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The protective effect of fat on the heat resistance of bacteria (I)*

A. F. SENHAJI† AND M. LONCIN‡

Summary

The effect of several oil/water systems on the heat resistance of spores of *Bacillus subtilis* and vegetative cells of *Pseudomonas fluorescens* has been investigated. Heat resistance was higher in the presence of oil and greatest without added water. An explanation of the phenomena observed is suggested in terms of differences in water activity between the systems.

Introduction

Many authors have suggested that fatty materials in the heating menstruum have an important effect on the heat resistance of microorganisms (Jensen, 1945; Zuccarro *et al.*, 1951; Hansen & Riemann, 1963; Molin & Snygg, 1967; Thuillot *et al.*, 1968; Zakula, 1969; Zaleski, Sobolewska-Ceronik & Ceronik, 1971). Most of these studies examined the heat resistance of organisms suspended in a single oily phase; in spite of the different methods of investigation almost all studies concluded that organisms in an oily phase were much more resistant than those in an aqueous phase. Various hypotheses, such as the low thermal conductivity of fats or the absence of water have been put forward to explain the effect, but until now no detailed study has been made.

Studies of two phase oil/water systems have yielded conflicting results (Molin & Snygg, 1967; Thuillot *et al.*, 1968; Zaleski *et al.*, 1971). This study, therefore, attempts:

(1) to determine under what conditions the protective effect occurs;

* The findings reported in this and the following paper formed the basis of a thesis submitted by A. Senhaji to the University of Paris to obtain the degree of Docteur Ingenieur. Some of the material has been published in *Industries Alimentaires et Agricoles* (1975) **92**, 622 and (1976) **93**, 13.

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(2) to suggest, with a mathematical model, the mechanism of the effect and to verify the theory by comparing the predicted with the experimental results.

Materials and methods

Organisms and preparation of suspensions

Spores of *Bacillus subtilis* NCIB 8054 and vegetative cells of *Pseudomonas fluorescens* SIK W1 were used, representing heat resistant and heat sensitive organisms respectively.

The spores were grown for two days at 30°C on the surface of a medium containing: nutrient broth (Difco) 0.8% w/v; yeast extract (Difco) 0.4% w/v; $MnCl_2.H_2O$ 10 ppm; agar (Difco) 2% w/v; pH 7.2. The organisms were harvested by scraping off the agar medium and putting into either sterile soya bean oil (A. B. Karlshamns Oljefabriker, Sweden) or into sterile 0.1 M phosphate buffer, pH 7.2. The two suspensions were homogenized (the one in oil with an Ultra-Turrax (Janke & Kunkel Kg)) and stored at 4°C until required. Microscopic examination showed less than 10% vegetative cells.

Suspensions of *Ps. fluorescens* cells were prepared in a similar manner, growing overnight at 30°C on tryptone glucose yeast extract agar (Difco). These suspensions in oil or phosphate buffer, were also stored at 4°C.

Preparation of ampoules and definition of heating systems

One or 1.5 ml portions of the suspensions were heated in 2.5 ml freeze-dry ampoules, the organisms being suspended either in phosphate buffer, or oil, or in one of the three model-systems shown in Fig. 1. Model-system I (MS I) represents the situation where drops of oil are suspended in an aqueous phase (an emulsion of oil in water). model-system II (MS II) is a two phase system, with separate layers of oil and water, in the same proportion as in MS I, and model-system III (MS III) is similar to MS II, where the thickness of

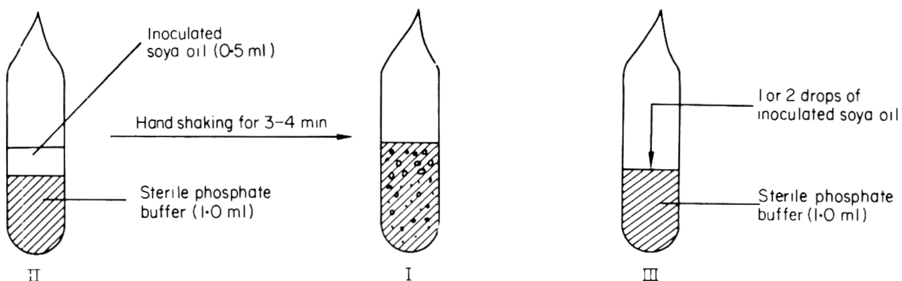

Figure 1. The three model-systems.

the oil is very small. Heat resistance of *B. subtilis* spores was examined in all systems; while *Ps. fluorescens* resistance was studied in phosphate buffer, oil and model-system II (MS II) only. Heating of the spores and vegetative cells was not carried out at the same temperature because of their widely differing heat resistance. The temperatures of heating were chosen so that survival curves could be conveniently determined.

Determination of heat resistance

A number of methods have been suggested for sterility testing or counting bacteria in fatty substances (Bullock & Keep, 1951; White, Bowman & Kirschbaum, 1968; Hambleton & Allwood, 1972). Most of these methods use solvents, which affect the bacteria to some extent. In order to compensate for this, the ampoules from all model-system suspensions were treated similarly, as follows.

The ampoules were opened aseptically and their entire contents transferred directly into the first dilution tube to give a 1:10 dilution. The diluent (0.1% w/v peptone water) contained 0.1% v/v Tween 80. The contents of the tube were homogenized with an Ultra-Turrax (20 000 rpm for 1 min) and the rest of the dilution series completed in the usual way using diluent without added Tween 80. Viable counts by surface plating were carried out on tryptone glucose yeast extract agar (Difco) enriched with 0.1% w/v soluble starch. Incubation was for two days at 30°C. Decimal dilutions were carried out in duplicate. Two ampoules were used to determine each point on the survival curves.

Treatment of results

The experimental results obtained by heating the bacteria in the five types of medium (phosphate buffer, oil and the three model-systems) were expressed as follows: N, the number per ml of viable organisms remaining, as a function of the time and temperature of heating, and the mean of counts from two ampoules; the initial counts (N_0) determined for each suspension remained remarkably stable even after storage at 4°C for more than three months. The results were expressed as surviving fractions (N/N_0) and for each heating temperature (T), time of heating (t) was plotted against log surviving fraction, and the linear parts of the curves were fitted by the method of least squares.

The decimal reduction times (D_T) and the inactivation velocity constants (k_T) were calculated from the slopes of the straight lines and their confidence limits at $P = 0.05$. Other pairs of values ($\log D_T$, T) and ($\log k$, $1/T$) were treated similarly when there was a linear relationship between the variables to give the values z and E (activation energy) respectively. From k_T, E and T it

was possible to calculate changes in free energy (ΔF^*), enthalpy (ΔH^*) and entropy (ΔS^*) of activation.

Results and discussion

The results obtained for *B. subtilis* spores in phosphate buffer are shown in Table 1. Table 2 compares the D values obtained in buffer with those obtained in oil and those obtained in MS II. Table 3 shows a similar comparison for *Ps. fluorescens*. The results for other systems appear in Figs 2–6 and Fig. 12

Table 1. Results of heating spores of *Bacillus subtilis* in phosphate buffer†

	Temperature (± 0.1°C)		
	85°C	95°C	105°C
Decimal reduction time (D_T)(min)	71±4	7.3±0.8	0.58±0.02
Correlation coefficient (r)	−0.993	−0.996	−0.999
Degrees of freedom	6	4	3
Shoulder (τ) (min)	408±2	21.5±0.5	1.5±0.1
$\tau + 4D_T$ (min)	(69±2)x 10	(5±1)x 10	3.8±0.2
k (sec^{-1})	5.37×10^{-4}	5.26×10^{-3}	6.62×10^{-2}
ΔF^* ($J\,K\,M^{-1}$)†	10.64×10^{7}	10.27×10^{7}	9.79×10^{7}
ΔH^* ($J\,K\,M^{-1}$)†	2.67×10^{8}	2.67×10^{8}	2.67×10^{8}
ΔS^* ($J\,K\,M^{-1}.\,{}^{\circ}K^{-1}$)†	4.48×10^{5}	4.46×10^{5}	5.26×10^{5}

$z = 9.5 \pm 0.5°$ C, $E = 2.70 \times 10^8$ J K M. † See text for definitions.

Table 2. Comparison of heat resistance of spores of *Bacillus subtilis* in phosphate buffer, oil and model-system II

	Temperature (±0.1°C)					
	85°C	95°C	105°C	110°C	115°C	120°C
Decimal reduction time (min) in phosphate buffer (D_p)	71	7.3	0.58	0.17*	0.05*	0.015*
Decimal reduction time (min) in oil (D_0)	1068	166.7	71.4	239.5	153.4	80.2
Decimal reduction time (min) in model-system II (D_{II})	100	47.6	38.5	—	—	—
ratio D_0/D_p	14.9	22.8	123	1409	3068	5347
ratio D_{II}/D_p	1.4	6.5	66.4	—	—	—

* Calculated values.

