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Analysis of pigments in intact beef samples

A simple method for the determination of oxymyoglobin and ferric myoglobin in intact beef samples using reflectance spectrophotometry

A. H. A. VAN DEN OORD AND J. J. WESDORP

Summary

Some basic aspects of the determination of the relative proportions of myoglobin pigments in meat by reflectance spectrophotometry are briefly reviewed. The potential of the Unicam SP 890 diffuse reflectance accessory for the quantitative determination of myoglobin forms in beef is assessed. The construction of the Unicam apparatus differs from most other reflectance units in that it has no integration sphere. The K/S values obtained with the Unicam apparatus and the requirements to be met by the samples correlate well with those for other spectrophotometric apparatus, except that the thickness of meat samples does not seem to affect the measured reflectance.

A simple and rapid method is described for the determination by reflectance spectrophotometry of the proportion of oxymyoglobin and ferric myoglobin in meat surfaces. If only negligible amounts of reduced myoglobin are present in the sample, then the absorbance at 580 nm minus the absorbance at 630 nm is linearly related to the percentage of oxymyoglobin, or ferric myoglobin. The theoretical basis for this linear relationship is outlined. A drawback of the method is that the difference in absorbance ($A_{580} - A_{632}$) is dependent on the myoglobin concentration in the meat sample. Nevertheless, the method is very convenient for comparing the pigment composition of meat samples with similar myoglobin contents and for following the course of pigment changes in a single meat sample.

Introduction

The colour of meat is determined by the concentration of the pigment myoglobin and the relative proportions of the three forms in which myoglobin can occur: red oxymyoglobin (oxyMb), purple myoglobin (Mb) and brown ferric myoglobin (Mb^+).

Meat colour can be expressed objectively in international CIE units or in terms of

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the three dimensions of colour, hue, saturation, and brightness, which are generally assessed with tristimulus colorimeters. Another possiblity is to describe the colour of meat in terms of the relative concentrations of the pigment forms. This is, of course, not an objective measure of the subjective colour impression, but rather a specification of the state of the myoglobin pigments in the meat. This can be achieved by either of two spectrophotometric methods. The first involves the extraction of the pigments from the meat and their subsequent analysis by absorption measurements (Broumand, Ball & Stier, 1958). In the second method the unextracted pigments are assessed by reflectance spectrophotometry (Snyder, 1965; Zimmerman & Snyder, 1969). The principles of the latter method are briefly summarized in this paper.

A simple method is described for the determination by reflectance measurements of the proportion of oxyMb and Mb^+–the two main pigment forms in bloomed raw meat. The method is based on accepted principles of pigment analysis by absorption and reflectance spectrophotometry. A Unicam SP 800 spectrophotometer was used for reflectance measurements. An evaluation of this instrument, the reflectance unit of which differs in construction from the units in most other spectrophotometers, is presented.

Aspects of the reflectance spectrophotometric analysis of meat pigments

Reflectance is a surface property, the measurement of which may be justifiably correlated with the subjective evaluation of colour. Furthermore, the measurement of reflectance involves a non-destructive procedure which is simple and rapid: meat samples can be re-used so that pigment changes can be measured at intervals with the same sample, and uncontrollable sample differences eliminated. For reflectance measurements, however, meat samples have to fulfil certain requirements. Meat slices, for instance, must be sufficiently thick. According to Elliott (1967) the minimum thickness for reflectance measurements below 575 nm should be 8 mm for pale muscle, and 12 mm for dark muscle. Above 575 nm, sample thickness should exceed 12 mm. Snyder & Armstrong (1967), in model experiments with myoglobins in a suspension of 5% non-fat dry milk, found the minimum tolerable sample thickness to be about 17 mm.

A second requirement for the meat sample concerns the muscle fibre orientation in the surface to be analysed. This should be the same in all samples in order to enable comparison of the reflectances of different samples. Samples with the fibres parallel to the surface give a higher reflectance than samples sliced perpendicularly to the fibres. The differences between the reflectances of the two types of samples decrease with decreasing wavelength (Elliott, 1967). Futhermore, attention should be paid to the fat content of the sample. Reflectance increases with increasing content of intramuscular fat, the effect being independent of wavelength (Elliott, 1967).

The translation of the reflectance spectra, or reflectances, into terms of relative pigment concentrations and, ultimately, the correlation of the results with visual

assessment of colour, is the most serious problem. Some authors considered the absorbance scale to be linear for reflectance measurements, just as is the case for absorption measurements in solution (Erdman & Watts, 1957; Naughton, Frodyma & Zeltlin, 1957). Snyder & Armstrong (1967), however, showed that this method gave erroneous results.

Meat may be considered opaque. Light falling on an opaque sample is partly absorbed, and partly scattered. The proportion of the light absorbed by the pigments to that scattered by the matrix decreases with increasing reflectivity. Reflectivity is the reflectance of a 'thick' sample, i.e. a sample for which no change in reflectance will occur upon further increasing its thickness. Reflectivity, $R\infty$, depends on that ratio of the absorption coefficient K and the scattering coefficient S in the following way:

$$\frac{K}{S} = \frac{(1 - R\infty)^2}{2R\infty}$$

The basic ideas that lead to this formula are described in the book by Judd & Wyszecki (1967). (The value of the ratio K/S as a function of reflectivity is given in Table D of the same book).

Reflectance spectra may therefore not be treated in the same way as absorption spectra, as done by some authors (Erdman & Watts, 1957; Naughton *et al.*, 1957). Even the application of the graphs of Broumand *et al.* (1958), using ratios of K/S values instead of ratios of absorbances, as Dean & Ball (1960) did in experiments with sliced beef, is not justified. The scattering coefficient, S, is dependent on wavelength, its value varying with the reciprocal of the fourth power of the wavelength. It is thus highly improbable that the ratio of K/S values at two wavelengths is identical to the ratio of the absorbances at the same wavelengths. We in fact confirmed the inequality of the two ratios during subsequent experiments.

A slightly modified approach was applied by Stewart, Zipser & Watts (1965) in reflectance measurements with minced meat. They used K/S ratios at two wavelengths, one of which, however, was 525 nm, an isosbestic point for all three myoglobin pigments. Reflectance at this wavelength is therefore independent of the relative proportions of the three pigments. They assumed that a plot of the ratio of two K/S values, one value taken at 525 nm and the other at an isosbestic point for two myoglobin pigments, against the relative concentration of the pigments would be a straight line. Thus, they predicted a linear relation between the percentage of Mb + oxyMb and the ratio of K/S values at 525 and 572 nm (the latter being an isosbestic point for Mb and oxyMb). Snyder & Armstrong (1967) proved this assumption to be valid by model reflectance experiments with known concentrations of oxyMb and Mb^+ in a 5% non-fat dry milk suspension. The same authors also found a linear relation between the total myoglobin concentration in their model suspensions and the K/S value at 505 nm, provided all the pigment was in the Mb^+ form.

Comparison of reflectance spectra from different samples is hampered by slight differences between the fat and moisture contents and the surface structures of the samples. These differences cause vertical displacement of the whole reflectance spectrum. Snyder (1965) therefore recommended that spectra of different samples be compared after adjustment of the reflectance data to an absorbance of 1·0 at 525 nm. This method has no theoretical basis and we did not use it.

Experimental

Reflectance was measured with a Unicam SP 800B spectrophotometer equipped with a Unicam SP 890 diffuse reflectance accessory.

Topside beef (*Biceps femoris*), 4 days post mortem, was used throughout the experiments. Slices with the myoglobin completely converted to oxyMb were obtained by blooming in an oxygen atmosphere at about 5°C for 1 hr. When necessary, the myoglobin pigments were converted to the ferric form (Mb^+) by spraying a 5% solution of potassium ferricyanide onto the meat to a level of 0·1 ml per 100 cm^2 of beef surface three times at intervals of 1 hr, the meat being kept at 5°C.

Minced beef with varying fat content was prepared by coarsely mincing topside beef in a Bauknecht Fleischwolf Type W6, mixing the mince with an appropriate amount of pre-comminuted beef fat, and then finely mincing the mixture. Blooming and oxidation of the pigment in the minces was performed as described for the slices.

Beef slices were retail-packed for a storage test by placing them on white polystyrene trays (Aphro-trays 'A-60', Delta Plastic, Ritterhude, Germany) which were subsequently wrapped in a film of high permeability to water and oxygen (Resinite RMF-61, The Borden Chemical Company (U.K.) Ltd.).

Percentages of oxyMb in meat extracts were determined as follows: Meat (approximately 40 g) was cut into small pieces and then mixed for 30 sec in a Waring blendor with two parts of ice water. The slurry was centrifuged ($\sim$ 16,000 × g) at 2°C for 10 min. The supernatant was filtered to remove fat particles and the absorption spectrum recorded between 400 and 700 nm with a Unicam SP 800 spectrophotometer using cells of 1-cm pathlength.

Only two forms of myoglobin, viz. oxyMb and Mb^+, are present in the supernatant: any Mb in the meat sample is converted into oxyMb during the extraction. The percentage of oxyMb can therefore be calculated from the quotient of the absorbances at two wavelengths, namely 580 and 525 nm, the former being an absorption maximum for oxyMb and the latter an isosbestic point for oxyMb and Mb^+. The value (p) of the quotient A_{580}/A_{525} is thus linearly correlated with the percentage of oxyMb, and is independent of the concentration of myoglobin pigment. For proportions of oxyMb of 0 and 100%, p has the values of 0·38 and 1·99 respectively. The percentage of oxyMb

corresponding to a given value of the quotient A_{580}/A_{525} may therefore be calculated as follows:

$$\% \text{ oxyMb} = \frac{p - 0{\cdot}38}{1{\cdot}99 - 0{\cdot}38} \times 100.$$

Comparison between results with the Unicam diffuse reflectance accessory and those with integration-sphere reflectance units

Reported reflectance spectra of meat have all been measured with spectrophotometers having integration-sphere reflectance units. These collect all the light that is reflected from the sample. The Unicam SP 890 diffuse reflectance accessory (UDRA) for the SP 800 spectrophotometer is of a different construction. Light which is diffusely reflected by the sample is collected by an ellipsoidal mirror over a range of angles of reflection between 35° and 55° approximately. The ellipsoidal mirror collects only about 30% of the total diffusely reflected light.

To evaluate the UDRA for reflectance measurements of meat, experiments were designed to investigate the influence of sample thickness, to determine the effect of fat content, and to compare ratios of K/S values at two wavelengths with those reported in the literature.

Influence of sample thickness

The influence of thickness was investigated on beef samples in which the myoglobin pigments had been completely converted to either oxyMb or Mb^+. The thickness of the meat slices was varied betweed 5 and 25 mm. The spectrum of each sample was recorded in turn against a white and a black background (Elliott, 1967).

With the pigments completely converted to Mb^+, we observed no difference between the spectra of a sample against a white background and against a black background in the range of sample thicknesses investigated. The reflectance spectra of the oxyMb samples, however, differed slightly above about 600 nm, although the differences were independent of sample thickness and were thus not a result of the background. The small differences which we observed we attribute to small variations in the oxyMb content or small deviations in the position of the samples in the sample holder of the UDRA.

These results are in contrast to those obtained with integration spheres by Elliott (1967) on meat samples, and by Snyder & Armstrong (1967) on Mb^+ in non-fat dry milk suspensions. The different construction of the UDRA is probably responsible for this discrepancy. For practical reasons we used a sample thickness of 10–12 mm in further investigations.

Comparison of ratios of K/S values with those reported in the literature

Slices of beef with their pigments converted into either oxyMb or Mb^+ were used in this experiment. In Table 1 our results are summarized and compared with those

TABLE 1. Comparison of ratios of measured K/S values with those reported in the literature. Our measurements were made with a Unicam SP 890 Diffuse Reflectance Accessory, whereas values from the literature are derived from measurements with integration-sphere reflectance units.

Myoglobin form in meat sample	$\frac{\lambda_1\ (nm)}{\lambda_2\ (nm)}$	Ratio of K/S values at two wavelentghs (λ_1, λ_2)									Ratio of adsorbances of myoglobin solutions at two wavelengths
		Stewart *et al.* (1965)			Snyder & Armstrong (1967)			This study			Broumand *et al.* (1958)
		No. of samples	Average $\frac{K_1}{S_1}/\frac{K_2}{S_2}$	Standard deviation	No. of samples	Average $\frac{K_1}{S_1}/\frac{K_2}{S_2}$	Standard deviation	No. of samples	Average $\frac{K_1}{S_1}/\frac{K_2}{S_2}$	Standard deviation	Average $\frac{A_1}{A_2}$
Ferric myoglobin (Mb^+)	$\frac{571}{525}$	19	0·56	0·04	35	0·608	0·038	4	0·55	0·03	
	$\frac{474}{525}$				35	0·962	0·020	4	1·06	0·02	
	$\frac{507}{573}$							4	2·13	0·13	3·30
	$\frac{473}{597}$							4	2·26	0·13	2·55
Oxymyoglobin (oxyMb)	$\frac{571}{525}$	20*	1·40	0·16	35	1.327	0·071	12	1·49	0·04	
	$\frac{474}{525}$				35	0·957	0·038	12	0·91	0·01	
	$\frac{507}{573}$							12	0·40	0·02	0·50
	$\frac{473}{597}$							12	3·51	0·23	2·55

*These samples were not bloomed and contained predominantly reduced myoglobin. 571 nm is an isosbestic point for Mb and oxyMb; these two pigment forms cannot be distinguished at this wavelength by measurement of reflection.

of Stewart *et al.* (1965), Snyder & Armstrong (1967), as well as the absorbance ratios of Broumand *et al.* (1958). It can be seen from Table 1 that ratios of the K/S values at two given wavelengths differ from the ratio of absorbances at the same wavelengths. This substantiates the conclusion that the method of pigment analysis of Dean & Ball (1960) will give erroneous results, as already suggested.

Our ratios of K/S values are in good agreement with those of Stewart *et al.* (1965) and of Snyder & Armstrong (1967), although it should be remembered that the latter authors worked with model suspensions. Ratios of K/S values obtained from reflectance measurements with the Unicam apparatus are, therefore, similar to those obtained from measurements with spectrophotometers equipped with integration spheres.

Effect of fat content

Minced beef containing 0, 15 and 30% added beef fat was used in this experiment. The myoglobin pigments were converted to oxyMb or Mb$^+$ beforehand. The reflectance spectra for oxyMb samples are shown in Fig. 1. It can be seen that a higher fat content

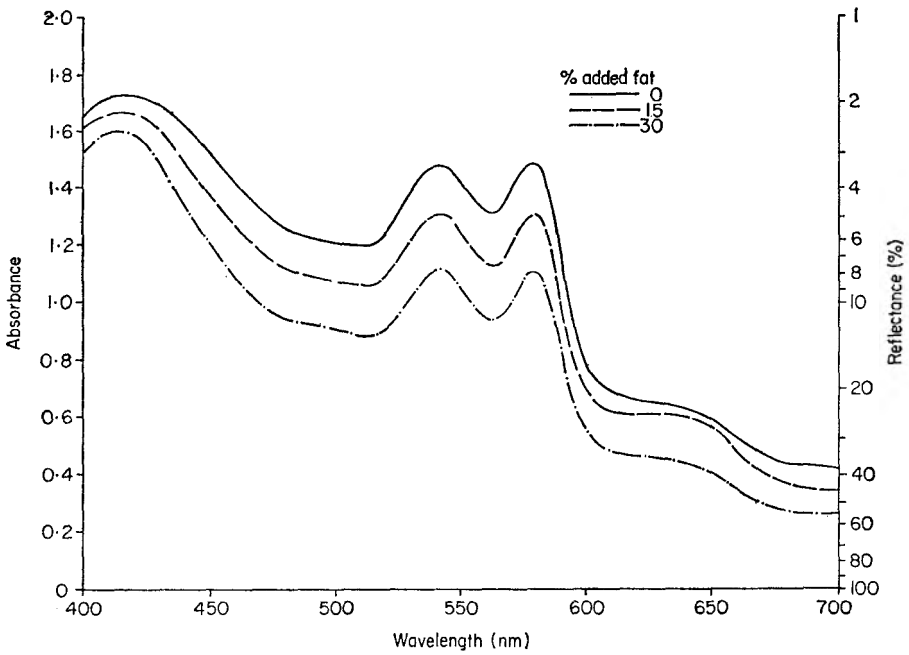

FIG. 1. Influence of added fat on the reflectance spectra of minced beef. Spectra were recorded 1 hr after mincing: pigment predominantly in the oxyMb form.

results in higher reflectances, although the shape of the reflectance curve remains very much the same between 400 and 700 nm, being subject only to a vertical displacement. Conversion to oxyMb was not complete, as is shown by the deviations around 630 nm,

a characteristic reflectance peak for Mb^+. Similar results have been obtained by Elliott (1967) in experiments with meat slices containing cores of fat of varying diameter. The effect of fat content on ratios of K/S values is summarized in Table 2. Only results for samples completely converted to Mb^+ are given, since it proved impossible to convert the pigments of minced meat to 100% oxyMb.

TABLE 2. Effect of fat content on the ratios of K/S values. Reflectance of minced beef with varying fat content and with all pigments converted to Mb^+.

$\frac{\lambda_1\ (nm)}{\lambda_2\ (nm)}$	No. of samples	Ratio of K/S values at two wavelengths (λ_1, λ_2)					
		0% fat		15% fat		30% fat	
		Average $\frac{K_1}{S_1}/\frac{K_2}{S_2}$	Standard deviation	Average $\frac{K_1}{S_1}/\frac{K_2}{S_2}$	Standard deviation	Average $\frac{K_1}{S_1}/\frac{K_2}{S_2}$	Standard deviation
$\frac{571}{525}$	4	0·56	0·01	0·59	0·01	0·60	0·01
$\frac{474}{525}$	4	0·98	0·01	1·03	0·02	1·08	0·01

The ratios of K/S values for both sets of wavelengths increase slightly with increasing fat content of the sample. The difference between the ratios for minced beef with markedly different fat contents, however, is small. Thus, for estimating relative pigment concentrations in beef with a limited variation in fat content, no large error will be introduced if the effect is neglected.

Rapid determination of the relative proportions of oxyMb and Mb^+ from reflectance spectra of beef containing negligible amounts of Mb

Analysis of the pigment composition of a meat sample by the K/S ratio method described above is rather laborious and we therefore looked for a simpler method of interpreting reflectance spectra. Scrutiny of a great number of reflectance spectra showed that most packed meat samples contain a negligible amount of Mb, if any. The colour of retail packed raw meat is determined predominantly by the pigments oxyMb and Mb^+. In most cases, therefore, we are interested in this two-component pigment system and the analysis can be simplified accordingly.

Fig. 2 shows typical reflectance spectra for fully-bloomed and fully-oxidized meat samples, i.e. with all the pigment in the oxyMb form and Mb^+ form respectively. Reflectance at the isosbestic point, 525 nm, has been taken as 10%. Characteristic peaks are at 580 nm for oxyMb and at 630 for Mb^+. These peaks also occur in the absorption spectra of the pigments. It was found that changes in meat colour correspond closely to changes in reflectance at these two wavelengths. We therefore checked

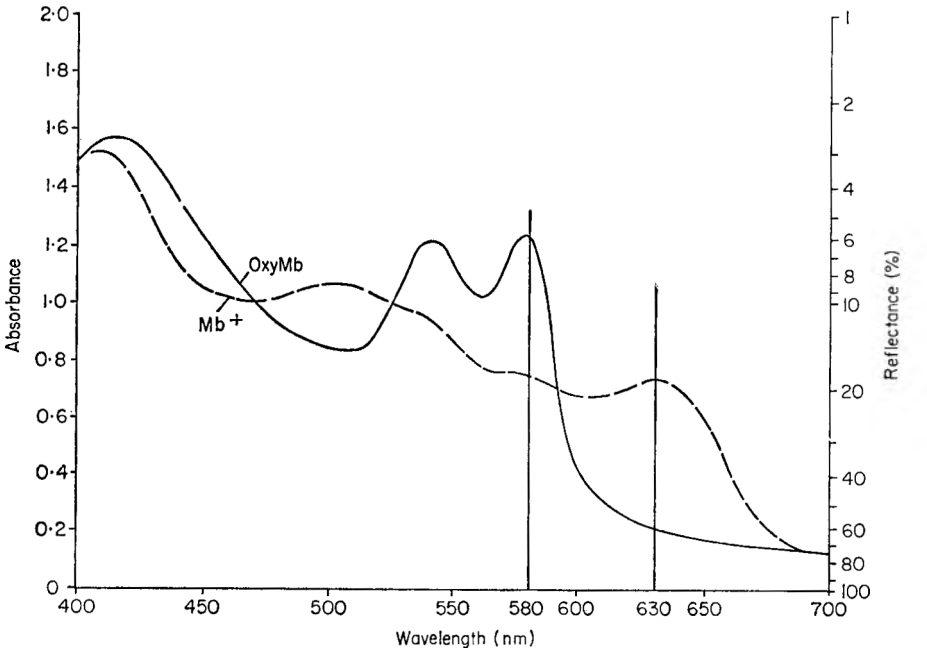

FIG. 2. Reflectance spectra of beef slices with pigments completely converted into oxyMb or Mb^+. Reflectance at 525 nm has been equated to 10%.

whether the difference between the absorbances at 580 and 630 nm could be used as a measure for the percentages of oxyMb and Mb^+ in a meat slice. (The Unicam SP 800 has a linear absorbance scale which was used for this analysis.) As can be seen in Fig. 2, this difference is a maximum for bloomed meat ($A_{580} > > A_{630}$ for oxyMb) and approaches zero for meat with fully-oxidized pigments ($A_{580} \cong A_{630}$ for Mb^+).

The procedure was as follows. The absorbance at 580 nm for a typical slice of beef, in which the pigments are completely converted to oxyMb, is 1·245 (Fig. 2). The corresponding K/S value is 7·81. For the same slice, with all pigments converted to Mb^+, the absorbance is 0·755 and the corresponding K/S value is 1·929. Absorbance and K/S values at 630 nm can be derived similarly.

It now remains to be established how intermediate values of absorbance can be related to the relative concentrations of the two pigment forms. According to Snyder & Armstrong (1967), there is, for any wavelength, a linear relation between K/S values and the relative composition of pigments in a two-component pigment system. We can therefore draw two straight lines, one for 580 and one for 630 nm, relating K/S value and pigment composition, as shown in Fig. 3(a). The corresponding absorbance lines can be deduced from these lines by first converting K/S values into reflectances (Judd & Wyszecki, 1967, Table D) and then the reflectances into absorbances, absorbance being

the negative logarithm of reflectance. This results in the two dotted curves shown in Fig. 3 (a). If the difference between the absorbances, $A_{580} - A_{630}$, as read from Fig. 3 (a), is now plotted against percentage oxyMb (for a system made up of the two pigments oxyMb and Mb^+), the result is a straight line, or nearly so (see Fig. 3(b). This means that the $(A_{580} - A_{630})$ value is a convenient measure of the relative

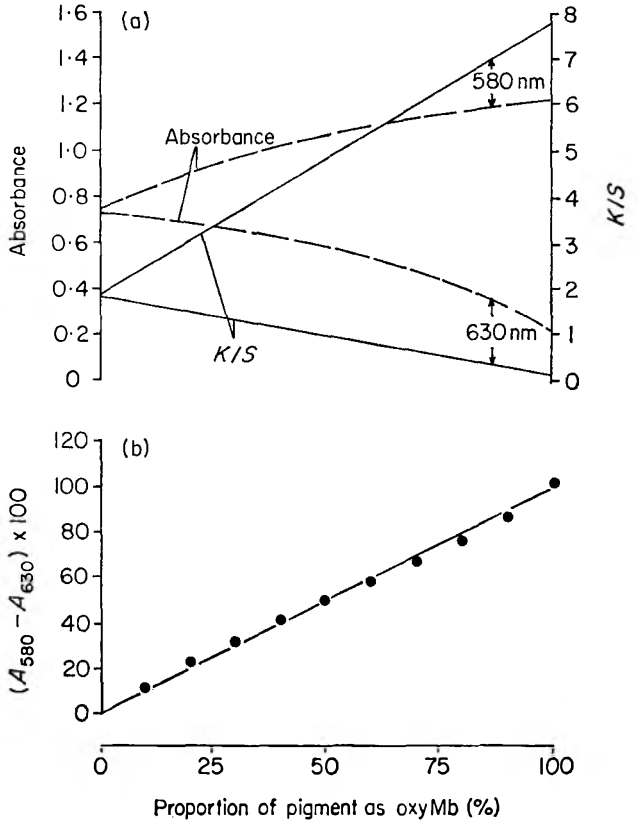

FIG. 3(a). Correlation of *K/S* value and adsorbance at 580 and 630 nm with the relative concentrations of oxyMb and Mb^+.

FIG. 3(b) Correlation between the relative concentrations of oxyMb and Mb^+ and the difference in adsorbance at 580 and 630 nm. $(A_{580} - A_{630})$ values are obtained from the dotted plots in Fig. 3(a).

proportions of oxyMb and Mb^+. The difference $A_{580} - A_{630}$, however, is dependent on the myoglobin content of the meat sample. This does not affect the applicability of the method to following changes in the relative proportions of Mb^+ and oxyMb, but for the determination of absolute pigment contents one must know the $(A_{580} - A_{630})$ value for meat containing all pigment in the oxyMb form. Beef slices can be

bloomed so that they contain almost 100% oxyMb, but this is impossible for minced raw beef, the pigment in which is very rapidly oxidized to Mb^+. In this latter case the percentage of oxyMb in the fully-bloomed sample can be calculated from the K/S ratio. The $(A_{580} - A_{630})$ value corresponding to 100% oxyMb in the sample can be calculated as follows:

The actual percentage of oxyMb is determined by measuring the reflectances of the meat sample at 525 and 572 nm: the reflectances are converted into the corresponding K/S values. The ratio, r, between the two K/S values is linearly related to the percentage of oxyMb (neglecting small amounts of Mb). For 0 and 100% oxyMb the values of r are 0·55 and 1·49, respectively. The percentage of oxyMb corresponding to a given value of r may thus be calculated as follows:

$$\% \text{ oxyMb } (s) = \frac{r - 0{\cdot}55}{1{\cdot}49 - 0{\cdot}55} \times 100.$$

Consequently, if the value of $(A_{580} - A_{630})$ for the same meat sample is q, the maximum value of $(A_{580} - A_{630})$, corresponding to 100% oxyMb, is $\frac{q}{s} \times 100$. Maximum $(A_{580} - A_{630})$ values were calculated from the results obtained with a number of types of beef:

Type of beef	Calculated maximum $(A_{580} - A_{630}) \times 100$	Standard deviation
Biceps femoris, A	115	6
Biceps femoris, B	114	5
Psoas major	109	4
Longhead Triceps	111	2

The maximum value is very much the same for the three types of beef. A value of 112 (the average) can normally be used in most experiments with beef.

It should be borne in mind that colour changes in a single slice of beef, or colour differences between beef samples of similar myoglobin content, can be expressed simply as $(A_{580} - A_{630})$ values. The $(A_{580} - A_{630})$ value is extremely useful for following the discoloration of meat slices in which the reduced form of myoglobin, Mb, is unlikely to be present.

In Fig. 4 the percentages of oxyMb as determined by our reflectance method are compared with those determined by absorption spectrophotometry in the pigment extracts from the same samples. The colour changes in beef slices during storage at various temperatures were followed. The percentages of oxyMb determined via

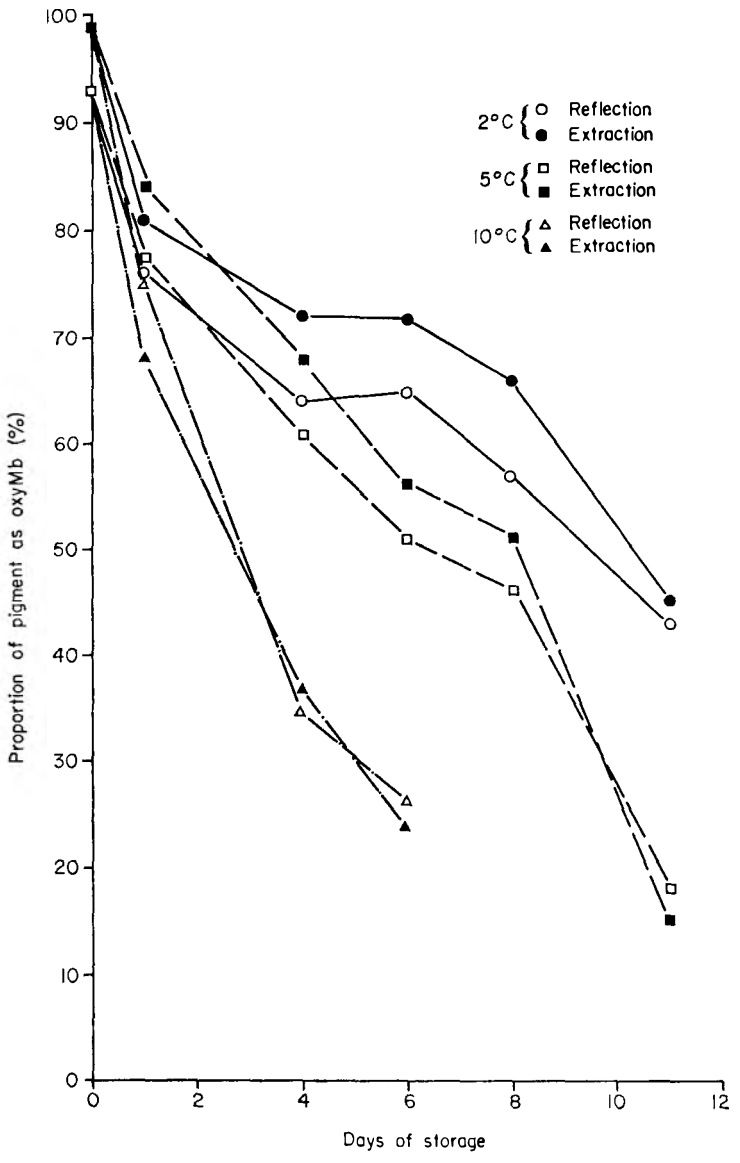

FIG. 4. Comparison of the relative concentrations of oxyMb in chilled retail-packed beef as determined by reflectance measurements and by the extraction procedure. Experiments were with topside of beef: all samples were cut from a single topside which was immersed in boiling water for 30 sec to reduce bacterial contamination. All points in the figure are the average of three determinations, one at each of three locations on each sample.

reflectance measurements are somewhat lower (by a maximum of 8% absolute) than the values determined by the extraction procedure. The reflectance method, however, emphasizes the composition in the surface layer, while the extraction method determines the composition of the pigment in the whole of the beef slice. The surface layer may well contain a lower level of oxyMb than the interior parts. Inside the beef slice some purple Mb may be present; this is determined as oxyMb in the extraction method but to a far lesser extent by reflectance measurements. Purple Mb may play a role in the determination of oxyMb by reflectance up to 2 hr after packing but its role will decrease quickly thereafter.

