2 to 3 hr after baking, i.e. at the usual time for slicing. However, the crumb is then still soft and relatively plastic, and consequently any method of measuring its properties must be sensitive enough to give results using small applied forces to minimize crumb deformation and internal cohesion. It is to be noted that when bread is taken from the oven and allowed to cool, the crumb rapidly loses its doughy consistency and increases in mechanical strength, so that crumb characteristics important to the consumer are usually measured some 24 hr after baking.

The literature on the long term staling of bread is extensive, but very little work has been done with very fresh bread crumb. Cornford & Coppock (1950) have reviewed the techniques used for the measurement of the physical properties of bread crumb,

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and Cornford, Axford & Elton (1964) describe the use of the cone indenter to measure the firmness of fresh crumb.

Tunger & Porchmann (1963) determined the stickiness of 24 hr old bread crumb using an apparatus developed by Kulman (1953) in which loads of 1–3 kg were applied to the crumb.

This paper describes a new apparatus which measures in one sequence the stickiness, compressibility and resilience (springiness) of the crumb at a particular position on the surface of a slice of bread. The relatively small loads applied to the crumb, allow the apparatus to measure successfully the properties of fresh crumb, whilst examination of more stable (24 hr old) crumb supplements the information from currently available instruments.

Experimental

Measurement of crumb properties

The apparatus shown in Fig. 1, is a modified free swinging balance.

A $1\frac{1}{2}$ in (3·8 cm) diameter Perspex disc (A) is fixed below the platform (B), and exactly counterbalanced by a small beaker (J) and appropriate weights on pan (C). A slice of bread, cut thick enough (3–4 cm) to allow unrestricted compression under the chosen load, is placed on the platform of the adjustable lab-jack (D), and raised until contact is made with the Perspex disc. A small weight (5 g) is placed on B to maintain this contact whilst D is again adjusted until the pointer (E) is at the centre of the specially prepared scale (F), on which 2×1 mm scale divisions correspond to a compression by the Perspex disc of 1 mm. Weight (G), (200 g unless stated otherwise) is placed on B, and is allowed to compress the crumb for 1 min, after which time the position of the pointer on the scale is noted and this reading divided by two to give the *crumb compressibility* in millimetres. Weight G is then removed, and after a further 1 min, the position of the pointer again noted, and the *crumb resilience* is calculated as the percentage of the original depth of compression that is recovered. The 5 g weight is then removed from B, and water from the burette (H) added one drop at a time into beaker (J) until the Perspex disc is just pulled from the crumb surface. The volume, and thus the weight, of water required is noted, and this quantity provides the value for the *adhesion*, or *stickiness*, of the crumb, i.e. as grams.

For maximum reproducibility of results, it was necessary to position the Perspex disc as far as possible exactly at the same position on each slice of bread, whilst allowing for the presence of small holes and other irregularities in the crumb texture.

A razor-sharp knife was used for slicing the bread to avoid compressing the bread crumb, and to obtain a smooth surface free from partially-detached fragments of crumb.

When measuring the properties of fresh, warm bread, the slices were placed in an insulated close-fitting, shallow polystyrene box (1 in thick). The temperature of the

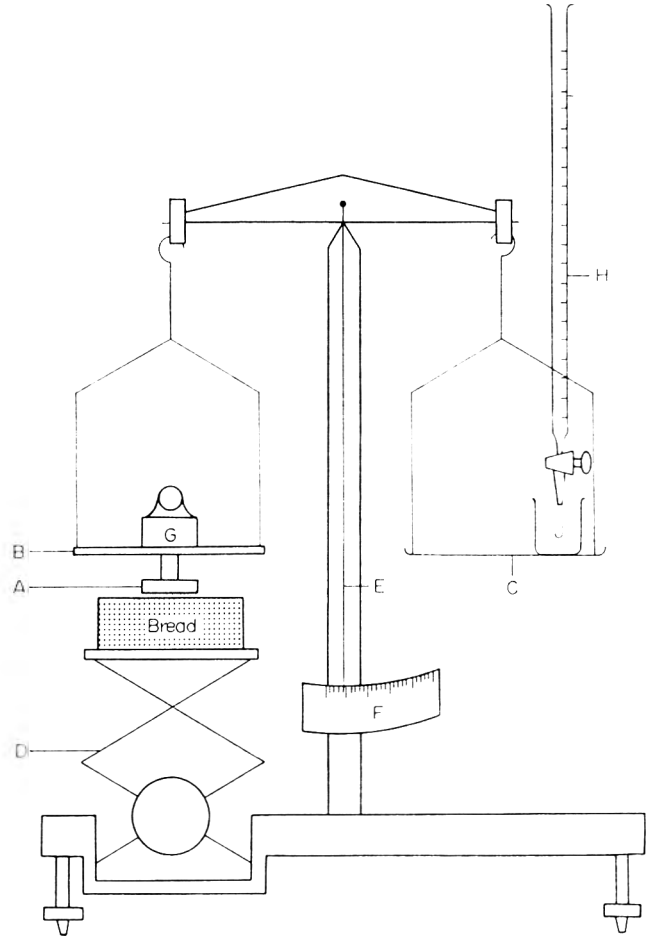

FIG. 1. Apparatus for measuring the stickiness, compressibility and resilience of bread crumb.

crumb was then determined by inserting the thermocouple of a 'Thermophil' into the slice as near as possible to the position of the Perspex disc. Values reported later are the means of temperatures recorded before and after the measurement of crumb properties.

Baking procedure

Bread was made by the Chorleywood Bread Process as follows.

Recipe.	Flour	7000 g	Fat	50 g
	Yeast	150 g	Water	4350 g
	Salt	125 g	Ascorbic acid	0·525 g

Malt flour, when required, was an addition to this recipe. 25 g malt flour = 1 lb malt/sack (280 lb), this amount increasing the α-amylase activity of the flour by approximately 1 Jongh Unit (Jongh, 1957).

The ingredients were mixed and developed to 5 W hr/lb, in a Tweedy 35 dough mixer. The dough was divided into 2 lb pieces and moulded to a ball-shape by hand. After an intermediate proof for 10 min at room temperature in proving boxes, the final mould was made by a Sorensen Universal Mark II commercial moulder. The dough pieces were proved at 105°F (40·5°C) to a height in the tin of 12 cm with sufficient humidity present to prevent skinning, and then backed at 460°F (238°C) in a commercial reel oven for 30 min.

Results

Reproducibility

The apparatus was first tested on bread 20 hr after baking in order to evaluate the reproducibility of the measurements of stickiness with reasonably stable crumb. Table 1 gives the values for stickiness obtained at the central position on the surfaces (a–e) of five slices (4 cm thick), cut, as illustrated in Fig. 2, from two separate batches of five loaves baked from flour containing 2 lb malt/sack (Bakes 1 and 2).

TABLE 1. Reproducibility of stickiness measurements

	Stickiness (g)										
	Bake 1					Bake 2					
Slice surface	Loaf					Slice surface	Loaf				
	1	2	3	4	5		1	2	3	4	5
a	5·5	5·4	5·7	6·0	5·0	a	5·8	5·3	5·9	6·0	5·5
b	6·7	7·7	7·2	7·7	6·3	b	6·9	6·5	6·8	6·9	6·5
c	8·2	8·0	7·6	8·4	7·5	c	7·2	7·5	8·0	8·2	7·8
d	7·0	7·1	6·6	6·2	6·5	d	6·8	6·7	6·9	7·0	6·8
e	0·8	0·8	1·0	0·5	0·2	e	0·5	0·2	0·8	0·8	0·5

Values for stickiness obtained for crumb surfaces at position e were much lower than those from the other positions. One contributing factor to this phenomenon could be that the crumb is less moist at this position, the moisture content ranges from 35% at a position 0·5 cm under the crust to 42% in the centre of the loaf. However, the more likely explanation for the lack of stickiness is that the very rapid heat penetration at this position considerably reduces the time during which the α-amylase can act on the starch. For Bake 1, the values obtained for surfaces at position e ranged from 0·2 g to 1·0 g around a mean value of 0·66 g, compared with those for positions a–d which

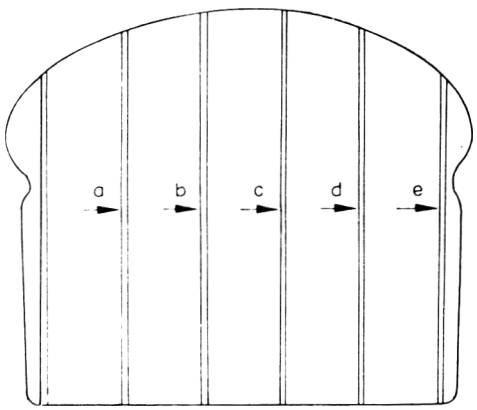

FIG. 2. Position within the loaf of slice surfaces used to obtain measurements in Table 1.

ranged from 5·0 g to 8·4 g with a mean of 6·81 g. The measurements for surfaces at position e were therefore excluded from the two-way analysis of variance of the stickiness results for slices a–d.

Values for stickiness obtained for different slice surfaces, and thus for different positions through the loaves of Bake 1, were well outside the experimental error, indicated by the interaction term ($\delta E = \pm 0{\cdot}38$, with 12 degrees of freedom, D.F.), and significantly greater than the differences between the five replicate loaves. At the 5% level of significance, the means for the five surface positions, using the procedure described by Snedecor (1956), are as follows: Surface c (7·94 g) > surfaces b and d (7·12 g and 6·88 g) > surface a (5·52 g) > surface e (0·66 g).

The loaves baked from another batch of the same flour (Bake 2), gave similar results; again, surfaces b and d were not separable, and the new overall mean at 6·75 g was almost identical with that for Bake 1 (6·81 g). Furthermore, the correlation between the two sets of means for the five slice surfaces is highly significant at $r = +0{\cdot}996$ with 3 D.F. ($P < 0{\cdot}1\%$).

Variation in properties throughout the loaf

Figure 3 illustrates diagrammatically the variation in stickiness and resilience for loaves baked from flour containing 1 lb malt/sack, 20 hr after baking. Measurements were made at three positions on the surfaces of 3 cm-thick, horizontal and vertical slices. Maximum stickiness occurred at the central position of the 'break' level (the horizontal level of the loaf corresponding to the top of the baking tin), and the lowest stickiness values were again found at positions close to the crust. The crumb resilience varied even more throughout the loaf, highest values being obtained at the centre of that part of the loaf within the baking tin, and also at positions near to the crust. Short & Roberts (1971) showed that bread crumb firmness varied throughout a loaf, the maximum value being found at the centre.

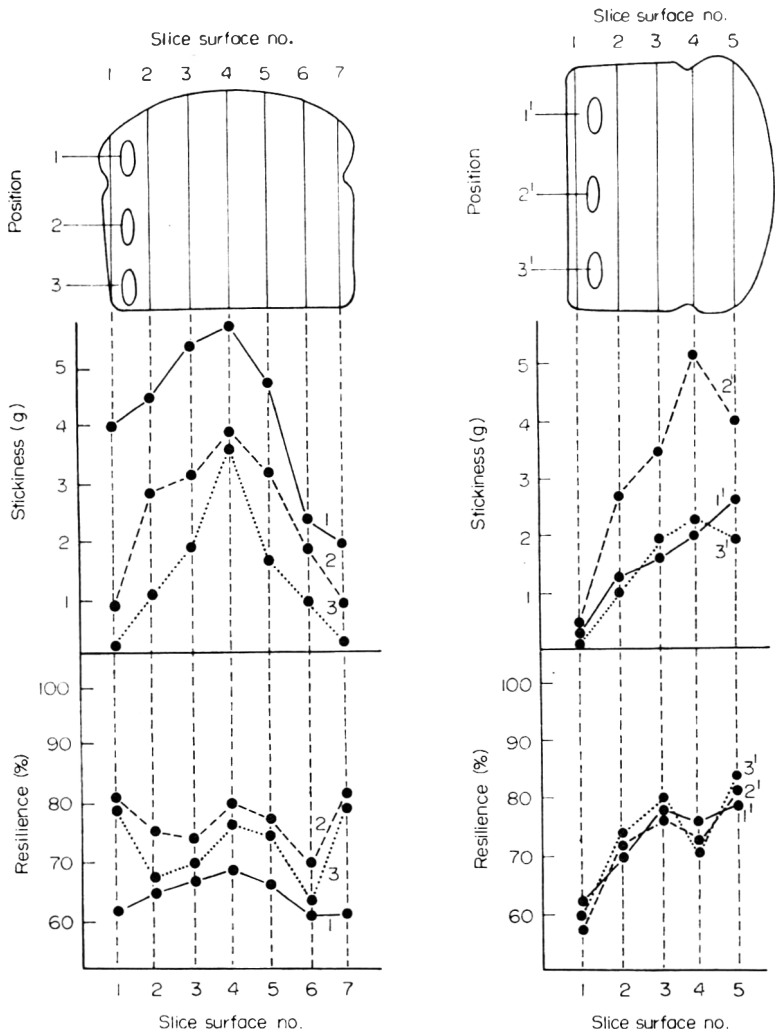

FIG. 3. Variation in crumb properties throughout a loaf (20 hr after baking).

Effect of increasing levels of malt, and of applied load

Figure 4 illustrates the results obtained for bread made from flour with increasing additions of malt, 20 hr after baking. The weight applied to the crumb surface was increased in 100 g increments from 200 g to 500 g. Values for crumb compression and resilience, measured at the same position and time as the stickiness measurement, are also shown. Although the stickiness of the crumb increases with extra additions of malt, the values for each level of malt are fairly steady over the range of applied weights, and not unduly influenced by the depth of crumb compression that increases steadily

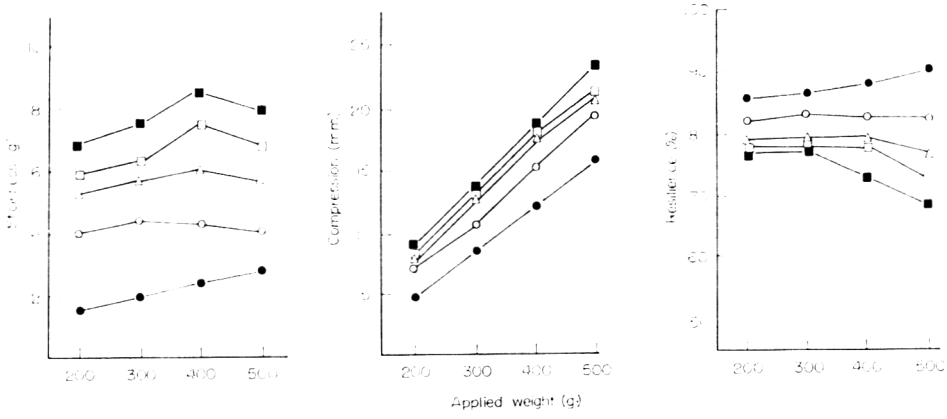

FIG. 4. Effect of increasing additions of malt on crumb properties (20 hr after baking), and the effect of using increasing loads on the slice surface. ●, 0 lb malt/sack; ○, 0·5 lb malt/sack; △, 1·0 lb malt/sack; □, 1·5 lb malt/sack; ■, 2·0 lb malt/sack.

over this range. Similarly, there is little change in crumb resilience except for the higher levels of added malt, where the crumb breaks down and loses some of its springiness.

Properties of very fresh bread crumb

Figures 5 and 6 show the properties of bread crumb over the critical period during which the bread is normally sliced. In these experiments the loaves were placed about 2 in apart on wire-mesh trays and allowed to cool to room temperature. In the first hour, the loaves cool to about 40°C, and slices cut during this time were placed in a heat-insulating polystyrene box and the crumb temperature measured as described above. As the bread was not cooled under strictly controlled conditions, the results given here for very fresh crumb illustrate only the general pattern of change in crumb properties during the early stages of cooling, and not the absolute changes.

Figure 5 shows that the stickiness of the crumb after cooling for 1 hr is essentially independent of the amount of malt added, whereas after 3–5 hr, the decrease is clearly related to the level of α-amylase activity. Crumb compressibility decreases steadily with time, whereas crumb resilience increases steadily after a rapid increase over the first 2 hr.

In Fig. 6 the pattern of change in crumb properties over a 3 hr cooling period is shown in greater detail for the control and the addition of 1 lb malt/sack. Crumb temperatures at the time of measuring the properties are shown on the graphs. The resilience of the control crumb increases very rapidly, reaching about 90% of its 3 hr value in the first 30 min (temperatures about 50°C), whereas the 1 lb malt/sack crumb reaches only 60% of its 3 hr value in the same time. The decrease in crumb compressibility again illustrates the improvement in crumb stability on cooling, but the malt

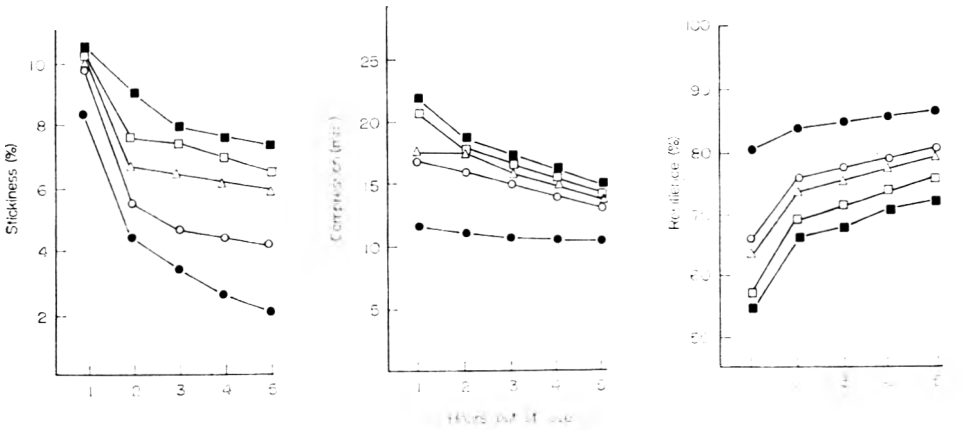

FIG. 5. Variation in crumb properties with time after baking, and the effect of increasing additions of malt. ●, 0 lb malt/sack; ○, 0·5 lb malt/sack; △, 1·0 lb malt/sack; □, 1·5 lb malt/sack; ■, 2·0 lb malt/sack.

crumb retains a high level of compressibility much longer than the control crumb. In contrast, the decrease in stickiness of the two crumbs from their very high initial values follow almost identical curves during the first hour's cooling (temperature about 40°C), and only then, as seen previously in Fig. 5, do the stickiness values for the control and malt crumbs begin to differ significantly.

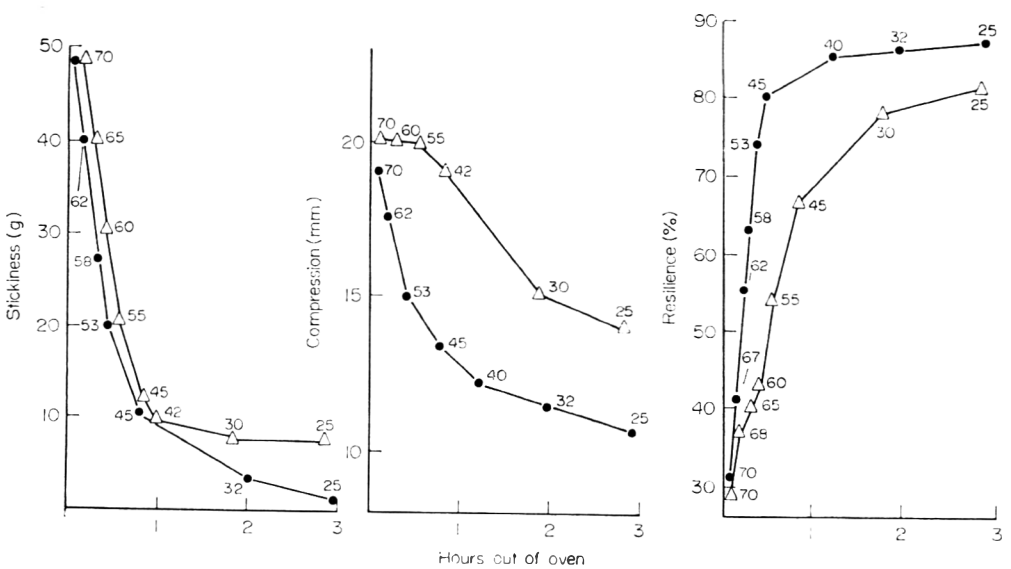

FIG. 6. Variation in the properties of very fresh crumb with time after baking and crumb temperature. ●, 0 lb malt/sack; △, 1·0 lb malt/sack. Number at symbol is the crumb temperature (°C) at that time out of oven.

Discussion

It would seem logical to relate the measured increase in stickiness of loaves with added malt flour with the presence of increased levels of dextrins and sugars resulting from the effect of the increase in α-amylase activity. However, unpublished work has shown that the distribution of these products throughout the loaf is remarkably uniform, and does not parallel or explain the variations in stickiness or the other physical properties of the crumb found in this study. These variations in crumb properties could result from differences in the rates of temperature rise within the dough, affecting the extent by which the starch granules swell or are gelatinized during baking. α-amylase action on starch during baking may not only produce dextrins and sugars in the bread, but also may modify the swelling and gelatinization pattern of the starch granules as they are heated in the dough. In this manner the crumb properties, and the way these properties change as the bread cools, may be influenced.