Table 3. Comparison of heat resistance of *Pseudomonas fluorescens* cells in phosphate buffer, oil and model-system II

	Temperature (±0.1°C)				
	45°C	50°C	60°C	65°C	70°C
D_p* (min)	19.2±0.6	0.94±0.06	—	[3.7×10^{-5}]†	[1.4×10^{-6}]†
D_0* (min)	667±54	—	53±3	129±10	38.6±0.9
D_{II}* (min)	154±18	67±2	—	59±3	25.1±0.9
ratio D_0/D_p	34.7	—	—	35×10^5	27×10^6
ratio D_{II}/D_p	8.0	71.3	—	16×10^5	18×10^6

* See Table 2 for explanation of symbols, † calculated values.

for *B. subtilis* spores, and Figs 7–12 for cells of *Ps. fluorescens.* Senhaji (1973) gives the results in full.

Shape of the survival curves

Thermal death of microorganisms is often considered to be a first order reaction, but many authors have observed deviations in practice (Roberts & Hitchins, 1969; Russell, 1971).

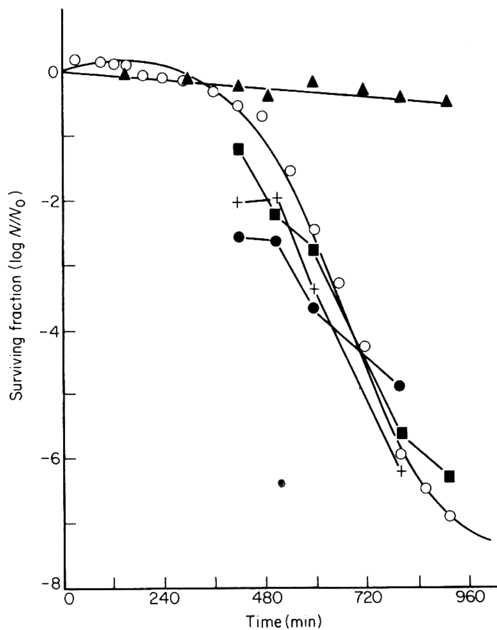

Figure 2. Heat survival curves of *Bacillus subtilis* spores at 85°C in phosphate buffer (○); oil (▲); model-system I (+); model-system II (■); model-system III (●).

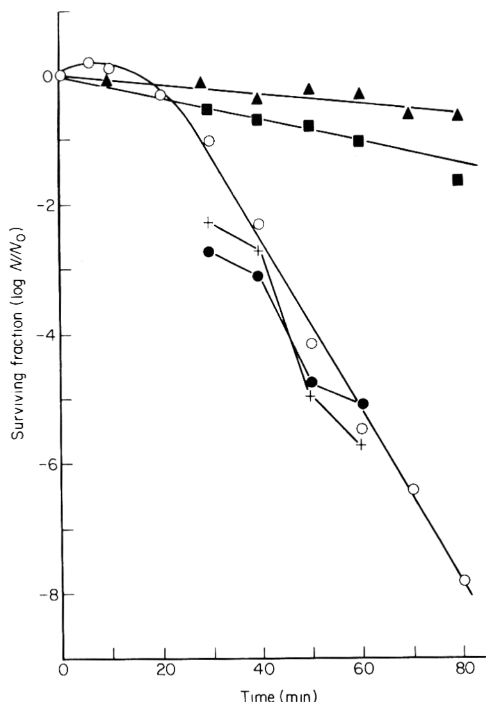

Figure 3. Heat survival curves of *Bacillus subtilis* spores at 95°C in phosphate buffer (○); oil (▲); model-system I (+); model-system II (■) and model-system III (●).

B. subtilis spores. Survival curves of *B. subtilis* spores in phosphate buffer (Figs 2, 3 and 4) showed deviations ('shoulders') on the initial parts of the curves. These shoulders could have resulted from activation of the spores (increased germination rate) (Shull & Ernst, 1962), a transition period from a heat resistant to a heat sensitive spore form (Komemushi, Okubo & Terui, 1963) or attributed to the presence of spores of differing heat resistance within the population (Levinson & Hyatt, 1971). The presence of clumps of organisms could also be a cause (Stumbo, 1965), but microscopic examination showed this to be insignificant during this investigation. The length of the shoulders seemed to depend on the temperature of heating: the higher the temperature the shorter the shoulder. In Fig. 2 the survival curve of the spores in phosphate buffer also shows a deviation concave upwards, in the later part of the curve, after the linear portion ('tailing'). One theory says that this tail indicates a variation in resistance within the population, another that there is a progressive accumulation of protective substances released from killed cells; no certain explanation has yet been given. It is however, interesting to note that these three parts of the curve gave it a sigmoid shape, which in our opinion should be the normal shape, at least in aqueous media. Linear curves observed after heating at high temperatures must also be sigmoid, but the shoulders and tails would be so small that they are difficult to observe. This hypothesis is in

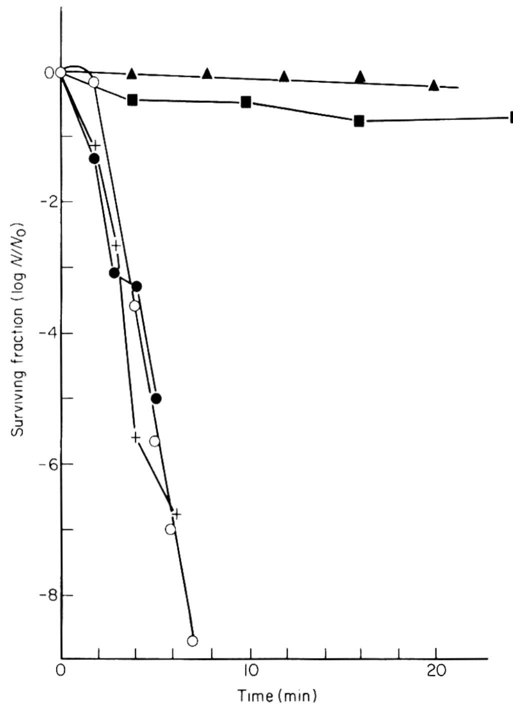

Figure 4. Heat survival curves of *Bacillus subtilis* spores at 105°C in phosphate buffer (○); oil (▲); model-system I (+); model-system II (■); and model-system III (●).

accord with the conclusions of a number of studies on thermal denaturation of enzymic proteins (Casey & Laidler, 1951; Dupont, 1965). The survival curves in soya oil at 85, 95 and 105°C (Figs 2, 3 and 4) are different. Given the high heat resistance of the spores in this medium, it is difficult to distinguish the shoulder, which must merge with the linear part of the curve.

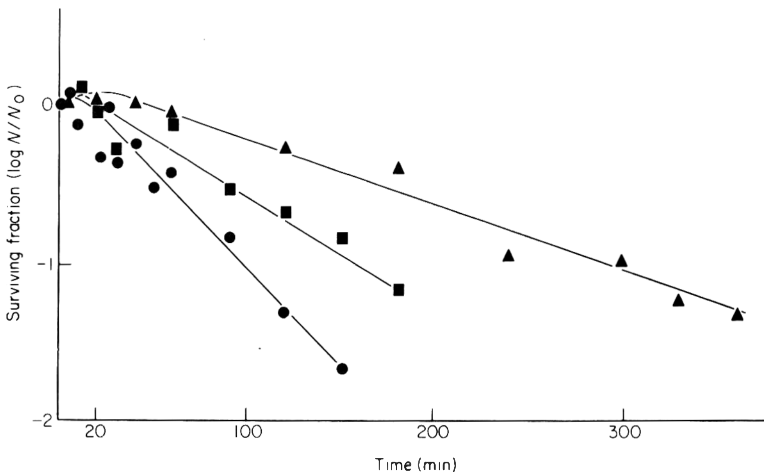

Figure 5. Heat survival curves of *Bacillus subtilis* spores in oil at 110°C (▲); 115°C (■) and 120°C (○).

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On the other hand, at higher temperatures, 110, 115 and 120°C (Fig. 5), with much higher resistance than in phosphate buffer, the curves again show indications of shoulders and tails. It seems from the survival curves that spores suspended in oil behave in the same way as those suspended in water, except for a large shift in the temperature scale.

The shape of the survival curves obtained in the three model-systems was the same as or similar to one or other of the curves described above. For instance, analysis of co-variance on the straight lines calculated for the survival curves of spores in phosphate buffer and in MS I, II and III at 85°C (Fig. 2) showed that they did not differ statistically from a mean straight line. Similarly for the inactivation curves in phosphate buffer and MS I and III at 95 and 105°C (Figs 3 and 4). However, the curves of MS II were similar to those in oil, but always with slightly greater slope, indicating that the resistance of spores in MS II at 95 or 105°C was much nearer that in oil than that in phosphate buffer.

Ps. fluorescens *cells.* These organisms gave acceptable straight line survival curves when heated in buffer (Fig. 7), but curves when heated in oil or MS II tended to be biphasic (Figs 8–10), showing an initial rapid death rate, followed by less rapid linear death rate, from which D values were calculated. In our opinion these biphasic survival curves occurred because the cells were surrounded initially by a layer of water, which rapidly diffused into the surrounding oil when the ampoule was heated, thus changing the environment of the cells from that similar to the buffer, in which the cells are heat sensitive, to a dry oil in which the cells are heat resistant. This phenomenon was not observed with the spore survival curves, perhaps because of their initial shoulder.