Standard deviation of the determinations by reflectance spectrophotometry of the percentage of oxyMb in individual beef slices (reflectance spectra recorded at three locations) was 3% relative for high (> 80%) and low (< 40%) levels, while at intermediate values the standard deviation was 5% relative. Much the same was found for the mean values of a series of five slices, i.e. of fifteen determinations.

Although the method does not give a comprehensive indication of the colour of the meat, a good correlation was found between the $(A_{580} - A_{630})$ value and the subjective colour evaluation by a panel. The technique is very suitable for studying artificially induced changes in pigment composition.

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Colour rating and pigment composition of beef

Relation between colour measurement by reflectance spectrophotometry, subjective colour evaluation, and the relative concentration of oxymyoglobin and ferric myoglobin in chilled retail-packed beef

A. H. A. VAN DEN OORD AND J. J. WESDORP

Summary

Reflectance measurements and subjective colour evaluations of retail-packed beef were recorded and compared. Subjective colour evaluation, which was found to be closely linked to the visual assessment of the overall quality of the meat, is largely determined by the percentage of oxymyoglobin (red) relative to ferric myoglobin (brown). The reflectance colour value, which is the difference in reflectances (expressed in absorbance units) at 580 and 630 nm, is linearly related to the percentage of oxymyoglobin and to the subjective colour score. The correlation between objective and subjective colour evaluation is statistically highly significant (correlation coefficient = 0·94).

Reflectance spectrophotometry is thus shown to be a convenient and reliable method for objective colour measurement and for the determination of the pigment composition in the surface zone of intact beef samples.

Introduction

The colour of meat is determined by the concentration of the pigment myoglobin and by the relative proportions of its three forms, oxymyoglobin (oxyMb), myoglobin (Mb), and ferric myoglobin (Mb^+). The concentration of total pigment is a factor inherent to the type of muscle and is more or less invariable for a specific type of meat. Time and temperature of storage, as well as the quality of the meat, largely determine the proportions of the three myoglobin forms. Objective colour evaluations, in terms of acceptability or quality, are thus related to the relative contents of the three pigment forms in meat.

In meat packed in oxygen-permeable films, as for the retail trade, only two pigments, oxyMb and Mb^+, are of importance for the colour and its objective and subjective evaluation. The relative proportions of oxyMb and Mb^+ in the surface of meat samples containing little or no Mb can be determined by reflectance spectrophotometry. The difference in absorbance at 580 and 630 nm ($A_{580} - A_{630}$) is linearly related to the

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percentage of oxyMb in such samples (Van den Oord & Wesdorp, 1970). We suggested, on the basis of these results, that there should be a similar relationship between the $A_{580} - A_{630}$ values and the subjective evaluation of meat colour.

Pfau (1968) and Elliott (1968) used tristimulus colorimetry to determine the colour of pork, and in a subsequent paper Elliott (1969) established a correlation between tristimulus colour values and the subjective evaluation of the colour of pork. Neither of these investigators, however, attempted to establish a correlation between the objective measurement of colour and the pigment composition of the meat, and thus no correlation was established between pigment composition and subjective colour evaluation. As mentioned above, we had good reason to believe that such a relationship should exist. Work described in this paper indeed confirms our hypothesis.

Experimental

Meat

The experiments were begun with beef which was 3 or 4 days post mortem. Topside of beef (*Biceps femoris*) was used in most of the experiments. In one series of experiments topside, *Psoas major*, and *Longhead triceps*—all from the same cow—were included to obtain some comparative results.

Bacterial contamination, with the risk of local surface discoloration of the beef slices, was greatly reduced by dipping the complete muscle in boiling water for 30 sec. The meat was then sliced with a sterile knife. The slices, about 1 cm thick, were placed on white polystyrene trays (Aphro-trays 'A–60', Delta Plastic, Ritterhude, Germany), and the whole was wrapped in a film of high permeability to water and oxygen (Resinite RMF–61, The Borden Chemical Company (U.K.) Ltd.) such that the film was not in contact with the beef surface. The packed slices were placed in a cold store at 2°C for about 2 hr in order to bloom the beef. The first colour evaluations were of the freshly-bloomed samples; reflectance spectra were recorded immediately thereafter. After this initial assessment (day 0), appropriate numbers of samples were placed in stores at 2, 5 and 10°C.

Determination of the percentage of oxyMb by reflectance measurements

Reflectance spectra in the range 400–700 nm were recorded at three locations on each slice of beef using a Unicam SP 800 spectrophotometer equipped with the SP 890 Diffuse Reflectance Accessory. The method for the determination of the relative proportions of oxyMb and Mb^+ (neglecting small amounts of Mb) by reflectance spectrophotometry, as well as an appraisal of the Diffuse Reflectance Accessory, were presented in an earlier report (Van den Oord & Wesdorp, 1971). The principle of the method is that the difference in absorption at two wavelengths (580 and 630 nm) is linearly related to the relative proportions of the two pigment forms in the surface of the beef sample.

The standard deviation in the percentage of oxyMb in triplicate determinations of individual beef slices was 3% relative for high (>80%) and low (<40%) values; at intermediate values the standard deviation was 5% relative. Much the same results were obtained for the mean values of five slices (i.e. of fifteen determinations).

Subjective colour evaluation

The colour of the beef in the intact packs was evaluated by an eight-member trained panel. The packs were viewed under fluorescent lamps (Philips TL F/57 'colour judgement' lamp). The panel members were asked to express all visual aspects of the samples which might influence the colour judgement and to combine them with the colour score to give a 'total score'. Colour was ranked on a ten-point scale, 1 being extremely bad, 10 extremely good. Samples given a mean score of less than 5·5 were considered unacceptable. Ten-point scales were also used to score for appearance, impression, and 'total score' (including all visual aspects).

Colour rating by an eight-member panel on a ten-point scale gave satisfactory results. The standard deviation (for a single sample) from the mean was 0·6; this applies for the whole scale. The colour scores were generally very much like the 'total score', which represents an overall evaluation of all visual aspects, while the ratings for appearance and impression tended to show slight deviation.

Results

Typical results for a single complete series of experiments are shown in Fig. 1(a) and (b): the lines connect the mean values for each series. In Fig. 1 (b) the reflectance values ($A_{580} - A_{630}$) and the corresponding oxyMb percentages are recorded during the storage of three series of topside of beef at three temperatures: Fig. 1(a) represents the corresponding panel scores. There is considerable variation in the colour of the samples, even those from one muscle. This variation is demonstrated in Fig. 2(a) and (b) in which each individual sample is represented. The correlation between the two colour evaluations is represented in Fig. 3, in which all individual samples are included: the score for each sample is the mean of eight scores, and the oxyMb content of each sample is the mean of three reflectance measurements. There is a statistically highly significant correlation ($r = 0{\cdot}94$) between the subjective 'colour score' and the oxyMb content of beef samples (the effect of temperature was not considered): the equation of the linear regression line is $y = 7{\cdot}38x + 7{\cdot}59$; the residual standard deviation (s_d) is 4·06. The spread of data around the regression line is determined predominantly by the spread in the reflectance measurements. The same statistical results were obtained when we considered only samples with a score greater than 5, the so-called 'commercial range' (Kramer, 1969): practically the same correlation was obtained with the other types of beef. A remarkable feature is that for each of the types of beef tested, the point of change-over from acceptable to not-acceptable occurs when the pigment in the meat surface comprises approximately 50% oxyMb.

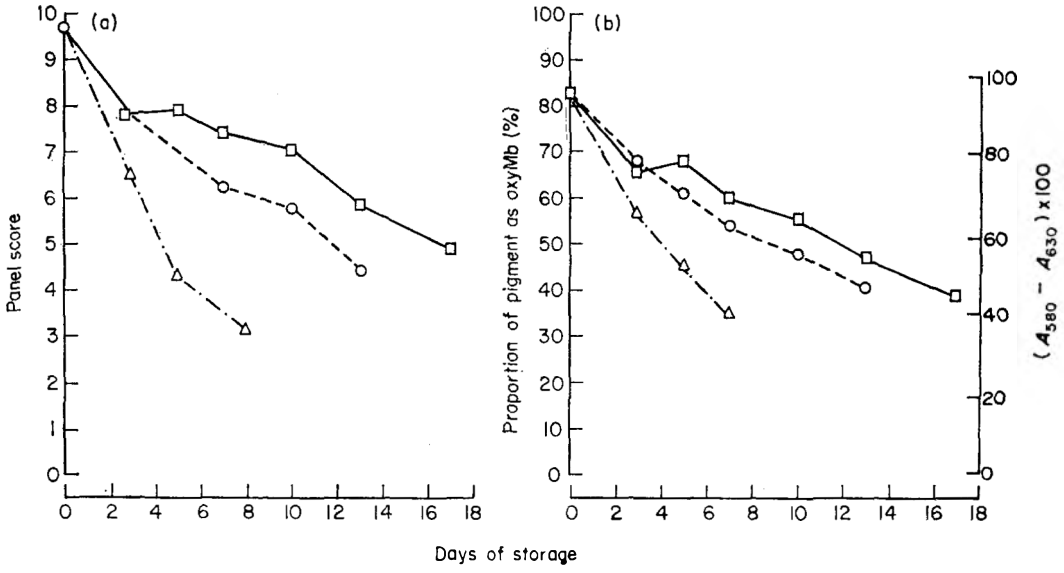

FIG. 1. Subjective colour evaluation (a) and reflectance measurements (b) of topside of beef stored at various temperatures. The lines connect the mean values of five (2° and 5°C) or three (10°C) slices. Reflectance values are expressed as $(A_{580} - A_{630}) \times 100$: the relative percentage of oxyMb is linearly related to the reflectance value. Storage Temperature □ 2°C; ○ 5°C; △ 10°C.

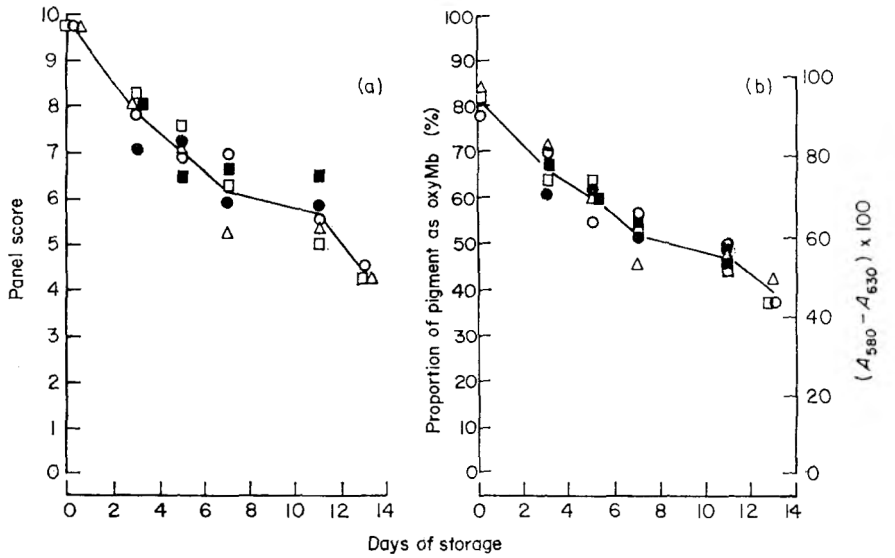

FIG. 2. Subjective colour evaluation (a) and reflectance measurements (b) of topside of beef stored at 5°C. The lines are the same as those for the 5°C samples in Fig. 1. The panel score and the relative percentage of oxyMb for each sample is given. For a given day of examination the symbol used to represent the results for a given slice is the same in (a) and (b), but there is no correspondence of symbols between days.

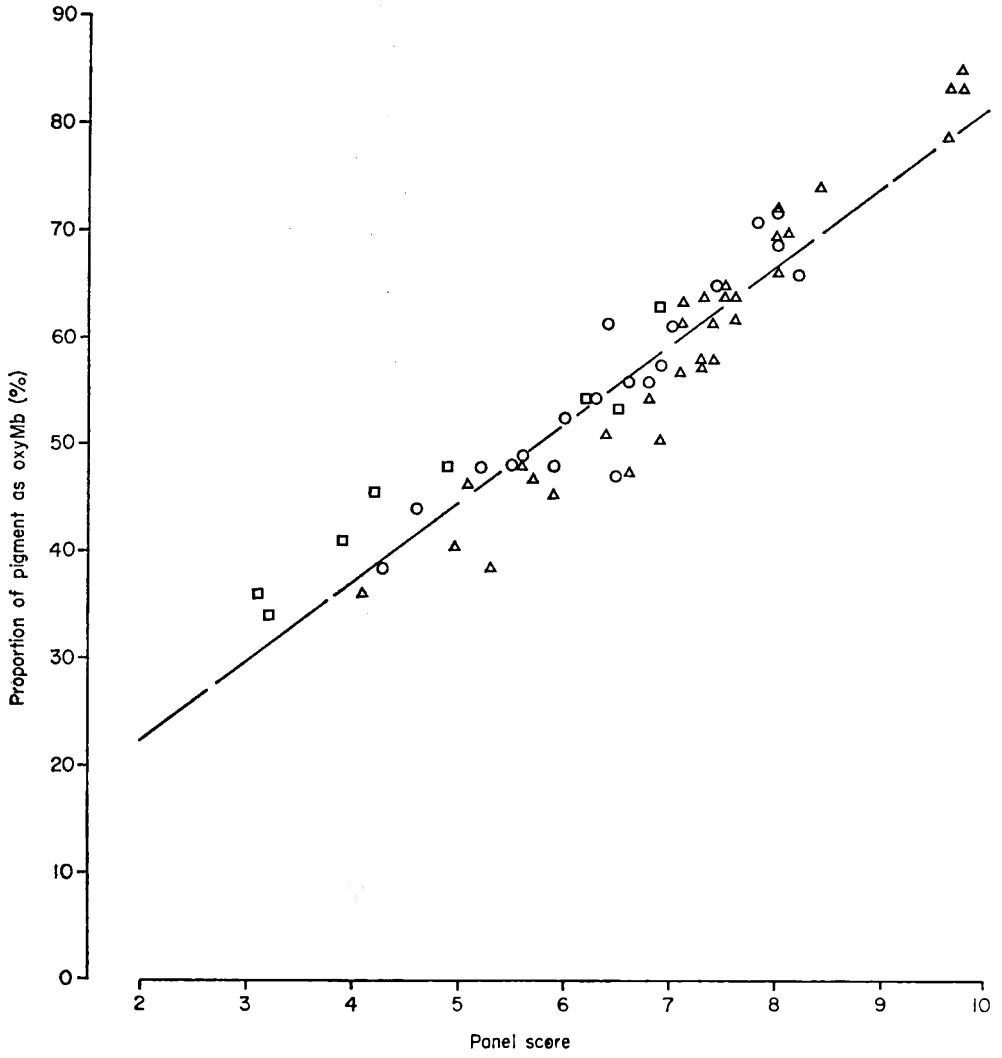

FIG. 3. Correlation between objective and subjective evaluation of the colour of topside of beef. The points are taken from Figs. 1 and 2. Objective colour measurements are expressed as relative percentages of oxyMb. The broken line is the calculated regression line. Storage temperature △ 2°C; ○ 5°C; □ 10°C.

Discussion

In an earlier paper (Van den Oord & Wesdorp, 1971) we established that there is a linear relation between the $(A_{580} - A_{630})$ reflectance value and the oxyMb content of beef slices. Reflectance spectrophotometry was shown to be suitable for the non-destructive determination of the percentage of myoglobin in the oxyMb form in the

surface zone of a beef sample. We have now shown that there is a statistically highly significant correlation between the subjective evaluation of colour and the reflectance value (i.e. the percentage of the myoglobin in the oxyMb form). It thus follows that reflectance measurement, and interpretation of the reflectance spectra by the indicated method, is a convenient and reliable objective colour measurement for beef. The results of reflectance measurements on a range of types of beef can be recorded on a single scale—the maximum $(A_{580} - A_{630})$ value being the same provided the samples have approximately the same myoglobin content.

The measurement of reflectance is extremely suitable for the experimental study of the effect on the colour of intact beef samples of such factors as storage temperature, oxygen partial pressure, permeability of wrapping material, etc. Furthermore, the results can be expressed in terms of pigment composition. It should be noted, however, that sufficient samples must be examined, and sufficient measurements per sample recorded, to level out natural variations in the meat.

It is worth noting that the subjective rating for colour and overall impression is very much the same. The overall impression greatly determines the consumer's decision of acceptable or not acceptable. We may therefore conclude that the contribution of the colour of beef is a most important factor in this decision.

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Application of the 'D-concept' to heat treatments involving curing salts

M. INGRAM AND T. A. ROBERTS

Summary

Canned cured meats are as safe as uncured meats which have received a 12–D heat process ('botulinum-cook') after heat treatments approximately one-fifth as great. Preliminary findings in laboratory media indicate that the D-concept may be equally applicable to cured meats, with a correspondingly smaller value of D. A means of calculating the effect of curing salts is described and suggestions are made for further study.

Introduction

The heat processing of normal foods in order to protect against *Clostridium botulinum* is based, as described in detail elsewhere (Schmidt, 1961; Ingram, 1969), on the 12–D concept which is briefly as follows. First, it is supposed (from extensive experimental evidence) that the period of heating needed to reduce the number of surviving spores by a factor of ten (a 'decimal reduction') remains constant (the '*D* value') as the number of surviving spores falls (a relation called 'exponential'); in which case the graph of the logarithm of the number of surviving spores against time of heating is a falling straight line, of slope $^{1}/_{D}$ if logarithms are to the base 10. Second, it is suggested (Perkins, 1964), mainly on the basis of experience, that there is satisfactory security from *Cl. botulinum* if the treatment is such as to destroy 10^{12} spores, i.e. produces twelve decimal reductions, implying a treatment of twelve times *D*. It is further supposed, following the D-concept, that the treatment needed to destroy 10^{12} spores in one container would be the same if these spores were much more thinly distributed, even at levels well below one per container; the importance of the D-concept is that it is the only generally accepted basis for making this extrapolation. It is also generally agreed that, to produce this twelve-decimal reduction of the most resistant *Cl. botulinum* spores, the necessary heat treatment is about 3 min at 121°C or some equivalent combination of time and temperature (Townsend *et al.*, 1938, 1954).

In the case of cured meats, decades of commercial experience have shown that an at least equal degree of freedom from *Cl. botulinum* is attained by heat treatment only of the order one-fifth as great, i.e. about equivalent to 0·5 minutes at 121°C. If it be

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accepted that this degree of protection results from a particular degree of inactivation of *Cl. botulinum* spores in uncured foods, one must assume a similar degree of inactivation in cured foods, and the inference is that the influence of a given heat treatment is multiplied some five-fold in cured meats. There is much experimental evidence that curing salts exert this kind of augmenting influence; but complex inter-actions have been revealed between salt (NaCl), nitrite, pH and number of spores (reviewed by Spencer, 1966). Knowledge of these complexities has hitherto discouraged attempts to put the relations on a quantitative basis, and the heat treatments used for canned cured meats have been applied by rule of thumb based on experience.

Riemann (1967) and Spencer (1969) have both considered the application of the D-concept to canned cured meats, but neither included the interaction between heat treatment and inhibitors. Application of the D-concept, presumably with an appropriately changed value of D, would permit more rational interpretation of the processes applied in canning cured meats. Moreover, there have long been published data which show that addition of salt produces a change of the kind in question; e.g. in the heat treatment of peas containing spores of *Cl. botulinum* the D value was changed, but the relation between number of survivors and heating time remained exponential (see Fig. 1, Viljoen, 1926).

We have therefore made experiments to see whether more complex mixtures of curing salts might still permit the use of the D-concept.

Materials and methods

Organism used and preparation of spore suspension

The organism used was *Cl. botulinum* type A (strain 33A) received from D. Berkowitz, U.S. Army Natick Laboratories, Natick, Mass. Spores were produced by adding 25 ml of 35°/24hr culture to 180 ml of freshly prepared medium comprising (g/l) Trypticase (B.B.L.) 50, Bacto-peptone (Difco) 5; ammonium sulphate 10; yeast extract ('Oxoid') 2, and cysteine HCl 0·5, in distilled water. After incubating at 35°C for one week, spores were harvested by centrifugation at 1°C, resuspended in sterile distilled water, and stored at 1°C. No heat-shock or enzyme treatment was used during the preparation of the spore suspension.

Determination of heat resistance

3·5 ml of spore suspension was heated at 95° in 1 oz screw-cap bottles totally immersed in a thermostatically controlled water bath. In view of the comparative nature of the experiment and the relatively long heating times (20–120 min), the come-up time was not determined accurately, 5 min being added to the nominal heating times.

Spores were heated in water, or in water containing sodium chloride (40 g/l) plus sodium nitrite (200 mg/l). Viable counts of spores surviving the heat treatment were made in two media both basically Reinforced Clostridial Agar (RCA) ('Oxoid') adjusted to pH 6·0, but containing 1 g/l sodium bicarbonate added to molten agar at

50°C as a 10% aqueous solution after sterilization by membrane filtration. The first 'normal' counting medium contained no further additions, but to the second was added 20 g/l sodium chloride and 50 mg/l sodium nitrite (the latter also added to sterile molten agar at 50°C as filter sterilized solution). After heating, samples of spore suspension were cooled rapidly in ice-water, and decimal dilutions prepared in maintenance medium (peptone 1 g/l; sodium chloride 5 g/l). Duplicate 1/50 ml drops of each dilution were placed in 5 cm plastic Petri dishes and 10–12 ml of molten medium added to give poured plates, which were incubated under hydrogen at 35°C. In the case of RCA + bicarbonate, colonies were counted after incubation for 42 hr. The presence of sodium chloride and sodium nitrite slowed growth, and colony counts were made after 4–5 days. All plates showing no colonies or low numbers of colonies were reincubated for a further 4 weeks at 35°C, but no increase in the number of colonies was evident.

Results

Viable counts in the four situations studied are listed in Table 1. The addition of 4% NaCl and 200 ppm $NaNO_2$ to the heating medium had little effect on heat resistance (comparing column 2 with column 4, and column 3 with column 5). The presence of

TABLE 1. The effect of sodium chloride and sodium nitrite on heat resistance and recovery of spores of *Cl. botulinum* 33A

	$\log_{10}$ viable count			
	Heated in water		Heated in water containing 4% NaCl and 200 ppm $NaNO_2$	
	Plating medium			
Min at 95°C	RCA/ bicarbonate	RCA/bicarbonate + 2% NaCl + 50 ppm $NaNO_2$	RCA/ bicarbonate	RCA/bicarbonate + 2% NaCl + 50 ppm $NaNO_2$
0	8·36	8·36	8·42	8·38
20	7·41	6·73	7·45	6·89
40	6·39	5·05	6·98	5·54
66	5·75	3·33	5.95	3·61
80	5·42	2·72	5·20	2·66
100	4·05	—	4·19	—
120	3·52	—	—	—
$D_{95°C}$(min)	26·3	14·9	24·3	14·1

2% NaCl and 50 ppm $NaNO_2$ in the plating medium markedly reduced recovery of spores, (compare column 2 with 3 and 4 with 5); but it did not destroy the exponential relation between survivors and heating time (Fig. 1) though it reduced the apparent D value from about 25 to about 15 min. This reduction in D value was not affected by the presence during heating of sodium chloride and sodium nitrite (compare columns 2 and 3 with 4 and 5, in Table 1).

A potent inhibitor of clostridial growth is formed when sodium nitrite is heated with certain components of bacteriological media (Perigo, Whiting & Bashford, 1967; Perigo & Roberts, 1968). Heating 50 mg/l $NaNO_2$ in RCA adjusted to pH6 for 15 min at 115°C rendered the medium much more inhibitory than when filter-sterilized nitrite was used, the $D_{95°C}$ then being less than 5 min.

Discussion

First, these observations confirm what has already been shown before: that the apparent effects of heating are much greater when curing salts are present; but that this results from inhibition of the development of heated spores by curing salts in the medium after heating, the direct effects of those salts on the heat treatment being negligible (Roberts & Ingram, 1966; Roberts, Gilbert & Ingram, 1966; Duncan, 1970).

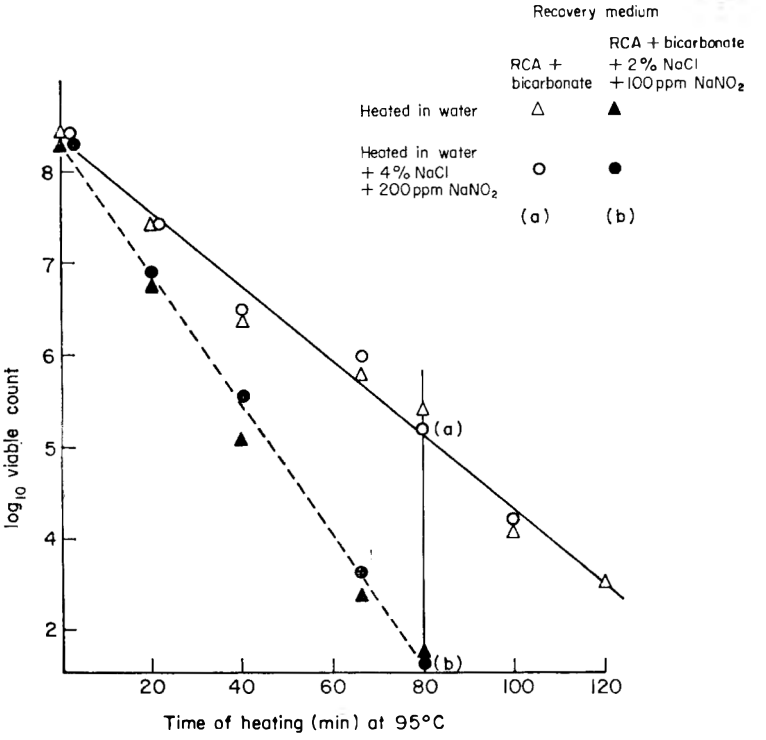

FIG. 1. The effect of sodium chloride and sodium nitrite on heat resistance and recovery of spores of *Cl. botulinum* 33A.

Next, considering in that light the relation between two observations corresponding to the same time of heating, such as (a) representing the count on a normal medium and (b) that on the medium with curing salts in Fig. 1, it is evident that the difference between (a) and (b) represents this effect of the curing salts in the medium. More strictly, since the ordinate in Fig. 1 is logarithmic, the difference between (b) and (a) represents that proportion (viz. $c\ 10^{2.5} : 1$) of those spores 'damaged' by the given degree of heating which remain 'viable' (i.e. capable of growth on an adequate medium) but which are prevented from growing (i.e. remain 'dormant') by the presence of the curing salts in the medium. It is a noteworthy feature of the observations in Fig. 1 that this proportion appears to rise systematically with the degree of heating.

Fig. 1 does not display the effect of time of incubation which is important because dormant spores could, in time, conceivably germinate and grow out, as occasionally happens during storage of canned foods in practice (Schmidt, 1954). That is to say, the count (b) in Fig. 1 might increase with sufficiently long periods of incubation. In fact the counts on the lower curve in Fig. 1 were obtained after 5 weeks incubation, at which time they were unchanged from the values at 1 week. The indication is that delayed germination might, nevertheless, result ultimately in an exponential relation, though with a slightly larger value of D than would be indicated initially. Moreover, the mathematical nature of the relation is such that, where large degrees of inactivation are in question, the initially apparent number of survivors would need to be increased (through this breaking of dormancy) many-fold before it would significantly upset the suggested relation.

We are well aware that much wider acquaintance will be needed, with results from much longer periods of incubation, before the foregoing indications can be accepted with confidence. For purposes of discussion, however, the following assumes that the exponential relation holds in the presence or absence of curing salts, as Fig. 1 has indicated.

Consider Fig. 2, which repeats Fig. 1 schematically. D corresponds to the counts in normal medium (the cells which are not 'killed') i.e. the D value in the accepted sense. D' corresponds to counts in the salts medium (the cells which are not 'inactivated' i.e. neither 'killed' nor inhibited), i.e. it is a pseudo- D value. If $D' = \alpha D$ for one decimal reduction, it follows that to achieve an inactivation of n decimal reductions, where the required treatment is nD minutes for the complete medium, the required dose in the salts medium is αnD minutes.

It is, however, more illuminating to consider the differences between corresponding counts on the different media for a given treatment. A treatment of nD minutes, giving n decimal reductions on the normal medium, produces on the salts medium an aggregate inactivation of n' decimal reductions. Now it follows that $n = \alpha n'$ (where $D' = \alpha D$) hence $n' = \frac{n}{\alpha} = n.\ \frac{D}{D'}$.

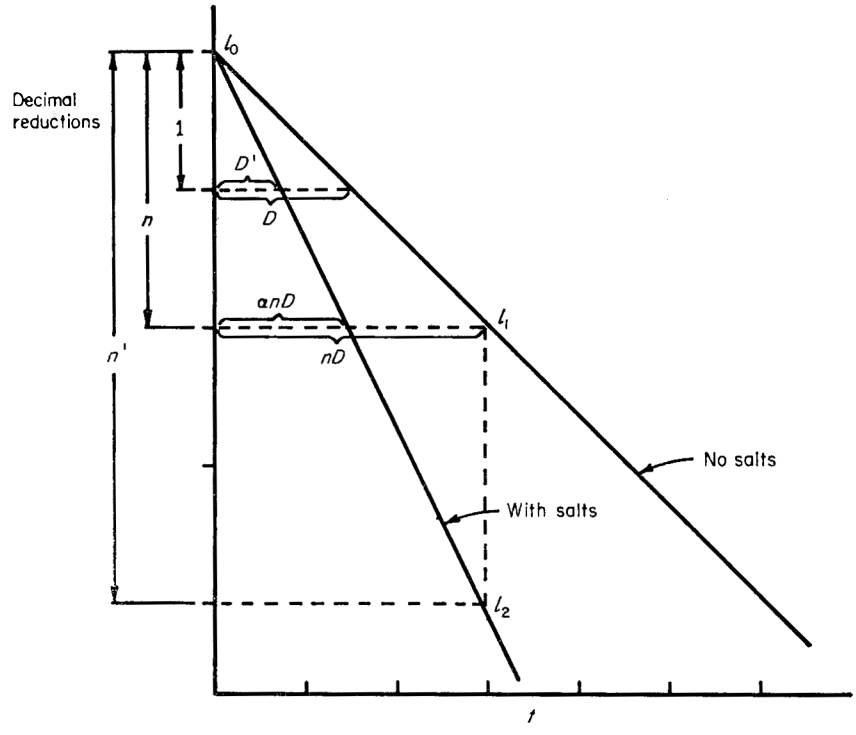

FIG. 2. Schematic representation of data such as those in Fig. 1.

D & D'	=	apparent decimal reduction times;
n & n'	=	number of decimal reductions;
nD & αnD	=	required treatment in min;
l_0	=	$\log_{10}$ initial count;
l_1 & l_2	=	$\log_{10}$ survivor counts;
t	=	time of heating in min.

The n decimal reductions represent the cells which are 'killed' by the heating, i.e. do not grow if transferred to normal media; the additional n'–n decimal reductions represent the proportion of still viable cells ($10^{n'-n} : 1$) which are 'inhibited' or 'dormant', i.e. presumably would grow if in a complete medium, but do not grow in the salts medium. Obviously the (logarithmic) proportion of viable cells inhibited is

$$n'-n = n.\frac{D}{D'} - n = n\left(\frac{D}{D'} - 1\right)$$

and this represents the extra inhibitory effect of the presence of the curing salts. This proportion (n'–n) is directly proportional to n (which is itself directly proportional to the period of heating), i.e. the proportion inhibited increases systematically with the heat treatment, as noted earlier.