Conclusions

The new apparatus has proved very suitable for measuring the surface stickiness of slices of bread, particularly when they are very fresh. Crumb stickiness has been shown to vary with position within the loaf and to remain fairly steady from 5 to 20 hr after baking. Measured values are related directly to the level of added malt flour. The crumb of bread made from flour of very low α-amylase activity possesses measurable stickiness 20 hr after baking. This fact, together with the very high initial stickiness of all the bread crumb tested, control and malted loaves, indicates the occurrence of a 'natural' crumb stickiness which is possibly related directly to the nature of swollen or gelatinized starch granules.

Acknowledgments

The author is grateful to Dr J. B. Hutchinson for the statistical analysis, and to Mr T. H. Collins and his colleagues for supplying the bread required in this study.

References

Cornford, S.J., Axford, D.W.E. & Elton, G.A.H. (1964) *Cereal Chem.* **41,** 216.
Cornford, S.J. & Coppock, J.B.H. (1950) *Research*, **3,** 558.
Jongh, G. (1957) *Chem. Weekbl.* **53,** 597.
Kulman, A.G. (1953) *Kolloide in der Brotbäckerei.* Moskau.
Short, A.L. & Roberts, E.A. (1971) *J. Sci. Fd Agric.* **22,** 470.
Snedecor, G.W. (1956) *Statistical Methods*, 5th edn, p. 253. Iowa State College Press, Ames, Iowa.
Tunger, L. & Porchmann, W. (1963) *Esnäkrungsforschung*, **8,** 70.

(*Received* 9 *July* 1974)

J. Fd Technol. (1974) **9**, 195–203.

The effect of dents on the vacuum of tin cans*

H. B. BASKER AND I. BEN-GERA

Summary

A method is presented for measuring the extent of dents in tin cans. The effect of such dents on 'vacuum' and headspace measurements is discussed. The effect of temperature variations during testing is considered. A simple, accurate method for the determination of the capacity of tin cans is also presented.

Introduction

In the food canning industry, cans are often sealed when their contents are hot, after the resultant flow of water vapour has driven out a proportion of the air in the headspace above the liquid surface. When the sealed can cools to storage temperature, a 'vacuum'—the difference between the pressure inside and the pressure outside the can—is created (Townsend *et al.*, 1956). Adequate 'vacuum' is considered a factor in assessing the quality of the product (Townsend *et al.*, 1956), and a vacuum test is part of any routine quality control procedure (Dickinson & Goose, 1955).

The Israel Standard for canned fruits and vegetables (Israel Standard, 1964) includes minimum vacuum specifications and maximum headspace specifications (this latter in the form of minimum fill requirements), but ambient conditions at the time of testing are not specified. Dents, panels and distortions render the measurement of vacuum and headspace inaccurate (Townsend *et al.*, 1956) and may also influence saleability: the presence of dents resulting from maltreatment is a common commercial complaint, but no objective method appears to be used to differentiate minor dents and panels from major damage. Measurement of the extent of any denting would assist in such differentiation.

Theoretical considerations

The ambient temperature at the time of measuring vacuum and headspace is considered for the range 10°–30°C, with standard reference temperature 20°C, for a model system consisting of a rigid right cylindrical can containing water, with an ideal gas and water vapour in the headspace.

* Contribution from the Volcani Center, Agricultural Research Organization, Bet-Dagan, Israel, 1972, Series No. 2133-E.

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Put subscripts $_s$ and $_t$ for standard temperature and test temperature, respectively, to

P = pressure in can (mmHg),

P' = 'vacuum' = $P_A - P$,

where

P_A = ambient atmospheric pressure (mmHg),

T = temperature (°A),

P_w = water vapour pressure (mmHg),

V_c = internal volume of can (ml),

V_w = volume of water in can (ml),

ΔV_c = change of volume of can at T_t.

Then, from the gas laws:

$$P_t = (P_s - P_{ws}) \left(\frac{V_c - V_{ws}}{V_c - V_{wt} + \Delta V_c} \right) \left(\frac{T_t}{T_s} \right) + P_{wt},$$

and rewriting in terms of 'vacuum',

$$P_s' = (P_t' - P_{ws} k_t + P_A k_t + P_{wt} - P_A) \div k_t, \tag{1}$$

where

$$k_t = \frac{V_c - V_{ws}}{V_c - V_{wt} + \Delta V_c} \cdot \frac{T_t}{T_s}.$$

ΔV_c is a function of

D = diameter of can (cm),

H = height of can (cm),

f_c = coefficient of thermal expansion of can construction material per °C.

Then

$$\Delta V_c = \frac{\pi}{4} D_t^2 H_t - \frac{\pi}{4} D_s^2 H_s,$$

where

$$D_t = D_s + f_c D_s (T_t - T_s),$$
$$H_t = H_s + f_c H_s (T_t - T_s).$$

Disregarding higher powers of f_c as negligibly small,

$$\Delta V_c = \tfrac{3}{4} \pi f_c D_s^2 H_s (T_t - T_s). \tag{2}$$

Also

$$V_{wt} = \left(V_c - \frac{\pi}{40} h D_s^2 \right) \frac{G_s}{G_t}, \tag{3}$$

where

h = headspace (mm)*

and

G = specific gravity of water.

Approximate solutions to equation (1) may be obtained graphically. Using the common 'A2' can (307 × 408) as an example, the corrections to be added to the vacuum at 10°, 15°, 25° and 30°C, at various headspace heights, are shown in Figs 1–4, respectively, to obtain the vacuum at 20°C, when $P_A = 760$ mm. ΔV_c attains $\mp 0{\cdot}21$ ml at 10° and 30°C, respectively (from equation (2)). The change of V_{wt} attains $-0{\cdot}8$ ml

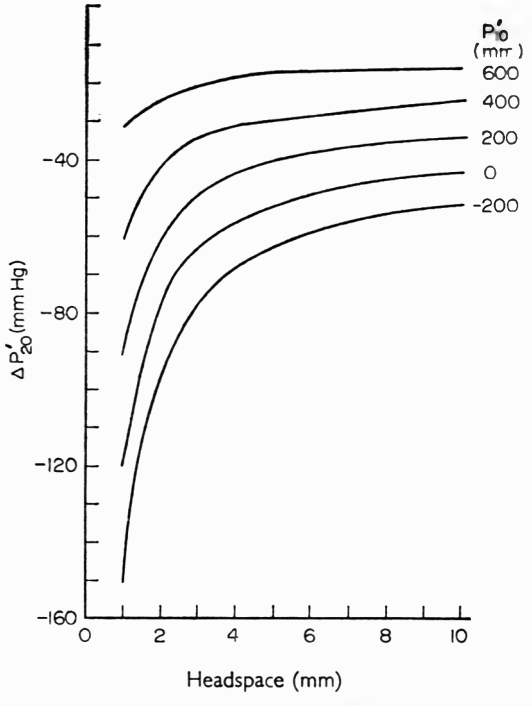

FIG. 1. Corrections to be added to the vacuum at 10°C in order to obtain the vacuum at 20°C, at various headspace heights: A2 cans.

and $+1{\cdot}5$ ml at 10° and 30°C, respectively, at 1 mm headspace (from equation (3)). Using the common 'A10' can (603 × 700) as a further example, ΔV_c attains $\mp 1{\cdot}07$ ml, and the change of V_{wt} attains $-4{\cdot}6$ ml and $+7{\cdot}8$ ml at 10° and 30°C, respectively, at 1 mm headspace.

The effect of physical dents to the cans is considered. Put subscripts $_0$ and $_d$ to

* In terms of the nomenclature below, this is h_d where d may be zero.

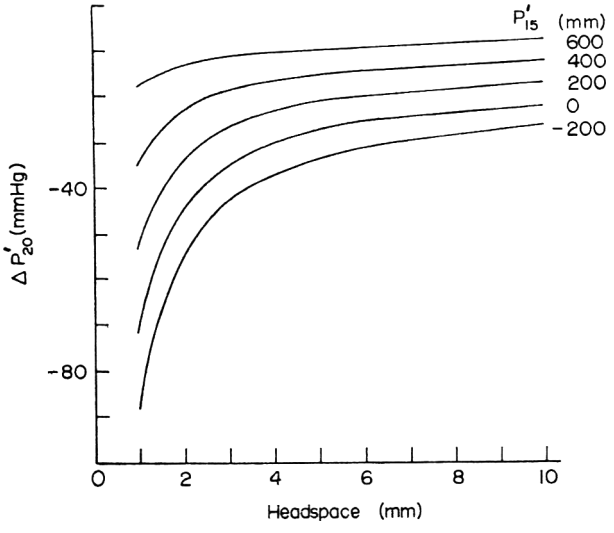

FIG. 2. Corrections to be added to the vacuum at 15°C in order to obtain the vacuum at 20°C, at various headspace heights: A2 cans.

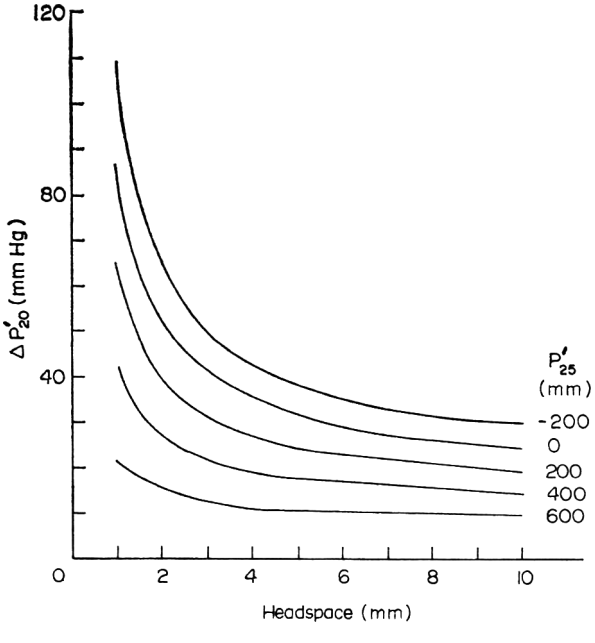

FIG. 3. Corrections to be added to the vacuum at 25°C in order to obtain the vacuum at 20°C, at various headspace heights: A2 cans.

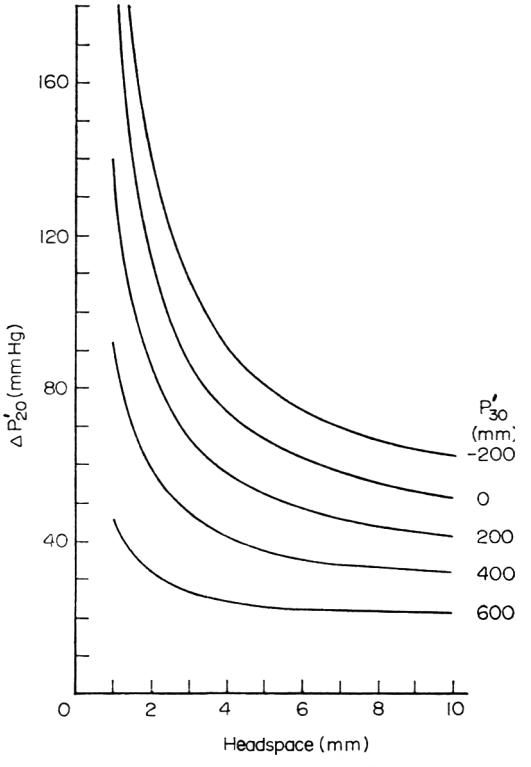

FIG. 4. Corrections to be added to the vacuum at 30°C in order to obtain the vacuum at 20°C, at various headspace heights: A2 cans.

denote undented cans and cans with a given size dent d (ml), respectively. Then

$$h_0 = h_d + \frac{40d}{D_s^2\pi}, \tag{4}$$

and

$$P_d = P_0 k_d,$$

where

$$k_d = \frac{\pi D_s^2 h_0}{\pi D_s^2 h_0 - 40d},$$

and thus

$$P_0' = (P_A(k_d - 1) + P_d') \div k_d. \tag{5}$$

Again using A2 cans as an example, equation (4) may be solved by the use of a nomograph (see Fig. 5), as may equation (5) (see Fig. 6), when $P_A = 760$ mm.

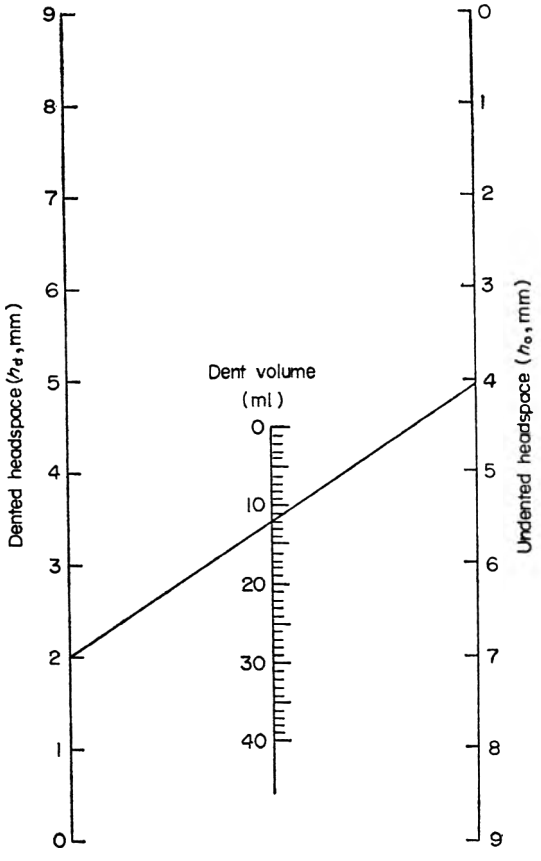

FIG. 5. Nomograph for the headspace of A2 cans with dents.

Methods

The loss of weight occasioned by suspending an object in water is the Archimedean principle for determining its volume. For the present tests, sixty-eight consecutive 'A2' cans (307 × 408) were taken from the production line of a food canning factory and were stored at room temperature for several months. The cans themselves consisted of part of a single delivery from a particular can manufacturer, and a single product was packed therein. All weighings were performed to an accuracy of 1 g. Vacuum was measured destructively at the periphery with a puncture-type gauge, and is expressed in millimetres of mercury: the gauge was calibrated to read both vacuum and pressure. Headspace was measured with a depth gauge, at the side of the can, from the level of the liquid to the underside of the lid, with an accuracy of 1 mm. The effective capacity of the cans was estimated as the weight of water required to fill the empty cans to the height of the underside of the lid; this position was marked by leaving

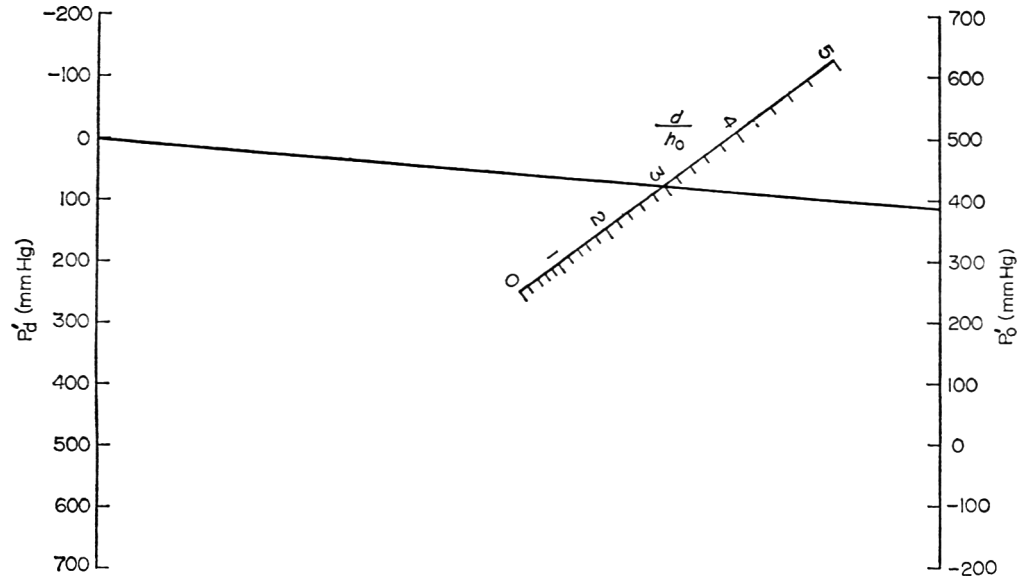

FIG. 6. Nomograph for the vacuum of A2 cans with dents.

one side of the lid attached to the body when cutting the can. The volume occupied by the tinplate was also determined on the empty cans by the Archimedean principle.

Ambient temperature was 20°C throughout.

Results and discussion

During the preliminary examinations, fifteen of the cans were found to have suffered small dents, presumably from collisions with other cans along the conveyor lines. The mean difference in volume between the dented cans and the undented cans was 1·9 ml.

No difference was found between the mean headspace of the dented cans and the mean headspace of the undented cans. (See Table 1). Applying equation (4) (or Fig. 5) above to the data of the individual dented cans gave a mean 'corrected' headspace (h_0) of 7·9 mm, which does not differ significantly from the 'uncorrected' headspace (h_d) of these cans ($t = 1{\cdot}064$, $0{\cdot}1 < p$), or from the headspace of the undented cans ($t = 1{\cdot}27$, $0{\cdot}1 < p$).

The difference between the vacuum readings of the dented cans and the vacuum readings of the undented cans (see Table 1) was found to be highly significant ($t = 4{\cdot}7$, $p < 0{\cdot}001$). Applying equation (5) (or Fig. 6) above to the data of the individual dented cans 'corrects' their mean vacuum (P_d') from -10 mm to $+20$ mm (P_0'). This figure still differs with some significance from the vacuum of the undented cans ($t = 2{\cdot}30$, $0{\cdot}02 < p < 0{\cdot}05$) and so indicates the limit to which the physical model is valid.

TABLE 1. Means and variations between cans

	Number of cans	Mean	Standard deviation	Remarks
Headspace (mm)	53	7·5	1·10	Undented cans
	15	7·5	1·04	Small dents
Vacuum (mmHg)	53	69	80	Undented cans
	15	−10	49	Small dents
Net Archimedean volume (ml)	53	574·2	1·85	Undented cans
Effective water capacity of cans (g)	53	575·1	2·12	Undented cans

The remaining difference between P_0' and the vacuum of the undented cans must be ascribed to other causes.

When the vacuum is high, the ends of undented cans are drawn inward, and the gross volume is significantly decreased. The correlation coefficient found between vacuum and gross volume was −0·37 ($p < 0{\cdot}005$). Interpolation at zero vacuum, and deducting the volume of the tinplate, provided a measure of the effective capacity of the cans, 574·8 g of water, with standard error of the estimate equal to 1·74 g of water. This value of the effective capacity does not differ significantly from the result obtained by the 'water-capacity' method (see Table 1), but it has greater accuracy. The value given by the Israel Standard (1966) at 20°C is 574·0 g of water.

The method presently used to determine the effective capacity of cans requires access to unused cans and to can-sealing equipment (Israel Standard, 1966). The 'Archimedean' method, at the zero-vacuum interpolation, may therefore be used as an alternative, particularly when unused cans are not available.

As a means of testing the vacuum of cans non-destructively after amassing sufficient data from destructive testing to obtain the regression equation of vacuum on Archimedean volume, the method above is rather inaccurate. The standard error of the estimate of vacuum found was 73 mmHg, which is too large for most practical purposes.

The mean net 'Archimedean' volume found was 0·6 ml lower than the zero-vacuum interpolating value. The former figure should thus be used as the datum value for determining the extent of denting of cans of common origin. The determination of the extent of large dents is an elementary exercise; dents of 20 ml and more have been measured, as well as swells (blown cans) of over 40 ml.

For routine determinations of the Archimedean volume, the construction of a hydrometer is feasible: a can totally immersed in water could be suspended from the hydrometer; the gross Archimedean volume of the can could then be found as a function of its gross weight and the extent of immersion of the hydrometer stem.

Put

weight of hydrometer = W_1 (g),

volume of hydrometer bulb permanently submerged = V_0 (ml),

volume of stem submerged with no load on hydrometer = V_1.

Load hydrometer with can, of (known) weight = W_2

and (unknown) volume = V_2;

volume of stem now submerged = V_3.

Now, considering the specific gravity of water as unity,

$$V_0 + V_1 = W_1,$$

and

$$V_0 + V_2 + V_3 = W_1 + W_2.$$

Subtracting,

$$V_2 + V_3 - V_1 = W_2,$$

$$V_2 = W_2 - (V_3 - V_1). \qquad (6)$$

The value of $(V_3 - V_1)$ required for the solution of equation (6) can be obtained by graduating the hydrometer stem in millilitres.

Conclusions

A method is presented for estimating the extent of dents and panels on tin cans. The method is based on the difference of the gross volume between dented and undented cans. The volume of the cans is determined by the Archimedean principle.

An accurate method for the determination of the total capacity of tin cans is presented.