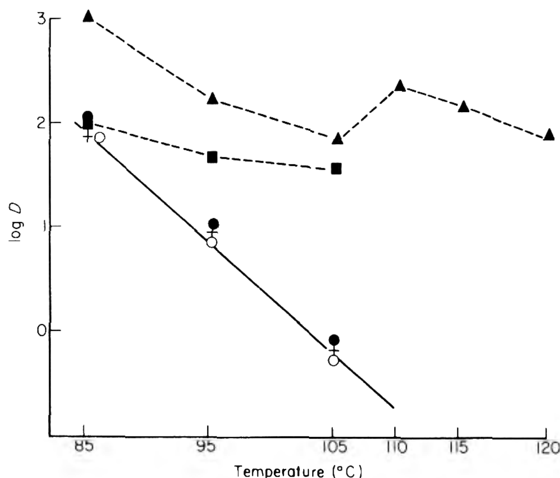

Figure 6. Thermal resistance curves for *Bacillus subtilis* spores ($D = f(T)$) in phosphate buffer (○); oil (▲); model-system I (+); model-system II (■); and model-system III (●).

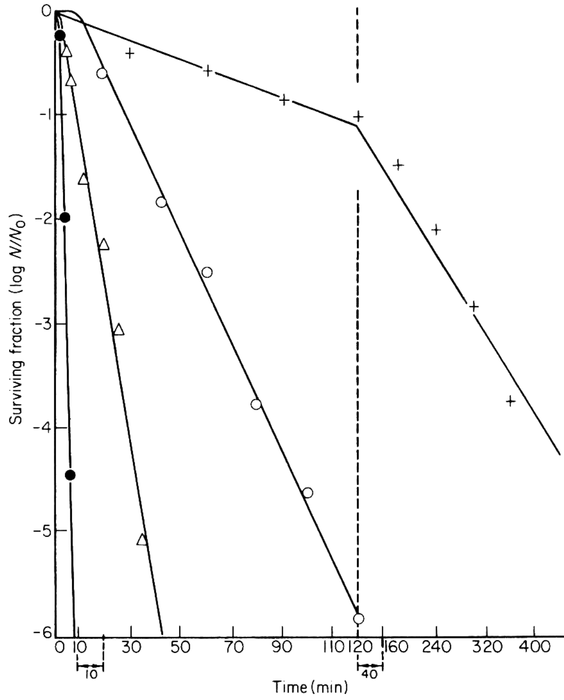

Figure 7. Heat survival curves of *Pseudomonas fluorescens* in phosphate buffer at 43°C (+), 45°C (○), 47°C (△) and 50°C (●).

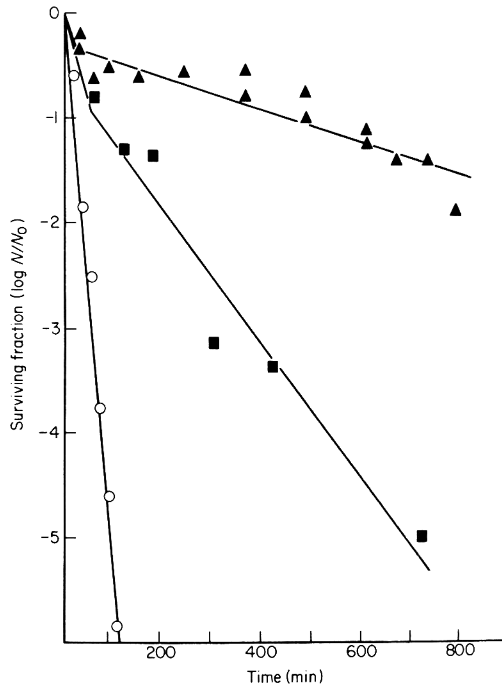

Figure 8. Heat survival curves of *Pseudomonas fluorescens* at 45°C in oil (▲), model-system II (■) and phosphate buffer (○).

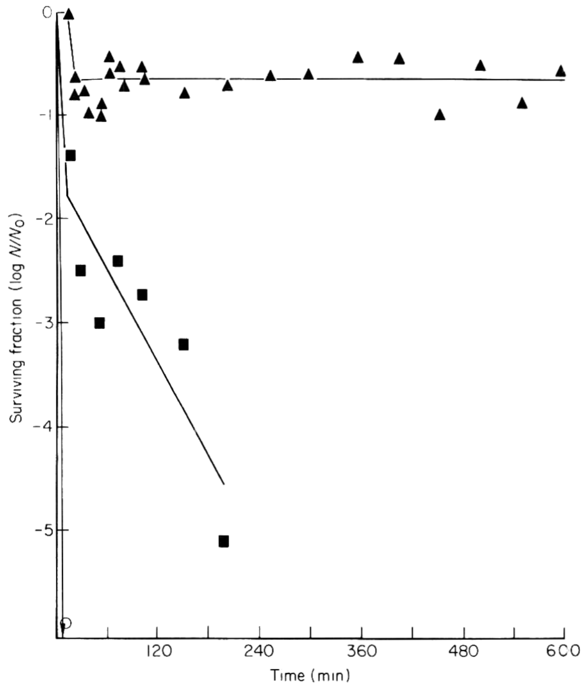

Figure 9. Heat survival of *Pseudomonas fluorescens* at 50°C in oil (▲), model-system II (■) and phosphate buffer (○).

In contrast to the results with *B. subtilis* spores, differences were observed, even at the lowest temperatures used, between heat resistance of *Ps. fluorescens* cells in phosphate buffer and MS II (Fig. 8). As the temperature of heating was raised, the resistance in MS II approached (Fig. 9) and almost equalled (Fig. 10) that in oil. The trend was similar to that exhibited by the spores (Figs 2–4).

In summary, similar trends in heat resistance in the various systems were

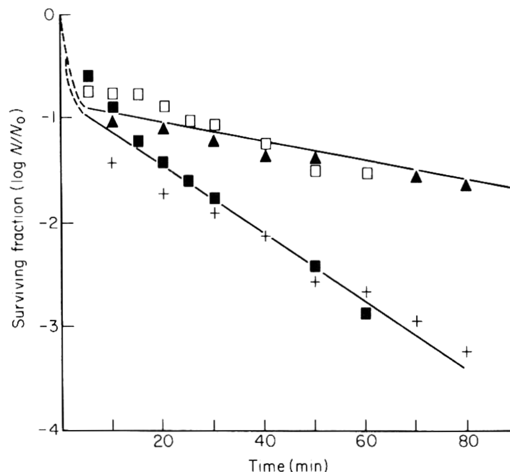

Figure 10. Heat survival of *Pseudomonas fluorescens* in oil at 65°C (▲) and at 70°C (+); in model-system II at 65°C (□) and 70°C (■).

observed with both strains of bacteria, although the spores were studied over a higher temperature range owing to their intrinsically higher resistance to heat.

Variation in heat resistance

(a) *Inactivation in oil.* The values of D_0 compared to D_p (Tables 2 and 3) show clearly, contrary to the statements of certain authors (Thuillot *et al.*, 1968) the important protective effect of oil, even though the oil used was not entirely free of water. This protective effect, measured by the ratio D_0/D_p increased considerably with temperature. Plotting log D against temperature (Figs 6 and 11) gave good straight lines for data from phosphate buffer, MS I and III, but not for the oil. Similarly for log k against $1/T$ (Fig. 12). Because of the non-linearity of these relations it was not possible to calculate values for z, nor those for activation energy and the other parameters that depend upon it. It was only possible to calculate changes in free energy of activation, F^* (from 26.8 to 28.4 kcal/M for *B. subtilis* in oil), which were of the same order as those calculated for inactivation in phosphate buffer (23.4 to 25.4 kcal/M). These figures seem to confirm quantitatively that the thermodynamics of heat destruction of microorganisms in aqueous media resembles that of proteins (Komemushi *et al.*, 1963; Rosenberg *et al.*, 1971), and that this also seems to be true in fatty media.

(b) *Inactivation of* B. subtilis *spores in the three model-systems.* The heat destruction of *B. subtilis* spores in MS I and III does not differ from that in phosphate buffer at any temperature, as can be seen in Figs 2, 3, 4, 6 and 12. The opposite is true for MS II, with the exception of the results at 85°C (Fig. 2). Table 2 shows that the D values for MS II (D_{II}) are much greater than those

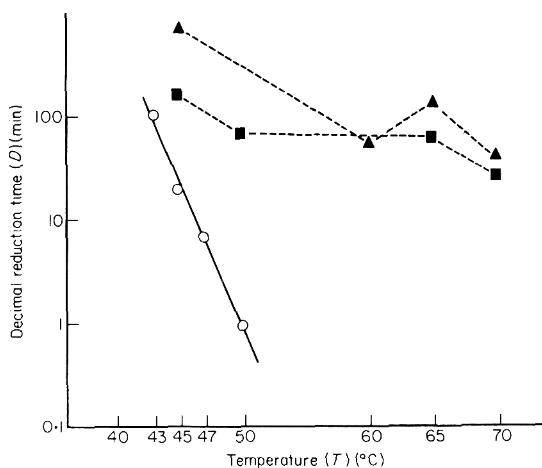

Figure 11. Thermal resistance curve ($D = f(T)$) for *Pseudomonas fluorescens* in oil (▲), model-system II (■) and phosphate buffer (○).