The above equation may also be written

$$n'-n = n\left(\frac{l_o - l_2}{l_o - l_1} - 1\right) = n\left(\frac{l_1 - l_2}{l_o - l_1}\right)$$

where l_o is the logarithm of the initial count in normal medium, and l_1 and l_2 are logarithms of counts after a given time of heating in the normal and salts media respectively. This form of expression would be most convenient for rapid experimental exploration of the effects of salt concentration etc., once the basic principle was established.

From the foregoing it will be clear that, if exponential relations between surviving spores and heating-time can be shown to prevail for spores in cured meats as in uncured foods, the pseudo-*D* values so derived could be used like normal *D* values to interpret the total inactivation resulting from heat treatment. Further, measurement of pseudo-*D* would, at the same time, provide the parameter needed for comparative evaluation of the effect of changing combinations of salt, nitrite, pH etc. Moreover, it would be possible to distinguish spores killed from spores viable but inhibited, by simple calculations based on 'normal' and 'pseudo' *D* values. We realize that we do not have enough evidence to assert confidently that these possibilities exist, but the results of preliminary examination seem so hopeful as to call for further exploration on a much wider, and a larger and longer scale than we can give.

In any case, our data give a plain indication that the presence of curing salts reduces the effective *D*-value. In so far as the D-concept may be applied to the data in Fig. 1, a 12-D treatment in the salts-free system would be approximately 300 min, while in the system with salts a similar degree of inactivation would be achieved with a treatment of 175 min—roughly half as great. Similar indications have been obtained in comparing the 'sterilizing doses' needed when using ionizing radiation instead of heat: for uncured meat the value is approximately 5 Mrad and for cured meat approximately 2·5 Mrad was suggested (Krabbenhoft *et al.*, 1964; Anellis *et al.*, 1965). In such cases, the diminished process has sometimes been described as a '6-D' treatment, or whatever other fraction of 12-D might be appropriate. The true situation apparently is that the effective value of D has been reduced in corresponding proportion to about one half, and that an approximately 12-D process has in fact been given. It is therefore suggested that such descriptions should cease, as they are likely to be undesirably misleading.

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Development of marinated herring

R. McLAY AND R. PIRIE

Summary

A marinated herring has been produced which has a higher acceptability than some commercially produced packs. It has a storage life of at least 4 months in the first pickle and 3 months in the second pickle.

Introduction

The term marinating as used in this paper means curing of herring fillets in a solution containing acetic acid and salt. Cold marinades of this type fall into the category of semi-preserves, that is they are not sterile and thus have a limited storage life. In semi-preserves bacterial activity is reduced or eliminated by a sufficiently high concentration of acid and/or salt. British marinades are normally preserved with acetic acid and salt, although lactic or tartaric acids may be used to substitute for some of the acetic acid in order to impart a less astringent taste. For marinades of this type, the essential factor for preservation is the acid. However, without an adequate proportion of salt the softening process, which is an additional effect of the acid, would proceed too far. In all cold marinades hydrolysis and enzymic digestion of the protein proceed steadily, the rates of these processes being governed by the concentrations of acid and salt used and upon the temperature of storage (Meyer, 1965).

Industrially, marinated herrings are usually prepared by a two-stage process. In the first stage fillets are immersed for up to three weeks in a relatively strong acetic acid and salt solution (for example 7% acid 14% salt) in the proportion of three parts of pickle to four parts of fish. This stage may be carried out in tanks or barrels.

At the end of the preliminary stage, the flesh of the herring is white, opaque and firm in texture. The fish are removed from the tanks or barrels, drained, and packed into suitable containers, usually glass jars. The fish are then immersed in a pickle containing $1\frac{3}{4}$–2% of acetic acid and 2–4% salt, flavoured with sugar and spices. At this stage there are a great number of variations in the manner of preparation, the packs being designated accordingly, as for example, Bismarks, rollmops, etc. Onion and other vegetables, sour cream, bay leaves, sauce made from herring milts, etc., also may be added at this stage.

To increase the consumption of marinated herrings in the U.K. it was thought necessary to add to the existing range of home produced marinades a milder cured pack which

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would have a wider appeal. U.K. marinades marketed in this country are cured normally in such a way that bacterial and/or chemical proteolysis does not occur during the retail storage life which may be up to six months. This type of marinade has a firm texture and a strong acid taste. (The techniques used to produce this type of pack are kept secret and are unknown to us.) Preliminary experiments comparing commercial packs with mild-cured marinades prepared in the laboratory showed that an overwhelming majority of tasters found the laboratory packs to be preferable to commercial packs.

The following is an account of the development of a mild-cured marinade which could be produced commercially. It was visualized that this marinade would be kept at chill temperature in the retail outlet and would have an expiry date of fourteen days from the time it left the manufacturer. The main problem was to find conditions which would allow the marinade to be stored for a sufficient time before dispatch to the retailer in order to ensure a smooth production flow independent of seasonal fluctuations in the supply and fat content of the raw material.

Methods

A preliminary investigation was carried out to determine the minimum acid and salt concentrations in the first pickle which would produce an acceptable product. To do this marinades were made from fresh herring in a one stage process. Five different pickles were used containing 10% salt and 1–5% acetic acid. Single herring fillets weighing, on average, 100 g, were immersed in 100 ml of these pickles, in closed jars. Some of the jars were stored at 3°C and some at 21°C. Samples were examined organoleptically after 21 and 35 days storage at 21°C, and after 50, 91 and 112 days storage at 3°C.

After the results of the preliminary investigation were assessed a second more detailed experiment was carried out as follows:

A batch of fresh herrings, of oil content 7%, was divided into two: one batch was marinated immediately and stored at 3°C, the other was frozen and stored at −30°C. A two stage marinating process was used. For the first pickle the following variations in acid and salt content were used:

No. 1 3% acetic acid 10% sodium chloride
No. 2 4% acetic acid 10% sodium chloride
No. 3 7% acetic acid 14% sodium chloride.

The strengths of pickles 1 and 2 were based on the findings of the preliminary experiment as described below. No. 3 was selected to provide a stronger cure; it was derived from the literature as being representative of Scandinavian commercial practice (Meyer, 1965). Single herring fillets were filled into 10 kilo jars, containing the pickle, so that the individual fish were in contact with the pickle throughout the process. The ratio of fish to pickle was 3:2.

The fresh fish were analysed for moisture content by drying to constant weight in

TABLE 1. Chemical analyses of marinades in first pickle—fresh fish 7% oil content

Type of pickle	Storage time in pickle													
	4 weeks							16 weeks						
	Flesh				Pickle			Flesh				Pickle		
	H_2O	N_2	NaCl	Acid	N_2	NaCl	Acid	H_2O	N_2	NaCl	Acid	N_2	NaCl	Acid
10% NaCl 3% acid	63·56	2·76	3·28	2·13	0·72	4·38	1·53	66·00	2·37	3·71	2·06	0·63	3·94	1·50
10% NaCl 4% acid	65·36	2·85	3·56	2·66	0·66	4·38	2·06	67·97	2·42	3·73	2·41	0·59	3·98	1·81
14% NaCl 7% acid	69·07	3·35	5·05	3·52	0·47	6·14	2·90	68·35	2·93	5·23	3·46	0·49	6·19	2·96

TABLE 2. Chemical analysis of marinades in the second pickle

Herring flesh prior to processing	Marinades after 2 weeks in second pickle																							
	Time of storage in first pickle (weeks)																							
				4				8				10				12				16				
Type of first pickle	H_2O	N_2	Fat	H_2O	N_2	NaCl	Acid	H_2O	N_2	NaCl	Acid	H_2O	N_2	NaCl	Acid	H_2O	N_2	NaCl	Acid	H_2O	N_2	NaCl	Acid
Fresh																								
10% NaCl 3% acid	73·66	2·93	6·96	68·75	2·52	2·10	1·32	69·74	2·54	2·28	1·52	72·12	2·58	2·36	1·51	69·18	2·43	2·09	1·62	71·21	2·34	2·38	2·01
10% NaCl 4% acid				69·27	2·45	2·39	1·65	64·77	2·52	2·44	1·61	70·09	2·60	2·33	1·62	69·82	2·45	2·30	1·82	67·06	2·22	2·29	2·11
14% NaCl 7% acid				70·21	2·81	2·51	1·82	72·83	2·92	2·53	1·97	72·87	2·81	2·65	1·77	69·97	2·65	2·54	2·11	67·59	2·45	2·74	2·65
Frozen 4 months																								
10% NaCl 3% acid	73·66	2·93	6·96	76·20	2·65	2·46	1·41													71·21	2·34	2·38	2·01
10% NaCl 4% acid				68·43	2·62	2·10	1·57													67·06	2·22	2·29	2·11
14% NaCl 7% acid				66·07	2·63	2·54	2·10													62·59	2·45	2·74	2·35
Fresh																								
10% salt 3% acid	64·37	2·66	17·72	62·32	2·31	2·12	1·43													60·52	2·15	2·16	1·91
10% salt 4% acid				63·51	2·41	2·29	1·79													56·27	2·08	1·89	1·77
14% salt 7% acid				64·01	2·49	2·54	2·31													61·18	2·36	2·47	2·39
Frozen 4 months																								
10% salt 3% acid	64·37	2·66	17·72	63·62	2·28	2·11	1·63													61·92	1·73	2·48	1·74
10% salt 4% acid				59·33	2·17	1·93	1·77													61·24	1·79	2·38	2·00
14% salt 7% acid				63·12	2·51	2·53	2·31													62·13	2·01	2·59	2·11

a vacuum oven at 75°C, for nitrogen content by the macro Kjeldahl method, and for oil content by the Soxhlet ether extraction method. After 4 and 16 weeks in the first pickle, samples of fish were removed, drained in a filter funnel for 10 min and their moisture and nitrogen contents determined as above. The sodium chloride content was determined by Volhard's method and the total acid content by titration of an aqueous extract of the flesh.

At about monthly intervals samples were removed from the first pickles and packed into 500 g jars containing the second pickles. At the same time an equal weight of pickle was removed to maintain a constant fish to pickle ratio. The second pickle contained 1% acetic acid and 2% salt. The ratio of fish to pickle was approximately one to one. To each jar was added 1 bay leaf, 1 whole clove, 1 red pepper, 1 white pepper, 2 coriander seeds and 30 g of sliced onion. The finished marinades were stored in the small jars at 3°C for 14 days, after which they were assessed for acceptability by a taste panel of ten, using a hedonic scale:

9–like extremely; 8–like very much; 7–like much; 6–like slightly; 5–neither like or dislike; 4–dislike slightly; 3–dislike much; 2–dislike very much; 1–dislike extremely. When samples were assessed by the taste panel, an aliquot of the pickle and of the drained fish were analysed for moisture, nitrogen, sodium chloride and acid content.

After four months storage at −30°C the remainder of the batch of herrings was marinated in three first pickles as above. After 4 and 16 weeks, aliquots were removed and immersed in the weaker second pickle, tasted and analysed as for the batch above. The whole of the second detailed experiment was then repeated on a second batch of herring with a mean oil content of 18%. Tasting and analyses were carried out after 14 days in the second pickle subsequent to storage for 4 and 16 weeks in the first pickle.

Samples from two commercial brands of marinades, obtained locally at monthly intervals, were tasted alongside laboratory samples. Analyses of the fish were performed as for the laboratory samples.

Results

Organoleptic examination of samples in the preliminary experiment showed that after 35 days storage at 21°C samples in 1, 2 and 3% acetic acid and 10% sodium chloride were spoiled. Samples in 4 and 5% acetic acid and 10% sodium chloride were considered acceptable. Samples stored at 3°C were all acceptable after 50 days storage. After 91 and 112 days storage at 3°C those in a 1% acetic acid, 10% salt pickle were spoiled, those in 2% acetic acid and 10% salt were slightly spoiled, while those containing 3, 4 and 5% acetic acid and 10% salt were acceptable. It appeared from these results that a pickle containing 3% acetic acid and 10% salt was the minimum acid content which could be used for a first stage marinade. Therefore the more detailed investigation was undertaken as described above.

Tables 3, 5 and 6 give the taste panel assessment of laboratory and commercial

TABLE 3. Taste panel assessment of marinades

Type of marinade	Mean score									
	Time in first pickle									
	4 weeks		8 weeks		10 weeks		12 weeks		16 weeks	
	Texture	Flavour	Texture	Flavour	Texture	Flavour	Texture	Flavour	Texture	Flavour
Fresh (7% fat)										
10% salt 3% acid	6·3	6·8	6·5	6·5	7·0	7·0	6·3	7·2	6·7	6·8
10% salt 4% acid	6·2	6·8	6·6	7·0	7·1	6·8	6·1	7·1	6·6	6·9
14% salt 7% acid	6·0	6·5	6·2	6·4	6·5	6·8	6·3	6·4	6·3	6·5
Frozen 4 months (7% fat)										
10% salt 3% acid	6·9	7·1							6·6	6·8
10% salt 4% acid	6·6	6·8							6·6	7·0
14% salt 7% acid	6·2	6·6							6·2	6·4
Fresh (17% fat)										
10% salt 3% acid	6·6	6·7							6·1	6·5
10% salt 4% acid	6·6	6·9							6·1	5·0
14% salt 7% scid	6·8	6·6							6·2	5·9
Frozen 4 months (17% fat)										
10% salt 3% acid	6·5	6·3							6·5	5·0
10% salt 4% acid	6·7	6·6							6·3	6·3
14% salt 7% acid	6·3	6·2							6·0	5·4

TABLE 4. Chemical analyses and taste panel assessment of commercial samples

Sample	H_20	N_2	NaCl	Acid	Mean taste score	
					Texture	Flavour
Brand A No 1	65·21	2·93	4·10	2·20	4·2	3·9
,, 2	68·66	3·15	3·06	2·21	5·0	5·3
,, 3	68·73	3·01	4·30	2·50	4·7	4·1
,, 4	69·64	3·07	3·96	2·33	4·1	4·3
,, 5	69·59	3·07	3·47	1·94	3·8	4·7
,, 6	66·40	3·36	4·48	2·50	3·8	3·2
,, 7	69·28	3·02	3·36	2·36	5·0	4·4
Brand B No 1	68·10	2·75	3·94	1·85	3·4	2·4
,, 2	67·08	2·92	4·27	2·12	3·0	2·3

TABLE 5. Comparison of marinades and commercial samples by taste panel assessment

		Texture	Flavour
Marinades	(Mean	6·382	6·533
(33 samples)	(variance	0·1384	0·2879
Commercial	(Mean	4·371	4·271
samples	(variance	0·2757	0·4290
(7 samples)			
Difference between means (Marinade minus Commercial)		2·011	2·262
Standard error of difference between means		0·224	0·265
$t = \frac{\text{difference between means}}{\text{standard error of difference}}$		8·98	8·54
Significance		0·1	0·1

TABLE 6. Comparison of various laboratory samples by taste panel assessment

Texture	4 weeks	8 weeks	10 weeks	12 weeks	16 weeks	Means
10% salt 3% acid	6·3	6·5	7·0	6·3	6·7	6·56
10% salt 4% acid	6·2	6·6	7·1	6·1	6·6	6·52
14% salt 7% acid	6·0	6·2	6·5	6·3	6·3	6·26
Means	6·17	6·43	6·87	6·23	6·53	6·45

Analysis of variance

Source of variation	DF	Sum of squares	Mean square	F
Salt/acid content	2	0·2653	0·1326	4·87 (*)
Storage	4	0·9239	0·2309	8·48 (**)
Interaction	8	0·2181	0·0272	
Total	14			

To compare the means for each salt/acid content, the 'standard error of difference between two means' is 0·10.

The average for 14% salt, 7% acid differs from the other two by more than twice the standard error, so it is significantly lower than the other two. However, the two means for 10% salt are not significantly different.

Flavour	4 weeks	8 weeks	10 weeks	12 weeks	16 weeks	Mean
10% salt 3% acid	6·8	6·5	7·0	7·1	6·8	6·86
10% salt 4% acid	6·8	7·0	6·8	7·1	6·9	6·92
14% salt 7% acid	6·5	6·4	6·8	6·4	6·5	6·52
Means	6·70	6·63	6·87	6·90	6·73	6·77

Analysis of variance

Source of variation	DF	Sum of squares	Mean square	F
Salt/acid content	2	0·4654	0·2327	6·32 (*)
Storage	4	0·1534	0·0383	1·04 (ns)
Interaction	8	0·2946	0·0368	
Total	14	0·9134		

Standard error of difference between two salt/acid means = 0·12.
Again, 10% salt 3% acid and 10% salt 4% acid are not significantly different, but 14% salt, 7% acid is significantly lower than both.

samples. The samples prepared with 10% NaCl were significantly better than those prepared with 14% NaCl in both flavour and texture. On both texture and flavour, the laboratory samples were significantly better than the commercial samples. Tables 1, 2 and 4 give the results of the chemical analyses on both laboratory and commercial samples.

In the first pickles the acid and salt reached equilibrium within 4 weeks. In fact both salt and acid exceeded the theoretical estimates of the equilibrium state. This has been reported for acetic acid (Meyer, 1965) but not for sodium chloride. After 4 weeks storage there was an excess of sodium chloride (estimated as chloride) of about 15%. This excess had increased to about 30% after 16 weeks storage at 3°C in the case of 3 and 4% acid, but in 7% acetic acid and 14% salt no significant change occurred after 16 weeks storage.

Analyses were carried out in the second pickle after 14 days storage at 3°C. Commercial practice was followed, in that whole fillets were used, with the result that the ratio of fish to pickle varied in order to allow the fillets to be completely immersed in pickle. There was therefore some slight variations in salt and acid concentrations in the flesh. Analyses of the pickle and fish showed that sodium chloride and acid had migrated from the flesh to the weaker pickle; however, an excess over calculated equilibrium levels of acid and salt in the flesh remained.

There was a range of salt content in the various laboratory samples of 2·09 to 2·74% and a range of acid content of 1·32 to 2·38%. The taste panel showed a slight preference for marinades prepared with the weaker pickles. In the commercial samples the salt content varied from 3·06 to 4·48% and the acid content from 1·85 to 2·50% but there was no correlation between chemical composition and taste panel assessment. On average the amounts of salt and acid in the commercial samples fell between those for the laboratory samples, but nevertheless the former always scored much lower. Thus it seems that the difference between the commercial and laboratory samples cannot be solely due to differences in either salt or acid content. To check whether the differences were due to variation in storage time in the second pickle a batch of laboratory samples was kept in the second pickle at 3°C and tasted at monthly intervals for 3 months. There was no significant difference in the taste score during 3 months of storage compared with previous samples kept for 14 days in the second pickle.

It was not possible to obtain details of the methods of preparation of commercial samples, therefore the reasons why the taste of the commercial samples was less acceptable are open to speculation.

Conclusions

Using the technique described above for the manufacture of cold marinades, it is possible to produce products which have a higher acceptability than some commercially available marinades, provided a shelf-life as long as that obtained for commercial samples is not required.

The herring used can vary in oil content from 7 to 18% and can be used successfully to produce marinades after at least 4 months storage at −30°C. The herring can be kept in the first pickle for up to 4 months and for up to 3 months in the second pickle, both at 3°C. Thus, it is possible to exceed by a considerable margin the planned retail shelf life. It should be possible using a combination of cold stored, marinated and fresh herring to produce continuously throughout a full year. After prolonged storage of herring with a high oil content in the first pickle, oil may be released and form a layer at the surface of the liquid. Such a layer of oil in contact with the air would readily become rancid. However this layer of oil can be floated off after adding excess pickle of suitable concentration.

Acknowledgment

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The threshold value for physiological action of ethylene on apple fruits

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Summary

In fruit picked at various stages of development and stored at 12°C, the threshold concentration of applied ethylene required to cause an increase in respiration decreased as the fruit matured. In young fruits 10 days elapsed between the application of 10 ppm ethylene (for the first 3 days) and an increase in respiration. By contrast, in mature fruit, close to the respiration climacteric, as little as 0·1 to 1 ppm ethylene induced an increase in respiration within a few days. Relatively massive production of ethylene and the development, in discs of peel taken from the fruit, of a malate effect (excess CO_2 produced on addition of malate) occur some time after increase in respiration. The implications of these results in the control of the respiration climacteric are discussed.

Introduction

It has been clear for some time that, as with the effect of ethylene on growth and abscission (see Mapson & Hulme, 1970), the threshold value for the physiological effect of the gas on the 'ripening' of fruits of the climacteric type is dependent on the age of the tissue (Burg & Burg, 1965). To obtain specific data on this changing susceptibility with maturity, samples of Cox's Orange Pippin apples were picked at different stages of development from 58 days after petal fall up to full maturity, and treated for 3 days with 0·1, 1·0, 10·0 and 100 ppm of ethylene. The respiration rate (CO_2 output) and ethylene production of the fruit were measured for periods of up to 16 days after the ethylene treatment and the number of days from the ethylene treatment until the onset of a rise in CO_2 and ethylene production was noted.

Materials and methods

Pre-climacteric Cox's Orange Pippin apples were picked at intervals from 28th July, 1969, from trees at the Burlingham Horticultural Station, Norfolk, and subsequently stored in glass vessels at 12°C. A stream of CO_2-free air was passed through the vessels

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at a constant rate. Three-way taps were included in the flow system to enable samples of gas to be removed for analysis of carbon dioxide, by the method of Hulme, Rhodes & Wooltorton (1970), and ethylene by the procedure of Galliard & Grey (1969).

Applications of the various concentrations of ethylene were carried out during the first 3 days of storage. A beaker, lined with filter paper and containing 30% potassium hydroxide solution (to remove CO_2) was placed in each glass vessel. The air flow was stopped and an appropriate volume of pure ethylene injected into the flask via a rubber serum cap. After 16 hr the KOH solution was removed and the air flow re-connected for a period of 8 hr. Treatment with ethylene was repeated on the second and third days, thereafter a constant flow of air was maintained.

The preparation of the discs of peel from the fruit at various stages of the climacteric in fruit picked on 4th September and subsequently stored with and without ethylene (44 ppm) and the measurement of the respiration by the Warburg method (20 discs = 1g, per Warburg flask) at 25°C was as described by Neal & Hulme (1958). Ethylene production by the discs was estimated by the method of Galliard *et al.* (1968).

Results

The overall results are given in Table 1 and a typical series of results for the response of immature fruit and fruit approaching maturity are shown in Fig. 1.

TABLE 1. The effect of the external application of ethylene on the respiration rate and ethylene production of apples

Pick	Date	Days from petal fall	Control		0·1 ppm ethylene		1 ppm ethylene		10 ppm ethylene		100 ppm ethylene	
			R	*E*	*R*	*E*	*R*	*E*	*R*	*E*	*R*	*E*
1	28 July	58	18	17	>14	>14	>14	>14	14	14	11	11–14
2	25 Aug.	86	13	12	13	11	13	13	12	12	9	8
3	9 Sept.	101	10	9	10	8	5	5	2	5	2	<4
4	22 Sept.	114	7	7	—	—	6	5	3	4	—	—

R = days to commencement of respiration climateric
E = days to commencement of measurable ethylene production

The results show that up to 86 days from petal fall (25th August) 100 ppm of ethylene were required to reduce appreciably the time taken for the onset of the climacteric. By 101 days from petal fall (9th September) 1 ppm of ethylene was effective in reducing the lag period to the climacteric; at this point, 100 ppm of ethylene brought an immediate response. By the 22nd September, 10 ppm induced a virtually immediate effect. Of particular interest is the fact that in the young fruit (58 days from petal fall) 0·1,

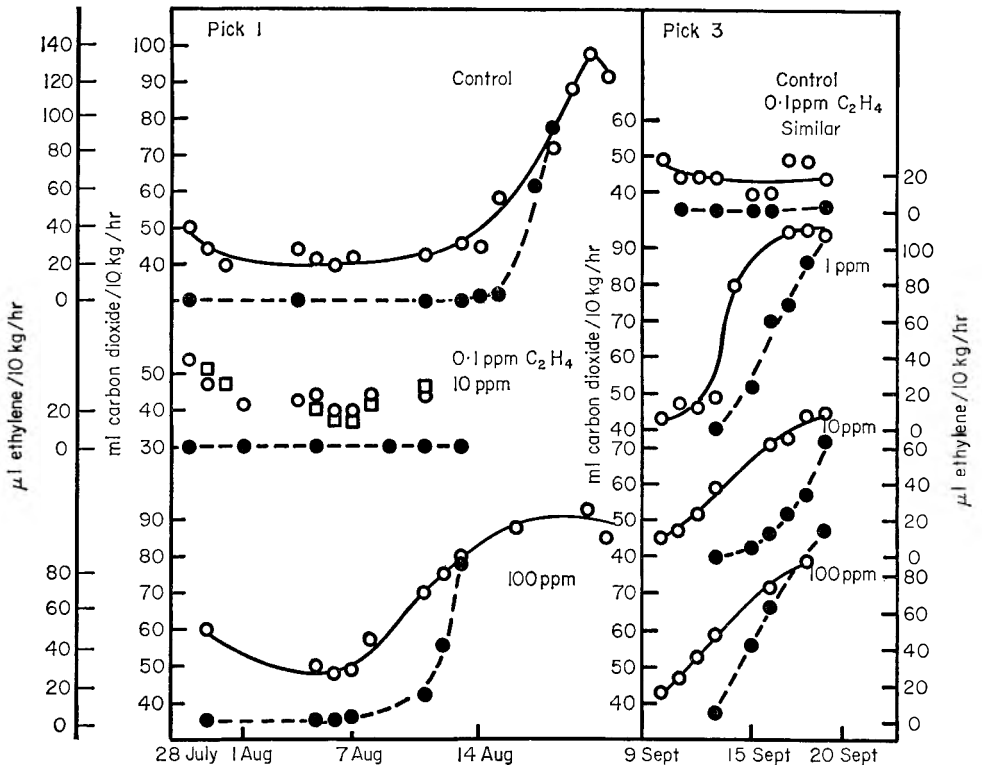

FIG. 1. CO_2 and ethylene production of fruits picked on 28th July (Pick 1) and on 9th September (Pick 3), treated with various concentrations of ethylene. Pick 1, respiration (open symbols and continuous line), control, 0·1 ppm, 100 ppm ethylene (O), 10 ppm ethylene (□). Ethylene production (●); 0·1 ppm and 10 ppm treatment, nil throughout. Pick 3, respiration (O); ethylene production (●).

1·0 and 10 ppm of ethylene produced no stimulation for 10 days after the ethylene treatment.

In these early samples the fact that although there is no increase in respiration or ethylene production for some time after the ethylene treatment with 100 ppm, yet the subsequent increase in respiration and ethylene production occurs several days before that of the comparable, untreated fruits, is an important one. It indicates that the ethylene has induced some, as yet unknown, change in the state of the tissue which does not result in the onset of the climacteric until some other, complementary, change in the state of the fruit has been achieved.

When the fruit is nearing the climacteric period on the tree (Fig. 1, pick 3) and its sensitivity to ethylene is increasing, there is little difference in the effect of the application of 1 or 10 ppm. At 1 ppm there is a short lag phase in the development of the respiration increase, but this is followed by a greater increase in the rate of respiration.

A more thorough study of the behaviour of fruit picked several days earlier than pick 4 of the main series of experiments was made. Here two sets of fruit were taken, one untreated and one given 44 ppm of ethylene for 3 days. In addition to the determination of the ethylene production and respiration rate, peel discs were prepared from the two sets of fruit and their ethylene production and response to the addition of malate were measured. The response in terms of extra CO_2 (ΔCO_2) to the addition of malate—the malate effect—increases rapidly during the climacteric phase (Flood, Hulme & Wooltorton, 1960) and serves as an additional criterion of the physiological action of ethylene. The results are shown in Fig. 2. If we consider the results for the untreated fruit, there is a lag of 7–8 days from the time the fruit was placed at 12°C until the onset of the climacteric in terms of respiration rate. There is a small, but physiologically significant production of ethylene by the fruit 1–2 days before the respiration begins to rise and ethylene production increases exponentially as the autocatalytic stage of production develops. Measurable production of ethylene by the peel discs is also delayed until the autocatalytic phase begins. It should be pointed out that the sensitivity of the method of measuring ethylene is not sufficient to register very small quantities of ethylene produced by the discs (1g). As previously shown (Hulme *et al.*, 1968), the development of the malate effect does not commence until the respiration climacteric has already begun. With the ethylene treated fruit, the ethylene production, respiration and malate effect are brought forward.

Discussion

The results described give support to the view that the action of ethylene as the ripening hormone in fruits may be separated into two distinct processes: the first is the initiation of ethylene synthesis, the second the physiological response to its presence. Until the tissue is in a responsive state, 'physiological concentrations' of the gas, whether produced by the tissue itself, or given off exogenously, will not produce a response in terms of autocatalytic production of ethylene and the consequential initiation of certain characteristic phenomena associated with ripening as a whole.

The lag in the early stages of development, between ethylene treatment and increase in respiration, suggests a change in the tissue without immediate response. This is in striking contrast to the effect of ethylene on the respiration of non-climacteric fruits (e.g. citrus fruits—Biale, 1961) and certain storage organs such as potatoes (Reid & Pratt, 1970) and swedes (Rhodes & Woolterton, 1971).

Since ethylene production by fruits during the climacteric period is considered to be an autocatalytic reaction (Kidd & West, 1945), the rate of production should rise exponentially, and this is consistent with the rapid increase in the ethylene evolution, when *measurable* evolution has begun (Fig. 1, Fig. 2, middle curve). The same argument applies to the evolution by the peel discs (Fig. 2, top curve).