Measurements of vacuum on even slightly dented cans are shown to give misleading results unless corrections are applied based on the volume of the dents and the headspace.

A method is given for the correction of vacuum determinations to standard temperature and pressure.

A simple hydrometric method for the routine determination of can volumes is suggested.

References

TOWNSEND, C.T., SOMERS, I.I., LAMB, F.C. & OLSON, N.A. (1956) *A Laboratory Manual for the Canning Industry*, 2nd edn. National Canners Association, Washington, D.C.

DICKINSON, D. & GOOSE, P. (1955) *Laboratory Inspection of Canned and Bottled Foods*. Blackie and Son Ltd, London.

ISRAEL STANDARD (1964) *Canned fruit and vegetables* S.I. 143.

ISRAEL STANDARD (1966) *Tin cans for food*. S.I. 136.

(*Received* 28 *July* 1973)

J. Fd Technol. (1974) **9**, 205–211.

Experiments with freezing of melon cubes in Israel*

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Summary

Freezing experiments with Israeli varieties of melons were carried out to study the effects of presoaking and different methods of freezing, packing and defrosting on the organoleptic properties of defrosted cubes.

Presoaked fruit was preferred to the controls. Vacuum-packed fruit was compressed, soft and watery. Nitrogen packed fruit was superior in taste, texture and appearance to the air packed. Fast defrosting resulted in a firmer, tastier product.

Introduction

The following types of melons are grown in Israel.

Honey Dew (*Tal Dvash*), a white fleshed melon with a tinge of pink near the seed cavity. It matures in May and is picked at 6–7% T.S.S. for export. There is little odour and the flesh is firm.

Haogen, a green, soft fleshed variety of moderate aroma. It has a long picking season —from April to December—and is picked at 10% T.S.S. for export.

Several species of Mediterranean melons, grouped under the variety name *Ananas*, are grown throughout the summer and fall for the local market. Two species of these varieties were included in these experiments: *En Dor Industrial*, a very large melon which has an orange, very firm flesh and a T.S.S. of 9–10% at maturity, and *En Dor Amid*, a white fleshed variety with soft flesh and a T.S.S. of approximately 11% at maturity.

Cantaloup—an orange fleshed variety called *Sharon* is raised to a limited extent. It matures in May and reaches 12–13% T.S.S.

The objective of these experiments was to compare changes in taste, appearance and structure following freezing and thawing of melon cubes which were variously pre-treated, frozen and packed. Such studies are necessary for the development of efficient processing techniques for the freezing industry and for the selection of suitable freezer varieties.

* Contribution from the Agricultural Research Organization, Volcani Center, Bet Dagan, Israel, 1973 Series, No 307-E.

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Materials and methods

Varieties

The varieties included in these experiments were Honey Dew (Tal Dvash), Haogen, En Dor Industrial, and En Dor Amid. Cantaloups, which are grown to a limited extent for export, were not included in the present experiments.

Preparation of samples

The melon cubes for freezing were prepared by cutting the melons in longitudinal slices, removing the seeds from the cavity carefully by hand, peeling, and then stamping out 1 cm cubes with a hand-operated french-fry cuber.

The cubes of several melons were carefully mixed and the % T.S.S. was determined with a refractometer.

A part of the cubes were soaked for 3 min in a sugar solution of the same % sugar as the T.S.S. of the melons. A second aliquot was soaked in the same sugar solution with the addition of 10% ascorbic acid and 0·25% malic acid, according to Winter & Sherman (1971). After soaking, the cubes were drained for 2 min. A third aliquot, which was not soaked, served as the control. From the three aliquots, 100 g samples were weighed for freezing for each of the treatments described below; five repetitions were included for each treatment.

Freezing

Two types of freezing were compared: air blast freezing (Frigoscandia Laboratory Blast Freezer, Sweden), which brought the samples down to −40°C within 30 min; and plate freezing (laboratory plate freezer), which brought the samples down to −40°C within 1½ hr.

Single layers of cubes were placed on cellophane or polyethylene squares on the perforated metal screens in the freezers to prevent sticking, and for uniform rapid freezing. The samples were bagged immediately upon completion of the freezing.

Packaging

Three types of packages were compared.

(a) Polyethylene—regular pack in air.
(b) PVC laminate—vacuum pack at 30 in of mercury.
(c) PVC laminate—nitrogen pack following evacuation.

The polyethylene bags were heat-sealed as soon as the cubes were placed in them and the excess air was 'squeezed out' as much as possible. The vacuum and nitrogen packs were prepared in an automatic machine (Minivac 300, manufacturer Negro, Italy) which evacuates and seals or evacuates, adds N_2, and then seals.

Storage

All the bags were stored at −20°C for 1 month.

Defrosting

Three methods of defrosting were compared: one was fast and the others were slower.

(a) Defrosting by submerging the sacks in 70°C water for 3 min, until the samples reached 4°C.

(b) Defrosting the sacks at room temperature (25°C) until internal temperature reached 4°C. This usually took $1\frac{1}{2}$ hr.

(c) Defrosting overnight in a 4°C refrigerator.

Subjective and objective tests

The samples were tested approximately 1 month after frozen storage, and tasting was done upon defrosting.

Taste. The panel consisted of four laboratory personnel, two of whom have worked with melons for many years. Two to four samples were presented to the panel at one sitting, and the difference preference method of evaluation was used. In the majority of cases the panel was unanimous in its judgment as it happened that the differences between the samples were usually very marked.

Shear tests and drip loss measurement. Upon defrosting, the sack contents were poured into a funnel suspended in a graduated cylinder. The amount of liquid in the sack was recorded as drip loss and the cubes were arranged in the standard shear cell, horizontal to the bars. Shearing was performed using the Alloe Kramer recording shear press fitted with a 3000 lb ring.

Histological examinations of the melon cubes. Ten micron cryostat and paraffin sections were prepared from fresh Haogen and Honey Dew (Tal Dvash) and from defrosted Honey Dew (Tal Dvash), Haogen and En Dor cubes. The cryostat sections were affixed directly from the −20°C knife to slides coated with Haupts adhesive and then treated with formaldehyde vapours for adhesion. The sections were stained with safranin-fast green according to Jensen (1962).

In identical slides, following affixation to the slides, the degree to which pectin was present in the cell walls was determined by staining with a 1 : 10,000 aqueous ruthenium red solution, according to Lillie (1965).

Results and discussion

Subjective evaluations

Following defrosting, the appearance of cubes from the various varieties differed considerably. En Dor Industrial and Honey Dew (Tal Dvash) maintained a fresh-like appearance, while Haogen turned flaccid and En Dor Amid became mushy. For all the varieties, the vacuum pack resulted in compression following thawing and the cubes appeared soft.

Taste tests showed that the four varieties reacted similarly to the various treatments. Following 1 month of storage, there was a loss of flavour in the polyethylene packs

or, sometimes, there developed an off-flavour while the cubes in the nitrogen and vacuum packs retained their flavour and did not develop off-flavours. This points to the elimination of oxygen as a necessary step in preserving flavour during storage and following defrosting. Oxidative processes probably are also responsible for the development of off-flavours.

For all the varieties tested, the melon cubes were noticeably softer in the vacuum pack than in the nitrogen pack. Among the three types of packs tested, the nitrogen pack was given first preference as taste and firmness.

Tasters generally preferred melons which had been frozen more rapidly, by the blast process, to the slower freezing by the plate freezer. These results confirm earlier findings by Durif (1969) that tasters preferred melons frozen by liquid nitrogen to those frozen by slower methods.

Tasters found the melon cubes defrosted by immersing the sacks in 70°C water for 1–3 min (the time needed to defrost depending on variety or the pack) to be firmer and preferable in taste to cubes which had been defrosted more slowly. The tasters also indicated that the cubes which had been soaked in sugar solutions had a more desirable flavour and aroma than non-dipped cubes, and those dipped in sugar solutions containing ascorbic and malic acids were preferred over those dipped in sugar solutions alone.

Objective tests

(a) *Shear and drip loss.* Shear tests generally confirmed the panel results.

As can be seen in Table 1, melon cubes in bags defrosted within minutes in hot water at 70°C showed greater resistance to shear and less water loss than melon cubes defrosted in the bags at room temperature.

Tables 2 and 3 show that melon cubes packed in polyethylene bags or in PVC bags with nitrogen gas lost less water than the vacuum-packed melons, and the vacuum-packed melon cubes appeared compressed. Thus, the vacuum pack resulted in a physical expression of liquid upon defrosting.

Blast freezing, which froze the cubes twice as fast as the plate freezer, also resulted in a smaller loss of water by the cubes, as can be seen in Tables 2 and 3 for En Dor Amid and Honey Dew-Tal Dvash. This was not the case for Haogen, however.

There was a greater resistance to shear for the cubes which had been soaked in sucrose solution containing ascorbic acid and malic acid than for the non-soaked, as can be seen in Tables 1, 2 and 3. Tables, 1, 2 and 3 show that for the soaked cubes shear was generally about three times as high. Durif (1969) who compared quick freezing of melon halves by liquid nitrogen with slower 2 hr freezing also found less drip in the quicker frozen melons.

From these studies it appears that fast freezing preserves textural properties better than slow freezing. Liquid nitrogen freezing is, however, expensive. Cubing the melon halves reduces thickness and exposes a larger surface area, thus decreasing freezing time.

TABLE 1. Shear press readings and drip loss following fast defrosting at 70°C and slow defrosting at room temperature (25°C)

Variety	Treatment and packing		Defrosting at 70°C		Defrosting at room temperature (25°C)	
			Shear force (lb)	Drip loss (ml)	Shear force (lb)	Drip loss (ml)
Honey Dew (Tal Dvash)	Soaked in sucrose solution + ascorbic acid	Poly-pack	108	11·5	108	17
		PVC vac. pack	69	27	61	25
Haogen	Non-soaked	PVC vac. pack	24	24	28·5	27
		PVC nitr. pack	28	22	26	29
	Soaked in sucrose solution + ascorbic and malic acids	PVC vac. pack	70	19	34·5	27
		Nitrogen pack	95	9	36	18
En Dor Industrial	Non-soaked	PVC vac. pack	44	22·5	27	27
	Soaked in sucrose solution + ascorbic acid	Vacuum pack	123	11	36	23
En Dor Amid	Non-soaked	Poly-pack	36	10	30	14·5
		PVC vac. pack	35	16	28·5	16

(b) *Histological changes.* All the histological observations are summed up in Table 4.

(i) Structural changes. The paraffin sections maintained cell structures better than the cryostat sections and also stained more intensely.

Fresh melons which were not completely ripe retained better shaped cells and cell walls than mature melons. Following defrosting of frozen cubes, there was a

TABLE 2. Shear force and drip loss of polyethylene *v.* PVC vacuum pack following defrosting at 70°C

Variety	Treatment	Method of freezing	Poly-pack		PVC vacuum pack	
			Shear force (lb)	Drip loss (ml)	Shear force (lb)	Drip loss (ml)
Honey Dew (Tal Dvash)	Soaked in sucrose solution + ascorbic acid	Blast	108	11·5	69	27
		Plate	99	19·5	67·5	29
En Dor Amid	Non-soaked	Blast	33	10	36	16·5
		Plate	34·5	12	34·5	16
	Soaked in sucrose solution + ascorbic and malic acids	Blast	120	10	102	15
		Plate	126	10	120	21

TABLE 3. Shear force and drip loss of PVC vacuum pack *v.* PVC nitrogen pack following defrosting at 70°C (Var. Haogen)

Treatment	Method of freezing	PVC—vacuum pack		PVC—N_2 pack	
		Shear force (lb)	Drip loss (ml)	Shear force (lb)	Drip loss (ml)
Non-soaked	Blast	24	24	28	20
	Plate	30	29	39	20·5
Soaked in sucrose solution + ascorbic and malic acids	Blast	70	19	95	9
	Plate	/	36	65	15

TABLE 4. Structural and chemical changes in fresh melon cubes and following various freezing and packaging treatments

Variety and treatment	Structural changes*		Depth of ruthenium red stain for pectin†
	Compressed cells	Broken cell walls	
HDT‡, fresh mature ripe fruit	−	−	+ + + +
HDT, fresh very ripe	−	+	+ +
Haogen, fresh, mature ripe	−	−	+ + +
Haogen, fresh, very ripe	−	+	+
HDT, sucrose sol. soaked, vac. pack, blast-frozen	+ + +	+	+ + to + + +
HDT, sucrose sol. soaked, vac. pack, plate-frozen	+ + +	+	+
Haogen, sucrose sol. soaked, vac. pack, blast frozen	+ + +	+	+ + to + + +
Haogen, sucrose sol. soaked, vac. pack, plate-frozen	+ + +	+ + +	+
En Dor Amid, sucrose sol. soaked, vac. pack, blast-frozen	+ + +	+	/
En Dor Amid, sucrose sol. soaked, vac. pack, plate-frozen	+ + +	+	/
HDT, sucrose sol. soaked, poly pack, blast-frozen	−	+	+ +
HDT, sucrose sol. soaked, poly pack, plate-frozen	−	+	+
Haogen, sucrose sol. soaked, N_2 pack, blast-frozen	−	+	+ + +
Haogen, sucrose sol. soaked, N_2 pack, plate-frozen	−	+	+ +

* −, none; +, low; + +, up to 50%; + + +, over 50%.

† −, none; +, light stain; + +, moderate stain; + + +, well stained; + + + +, deeply stained; / not determined.

‡ HDT, Honey Dew (Tal Dvash).

breakdown in structure, the extent depending on treatment. This can be seen from Table 4. Cells of vacuum-packed melons were markedly compressed, while this did not appear to be the case for nitrogen-packed cubes. The structure of the cells of the blast-frozen (faster) cubes did not differ from that of the plate-frozen cubes. This confirmed the shear press results, as can be seen in Table 3.

(ii) Histochemical changes. While structurally no differences could be observed between sections of the blast- and the plate-frozen fruit, the staining with ruthenium red revealed a greater uptake of the stain in the cells of the blast-frozen fruit. This indicates a possible decrease in the pectolytic enzyme activity in the blast-frozen fruit. This fact is supported by the observation that the less mature fresh melons showed a greater uptake of the ruthenium red stain than did the mature melon cells.

There was also a greater stain for pectin in the nitrogen packed fruit than in the vacuum pack. This may have come about as a result of the leaching of the pectin from the fruit in the greater drip that was present in the vacuum packed fruit.

References

DURIF, E. (1969) *Revue gén. Froid. Ind. frigor.* **60,** 49.

JENSEN, WILLIAM A. (1962) *Botanical Histochemistry,* W. H. Freeman and Cc., San Francisco.

LILLIE, R.D. (1965) *Histopathologic Technic and Practical Histochemistry.* 3rd edr.. McGraw Hill, New York.

WINTER, FRANK W. & SHERMAN, LEONARD (1971) Dip process for IQF fruit eliminates syrup but retains flavor, color fresh. *Quick Frozen Foods,* **33,** 109.

(*Received* 7 *November* 1973)

J. Fd Technol. (1974) **9**, 213–221.

The influence of hygiene in catch handling on the storage life of iced cod and plaice

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Summary

Investigations of commercial fisheries in the North Sea and the Kattegat show that the bacterial load of the fish as caught is generally low. Substantial bacterial infection may occur, however, during sorting, gutting and other fish handling on deck, depending on the hygienic standard on board.

The influence of the hygiene in catch handling on the storage life of iced cod and plaice was studied at all seasons of the year.

During the first week of iced storage, no difference was found in the eating quality and in the contents of TVA in plaice and TVB in cod, whether the initial bacterial infection was low, medium or high. The three levels of infection were the result of very clean, reasonably clean and very dirty handling conditions, respectively. During the second week of storage, little difference was found between fish handled under very clean or reasonably clean conditions. Very dirty conditions, however, resulted in a slightly faster decrease of quality and in 1–3 days reduction in the total storage life in ice.

Introduction

Fish, as has been said, are more perishable than most other protein foods (Burgess & Shewan, 1970). Even at chill temperatures (0°C) rapid spoilage is observed in wet fish, and it is generally accepted that spoilage of lean fish is mainly due to the action of endogenous tissue enzymes (autolysis) and certain groups of gram negative psycrophilic bacteria (Herbert *et al.*, 1971). Further it has often been claimed that the dominant role in this process is played by bacteria (Laycock & Regier, 1971; Shaw & Shewan, 1968; Liston, 1965; Partmann, 1966), and some people even regard bacteria as the number one enemy of the fishing industry (Tarr, 1964).

Recent investigations seem to indicate that the fish caught in unpolluted water only carry few micro-organisms on the surface and in the guts. Most of the bacterial contamination found on the landed fish appears to be related to unhygienic handling of the fish and bacterial growth during storage on board The present study has been under-

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taken to determine the quality and keeping time of cod and plaice in relation to the applied hygienic precautions during the handling of the fish on board.

Materials and methods

The experimental material was cod and plaice caught in the North Sea and the Kattegat. To rule out any possible seasonal variations one storage experiment was conducted each month during a whole year with each of the two fish species caught by commercial fishing vessels in the two selected areas.

Each experiment included three lots of fish representing three different methods of handling on board. One lot was handled with extreme care. The fish were never in direct contact with deck, equipment and utensils on board or any other potential source of contamination, but were always kept in clean chlorine-disinfected plastic boxes. Gutting was carried out with a sterile knife, and the operator was wearing sterile gloves. The fish were carefully washed with clean sea-water and finally iced in clean disinfected plastic boxes.

Another lot of fish was handled by the fisherman in the normal way and no special care was taken to avoid contamination. The fish were landed direct on the deck, sorted, gutted by hand, washed with the deck hose and finally iced in clean plastic boxes.

The third lot of fish was handled the same way as the second lot, but all containers used in the handling of this fish were old wooden fish boxes. The way of handling the fish thus represented three levels of hygiene and also presumably three levels of initial contamination from the very low (L-fish) over medium (M-fish) to a high (H-fish) level. During storage the fish were at all times completely surrounded with ice thus making sure that the storage temperature was 0°C and also avoiding the special type of deterioration seen when fish are stored in direct contact with dirty wood (McLean & Castell, 1956).

The fish were landed on the day of catch or the day after catch, transported to the laboratory and immediately placed in the chill room (+0·5°C). During storage the decline in the quality of the fish was determined.

The organoleptic assessment was carried out on the cooked material by a team of eight trained persons. The material was prepared by immersion for 15 min in 2% salt water at 80°C. Immediately after cooking, the odour, flavour and texture were evaluated according to a hedonic scale from 0 to 10 with 4 as the limit of acceptability. The total aerobic counts were expressed as the logarithm of the numbers of bacteria per gram. The counts of the day of examination were calculated as the geometric mean of six individual samples, taken from six different fish, each sample consisting of 10 g of material. In the case of cod, the flesh sample was taken from the belly flap, including both the skin and the belly wall. Flesh samples of plaice, however, consisted of a cross-section of the fish immediately anterior to the tail, including two skin surfaces and the intervening meat and bone. Each sample was homogenized in 90 ml of sterile

physiological saline solution containing 0·1% peptone, and serial dilutions were spread in Plate Count Agar. The plates were incubated for 5 days at 20°C.

The volatile bases in cod were determined by the method of Conway & Byrne (1933) and the volatile acids in plaice by the method of Steinhauer & Dawson (1969).

Results and discussions

Initial bacterial contamination of the fish

Several workers have provided evidence of a seasonal variation of the bacterial flora of newly caught fish (Liston, 1956; Georgala, 1958; Shewan & Hobbs, 1967). A detailed analysis of the monthly counts in our work did not confirm these findings, thus Table 1 summarizes the results of the twenty-four storage experiments carried out.

TABLE 1. Bacteriological examination of newly caught fish. Initial count on gutted fish handled in three different ways (see text)

	Log total count/g			
	L-fish	M-fish	H-fish	$t \times s/\sqrt{n}$
Cod	3·39	4·00	5·50	0·15
Plaice	2·71	3·64	5·42	0·37

The results confirm earlier findings that fish caught in unpolluted areas only carry few organisms, and by means of strict sanitary measures during handling on board it is possible to keep the number of bacteria on the fish at a low level. However, direct contact with deck, equipment and old fish boxes is immediately followed by a 10–100-fold increase of the number of bacteria found on the fish.

During storage a steady increase in the bacterial count is seen in both cod and plaice as shown in Figs 1 and 2.