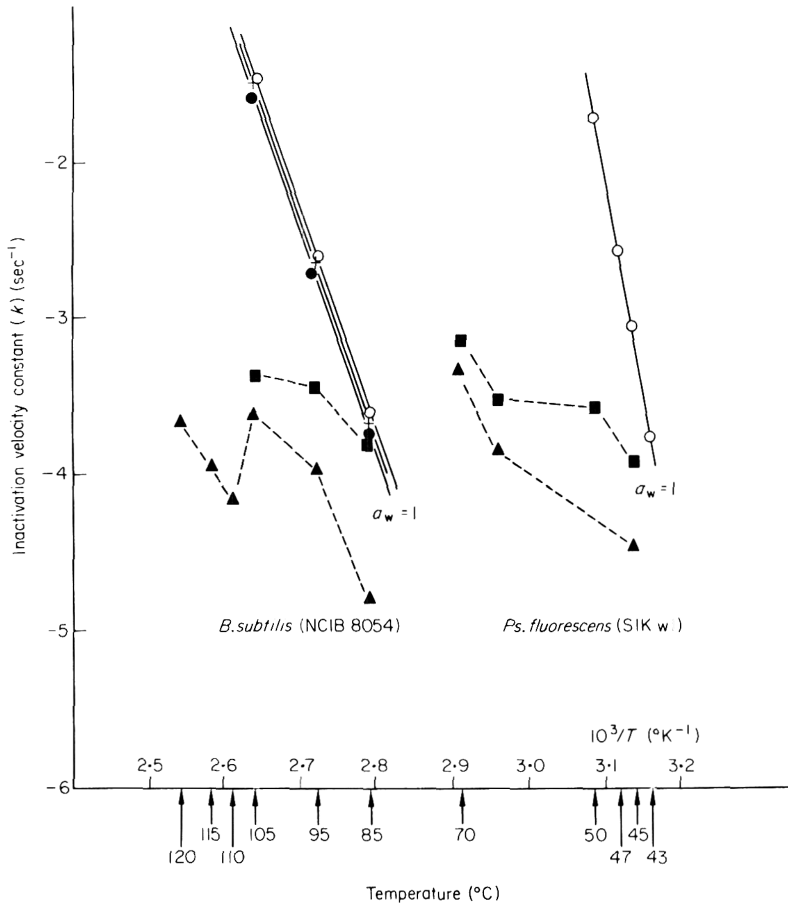

Figure 12. Plot of k = f(1/T) (see text for explanation of symbols) for *Bacillus subtilis* spores and *Pseudomonas fluorescens*, in phosphate buffer (○), oil (▲), model-system I (+), model-system II (■) and model-system III (●).

for phosphate buffer, although lower than those for oil. (The same is true for *Ps. fluorescens* (Table 3)).

Although it was possible to construct acceptable straight lines for log D_{II} as a function of T (Fig. 6) or for log k as a function of $1/T$, we consider, as with oil alone, that MS II changes during heating. A clue to the reason for these changes can be found in the difference in heat resistance observed between MS II and oil (Table 2). The sole difference between the two systems is the presence of free water in one and its absence in the other. If the penetration of heat is assumed to take place at about the same rate, and the composition of the media in the two systems is the same, then there are two possible alternative explanations.

1 *The faster death rate in MS II is due to microorganisms passing from the oily to the aqueous phase.* If the number of spores transferred to the aqueous phase is accepted to be a function of time, then this explains the observed

results – at high-temperature-short-time the inactivation approaches that in oil, while at low-temperature-long-time it approaches that in water. However, closer examination of the survival curves shows that this theory is not satisfactory. Take, for example, the survival curves of spores in MS II at 95 and 105°C (Figs 3 and 4): for the same time of heating (20 min) the number of spores transferred from one phase to the other should be the same in both cases; likewise, the behaviour (difference) in the destruction in phosphate buffer at these two temperatures should have been exactly the same.

2 *It could be a direct effect of free water present in MS II.* Solubility of water in oil increases with increasing temperature (Parsons & Holmberg, 1937; Mills & McClain, 1949; Loncin, 1955; Hilder, 1968, 1971).

On this basis, if we consider the spore suspension in oil, dissolved water is present at a concentration C_0 which corresponds to saturation in oil at the initial temperature; when this suspension is heated, the solubility of water in oil increases and the concentration at saturation, C_s, is raised (higher than C_0). To relate this to water activity (a_w), $a_w \triangleq C/C_s$ (shown experimentally by Loncin (1955) and proved theoretically by Van Ness (1964)), C being the actual concentration of water (expressed as molar fraction) and C_s the saturation concentration at the temperature in question. Thus, it would appear that a_w in oil starts at a value of 1 (if initially saturated with water), and becomes lower as the temperature is raised. For instance, an oil with an initial water content of 0.25% at ambient temperature, heated to 85°C would have a saturation concentration of 0.33% and therefore an a_w value of about 0.75. Heating the same solution to 105°C, the saturation concentration would be 0.56% and the $a_w \triangleq 0.45$. Heat resistance of spores and vegetative bacteria increases as a_w is reduced and is maximum for spores at values of a_w between 0.4 and 0.2 (Murrell & Scott, 1966). This effect could explain satisfactorily the abnormally high heat resistance observed with these spore suspensions and this is consistent with a maximum of D for spores at about 105°C in oil alone, but not in any mixture with water, and can equally explain the similar effect observed with cells of *Ps. fluorescens.* This hypothesis remains to be verified (see Senhaji, 1977).

Acknowledgments

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The protective effect of fat on the heat resistance of bacteria (II)

A. F. SENHAJI

Summary

The experimental results of studies on the heat resistance of *Bacillus subtilis* spores in the model-systems described in the first part of this study (Senhaji & Loncin, 1977) are shown to be in accord with the results of numerical simulation. This confirms the hypothesis that the heat protection phenomenon of fats is due to reduced water activity in the fat during heating.

A second and direct experimental verification allows the presentation of guidelines permitting the occurrence of the phenomenon to be predicted.

Introduction

The hypothesis advanced in the first part of this study (Senhaji & Loncin, 1977) to explain the phenomena observed, needs to be verified by a numerical simulation. In order to achieve this, a number of constants occurring in laws of dynamics, besides various equilibrium coefficients, must be known.

Determination of constants and equilibrium laws

Physical constants. These constants, involved in the partial differential equations of Fick and Fourier, permit prediction of the changes of temperature and water concentration in relation to time and the geometry of the system considered (e.g. see Bird, Stewart & Lightfoot, 1960; Crank, 1967; Loncin, 1971).

Lack of information in the literature on food products, and lack of theory for prediction of these constants, obliged us to carry out our own experimental determinations. A review of the methods available and a description of those used in this study to measure thermal diffusivity ($\kappa/\rho C_p$) and the diffusion of

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water in certain vegetable oils ($\mathscr{D}$) can be found in full elsewhere (Senhaji, 1973a).

The values determined experimentally for soya bean oil are as follows:

$$\frac{\kappa}{\rho C_p} = 1.7 \pm 0.3 \times 10^{-7} \text{ m}^2/\text{sec}$$

$$\mathscr{D} \text{ at } 30°\text{C} = 2.6 \pm 0.3 \times 10^{-10} \text{ m}^2/\text{sec}$$

(where: κ = thermal conductivity of soya bean oil, ρ = specific gravity of soya bean oil, and C_p = specific heat of soya bean oil).

The laws of equilibrium. In order to follow the changes in physical, thermodynamic and biological constants in the system, and to correct their values according to the mathematical constants in the system, and to correct their values according to the mathematical model, a number of equilibrium relations were examined. The coefficient of the relation giving viscosity as a function of temperature was determined as follows:

$$\eta = \eta_0 \exp[3755(1/T - 1/T_0)]$$

(where η = dynamic viscosity at temperature T; η_0 = viscosity at temperature T_0) to be used in the Stokes–Einstein relation: $T/(\eta\,\mathscr{D})$ = constant. The expression for the saturation concentration of water in oil established by Hilder (1968, 1971), was verified:

$$\ln X_s = -7.118 - (1222)/T + (1.459 \ln T)$$

(where T = temperature (°K)). The approximation of water activity (a_w), as the ratio between the actual and the saturation concentration of water in oil X_E, expressed as molar fraction ($a_w = X_E/X_s$, where X_s is the saturation concentration at the same temperature) was established (Senhaji, 1973b). Finally, the relation between decimal reduction time (D) and a_w was established by a polynomial approximation from the experimental results (the influence of a_w on the heat resistance of bacterial spores has been studied thoroughly by Murrell & Scott (1966) and Alderton & Snell (1970)).