The apparent lag in ethylene production in the sensitive pre-climacteric phase combined with the increase in respiration is most probably because ethylene is accu-

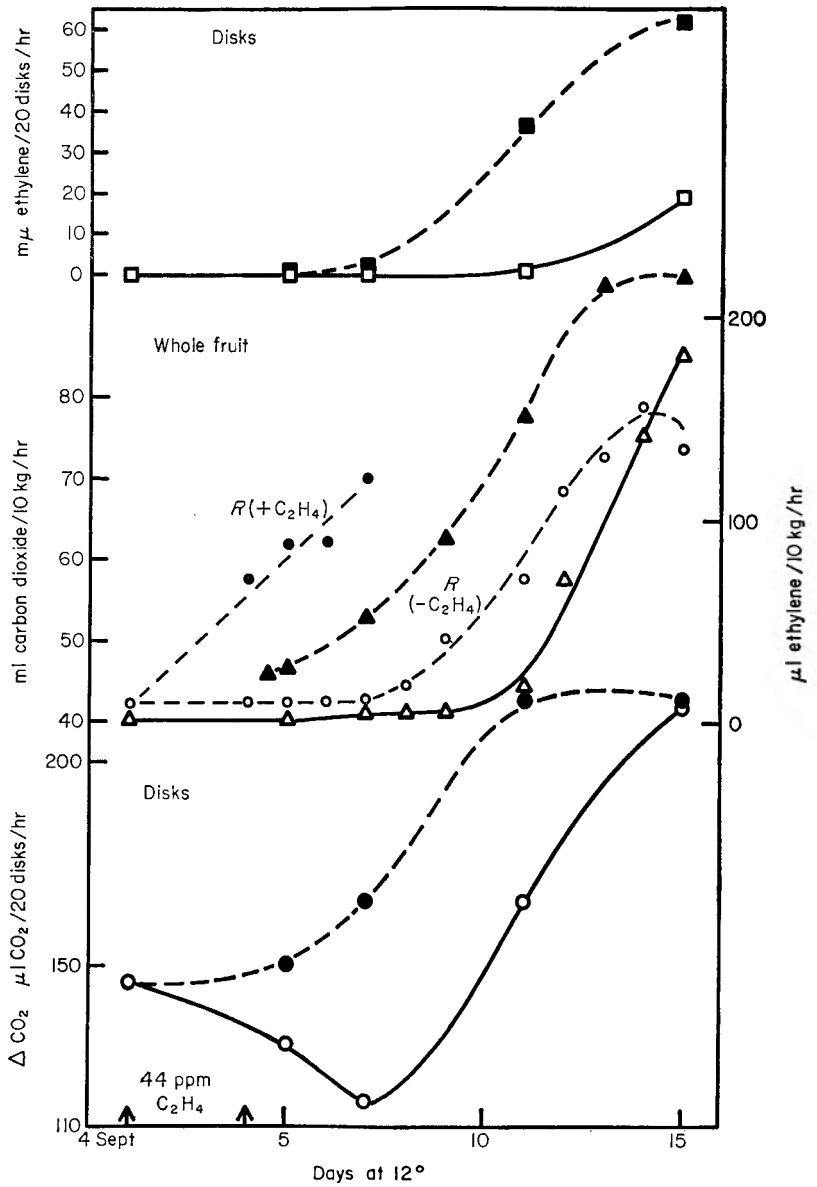

FIG. 2. Respiration and ethylene production of whole apples, and ethylene production and malate effect of peel disks prepared therefrom, for apple fruit with and without the addition of 44 ppm ethylene for 3 days, as indicated by arrows.

Upper set of curves. Ethylene production of disks from control (□) and ethylene treated (■) fruits.

Middle set of curves. Whole fruit respiration of control (O) and ethylene treated (●) fruits. Ethylene production of control (△) and ethylene treated (▲) fruits.

Lower set of curves. CO_2 produced ($\triangle CO_2$) by addition of malate to disks of untreated (O) and ethylene treated (●) fruits.

mulating within the tissue at the physiologically active level, before the level outside the fruit reaches measurable amounts. Burg & Burg (1962) showed that, for several fruits appreciable quantities of ethylene may be present within the cells before measurable amounts are evolved by the fruit. Pratt & Goeschl (1968) found for the Honeydew melon that *internal* concentrations of 3 ppm obtained just before the respiration rate was stimulated into the climacteric rise.

There appear to be at least two possible reasons why the threshold value of ethylene for physiological activity decreases as the fruit develops. It could be that an inhibitor of ethylene action, probably entering the fruit from the tree, could decrease as the fruit matures on the tree. Alternatively, it could be that for its physiological action ethylene must combine with an 'effector' which only appears when the tissue is in a receptive state. The former supposition is supported by the fact that the respiration climacteric, at comparable temperatures, develops much more slowly on the tree than after detachment. On the other hand, it would appear that some 'effector' is required since the action of ethylene is not just a switching on mechanism at all stages of development. Furthermore, it has been shown that ethylene acting on discs of peel from pre-climacteric apples initiates RNA and protein synthesis (Hulme *et al.*, 1971). It is of course possible that both factors are operative, or even that there is some, as yet unknown, change in the 'physiological state' of the tissue.

It is relevant here to mention that ethylene produced in low O_2 (3% O_2; 97% N_2) or given to fruit maintained in low O_2 only *temporarily* raises the respiration rate and malate decarboxylation by discs of peel tissue (Hulme *et al.*, 1970). When, however, the O_2-tension is raised to that of air the rate of respiration, ethylene production and the development of the malate effect increase quite rapidly to the full, ethylene stimulated level as in air. It could be that the ethylene 'effector', removal of inhibitor, or whatever is involved in the move to 'receptive state' (as well as the production of small amounts of ethylene) is developed in low O_2 but that higher concentrations of O_2 are required for its operation. Such a theory provides a working hypothesis for further experiments on the action of ethylene in the ripening of apples.

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Protein quality and quantity: A rheological assessment of their relative importance in breadmaking

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Summary

It is suggested that the terms 'strong' and 'weak' derived from bakery experience may not always be directly applicable to the quality of gluten present in such flours, and may be more related to gluten quantity than hitherto believed. It is clearly demonstrated in this work that the quality of glutens derived from 'strong' and 'weak' flours may be similar, although the glutens may be present in dissimilar quantity. There are, of course, certain flours which have poor glutens but until these are detected by precise rheological measurements, it is wrong to assume that the empirical rule 'weak flour means poor gluten quality' holds true.

Introduction

Rheological measurements have traditionally been used to give some indication of the probable baking quality of a dough. These measurements have often been interpreted in terms of protein quantity and quality: the latter being ill-defined. While the importance of both protein quality and quantity is generally accepted, data from which to judge the relative importance of these two factors is tenuous.

It is known that for the production of a commercially acceptable loaf, the flour must contain not less than, say, 10% protein but it is said that this protein must be of good quality (Kent-Jones & Amos, 1967).

Any investigation into flour protein quality using the rheological properties of a dough must of necessity take into account the quantity of protein present. Thus Aitken & Geddes (1939) showed that when freeze dried gluten, obtained from a flour of known breadmaking quality, was used to raise the protein content of the parent flour, a linear relationship was followed between loaf volume and the protein content of bromated doughs.

Recently it has been shown that the physical properties of a dough, appear very largely to be a reflection of the properties of its gluten (Heaps *et al.*, 1968). While it was realized that the gluten content and the protein content of a flour are not equal, and that the water soluble proteins may play an important role in the baking process, it

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was nevertheless felt that an investigation into gluten properties would be instructive in assessing the baking quality of the parent flour since often the gluten represents more than 75% of the total protein.

Various methods of determining the character or quality of gluten appear in the literature, each method necessarily incorporating its own definition of gluten quality. For example, Berliner & Koopman (1929) related gluten quality to the imbibitional or swelling power of the gluten while Baker, Mize & Parker (1943) denoted gluten quality in terms of the force required to rupture a ball of gluten using a spherical headed probe. Hyldon (1964) devised the term 'Gluten Efficiency Factor' to indicate gluten quality when using a Farinograph method based on comparison with the performance of a selected gluten.

With the variety of methods comes a variety of conclusions drawn regarding gluten composition. Wood (1907) described experiments with gluten in dilute acids and salt solutions and suggested that 'the variations in coherence, elasticity, and water content observed in gluten extracted from different flours are due rather to the varying concentrations of acids and soluble salts in the natural surroundings of the gluten than to any intrinsic difference in the composition of the glutens themselves'. Bungenburg de Jong & Klaar (1952) considered that perhaps the physical properties of gluten could be represented by a simple mixture of gliadin and glutenin—a view which Fleurent proposed as early as 1896. Sullivan (1954), however, considered that the difference between these two protein fractions was too small to explain adequately the observed variations in the physical properties of gluten while Bungenburg de Jong and Klaar found that outside a definite pH region, the law of additive properties was obeyed; but within the pH region, a complex system involving interactions between the two fractions was observed. A further view on gluten properties has been proposed more recently by Kaminski & Halton (1964) who from results obtained using an Extensometer on gluten-starch doughs suggested that glutens differed in their state of oxidation rather than in intrinsic strength. Referring to the failure of immunoelectrophoresis studies to show any marked qualitative differences between proteins from two widely different wheat samples, Binger (1965) stated 'while we cannot yet be absolutely certain that qualitative differences do not exist among proteins of different wheats, there is quite a high degree of certainty to this conclusion'.

A similar range of opinion is obtained when reference is made to the more specific comparison of glutens from strong and weak flours. Fisher & Halton (1933) commenting on results obtained by Berliner and Koopman mention the extent to which glutens from different flours swelled in acid solutions. Those from strong flours showed considerable swelling whilst those from weak flours little or no swelling. It is further stated that gluten from a weak flour not only imbibes water at a slower rate than a strong gluten, but it also changes more rapidly from the gel to the sol condition. Recently Matsuo & McCalla (1964) in a study of glutens from three different types of wheat concluded that 'differences among the three glutens could not be detected by sedimentation velocity,

distribution of sedimentation coefficients, molecular weights, diffusion, and amino acid composition studies'. Tracey (1967) has noted that although a number of attempts have been made to relate the constituent proteins of gluten to wheat quality, these attempts have in general been unsuccessful.

Thus, in view of the widely found similarity in chemical structure between proteins from different wheat flours, it would appear that the property called quality is perhaps more related to the physical state of the gluten than to its chemistry.

Perhaps the foregoing references become more comprehensible when one reads Farrand's article 'Bread Production Using Soft Flour' (Farrand, 1967). After mentioning the widely held belief that soft wheats give weak glutens and hard wheats give strong glutens, he goes on to say 'However, if glutens from hard and soft wheats are washed out and compared, frequently no significant difference between the physical characteristics can be objectively established. On the other hand, glutens obtained from two soft wheats or two hard wheats may differ significantly'. In other words, it may be that no hard and fast relationship exists between the baking performance of a flour and its gluten quality. With this in mind, the present work was initiated with the object of measuring both the quality and quantity of glutens obtained from as varied a range of flours as possible so as to determine, if possible, the relative importance of these two factors in breadmaking.

Materials and methods

Fourteen flours covering a broad spectrum of protein content were examined in this investigation. The analyses for protein content, water absorption, moisture content and acidity are shown in Table 1. The acidity analyses were obtained from aqueous suspensions of the flours and are recorded as percentage titratable lactic acid present. All wheats were milled in a Buhler M.C.K. automatic mill.

The gluten contents of the flours were determined by washing out with tap water as much starch as possible from a known quantity of flour using a Henry Simon gluten-washer. This method was considered to produce a minimum degree of devitalization of the gluten. The gluten was freeze dried, weighed and its protein content determined by a micro-Kjeldahl technique.

Rheological tests

About 220 g of gluten from each flour were prepared for testing. Initial rheological tests on the glutens were carried out as follows: five 10 g balls of gluten were kept under water overnight, at 5°C, to ensure that any stresses introduced during moulding had relaxed. They were warmed up to 30°C for 45 min and their relaxation times (defined as the time taken to relax, under constant strain, from 90 g compressive load to 60 g compressive load) were measured on an Instron tester at 30°C $\pm$ 1°C using a cross-head speed of 1 cm/min. The remainder of the gluten was freeze dried, ground and

TABLE 1. A brief analysis of the flours

	Flour	Moisture content (%)	Protein content (%)	Water absorption 'as received' (%)	Acidity as lactic acid (%)
1	Russian	15·2	13·8	57·0	0·24
2	N.S.W. Australian Prime Hard	14·0	13·1	60·6	0·27
3	No. 2 Atlantic Manitoba	15·2	12·7	56·8	0·25
4	Russian Spring White	15·5	11·3	57·4	0·23
5	N.S.W. Australian Northern Area	14·3	11·2	57·0	0·23
6	Mildress	14·0	11·5	53·2	0·26
7	Hungarian	14·5	10·0	56·3	0·20
8	Hybrid 46	14·0	9·8	53·8	0·25
9	Spanish High Protein (b)	13·8	9·5	54·7	0·22
10	West Australian	13·8	9·0	55·5	0·20
11	English Biscuit	13·0	9·0	52·7	0·22
12	Spanish High Protein (a)	13·9	8·9	53·8	0·20
13	American Soft White	13·9	8·2	49·5	0·20
14	French	14·1	7·4	49·5	0·20

stored pending further use. The effect of the freeze drying/rehydration process upon gluten relaxation times was studied by rehydrating the powdered 'gluten' and testing as above. The same test pieces were then freeze dried a second time to observe any further effect on gluten properties.

Doughs from each flour were mixed at a constant dough weight of 470 g in a stainless steel clad Farinograph bowl at 30°C ± 1°C attached to a modified Brabender Do-Corder. The recipe used was 2% salt, based on the weight of flour, plus water added to give the 'as received' water absorption of the flour less 4% to facilitate dough handling.

The doughs were mixed at a constant rate of 0·2 h.p. min/lb/min in a work input range 0–3·6 h.p. min/lb. The relaxation times (180 g compressive load to 100 g compressive load using a crosshead speed of 1 cm/min) of the different samples were determined by moulding five 10 g balls, storing for 20 min in a humidified cabinet at 30°C ± 1°C and testing on the Instron tester. Extensibility (E_{max}) was determined by moulding a 150 g dough piece and allowing it to rest for 45 min in a humidified cabinet before stretching on the Extensograph. The values of E_{max} being those which correspond to the maximum force observed.

Baking tests

The baking tests were carried out on the flours in the following manner:

(i) A control bake on each flour using the following standard recipe: yeast 6 lb/

sack (2·14%), salt 5 lb/sack (1·79%), Soyswift* 3 lb/sack (1·07%) plus water to give a satisfactory final consistency in mixing.

(ii) To those flours from wheats which are not normally regarded as being of bread baking quality, namely, Mildress, Hybrid 46 (English wheat varieties), Hungarian, Spanish High Protein (a) and (b) (both Mara type), West Australian, English Biscuit (predominantly Cappelle variety), American Soft White and French, was added their own freeze dried 'gluten'. The protein contents of the flours were raised to 12·2% approximately; this being about the average protein content of the bread baking flours. The standard recipe was used except that the quantity of water added was that percentage used in bake (i) plus water equal to twice the weight of the added dried 'gluten', since 'washed-out' gluten contains about two-thirds water.

The flours from breadmaking wheats, namely, Russian, N.S.W. Australian Prime Hard, No. 2 Atlantic Manitoba, Russian Spring White and N.S.W. Australian Northern Area, were mixed according to the standard recipe without the addition of gluten, and were used as process controls.

(iii) In view of the observed changes in relaxation time of gluten with freeze drying, reported later, and its possible effect on baking test (ii), it was considered necessary to carry out a further baking test comparing doughs supplemented with (a) freeze dried gluten, and (b) 'washed-out' gluten (i.e. material which had not been subjected to the lyophilization process).

The level of work introduced in all cases was 0·4 h.p. min/lb at a rate of 0·2 h.p. min/lb/min. The mixing bowl was kept at 26°C in order to produce a final dough temperature of 30°C $\pm$ ½°C. Three 150 g pieces of dough were handed up in a Mono Universal Moulder, rested for 10 min in a humidified cabinet at 30°C $\pm$ 1°C and moulded again. The moulded dough was proved for 50 min at 110°F $\pm$ 5°F at 70% $\pm$ 5% relative humidity and baked for 30 min at 450°F.

The specific volumes of the resulting loaves were determined and photographic records kept of both loaf and crumb appearance.

Rheological tests were carried out on the doughs (without yeast and Soyswift) corresponding to all the bakes except bake (iii).

Results and discussions

Gluten relaxation times which cover a wide range of values are shown in Table 2 together with the experimental recovery of glutens from their flours. These results show that all flours which are acknowledged to be of good breadmaking ability yield glutens of high relaxation time. The converse is not necessarily true: some of the so-called 'weak' flours, e.g. American Soft White, French, West Australian, Spanish High

*A commercial bread improver containing yeast food, oxidants, fat and soya flour.

TABLE 2. Showing the relaxation time and percentage recovery of the gluten from each flour.

	Flour	Protein content (%)	Gluten relaxation time (sec)	Gluten recovered	
				Flour basis (%)	Protein basis (%)
1	Russian	13·8	17·1 ± 1·0	11·4	82·6
2	N.S.W. Australian Prime Hard	13·1	14·7 ± 0·3	10·9	83·2
3	No. 2 Atlantic Manitoba	12·7	19·4 ± 0·8	10·3	81·1
4	Russian Spring White	11·3	17·5 ± 0·6	9·2	81·5
5	N.S.W. Australian Northern Area	11·2	20·0 ± 0·3	9·0	80·4
6	Mildress	11·5	3·3 ± 0·2	9·1	79·1
7	Hungarian	10·0	3·2 ± 0·2	8·1	80·9
8	Hybrid 46	9·8	10·7 ± 0·3	7·6	77·9
9	Spanish High Protein (b)	9·5	19·8 ± 0·4	6·7	70·5
10	West Australian	9·0	23·9 ± 0·7	6·9	76·7
11	English Biscuit	9·0	14·0 ± 1·4	6·8	75·6
12	Spanish High Protein (a)	8·9	1·6 ± 0·1	6·7	75·3
13	American Soft White	8·2	26·8 ± 1·3	6·2	75·8
14	French	7·4	20·2 ± 1·0	6·0	81·0

Protein (b), giving rise to glutens with relaxation times at least as high as the highest observed amongst the breadmaking flours. The good quality glutens in these four flours appeared in low quantities, suggesting that it may be only a deficiency in the quantity of gluten that prevents a good loaf from being obtained.

A further observation from Table 2 is that a particular flour type does not have a specific gluten quality. In fact, it was found that the gluten quality obtained was related only to the particular sample under test. This was exemplified by the extreme gluten relaxation times (19·8 and 1·6 sec) obtained from the two Spanish High Protein flour samples. (The following relaxation data have been obtained in earlier unpublished work on glutens from nominally similar flours and although they do not bear directly on the present paper, they do provide further evidence in support of this observation: (a) N.S.W. Australian Northern Area, 10·9, 12·5, 13·0, 15·6, 20·0 sec, (b) Russian, 17·1, 30·4 sec, (c) No. 2 Atlantic Manitoba, 19·4, 22·8, 43·6 sec, (d) English Biscuit, 8·4, 15·2 sec, (e) French, 16.1, 20·2 sec).

Tables 3 and 4 summarize the results of the rheological tests performed on the flour–salt–water doughs. The determinations of dough relaxation time (which, as with gluten, is being taken to be synonymous with strength) and extensibility here revealed two quite distinct types of response to mechanical work input as follows:

1. Most of the flours, including all recognized breadmaking flours, exhibited an increase in dough strength to a maximum with increasing work input. At the

TABLE 3. The dependence of maximum extensibility (E_{max}, cm) upon mechanical work input (Rate of work input = 0·2 h.p. min/lb/min.)

	Flour	Total work input (h.p. min/lb.)							
		0·1	0·6	1·1	1·6	2·1	2·6	3·1	3·6
1	Russian	30·5	25·0	17·5	15·3	13·9	12·1	11·0	10·1
2	N.S.W. Australian Prime Hard	28·9	20·4	17·4	12·4	9·7	11·1	9·0	8·2
3	No. 2 Atlantic Manitoba	28·2	24·9	17·2	13·1	11·7	9·3	10·8	7·5
4	Russian Spring White	28·7	15·3	11·4	10·0	8·9	8·4	8·2	7·8
				12·4	10·9				
5	N.S.W. Australian Northern Area	25·8	21·8	14·5	11·0	10·2	7·7	7·8	6·9
9	Spanish High Protein (b)	19·7	15·3	11·3	9·3	8·0	7·0	6·0	6·1
10	West Australian	18·4	15·3	11·4	8·3	6·4	5·7	5·5	6·3
12	Spanish High Protein (a)	11·1	11·4	6·8	6·4	5·6	5·0	4·7	—
			11·4						5·1
13	American Soft White	13·8	12·4	11·9	9·2	8·0	7·5	5·9	4·2
14	French	12·9	11·9	8·8	7·9	6·7	6·0	5·5	5·1
									5·3
7	Hungarian	18·5	16·1	10·4	8·2	7·9	7·2	6·4	5·5
		0·05	0·1	0·3	0·4	0·6	0·8	1·1	1·6
6	Mildress	5·3	3·1	4·4	—	4·4	—	—	—
			3·6						
8	Hybrid 46	10·7	15·9	16·9	—	14·5	6·0	3·1	—
11	English Biscuit	14·9	16·8	—	18·0	19·2	18·3	16·2	12·4
		14·0							

same time, a general decrease in E_{max} was observed for these flours. This type of response has been reported earlier (Webb *et al.*, 1970).

2. In this category, which includes four flours, namely Hybrid 46, Mildress, American Soft White and English Biscuit, little change in dough strength with work input was observed, whereas the response of E_{max} ranged from the decrease reported under (1) to an increase to a maximum.

These results would appear to be properties related to the dough as a whole rather than specifically to gluten quality or quantity. It is possible that the presence of water-soluble proteins or enzymes (proteolytic or amylolytic) may affect the properties of the dough but which would be washed away in the preparation of gluten for quality determination.

TABLE 4. The dependence of dough relaxation time (R.T., sec) upon mechanical work input
(Rate of work input = 0·2 h.p. min/lb/min)

	Flour	Total work input (h.p. min/lb)							
		0·1	0·6	1·1	1·6	2·1	2·6	3·1	3·6
1	Russian	4·9 ± 0·2	9·4 ± 0·3	15·9 ± 0·5	18·0 ± 0·8	18·4 ± 0·2	19·1 ± 0·7	14·9 ± 0·2	13·9 ± 0·3
2	N.S.W. Australian Prime Hard	6·9 ± 0·2	10·2 ± 0·3	12·9 ± 0·4	17·6 ± 0·5	16·5 ± 0·7	14·3 ± 0·7	13·3 ± 0·8	12·0 ± 0·9
3	No. 2 Atlantic Manitoba	5·1 ± 0·1	7·2 ± 0·2	11·4 ± 0·4	15·1 ± 0·4	15·6 ± 0·8	15·3 ± 1·0	14·5 ± 0·5	12·1 ± 0·3
4	Russian Spring White	5·5 ± 0·4	17·8 ± 0·6	25·5 ± 0·6 22·5 ± 0·5	24·1 ± 0·1 24·4 ± 0·7	24·8 ± 0·5	20·0 ± 0·7	15·1 ± 0·2	14·5 ± 0·2
5	N.S.W. Australian Northern Area	6·3 ± 0·3	7·6 ± 0·2	11·3 ± 0·2	13·8 ± 0·3	15·2 ± 0·6	17·1 ± 0·4	14·1 ± 0·4	11.9 ± 0·3
9	Spanish High Protein (b)	5·8 ± 0·4	6·8 ± 0·3	8·6 ± 0·2	10·4 ± 0·3	11·3 ± 0·5	12·0 ± 0·2	12·9 ± 0·3	12·4 ± 0·5
10	West Australian	4·6 ± 0·1	6·1 ± 0·2	8·7 ± 0·2	12·6 ± 0·3	13·9 ± 0·5	17·3 ± 0·3	14·6 ± 0·2	12·6 ± 0·4
12	Spanish High Protein (a)	4·4 ± 0·1	4·5 ± 0·6 4·3 ± 0·3	6·8 ± 0·4	7·7 ± 0·3	9·7 ± 0·3	10·4 ± 0·2	10·0 ± 0·5	11·0 ± 0·3 9·5 ± 0·4
13	American Soft White	4·2 ± 0·2	3·9 ± 0·3	4·1 ± 0·2	4·1 ± 0·3	4·6 ± 0·2	3·5 ± 0·3	3·9 ± 0·2	3·6 ± 0·2
14	French	3·9 ± 0·2	5·7 ± 0·5	8·1 ± 0·6	8·7 ± 1·0	10·1 ± 0·6	9·0 ± 0·6	8·6 ± 0·7	10·1 ± 0·7 9·0 ± 0·4
7	Hungarian	4·5 ± 0·1	6·9 ± 0·2	9·6 ± 0·3	11·7 ± 0·2	13·5 ± 0·6	12·7 ± 0·3	11·8 ± 0·2	9·5 ± 0·3
		0·05	0·1	0·3	0·4	0·6	0·8	1·1	1·6
6	Mildress	1·00 ± 0·04	0·96 ± 0·02 0·82 ± 0·02	1·26 ± 0·04	—	1·5 ± 0·1	—	—	—
8	Hybrid 46	3·3 ± 0·2	2·7 ± 0·1	2·1 ± 0·1	—	1·34 ± 0·02	1·40 ± 0·03	1·32 ± 0·04	—
11	English Biscuit	5·0 ± 0·4 3·5 ± 0·2	3·8 ± 0·1	—	3·3 ± 0·1	3·2 ± 0·1	3·6 ± 0·1	3·3 ± 0·3	2·6 ± 0·1

Figs. 1 and 2 show the dependence upon mechanical work of relaxation time and maximum extensibility respectively, for the two different types of response to work input.

In order to test the possibility that it may be only a deficiency in the quantity of gluten that prevents the production of a good loaf from some weak flours, it was decided to bake doughs in which the natural protein content of the flours had been supplemented with their own freeze dried gluten. However, before discussing the results of the baking tests, a number of points should be made relating to the changes in gluten properties occurring as a result of the freeze drying/rehydration cycle.

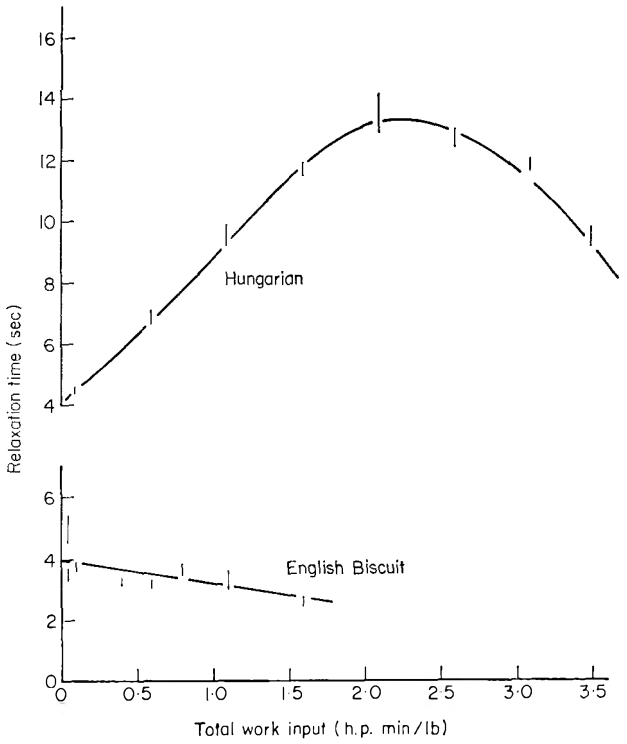

FIG. 1. The dependence of dough relaxation time upon total work input for Hungarian and English Biscuit flours. (Added water = 52·3% and 48·7% for Hungarian and English Biscuit flours respectively; rate of work input = 0·2 h.p. min/lb/min)

The freeze dried glutens were rehydrated and tested, as described earlier, on the Instron tester. The results, which include materials taken twice through the lyophilization/rehydration cycle, are summarized in Table 5.

The relaxation times of all glutens were seen to increase during the first cycle. Of

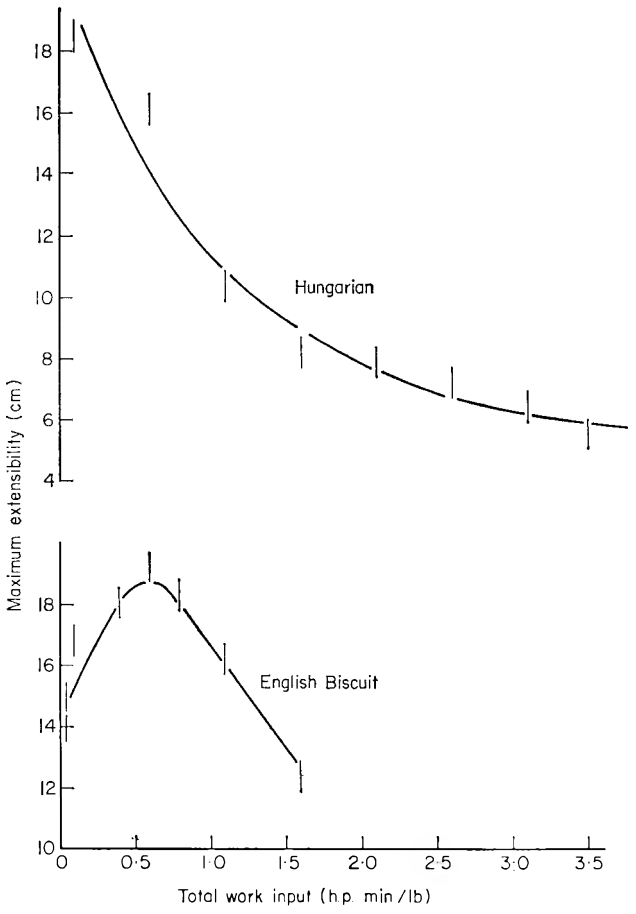

FIG. 2. The dependence of maximum extensibility upon total work input for Hungarian and English Biscuit flours. (Added water = 52·3% and 48·7% for Hungarian and English Biscuit flours respectively; rate of work input = 0·2 h.p. min/lb/min)

the nine flours which were considered to require supplementation, two (American Soft White and French) were outstanding.

As a result of the second cycle, those glutens which were originally noted as of good quality were improved further, whilst the relaxation times of the poor glutens were reduced in some cases to values below those of their 'washed-out' glutens.

Four aspects of the freeze drying/rehydration cycle were considered as possible causes of the observed changes, i.e. reduction in temperature, removal of water, grinding and replacement of water. These possibilities were examined by washing out approximately 180 g of gluten from three unbleached, untreated flours: A, being a bakers' grade flour, B and C being two weak flours, an English Biscuit and a Finnish flour respectively. 60g of the gluten sample were prepared for relaxation time measure-

TABLE 5. Showing the effect of freeze drying and rehydration on gluten relaxation time.