Development of volatile acids and bases

Figures 3 and 4 show the development of volatile acids (TVA) in plaice and volatile bases (TVB) in cod. It is remarkable that irrespective of the handling practice on board and the initial number of bacteria present on the fish, there is no difference in the development of volatile compounds during the first week of storage in ice. The content of TVA and TVB is low and only slightly increasing. After one week of storage, when the number of bacteria is about 10^4/g of L-fish and 10^7/g of H-fish, an increase in the development of TVA and TVB is seen, the increase being only slightly more pronounced in the H-fish. It is not known, however, whether there has been any variation in the qualitative aspects of the bacterial flora on the three groups of fish, but it may be said then that the steps taken in those experiments to improve hygiene have had only

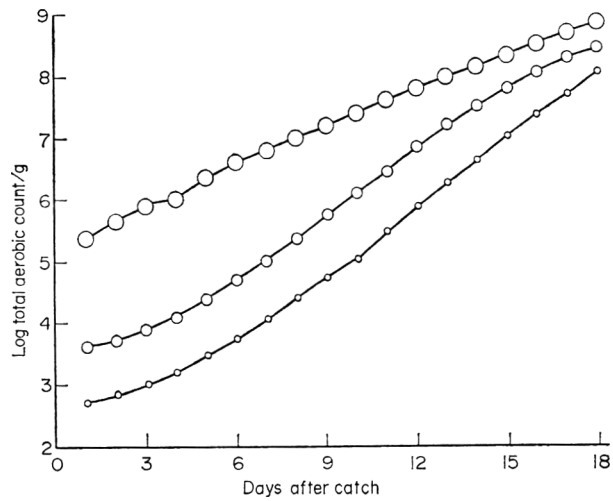

FIG. 1. Bacterial growth in iced plaice with an initial low (small circles), medium (medium size circles) and high (large circles) bacterial count. Graphs are mean values based on twelve storage experiments. Half confidence limit ($t \times s/\sqrt{n}$) on the calculations is 0·23 log units.

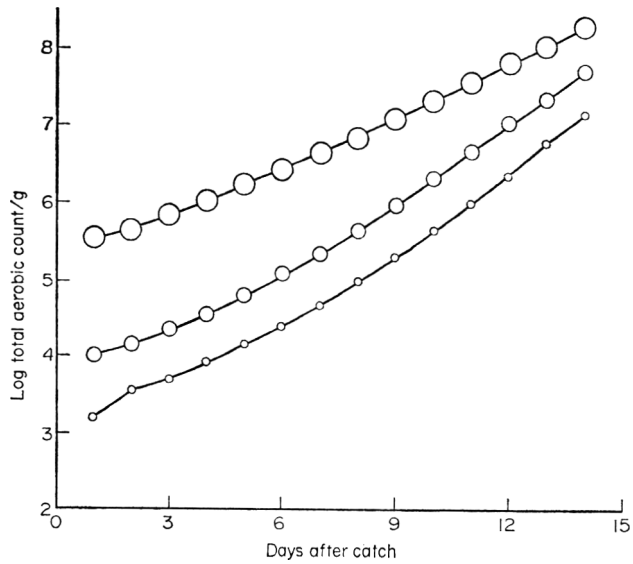

FIG. 2. Bacterial growth in iced cod. Same symbols as in Fig. 1. Graphs are mean values based on twelve storage experiments. Half confidence limit ($t \times s/\sqrt{n}$) on the calculations is 0·15 log units.

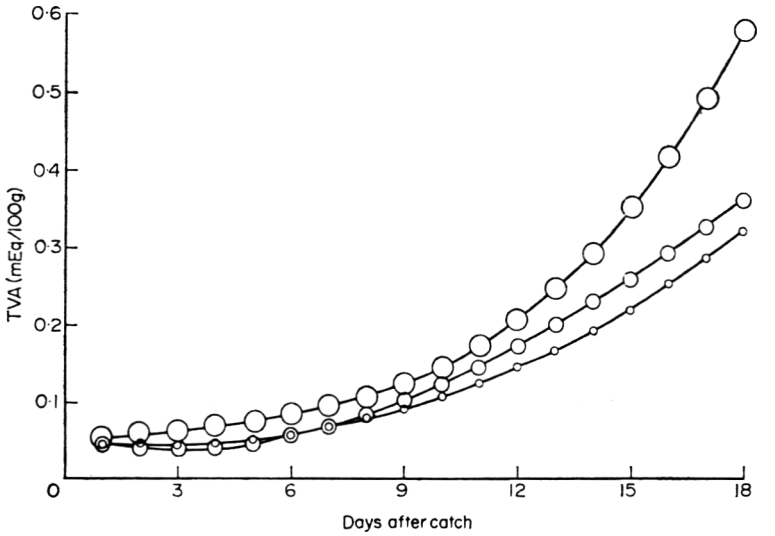

FIG. 3. Development of volatile acids in iced plaice. Same symbols as in Fig. 1. Graphs are mean values based on twelve storage experiments. Half confidence limit ($t \times s/\sqrt{n}$) on the calculations is 0·05 mEq/100 g.

a slight effect on the development of what is normally regarded as chemical indices of spoilage.

The figures recorded are the geometric mean calculated from twelve storage experiments with each fish species. $t \times s/\sqrt{n}$ is an expression of half confidence limit, where t is the t-value, s is the standard deviation and n is the number of observations.

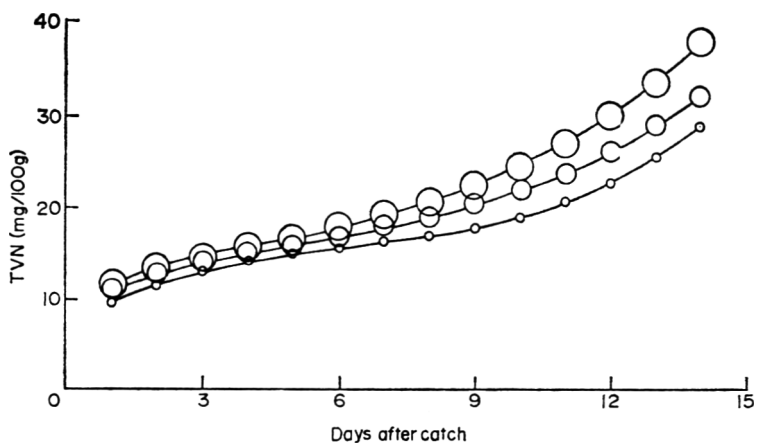

FIG. 4. Development of volatile bases in iced cod. Same symbols as in Fig. 1. Graphs are mean values based on twelve storage experiments. Half confidence limit ($t \times s/\sqrt{n}$) on the calculations is 1·04 mg/100 g.

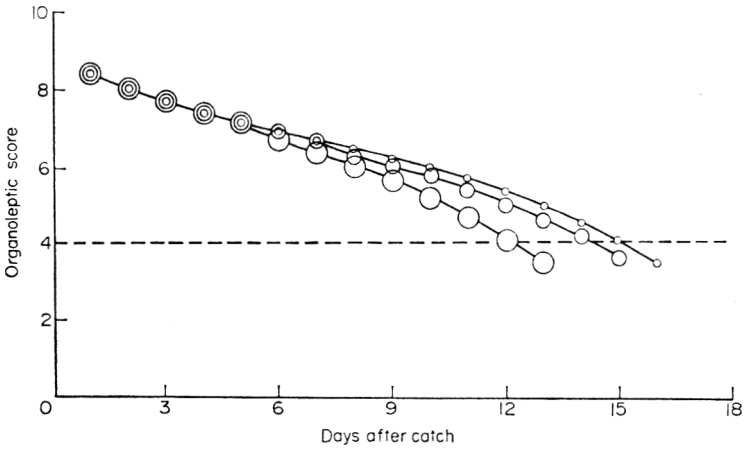

FIG. 5. Quality scores for iced plaice. Same symbols as in Fig. 1. Graphs are mean values based on twelve storage experiments. Half confidence limit ($t \times s/\sqrt{n}$) on the calculations is 0·38 units.

Organoleptic assessment

The decrease in organoleptic quality of the fish during storage in ice is illustrated in Figs 5 and 6. Again it is noted that the handling practice on board and the initial bacterial contamination have no effect on the quality during the first week of storage. During the second week of storage this H-fish are rated a little lower than M- and L-fish, which also results in a difference of a few days in the keeping time of the three lots of fish. There does appear then to be a very good correlation between the measured

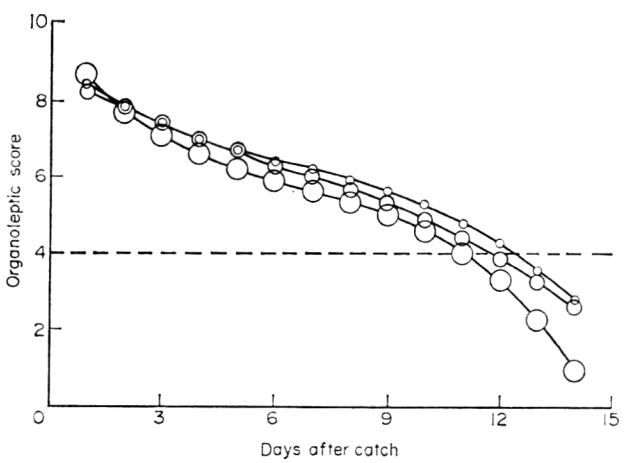

FIG. 6. Quality scores for iced cod. Same symbols as in Fig. 1. Graphs are mean values based on twelve storage experiments. Half confidence limit ($t \times s/\sqrt{n}$) on the calculations is 0·37 units.

chemical indices of spoilage and the eating quality of the fish, the plaice being rejected when the TVA content reaches 0·20–0·25 mEq/100 g, and the cod being rejected when the TVB content in the flesh is 25 mg/100 g fish. It should be emphasized that the figures quoted are the mean from a large number of samples, and considerable variations may be found among the individual samples of fish.

The correlation between the eating quality and the total number of bacteria found on the fish is rather poor at all stages during storage. The relationship between the quality of the three lots of fish during storage and the handling practice, as expressed by the three levels of initial bacterial contamination, is shown in Figs 7 and 8. Only beyond 10–11 days of storage the bacteria are significantly influencing the organoleptic score. Since the quality of the cod is already at that time near the border of acceptability this means in practice that the storage life of the plaice only is influenced significantly by the application of strict hygiene on board the fishing vessels. This agrees very well with the results of Castell, MacCallum & Power (1956), who stated from a series of experiments that there was very little evidence to indicate that extreme care in gutting,

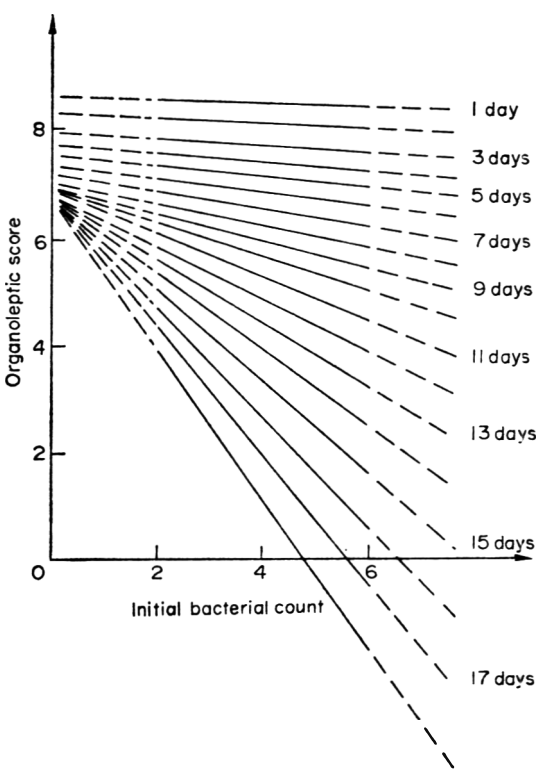

FIG. 7. The relationship between the initial bacterial contamination and the organoleptic score of plaice during storage at 0°C. The lines are calculated as the mean of the results obtained in each of twelve individual experiments.

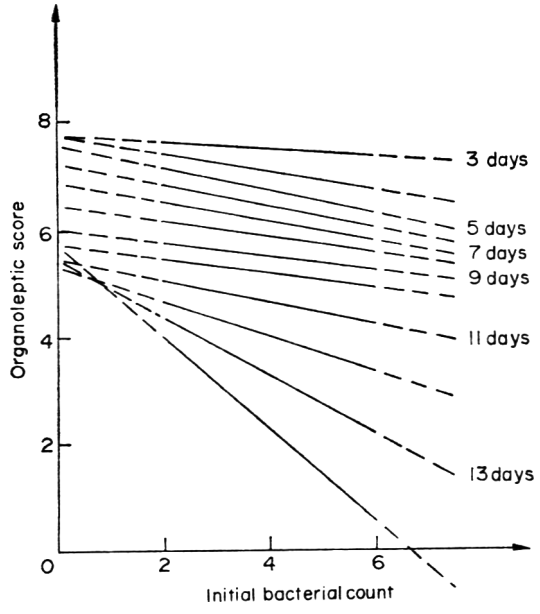

FIG. 8. The relationship between the initial bacterial contamination and the organoleptic score of cod during storage at 0°C. The lines are calculated as the mean of the results obtained in each of twelve individual experiments.

washing and handling the fish on deck would add very much to the normal keeping time as they are now handled at sea.

Conclusions

The living fish normally carry few psychrophilic micro-organisms, but immediately after catch the fish are contaminated due to contact with deck, fish boxes and other equipment. By applying strict hygiene during the handling of the catch it is possible to keep the contamination at a low level, but the quantitative variation in the bacterial contamination brought about in this way is not having any considerable influence on the quality of iced cod and plaice.

The storage lives of cod and plaice were about 12 and 14–15 days, respectively, when iced in clean plastic boxes, no significant difference being seen between extremely clean and reasonably clean handling conditions. Storage in dirty wooden boxes, on the other hand, reduced the storage life of cod by about 1 day and that of plaice by about 3 days.

These results indicate that ordinary good cleanliness on board and the use of clean fish boxes are desirable, while going to extremes in hygiene requirements is not warranted by the gain in quality and keeping time of well-iced fish. Comparing these

results with other known factors, it is evident that insufficient chilling still remains the major cause of loss of quality (Castell *et al.*, 1956; Huss, 1971; Shewan & Hobbs, 1963).

The single total viable count at 20°C appears to be a doubtful figure in terms of expressing quality and predicting keeping time, but is does give an indication of the hygiene applied during the handling of the fish. TVB in cod and TVA in plaice, expressed as the mean of a rather large number of samples, are useful as chemical indices of spoilage, but only for fish stored in excess of 1 week. Of all the methods used in this work, only the organoleptic assessment can be used to evaluate the initial changes in quality of cod and plaice during the first week of storage in ice.

References

Burgess, G.H.O. & Shewan, J.M. (1970) *Torry Memoir* No. 354. Torry Research Station, Aberdeen.

Castell, C.H., MacCallum, W.A. & Power, H.E. (1956) *J. Fish. Res. Bd Canada*, **13,** 21.

Castell, C.H., Dale, J. & Greenough, M.F. (1959) *J. Fish. Res. Bd Canada*, **16,** 223.

Castell, C.H. & Giles, J.G. (1961) *J. Fish. Res. Bd Canada*, **18,** 295.

Conway, E.J. & Byrne, A. (1933) *Biochem. J.* **27,** 419.

Georgala, D.L. (1958) *J. gen. Microbiol.* **18,** 84.

Herbert, R.A., Hendrie, M.S., Gibson, D.M. & Shewan, J.M. (1971) *J. appl. Bact.* **34,** 41.

Huss, H.H. (1971) *Fish Inspection and Quality Control* (Ed. by R. Kreuzer), p 60. Fishing News (Books) Ltd, London.

Jason, A.C. & Lees, A. (1971) *Estimations of Fish Freshness by Dielectric Measurements.* Torry Research Station, Aberdeen.

Laycock, R.A. & Regier, L.W. (1971) *J. Fish. Res. Bd Canada*, **28,** 305.

Liston, J. (1956) *J. gen. Microbiol.* **15,** 305.

Liston, J. (1965) *The Technology of Fish Utilization* (Ed. by R. Kreuzer), p. 53. Fishing News (Books) Ltd, London.

Love, R.M. (1965) *The Technology of Fish Utilization* (Ed. by R. Kreuzer), p. 102. Fishing News (Books) Ltd, London.

McLean, N. L. & Castell, C. H. (1956) *J. Fish. Res. Bd Canada*, **13,** 861.

Partmann, W. (1966) *Z. Lebensmittelunters. u.-Forsch.* **129,** 205.

Shaw, B.G. & Shewan, J.M. (1968) *J. appl. Bact.* **31,** 89.

Shewan, J.M. & Hobbs, G. (1963) *Fish. News Int.* **2,** 103.

Shewan, J.M. & Hobbs, G. (1967) *Prog. ind. Microbiol.* **6,** 169.

Steinhauer, J.E. & Dawson, L.E. (1969) *J. Fd Sci.* **34,** 359.

Tarr, H.L.A. (1964) *Developments in Industrial Microbiology*, Vol. 5, p. 135. American Institute of Biological Sciences, Washington.

(*Received* 4 *September* 1973)

J. Fd Technol. (1974) **9**, 223–233.

Technological investigations into Cape hake

I. Chilling at sea for freezing ashore

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C. K. SIMMONDS* AND G. D. STROUD

Summary

Cape hake were treated in a variety of ways before stowage in ice or refrigerated sea water at sea and before handling and freezing ashore. The adverse effects on colour and gaping of delays before chilling, of holding ungutted and of storage before freezing are described. The qualities of a selection of end products —fillets cut from wet fish, fillets cut from thawed fish, thawed fillets, steaks— have been assessed and compared.

Introduction

During the past 20 years or so many aspects of the processing and quality of Cape hake (*Merluccius capensis*, *M. paradoxus*) have been studied at the Fishing Industry Research Institute, Cape Town, and the exercise reported here and in the accompanying two papers was designed to take account of the results obtained so far, to confirm some of them and to cover new ground. A large number of experiments was carried out using several tons of hake and the accounts given here and in the accompanying two papers are in the nature of summaries. Statistical evaluations have been used wherever appropriate and possible.

In common with most other species of hake, the Cape hake have an inherently soft flesh and their early and rapid further softening has long been recognized as one of the main problems in handling them (Rowan & Marks, 1951). This softening was thought to be responsible for the amount of gaping or break up (the disruption of the musculature which results in the muscle blocks separating from each other) shown by fillets cut from chill stored fish. However other attributes of quality are also important facets of commercial acceptability and colour, flavour, texture and odour were all investigated in this study. Comparatively recent results on the involvement of blood

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coagulation and of rigor mortis (Jones, 1964; Kelly, 1969; Kelly & Little, 1966) in the ultimate quality of frozen fillets were taken into account and the effects of semi-tropical conditions on these factors were examined.

The experiments described in this paper were designed to investigate and assess the effects of some variations in prefreezing treatment on the quality, before freezing ashore and after thawing, of hake chill stored at sea in ice or kept in refrigerated sea water (rsw). The factors investigated were (a) length of delay ungutted at deck temperatures before handling, (b) period of chill storage before freezing, (c) whether the fish were boxed or shallow bulked, (d) the form in which the chill stored fish were held (gutted, ungutted or gutted with tails cut), (e) the form of the product, whether frozen whole (heads off, gutted), as skin on fillets or as skin off fillets, and (f) the effect on quality of time of cold storage.

Materials and methods

Series I

Fish were taken at sea from the catch of a commercial trawler fishing off Luderitz (27° 48′ S to 27° 40′ S; 110–160 m depth) during fishing days 1–4 and off Point Columbine (32° 22′ S; approximately 90 m depth) during fishing day 5 of a 7 day voyage at the end of the month of January. Air temperatures were 17–21°C, surface water temperatures were 17–20°C and the temperatures of the fish at the time of landing on deck were between 12·5° and 19°C.

(a) During each of 4 days trawling, three groups of ten fish each were removed from the catch as soon as the cod end was emptied and also after a period of 2–4 hr had elapsed during which time the fish remained ungutted in the pounds on a shelter deck. The fish were stowed in ice ungutted, gutted and washed, or gutted and washed with tails cut off.

(b) Each day, further batches of fish were selected from the stream of gutted, headed and washed fish entering the hold. These were stowed away boxed in ice. When the boat discharged strictly comparable (as regards delay before handling) fish were retrieved from the pounds where they had been shallow bulked by the crew.

Further processing followed ashore by which time the fish were gutted and all were headed and trimmed. Half the number of fish in each batch was frozen on the bone, the remainder being filleted by hand. The fillets were assessed for discolouration and gaping, one from each fish was skinned by machine, and all were frozen in approximately 2 kg blocks, interleaved and wrapped in polythene film.

The fish were held in cold storage at −30°C or below except during shipment to the United Kingdom when the temperature was −15°C for a period of about 3 weeks. Samples were thawed in still air and assessed on various occasions during the first 2 months of cold storage both in South Africa and in the United Kingdom. A few samples were also left for just over a year before they were assessed but this time for eating quality only.

Series II

Fish which had been held chilled under a variety of conditions were taken from the catches of commercial trawlers during discharge ashore in the month of May. These fish had also been caught on the two grounds mentioned above and it was established that the batches sampled had received the following treatments.

(a) Storage ungutted in rsw for less than 1 day, for between 3 and 4 days or for 7 days.
(b) Storage ungutted, boxed in ice for less than 1 day or for 3 days.
(c) Storage gutted, bulked in ice for between 4 and 5 days or for between 10 and 12 days in ice.

All the fish were headed, gutted and descaled mechanically. Filleting was carried out mainly by machine, but a few were cut by hand for comparison. After assessment, the fillets were frozen in 2 kg packs as described above. Some of the fish were cut into steaks which were frozen packed in polythene in standard fillet trays and some fish were frozen on the bone in 10 or 20 kg blocks. Assessments were made after different periods of time in cold storage for up to a year at −30°C. Shipment to the United Kingdom at −15°C took place before the 18 week assessment. Thawing of samples was carried out in still air over a period of 16 hr.