Investigation of the relationship between D and a_w on our spores was carried out according to the method of Harnulv & Snygg (1972) and with their collaboration. (Water activities were controlled by use of equilibrating solutions of LiCl, sealed into the same ampoule, but not in direct contact with freeze-dried spores.) Table 1 shows the results obtained. At 95°C, at a_w values below 0.48 it was almost impossible to determine D-values because of the very long heating times required. For this reason they were estimated by extrapolation from the measurements made at higher temperatures. Similar treatment of cells of *Pseudomonas fluorescens* gave a similar relationship, but because of the sensitivity of this organism to freeze-drying, even when suspended in skimmed milk, and the variability of the initial levels of viable organisms the results were considered to be unreliable, and therefore the numerical simulation was carried out only for spores of *B. subtilis*.

Table 1. Heat resistance of spores of *Bacillus subtilis* at various a_w levels

a_w	z (°C)	Decimal reduction time (min) at				
		95°C	105°C	115°C	125°C	135°C
1.00	9.3	4.8	—	—	—	—
0.93	9.3	9.1	—	—	—	—
0.77	9.3	76	—	—	—	—
0.48	13	4800*	—	150	27	4.7
0.18	19	10000*	—	—	260	91
0.00	19	1100*	330	97	—	—

*Extrapolated values.

The results with spores confirmed those of Murrell & Scott (1966) who found that spores of *B. megaterium* have a maximum heat resistance at a_w levels between 0.2 and 0.4, and not 0 as was previously generally supposed. Figure 1 shows the smoothed curve of $D_{95} = f(a_w)$ for which approximation by polynomial regression gives the equation:

$$D_{95} = \exp\,[14.8(a_w)^4 - 6.7(a_w)^3 - 31.5(a_w)^2 + 18.1(a_w) + 7.0]$$

First verification of the hypothesis

For each experimental a_w value the corresponding thermal reduction time curve was plotted, either from values of D_{95} and z, or from two values of D

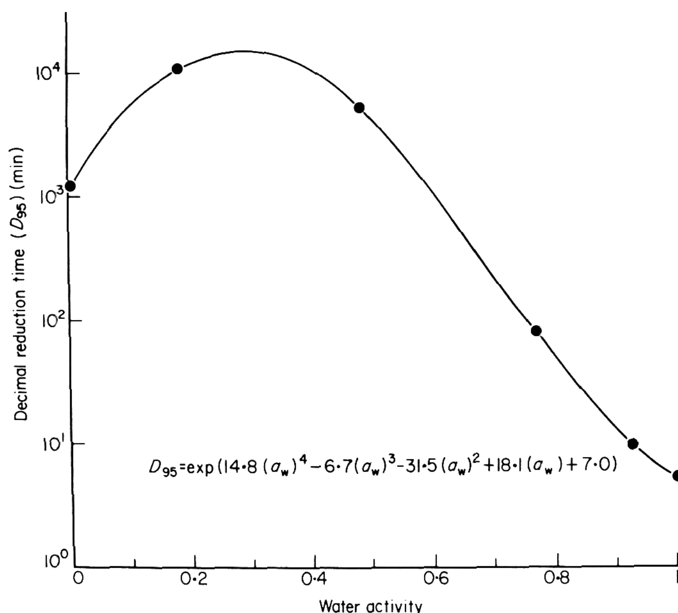

Figure 1. The effect of water activity on the heat resistance of *Bacillus subtilis* NCIB 8054 spores at 95°C ($D = f(a_w)$).

at two different temperatures, given that one is dealing with an 'invariable' medium to which the second law of thermal destruction applies. ($\log D_{T1}/D_{T2} = (T_2 - T_1)/z$, where D_{T1} = decimal reduction time at temperature T_1 and D_{T2} = decimal reduction time at temperature T_2.) In this way a series of TRT curves were obtained with parameters according to a_w.

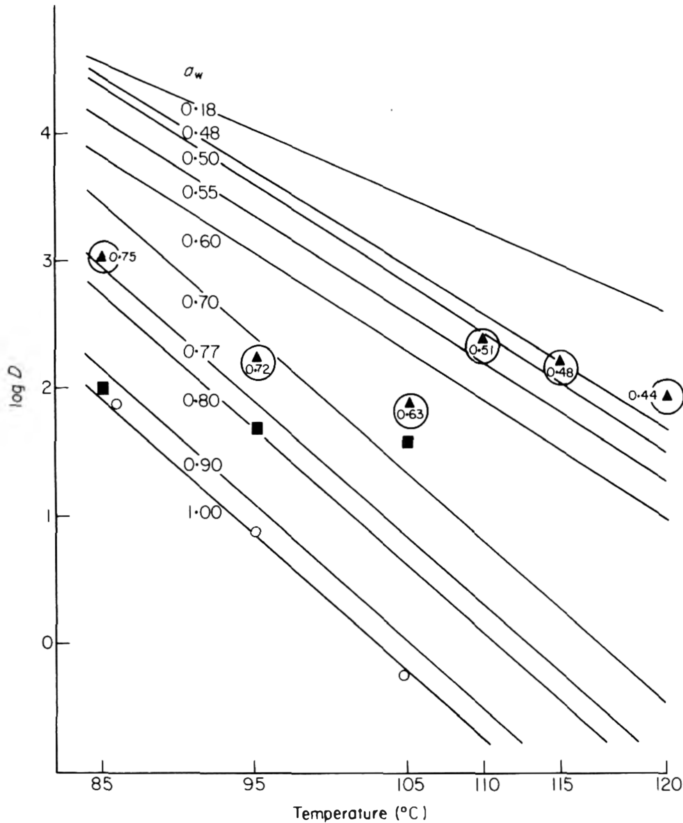

Figure 2. Thermal reduction time (TRT) curves ($D = f(T \times a_w)$), and experimental values for spores of *Bacillus subtilis* NCIB 8054 in soya bean oil (▲); model-system II (■); and phosphate buffer (○).

If the experimental values of D_T for the heat destruction of the same spores in phosphate buffer, oil and the model systems are located among the series of TRT curves (Fig. 2), the following three statements can be made:

1 The experimental points corresponding to the death rate in buffer and model-systems I and III fall near the TRT line for $a_w = 1$.

2 For destruction in oil, the approximate a_w values could be calculated in each case. From the initial water concentration in oil, determined by use of the Karl Fischer reagent, and using the diagram of Loncin (1955) relating to the solubility of water in oil, the approximate a_w values in the suspension at the experimental temperatures were obtained. They are given in Table 2.

Table 2. The relationship between the initial water content, the water activity during heating and the decimal reduction time (D-value) at various temperatures for spores of *Bacillus subtilis* suspended in soya bean oil

Temperature (°C)	Initial water content (% w/w)	a_w of the suspension during heating	D-value (min)
85	0.26	0.75	1068
95	0.28	0.72	167
105	0.28	0.63	71
110	0.25	0.51	239
115	0.25	0.48	153
120	0.25	0.44	80

Figure 2 shows that the experimental points for D fall near the TRT curves whose a_w levels most nearly correspond to those calculated in Table 2.
3 The points corresponding to heat destruction in model-system II (MS II) fall within the series of TRT curves (Fig. 2), but because the a_w during heating is variable, no estimation can be made. The points do not fall on any particular TRT curve; they represent an average value for D as a result of a continuous change in a_w values during heating (the values move vertically across the TRT lines, at constant temperature).

The same conclusions emerge from representation of the velocity constants, k, as a function of temperature and of a_w. This allows calculation of the energy of activation (E) from the Arrhenius equation, as a function of a_w (Senhaji, 1973c).

Second verification of the hypothesis

To confirm the effect of a_w the following experiment was carried out: ampoules, already containing 25 μl of sterile water, were filled with 1 ml of a suspension of *B. subtilis* spores in oil. For each treatment, two ampoules were heated statically and two others were agitated continuously during heating, with the aid of a vibrator. This allowed dispersal of the water throughout the oil in small droplets, so that the oil was permanently saturated with water during heating, maintaining the a_w of the oil close to 1. In the static ampoules the drop of water stayed at the bottom of the tube and movement of the water took place only by diffusion through the oil. A small quantity of water was chosen, sufficient to saturate the oil at the maximum experimental temperature, but avoiding the problem of the passage of spores from the oil to the water.

The results, two of which are shown as examples in Fig. 3, are unequivocal: destruction in the vibrated ampoules followed remarkably closely the pattern of destruction in phosphate buffer, while the static ampoules showed the

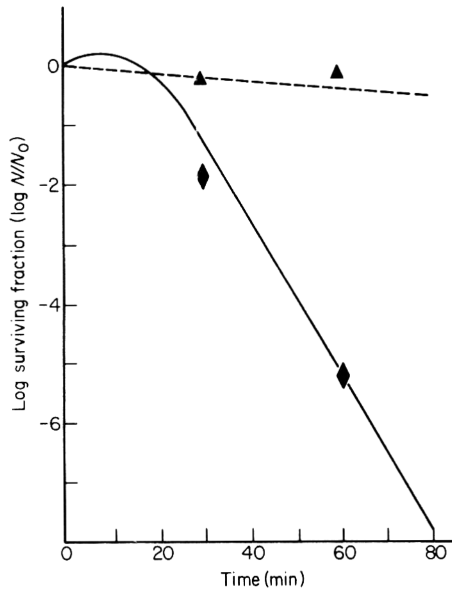

Figure 3. The effect of heating spores of *Bacillus subtilis* NCIB 8054 at 95°C in oil and 2.5% water, agitating (♦) and not agitating (▲), compared with heating in oil without added water (---------) or heating in phosphate buffer (————).

same inactivation rate as those with oil. This shows in a simple manner, that the protective mechanism of this system depends on a_w and its level during heating.