	Flour	'Washed-out' gluten R.T. (sec)	Freeze dried and rehydrated once R.T. (sec)	Freeze dried and rehydrated twice R.T. (sec)
1	Russian	17·0 ± 1·0	23·5 ± 0·3	61·8 ± 4·2
2	N.S.W. Australian Prime Hard	14·7 ± 0·3	22·1 ± 0·4	41·7 ± 1·4
3	No. 2 Atlantic Manitoba	19·4 ± 0·8	26·1 ± 1·6	76·5 ± 9·6
4	Russian Spring White	17·5 ± 0·6	52·5 ± 1·8	84·0 ± 4·2
5	N.S.W. Australian Northern Area	20·0 ± 0·3	27·8 ± 1·2	51·8 ± 3·0
6	Mildress	3·3 ± 0·2	4·1 ± 0·1	2·6 ± 0·02
7	Hungarian	3·2 ± 0·2	5·4 ± 0·1	1·8 ± 0·1
8	Hybrid 46	10·7 ± 0·3	16·1 ± 0·4	9·0 ± 0·5
9	Spanish High Protein (b)	19·8 ± 0·4	28·6 ± 1·5	59·4 ± 6·0
10	West Australian	23·9 ± 0·7	29·9 ± 1·2	69·8 ± 1·6
11	English Biscuit	14·0 ± 1·4	20·9 ± 1·8	25·5 ± 0·3
12	Spanish High Protein (a)	1·6 ± 0·1	3·8 ± 0·1	2·3 ± 0·04
13	American Soft White	26·8 ± 1·3	61·2 ± 0·8	94·8 ± 2·4
14	French	20·2 ± 1·0	40·0 ± 0·4	53·1 ± 2·4

ment – the remainder was freeze dried. Half of the freeze dried sample was rehydrated under liquid nitrogen according to the method of Davies, Daniels & Greenshields (1969), thus eliminating any heat of hydration, while the second half was rehydrated at room temperature with an excess of water; this excess being removed by placing the mixture into the gluten washer for a short while. All rehydrated glutens were then prepared and tested in the usual manner. The results of the test are shown below:

Process	Relaxation Time (sec)		
	Flour A	Flour B	Flour C
1. 'washed-out' gluten	15·9	16·5	13·3
2. rehydrated at room temperature	34·0	27·7	26·0
3. rehydrated under liquid nitrogen	62·0	32·4	26·9
S.E. mean	1·296	1·251	0·891
L.S.D. (P = 0·05)	3·96	3·87	2·73
(= 0·01)	5·52	5·40	3·78
(= 0·001)	7·74	7·65	5·31

Relaxation times of the rehydrated glutens (processes 2 and 3) for all three flours were very significantly higher than those of the corresponding 'washed-out' glutens (process 1). (2) was very highly significantly lower than (3) on flour A but was just significantly lower on flour B and on flour C there was no difference at all. Having

eliminated the heat of hydration in process (3), the only other aspect of the freeze drying/rehydration process to be duplicated is that of reduction in temperature. These results would suggest, therefore, that severe cooling of gluten could account for the observed changes in relaxation time: (2) involved freezing the gluten once, (3) involved freezing the gluten once and also severely cooling the powdered 'gluten' with liquid nitrogen ($-196°C$).

It has been shown that it is possible to increase gluten relaxation time by the application of mechanical work to dough systems (Heaps, *et al.*, 1968), furthermore, the mechanism of the Chorleywood Bread Process is said to be the scission of disulphide bonds allowing a reformation of a more orderly system to produce a stable, expandable protein network (Axford & Elton 1960). It seems possible that the severe cooling of gluten may cause rupture of disulphide bonds enabling a reformation of bonds to effect the changes in relaxation times observed. The decreases in relaxation times with the second cycle could then be an effect of over-development or breakdown.

Baking tests

The specific volumes of all the loaves baked and the additions of water used in baking are shown in Tables 6 and 7. Table 6 illustrates the changes in specific volumes of the loaves from the nine weak flours with the addition of their own glutens (bake (ii)), gluten which had been freeze dried once only being used for supplementation.

In the supplementation experiments, the effect of the increased gluten relaxation times resulting from freeze drying would be expected to be maximal for the two outstanding cases referred to in view of (a) the relatively high level required for supplementation and (b) the especially high relaxation time of the freeze dried material. In other cases, the effect is considered to be less important.

When considering external appearance, crumb structure and colour as well as the specific volumes of the loaves, the results of the baking tests show that in baking test (i), the control bake, the best loaves, as expected were obtained from the recognized breadmaking flours; these being the only loaves of commercial standard. The poorest loaves, i.e. small uneven loaves with a grey close texture, were obtained from the Hybrid and Mildress flours. It is seen from Table 6 that only the extensibilities of the flour–salt–water doughs corresponding to the best loaves are within the suggested optimum range 20–28 cm (Heaps, Russell Eggitt & Coppock, 1965). With baking test (ii) there was a considerable improvement, in general, in the baking quality of those flours to which gluten was added. In particular the loaves from Spanish High Protein (b), American Soft White, French and West Australian flours were now considered to be of commercial quality together with the five process controls. Although there was a slight improvement in the loaves from Hybrid and Mildress flours, they were still poor having thick cell structure, a coarse crumb and a poor colour. It is seen from Table 6 that while generally there was little change in relaxation time,

TABLE 6. The specific volumes of the loaves from baking tests (i) and (ii)—together with the rheological properties of the corresponding flour–salt–water doughs.

	Flour	Baking test (i)—Flour alone					Baking test (ii)—Flour + Gluten				
		Total Protein (%)	Added water (%)	Specific volume (ml/g)	E_{max} (cm)	R.T. (sec)	Total Protein (%)	Added water (%)	Specific volume (ml/g)	E_{max} (cm)	R.T. (sec)
1	Russian*	13·8	58·8	4·41 ± 0·04	26·6	7·8			4·06 ± 0·02		
2	N.S.W. Australian Prime Hard*	13·1	62·4	3·81 ± 0·08	23·6	8·8			3·92 ± 0·03		
3	No. 2 Atlantic Manitoba*	12·7	58·6	4·28 ± 0·02	25·8	6·2			4·19 ± 0·03		
4	Russian Spring White*	11·3	59·2	4·17 ± 0·07	20·5	12·7			4·06 ± 0·02		
5	N.S.W. Australian Northern Area*	11·2	58·8	4·34 ± 0·08	22·5	7·0			4·01 ± 0·04		
6	Mildress	11·5	47·2	3·00 ± 0·01	1·4	4·0	12·0	48·4	3·38 ± 0·02	4·0	1·5
7	Hungarian	10·0	58·1	3·73 ± 0·06	16·0	5·7	12·0	62·1	3·79 ± 0·03	27·4	2·8
8	Hybrid 46	9·8	47·8	2·83 ± 0·03	16·3	1·8	12·2	52·7	3·40 ± 0·04	22·4	2·8
9	Spanish High Protein (b)	9·5	54·7	3·94 ± 0·06	16·7	6·3	12·0	60·6	4·56 ± 0·05	29·6	4·9
10	West Australian	9·0	55·5	3·98 ± 0·04	16·4	5·0	12·4	61·9	4·22 ± 0·02	32·1	4·1
11	English Biscuit	9·0	50·7	3·75 ± 0·04	18·0	3·3	12·4	57·4	4·28 ± 0·04	33·0	4·5
12	Spanish High Protein (a)	8·9	53·8	3·87 ± 0·02	9·8	4·2	12·2	60·6	4·18 ± 0·04	21·6	2·2
13	American Soft White	8·2	47·5	3·99 ± 0·02	13·0	4·0	12·4	56·1	4·67 ± 0·04	25·7	4·1
14	French	7·4	49·5	4·09 ± 0·03	11·5	4·8	11·9	58·8	4·55 ± 0·06	33·5	5·8

* Process control

i.e. little change in dough strength, following gluten supplementation, in all cases except Mildress, there was a considerable increase in extensibility, often being in excess of the suggested optimum (20–28 cm) whereas without supplementation the doughs were below this range.

Ideally the effect of gluten supplementation on the baking performance of a weak flour should be carried out using 'washed-out' gluten. Since the time involved in washing sufficient gluten from each flour created experimental difficulties, it was decided to use the gluten in its freeze dried form. In view of the 'improved' nature of the dried gluten and its possible effect, other than that of a straightforward increase in gluten content, on the loaves of baking test (ii), baking test (iii) was used to compare the loaves obtained from an English Biscuit flour and a Finnish flour, which had been supplemented with their own glutens in both the 'washed-out' and freeze dried forms. These loaves were also compared with that produced by a No. 2 Atlantic Manitoba flour. The results of the bake are shown in Table 7.

TABLE 7. Showing the baking performance of English Biscuit and Finnish flours supplemented with both wet and freeze dried gluten

	Flour/gluten mixture	Total protein content (%)	Added water (flour wt basis) (%)	Loaf specific volume (ml/g)
1	English Biscuit flour	8·6	50·5	4·62 ± 0·07
2	English Biscuit flour and wet gluten	12·0	50·5	4·82 ± 0·01
3	English Biscuit flour and freeze dried gluten	12·0	58·4*	4·87 ± 0·02
4	Finnish flour	10·1	56·0	4·16 ± 0·02
5	Finnish flour and wet gluten	12·0	56·0	4·35 ± 0·02
6	Finnish flour and freeze dried gluten	12·0	60·2*	4·37 ± 0·06
7	No. 2 Atlantic Manitoba flour	12·7	63·0	4·14 ± 0·04

* Flour + dry gluten basis

The relevant analyses of the three flours are shown below:

	Protein content (%)	Water absorption 'as rec'd' (%)	Gluten relaxation time (sec)	f.d./rehydrated gluten relaxation time (sec)
English Biscuit	8·6	50·5	16·5 ± 0·3	27·7 ± 0·5
Finnish	10·1	56·0	13·3 ± 0·7	25·3 ± 0·6
No. 2 Atlantic Manitoba	12·7	61·2	22·8 ± 0·6	—

Comparing the loaves obtained from the English Biscuit flour, the supplemented loaves were considered to be better than the control, differing only in crumb properties. The loaf supplemented with dried gluten was marginally inferior to that supplemented with wet gluten because of a slightly more open texture.

With the loaves obtained from the Finnish flour the supplemented loaves were considered to be of equal quality and an improvement on the control loaf, which had a more coarse and irregular crumb.

The Manitoba flour produced a very good loaf with an even, well-formed crumb and a very good colour. It was inferior only in respect of loaf volume.

These results would indicate that the net increase in gluten quality brought about by the addition of freeze dried, rather than wet, gluten was negligible.

Considering the extensibilities of the flour–salt–water doughs corresponding to baking tests (i) and (ii), it is clear that supplementation of the flours with gluten increases their extensibilities. Thus the larger loaf volume obtained with gluten supplemented loaves may be attributable to better dough extensibilities.

The four weak flours, American Soft White, West Australian, French and Spanish High Protein (b), which gave loaves of commercial quality, were the flours with the best gluten qualities as indicated by gluten relaxation time. Other flours, to which gluten was added producing loaves which had large enough specific volumes but too poor crumb properties to be of commercial quality, generally had gluten relaxation times below those of the five process controls. This may indicate that gluten quality, perhaps, has more influence on crumb structure than on loaf volume; quantity, however, having its main effect on the latter.

Recent work (Webb & Isitt, unpublished work 1970) has shown that wheat protein, extracted with 2M citric acid and freeze dried, can also be used successfully to supplement weak flours, producing loaves of commercial quality.

Conclusions

Using gluten relaxation time as an index of gluten quality, the following inferences have been made:

1. (a) The best glutens are not restricted to the recognized breadmaking flours; some so-called weak flours have glutens as good as those of the breadmaking flours and only require an increase in good gluten content to produce bread of acceptable commercial quality. (b) A flour type does not have a specific gluten quality – a quality determination relates only to the flour sample under test, see, for example, the extreme gluten relaxation times obtained from the two Spanish High Protein flour samples.

2. Gluten quantity appears to be related directly to loaf volume whereas the importance of gluten quality lies in its relationship to crumb properties (texture, colour, softness).

3. The results of the lyophilization/rehydration tests would indicated that severe cooling of gluten can have the same net effect as mechanical development, probably by denaturation (Davies, J. D., 1968).

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Microbiological assessment of surfaces

J. T. PATTERSON

Summary

The main methods in use in laboratories for the microbiological assessment of surfaces are examined, viz. (1) the swab-rinse, (2) the rinse, (3) the agar contact, and (4) the direct surface agar plating. The advantages and disadvantages of these are discussed and their suitability for particular purposes in the food industry. Methods employed in the author's laboratory are discussed for sampling surfaces in milk plants, farm dairies, canneries, meat and poultry processing plants. Consideration is given based on actual investigations of the use and limitations of the swab-rinse technique and its variations and the rinse and the agar sausage techniques. The importance of careful interpretation of and use of the results is stressed. Microbiological standards are suggested for cleaned surfaces, working surfaces in meat and poultry processing plants and for cattle, sheep and poultry carcases.

Introduction

The problem of obtaining a representative sample of a foodstuff for examination is often difficult, and the microbiological assessment of surfaces is no less a problem, particularly where the spread of surface contamination is uneven and the surface rough, as is the case with animal carcases. Microbiologists have been concerned with the detection and enumeration of microorganisms on surfaces for over 50 years. The problem is a complicated one and even using the very best available techniques only a proportion of the bacteria or other microorganisms will be recovered, and sometimes this proportion is exceedingly small.

Sampling methods available

Literature dealing with the microbiological sampling methods for surfaces have been reviewed by Favero *et al.* (1968). These workers described four basic methods for enumeration of bacteria on surfaces, viz. (1) the swab-rinse, (2) the rinse, (3) the agar contact, (4) the direct surface agar plating. Some of these methods have more application than others in the food industry, and there are many variations of the basic types.

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(1) *The swab-rinse*

This has many forms, and is possibly the most widely used method. Essentially, a sterile swab is rubbed over the surface of the object to be sampled (the swab is moistened with sterile fluid if the surface is dry) and then the tip of the swab is broken into a tube containing a sterile diluent, shaken, and the rinse fluid plated with or on to an appropriate culture medium. There is often poor recovery of bacteria from the surfaces sampled, either because of the nature of the surface, or the amount of pressure applied to the swab, or the time and the speed of application to the surface. Different people use swabs in different ways, so the results may not be reproducible between samples, or between laboratories. The cotton also retains some of the microorganisms, causing reduced counts.

Various modifications have been made to reduce these errors. A sterile metal template can be used to outline a known area, inside which the swabbing is done. The time of swabbing can be standardized, e.g. 15 sec, and also the size of the swab and the amount and type of material used to make the swab. Likewise replicate swabs are sometimes used on the same area. Another approach to aid the recovery of bacteria from the swabs is to use a known weighed quantity of calcium alginate wool to replace the cotton wool and the alginate can be dissolved in Ringer's solution containing 1% of sodium hexametaphosphate. Higgins (1950) stated that calcium alginate swabs will dissolve in most sodium salts to give the soluble sodium alginate, thus freeing all the organisms taken up on the swab and giving a more accurate quantitative recovery. A special alginate wool was specified, free from the bactericidal action possessed by alginate containing a quaternary ammonium compound used in textile processing.

However, there is some evidence that the alginate swab does not recover small numbers of organisms as well as cotton wool (Barnes, 1952), and a suggestion that the alginate or sodium hexametaphosphate may be inhibitory to some micro-organisms (Angelotti *et al.*, 1958; Strong, Woodburn & Mancini, 1961). Others have reported higher recoveries with the alginate swab (Higgins, 1950; Tredinnick & Tucker, 1951; Cain & Steele, 1953; Walter, 1955).

(2) *The rinse method*

With this method the contaminated surface is either immersed (as in the case of small objects) in a sterile fluid, or the fluid brought into contact with the surface being examined. This may require aseptic removal of part of the surface into the diluent. Though essentially more accurate since all the surface, or a known section of it, is being sampled directly by the diluent fluid, there are certain disadvantages. Recovery from the surface may be low if the surface is such that it tends to retain the bacteria. Thus with poultry skin, recovery by this method is low unless special precautions are taken to remove the bacteria, e.g. by shaking an area of skin with an abrasive material such as rough sand in the sterile diluent. Greasy surfaces are also difficult to sample by this method, and large objects or large areas of surfaces mean that only a small

proportion can be sampled, and the results may not be representative of the whole due to uneven spread of contamination.

(3) *Agar contact methods*

Although there are many modifications of the agar contact method, basically it involves pressing a sterile nutrient agar surface against the surface to be sampled. The agar is then incubated and the adhering microorganisms enumerated. By the very nature of the method, it is most useful for smooth flat surfaces, and since dilution is not possible, only small numbers of contaminants can be enumerated. Rough surfaces, heavily contaminated, or those contaminated by spreading bacteria or moulds are not suited to the method, if enumeration is required.

The agar sausage technique often gives a lower apparent recovery of microorganisms than is achieved by other sampling methods. The reasons for this have been discussed by Riddle (1967) who pointed out that since the organisms contaminating a surface are present as micro-colonies, the swabbing technique breaks these up and gives a measure of individual viable cells. The agar sausage method gives a mirror-image of the number and distribution of these loci of infection on the surface and these may consist of one or many microorganisms.

Pictorial methods of representing the results are useful when dealing with non-scientific staff. Such methods (cited by Riddle, 1967) are those of Hansen (1962), Ten Cate (1965), Buchli (1965), Van Schothorst, Mossel & Kempelmacher (1966) and Mossell, Kampelmacher & Van Noorle Jansen (1966). For more accurate assessment of the amount and spread of surface contamination the method of Hansen (1962) can be used. This is a statistical procedure for measurement of bacterial surface contamination which can be applied to the agar sausage technique (Wilson, pers. comm.). The method consists of taking ten replicate agar sausage impressions of a surface and plotting the colony counts graphically on probability paper so that the logarithmic mean and standard deviation can be calculated.

(4) *Direct surface agar plating*

Contaminants on surfaces can be detected *in situ* by the direct surface agar plate (DSAP) method (Angelotti & Foter, 1958; Angelotti *et al.*, 1958), where sterile melted agar is poured on to the surface to be sampled and left to solidify under a sterile cover. After incubation the colonies at the interface are counted. Small items can be placed in a petri dish and covered with agar. This is mainly a laboratory technique since food plant surfaces are generally large, fixed, and cannot be incubated at a desired temperature. It is not applicable to dirty surfaces, since growth becomes confluent.

Sampling of surfaces in food plants

Microbiologists have to assess microbial contamination on surfaces in a wide variety of food plants. In this Laboratory these include farm dairies, milk processing plants,

canneries (both meat and fruit and vegetable), poultry processing plants, and abattoirs. In the main, unless by special arrangement, samples are not taken of cleaned surfaces in abattoirs and poultry plants, but hygiene of working surfaces is assessed during working hours. At the same visit (to abattoirs) samples are also taken from recently slaughtered carcases and from those in the cooling-rooms. In the case of poultry, a number (not greater than twelve) are brought back to the laboratory for examination, though on occasions, when sampling for specific organisms such as *Salmonella* and *Clostridium*, a larger number may be sampled during processing. On the other hand work in the farm dairy and in the milk processing plants is often concerned with the assessment of cleaned surfaces.

Sampling of farm dairy equipment

The methods used are based upon Technical Bull. No. 17 'Bacteriological Techniques for Dairy Purposes' M.A.F.F. (1968). These are surface *rinses* for milk cans, buckets, strainers, surface coolers, milk bottles, teat-cup cluster assemblies, pipelines, bulk tanks etc., and *swabs* for equipment difficult to rinse, viz: milking machine bucket lid, in-can cooler, bottling plant, cocks, gaskets, sections of pipelines, blades of pumps, bulk milk tanks etc. When taking the rinse sample sterile R/4 solution is used, the quantity depending upon the size of the equipment being sampled, the solution swilled or shaken in a standard manner for a standard time, e.g. for a milk can 500 ml of the rinse solution is poured into the lid, the lid replaced, the can laid on its side and given twelve complete revolutions. It is allowed to stand for 5 min and the rolling repeated and then the rinse poured back into the original bottle, which is taken to the laboratory under refrigerated conditions and tested in a standardized manner within specified time limits. Swabs are specially prepared 14 inches long with 6 inches of 2 inches wide unmedicated ribbon gauze at one end, sterilized in 10 inches × 1 inch test tubes with 25 ml R/4 solution. Where possible, 1 ft^2 is swabbed with the moist swab, rotating the swab under heavy pressure until the whole area is covered twice and all parts of the swab make contact with the surface. Thereafter the technique of plating is a standardized one. Similar techniques are used in the milk processing plant. Table 1 gives details of bacteriological standards being applied in the dairy industry.

Sampling of poultry carcases

Two situations present themselves in the poultry plant. The first is when specific organisms are being recovered such as salmonellae. In this case, one would expect such organisms to be present in greatest numbers on eviscerated carcases on heavily contaminated areas inside and around the vent and in the body cavity. Recent work on the spread of contamination on carcases after processing has shown that, in addition to the vent area, the loose neck skin is often more heavily contaminated. Sampling for specific organisms is by means of a flat swab stick $5\frac{1}{2}$ inches long with $1\frac{1}{2}$ inches × 6 inches of unmedicated cotton gauze wrapped tightly around one end, stapled to the

TABLE 1. Bacteriological standards for surfaces (milk plants)

		Coliform organisms	
	Colony count per container	Absent	Present
Washed bottles*			
	Not more than 200	Satisfactory	Not satisfactory
	201–600	Fairly satisfactory	Not satisfactory
	More than 600	Not satisfactory	Not satisfactory
Can rinse†	The total bacterial colony count shall not exceed 50,000 per can.		
Tanker swab†	The total bacterial colony count shall not exceed 50,000 per ft^2.		

* Advisory standard
† Statutory requirement

wood. The vent region, inside the body cavity, and the neck skin are thoroughly swabbed (two swabs in all) and the cotton gauze end broken into sterile dry 1 oz Universal bottles for refrigerated transport to the laboratory. There is enough moisture and nutrients on the bird to make transport fluid unnecessary. In the laboratory the swabs are broken into 10 ml sterile nutrient broth which is incubated for 3 hr at 37°C before enrichment for the detection of salmonellae.

The method of sampling poultry skin to test for total microbial contaminants (the second situation) is somewhat different. Poultry skin is rough, somewhat greasy, with many feather follicles. Patterson & Stewart (1962) examined several methods such as scraping with a scalpel blade, swabbing, swabbing followed by shaking in peptone with rough sand to act as an abrasive, and cutting out followed by shaking with sand. The method finally adopted was to remove a 16 cm^2 area of breast skin which was shaken vigorously on a shaker for 5 min in 10 ml of 0·5% peptone diluent with about 3 g of sharp rough washed sand. However, with this type of surface recovery of bacteria is not complete. In this particular study one swab removed rather more than half of that obtained by swabbing followed by cutting and shaking. In addition a 16 cm^2 area was repeatedly swabbed with cotton-gauze swabs with the results given in Table 2. Obviously two or three swabs are needed to obtain a good recovery and no record was made of how many bacteria still remained on the skin. For certain work, where as good a recovery as possible is required without damaging the product (e.g. turkey carcases) two swabs are used on the same area to try to reduce the error. Patterson & Stewart (1962) also applied an inoculum of *Serratia marcescens* to poultry skin and only succeeded in removing about 30% of the organisms by the methods employed. Williams (1967) stated that cotton wool swabs removed less than 10% of the organisms present on raw meat surfaces, though no details were given of the size of

TABLE 2. Repeated swabbing of poultry skin

No. of swab	Total bacteria recovered from 16 cm^2 of skin	% recovery of total removed by 5 swabs
1	440×10^3	38
2	490×10^3	43
3	104×10^3	9
4	89×10^3	8
5	19×10^3	2

swabs or how the swab was used. The spray method of Clark (1965), designed to recover bacteria from poultry skin, has given low recoveries in our hands, compared to the cut and shake method.

Sampling of cattle and sheep carcases

Similar kinds of problems occur when sampling these, and are complicated by the fact that there are both fatty and muscle surfaces, a large total surface area, and the carcases cannot be transported to the laboratory for sampling. In addition a non-destructive method is essential.

In this Laboratory, the method used at first was similar to that described by Dyett (1963). As much as possible of the surface of the sides of the carcase was scraped with a sharp knife to give 2–3 g of scrapings, which was then examined by surface plating. It soon became obvious that contamination varied greatly on different parts of the carcase, and if some help was to be given in improving butchering hygiene, more critical sampling was essential. It was necessary first of all to establish which type of swab to use on each sampling area. Results are given in Table 3 of replicate cotton-gauze and alginate swabs used on fourteen areas of muscle and five fatty areas on cattle carcases, (Patterson, 1968).

The first swab in this case did recover a fairly high percentage of that recovered by all five—from muscle tissue 76% (cotton gauze and alginate) and from fatty tissue 93% (cotton gauze). An even greater recovery is achieved by the use of duplicate or triplicate swabs. Table 4 gives data obtained in a comparison between cotton gauze and alginate swabs used to swab adjacent areas on eleven cattle carcases at seven different sampling sites. Only on the brisket did the alginate swab recover a just significantly higher number of bacteria than the cotton gauze (data of Patterson, 1968).

TABLE 3.

TABLE 3. Mean recovery of bacteria by replicate swabs on muscle and fatty tissues of cattle carcases

No. of areas sampled	Type of surface	Type of swab	Log_{10} bacterial number per cm^2 recovered by swab no. 1	2	3	4	5
14	Muscle	Cotton gauze	3·04	2·13	2·04	1·90	1·52
Range			< 1·04–3·78	< 1·04–2·60	< 1·04–2·83	< 1·04–2·84	< 1·04–2·30
14	Muscle	Alginate	3·35	2·64	2·16	2·00	1·64
Range			< 1·04–4·38	< 1·04–3·61	< 1·04–2·87	< 1·04–2·70	< 1·04–2·34
5	Fatty	Cotton gauze	4·13	2·36	2·89	2·76	2·85
Range			2·49–4·82	< 1·04–2·62	< 1·04–2·36	< 1·32–2·25	< 1·04–3·54

TABLE 4. Mean recovery of bacteria by single cotton-gauze and alginate swabs from cattle carcases

No. of carcases sampled	Type of swab	Log_{10} bacterial number per cm^2 recovered from: Hindleg	Rump	Flank	Sirloin	Brisket	Foreleg	Neck
11	Alginate	2·58	2·72	2·37	2·52	4·05	2·98	2·02
11	Cotton-gauze	2·34	3·01	2·19	2·30	3·73	2·75	1·66
Mean difference		0·242	−0·297	0·179	0·221	0·318	0·224	0·359
S.E. of a mean difference		0·198	0·241	0·260	0·251	0·133	0·144	0·335

In subsequent hygiene investigations in abattoirs, a single cotton-gauze swab was employed to swab 16 cm^2 areas on the rump, brisket and foreleg of carcase, except in certain experiments where maximum recovery was necessary, when triplicate swabs were used. Large swabs ($1\frac{1}{2}$ inch × 10 inch cotton gauze tightly wrapped around the end of $7\frac{1}{2}$ inch wooden skewers) are sometimes employed when a whole carcase has to be swabbed, e.g. when sampling for the presence of salmonellae, using four per carcase. Less work has been done with sheep carcases but in a replicate swab trial, three swabs were found to recover more than 90% of the total number of bacteria recovered by five replicate swabs.

Sampling of meat cuts

When sampling cuts of packaged beef, the method described by Williams (1967) has proved very suitable. This method consists of scraping and washing the organisms directly from the meat surface into the diluent. An A1 size can body is pressed on to the surface of the meat giving a sampling area of 5·25 in^2, into which is poured 25 ml of 0·1% peptone water which is then stirred and the meat surface scraped with a sterile spatula to release the maximum number of organisms. A 1 ml sample is then removed for plating. Unfortunately this method is not suitable for carcases unless these can be laid flat.

Sampling of surfaces in poultry plants and abattoirs

Most of the work in meat and poultry processing plants is done while the plants are in operation, and the opportunity does not often arise for checking cleaned surfaces. Where this does arise two of the methods already discussed are used, viz: the swab-rinse, in this case using the small cotton-gauze swab to sample ca.1000 cm^2, or the agar-contact when a simple assessment is used rather than the statistical method of Hansen (1962). More often working surfaces have to be sampled and these are often

heavily contaminated. In this case we sample a known area, though this is not always possible with hands, and small items of equipment, and obtain a total count per unit area or per swab.

Conclusion

Many factors have to be considered when assessing the microbiological condition of surfaces. The contamination is more likely to be unevenly spread than evenly; it is essential with swabbing techniques to swab a known area, for a specified time with a standardized swab or swabs. The nature of the surface, the type of bacteria present, the presence or otherwise of bactericidal chemicals which may require neutralization are all important factors. Other techniques require different precautions. Having taken the sample, then the methods of transportation, e.g. in dry containers, or in a diluent, presence or absence of refrigeration, and finally methods of recovery are all critical. It can be seen, therefore, that the interpretation of the results of any assessment must be made in the light of the various limitations of methods, and in many cases only an

TABLE 5. Bacteriological standards for surfaces in meat and poultry plants

Cleaned surfaces		
Total count	per cm^2	$\not>$ 50
Faecal streptococci	,, ,,	$\not>$ 1
Working surfaces		
Total count	per cm^2	$\not>$ 1,000
Faecal streptococci	,, ,,	$\not>$ 100
Poultry carcases		
Total count	per 16 cm^2 breast skin	$\not>$ 250,000
Coli-aerogenes	,, ,, ,, ,, ,,	$\not>$ 1,000
Faecal streptococci	,, ,, ,, ,, ,,	$\not>$ 10,000
Staph. aureus	,, ,, ,, ,, ,,	$\not>$ 100
Salmonella		Absent
Cattle and sheep carcases (freshly butchered)		
Total count per 16 cm^2 on the	*rump*	$\not>$ 50,000
	brisket	$\not>$ 150,000
	foreleg	$\not>$ 50,000

opinion can be given. From this point of view, it is difficult to formulate bacteriological standards for surfaces, but the microbiologist must try to do this even in the knowledge that such standards must be under constant review. Table 5 gives examples of the advisory bacteriological standards to which we work in the meat industry. These have proved helpful in assessing hygienic practices in the poultry processing plants and cattle and sheep abattoirs regularly visited. Although they do not give a complete assessment of hygiene at any one visit, variations can be quickly noted, and remedial action suggested if necessary.

Acknowledgments

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J. Fd Technol. (1971) **6**, 73–83.

An accelerated system for screening of process variables and freshness indices of irradiated fishery products

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Summary

Incubation of fish homogenates at 30°C led to enhancement of microbial growth and other freshness indices like TMA and TVBN values enabling rapid screening of process variables. This accelerated test system was found to be successful when applied to shrimps and four species of locally available fish, viz. Indian Salmon (*Eleutheronesma tetradactylum*), Surmai (*Scomberomorus gettatus*), Pomfret (*Stomateus cinereus*) and Bombay duck (*Harpodon nehereus*); which were either untreated or subjected to gamma radiation with or without combination of sodium nitrite and benzoic acid.

Introduction

Bactericidal effects of ionizing radiation on microorganisms without causing appreciable changes in quality attributes of foods form the basic principle of the radiation process for preservation of flesh foods. However, since food-borne microorganisms vary in their radiation sensitivities (Lewis, Alur & Kumta, 1970) the selected dose required for elimination of specific types of bacteria should not cause any impairment of organoleptic attributes by degradation of food components. Radiation dose, therefore, becomes an important process parameter. Several physical and chemical combination treatments have been reported (Thornley & Ingram, 1963; Lee, Shiflett & Sinnhuber, 1965) for minimizing the radiation dose required for pasteurization as well as for sterilization processes.

Although certain chemical indices are better monitors of freshness than taste panels (Jones *et al.*, 1964) the acceptability of irradiated fishery products is determined mostly by sensory tests. Studies in this laboratory (Sawant *et al.*, 1967) on irradiated Bombay duck and shrimps as well as those of Spinelli, Eklund & Miyauchi, (1965) on petrale sole fillets indicated that TVBN value is a reliable index of quality. To define process variables and their effects on quality characteristics through storage studies, is cumbersome and time-consuming; an accelerated test system was therefore developed, based on the fact that growth of microorganisms and formation of metabolites like TVBN and TMAN are accelerated by increasing the incubation temperature (Alur, Lewis & Kumta, 1970).

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The present paper refers to studies on the effects of radiation with or without combination of sodium nitrite and benzoic acid on the keeping qualities of fish homogenates incubated at 30°C. The studies have been extended to four types of locally available fish, viz. salmon, pomfret, Bombay duck, surmai and shell-fish (shrimps) to examine the usefulness of the accelerated test system.