Assessments

The degrees of discolouration and of gaping were each marked on a 6-point scale, where 5 indicates best and 0 indicates worst quality, by a group of at least three experienced assessors. It had been established that the borderline for commercial acceptability lies between $2\frac{1}{2}$ and 3. As many samples as possible were cooked by steaming for 30 min in a casserole and tasted by a panel of untrained but experienced members.

Results

The colour scores given to the various batches of fillets treated as described for Series Ia above are shown in Fig. 1. It was established that there was no difference between the colours of fillets frozen skin on and skin off and assessments of corresponding batches have been pooled (Fig. 1b, e).

When assessed before freezing (Fig. 1a, d) all the fillets had a good colour regardless of length of time in ice for up to 5 days; regardless of whether the fish had been held gutted, ungutted, or gutted with tails cut; and regardless of delays of up to 4 hr at deck temperature before handling. A slight worsening of colour is noticeable at this time as a result of holding the fish ungutted for 5 days in ice (Fig. 1a). Delays at deck temperature (17–20°C) before chilling produce a slight worsening in colour which is more apparent in the fresher samples. Despite these trends all the batches would be classified as very good to excellent with regard to colour, and such differences would be of little significance at this stage.

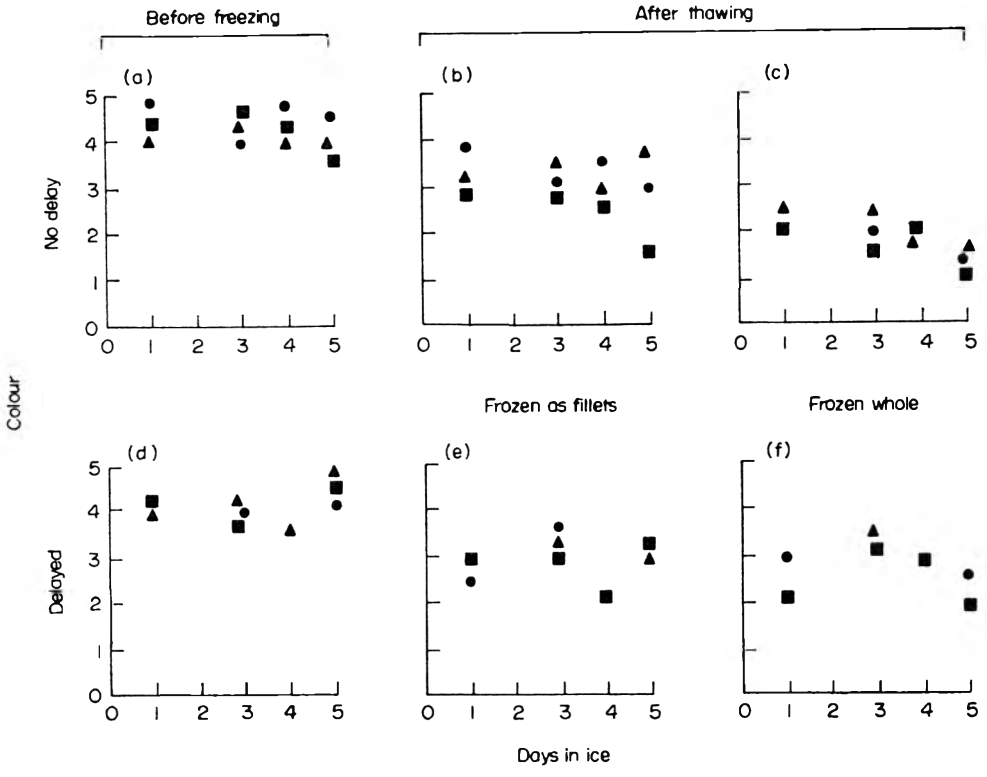

FIG. 1. Effect of gutting, bleeding method and length of storage in ice on the colour of fillets before freezing and after thawing (Series I). ●, Gutted; ▲, gutted and tails cut; ■, not gutted.

Freezing and thawing leads to a worsening of colour in all cases, the fillets prepared from ungutted fish suffering the biggest change. Increasing storage times in ice before filleting led to a progressive worsening of the colour of fillets from fish which had been stowed in ice immediately (Fig. 1b). Delays at deck temperature before chilling usually produced fillets (Fig. 1e) that were more discoloured than those cut from fish which had been iced immediately after capture (Fig. 1b) the biggest effect being noticed in the fresher fish. Fillets cut from frozen and thawed fish (Fig. 1c, f) on the whole had even worse discolourations than comparable frozen and thawed fillets (Fig. 1b, e). This is most noticeable in the cases of fish which had been chilled immediately after capture (Fig. 1c); the downward trend resulting from time in ice is again apparent in these fillets. With fish that had suffered a delay at that time (Fig. 1e, f), the form in which the flesh was frozen (i.e. on or off the bone) had little effect on fillet colour: the colour of fillets in these latter groups being mainly poor. Taking account of the results obtained after thawing (Fig. 1b, c, e, f) it is evident that the additional blood letting induced by tail cutting does not improve the colour of fillets cut from gutted

fish. What is noticeable though, is the generally much poorer colour of fillets from fish that had been held ungutted in ice for more than one day or so.

Figure 2 illustrates the amount of gaping exhibited before freezing and after thawing by fillets cut from hake that had been chilled with and without delays on board and then held in ice for up to 5 days, or cut from fish which had been frozen whole after undergoing the above treatments.

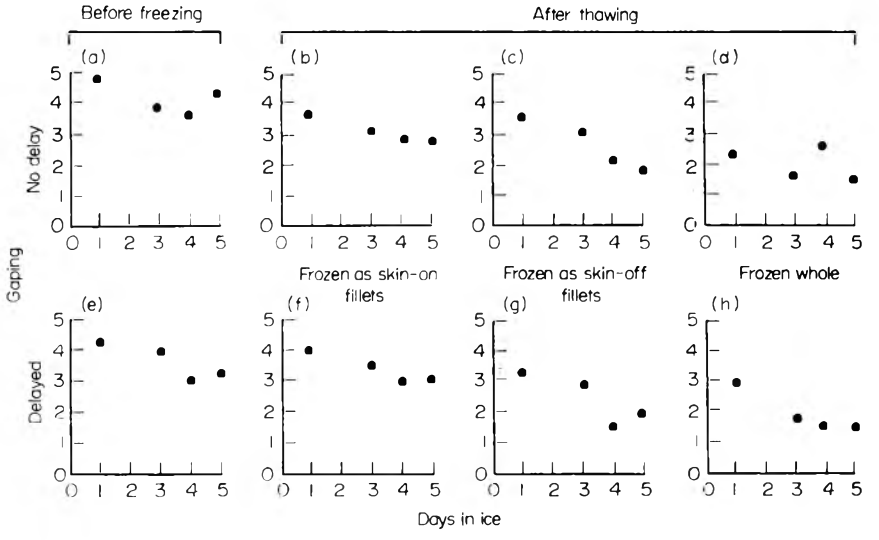

FIG. 2. Effect of length of storage in ice on the amount of gaping in fillets from chill stored hake before freezing and after thawing (Series I).

When they were assessed just prior to freezing (Fig. 2a, e) the fillets demonstrated that an increase in gaping results from longer storage times in ice, but there is some variation. With delays of up to 4 hr at deck temperature prior to handling and chilling, the deterioration ascribable to longer storage times in ice is more marked (Fig. 2e) Compared with the increased gaping resulting from a few days in ice, delays of up to 4 hr on deck before icing produced only a slight worsening (Fig. 2a, e).

Freezing and thawing produced a slight but consistent increase in the amount of gaping (Fig. 2a and b, e and f). Fillets frozen skin on (Fig. 2b, f) showed on balance the smallest increase in the amount of gaping, while fillets prepared from fish which had been frozen whole (Fig. 2d, h) showed the biggest increase as a result of freezing and thawing. Fillets cut before freezing from fresher fish withstood the extra manipulations involved in the skinning process better than did those from older ones. This emphasizes the correlation of gaping with time in ice before freezing which was observed regardless of whether the samples were frozen as fillets or on the bone. Differences ascribable to delays at deck temperature prior to handling were not consistent and only slight. Variations in bleeding techniques did not, as was expected, affect the

amount of gaping. Graphic presentation of the results of the comparisons between shallow bulking and boxing is not given since both at the point of filleting and after thawing no significant differences in colour between the two groups of fish were apparent over the whole storage period (Paired *t*-test; $P > 0{\cdot}05$). This conclusion is the same where all the relevant data are pooled as well as where the assessments obtained after thawing are considered alone.

Fish boxed at sea tended to show less gaping throughout the storage period in ice than did shallow bulked fish but the differences were not significant at the 95% level (Paired *t*-test). The advantage of boxing was retained to a lesser extent after thawing. The trends and differences apparent in Figs 1 and 2 in relation to the effects of freezing and thawing, of days in ice and of form when frozen on colour and gaping are also present in these samples.

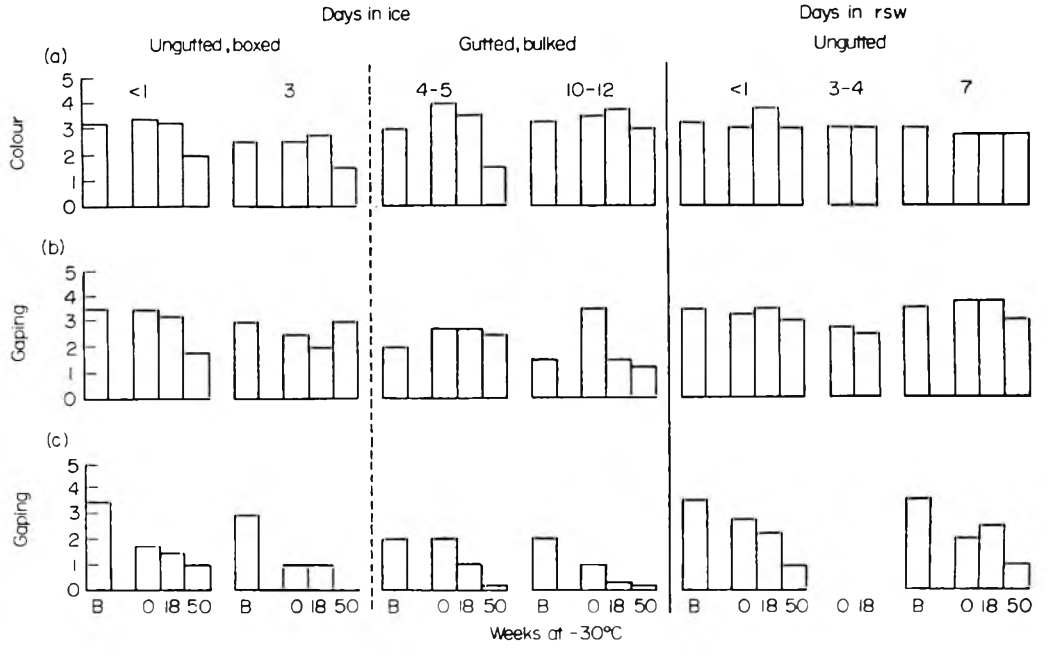

FIG. 3. Effect of length of storage in ice or rsw and of length of frozen storage on colour and gaping of fillets cut from chill stored gutted and guts-in hake (Series II). B, assessment before freezing.

Figure 3 shows the effects of different lengths of time in cold store on colour and gaping of fillets frozen as such or cut after thawing from the frozen headed and gutted fish of Series II. The prefreezing histories (i.e. iced or stored in rsw, gutted or guts in) received by each batch are detailed in the figure.

Fish which had been stored ungutted for 3 days in ice had colours (Fig. 3a) which were just on the borderline of acceptability but all the other treatments afforded

material with less discolouration when the assessments were made before freezing or after freezing and thawing almost immediately (1 day in cold store). The longer periods of cold storage generally caused an increase in discolouration of the fillets. This was most apparent in fish which had been stored in ice. Chill storage ungutted produced fillets which had discoloured belly flaps. These were removed at the time of filleting and the assessments show that the bulk of such fillets is not affected by this type of pretreatment.

Increases in length of time in ice produced a progressive increase in gaping at the time of first assessment (Fig. 3b). Freezing and thawing of blocks of fillets did not alone produce a worsening of the texture, and in some cases less gaping was observed in fillets that had been frozen and thawed than was seen before they were frozen. The effects on gaping of delay in ice before freezing were not seen after longer times in cold storage, but there did appear to be a correlation between gaping and time in cold storage for fish with comparable prefreezing histories.

Storage in rsw tended to produce firmer fish than did storage in ice, but this firmness did not necessarily correlate with less gaping. A trend of increased gaping with longer delay was not so apparent as in the case of iced fish and even after 7 days in rsw the fillets were less broken than those cut from fish stored in ice for 3 days.

Fillets cut from whole frozen fish also show a general increase in gaping with increase of time in cold store; but in this case freezing and thawing alone caused a marked increase in the amount of gaping (Fig. 3c).

The data presented in Fig. 3a, b were obtained from machine cut fillets and where comparisons with hand filleting were available the latter produced significantly less gaping. In Fig. 3c, with the exception of the first assessments which are the same as those in Fig. 3b, the data were obtained from hand filleted fish.

Steaks cut and frozen from the chilled fish retained their good colour on the upper side, but frequently the undersides of the steaks were badly discoloured. The colour of steaks cut from whole frozen fish corresponded with that of fillets with identical treatments. A more coherent product was obtained by steaking, when compared with equivalent fillets, whether the steaks were prepared from chilled fish and then frozen or prepared from thawed whole frozen fish.

All the samples which were tasted from Series I fish had the fresh sweet flavours characteristic of this species even after 1 year's cold storage when only slight cold storage flavours were detected. Included amongst those tasted were samples which had incurred a delay at deck temperatures of 6½ hr and had then been held in ice ungutted for 5 days. The main changes observed were in texture and these arose as a result of cold storage: after 2 months in cold store all samples had a generally soft and juicy texture which, at the end of a year was tougher, dry and fibrous. However, few of the textural descriptions given were indicative of extremes in character.

Those samples from Series II which were tasted showed a similar picture, but with a noticeable deterioration in flavour resulting from cold storage. Even with less than

1 day in rsw before freezing enough salt penetrated the muscle for these fillets to taste salty. After 18 weeks' cold storage most of the rsw samples had less desirable flavour attributes although they were slightly better liked, overall, than comparable iced fish. This is probably because their textures were more juicy and moist. During a year's cold storage strong salt-fishy off odours and flavours developed in most of the rsw samples. Another point which appeared in the limited tasting data indicates that skin off fillets tended to become tougher and dryer and were more prone to develop cold store flavours than the equivalent samples stored as skin on fillets or as whole fish.

Only low incidences of the parasite *Chloromyxum thyrsites* were present in the fish and there was no patent correlation between it and gaping. This organism is associated with the development of soft, pasty textures and milky appearance in hake and other species (Rowan, 1956; Patashnik & Groninger, 1964; Fletcher, Hodgkiss & Shewan, 1951; Dassow, Patashnik & Koury, 1970; Grinols & Tillman, 1970).

Discussion

Many of the results obtained from these series of experiments are in agreement with existing knowledge of the handling and processing characteristics of other species of white fish. The deleterious effects of delaying handling and chilling are noticeable in the results from Series I fish. It is interesting to compare these findings with those of Kordyl & Karnicki (1969) and of Wagenknecht & Horn (1967) who described the deteriorations in quality observable during delays at various temperatures and showed how much faster these occurred at deck than at chill temperatures. If, as in the experiments reported here, the fish are then gutted and chilled, it would appear that the differences between pregutting chilled and unchilled fish largely disappear when they are assessed 1–5 days later and that the damage done by delays at higher temperatures is possibly even reversed. However the benefits of early chilling become apparent once more if these fish are frozen and thawed. Love, Lavety & Garcia (1972) also report a similar phenomenon with cod connective tissue which is weakened by exposure to elevated temperatures but has its strength largely restored by subsequent chilling.

The effects of bleeding fish promptly and the fact that the method of bleeding used at chill temperatures is relatively unimportant (gutting alone compared with gutting and tail cutting) were consistent with the findings of Pienaar (1961).

In the Series I experiments, storage in ice of gutted fish for 5 days produced fillets with poorer colours than those stored for shorter periods, but in the Series II experiments chilled gutted fish stored in ice produced fillets with a good colour after 4 and 12 days. This disagreement between the two sets of results could be due either to seasonal variations or, more probably, to the fact that the storage times in the Series I experiments were not long enough, on the whole, for the expected bleaching due to prolonged icing to become established.

Fish iced with guts in, without any form of bleeding, had worse colours than comparable

bled fish, but the colours were still acceptable when assessed before freezing. Freezing and thawing always caused a worsening of the colour of fillets, the biggest change being recorded in fillets cut from fish stored ungutted before filleting, and consequently the colour of the thawed product cannot easily be predicted by assessing the colour just before freezing. This change in colour following freezing and thawing can be ascribed to lysis of red blood cells and the oxidation of the haemoglobin of residual blood to methaemoglobin (Kelly & Little, 1966; MacLean, 1971).

As expected, no significant differences in the colour of comparable fish stored either in boxes or shallow bulked in ice in the pounds were detected. No apparent differences in colour were observed when fish were frozen as steaks or as fillets, but discolouration of the underside of the steaks occurred as a result of blood draining from the vessels alongside the vertebrae as well as from vessels in the flesh and collecting in the bottom of the pack.

The results of the texture assessments confirmed earlier reports that the most serious quality defect in iced Cape hake is the rapid softening of the flesh caused by delays before handling and by extended storage before freezing and filleting. However, the findings that severe deterioration in texture occurs after 18 hr storage in ice (Wagenknecht & Horn, 1967) or after 100 hr in sea water cooled to between 3·5° and 0·5°C (Kordyl & Karnicki, 1969) were not confirmed, as acceptable fillets were produced from material stored in ice for 5 days and in rsw for 7 days. Incidentally, commercial boats make voyages of up to 12 days for iced fish and of 5–7 days for rsw fish producing fish which is commercially acceptable. It is also interesting to note that softness did not correlate very well with gaping: soft fish often produced fillets with little gaping and *vice versa.*

Comparisons of the degree of gaping exhibited by equivalent fish from Series I and Series II showed that fish from the latter tended to gape more. This is probably a reflection on the amount of damage caused to the fillet by the filleting machine, as comparisons between hand and machine cut fillets (Series II) showed that the latter always gaped more than the former. All the fish from the Series I experiments were cut by hand, and skilled hand filleting leaves a membrane round the anterior part of the fillet and this helps hold the flakes together. This membrane is not retained during the machine filleting operation. All filleting after freezing and thawing of Series II fish was performed by hand, and the very big increase in the amount of gaping when whole frozen fish were thawed and filleted would probably have been even greater had those fish been filleted mechanically.

Comparisons between the colours and the amount of gaping of fillets frozen skin on and fillets cut from thawed whole frozen fish of equivalent prefreezing histories showed that there was little difference in colour, but that the latter showed much more gaping. This result confirms the advisability of current practice of freezing iced fish as fillets rather than as whole fish for subsequent thawing and filleting. Fillets frozen skin off gave, as expected, lower scores for gaping. Where fillets are frozen in interleaved

polythene packs they occasionally show less gaping after thawing than before freezing, but this observation was often more apparent than real in that the polythene sheets tended to compress and smooth over the surfaces of the fillets and, apparently, to cement the broken flakes together.

The tasting data indicate that fish well handled before freezing keep well in the cold store for up to 1 year without developing any serious off flavours. Staler fish, particularly those which had been stored in rsw developed off flavours within that time and were rejected by the taste panel. Despite the fact that the muscle of Cape hake may have a fat content of up to 1·8% (Van Wyk, 1944) no traces of rancidity were found in the Series I fish after a year's frozen storage. Traces of rancidity were found, though, in some of the rsw samples. This difference can be ascribed to the catalytic action of salt on oxidative changes leading to fat rancidity (Banks, 1937), and the better frozen storage properties of hake in the round or as skin on fillets is most likely due to the fact that the bulk of the muscle fat lies in the layer of brown muscle just under the skin.

Where comparisons are available between fish of equivalent history but stored either in rsw or in ice before freezing, no significant differences were discerned with regard to colour, but the salt from the rsw appeared to have a firming action on the texture of the flesh. This property of salt on Cape hake has been reported for brine dipped fillets (Burt *et al.*, 1968; Pienaar, 1964) and for sea frozen, rsw-bled, whole fish (Burt *et al.*, 1974).

From this study a number of fairly definite conclusions have been drawn on the optimum conditions for processing chill stored hake, what the effects of delays before are and how long the frozen material may be stored before deterioration due to cold storage will take place. Delays at deck temperature before gutting should be kept to a minimum and early and rapid chilling is advisable. Chill storage in ice should be limited to 4 or 5 days to produce fish with acceptable colours and little gaping. Prolonged storage in ice does not affect the colour but does cause more gaping. Freezing and thawing always tend to aggravate these defects so assessment before freezing is not a good indicator of ultimate quality. Freezing fish whole after chill storage proved to be an unrewarding exercise since it consistently produced very broken fillets. No great differences in colour and gaping were shown between fish stored in ice or in rsw.