Simulation of the simultaneous transfer of heat and water, and the thermal destruction of microorganisms in the model-systems

Having determined the physical constants and verified or established the laws of equilibrium, all the data necessary were available to carry out calculations on model systems – to simulate the simultaneous transfer of heat and matter and to evaluate their effect on the thermal destruction of bacteria. The methods used for the calculation are not given here, but can be found in full in the complete report (Senhaji, 1973d).

Model-system I. The geometric model of this system (an oil–water emulsion) was satisfactorily defined as a spherical drop of fat, radius R_0, surrounded by an aqueous phase.

Simulation was carried out for the three experimental temperatures, 85, 95 and 105°C, for *B. subtilis* spores suspended in MS I and for fat droplets of various diameters. The initial temperature was taken in all cases to be 30°C. The numerical values of the physical constants were those determined above.

At 85°C, for the (unlikely) droplet diameter of 18 mm, saturation of water in oil is attained after 236 min, a much shorter time than the length of the shoulder (τ = 408 min), so that no protective effect would be observed in this case.

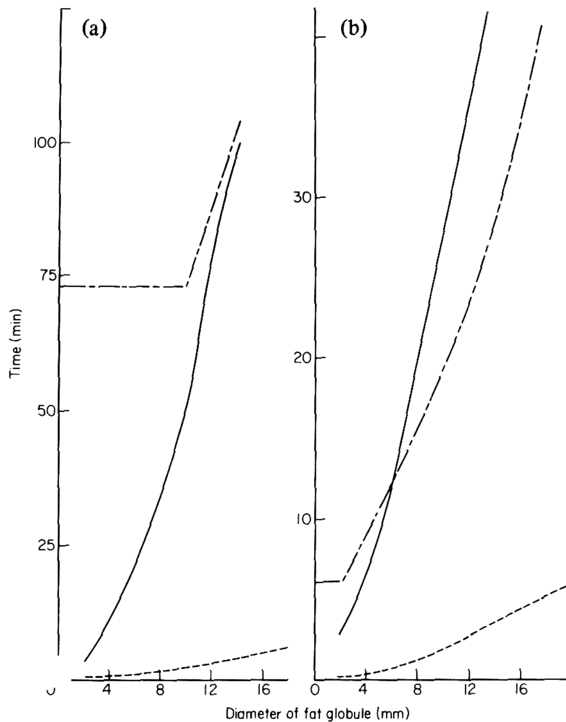

Figure 4. The effect of fat globule diameter during heating at 95°C (a) and 105°C (b) on the time to reach saturation with water (————); the time to reach thermal equilibrium (----------) and the time to reduce the initial spore population to 10^{-7} (—·—·—).

Figure 4 shows the time required at 95 and 105°C for droplets of various diameters to reach thermal equilibrium, to become saturated with water, and for the reduction of the initial spore population of the droplet to 10^{-7}. At 95°C, in fat droplets of diameters less than 10 mm, spore death rate is identical to that in buffer. Above this diameter the protective effect begins to appear, but droplets of this size would never occur in practice. At 105°C the limiting diameter beyond which the protective effect appears is 2 mm. Given that, even in emulsions with the largest fat globules, the diameters were much less than 2 mm (usually 0.1–30 μm), there would be little chance of a protective effect appearing at this temperature either.

These calculations explain the results obtained for MS I, where spore destruction followed exactly the same dynamics as that in phosphate buffer. It should be noted that, although the limiting diameter of fat droplets decreases as the temperature of heating is raised, this would certainly not pose any problem in food emulsions, provided there were sufficient water available in the environment of the droplets.

Model-system II. This system represents a layer of oil, thickness Δx, covering a layer of water. Heat transfer occurs radially in a cylinder, and in approxi-

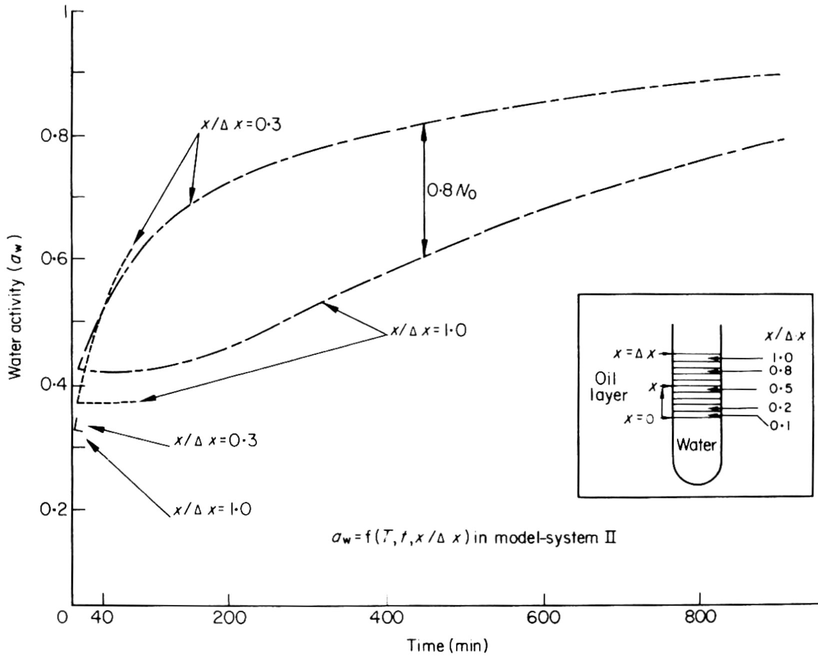

Figure 5. Change, at two levels in the oil layer, of water activity with time of heating of model-system II at 85°C (— — — —); 95°C (----------); and 105°C (————).

mately the same fashion in the two phases. Transfer of matter, however, (diffusion of water in oil) occurs in one direction only, from the lower to the upper layer across the oil–water interface. There are various possible models adaptable to this system; we chose the flat slab model for our calculations and the oily layer represents half its thickness. Figure 5 shows some of the results of simulations for this model-system, concerning the change of a_w with time in the oily layer.

Table 3 shows the a_w values at two levels in the oily layer at the beginning of treatment ('initial') and after a given time ('final') at various temperatures, and compares the survival of spores calculated from the simulation, or determined experimentally, with survival in aqueous medium alone, assuming that 80% of the spores are in the oily phase between the two levels. Before the temperature of the system has been raised the a_w level is 1.0, but Table 3 shows that during heat treatment the initial level (assuming heating-up is instantaneous) is in the region 0.3–0.4, where the heat resistance of spores is known to be near maximum. As the temperature of heating is increased the initial a_w levels are reduced. Conversely, as the temperature of heating is reduced and the treatment time increased, the a_w values at the end of the heating period approach unity. Figure 6, which represents a_w as a function of Fourier numbers, shows that the a_w depends less on the temperature than the time of treatment. An approximately equal treatment time should give a given a_w value

Table 3. Comparison of experimental surviving fraction of *Bacillus subtilis* spores in model-system II and aqueous medium (phosphate buffer) with the surviving fraction calculated from the water activity in the oily layer, after heating for various temperature/time combinations

Treatment	85°C – 900 min		95°C – 80 min		105°C – 7 min	
Position (x)	18 mm	3.6 mm	18 mm	3.6 mm	18 mm	3.6 mm
a_w* Initial	0.42	0.42	0.37	0.37	0.32	0.32
a_w* Final	0.79	0.90	0.37	0.62	0.32	0.33
Surviving fraction:						
Calculated	2.5×10^{-1}		9.1×10^{-1}		9.2×10^{-1}	
Experimental	10^{-6}		5×10^{-2}		5×10^{-1}	
In aqueous medium	7×10^{-8}		10^{-8}		10^{-9}	

* The values of a_w at the beginning and end of treatment represent the limits of variation in the oil layer between $x = 3.6$ mm ($x/\Delta x = 0.3$) and $x = 18$ mm ($x/\Delta x = 1$), $x = 0$ is at the oil–water interface. It is assumed that the oil zone contains 80% of the spore population and that the spores are evenly distributed in the oil.

in the oily layer. For instance, to attain $a_w \geqslant 0.75$ as a mean value for the oily phase requires 700 min at 85°C, 600 min at 95°C and about 500 min at 105°C. It should also be noted that for equivalent treatment, low temperature long time is more effective than high temperature short time.