Experimental

Five samples each, of Indian salmon (*Eleutheronesma tetradactylum*), surmai (*Scomberomorus gettatus*), pomfret (*Stomateus cinereus*), Bombay duck (*Harpodon nehereus*) and shrimps were obtained from a local market. These fish were beheaded, eviscerated, cleaned and filleted.

Preparation of homogenates

Ten percent homogenates of the pooled fillets from five samples of each fish species were prepared in three independent experiments with sterile normal saline and transferred asceptically into sterile test tubes. Each tube contained 9 ml of homogenate.

Process variables

(a) *Radiation.* Fish homogenates were exposed to gamma radiation at doses of 0·1, 0·25, 0·50 and 1·0 Mrad. Irradiation was carried out in a Co^{60} gamma-cell 220 (AECL), at a dose rate of 0·67 Mrad per hr. Unirradiated samples served as controls.

(b) *Sodium nitrite or Benzoic acid treatment.* A 0·1% solution of sodium nitrite or benzoic acid was prepared in sterile distilled water. 1 ml of this solution was pipetted into 9 ml of fish homogenate to obtain a final concentration of 0·01%. The samples prepared with and without benzoic acid or sodium nitrite were exposed to doses of 0·1 and 0·25 Mrad.

Incubation procedure

Samples of fish homogenates were incubated in a water bath maintained at 30°C. Samples were withdrawn after 0, 3, 6 and 24 hr for bacteriological and biochemical tests.

Bacteriological tests

Total bacterial counts of the samples were determined by the pour plate method using TGY agar. Plates were incubated at room temperature for 48 hr.

Biochemical tests

TVBN and TMAN contents of the homogenates were determined on TCA extracts of the homogenates according to the method of Farber & Ferro (1956) and Bethea & Hillig (1963).

Calculations

TVBN and TMAN contents of unirradiated and irradiated fish homogenates at 0 and 24 hr incubation at 30°C were determined. The increase in these levels after 24 hr incubation were considered as the net formation of the metabolites. Unirradiated samples showed a much higher increase than irradiated samples.

The suppression of these metabolites in irradiated samples by a particular radiation dose were calculated as per cent depression of the corresponding value for the unirradiated samples.

Results

Total bacterial count (TBC) of unirradiated and irradiated fish homogenates during incubation at 30°C

Fig. 1 shows the marked differences in the total bacterial load in unirradiated and irradiated (1·0 Mrad) homogenates of shrimp, Bombay duck, Surmai, Salmon and Pomfret during incubation at 30°C.

Initial bacterial counts of unirradiated homogenates ranged from 10^3 to 10^6/g. After 6 and 24 hr of incubation, counts increased rapidly to 10^8–10^9/g and 10^{10}–10^{11}/g respectively.

After exposure to 1·0 Mrad, the bacterial count of each of the different homogenates was approximately 10^2/g. During incubation however, certain differences were observed in growth patterns of microflora in different fish homogenates. Thus, in shrimp and salmon homogenates, there was a rapid increase in TBC to 10^4–10^5/g within 6 hr whereas a lag of 6 hr was observed in irradiated pomfret and surmai homogenates, followed by an increase in the counts to 10^3–10^5/g after 24 hr.

Chemical indices in unirradiated and irradiated fish homogenates during incubation at 30°C

TVBN and TMAN levels of unirradiated and irradiated (1·0 Mrad) fish homogenates during incubation at 30°C are shown in Fig. 2.

During incubation there was an increase in TVBN formation. Thus, initial value of 30 mg% for unirradiated shrimp increased to 75 mg% and 440·4 mg % after incubation at 30°C for 6 hr and 24 hr respectively. Further, increase in TVBN contents, however, was found to be dependent upon the type of fish; the net formation of TVBN in shrimps, surmai, salmon, Bombay duck and pomfret homogenates after 24 hr incubation at 30°C, were found to be respectively 440·4, 172·5, 241·8, 57·0 and 78·5 mg%.

Increase in TMAN values of unirradiated fish homogenates was also dependent on the type of fish. Although initial TMAN in all homogenates ranged from 0·06 to 0·55 mg%, after 24 hr incubation these values in shrimps increased to 5·1 mg% whereas in Bombay duck, it was only 1·3 mg%.

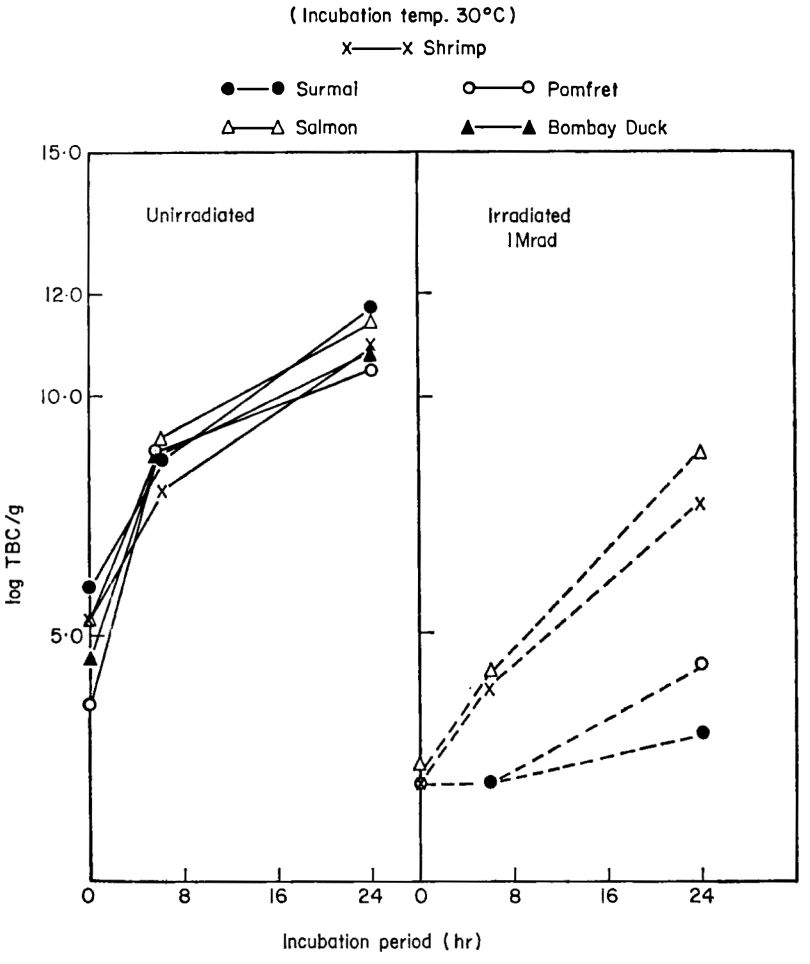

FIG. 1. *Effects of irradiation* (1 *Mrad*) *on the growth of microflora in fish homogenates* (*incubation temp.* 30°*C*);
10% homogenates of Bombay duck, salmon, surmai, pomfret and shrimp were incubated at 30°C with or without irradiation treatment (1 Mrad). Total viable bacterial counts (TBC) were determined in the samples at different intervals by pour plate techniques. Each value is the average of three independent experiments.

It is interesting to note that homogenates irradiated at 1·0 Mrad did not show any significant increase from the initial values in both TMAN and TVBN formation, throughout the incubation of 24 hr at 30°C. Maintenance of freshness levels in irradiated samples in contrast to the unirradiated homogenates can be considered as a unique feature.

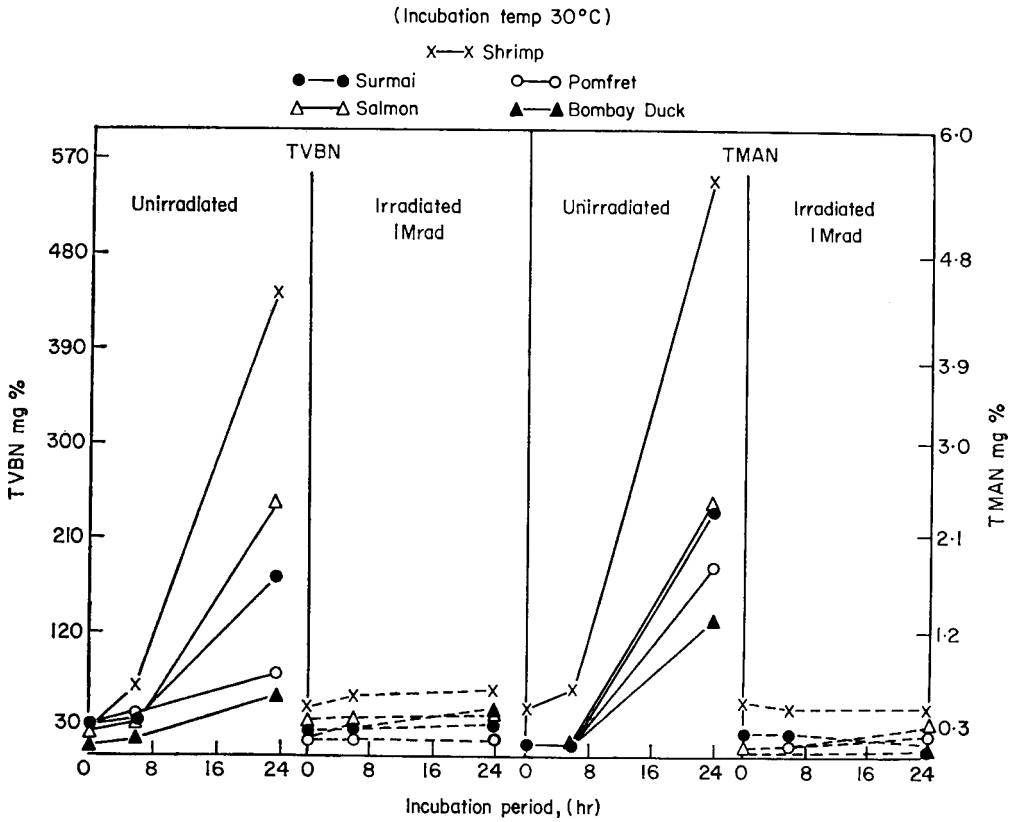

FIG. 2. *Effect of irradiation* (1 *Mrad*) *on TMAN and TVBN levels in fish homogenates* (*incubation temp.* 30°*C*):
10% homogenates of Bombay duck, salmon, surmai, pomfret and shrimp were incubated at 30°C with or without irradiation treatment (1 Mrad). Extent of formation of TVBN and TMAN were determined in the fish homogenate referred to in Fig. 1 as in the method described in the text. Each value is the average of three independent experiments.

Relationship between TBC and chemical indices in unirradiated and irradiated fish homogenates

Fig. 3 illustrates the relationship between TBC and formation of TMA and TVBN in unirradiated and irradiated (1·0 Mrad) homogenates, during incubation at 30°C.

Increase in bacterial counts to 10^8/g in unirradiated fish was not accompanied by substantial rises in TVBN and TMAN contents. However, further increase in TBC from 10^8/g to 10^{11}/g, was coincident with a rapid increase in TVBN as well as TMAN content (Fig. 3). The extent of increase in these values however, varied with the type of fish. Thus, although shrimps and Bombay duck homogenates show TBC of 10^{11}/g the corresponding TVBN and TMAN values indicate wide differences, viz. 440 and 57 mg% of TVBN and 5·1 and 1·3 mg% of TMAN respectively.

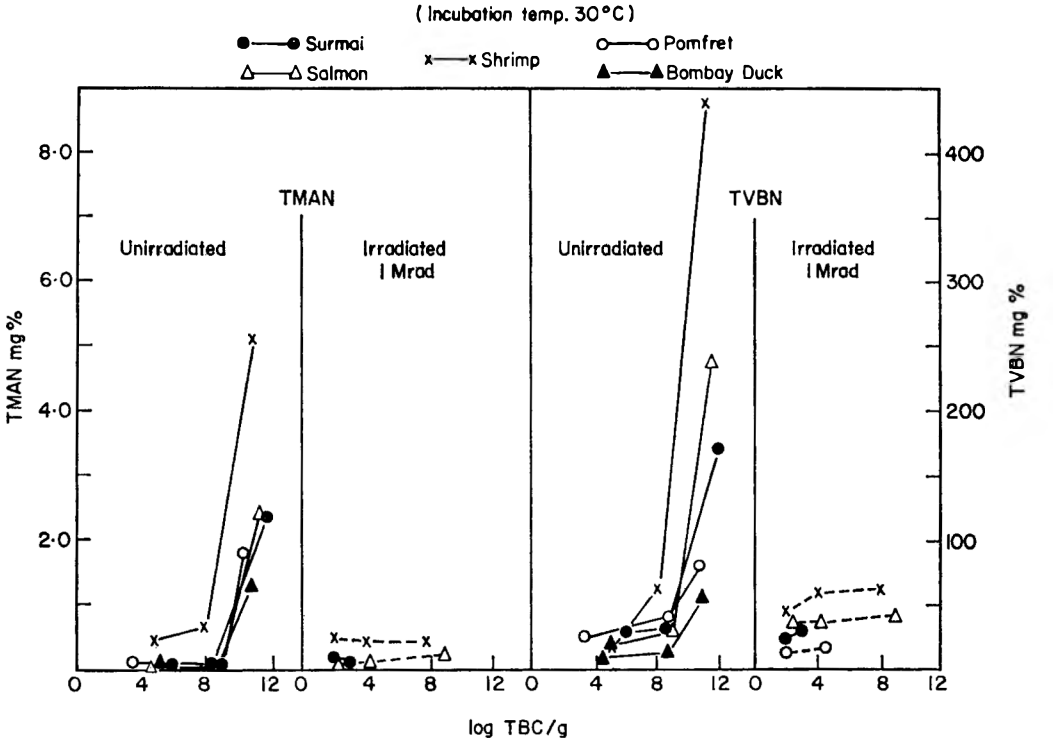

FIG. 3. *Relationship between TBC and chemical indices in fish homogenates (incubation temp. 30°C)*: TBC, TMAN and TVBN in fish homogenates were determined as for Figs. 1 and 2. Formation of TVBN and TMAN determined as a function of bacterial load of unirradiated and irradiated (1·0 Mrad) fish homogenates incubated at 30°C shows lack of correlation in rise in TBC with low levels of TVBN and TMAN. Each value is the average of three independent experiments.

It is interesting to note that in irradiated (1·0 Mrad) homogenates of salmon and shrimp, though the TBC increased by six logarithmic cycles from the initial counts of 10^2/g, there is no proportionate increase in TVBN and TMAN contents of the samples. This observation which points out that in irradiated fishery products TBC may not serve as a good index of spoilage status, has been consistently reported (Spinelli *et al.*, 1964; Sawant *et al.*, 1967, Kumta *et al.*, 1970a).

Suppression of chemical indices as a function of radiation dose

Fig. 4 illustrates the per cent depression in TVBN and TMAN formation by radiation doses varying from 0·1–1·0 Mrad.

The extent of radiation-induced depression, in the formation of TVBN and TMAN, varied with the different types of fish homogenates. Low dose irradiation (0·1 Mrad) of different homogenates resulted in significant depression from the corresponding

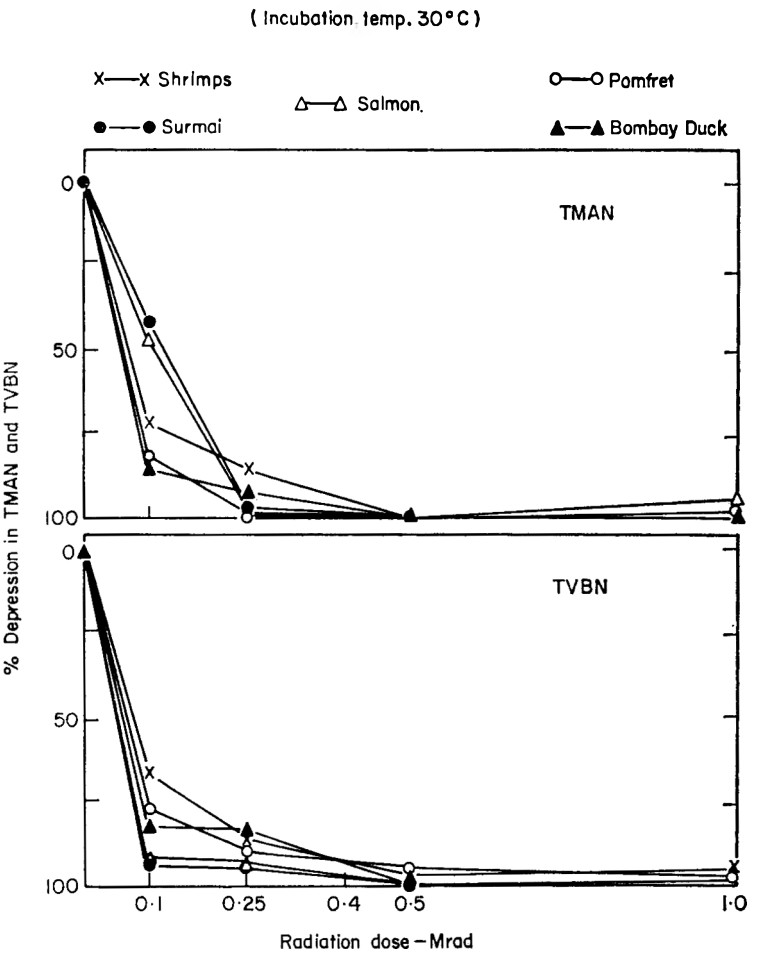

FIG. 4. *Radiation-induced depression in TMAN and TVBN of fish homogenates*: Suppression in TMAN and TVBN formation is observed in 10% fish homogenates referred to in figs. 1–3, with increasing doses of radiation. Each value is the average of three independent experiments.

control levels. The variation ranged from 15–58% for TMAN, and 6–33% for TVBN, for the five different fish homogenates tested. As may be seen from Fig. 4, by increasing the radiation dose from 0·1 to 0·5 Mrad, a maximum suppression of chemical indices is observed, there being no further reduction of TVBN and TMAN in samples exposed to 1·0 Mrad. This suggests that freshness indices could be maintained, and spoilage thereby delayed, by careful selection of the radiation dose.

Tables 1 and 2 summarize the effects of both radiation (0·1 Mrad and 0·25 Mrad) and sodium nitrite or benzoic acid added to fish homogenates at a concentration of 1000 ppm, on TMAN and TVBN values; as well as the combined effect of radiation

and chemical treatment on these freshness indices. Of the two chemical preservatives investigated, benzoic acid was found to have a much more pronounced effect than sodium nitrite in suppressing the formation of TMAN and TVBN metabolites, in all of the fish species used. A combination of radiation and chemical preservatives gave the best results—however, although 0·25 Mrad gave better results than 0·1 Mrad treatment, there was no further advantage in using 0·25 Mrad as compared with 0·1 Mrad, in combination with chemical treatment for the suppression of TMAN and TVBN values.

One unique advantage of the accelerated test system is that at elevated storage temperatures, metabolic products rapidly accumulate so that differences between irradiated and unirradiated fresh samples can be easily detected. Palmateer, Yu & Sinnhuber (1960) who applied this test system to fatty fish, reported good correlation with values of thiobarbituric acid (TBA) determined during test conditions as compared with those obtained at sub-room temperatures. Farber & Lerke (1961) incubated fish

TABLE 1. Combination effect of sodium nitrite and benzoic acid with irradiation on TMAN values in fish homogenates (incubation temperature, 30°C)

Treatments	Shrimps	Surmai	Salmon	Pomfret	Bombay duck
			TMAN mg%[a]		
Untreated	12·6 (0·20)	9·43 (0·55)	6·74 (0·06)	12·06 (0·06)	6·7 (0·06)
Sodium nitrite (0·01%)	10·5	8·5	6·4	10·7	5·3
Benzoic acid (0·01%)	1·7	0·6	2·5	0·5	0·6
Irradiation:					
0·1 Mrad	3·1	5·33	3·47	2·4	1·2
0·25 Mrad	2·23	0·45	0·13	0·25	0·66
Combination:					
Nitrite + 0·1 Mrad	1·6	5·8	0·4	0·6	0·5
Nitrite + 0·25 Mrad	0·5	0·9	0·4	0·4	0·5
Benzoic acid + 0·1 Mrad	0·5	0·5	0·8	0·6	0·5
Benzoic acid + 0·25 Mrad	0·4	0·3	0·8	0·4	0·4

Fish homogenates were incorporated with 0·01% benzoic acid or sodium nitrite solutions. Unirradiated homogenates served as control. Some of the samples were irradiated with 0·1 and 0·25 Mrad. All the samples were incubated at 30°C for 24 hr and TMAN values were determined, as in the method described in the text. TMAN values shown in the table are the differences between 24 hr and initial levels which are given in the parenthesis.

[a] Average of three experiments

TABLE 2. Combined effect of $NaNO_2$ and benzoic acid with irradiation on TVBN values in fish homogenates (incubation temperature, 30°C)

Treatments	Shrimps	Surmai	Salmon	Pomfret	Bombay duck
			TVBN mg%[a]		
Untreated	841·0 (21·5)	323·4 (15·4)	817·4 (29·6)	275·7 (21·5)	303·0 (16·9)
Sodium nitrite (1000 PPM)	544·3	298·5	671·3	253·5	223·0
Benzoic acid (1000 PPM)	97·0	61·6	46·2	5·0	4·1
Irradiation:					
0·1 Mrad	287	24·6	63·0	61·0	57·2
0·25 Mrad	164	24·6	63·0	30·5	57·2
Combination:					
Nitrite + 0·1 Mrad	110·9	35·4	48·6	20·0	44·7
Nitrite + 0·25 Mrad	100·1	16·9	30·2	18·5	23·0
Benzoic acid + 0·1 Mrad	10·8	1·8	1·2	1·5	1·5
Benzoic acid + 0·25 Mrad	10·7	0·3	1·2	1·4	0·4

Fish homogenates were incorporated with 1000 PPM benzoic acid or sodium nitrite solutions. Unirradiated homogenates served as control. Some of the samples were irradiated with 0·1 and 0·25 Mrad. All the samples were incubated at 30°C for 24 hr and TVBN values were determined, as in the method described in the text. Differences in TVBN levels after 24 hr and initial levels are shown in the table for all the samples. Initial levels of TVBN are shown in the parenthesis.

[a] Average of three experiments

for 3 to 12 hr at 31°C and compared TMA and VRS values with those obtained during prolonged storage at refrigerated temperatures—if the fish samples, mackerel and sardines produced high TMA and VRS during incubation at 31°C it was interpreted that the fish had already reached the stage of incipient spoilage, since fresh fish did not yield similar high TMA and VRS levels on incubation at 31°C. The accelerated system has also been used for calculating approximately the time elapsed between the catch and remaining storage life of raw shrimps by incubating at room temperature from 2 to 6 hr; Visweswariah *et al.*, (1959) reported that this system could be best used for tropical fishery products subjected to CTC and OTC treatments, so that the marketable life of fishery products could be predicted.

Selection of process variables and freshness indices

To evolve a suitable radiation dose for pasteurization of fishery products, two series

of tests could be conducted, based on: (i) dose response relationship in respect of retention of organoleptic attributes, (ii) dose required for suppressing bacterial spoilage. Without involving protracted periods of storage, the tolerance dose can be selected by conducting a sensory evaluation immediately after the fishery or meat products have been exposed to a wide range of radiation doses. The efficacy of this dose in suppressing the spoilage organisms can be further established from accelerated test studies by determining the extent of formation of end products of metabolism like TMAN and TVBN, by the surviving mixed flora.

Since sea-foods differ in their chemical composition and microbial patterns, certain qualitative and quantitative differences in their response to radiation treatment could be expected. As observed with the four varieties of fish and shrimps, the net formation of TMAN and TVBN at incubation temperature of 30°C would be different. However, freshness levels of these indices can be maintained for each of the fish species by appropriate selection of the process parameters. A number of combination treatments could be screened rapidly for this purpose. It is imperative that some of the effective ones should be then rigorously tested for specific fishery products, with respect to storage temperatures, packaging conditions and desirable quality characteristics peculiar to that product. For example, as irradiation alone is not effective in suppressing enzymes involved in autolysis and melanosis, blackening in radio-pasteurized shrimps can be effectively controlled by pre-blanching treatment (Kumta *et al.*, 1970a). The combination of blanching and irradiation, however, is not applicable to Bombay duck, since both these treatments lead to extensive exudation of drip and textural damage.

Conclusion

A combination of sodium nitrite or benzoic acid treatment with low dose irradiation; or integrating vacuum-packaging in the radiation pasteurization process, which effectively suppresses bacterial spoilage, may find application for treatment of fatty fish varieties which are prone to oxidative changes and degradation of heme pigments at higher radiation doses.

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A practical approach to water sorption isotherms and the basis for the determination of optimum moisture levels of dehydrated foods

M. CAURIE

Summary

The underlying principle for the determination of the optimum moisture content (M_o) for the stability of dehydrated foods and materials of different adsorption patterns is discussed. Sorptive forces have been suggested as playing a very important role in the determination of M_o values. The relation between M_o and heat of sorption has been established and graphically illustrated.

The effect of external conditions on the variation in sorptive as well as M_o values has been illustrated graphically. Alternative methods for the determination of M_o values have also been suggested.

Introduction

Water which controls the stability of dehydrated foods may exist in different states in the food depending on the conditions imposed. It may for example exist as bound, monolayer, multilayer and free water. These states of water do not only affect the stability of food in storage but the course and processes of dehydration as well.

Moisture content of foods (M) soon adjusts to the humidity (RH) exposed to it. The availability of this moisture for deteriorative reactions is now known to be numerically equal to the surrounding equilibrium relative humidity (ERH) expressed as a fraction or water activity (A_w).

The relationship between RH and M (moisture sorption isotherm) of food substances exhibits several characteristic curves which are generally sigmoid. Many equations, recently reviewed by Labuza (1968), have been developed to describe, and where possible to transform, these isotherms into linear forms to allow for easy mathematical analysis. None, however, has been found which is entirely satisfactory (Labuza, 1968; Caurie, 1970a) to describe the complex and variable nature of the physical and chemical properties of dehydrated food substances and natural products.

Rockland (1957, 1969) recognizing the apparent limitations of current models has developed a local isotherm (LI) concept based on the assumption that water sorption isotherms may not be smooth curves but a composite of three local or straight line isotherms. These local isotherms are claimed to correspond to definite A_w ranges which

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are characterized by certain chemical, physical and biological properties. This concept was substantiated by Bull's (1944) data for gelatin, salmine and silk.

Although the LI concept may be sound, recent work indicates that a simpler mathematical description of water sorption isotherms is possible. The application of the mathematical model (Caurie, 1970a) to the same data with which the LI concept was justified yielded the desired single straight lines instead of the three in LI concept.

The adsorption of moisture by hydrophilic substances is known to involve sorptive forces of great magnitude. The literature offers no definite records of the influence of these forces on the amount of moisture adsorbed in relation to food stability. Data made possible by the new model equation suggests that these sorptive forces play a very important role in the determination of optimal conditions for the storage of food substances.

This paper discusses the importance of these sorptive forces in the determination of the optimum moisture levels for the stability of dehydrated food substances and natural products. It propounds a view that the optimum moisture content (M_o) for optimal stability of food substances and natural products is that unique moisture level whose value is numerically equal to the energy, in kcal, with which it is bound to the macromolecular constituents of food and natural products.

The fraction of water involved in general water sorption isotherms

Most of the water in fresh or very wet food substances exerts a vapour pressure (VP) very nearly equivalent to that of pure water, i.e. unity (Labuza, 1968). This vapour pressure level is maintained until the moisture content of the food decreases to about 22·0%. The moisture level is then no longer able to sustain the VP of the food at unity and therefore begins to show a lowered VP as if it is in solution; the VP then rises with gains and falls with losses in moisture content.

It is the changes with atmospheric humidity of this last fraction (22·0%) of water in dehydrated foods which results in the characteristic sigmoid and other shapes of water sorption isotherms (Fig. 1).

The importance of these water sorption isotherms lies in their use to predict certain critical levels of moisture for which numerous equations have been proposed. The model equation (Caurie, 1970a) was developed to estimate the optimum moisture content (M_o), for the stable storage of dehydrated foods, from these water sorption isotherms.

Relation between the optimum moisture content (M_o) and water activity (A_w)

The optimum moisture content (M_o) is defined according to Caurie (1970a) by the relation

$$\ln C = \frac{1}{0{\cdot}045\ M_o} - A_w \ln r \qquad (1)$$

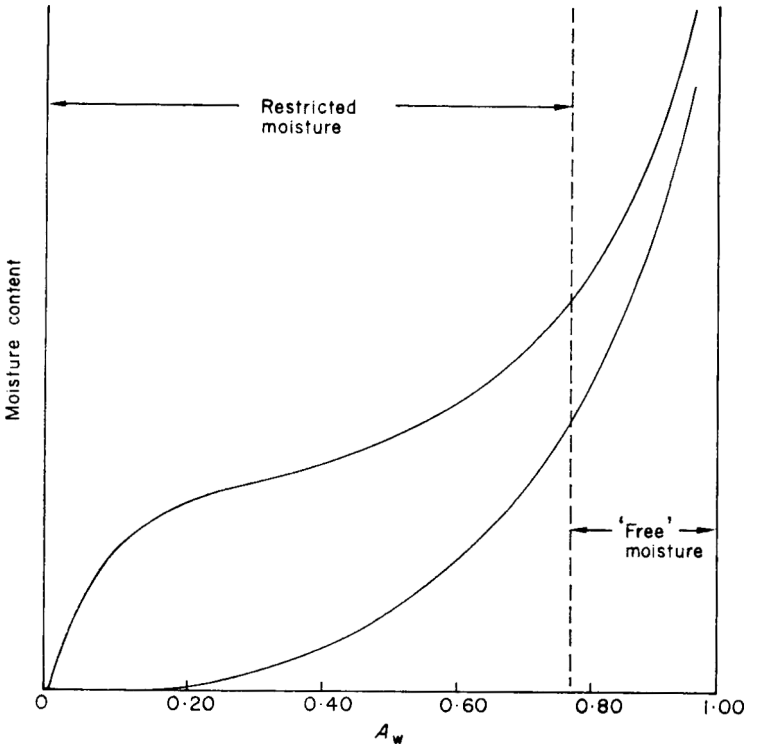

FIG. 1. Water sorption isotherms.

where $$C = \frac{100 - \%\ H_2O}{\%\ H_2O}$$

A plot of the calculated M_o against the corresponding A_w of the data in Table 1 and similar data from previous work (Caurie, 1970a) is shown in Fig. 2.

It may be observed that all the M:ERH co-ordinates fall on the regression line Y_c (A_w on M_o).

$$Y_c = 0{\cdot}784 - 0{\cdot}0693X \qquad (2)$$

except the two kernels which are foods containing high proportions of oil and those containing high sugar levels (orange crystals, raw cane sugar) which fall outside Y_c and form their own separate regression lines.