References

Banks, A. (1937) *J. Soc. chem. Ind., Lond.* **56,** 13 T.

Burt, J.R., Dreosti, G.M., Jones, N.R., Kelman, J.H., McDonald, I., Murray, J. & Simmonds, C.K. (1974) *J. Fd Technol.* **9,** 235.

Burt, J.R., Dresoti, G.M., Jones, N.R., Kelman, J.H., McDonald, I., Murray, J., Simmonds, C.K. & Stroud, G.D. (1968) *Fishing News Int.* 7, 39.

Dassow, J.A., Patashnik, M. & Koury, B.J. (1970) *U.S. Fish and Wildlife Service, Bureau of Commercial Fisheries*, Circular No. 332, p. 127.

Fletcher, L.I., Hodgkiss, W. & Shewan, J.M. (1951) *Fishing News*, (2007), 11.

Grinols, R.B. & Tillman, M.F. (1970) *U.S. Fish and Wildlife Service, Bureau of Commercial Fisheries*, Circular No 332, p. 1.

JONES, N.R. (1964) *Proceedings of a Meeting on Fish Technology, Fish Handling and Processsing*, p. 31. Scheveningen, O.E.C.D., Paris.

KELLY, T.R. (1969) In: *Freezing and Irradiation of Fish* (Ed. by R. Kreuzer), p. 64. Fishing News (Books) Ltd, London.

KELLY, T.R. & LITTLE, W.T. (1966) *J. Fd Technol.* **1,** 121.

KORDYL, E. & KARNICKI, Z. (1969) In: *Freezing and Irradiation of Fish* (Ed. by R. Kreuzer), p. 189. Fishing News (Books) Ltd, London.

LOVE, R.M., LAVETY, J. & GARCIA, N.G. (1972) *J. Fd Technol.* **7,** 291.

MACLEAN, C.M. (1971) *Discoloration of sea frozen fish fillets.* Ph.D. Thesis, University of Strathclyde.

PATASHNIK, M. & GRONINGER, H.S. JR (1964) *J. Fish. Res. Bd Canada*, **21,** 335.

PIENAAR, A.G. (1961) *Ann. Rept Fish. Ind. Res. Inst., Cape Town.* **15,** 20.

PIENAAR, A. G. (1964) *Ann. Rept Fish. Ind. Res. Inst., Cape Town*, **18,** 19.

ROWAN, A.N. & MARKS, C.F. (1951) *Ann. Rept Fish. Ind. Res. Inst., Cape Town*, **4,** 11.

ROWAN, M.K. (1956) *Ann. Rept Fish. Ind. Res. Inst., Cape Town*, **9,** 11.

VAN WYK, G.F. (1944) *J. Soc. chem. Ind., Lond.* **63**, 367.

WAGENKNECHT, W. & HORN, R. (1967) *Fisch.-Forsch.* **3,** 97.

(*Received* 14 *October* 1973)

J. Fd Technol. (1974) **9**, 235–245.

Technological investigations into Cape hake

II. Freezing at sea

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Summary

A study of the effects of various delay periods and handling conditions before freezing on Cape hake in the whole or eviscerated state has been made in order to assess the importance of these to the quality and acceptability of the final product. Increasing delays before gutting lead to increasing discolourations in sea frozen fillets and in fillets cut from sea frozen whole fish, even where a further delay for bleeding occurs before freezing. Increasing bleeding times beyond 30 min does not lead to further reductions in this discolouration which is minimized by holding the fish chilled from catching to freezing. The extent of gaping apparent in fillets from whole fish is related to delay before freezing and can also be reduced by holding at chill temperatures throughout the prefreezing period.

Quality deterioration was less in fillets than in fish on the bone during extended chill storage after thawing but freezing and thawing again produced little change in fillet quality.

Introduction

The problems associated with the production of either whole fish or fillets frozen at sea have been studied extensively in recent years. The effects of varying handling conditions before freezing have been reviewed (Jones, 1964) and the benefits obtained from holding the fish at chill temperatures throughout the period before freezing clearly shown, even though the species from which this conclusion was mainly derived was cod (*Gadus callarias*) which is normally found in relatively cold waters.

Cape hakes (*Merluccius capensis*, *Merluccius paradoxus*) have a reputation for being a 'soft' fleshed species and it was recognized that the elevated ambient temperatures

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might present a major problem in the production of high quality sea frozen material. The effect of this higher temperature on rigor mortis and on the escape of blood from the muscle of Cape hake after gutting were two specific factors reasoned to be of prime importance and where knowledge was lacking for this particular species.

Two separate studies on the technology of freezing Cape hake at sea have been published. Kordyl & Karnicki (1969) followed the sequence of deteriorative changes through which unfrozen hake progresses under various holding conditions and the products were also assessed after freezing and thawing. The quality attributes monitored in their work did not directly include two properties, colour and gaping, which are considered to be of major importance to the fish trade in the United Kingdom. On the other hand, Flechtenmacher (1967) reported mainly on the production at sea of blocks of fillets and of packs of steaks. The importance of careful handling of the frozen products was stressed, but the effects of variations in handling before freezing on ultimate quality were not investigated to any great extent.

Samples of Cape hake were obtained on board freezer trawlers during three separate voyages. Material for an initial group of experiments was obtained in the month of January. Experimentation was designed largely to test the relative efficiency of different methods of blood letting at various temperatures on the final colour of the products and to test the effects of holding, for up to 6 hr at ambient or chill temperatures, on gaping. For this, fish were frozen on the bone or as fillets, and in certain instances as steaks. A second group of experiments to investigate the possible effects of extended delays before gutting was carried out in the month of May and in this case only sea frozen whole fish were prepared. The relative merits of refrigerated sea water (rsw) and ice as coolants for holding ungutted fish and as media for bleeding purposes were also investigated on board another freezer trawler in the month of April, fillets and whole fish being prepared at the same time.

In addition to assessing the effects of handling at sea on various aspects of quality of this sea frozen fish, further experiments were done ashore on the thawed material in order to determine the effects of refreezing and of chill storage after thawing.

Materials and methods

Voyage 1

Bleeding the fish was started by one of three techniques, gutting, tail cutting or throat cutting immediately after landing on deck. Batches comprising twelve to thirty individual fish were left to bleed for periods of up to 6·5 hr in iced sea water, in sea water at 17–18°C or in air at 15–16°C. After these delays the fish were gutted if necessary, a part of most of the batches was filleted and frozen, the remainder being frozen on the bone. The actual delays and type of treatment accorded to each batch are summarized, along with some assessments in Fig. 1. A large number of steaks, or cutlets, were prepared from fish of varied prefreezing history by cutting 2 to 3 cm thick pieces from

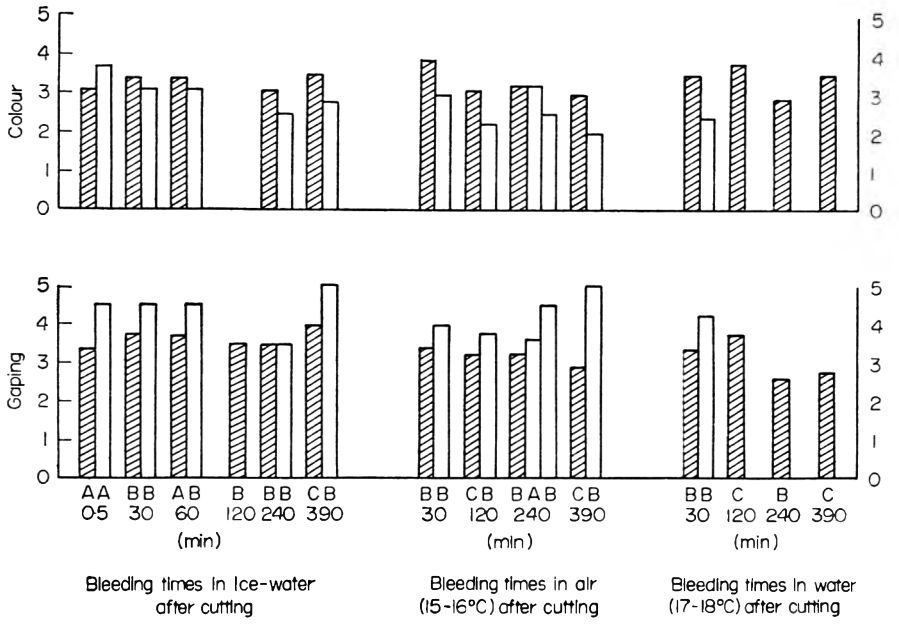

FIG. 1. Effect of bleeding method and time before freezing on colour and gaping of Cape hake. In each case, bleeding was started as soon as the fish were landed on deck. A, gutted; B, throat cut; C, tail cut. Shaded columns, frozen whole; open columns, frozen as fillets.

gutted, headed fish of at least 40 cm length. Individual steaks were laid between polythene films and frozen in a single layer.

Observations on the rigor status were made on whole fish at various times after catching. Additionally fillets were cut from some pre rigor fish and left at 16°C for up to 4 hr during which time the extent of contraction was noted.

Voyage 2

Batches of fish comprising twenty to forty individual fish were either gutted immediately and left to bleed for periods up to 33 hr in iced sea water or were placed immediately in iced sea water and held for various times up to 20 hr before being gutted and returned to bleed for 30 or 60 min. All batches were frozen on the bone in a vertical plate freezer. Approximately similar experiments were carried out on each of two separate fishing grounds (Lüderitz and Cape Point). The actual delays incurred before gutting and before freezing can be obtained by referring to the relevant data in Figs 2–4.

Voyage 3

Batches of ten fish were held ungutted in rsw or in crushed ice for periods of up to 10 hr and after gutting were returned to bleed for up to 8 hr in the same medium.

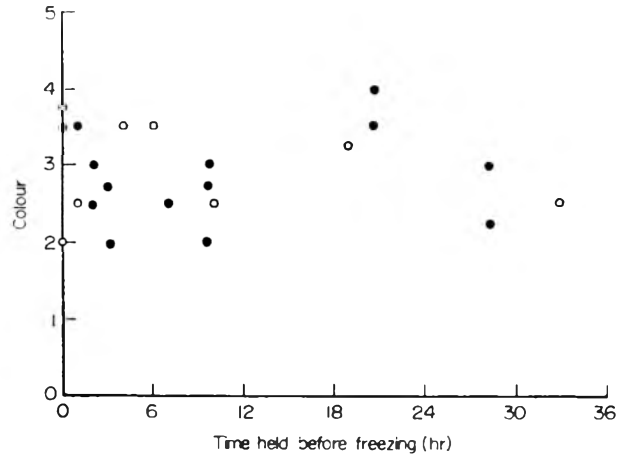

FIG. 2. Effect of bleeding time in iced sea water on colour of Cape hake frozen on the bone at sea. Bleeding was effected by gutting immediately after landing on deck. ○, Cape Point Grounds; ●, Lüderitz Grounds. Both groups were caught in May.

Approximately half the fish in each batch were filleted, the fillets were interleaved with polythene and frozen in an air blast freezer. The remaining fish from each batch were frozen on the bone in a vertical plate freezer. All fish in this voyage were obtained off Lüderitz.

Cold storage and thawing

Most material was held in cold store at −29°C until assessment took place, except during shipment to the United Kingdom when a temperature of −15°C prevailed.

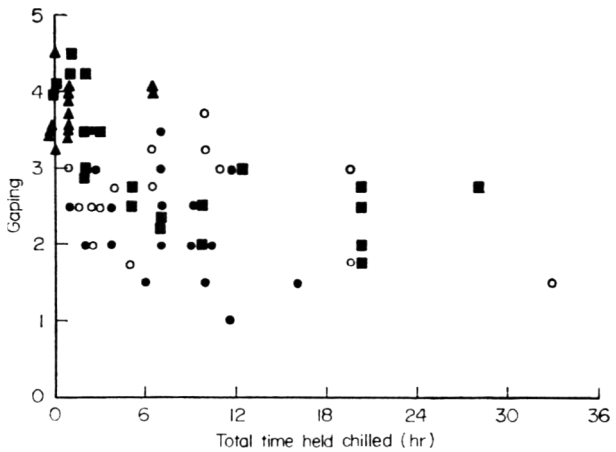

FIG. 3. Effect of delay at chill temperatures before freezing on gaping in Cape hake frozen on the bone at sea. ▲, Iced sea water; January, Lüderitz Grounds. ○, Iced sea water; May, Cape Point Grounds. ■, Iced sea water; May, Lüderitz Grounds. ●, rsw, 5°–8°C April, Lüderitz Grounds.

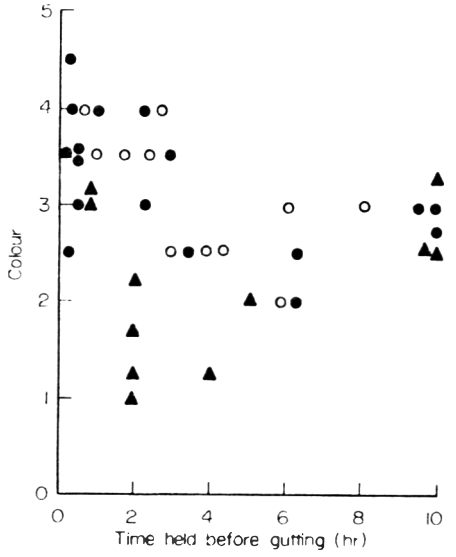

FIG. 4. Effect of delay at chill temperatures before gutting on colour of Cape hake frozen at sea. Fish were allowed to bleed for at least 30 min before freezing. ○, rsw, 5–8°C; frozen on the bone (Voyage 3). ●, rsw. 5–8°C; frozen as fillets (Voyage 3). ▲, iced sea water; frozen on the bone (Voyage 2).

Some duplicate batches obtained during Voyage 3 were held at −14°C for comparison with −29°C held material. All thawing occurred in still air overnight.

Assessments

Assessments of quality after thawing and filleting (if required) were carried out by noting the degree of discolouration and extent of gaping using a panel of at least five experienced members. Each parameter was scored on a 6 point scale where 5 indicates good and 0 indicates poor quality for each batch as a whole. As a general rule it has been found that fillets with ratings of ≥ 3 are acceptable commercially while those of $\leq 2{\cdot}5$ are not. Considerable within batch variation was occasionally encountered particularly when the batches contained large numbers of fillets, but in these cases also it was an overall impression that was rated. The means of the scores of the individual panel members appear in the figures. The commercial acceptability of some samples was also determined by representatives of the fish handling trade in South Africa and in the United Kingdom. In addition to assessments at intervals for colour and gaping certain samples were steam cooked and tasted by a number of experienced tasters. Descriptions of flavour and texture were obtained and, where relevant, attention was focused on the degree of saltiness and extent of rancidity detected.

Salt concentrations in the flesh were determined using the method of Volhard (Vogel, 1947) and peroxide values using the method of Banks (1937).

Experimental treatments ashore

The effects of refreezing and rethawing were investigated using a selection of fish from Voyage 2 which exhibited a wide range of qualities after initial thawing and filleting. The fillets were wrapped in polythene, air blast frozen and stored for one week at −29°C before thawing for reassessment.

Changes in quality during chill storage in ice of thawed whole fish and of fillets cut from the same type of material were followed. A large batch of similarly treated fish was taken and divided into four groups of ten fish each. One group of fish was filleted immediately after thawing while the other groups were stored in ice and filleted 3, 6 or 9 days later. The fillets cut on each occasion were packed in ice between sheets of polythene and reassessed at subsequent sampling times.

Results

The relative efficiency of the different blood letting techniques adopted initially can be seen from the colour ratings given in Fig. 1. Bleeding was started by one of the methods indicated immediately after the fish were landed on deck. No significant differences were seen in the colour of thawed fillets from whole frozen fish which had been allowed to bleed for between 30 and 390 min in iced sea water, in air at 15–16°C or in water at 17–18°C. It is also obvious that the method of blood letting does not produce marked differences in the colour quality of these same fillets. Regardless of variations in treatment within the limits specified in Fig. 1, and bearing in mind that bleeding was initiated in all samples immediately, all fillets cut from eviscerated whole frozen fish had colours which were judged to be commercially acceptable. On the other hand increasing the delays before freezing up to 390 min at deck temperatures (15–18°C) in air or water increases the extent of gaping though delays of this duration at chill temperatures in iced sea water made little difference to the extent of gaping apparent. Comparison of whole frozen and fillet frozen fish of identical prefreezing history shows that in all cases except one the colour of the thawed fillet from frozen whole fish was superior to that from the thawed frozen fillet. On the other hand the extent of gaping in the whole frozen material was never less and was usually appreciably more than that obtaining in the frozen fillet. While none of the sea frozen fillets scored highly for colour it is obvious that bleeding at chill temperature gives rise to considerably less discolouration. Bleeding times of greater than 30 min did not appear to offer any advantage in relation to colour of either whole or fillet frozen material and even in some instances longer bleeding times were associated with poorer colours.

It was noted that several fish were already in rigor when the net was emptied and that approximately 50% had reached this stage within 1–2 hr at deck temperatures. However problems associated with the onset of rigor mortis did not appear to be serious, only very slight contractions occurring in those fillets which had been allowed to enter rigor off the bone. Similarly the rigidity developed during the onset of rigor

on the bone was not great and difficulties were not encountered due to this during loading of the vertical plate freezers. Repeated flexing of in rigor fish did however lead to the production of badly gaping fillets after thawing.

Commercial acceptability ratings showed in general that fish frozen whole less than 3 hr after death were judged acceptable whereas all fillet frozen treatments except one produced unacceptable material largely on the basis of blood discolouration, the exception being produced from a batch throat cut immediately and held for 30 min in iced sea water before freezing. The cut surfaces of sea frozen fillets were significantly less glossy than those of fillets cut from sea frozen whole fish and this factor may have contributed to the lower acceptability of the former.

Additional data on the effect of various bleeding times on colour of fillets cut from whole frozen fish is presented in Fig. 2 which shows that no consistent relationship is evident for bleeding times of between 0 and 33 hr in iced sea water. It should perhaps be pointed out here that zero bleeding times as such were not achieved since a finite delay between gutting and freezing always occurred during loading of the freezers. The times quoted here and later do not include this interval of approximately 30 min.

In contrast with the findings from the experiments of Voyage 1, results were obtained which indicated that increasing delay at chill temperature before freezing leads to a progressive increase in gaping. Results shown in Fig. 3 obtained from a large variety of experimental treatments prepared at different times of the year indicate that gaping is related to total time held chilled before freezing and suggest that the initial results obtained may be atypical.

The results of the investigations into the effects of extended pregutting delays on colour of sea frozen fillets and of fillets from sea frozen whole fish are shown in Fig. 4. For fish which were allowed to bleed for a minimum time of 30 min in iced sea water or in rsw increasing the delay before gutting caused a progressive worsening in colour up to a maximum which is variable in the region of 2–6 hr. Further increases in the delay period before gutting usually led to a slight improvement in colour and samples from fish held ungutted for 10 hr before bleeding gave material which was regarded as just acceptable commercially. As with the experiments carried out on Voyage 1, longer bleeding times did not lead to less discoloured fillets and the data presented in Fig. 4 have been plotted without regard being paid to the different bleeding times the various batches received.

With the necessity for chilling the catch at all stages before freezing clearly established if high quality material was to be obtained it was decided to compare the relative merits of rsw and ice for this purpose. Batches of fish obtained on Voyage 3 which had been held for a variety of times pre- and post-gutting either in rsw or in ice showed that both media are equally effective. Statistical treatment of the colour and gaping scores (t-test for paired samples) indicated that there was no significant difference at the 5% level between the colours of whole frozen and fillet frozen fish held either in ice or in

rsw before freezing. Similar analysis showed no significant difference between the colour scores of fillets frozen as such and of fillets from whole frozen fish of identical pre-freezing treatments. The data for the rsw held fish is included in Fig. 4, that for the comparable iced fish is essentially similar and has been excluded for this reason. As noted earlier gaping increases as a result of increased delay before freezing (Fig. 3) but no significant difference could be seen (*t*-test for paired samples) in the relative amounts of gaping apparent as a result of holding in either rsw or crushed ice. Significant differences were seen ($P < 1\%$) however, between the extent of gaping in fillets cut from thawed whole fish and those frozen as fillets regardless of the chilling medium employed pre-freezing.

It was anticipated that salt penetration into the flesh would be greater during the bleeding period in rsw than during the pre-gutting delay but similar rates of salt uptake were found to occur regardless of whether the fish were held gutted or ungutted. Figure 5 shows the concentrations of salt in the muscle of fish as a function of total time immersed, regardless of the proportions of time held in the ungutted and gutted states. Since it was also considered possible that the presence of high concentrations of salt in the flesh of hake might enhance the rate of oxidation of muscle lipid, samples of rsw and ice chilled fish were held as fillets and whole fish at two cold storage temperatures ($-14°$ and $-30°$C) for periods up to 1 year. Tasting assessments at 22 and 50 weeks' storage showed that only slight rancid flavours had developed even at the higher storage temperature although fish were often judged unacceptable for reasons other than this, e.g. bitter flavours or tough texture. No relationship between rancidity as tasted and salt concentration as estimated analytically could be demonstrated and peroxide values measured on the lipid of the stored material similarly failed to correlate with salt concentration or indeed with the organoleptic evaluation of rancidity.