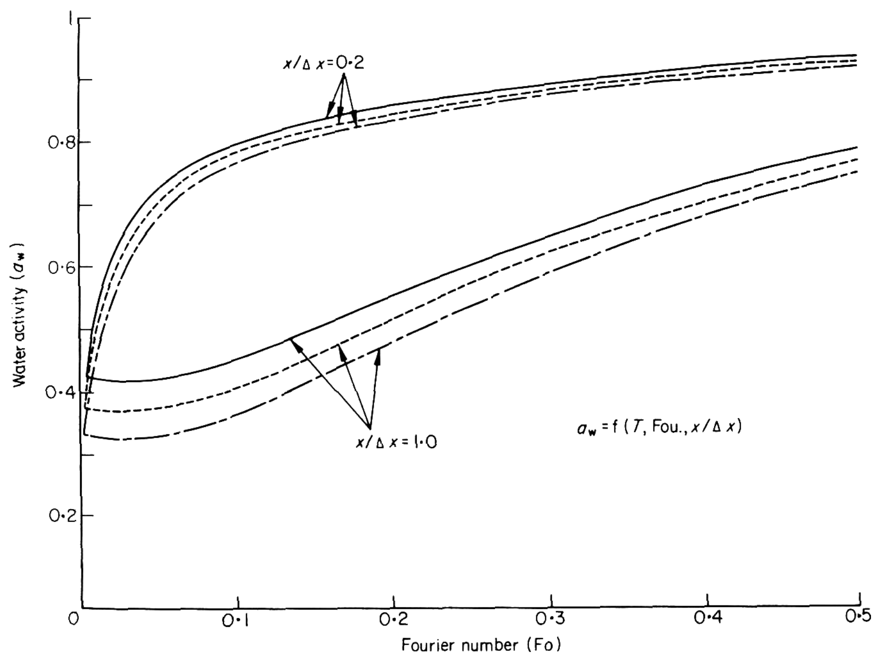

Figure 6. Change of water activity as a function of Fourier number at 85°C (————); 95°C (---------); 105°C (– – – – – –).

The simulated results and the experimental results for the thermal destruction of *B. subtilis* spores are compared in Figs 7, 8 and 9, for heating temperatures of 85, 95 and 105°C respectively. At 85°C simulation predicts a protective effect significant but less than that at high temperature short time (Figs 8 and 9), however, the experimental results for MS II are the same as those for phosphate buffer at this temperature. The divergence between theoretical and experimental seems at first surprising, but can be explained by the

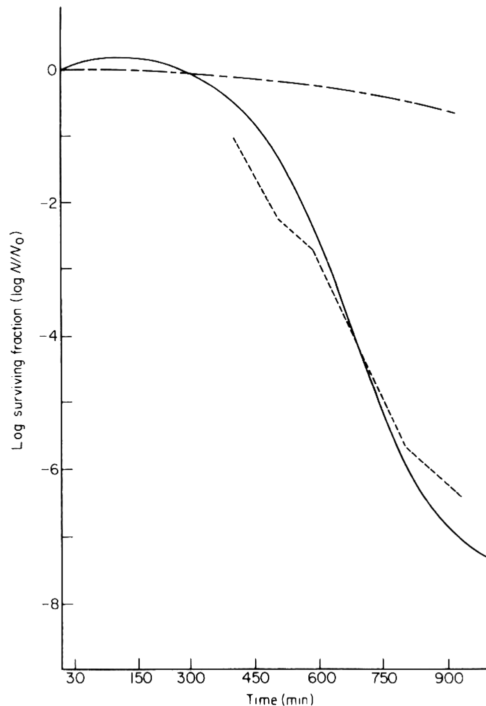

Figure 7. Simulated survival curves for *Bacillus subtilis* NCIB 8054 spores in model-system II (— - — - —); compared with experimental curves for phosphate buffer (————); and model-system II (----------) at 85°C.

fact that a transfer of spores occurs from the oily to the aqueous phase. Study of this transfer proved difficult, but it appeared to depend on the temperature of the system, the type of bacteria, the transfer surface and the contact time of the two phases, i.e. it was more important for treatments at 85°C when heating times were long. Most spores were destroyed in the aqueous phase and not in the oil, which explains the experimental results obtained.

On the other hand, at 95 and 105°C, the experimental and theoretical results agree well, considering the simplification of the hypotheses, the spore transfer factor and the limited accuracy of the determination of physical and biological constants. Thus the increased heat resistance of microbes in an oily layer is confirmed.

It follows that a protective effect of an oil layer, in the presence of water, can occur, and can be dangerous under the following conditions.

(1) The microorganisms must be present in the oil and not the aqueous phase; a chemical effect of protection by the oil have been shown to be absent (identical destruction in a tube containing an aqueous suspension of microbes and a drop of oil, and a tube with aqueous suspension only).

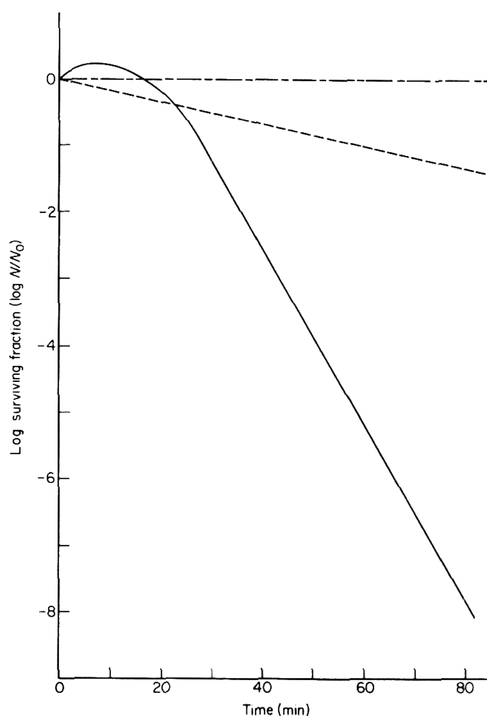

Figure 8. Simulated survival curves for spores of ***Bacillus subtilis*** NCIB 8054 in model-system II (— - — - —); compared with experimental curves for phosphate buffer (————) and model-system II (---------) at 95°C.

(2) The oil layer (containing the microorganisms) must have a thickness Δx (defined as the ratio of volume/surface contact with the water) such that the rate of diffusion of water into the oil limits the attainment of saturation.

Table 4 shows times to reach $a_w \geqslant 0.9$ at various temperatures and for various thicknesses of oily layer (Δx_L). If the protective phenomenon is considered to become significant when the a_w level is less than 0.9, then shorter treatment time with the same thickness of oily layer ($t < t_L, \Delta x_L$), or the same treatment of thicker layers of oil ($\Delta x > \Delta x_L, t_L$) would induce a protective effect.

Conversely, for longer treatment times ($t > t_L$) or for thinner layers of oil ($\Delta x < \Delta x_L$) there would be no protective effect. This explains the results obtained for the suspensions in MS III, the limit of MS II when Δx tends to zero.

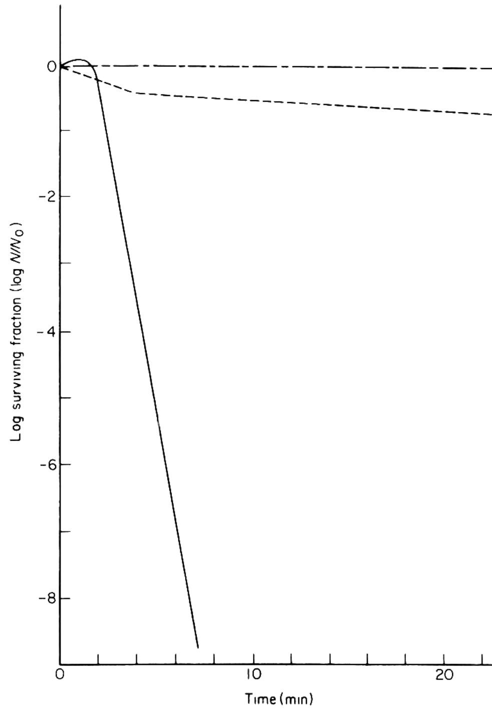

Figure 9. Simulated survival curve for spores of *Bacillus subtilis* NCIB 8054 in model-system II (—·—·—), compared with experimental curves for phosphate buffer (————) and model-system II (-----------) at 105°C.

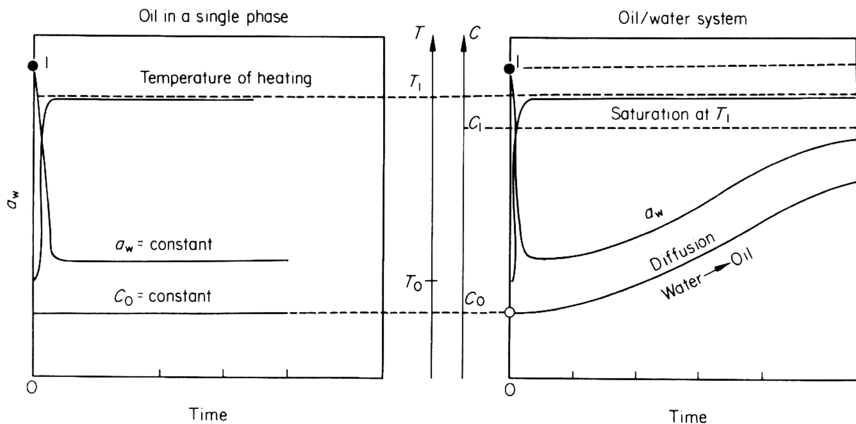

Figure 10. Schematic representation of the protective effect of oil on micro-organisms during heating. (T_0 = initial temperature; T_1 = temperature of heating; C_0 = initial concentration of water in oil; C_1 = saturation concentration of water in oil at temperature T_1.)