The regression line (A_w on M_o) for the high sugar products (Fig. 2) is given by the relation:

$$Y_s = 2{\cdot}577 - 0{\cdot}582X \qquad (3)$$

The suggested new model Equation (1) therefore differentiates between sugar and

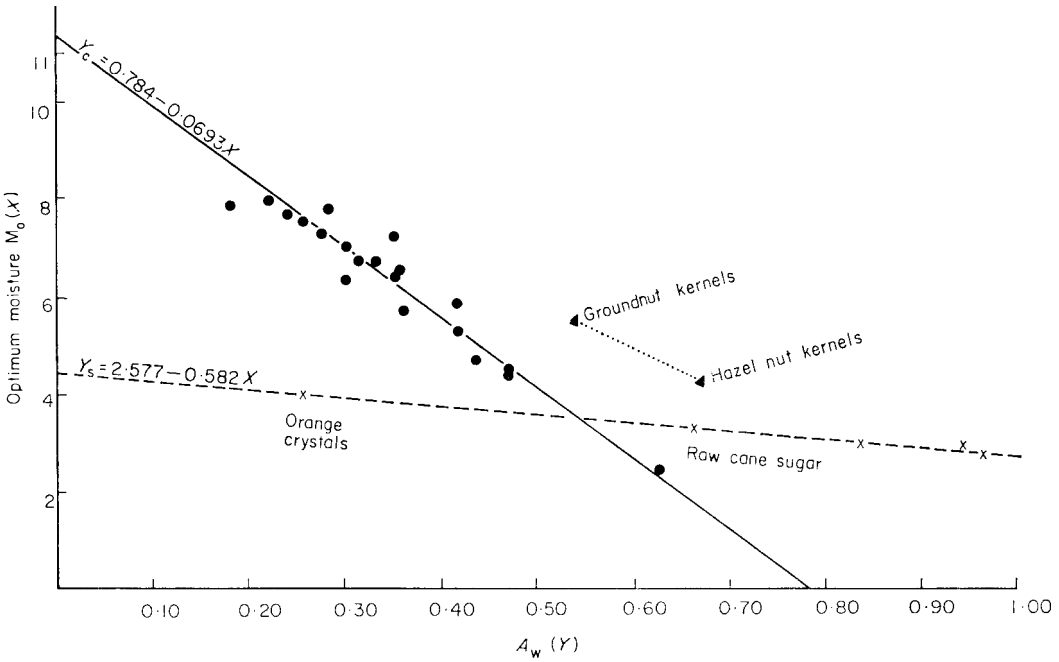

FIG. 2. The relationship between A_w and the optimum moisture content (M_o) of dehydrated foods.

TABLE 1. The effect of increasing levels of reducing sugar on the rate of hydration, optimum moisture content and A_w of raw sugar cane (from the data of Tilbury, 1966)

Group	Reducing Sugar	ln C_o	Optimum % H_2O (M_o)	A_w	Gradient ln r
I	0·16	8·00	2·78	0·967	0·046
II	0·24	7·67	2·90	0·945	0·044
III	0·73	7·60	2.92	0·836	0·049
IV	1·35	7·43	3·24	0·661	0·061
Orange* crystals	2.95	5·42	4·01	0·255	0·090

*Data from Karel & Nickerson (1964).

non-sugar foods and possibly oil foods as well (Fig. 2). The ability to separate different groups of food for which separate regression lines may be drawn is an important property of the new model equation (1) which permits its wide applicability to foods.

It may further be observed that these regression lines establish an unusual inverse relationship between the optimum moisture content (M_o) and corresponding A_w. Normally, increases in water activity result in proportional increases in moisture content of hygroscopic materials.

Sorptive or water-binding energy (Q) of hydrophilic systems, and the principle of the numerical equivalence of M_o and its sorptive energy (Q_c)

Van Arsdel (1963) has indicated that water begins to condense in the larger capillaries of capillary-porous products in the region of 0·80 A_w with no heat of sorption at all, i.e. the moisture at this activity is totally free. The value of 0·784 A_w recorded in this work corresponding to zero M_o in most dehydrated foods, is in sufficient agreement with 0·80 A_w to regard the sorption energy (Q) at this activity as zero. This means that when the heat of sorption (Q) is zero the optimum moisture content (M_o) is also zero at 0·784 A_w for most foods.

Moreover, the water binding energy (Q) of hydrophilic substances has been stated by Kuprianoff (1958) to vary between zero and the energy of a valence bond which varies between 2 and 10 kcal/mole. Labuza (1968) has also stated that for most foods, when bearing less than a complete monolayer of water, their heat of sorption or water binding energy varies between 2 and 10 kcal/mole.

We find in this work that for most foods M_o rises linearly (Fig. 2) to a maximum of 11·3% at zero A_w where the maximum water binding energy of one valence bond also occurs (Labuza, 1968; Kuprianoff, 1958).

From the close numerical agreement between M_o and Q at their minimum and maximum values, it is suggested that optimum moisture contents (M_o) are numerically equivalent in kcal to the sorptive energies (Q_c) with which they are bound. The optimum moisture content is therefore the unique moisture level which is bound by a force (Q_c) numerically equal in kcal to its own value.

The equivalence of M_o and Q_c explains the rather unusual inverse relationship recorded between A_w and M_o (Fig. 2). The recorded trend is explained if it is taken to indicate energy rather than a moisture relationship. It follows that the relationship between A_w and Q is linear and identical with the relationship between A_w and M_o.

Therefore for most foods (i.e. non-sugar foods) according to equation (2)

$$A_w = 0{\cdot}784 - 0{\cdot}0693\ Q$$

$$Q = 11{\cdot}3131 - \frac{A_w}{0{\cdot}0693} \qquad (4)$$

Similarly for high sugar foods according to equation (3)

$$Q = 4{\cdot}429 - \frac{A_w}{0{\cdot}582} \tag{5}$$

The regression line, Y_c, representing most foods thus delineates complete water sorption isotherms into two clear regions. Region 1, which may be described as the area of restricted moisture, starts from zero to 0·784 A_w, the term 'restricted' being preferred to 'bound' since the sorbed water in this range of A_w is not strictly bound (Caurie, unpublished data). Region 2 covers the range of A_w beyond 0·784 A_w. This is the area of 'free' or unrestricted water.

The spread of M_o: ERH co-ordinates along regression lines

The numerical equality between M_o and Q_c means that at any M_o: ERH co-ordinate the energy/moisture ratio is unity. This makes all values of M_o anywhere in Region 1 (0 – 0·784 A_w) on any isotherm of equal stability instead of the differential stability associated with the LI concept of Rockland (1969).

In practice, however, there is a limited range of optimum A_w and corresponding optimum moisture content within which most M_o:ERH co-ordinates lie. This limited spread of co-ordinates is imposed by the characteristics of the foods themselves. The range of optimum moisture content within this limitation has been found to vary between 4 and 8%. Indeed Charm (1963) has stated that one of the bases of food preservation by dehydration is the fact that below about 10% moisture content, microorganisms do not grow at appreciable rates.

It has been stated that with the new transformation local isotherms recognized by Rockland (1957, 1969) are not evident. In the absence of LI divisions therefore, it appears that the chemical, physical and biological properties attributed to each of the three LI's, should refer more to individual M_o:ERH co-ordinates instead of the LI's. This view agrees with that stated above that the M_o:ERH co-ordinates anywhere in Region 1 represent points of equal stability; and also to the view that most food products exhibit an optimum moisture phenomenon above and below which there is deterioration at a more rapid rate (Rockland, 1957; Rockland *et al.*, 1960, 1961).

Illustration of the principle of the equivalence of M_o and Q_c

The principle may be illustrated with Fig. 3 which shows the relationship between the percent moisture content of food substances, sorptive energy (Q) and A_w. The line AB is the regression line representing either equation (2) or (3) which shows the inverse linear relationship between A_w and Q_c or M_o. The three curves OS_1C, OS_oC and OS_2C show the normal sigmoid relationship between A_w and moisture content of food substances. By the suggested principle M_o lies at the point of intersection of the appropriate regression line and the isotherm curves, i.e. at S_1, S_o and S_2. These are the

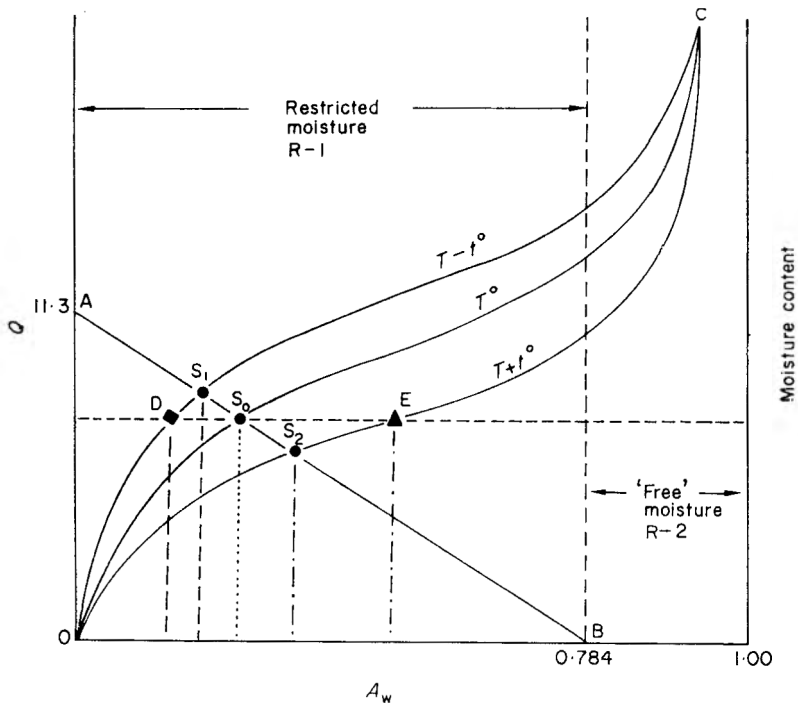

FIG. 3. Diagrammatic relationship between A_w, moisture content of food substances (M) and sorption energy (Q).

unique critical moisture levels predicted by equation (1) below and above which, on the appropriate isotherm curve, there is deterioration. The critical moisture level is higher but close to the B.E.T. monolayer (Caurie, 1970a). This means that M_o falls in the multilayer state of adsorbed water.

The effect of imposed external conditions on M_o or Q_c

It is known that for most foods the heat of adsorption (Q) is not constant (Labuza, 1968) and varies with the type of food, A_w, and with temperature (Ayerst, 1965) as well as with processing conditions. It also means that the heat of adsorption at the optimum moisture content is also not constant. The suggested principle allows for this variability in Q_c as is illustrated with Fig. 3. This illustration also offers a graphical method for the quantitative determination of Q.

Rockland (1969) discussing the effect of temperature changes on water sorption could not specify where the final M_o:ERH co-ordinates on any transposed isotherm may lie. These co-ordinates are, however, precisely predicted as described below.

An important characteristic of physical adsorption, which prevails in food substances, is that equilibration is both rapid and reversible. From an initial stable M_o:ERH

equilibrium condition at S_o on curve $T°$ (Fig. 3) a rapid drop in temperature to $T - t°$ will transpose the complete isotherm from $T°$ to curve $T - t°$. A new ERH will be rapidly but temporarily established at point D until equilibrium is re-established, at a higher moisture level, with the RH inside the storage facility. Under these conditions Fig. 3 shows that the M_o:ERH co-ordinates increase to S_1 where curve $T - t°$ cuts the energy regression line AB.

Similarly a rapid increase in temperature to $T + t°$ establishes the curve $T + t°$. A new ERH is again rapidly established at E temporarily until a final equilibrium condition is re-established with the RH of the storage facility at a lower moisture content of the product. At this higher temperature M_o:ERH co-ordinates are reduced to S_2 where the regression line AB cuts the transposed isotherm.

It is clear from these illustrations that increasing the temperature of the storage facility reduces the heat of sorption or M_o but raises the corresponding optimum ERH of storage. Thus temperature may be used to formulate convenient optimum M_o:ERH combination for the stable storage of dehydrated foods. It means that where the optimum A_w is inconveniently low, increased temperature may be used to raise it to a convenient level, with a corresponding reduction in Q_c or M_o, for stable storage. This should be of advantage in tropical food storage.

Alternative methods for measuring M_o or Q_c

From this work two alternative general methods suggest themselves to an investigator when the optimum moisture contents of dehydrated foods are desired. He may either use equation (1) alone, i.e. the mathematical method when only a few points on the isotherm, within the A_w range over which it is applicable, are known; or by the completely graphical or regression line method using the appropriate regression equation (Fig. 3) when the complete isotherm has been drawn up on the same numerical scale as for the regression line to find their point of intersection. The latter method does not require any equation to describe the water sorption isotherm.

Conclusion

The relationship between A_w and the optimum moisture content of dehydrated foods calculated with the author's model equation has been discussed.

A maximum of two distinct areas of the complete isotherm were identified. These were the areas of restricted water (R – 1) and the area of free water (R – 2).

The maximum attainable sorptive energy (Q_c) for optimum stability of dehydrated foods was found to be 11·3 kcal at zero A_w which is greater than one valence bond. This water binding energy reduces to zero at 0·784 A_w.

It is stated that the optimum moisture content for the stability of dehydrated foods is that moisture content which is bound to the macro-molecular constituents of the food with energy numerically equal in kcal to its own value. This critical sorptive energy is altered by temperature changes in the storage environment.

This principle has been illustrated and it is clear from the illustration that the proposed mathematical model can predict the optimum moisture levels of any adsorption pattern. Its general applicability is due to its ability to separate out groups of food for which separate regression lines can be drawn and used to estimate the optimal conditions for storage.

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Effects of oxygen diffusion on oxidation of some dry foods

DIETRICH QUAST AND MARCUS KAREL

Summary

The rate of oxygen uptake and the effective diffusivity of oxygen in some porous food products were determined. The effect of oxygen diffusion on the overall rate of oxygen uptake was investigated in these foods and in other food products for which approximate values of oxidation rate constants and of oxygen diffusivity existed in the literature. The results show that diffusion can be a rate-limiting factor under certain conditions of commercial processing and handling.

Introduction

Headspace oxygen has a deleterious effect on the quality of many products. To minimize this effect, the food industry uses processes such as deaeration, inert-gas packaging, inert-gas blanketing, vacuum-breaking with an inert gas, and packaging in materials with low permeability to oxygen.

Oxygen uptake by a food product may be due to respiration, enzymatic browning, lipid oxidation, or oxidation of proteins, vitamins, and many other food components. Lipid oxidation is probably the most common mechanism of oxygen uptake in dried foods and is responsible for the very high oxidation rates observed in such items as fish meal and potato chips. It may also result in significant flavour deterioration even when the oxygen uptake is relatively low, as in potato flakes (Buttery, Hendel & Boggs, 1961).

Marcuse (1967) and others have suggested that oxygen diffusion through the food product towards the reaction site may be a rate-limiting factor in some instances.

In this investigation, the diffusion of oxygen in foods was studied for conditions that may occur during processing, handling, packaging and storage.

Theoretical considerations

Diffusion and uptake of oxygen in foods

The unsteady-state diffusion of a gas into an infinite plate of a product with which it reacts in a first order reaction is given by the differential equation:

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$$D\frac{\partial^2 C}{\partial X^2} - \frac{\partial C}{\partial t} - k_1 C = 0 \tag{1}$$

where: D = oxygen diffusivity (cm^2/sec),
X = distance (cm),
C = concentration of oxygen ($moles/cm^3$),
t = time (sec),
k_1 = first order reaction rate constant (sec^{-1}).

Experimental results have shown that oxygen uptake by foods is frequently a first order reaction with respect to oxygen, especially at low concentrations (Marcuse, 1967; Karel & Labuza, 1969; Simon, 1969; Spiess, 1963). If the rate constant and concentration are very low, the last term becomes negligible compared to the other two and the diffusion process can be represented by Fick's law of diffusion:

$$D\frac{\partial^2 C}{\partial X^2} = \frac{\partial C}{\partial t} \tag{2}$$

If, however, the process occurs over very long periods, such as are found in storage, then the diffusion and reaction occur under practically steady-state conditions:

$$D\frac{\partial^2 C}{\partial X^2} - k_1 C = 0 \tag{3}$$

$$\frac{\partial^2 C}{\partial X^2} = \left(\frac{k_1}{D}\right) C. \tag{4}$$

In this case, the profile of the oxygen concentration inside the product does not change with time and is a function of the parameter $\frac{k_1}{D}$.

In the case of porous dry foods, D actually represents the effective diffusivity given by

$$D_{eff} = \frac{D_{12} \cdot \theta}{\tau} \tag{5}$$

where: D_{12} = diffusivity of binary $O_2 - N_2$ mixture,
θ = void fraction,
τ = tortuosity.

For small values of $\frac{k_1}{D}$ the concentration of oxygen inside the product becomes essentially uniform and equal to the outside concentration under steady-state conditions. However, for large values of $\frac{k_1}{D}$, a steep oxygen concentration gradient exists and the rate of uptake is limited by the rate at which oxygen is supplied by diffusion. The situation is identical to that existing for a porous catalyst, which was reviewed by Satterfield & Sherwood (1963). To establish whether diffusion is a rate-limiting factor, an effectiveness, η, is defined as the ratio of the observed reaction rate and the reaction rate when the concentration throughout the whole product is equal to the concentration at the boundary.

The relationship between η and the Thiele modulus, φ_L, is given graphically by Satterfield & Sherwood (1963) for several shapes. The Thiele modulus is defined as:

$$\varphi_L = \sqrt{\frac{kC^{m-1}}{D_{eff}}} \times L \qquad (6)$$

where: L = thickness of the infinite plate, sealed on one side (cm),
k = reaction rate constant,
m = order of the reaction.

From this relation, values of φ_L can be obtained for selected values of L, k, and D_{eff}. The corresponding values of η are obtained from Satterfield & Sherwood (1963).

Calculations

The rate of oxygen uptake by food products is often expressed as μl O_2 STP per unit of time and weight. To maintain a consistent set of units, the following conventions are used in the calculations:

R_m = rate of reaction based on mass $\frac{\mu l\ O_2\ STP}{g\ hr}$,

R_v = rate of reaction based on bulk volume of the product $\frac{cm^3\ O_2\ STP}{cm^3\ product \cdot sec}$,

ρ_b = bulk density (g/cm^3 product).

Since concentration of oxygen in the gas phase is directly proportional to partial pressure, calculations are based on P,

where: P = partial pressure of oxygen (atm).

With these units, the following relation holds:

$$R_v = 2{\cdot}8 \times 10^{-7} R_m \rho_b. \tag{7}$$

If the order of the reaction is known at any particular temperature, the overall rate constant can be calculated from the oxygen uptake.

If we assume that all the oxygen in porous dry foods is in the void space, then:

$$k_1 = \frac{R_v}{P_1 \theta} \tag{8}$$

$$k_0 = \frac{R_v}{\theta} \tag{9}$$

where: k_0 = zero order reaction constant $\frac{\text{atm}}{\text{sec}}$,

θ = void fraction,

P_1 = oxygen pressure at which rate R_v occurs.

For liquid and solid products:

$$k_1 = \frac{R_v}{PS} \tag{10}$$

$$k_0 = \frac{R_v}{S} \tag{11}$$

where: S = solubility of oxygen in the product

$$\frac{\text{cm}^3\ \text{O}_2\ \text{STP}}{\text{cm}^3\ \text{product atm}}.$$

Materials and methods

Materials

The rates of oxygen uptake of the following food products were determined:

1. Potato chips from the John E. Cain Co., Potato Chip Division, Cambridge, Massachusetts. This was a commercial product fried in sunflower oil with no antioxidant added. The oil content of the product was 39%. The experiments were started 1 day after production.

2. Fish meal obtained from anchovies in a commercial process at the Fisheries Research Institute, Santiago, Chile. The product was shipped by air in a sealed con-

tainer in inert gas. The experiments were started immediately after the package was opened, 2 weeks after the sample was collected.

3. Foam-spray-dried whole milk obtained from the Eastern Regional Research Laboratories of the United States Department of Agriculture in Philadelphia. Similar products have been described and studied by Tamsma *et al.* (1961). The experiments were started several days after production.

4. 'Bugles', a commercial snack food obtained from General Mills, Co., Minneapolis, Minnesota. This product contains corn grits, vegetable oil, sugar, salt, soda, BHA, BHT, citric acid, and monoglycerides. The product was well packaged in bags of aluminium foil laminate. Experiments were started 5 weeks after production.

Oxygen uptake

Two different techniques were employed. One was the Warburg manometric technique, which has been used extensively in this type of research for many years. The major disadvantage of the procedure is the dependence on atmospheric pressure.

The other method of measuring oxygen uptake involved use of the oxygen probe developed and described fully by Johnson, Borkowski & Engblom (1964). A known weight of product was transferred to a special test chamber with an inner volume of 380 cm^3. Thorough flushing of the chamber, containing the product, with an oxygen–nitrogen mixture allowed the headspace oxygen concentration to be adjusted to a desired level. A special device was developed to permit introduction of a small (5 mm diameter) oxygen probe at any time without gas leakage, as shown in Fig. 1. The probe was introduced from the left by pushing the rod to the right through the rubber seals.

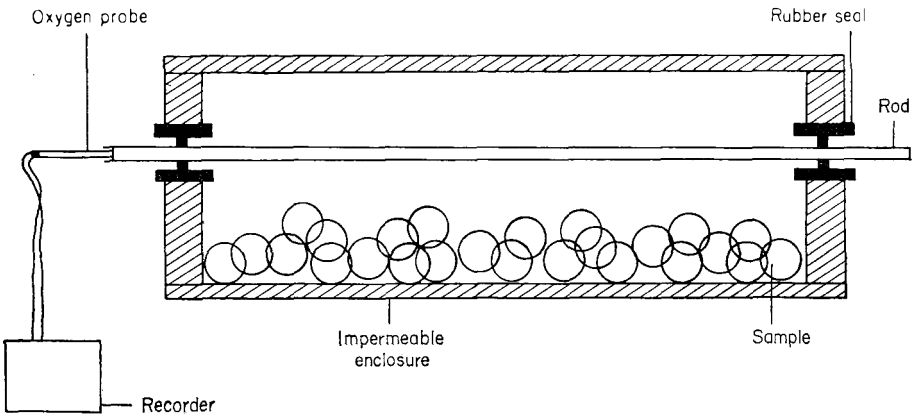

FIG. 1. Test chamber for measurement of oxygen uptake.

This technique allowed standardization of the probe prior to measurement; the significant change in the probe's output over a time interval of 1 to 6 months made standardization necessary. The oxygen pressure, P, could be determined within $\pm$ 0·001 atm. From the change of the headspace oxygen concentration with time, the rate of

oxygen uptake could be determined, since the total gas volume in the chamber was known.

Unlike the standard Warburg technique, this method suffers from no interference by the atmospheric pressure. It is suitable for high rates of oxygen uptake. For systems that react slowly, the change in headspace oxygen concentration is small even at a time when significant flavour deterioration has occurred.

Effective diffusivity

The effective diffusivities for several porous products were obtained by measuring the steady-state transfer of oxygen through a porous bed of known cross-section and thickness:

$$N = (A)\ (D_{eff})\ \frac{\Delta P}{\Delta X} \times \frac{273}{T} \qquad (12)$$

where: N = rate of oxygen transfer (cm^3 O_2 STP/sec),
A = cross-sectional area (cm^2),
D_{eff} = effective diffusivity at temperature T (cm^2/sec),
ΔP = partial pressure differential through the bed of thickness ΔX (atm),
ΔX = bed thickness (cm),
T = temperature (°K).

Oxygen partial pressures were measured with probes, and the rate of oxygen transfer was calculated from the oxygen concentration in the lower chamber and the gas flow rate (see Fig. 2).

Results and discussion

Effects of diffusion resistances on rates of oxidation in food products

The effectiveness parameter, η, was calculated for several food products on the basis of either experimental results or properties estimated from values in the literature (Table 1). Experimental values of the properties required for calculation of η were obtained for potato chips, fish meal, and foam-spray-dried milk. The results for other food systems were calculated on the basis of results reported in the literature. The effectiveness parameter, η, was estimated from the relationships developed by Satterfield & Sherwood (1963).

Additional assumptions made are discussed below for each product.

(A) *Potato chips.* Experiments conducted on this product gave the following average properties: bulk density = 0·1 g/cm^3, void fraction = 0·91.

The rate of oxygen uptake of 176 μl O_2 STP/g hr at 37°C was observed during the post-induction period, when the product was significantly rancid.

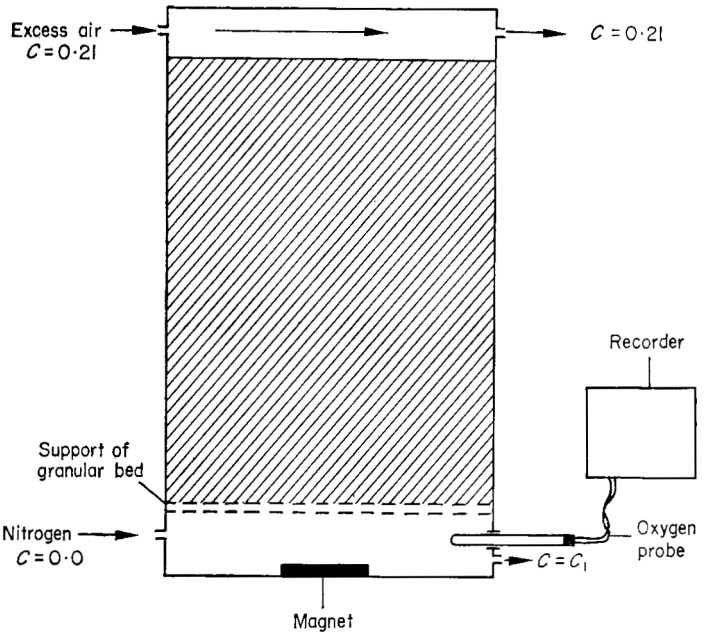

FIG. 2. Apparatus for determining effective diffusivity in porous foods.

During the induction period, which lasted more than 2 months at 37°C, the rate was approximately one-hundredth of the rate observed later (Fig. 3). The activation energy for oxygen uptake was 13 Kcal/g mole as compared to 2 Kcal/g mole in the postinduction period.

In the induction period, the rate of oxygen uptake was zero order with respect to oxygen partial pressure down to $P = 0{\cdot}05$. At lower concentrations, the reaction gradually approximated first order behaviour.

During the postinduction period, the rate of oxygen uptake was zero order down to $P = 0{\cdot}04$ (Fig. 4). Even during this period of rapid oxygen uptake, oxygen diffusion was not limiting, as manifested by a value of 1 for η even for a bed depth of 1 meter (Table 1). However, at low concentrations where the first order mechanism holds, oxygen diffusion is more likely to become a rate-limiting factor, as shown by a value of 0·3 for η.

For practical purposes, oxygen diffusion is not a rate-limiting factor for oxygen uptake since package dimensions are considerably smaller than 100 cm and the product should reach the consumer before leaving the induction period.

(B) *Fish meal.* Experiments with this product indicated a bulk density of 0·59 g/cm^3, a void fraction of 0·59, and an effective diffusivity of 0·05 cm^2/sec at 37°C. The rate of oxygen uptake was 24 μl O_2 STP/g hr at 37°C, with no induction period. The activation energy for oxygen uptake was 10 Kcal/g mole. Fig. 5 shows the change of oxygen concentration with time in two test chambers containing fish meal. After

TABLE 1. Kinetic and transport parameters for several food products

Product	Temp. °C	R_m µl O_2STP / g hr	ρ_b g/cm³	R_v cm³ O_2 STP / cm³ prod sec	θ	S (Solubility) cm³ O_2 STP / cm³ atm	P atm	k_1 sec⁻¹	k_o atm / sec	D_{eff} cm² / sec	L cm	φ	η
Potato chips, after	37	176	0·1	$4{\cdot}9\times10^{-6}$	0·91	—	0·21	—	$5{\cdot}4\times10^{-6}$	2×10^{-1}	10^2	1·14	1
induc-tion	37	176	0·1	$4{\cdot}9\times10^{-6}$	0·91	—	0·02	$2{\cdot}7\times10^{-4}$	—	2×10^{-1}	10^2	3·7	0·3
Fish meal	37	24	0·59	4×10^{-6}	0·59	—	0·21	$2{\cdot}3\times10^{-5}$	—	8×10^{-2}	10^1	0·2	1
during curing						—				8×10^{-2}	10^2	2·0	0·46
compressed						—				5×10^{-2}	10^2	2·5	0·40
Banana purée	23	—	—	$1{\cdot}2\times10^{-2}$	—	0·022	0·21	—	0·55	$2{\cdot}4\times10^{-5}$	10^{-3}	0·33	1
										$2{\cdot}4\times10^{-5}$	10^{-2}	3·3	0·47
										$2{\cdot}4\times10^{-5}$	10^{-1}	33	0·03
Butter	—	—	—	—	—	—	0·21	$1{\cdot}6\times10^{-8}$	—	$2{\cdot}2\times10^{-7}$	10^1	2·7	0·42

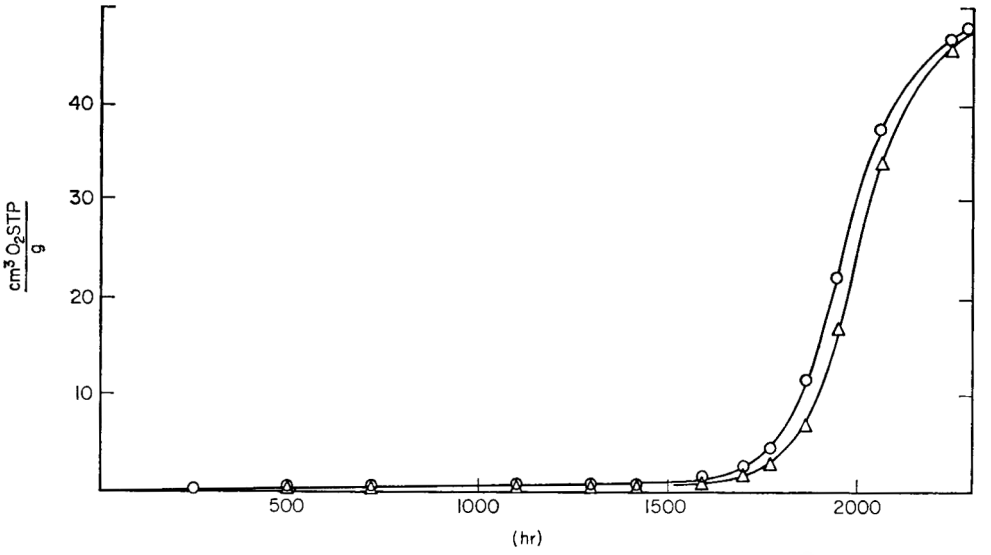

FIG. 3. Oxygen uptake of potato chips at 37°C, 0·21 atm O_2.