The effect of refreezing fillets cut from whole frozen fish was found to be slight and

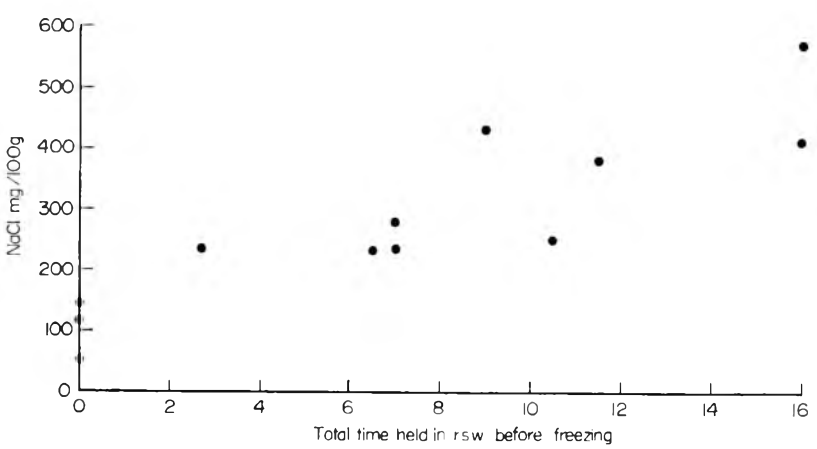

FIG. 5. Effect of holding in rsw, 5–8°C, on uptake of salt into the muscle of Cape hake. (Time in hr.)

colour and gaping scores were on average the same pre- and post-refreezing and thawing.

Chill storage of thawed fish showed that there was little difference between samples held as fillets and those which had been stored on the bone and freshly filleted on each sampling day. The latter however did show an increasing incidence of staining in the belly flaps and in the parts of the fillets which had been contiguous to the backbones. In general both discolouration and gaping increased on both types of material with time held after thawing and off odours were apparent in both fillets and whole fish after 9 days' storage in ice.

Steaks were not rated systematically for colour and gaping, but in general the quality of steaks produced was high with very little gaping evident regardless of prefreezing treatment. A slightly increased discolouration in the muscle near the backbone was seen in most packs produced but overall the colour was regarded as good.

Discussion

Rapid deterioration of quality occurs when hake are exposed immediately after catching to high ambient temperatures (Wagenknecht & Horne, 1967). To the extent that the muscle of freshly caught hake has also a very soft consistency maintenance of the high quality of this material is made even more difficult if conventional icing techniques are used for preservation. For these reasons it was thought that freezing at sea would be likely to introduce even greater benefits than are normally expected, but recognition of problems which might arise would be essential. This is particularly true for a fillet freezing operation which, for certain reasons, might appear to be the logical choice of process.

In dealing with attempts to assess the possibility of reducing the extent of discolouration on the final fillet either frozen as such or from thawed whole fish it is apparent that there is no outstanding benefit to be obtained from the use of one particular method of blood letting. In certain circumstances where fillets are to be frozen at sea it may be preferable at times of high catching rates to initiate bleeding in a larger number of fish by the simpler expedient of throat cutting as opposed to gutting smaller numbers of fish in the same limited time, thus ensuring a higher proportion of the catch would be acceptable in terms of the colour of the thawed fillet. Bleeding times of greater than 30 min do not appear to offer any advantage for colour of fillet, but is important that this bleeding takes place at chill temperatures if discolouration is to be minimized. These conclusions, in general, are in agreement with those obtained when colder water species of fish have been investigated (Jones, 1964; Kelly, 1969) with the exception of the findings that extending bleeding time does not lead to improvement in colour quality (Kelly, 1969).

Chilling in addition to being an aid to bleeding also retards the rapid drop in quality which hake undergo if left for only short periods at elevated temperatures. A marked

drop in quality has been noted after only 3 hr at deck temperatures while machine filleting was considered to be impractical in fish stored in ice for as little as 12 hr after capture because of softness and a consequential low, uneconomic yield (Wagenknecht & Horn, 1967). Essentially similar results have been obtained in the course of the present work with frozen fish where progressive increases in the extent of gaping are apparent in fillets from fish which had been held chilled before freezing whole, but this deterioration is small when compared with that experienced when the fish are held unchilled. In view of the ease with which the flesh of this species gapes it would be interesting to determine whether the pH of the flesh has an effect on gaping in Cape hake similar to the one it has in certain other fish (Love & Haq, 1970).

It is therefore obvious that even in a freezing at sea operation chilling of the catch is necessary as soon as possible after catching if its inherent advantages are not to be lost pre-freezing. In the practical situation where it is not possible to deal with the catch as a unit immediately, results obtained from whole and fillet frozen fish held for limited periods ungutted before blood letting show that increased discolouration can be expected as the result of this delay. Comparison of the colour scores of whole and fillet frozen fish of similar prefreezing treatment shows, perhaps surprisingly, that in one set of experiments similar amounts of discolouration were evident in both types of material. In general the findings from Voyage 1, where marked differences between the colours of both types of fillet were evident, are more in agreement with data obtained from a variety of other species. However the two sets of experiments on Cape hake being compared here were carried out at different times of the year and it is possible that this along with the wide variations present even between fish of the same history could account for the differing results. In addition to the possible colour problems associated with sea frozen fillets, this type of material exhibits a lack of surface gloss on the cut surfaces (Jones, 1966) and therefore will not yield a smoke cure with a satisfactory appearance. As noted earlier however problems of shrinkage and toughening of these fillets will not arise since rigor is a weak condition in hake, a finding in agreement with that reached by Wagenknecht & Horn (1967). Vigorous manipulation and rough handling of the whole fish should nevertheless be avoided since this greatly increases the incidence of broken fillets.

In deciding the means to be adopted for chilling the catch pre-freezing rsw would appear to be the method of choice since it was found to be as effective as the other methods investigated in promoting bleeding and reducing gaping. Further, no adverse effects have been shown to follow from its use particularly in relation to salt levels tasted or enhancement of rancidity. Rates of development of rancid flavours in cold stored fish which had been held for a maximum of 16 hr in rsw were not found to be greater than those in similar material held in ice before freezing although extended holding times in rsw have been shown to accelerate the production of certain undesirable flavours and odours (Burt *et al.*, 1974). The extent of salt tasted was similarly not found to be objectionable from fish held in rsw for up to 16 hr and since delays of this

length are not likely to occur even when catching rates are very high this factor is not considered likely to affect the eating quality of fish from normal commercial production if such a system is used. In general the extent of rancid flavours found in our material appeared to be lower than that obtained from similarly cold stored Pacific hake (*Merluccius productus*) (Dassow, Patashnik & Koury, 1970). Whether this difference reflects the fact that different species were used in the two investigations or is related to the form in which the fish were stored (glazed skin on fillets and whole fish in the present work) must remain a matter for speculation. These considerations apart, the use of rsw for immediate chilling of the catch has much to commend it and it is perhaps significant that several vessels equipped with rsw systems are now operating from South African ports (Anon., 1970).

To conclude, the advantages of holding Cape hake chilled at all stages before freezing at sea have been demonstrated. Discolourations will be minimized by bleeding, and this should be started soon after capture. Although the method of blood letting and bleeding times are not critical extended delays before freezing will lead to increased gaping. Provided the temperature is kept low the bleeding environment is also relatively unimportant.

The above considerations apply more or less equally whether the fish is to be frozen on the bone, as fillets or as steaks. It is the outlet which will determine which form is most suitable since gaping can be accepted more readily than discolourations in some products but the reverse will be true in others.

References

Anon. (1970) *S. Afr. Shipping News and Fishg. Indust. Rev.* **25,** 66.

Banks, A. (1937) *J. Soc. chem. Ind. Lond.* **56,** 13T.

Burt, J.R., Dreosti, G.M., Jones, N.R., Simmonds, C.K. & Stroud, G.D. (1974) *J. Fd Technol.* **9,** 223.

Dassow, J.A., Patashnik, M. & Koury, B.J. (1970) *U.S. Fish and Wildlife Service, Bureau of Commercial Fisheries,* Circular 332, p. 127.

Flechtenmacher, W. (1967) *Inform. für die Fischwirtshcaft.* **14,** 131.

Jones, N.R. (1964) *Proceedings of a Meeting on Fish Technology, Fish Handling and Preservation,* p. 31. Scheveningen, O.E.C.D., Paris.

Jones, N.R. (1966) *Proceedings, Conference on the design of Fishing Vessels and their Equipment in Relation to the Improvement in Quality,* p. 81. Grampian Press, London.

Kelly, T.R. (1969) In: *Freezing and Irradiation of Fish* (Ed. by R. Kreuzer), p. 64. Fishing News (Books) Ltd, London.

Kordyl, E. & Karnicki, Z. (1969) In: *Freezing and Irradiation of Fish* (Ed. by R. Kreuzer), p. 189. Fishing News (Books) Ltd, London.

Love, R.M. & Haq, M.A. (1970) *J. Fd Technol.* **5,** 241, 249.

Vogel, A. (1947) In: *Quantitative Inorganic Analysis,* p. 92. Longmans Green and Co. London.

Wagenknecht, W. & Horn, R. (1967) *Fisch.-Forsch.* **3,** 97.

(*Received* 23 *October* 1973)

J. Fd Technol. (1974) **9**, 247–253.

Effect of deep fat frying on the availability of lysine in fish fillets

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Summary

An examination has been made of the effect of the state of thermal oxidation of oil used in deep fat frying on the available lysine in fillets of cod, haddock, coley, plaice, skate and dogfish. Corn oil, groundnut oil and olive oil were used under controlled conditions.

Results indicated that the loss of available lysine when using oxidized oil was about 10% higher than when fresh oil was used.

Introduction

Foods of animal origin such as meat, fish, eggs, milk and cheese are good sources of lysine, and there is no evidence that lysine deficiency is prevalent in Western countries in which these commodities are common dietary items. Nevertheless because of the high lysine content of fish and the popularity of deep fat frying in Britain the effect of this form of cooking on the availability of fish lysine is of nutritional interest.

It has been established that linkages between certain amino acids and other food components can be formed during heating. The complexes produced cannot be broken down by digestive hydrolysis and the amino acid involved is no longer available (Lowry & Thiessen, 1950). During 1951 Bieley, March & Tarr showed that the protein in herring meal was damaged by commercial drying processes as a result of interaction with oxidized fish oils under fairly severe heating conditions. Similarly lysine damage after heating herring meal was reported by Lea, Parr & Carpenter in 1958, and later this was partly attributed to reaction with autoxidized fat (Lea, Parr & Carpenter, 1960).

The present investigations were carried out to determine the loss of available lysine after deep frying fresh and deep frozen fish in fresh and thermally oxidized cooking oils in general use in European countries. In addition the possible influence of portion size and batter coating was investigated.

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Materials and methods

Choice of fish and sample preparation

The principle factors influencing choice of fish to be used in the investigations were local availability (in both fresh and frozen condition) and the selection of species commonly used for commercial and domestic deep frying. Freshly caught fish was available from the nearby fishing ports of Dovercourt and Brightlingsea and ordinary retail packs of deep frozen fish were obtained from local supermarkets. The species used were cod, haddock, coley, plaice, skate and dogfish (rock salmon), although the last two were not available frozen.

All material was used as soon as possible after purchase, fresh fish being filleted and stored in slush ice and frozen fish being placed in a deep freeze cabinet until required. Samples for analysis free of skin and bone were obtained by selecting flakes from the middle of each fillet or in the case of frozen material by rasping off the required amount using a household grater.

Total nitrogen and 'theoretical' lysine

Total nitrogen content was determined by the Kjeldahl process, 1 g samples of fish being used in each case. All determinations were carried out in triplicate and the mean values recorded. A conversion factor of 6·25 was used to convert the mean readings into total protein (McCance & Widdowson, 1967) and the 'theoretical' lysine value of the raw fish samples calculated from this using the appropriate FAO factors (FAO, 1970).

Determination of available lysine

(a) *Fresh fish samples.* A modification of the technique devised by Carpenter was used in all determinations (Carpenter, 1960).

Three 1 g uncooked samples of each fish fillet examined were mechanically shaken for 10 min in stoppered 50 cm^3 flat-bottomed flasks with 3 g of silica anti-bumping granules and 8 cm^3 of 8% sodium hydrogen carbonate solution. After allowing to stand for 5 min, 12 cm^3 of a freshly prepared 2·5% v/v ethanolic solution of 1-fluoro-2,4 dinitrobenzene was added using a safety pipette. The stoppered flasks were then mechanically shaken for 2 hr and subsequently placed in a boiling water bath to volatilize the ethanol, 24 cm^3 of 8·1 M hydrochloric acid was then slowly added to each flask, care being taken not to lose any of the contents as a result of effervescence.

The flasks were fitted with 30 cm water condensers and the contents refluxed for 16 hr. After refluxing and allowing to cool the condensers were rinsed into the flasks with distilled water and the latter stoppered and placed in slush ice for 1·5 hr. The chilled flask liquor was then filtered and made up to 200 cm^3 after repeatedly rinsing the flasks with chilled distilled water, the rinsings being added to the filtrate.

Three 3 cm^3 samples were taken from each solution, one being pipetted into a 25 cm^3 conical flask and the remainder into two stoppered 10 cm^3 calibrated test tubes. After twice extracting the contents of the two test tubes with 5 cm^3 of ethoxyethane and discarding the other washings, the residue of the ether was driven off by immersing the tubes in boiling water for 2 min. One tube (A) was then filled to the 10 cm^3 mark with M hydrochloric acid and the other (B) retained for treatment with methylcarbonyl chloride using the method described by Carpenter (1960).

The contents of tubes A and B were transferred to two 1 cm quartz cells and their optical densities measured at 440 nm using distilled water as a blank. The difference between the two readings ($E_A - E_B$) was used to calculate the available lysine, reference being made to a standard calibration curve. A mean of three available lysine determinations was recorded for each fish sample.

(b) *Fried fish samples.* Ten-gram samples of each of the fish fillets used in the lysine determinations described above were fried in fresh and thermally abused samples of corn oil, groundnut oil and olive oil for 4 min at 180°C. To avoid sample loss during frying each fish piece was stitched into a muslin sheath before lowering into the frying bath. The sheath also aided identification and rapid retrieval of the sample after frying. The thermally abused oil samples had been used for frying potato chips for an overall period of 48 hr at 180°C prior to the experiment.

After frying the samples were drained on filter paper for 2 min and after breaking into pieces by gentle pressure were transferred to a paper thimble and extracted for 2 hr with petroleum ether in a Soxhlet. The oil free samples were then air dried overnight and 1/10 of each sample by weight analysed for total protein and available lysine as before. A correction factor was introduced into the calculation of available lysine to allow for damage during extraction. This was found by experiment to be equivalent to a mean loss of 6·7% for all species. As in the case of the uncooked samples a mean of three available lysine determinations was recorded for each fried sample of fish.

Effect of battering and sample size

Commercially produced frozen fish fingers were fried in fresh and 48 hr abused samples of corn oil with and without their batter coating to assess the effect of battering on available lysine loss. Similarly whole cod fillets were fried in fresh and 48 hr abused samples of corn oil to take into account the smaller degree of oil/fish contact when frying pieces of fish of the size normally used in domestic and catering operations.

The fillets were weighed and a 5 g sample analysed to determine FDNB available lysine. After frying for 4 min at 180°C the fish was allowed to drain for 2 min and then weighed and comminuted in a domestic blender. A sample equivalent to 5 g fresh weight was then extracted with petroleum ether for 2 hr and 1/5 aliquot samples by weight analysed to determine the FDNB available lysine. All determinations were carried out in triplicate and mean values recorded.

Heat treatment of frying oils

Four-litre samples of corn oil, groundnut oil and olive oil were heated to 180°C in a thermostatically controlled aluminium frying bath. Water rinsed potato chips were added continuously during each heating cycle of 6 hr and removed after 8 min. Each oil sample was subjected to eight frying cycles. After each cycle the oil was allowed to cool and then filtered to remove debris. When not in use the oil surface was covered by a disc of aluminium foil.

Before use the fresh oil was stored at 4°C in tinned metal drums under an atmosphere of nitrogen.

Preparation of ε-DNP lysine standard

Reference standard solutions of the ε-DNP lysine salt were prepared by dissolving ε-(2,4-dinitrophenyl)-L-lysine in M hydrochloric acid solution. The lysine salt was chromatographically homogenous and was purchased from BDH (Poole).

Optical densities at 440 nm of a range of concentrations between 0·3 mg and 4·5 mg per 100 cm^3 of solution were determined using an SP 600 Pye Unicam spectrophotometer. Quartz cells of 1 cm cross-section were used and the instrument zeroed with distilled water.

A linear relationship between concentration and optical density was established over the range of solutions used and a median value was selected as a reference standard. All readings were increased by a factor of 0·436 to allow for incomplete conversion of fish lysine to the ε-DNP salt ($\times 1{\cdot}09$) and the proportion of lysine in the compound ($\times 0{\cdot}40$) (Carpenter, 1960).

Results and discussion

Data obtained are summarized in Tables 1 and 2.

Theoretical lysine values obtained by calculation from the total protein values of raw fish samples were in good agreement with published values (FAO, 1970). The values for available lysine obtained by Carpenter's (1960) method were up to 9% lower than the calculated values. This was mainly ascribed to incomplete recovery of DNP lysine and the presence of interfering coloured DNP complexes of histidine and hydroxylysine.

A mean overall loss of 17·9% FDNB available lysine was observed in fish samples fried for 4 min at 180°C in fresh corn oil, groundnut oil and olive oil. The mean loss of FDNB available lysine for all samples increased to 28·9% when frying in oil which had been thermally oxidized by intermittent frying over a period of 48 hr thus representing a further lysine loss of 11%. This indicated lysine binding reactions between oxidation products of the thermally abused oils (Carpenter *et al.*, 1962) in addition to the reduction in lysine normally experienced at frying temperatures due to polymerization and other reactions (Heinrich, Rohlfing & Bugna, 1969).

TABLE 1. Protein and lysine values for frozen and fresh fish fillets

Species		Total protein (g/100 g)			Lysine (g/16 g N)			
		FAO (1970)	Kjeldahl	Mean*	Calculated	Mean*	Experimental (Carpenter 1960)	Mean*
Cod	(Fresh)	17·00	17·24		10·81		10·35	
				17·38		10·89		10·17
	(Frozen)	—	17·53		10·97		10·00	
Haddock	(Fresh)	17·00	17·10		10·72		10·49	
				17·83		11·17		10·55
	(Frozen)	—	18·56		11·62		10·70	
Coley	(Fresh)	17·00	17·80		11·16		10·07	
				18·20		11·42		10·46
	(Frozen)	—	18·61		11·69		10·86	
Plaice	(Fresh)	16·00	15·75		10·04		9·78	
				15·90		10·15		9·96
	(Frozen)	—	16·05		10·27		10·15	
Skate	(Fresh)	20·00	20·02	—	13·43	—	12·40	—
Dogfish	(Fresh)	20·00	20·65	—	12·39	—	11·45	—
Mean all species		18·80	17·93	—	11·31	—	10·63	—

* Mean value for fresh and frozen samples.

All replicates were in good agreement although fairly wide differences in percentage losses of available lysine were obtained in individual experiments. This was to be expected due to the varying quality of both fresh and frozen fish, differences due to the varying quality of both fresh and frozen fish, differences due to species and season, and the diverse chemical composition of the oils used. Nevertheless the mean differences in available lysine resulting from frying in fresh and thermally oxidized oils were in good agreement. Values ranged fairly equally around the overall mean of 11·0% (olive oil 10·3%, corn oil 11·0%, groundnut oil 12·4%) although it was not possible to relate this order to chemical features such as linoleic acid content, or any of the parameters such as viscosity, colour darkening, iodine number or peroxide value commonly used to indicate thermal degradation. It is interesting to note that with the exception of coley the mean loss of available lysine from frozen fish was appreciably less than from fresh fish. This indicates that the condition of the fish before frying could affect lysine loss.

The available lysine value of the fish fingers as bought was about 11% below the mean for fresh and frozen cod which was expected as the fingers are partly fried during processing. The available lysine was only 2–3% lower after frying in fresh corn oil both with and without batter. There was a further fall in available lysine after frying in

TABLE 2. Reduction in FDNB available lysine after frying in vegetable oils at 130°C for 4 min

Species		FDNB available lysine (g/16 g N)							
			Corn oil fried		Groundnut oil fried		Olive oil fried		Mean % difference
		Raw	*a* Fresh	*b* Abused	*a* Fresh	*b* Abused	*a* Fresh	*b* Abused	(*a* − *b*) All oils
Cod	(Fresh)	10·35	7·30	6·95	7·45	6·40	9·05	7·25	10·2
	(Frozen)	10·00	8·25	7·26	8·50	7·50	8·31	7·69	8·8
		(10·17)*	(7·77)	(7·10)	(7·97)	(6·95)	(8·68)	(7·47)	(9·5)
Haddock	(Fresh)	10·49	8·65	6·38	8·91	7·75	6·97	6·72	11·8
	(Frozen)	10·70	9·35	9·02	10·00	9·09	8·22	6·20	10·2
		(10·59)	(9·00)	(7·70)	(9·45)	(8·42)	(7·59)	(6·46)	(11·0)
Coley	(Fresh)	10·05	8·60	8·32	9·98	8·98	7·81	6·05	10·1
	(Frozen)	10·80	10·75	8·64	10·90	8·22	8·61	7·00	16·8
		(10·42)	(9·67)	(8·48)	(10·44)	(8·60)	(8·21)	(6·52)	(13·4)
Plaice	(Fresh)	9·78	7·89	6·14	8·78	7·32	8·79	8·36	12·5
	(Frozen)	10·15	9·16	8·75	9·80	8·65	6·91	5·82	8·7
		(9·96)	(8·52)	(7·44)	(9·29)	(7·98)	(7·85)	(7·09)	(10·6)
Skate	(Fresh)	12·40	9·45	7·00	10·21	7·94	7·82	7·50	13·5
		(—)	(—)	(—)	(—)	(—)	(—)	(—)	(—)
Dogfish	(Fresh)	11·45	9·10	8·00	9·31	8·42	8·61	8·12	7·5
		(—)	(—)	(—)	(—)	(—)	(—)	(—)	(—)
All species		10·63	8·86	7·65	9·38	8·03	8·11	7·70	11·1

* Figures in parentheses are mean values for fresh and frozen samples.

thermally abused corn oil (4·8%) which was substantially greater in the case of de-battered samples (13·4%).

This suggests that battering provides an adequate barrier against frying oil degradation products which might otherwise interact with the lysine. In the case of pre-fried battered products the oil adsorbed by the batter also presumably hinders the ingress of oil from the frying bath.

In the case of whole cod fillets the mean loss of available lysine during frying in fresh corn oil (26·3%) was appreciably lower than when using the small experimental samples referred to in Table 2 (29·2%). When frying in thermally abused corn oil the lysine loss was virtually unchanged (27%) compared to the equivalent loss suffered by the experimental samples (32·6%). This indicates that losses of available lysine during domestic or restaurant frying of whole fillets are likely to be approximately 12% lower when using fresh corn oil and 25% lower when using thermally degraded corn oil, than during the experimental trials.

From these results it may be concluded that during frying the major source of available lysine loss is heat damage rather than complexing with thermal oxidation products in the frying oil. In the case of comminuted fish products such as fish balls, fish cakes and fish sausage however, which have a comparatively large surface area of contact with the frying oil and are unprotected by batter the effects of complex formation might be more pronounced.

Acknowledgment

The authors acknowledge the help of Miss S. M. Fairbairn who assisted with the lysine determinations.

References

Biely, J., March, B.E. & Tarr, H.L.A. (1951) *Pacific Biological Station (Progress Reports)* **89** (December).
Carpenter, K.J. (1960) *Biochem. J.* **77,** 604.
Carpenter, K.J., Morgan, C.B., Lea, C.H. & Parr, L.J. (1962) *Br. J. Nutr.* **16,** 451.
F.A.O. (1970) *Nutritional Studies*, No. 24.
Heinrich, M.R., Rohlfing, D.L. & Bugna, E. (1969) *Archs Biochem. Biophys.* **130,** 441.
Lea, C.H., Parr, L.J. & Carpenter, K.J. (1958) *Br. J. Nutr.* **12,** 297.
Lea, C.H., Parr, L.J. & Carpenter, K.J. (1960) *Br. J. Nutr.* **14,** 91.
Lowry, J.R. & Thiessen, R. (1950) *Archs Biochem. Biophys.* **25,** 148.
McCance, R.A. & Widdowson, E.M. (1967) *The Composition of Foods*, Special Report Series 297. H.M.S.O., London.

(*Received* 12 *September* 1973)

J. Fd Technol. (1974) **9**, 255–258.

Technical note: The effect of ultimate pH (pH_u) of pork on some characteristics of vacuum packaged bacon

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The inherent variability of pork likely to affect the quality of bacon includes, among other factors, the rate of acid formation in muscle after death. Many workers (Callow, 1936; Bate-Smith, 1938; Gibbons & Rose, 1950; Rose & Peterson, 1951) have shown that the ante mortem physiological condition of pig muscle influences the properties of the cured meat. The final or ultimate pH (pH_u) is that which is attained in muscle when post mortem breakdown of glycogen has ended and is usually measured 24 hr after death. Pork of high ($>6{\cdot}0$) ultimate pH is more susceptible to bacterial spoilage than pork of low ($<6{\cdot}0$) ultimate pH because the higher concentration of lactic acid in the latter material retards bacterial growth (Ingram, 1948; Kitchell & Ingram, 1965). In view of these findings, it was considered of value to determine the effects of pH_u on some properties of prepackaged bacon.

Experimental

Eight sides of pork were selected on the basis of pH 24 hr after killing. The pH was measured in the lumbar region of the longissimus dorsi muscle at the position of the last rib using a Radiometer type D24 meter with spear electrodes. Four sides of low pH ($<6{\cdot}0$) and four sides of high pH ($>6{\cdot}0$) pH_u were selected and cured in the same Wiltshire brine. After maturation, the pH of the same area of the muscle was again measured. A portion of the mid-rib area of each side was then sliced and the rashers placed in pouches of Saran coated cellulose/polythene (Robinson Waxed Paper Co., Bristol.) A total of sixty-four packs were prepared, eight from each middle. The packs were stored at 0°C for 7 days and were then transferred to a refrigerated incubator ($10° \pm 1°C$) for 21 days. These conditions simulated those used in commercial practice.

The bacteriological and organoleptical analyses of the bacon have been described elsewhere (Dempser, 1972). Initial analyses were made by sampling duplicate packs within 2 hr of packaging. The bacon was subsequently tested at weekly intervals for 4 weeks. Chemical analyses were carried out at the same time and were made as follows: the lean from five rashers was minced and duplicate samples (5 g) were dried for 18 hr at 100°C and ashed for a further 18 hr at 550°C. Chloride was determined on the ash by Volhard's method. Nitrite (NO_2) was estimated by the method of Follet & Ratcliffe

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(1963) using 10 g of minced lean. The pH values of the homogenates were measured with a glass electrode.

An analysis of variance was performed on the NaCl, $\log_{10}$ nitrite and odour values. The data were analysed as a split plot design with pH as the main plot factor and time of storage as a sub plot factor. The *t*-test was used for all tests between individual means. When the term 'significant' is used in the text it denotes significance at the 5% level.

Results and conclusions

The pH measurements of the eight sides 24 hr post mortem and after 8 days are presented in Table 1. The mean salt (NaCl) in the aqueous phase (% w/w) of both types of bacon ranged from 6·77 to 7·02 (low pH_u) and from 6·18 to 6·84 (high pH_u). Neither pH nor time had a significant effect on the level of salt (Table 2). At 0 hr the differences in nitrite content between low and high pH_u bacon were not significant. However, at all other sampling times there were significant differences.

TABLE 1. pH measurements of eight sides of pork before and after curing and maturation

Time	Side number							
	Low pH_u				High pH_u			
	1	2	3	4	5	6	7	8
24 hr post mortem	5·75	5·70	5·60	5·70	6·60	6·45	6·45	6·80
8 days post mortem	5·75	5·60	5·55	5·60	6·45	6·00	6·10	6·45

TABLE 2. Mean % (w/w) NaCl and $\log_{10}$ nitrite (μg/g) content in low and high pH_u bacon

		Storage time (days) and temperature (°C) of incubation			
		0	7/0	14/10	21/10
% NaCl	Low pH	6·768	7·128	6·843	7·018
	High pH	6·183	6·863	6·003	6·845
		s.e. of difference between two means	{ pH constant = 0·535 otherwise = 0·848		df = 18 df = 8
$\log_{10}$ nitrite	Low pH	2·034	1·973	2·201	2·463
	High pH	2·133	2·315	2·465	2·681

s.e. of difference between two means. (i) pH constant = 0·079, df = 18; (ii) otherwise = 0·095, df = 9.

TABLE 3. Mean odour scores for pH and time treatments

	Storage time (days)				
	0	7	14	21	Mean (*A*)
Low pH	3·55	3·40	3·075	3·325	3·3375
High pH	3·575	3·30	2·80	2·925	3·1625
Mean (*B*)	3·5625	3·35	2·9625	3·125	

s.e. of difference between means (*A*) = 0·1005, df = 6.
s.e. of difference between means (*B*) = 0·1346, df = 18.
s.e. of difference between means within body of table. (i) pH constant = 0·1904, df = 18; (ii) otherwise = 0·1931, df = 12.

TABLE 4. Comparison of $\log_{10}$ bacterial counts on three media of bacon of low (< 6·0) and high (> 6·0) pH_u

Medium	Low pH	High pH	s.e. of mean	*F* value
PCA + 4% NaCl	3·031	3·422	0·116	5·70
PCA + 10% NaCl	2·397	2·75	0·099	3·96
MRS	2·258	2·525	0·246	0·59

Degrees of freedom = 6.

High pH_u bacon gave a significantly lower odour score than low pH_u bacon. There was no significant pH × time interaction on odour score (Table 3). Still as time increased the differences in odour score between low and high pH_u increased from nearly zero to about 0·4. This trend might have reflected a real time × pH interaction but more replication would be necessary to detect it as significant. At no time were there significant differences (5% level) between high and low pH_u bacon for the bacterial counts on three media (Table 4). However, at the 10% level differences did exist on the two salt media (4 PCA and 10 PCA).

The findings reported here are based only on a small number of replicates at each pH level. Nevertheless, certain trends are evident. A further experiment, based on a greater number of replications, is currently being carried out to determine whether a real effect of pH exists on bacteriological, chemical and organoleptic analyses of vacuum packed bacon.

Acknowledgments

Thanks are due to Mr S. N. Reid, F.I.M.L.T. and Miss C. Murphy for technical assistance. The co-operation of the management of the bacon factory is also gratefully acknowledged. To Mr J. F. Connolly, my thanks for the statistical analysis of the results.

References

Bate-Smith, E.C. (1938) *Ann. Rep. Fd Invest. Bd*, p. 22. H.M.S.O., London.
Callow, E.H. (1936) *Rep. Fd Invest. Bd*, p. 75. London.
Dempster, J.F. (1972) *J. Fd Tech.* **7,** 271.
Follet, M.J. & Ratcliffe, P.W. (1963) *J. Sci. Fd Agric.* **14,** 138.
Gibbons, N.E. & Rose, D. (1950) *Can. J. Res.* F **28,** 438.
Ingram, M. (1948) *Ann. Inst. Pasteur, Lille*, **75,** 139.
Kitchell, A.G. & Ingram, M. (1965) *Proc. 1st Int. Congr. Fd Sci. Tech.* Gordon & Breech, London.
Rose, D. & Peterson, R. (1951) *Can. J. Tech.* **29,** 421.

(*Received* 9 *November* 1973)

J. Fd Technol. (1974) **9**, 259–262.

Book Reviews

Living Nutrition. By F. J. STARE and MARGARET MCWILLIAMS.
New York, London, Sydney, Toronto: John Wiley & Sons, Inc., 1973. Pp. vii + 467. £5.10.

Stare and McWilliams wrote *Living Nutrition* to 'stimulate enthusiasm for nutrition among students who will become tomorrow's nutrition educators, citizens and parents'. This admirable book fulfils this intention. It is refreshing to find the subject treated from both the sociophysiological and physiological viewpoints. Over 200 pages are devoted to nutrition in underdeveloped countries and in affluent societies, to health foods and to the feeding of all age groups from the unborn child to the adult. The influences of cultural and religious practices on inadequate diet in other countries is also covered and the authors go into considerable detail on the impact of sociological changes on our daily food. Numerous tables summarize the daily nutritional requirements for people of all ages. As the authors are American, much of the sociophysiological work is based upon investigations carried out in the States, but this American bias should not deter non-American readers. It is a pity that the use of novel proteins has not received more attention.

The second half of the book is a sound presentation of the role of carbohydrates, fats, proteins, minerals, water and vitamins in our diet. Daily requirements, metabolic pathways and deficiencies of these substances receive consideration, and here again very adequate tables, diagrams and lists of references add to the value of the book. In the Appendix an extensive list of the nutritional values of various foods is given. Where known, figures for preserved and cooked foods are included. The values are generally expressed as the amount of nutrient found in one cup or one helping of the product.

The attractiveness of this very readable book is enhanced by many photographs and the use of coloured print. Throughout the work adequate charts substantiate the text and numerous references pave the way for further reading. The book should be a useful background work for those studying Food Sciences and Home Economics, and it will also be of value as a reference book in libraries, schools and colleges.

M. A. HUDSON

Toxicants Occurring Naturally in Foods. COMMITTEE ON FOOD PROTECTION, FOOD AND NUTRITION BOARD, NATIONAL RESEARCH COUNCIL.
Washington, D.C.: National Academy of Sciences, 1973. 2nd edution. Pp vii + 624. $10.50.

At first sight this book might appear to be written for the purpose of warning the reader to 'stop eating—it might damage your health', but any such impression is disclaimed

by Frank M. Strong's introduction which, space allowing, would merit quoting in its entirety.

Although it is only 7 years since the appearance of the first edition this is an entirely different book. Not only is it twice as long, it is comprehensive in its coverage of the kinds of substances occurring in plants and animals that actually do, or conceivably might, produce toxic effects. Many of these are admittedly unrealistic. Carp viscera, for instance, contain an antithiamine factor, but while silver foxes fed raw carp have suffered on this account, this is not likely to be a risk to the human population.

The book contains information of a kind not usually accessible to the food technologist, much of which makes interesting reading. There are twenty-six chapters, and judging from those with which the reviewer is most familiar, they are exceedingly competent. The price is such that it can be recommended to the food scientist without hesitation as a soft option, either as an authoritative work for reference purposes, or as entertaining reading.

E. C. BATE-SMITH

Nutritive Value of Triticale Protein (and the proteins of wheat and rye). By JOSEPH H. HULSE and EVANGELINE M. LAING.
Ottawa: International Development Research Centre, 1974. Pp. 183. Can.$7.50.

The shortened title on the cover of this book—*Nutritive Value of Triticale Protein*—does not do justice to the aims of the authors. They have not only surveyed the literature concerning the biological value of the protein of triticale but have related this to the comparable characteristics in its wheat and rye parents.

The literature is presented under the following chapter headings. 'Methodology for evaluation of plant proteins for human use' (contributed by J. M. McLaughlan and J. A. Campbell); 'Genetic, varietal, environmental and agronomic factors'; 'Nutritional inhibitors and toxic factors'; 'Processing and supplementation with other protein sources'.

The survey is extensive but confusion has been avoided by division into well-chosen section headings. A useful feature is a summary of the important points from each of the chapters.

The call for internationally standardized methodology and terminology in the evaluation of biological quality and amino acid analysis is welcomed by the reviewer.

The lack of reliable tryptophan analyses could have had more discussion especially as two references were given to it being the first limiting amino acid in rye, although it is clear from other references that lysine has been generally accepted as first limiting in this cereal. One notable omission in the 'Methodology' chapter is the Silcock method for available lysine.

In summary the authors have presented an extensive survey which, although mainly

non-critical, should prove of value to plant scientists, nutritionists, food scientists and technologists, particularly those who may be concerned with triticale.

D. G. REDMAN

Nutrition and Technology of Foods for Growing Humans. Ed. by J. C. SOMOGYI. (Bibl. 'Nutr. Diet'. No. 18.)
Basel: S. Karger, 1973. Pp. viii + 288. £11.65.

This book contains the proceedings of a symposium held from 13 to 15 October 1971 jointly by the International Union of Nutritional Sciences, the International Union of Food Science and Technology and the Institute for Nutrition Research, Rüschlikon—Zürich.

Three of the papers, 'Protein and energy requirements of children' (J. C. Waterlow), 'Recommended intakes of vitamins for normal growth and development', including discussion on the terms optimum and minimum requirements and recommended allowances and intakes (E. Kodicek) and 'Mineral and trace element requirements for normal growth and development' (D. P. Cuthbertson) are concise reviews of the subjects indicated by their titles. Other papers dealing with the development of children discuss the relation of nutrition to physical and mental development, with particular emphasis on the apparent relationship between severe malnutrition early in life and psychological retardation (M. Winick), overfeeding and obesity in infants and children leading to the conclusion that prevention of obesity is better than cure (J. T. Dwyer and J. Mayer), the adverse effects of infection on nutritional status (N. S. Scrimshaw) and, in summary only, inborn errors of metabolism (A. Prader).

Foods rather than children are dealt with in other papers, including a comprehensive and interesting survey of the technology of protein synthesis and protein-rich foods (J. Mauron), which comes to the increasingly well known conclusion that '. . . none of the protein-rich foods developed so far have reached the target population to any considerable extent. The reason is . . . purely economic and very simple: the most vulnerable groups . . . at subsistence level in agriculture and in the urban slums have no extra income above mere subsistence and cannot raise an effective demand for more calories and more protein. Only radical, political and economic changes . . . can be successful in alleviating malnutrition in the most vulnerable groups . . . , and not the development of more and fancier protein sources'. Another paper deals with the loss, and prevention of loss, of vitamins in industrially handled foods (A. E. Bender) and comes, as such reviews usually do, to the reassuring conclusion that advances in food technology, which usually require increased control and more gentle methods of processing, lead to reduced losses of vitamins, but that a watch must be kept.

The problems of designing foods for special dietary purposes are discussed in a number of papers: dietetic foods (E. J. Bigwood), low calorie foods (A. Krieger), foods for the treatment of inborn errors of amino acid and carbohydrate metabolism (H.

Bickel, H. Schmidt and L. Schürrle) and supplementary foods for infants and young children, in which is made a strong plea for adequate nutrition education of all those responsible for feeding young children (B. Vahlquist), problems relating to sodium chloride (W. Droese, H. Stolley, C. Schlage and B. Wortberg) and enrichment and fortification of foods (W. Kübler). This set of papers is rounded off with a review of the nutritional value of commercially produced foods for infants (H. P. Sarett), which comes to the modest conclusion that, though many steps have been taken towards designing and testing 'infant formulas', we must 'not assume that we know all the answers at this time', and which ends with a somewhat indirect reminder that the aim is 'to help start the infant on the road to optimal health and well-being throughout life'. The book ends with two papers in which school feeding programmes in Japan (N. Shimazono) and some European countries (R. Tarjan) are evaluated.

Altogether, this is an interesting and useful collection of papers, most of which conclude with comprehensive lists of references from the world literature.

DOROTHY HOLLINGSWORTH

Books received

Food. Readings from the 'Scientific American'. Ed. by J. E. HOFF and JULES JANICK.
San Francisco: W. H. Freeman & Co, 1973. Pp. 268. £5.30 clothbound, £2.40 paperback.

A collection of articles from the *Scientific American* giving an elementary introduction to the fields of food, food production and nutrition.

Wine and Must Analysis. By M. A. AMERINE and C. S. OUGH.
New York: John Wiley & Sons, 1974, Pp. viii + 121. £5.30.

A collection of methods of analysis of wine and must, mainly recommended by AOAC and other official sources. Contains an extensive bibliography.

Induced Mutations in Vegetatively Propagated Plants. INTERNATIONAL ATOMIC ENERGY AGENCY.
Vienna: IAEA, 1973. Pp. 222. £2.40.

Proceedings of a panel meeting organized by the Joint FAO/IAEA Division of Atomic Energy in Food and Agriculture. Available from HMSO.

JOURNAL OF FOOD TECHNOLOGY: NOTICE TO CONTRIBUTORS

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Arrangement. Papers should normally be divided into: (a) Summary, brief, self-contained and embodying the main conclusions; (b) Introduction; (c) Materials and methods; (d) Results, as concise as possible (both tables and figures illustrating the same data will rarely be permitted); (e) Discussion and conclusions; (f) Acknowledgments; (g) References.

References. Only papers closely related to the author's work should be included; exhaustive lists should be avoided. References should be made by giving the author's surname, with the year of publication in parentheses. When reference is made to a work by three authors all names should be given when cited for the first time, and thereafter only the first name, adding *et al.*, e.g. Smith *et al.* (1958). The '*et al.*' form should always be used for works by four or more authors. If several papers by the same author and from the same year are cited, a, b, c, etc., should be put after the year of publication, e.g. Smith *et al.* (1958a). All references should be brought together at the end of the paper in alphabetical order. References to articles and papers should mention (a) name(s) of the author(s); (b) year of publication in parentheses; (c) title of journal, underlined, abbreviated according to the *World List of Scientific Publications*, 4th edn and supplements; (d) volume number; number of first page of article. References to books and monographs should include (a) name(s) and initials of author(s) or editor(s); year of publication in parentheses; (b) title, underlined; (c) edition; (d) page referred to; (e) publisher; (f) place.

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'.

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Journal of Food Technology
Volume 9 Number 2 June 1974

Contents

Printed by Adlard and Son Ltd, Bartholomew Press, Dorking