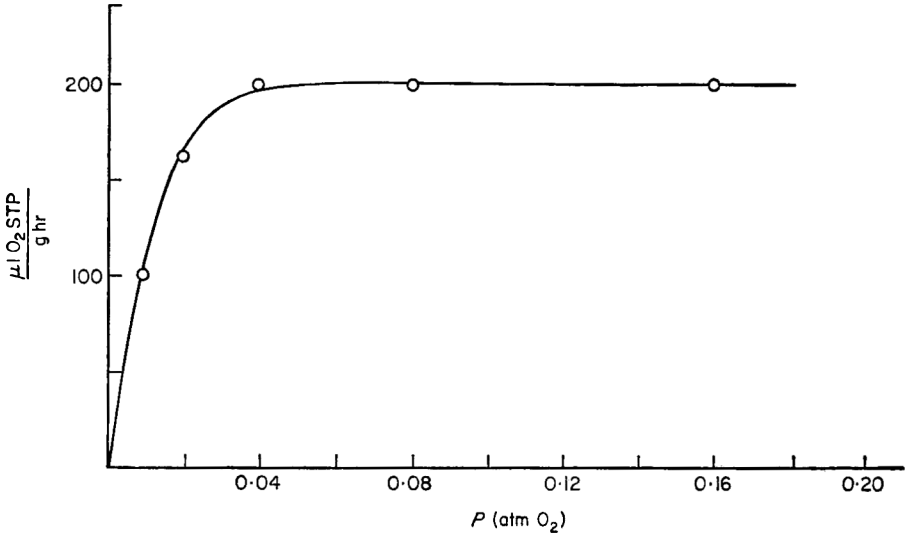

FIG. 4. Rate of oxygen uptake of potato chips in postinduction period at 50°C.

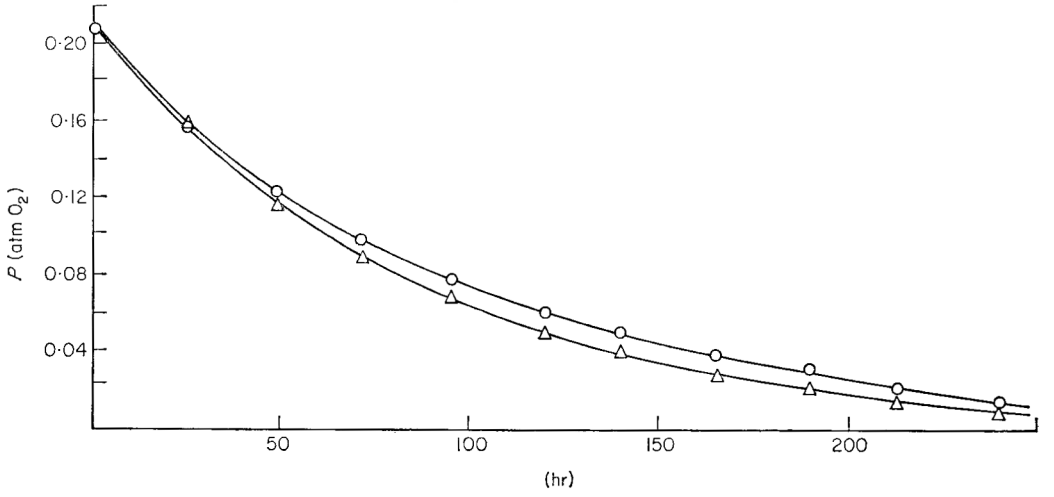

FIG. 5. Decrease of oxygen concentration in test chamber containing fish meal at 23°C.

240 hr the initial oxygen pressure was again adjusted to 0·21 atm and a similar curve was obtained. This consistency indicated that the effect of change of substrate concentration on the rate was negligible. Since the net internal volume, sample weight, and temperature were known, the rate of oxygen uptake at any pressure could be calculated from the slope at this concentration. The results are shown in Fig. 6. Unlike the results with potato chips, the reaction was first order over a wide range of oxygen partial pressures.

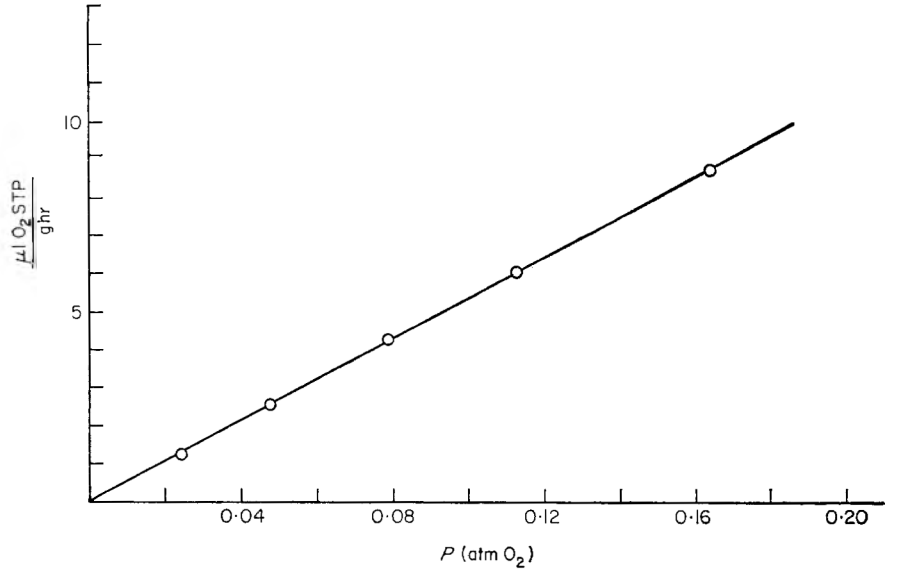

FIG. 6. Rate of oxygen uptake of fish meal at 23°C.

Fish meal is often 'cured' (i.e. autoxidized by exposure to air) to prevent combustion during shipping. Curing is usually done in bags where the path for oxygen diffusion is less than 20 cm long. Thus, diffusion is usually not a rate-limiting factor. However, diffusion becomes rate-limiting once the depth of the bed is about 100 cm (Table 1). This situation is likely to occur if the product is kept in silos or in bulk piles. Here the fish meal will oxidize significantly faster at the surfaces exposed to atmospheric air than in the bulk.

(C) *Whole milk and 'bugles'*. In both cases, the rate of oxidation was constant and very low at 37°C over a period of 100 days. At atmospheric oxygen pressures, the rate of oxygen uptake for both products was approximately 0·06 μl/g hr. The effective diffusivity of oxygen in the milk was 0·07 cm^2/sec. Diffusion is not likely to be a rate-limiting factor in the oxidation of these products except possibly at the almost microscopic level where 'glassy' structures may occur.

(D) *Other products*. After evaluating experimentally the problems of oxygen diffusion in some porous foods, we found it interesting to apply similar considerations to other kinds of foods for which sufficient information was available in the literature.

For banana purée, Palmer (1970, personal communication) found that the rate of oxygen uptake was approximately 0·7 cm^3 O_2 per cm product per min. According to Corse (1965), the Michaelis Menten constant for banana polyphenol oxidase (PPO) is 0·015 atmospheres. For other mechanisms of oxygen uptake, this constant is even lower. Thus, the reaction can be considered zero order for most situations occurring during processing and storage. Diffusion is a rate-limiting factor even for a distance of only 0·01 cm (Table 1).

The diffusion of oxygen in fat-containing foods under unsteady-state conditions was studied by Hintze, Becker & Heiss (1965). Their experimental results for butter were used to evaluate the effectiveness of oxidation under steady-state conditions. For certain packaging conditions, oxygen diffusion may be rate-limiting.

Certain foods, like candies, have oxygen diffusivities much lower than water. It is clear that in these foods oxygen diffusion can be a significant factor in reducing the overall rate of oxygen uptake.

Acknowledgment

The research was supported by U.S. Public Health Service Grant No. FD-00140 (formerly UI-00385).

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J. Fd Technol. (1971) **6**, 107–109.

Technical note: Temperature control of food

J. W. PEACOCK AND E. E. FITZGERALD

Concern exists that where a food provides a good medium for the growth of organisms and there is a risk of hazard to health, the food should be kept at a temperature at which organisms will not readily grow. The risk of hazard to health is normally considered not to exist if such food is maintained outside the temperature range 50°F (10°C)–145°F (62·7°C).

However there is little published evidence on bacterial growth at the relevant temperatures to support the choice of these limits. The extent of the risk must depend on contamination with pathogens and the ability of the pathogens to multiply to a level where a hazard to health would result.

This note presents the result of a 'limited' investigation into bacterial growth rates within the temperature ranges of 50°F (10°C)–70°F (21°C) and 120°F (49°C)–145°F (62·7°C). Using laboratory media and two strains each of *Salmonella* and *Staphylococci* we have found that all four organisms fail to multiply at 122°F (50°C), or higher temperatures, in fact they die out steadily at these temperatures. At 70°F (21°C) the shortest generation time, in the first 8 hr, for any of these strains was 4 hr so that in 8 hr the count had increased no more than four-fold.

These results indicate the need for more careful consideration before we accept or impose control of the temperature of storage of food, even for a short period, within limits which may not be justifiable on scientific evidence.

It is intended to extend the present investigation to the study of other strains of pathogenic bacteria.

Experimental

Cultures	*Salmonella heidelberg*	N.C.T.C.	5717
	Salmonella typhimurum	,,	74
	Staphylococcus aureus	,,	6571 (Oxford)
	Staphylococcus sp.	,,	10652
Media	Nutrient Broth No. 2	(Oxoid CM 67)	
	Plate Count Agar	(Oxoid CM 183)	

Procedure 100 ml amounts of nutrient broth were inoculated from young broth cultures of the organisms. All cultures were incubated in thermostatically controlled

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waterbaths. Samples of broth were withdrawn for plate counts initially and after 2, 4, 8, 16 and 24 hr. It was intended that the inocula should be of the order of 1000 organisms per ml and the results have been adjusted to an initial count of 1000 per ml in each test.

TABLE 1. *Salmonella heidelberg* N.C.T.C. 5717

Incubation temperature	Total count per ml at 37°C				
	10°C (50°F)	15·5°C (60°F)	18·3°C (65°F)	21°C (70°F)	50°C (122°F)
Initial	1000	1000	1000	1000	1000
2 hr	—	1050	1140	1200	800
4 hr	1080	1070	1140	1230	230
8 hr	1020	1670	1800	1940	< 10
16 hr	866	2970	3710	6150	< 10
24 hr	1060	5000	7600	17400	< 10

TABLE 2. *Salmonella typhimurium* N.C.T.C. 74

Incubation temperature	Total count per ml at 37°C				
	10°C (50°F)	15·5°C (60°F)	18·3°C (65°F)	21°C (70°F)	50°C (122°F)
Initial	1000	1000	1000	1000	1000
2 hr	—	1320	1050	1210	380
4 hr	930	1330	1490	1910	190
8 hr	1000	1670	3300	3820	150
16 hr	910	5330	16700	19100	< 10
24 hr	920	11600	87000	148000	< 10

TABLE 3. *Staphylococcus aureus* N.C.T.C. 6571

Incubation temperature	Total count per ml at 37°C				
	10°C (50°F)	15·5°C (60°F)	18·3°C (65°F)	21°C (70°F)	50°C (122°F)
Initial	1000	1000	1000	1000	1000
2 hr	—	900	1200	1460	282
4 hr	1150	1450	1550	1460	254
8 hr	1200	1800	2000	1820	70
16 hr	1200	3600	5000	7300	< 10
24 hr	1150	8000	15000	22500	< 10

TABLE 4. *Staphylococcus sp.* N.C.T.C. 10652

Incubation temperature	Total count per ml at 37°C				
	10°C (50°F)	15·5°C (60°F)	18·3°C (65°F)	21°C (70°F)	50°C (122°F)
Initial	1000	1000	1000	1000	1000
2 hr	—	1120	1200	1300	530
4 hr	1040	1260	1550	1860	430
8 hr	600	2000	2500	2310	<200
16 hr	900	3980	12500	14700	<20
24 hr	800	11200	69000	110000	<10

J. Fd Technol. (1971) **6**, 111–117.

Book Reviews

Carotenoids other than Vitamin A. Second International Symposium, New Mexico, May 1969.

London: Butterworths, 1970. Pp. 188 + v. £3.40.

This continues the work of an earlier symposium (*Pure & Applied Chemistry*, 1967, Vol. 14).

The term 'carotenoid' referred at one stage only to C_{40} polyisoprenoid compounds but today it is used, perhaps too loosely, to include a wide range of natural and synthetic substances containing from twenty to sixty carbon atoms. The provenance of carotenoids includes micro-organisms, plants and animals and the several hundred members of the family have trivial names reflecting, in the main, the original sources. Most of the known carotenoids have been characterized and many have been synthesized.

As far back as 1921–1924 research students working with Baly and Heilbron at Liverpool were isolating 'carotene' and 'xanthophyll' as part of investigations of photosynthesis—a research which paid the price of being too far ahead of its time. The main impression made by the present volume is that the time was ripe for a flood of new knowledge made possible largely by the skilful exploitation of new techniques.

Between the two Wars there was a gradual build-up of research on carotenoids in step with general advances in organic chemistry, spectroscopy and biochemistry. Interest was stimulated by the recognition of carotenoid precursors of vitamin A and it was realized that if the vitamin could be synthesized carotenoids might soon follow suit. The advent of commercial photoelectric spectrophotometers facilitated distribution studies and a great deal was achieved. A later climax was reached by sustained ingenuity in organic synthesis and improved methods of separation. Deployment of proton magnetic resonance spectroscopy and mass spectrometry made structural studies easier while the use of isotopes made possible much fine work on biosynthesis. A large debt to the designers of instruments needs to be generously acknowledged.

Physical methods are discussed by U. Schwieter and his colleagues. The relevance of absorption spectroscopy in the infra-red, visible and ultra-violet regions is clearly brought out. The value of n.m.r. spectroscopy is illustrated and the superiority of the expensive 220 MHz instrument over its 100 MHz predecessor is displayed over some difficult problems. Mass spectrometry is discussed with regard both to the determination of molecular weights and to molecular fragmentation. C. R. Enzell adds important information on the utility of fragmentation patterns in structure determinations.

S. Liaanen-Jensen contributes a clear account of her work on structures, particularly in respect of some recent examples. C. Bodea discusses the cyclization of carotenoids

in vitro as an aid to the study of *in vivo* processes. He deals with epoxides and notes how formation of carotenoid epoxides is favoured in plants grown at high altitudes. B. H. Davies discusses the carotenoids obtained from a photosynthetic bacterial species grown in the presence of an inhibitor that favours the accumulation of biosynthetic intermediates.

B. C. L. Weedon contributes a concise survey of some recent advances in total synthesis, particularly of the newer allenic and acetylenic carotenoids. His treatment brings out a surprising range of end-groups. The technical mastery shown by organic chemists now reveals how vast are the areas for biological research.

T. W. Goodwin summarizes the work of his group on the use of [2-^{14}C]mevalonic acid stereospecifically labelled with tritium at C–2, C–4 and C–5 to disclose details of carotenoid biosynthesis in chloroplasts, root tissue slices and fungi. Biochemical mechanisms are advanced for the formation of phytoene and the cyclization of acyclic precursors of α- and β-carotene derivatives and for the formation of 3-hydroxy-carotenoids.

J. W. Porter discusses the enzymic synthesis of carotenes and related substances by the plastids of tomato fruits. He demonstrates that given mevalonic acid a soluble extract of an acetone powder of tomato fruits catalyses numerous steps in accepted biosynthetic pathways.

Although not all the information in this book is very new, the symposium is valuable and interesting. Almost every way of building C_{40} carotenoids has been explored and many aspects of carotenoid chemistry are now in a highly developed state thanks to the work of Isler and others. Biosynthesis too has advanced considerably but much remains to be discovered about functions.

R. A. MORTON

Infrared Vapour Spectra. By D. WELTI.

London: Heyden and Son Ltd, 1970. Pp. xi + 211. £8.50.

This book will be valuable to both infrared (IR) spectroscopists and to those engaged in vapour phase chromatography. IR absorption is an important method for the identification of the components eluted from a chromatographic column. The IR spectrum can be used to 'finger-print' the component and this 'finger-print' can then be compared with reference spectra of known components. It is often convenient to measure the IR absorption of the chromatographic fraction in its vapour state and, as the title implies, this book is primarily concerned with vapour phase spectroscopy.

The first four chapters deal with the theoretical and practical aspects of IR spectroscopy and its combination with gas chromatography. Chapter 1 is concerned with the problems of sample handling and the choice of infrared cells of suitable path-length

and volume. Chapter 2 is entirely devoted to correlations of specific chemical groupings with the position of the absorption band. For most grouping normal frequency correlations apply equally well for liquid and vapour spectra; the main exceptions are those groups which can take part in hydrogen bonding. The third chapter gives a brief introduction to some of the theoretical aspects of IR spectroscopy, while Chapter 4 summarizes the techniques for trapping samples and enumerates the advantages of the direct combination of chromatography and spectrometer. An extensive collection of over 300 vapour phase IR spectra of organic compounds with boiling point up to ~ 270°C is given in Chapter 5. The spectra are drawn in the region 650–4000 cm^{-1}. To aid the search for other published data an index is provided to the vapour phase spectra to be found in the commercial reference spectra and in the Wyandotte/ASTM punched cards.

Some illogicality exists in the order of the chapters which vacillate between theory and experiment. That on the combination of IR and gas chromatography contains much valuable information, although in places it is difficult to decide whether it is liquid or vapour spectra that are under consideration. The paragraph giving a discussion of mass spectrometry seems somewhat out of place.

The book is well presented on good quality paper with hard covers and is a must for laboratories engaged in the IR spectroscopy of vapours.

W. R. LADNER

The Yeasts. Vol. 3: Yeast Technology. Ed. by A. H. ROSE & J. S. HARRISON London and New York: Academic Press, 1970. Pp. 590, £7·50.

It has been the aim of the editors to bring together and relate the vast amount of information concerned with the study of yeast. Much of this information could be found previously only by reference to original communications. The editors have taken account of the practical and academical aspects of such information and they were sufficiently wise to recognize that justice could not be done to the study of yeasts in a single manageable book. Accordingly, the material has been divided into three volumes. The first volume deals with the biology of yeasts, the second will deal with the physiology and biochemistry, and the third volume deals with the technological use of yeasts. The aim of the editors was to arrange for 'each volume to be sufficiently complete in itself to be perused separately'. To a large extent this last objective has succeeded, but for anyone wishing to delve more deeply into some of the physiological and biochemical aspects of a number of topics dealt with in Vol. 3 (the topic of this review), more detailed information may be required, hence it is regrettable that circumstances have delayed the publication of Vol. 2, in which much of the additional information will undoubtedly be found.

The editors choice of contributors was sound, each chapter being the work of an outstanding authority on his topic. The high standard of presentation, as set out by the editors in their 'Introduction', has been maintained throughout this volume. All chapters are concise and clear, and what is more, each contributor, while maintaining his own style of writing, maintains the reader's interest. Tables and figures are easily understood, and all are essential to the text. The editors are to be congratulated on the comprehensive Author Index and Subject Index.

It would be difficult, in such a review as this, to attempt to do more than indicate the coverage of this volume, this is probably more effectively done by quoting the titles of the Chapters which are as follows: 'Yeast in Wine-making' by R. E. Kunkee and M. A. Amerine, 'The Role of Yeast in Cider-making' by F. W. Beech and R. R. Davenport, 'Brewer's Yeasts' by C. Rainbow, 'Saké Yeast' by K. Kodama, 'Yeasts in Distillery Practice' by J. S. Harrison and J. C. J. Graham, 'Baker's Yeast' by S. Burrows, 'Food Yeasts' by H. J. Peppler, 'Yeast as Spoilage Organism' by H. W. Walker and J. C. Ayres, 'Miscellaneous Products of Yeasts' by J. S. Harrison.

In dealing with yeast technology, it is inevitable that the bulk of the material deals with the role of yeasts in the production of alcoholic beverages. The various contributors have managed to avoid extensive repetition of data. Where repetition of essential data occurs, the emphasis is different, hence a wide picture is presented which enables the reader to appreciate the subtle differences in mechanisms of the various yeasts used in the production of different products. It is pleasing to note that the opening Chapter 'Yeast in Wine-Making' supplies an interesting excursion into the historic background of yeast technology, as well as bringing the reader up to date with recent developments. This chapter, and that on 'Saké Yeast' reveals that there are many problems still to be resolved before the yeast technology in the production of wine and saké have reached the stage of scientific know-how and development such as has been achieved in brewing and cider production. To many readers in the Western part of the World, the unfolding of the mysteries of the art and science of producing Saké will be novel, as will the quaint terminology which provides additional colour to this intriguing aspect of yeast technology.

So far as the food scientist and technologist is concerned, the chapters of greatest interest are likely to be those concerned with baker's yeast and with yeasts as spoilage organisms. The chapters on 'Baker's Yeasts' and 'Food Yeasts' clearly shows that it is only as a result of extensive research and development programmes that these yeasts can be produced efficiently and cheaply. The chapter on 'Yeast as Spoilage Organisms' is one of the most comprehensive reviews on this topic that the present writer has encountered, however, it is a pity that more mention was not made to the techniques available for the isolation of yeasts from mixed populations. Anyone concerned with spoilage of food should find this chapter justifies the expense of purchasing this volume.

It is possible that the concluding chapter on miscellaneous products of yeasts will

stimulate thought on the use of yeasts in the development of new processes.

This volume should find wide usage among food scientists concerned with microbiological aspects of their subject, teachers, advanced level undergraduates and post graduate students. Those interested in yeasts in general, will certainly find all three volumes a valuable, if not indispensable, addition to their library.

E. O. MORRIS

The Chemical Analysis of Foods, 6th Edition. By DAVID PEARSON. London: J. & A. Churchill, 1970. Pp. xii + 604. £6.

Although the 6th edition of the late H. E. Cox's original book, it is the second time which Mr D. Pearson has brought it up to date. Much of Cox's original material was left out of the preceding edition and little now remains in the new edition. Few practising analytical chemists specializing in food analysis will mourn the loss, since the book has been greatly extended and now offers a comprehensive collection of methods, supported by appropriate technological information and set against the background of food legislation and the Codes of Practice in being in this country up to the date of publication.

Comparison with the previous edition, published in 1962, shows that all of the sections have been revised and in many instances much more detail is given in them. Chapter 3 on additives and contaminants has been extended to include emulsifying and stabilizing agents, solvents, pesticide and catty odours, and the determination of aflatoxin in peanuts is now included in Chapter 7. Antibiotics in milk are also now covered, and the number of commodities has been extended to include sugar confectionery, biscuits, marzipan, canned cereal milk puddings, pickles and sauces, peanut butter, vodka, meat pies, sausage rolls, monosodium glutamate and yoghourt. Some indication of the extent to which new material has been included is illustrated by the fact that the 6th edition contains 140 pages more than the 5th edition, and 260 pages more than the 4th edition, which was the last which H. E. Cox himself revised.

The analytical methods described in detail must of necessity reflect the personal choice of the author, but it is likely that experienced food analysts will not find themselves querying many of the chosen methods, and in that they do, it will stem from the difference in emphasis which their own experience imposes on the subject. The book, in fact, would form a very useful basis on which to bring such analysts together in order for them to discuss the relative strengths and weaknesses in many of the methods in common use and in doing so exchange useful information, based on practical experience, which might not otherwise be publicized. For example, not every batch of the recommended grade of Fuller's earth is suitable for use in Chapman, Fogden and Urry's method for cacao alkaloids which is described in the section on flour confectionery. Not only is it necessary to check the active earth by carrying out appro-

priate recovery tests, but also the blank should include the active earth and not just the steps in the method immediately following elution as the original authors suggest.

Although, the G.L.C. method for determining the butyric acid content of fatty mixtures containing butterfat is described in the book, there is only a passing reference, in the section on lard, to the use of G.L.C. in the examination of edible oils and fats. This is a somewhat surprising omission in view of the extensive use of this technique for such purposes but, in view of the different types of laboratory in which the book will be used, the author could not have found the task of deciding what to include and what to exclude very easy, and, on the whole, it has been well done.

Both the text and the methods are well backed by references, and it is particularly pleasing to note that the author makes a point of including references to the industrial processes by which are prepared the various materials and food commodities dealt with in the book, since it is equally important that analysis is carried out against the background as well as for ensuring compliance with legal requirements. Also important, of course, is sampling, and this aspect might well have been given rather more emphasis.

The book is remarkably free from errors and is well indexed, so that the proof readers assisting the author are to be congratulated on the results of their labours. At £6 it is unlikely to be bought by many individual analysts, although on present-day standards it is value for money. The reviewer has no hesitation in recommending laboratories engaged in work involving food analysis to invest in a copy for their shelves. The 'nature, substance and quality' of the book is such that analysts in those laboratories are unlikely to let it stay there for very long periods.

A. TURNER

Food Science & Technology. By MAGNUS PYKE. 3rd edition
London: John Murray, 1970. Pp. xviii + 228. £2.50.

In his introduction to the First Edition, Professor John Hawthorn states that Dr Pyke has made an attempt to survey the subject of Food Science and Technology as a whole and that he has done so in terms which an educated layman will understand. Dr Pyke has indeed done so and done it well. This easily readable book can be read by the educated layman and—even more to the point—by educated Directors, Executives and Plant Managers in the Processed Food Industries. The major areas of the Processed Food Industry are adequately covered (cereals; meats; fish and poultry; milk, cheese and eggs; fats and oils; and sugars, syrups and soft drinks).

The science and technology of fresh fruits and vegetables is perhaps not as well covered as the others and the new developments on storage, 'out-of-season' and long-distance transportation by the 'cold-chain' technique only just touched upon.

The processes of canning, quick-freezing, accelerated freeze-drying and dehydration are dealt with adequately. Insufficient attention is, however, paid to Food Packaging—a rapidly developing industry on its own, particularly as the use of the wrong

type of packaging can make the food wrapped therein unsaleable either because of deterioration in quality or absorption of 'taints' or 'off-flavours' from the packaging materials. It was also rather surprising not to find included a select Bibliography of recommended books to be read by those who wish to read further and deeper in a particular field. It is, nevertheless, a book to be recommended to Students, young Graduates, 'educated laymen' and all non-technical Executives in all branches in the Processed Food Industries.

N. GOLDENBERG

Books Received

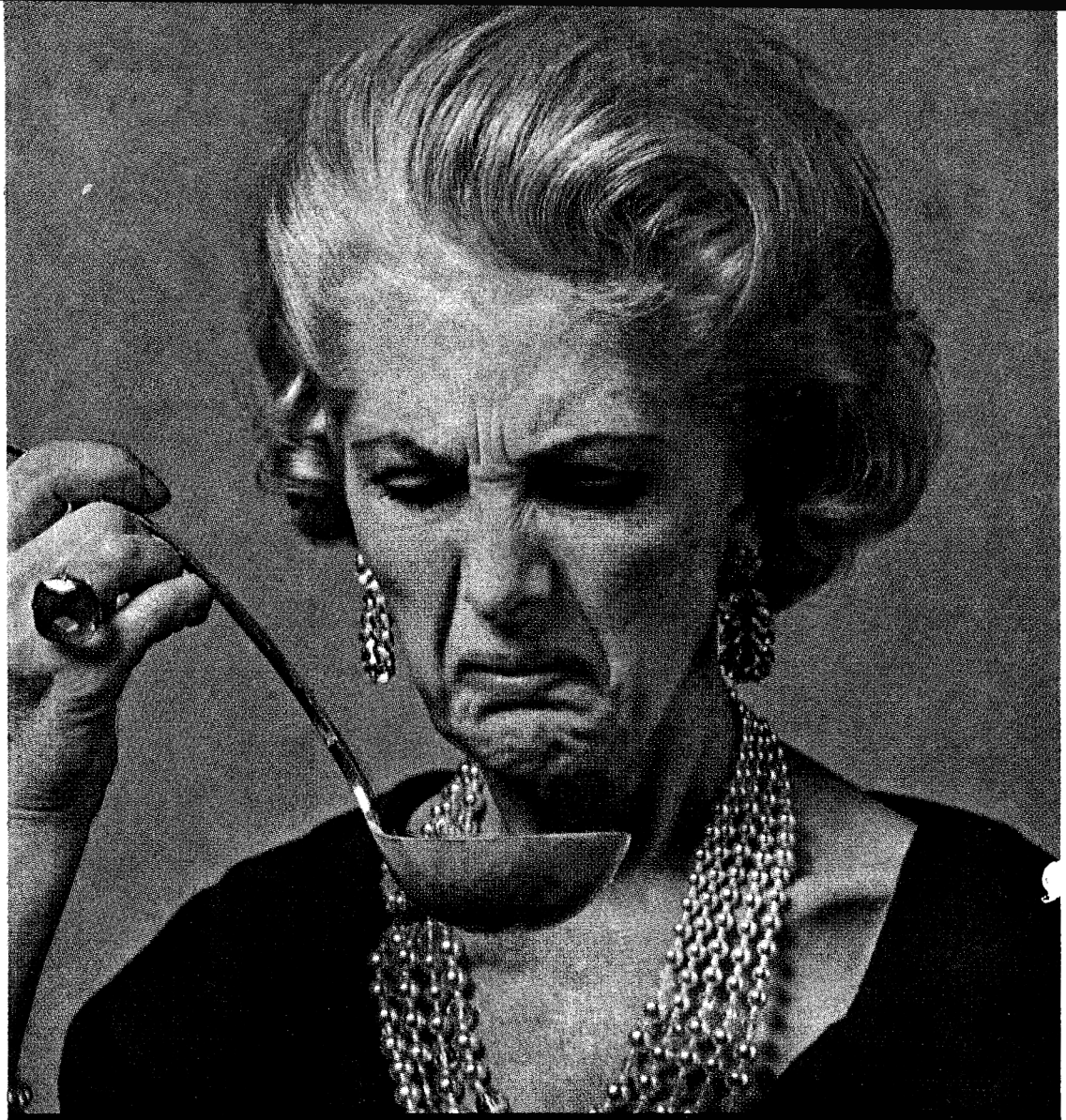
Chemistry of Natural Products. Sixth International Symposium, Mexico City, 1969
London: Butterworths, 1970. Pp. v + 123–293. £4·50.

Rice and Bulgur Quick-Cooking Processes. By ROGER DANIELS.
U.S.A.: Noyes Data Corporation, New Jersey, 1970. Pp. vi + 267. $35.

Gas Chromatography (Laboratory Instruments & Techniques Series). By C. SIMPSON.
London: Kogan Page, 1970. Pp. 117. £2·50.

Electron Microscopes (Laboratory Instruments & Techniques Series). By J. A. SWIFT
London: Kogan Page, 1970. Pp. 88. £2·00.

pH Meters (Laboratory Instruments and Techniques Series). By A. WILSON.
London: Kogan Page, 1970. Pp. 119. £2·00.



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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 of the commoner units are given below. The abbreviation for the plural of a unit is the same as that for the singular unless confusion is likely to arise.

gram(s)	g	second(s)	sec
kilogram(s)	kg	cubic millimetre(s)	mm^3
milligram(s)		millimetre(s)	mm
(10^{-3} g)	mg	centimetre(s)	cm
microgram(s)		litre(s)	l
(10^{-6} g)	μg	millilitre(s)	ml
nanogram(s)		pound(s)	lb
(10^{-9} g)	ng	gallon(s)	gal
hour(s)	hr	milliequivalent	mEq
minute(s)	min	R_F values	R_F

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. Letters and numbers must be written lightly in pencil. Whenever possible, the originals of line diagrams, prepared as described above, should be submitted and not photographs. The legends of all the figures should be typed together on a single sheet of paper headed 'Legends to Figures'.

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

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