Table 4. Time required to attain $a_w \geqslant 0.9$ at various depths in the oily layer of model-system II with various temperatures of heating

Temperature (°C)	Time to reach $a_w \geqslant 0.9$, t_L (min)	Thickness of the oily layer, Δx_L (mm)
85	900	5.4
95	90	1.8
	720	5.4
	900	9.0
105	30	1.8
	540	5.4
	900	18.0

Conclusion

It has been demonstrated clearly from this study that the phenomenon of protection of microorganisms by fat against thermal destruction does exist:

1 for bacterial suspensions in a single oily phase in all cases;

2 for suspensions in oil–water systems under the following conditions: (a) the bacteria must be situated in the oil, and (b) the geometric form of the oil in the system must present a ratio volume/surface of contact with the water such that the diffusion of water into the oil is a limiting factor for the attainment of saturation at the temperature of treatment.

The phenomenon can be explained graphically, as shown in Fig. 10. The rapid transfer of heat causes the solubility of water in oil to increase, and consequently the a_w level in the oil decreases, so that the heat resistance of organisms in the oil is greatly increased. The a_w remains at this level or, if water is able to diffuse into the oil, approaches asymptotically $a_w = 1.0$ during treatment. Whether or not the phenomenon appears depends on the speed of diffusion.

The fat protection effect would not occur in most food emulsions because of the small size of the fat droplets in suspension. However, attention should be paid to their stability before sterilization – coalescence of the droplets could produce a situation similar to that in MS II.

For other types of preserved food (tinned foods, sauces, etc.) the occurrence of fat protection depends on the various factors mentioned above. The phenomenon could cause, at least, a latent danger; if microbes surviving in the oil stay in that phase, the failure of sterilization remains invisible, but if the survivors (one only is enough) migrate into the aqueous phase as a result of mechanical action or some other cause, multiplication could occur. It should be noted that when the aqueous phase contains solutes (salts, sugars, spices, etc.) they lower its a_w level and render the water less readily available to the oil, and at the same time increase the possibility of unwelcome protection.

Acknowledgments

I should like to thank Dr J. Corry and Professor M. Ingram for translating this paper into English.

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The effects of suspension method, chilling rates and post mortem ageing period on beef quality

R. L. JOSEPH AND J. CONNOLLY

Summary

The combined effects of 'tenderstretch' (pelvic) suspension, slow chilling and extended post mortem ageing on beef quality in six hind quarter muscles have been investigated using young Hereford cross heifers.

In *M. longissimus dorsi* at two days post mortem, tenderstretched fast (commercially) chilled meat was more tender than meat from normally suspended carcases. Slow chilling produced still greater tenderness in both normal and tenderstretched carcases. At seven days tenderstretched fast chilled meat had become as tender as slowly chilled meat but normally suspended fast chilled meat was not as tender, although it had undergone considerable tenderizing. These differences persisted to fourteen days post mortem without any significant increase in tenderness occurring. Sarcomere lengths were increased by tenderstretching but were not reduced by fast chilling.

In *M. psoas major* tenderstretching produced a slight toughening and shorter sarcomeres. Slow chilling had no effect, and ageing produced a slight reduction in shearforce.

In *M. biceps femoris* tenderstretching greatly lengthened the sarcomeres but produced only a small tenderizing effect. Slow chilling and ageing had no effect.

In *M. semitendinosus* tenderstretching increased sarcomere lengths. A slight fall in shearforce was associated without interaction with both tenderstretching and ageing.

In *M. semimembranosus* slow chilling had no apparent effect while tenderstretching increased sarcomere length and produced meat that was more tender at two days than meat from normally suspended sides at fourteen days. Ageing increased tenderness in both suspension treatments to about the same extent.

In *M. gluteus medius* tenderstretch suspension produced a marked tenderizing effect which persisted to fourteen days. Ageing increased tenderness and, unlike other muscles continued to be effective between seven and fourteen days.

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No experimental treatment had any deleterious effect on meat colour, drip loss or cooking loss. However under slow chilling both carcase weight loss and bacterial growth, at two days post mortem, were enhanced, making slow chilling less attractive commercially.

Introduction

Hostetler *et al.* (1970) have shown that if a beef side is allowed to go into rigor mortis whilst suspended from the obturator foramen, as in the 'tenderstretch' (R) process, a number of muscles on the outside of the hip joint are prevented from shortening and are subsequently more tender than muscles from control sides suspended normally, from the Achilles tendon.

Smith, Hostetler & Carpenter (1973) found that in a slowly cooled beef side the tenderness of *M. longissimus dorsi* at two days post mortem was enhanced. Additional improvement was obtained by tenderstretch suspension. Parrish *et al.* (1973) have reported that tenderness increases generally if beef is 'conditioned' at higher temperature during rigor onset.

Hostetler *et al.* (1975) reported on the effects of slow chilling and tenderstretch suspension on nine beef muscles aged on the carcase for seven days. Slow chilling did not produce enhanced panel tenderness scores but did produce significantly lower shear force values. Suspension via the obturator foramen had the greatest effect on tenderness, and this combined with elevated storage temperature produced the most tender beef.

Since post mortem ageing enhances tenderness (Deatherage & Harsham, 1947; Sleeth, Kelley & Brady, 1958; Bouton, 1968; Joseph & Connolly, 1974) it is possible that improvements effected by slow chilling or tenderstretch might not be detected if testing were not carried out at several suitable intervals post mortem. The present study was therefore undertaken to measure the effects of tenderstretch suspension, slow chilling and post mortem ageing in combination on meat tenderness, and also on other meat and carcase qualities of commercial importance.

Materials and methods

Material

Twenty-seven Hereford cross heifers, (with two adult incisor teeth), were slaughtered by stunning, pithing and sticking. They were dressed by conventional methods. Hot carcase weights were within the range 167–242 kg.

Suspension procedures

After dressing, left or right sides of the carcase were assigned at random to the two procedures. In the tenderstretch procedure a stainless steel hook

previously rinsed in 'Chloros', (20 ppm Cl_2) was inserted under the exposed face of the pubic symphysis into the obturator foramen. The other side was suspended normally from the Achilles tendon. Both sides were weighed and then washed down with water containing 20 ppm chlorine at 50°C.

Microbiological swabbing

Three sites on each side were swabbed using a 10 cm^2 template. Swabs were taken, after washing, 2 hr post mortem on the outer round near the base of the tail, on the surface of the back near the 11/12 rib, (the 'quartering point'), and on the surface of the neck in front of the shoulder. Swabs were repeated at 48 hr on adjacent sites. The swabs were placed in Ringers diluent with 0.1% peptone added, shaken and plated out in plate count agar (Oxoid). Total viable counts were made after incubation at three days at 25°C.

Chilling procedures

Three chilling procedures were defined as 'fast', 'medium' and 'slow'. Animals were assigned at random with the constraint that in every three successive animals one was assigned to each of the three procedures. 'Fast' was obtained by putting the sides into the laboratory chill at about 2½–3 hr post mortem. Air at 0 ± 1°C was circulated over the sides until 48 hr post mortem. 'Medium' was obtained by leaving the sides hanging in the laboratory slaughter room at ambient temperatures, 14–18°C, until 24 hr post mortem and then transferring them into the chill for a further 24 hr. 'Slow' was obtained by surrounding both sides as they hung on the rail in the room in a box of expanded polystyrene 2.5 cm thick lined with polythene film, of dimensions $1.2 \times 1.2 \times 2.4$ m. The humidity inside rose rapidly to 100% R.H. and the temperature was approximately 2–3°C above ambient. At 24 hr post mortem the box was opened and the sides placed in the chill for 24 hr.

Temperature

Temperature was measured at four sites on each carcase. As soon as practicable after washing, (about 2–2½ hr post mortem) copper/constantan thermocouples, linked to a Honeywell Elektronik 15 recording thermometer, were inserted into the centre of the round, in *M. semimembranosus* and the centre of *M. long. dorsi,* at the 11/12 rib level, the quartering point. The centre point of the round was determined by bisecting the distance from the pubic symphysis, to the outside of the round measured perpendicularly to the body axes. Other thermocouples were inserted just under the surface, on the top of the round and at the quartering point on the back.

pH

pH was measured at 2, 5, 24 and 48 hr post mortem. A Radiometer combined glass/reference electrode was inserted into (1) a gash cut in the *M. semimembranosus* at the pubic symphysis and (2) a gash in the *M. long. dorsi* in front of the 11/12 rib level. The mean of three individual readings was taken. The electrode was withdrawn and rinsed between readings. The ultimate pH was taken at 48 hr in the six excised muscles.

Excision of muscles

Forty-eight hours post mortem the sides were removed from the chill. After weighing, swabbing and taking pHs, the following muscles were dissected out: *M. longissimus dorsi*, 11/12 rib to the posterior end, (LD), *M. psoas major* (PM), *M. biceps femoris* (BF), *M. semitendinosus* (ST), *M. semimembranosus* (SM) and *M. gluteus medius* (GM) (Naudé & Joseph, 1970).

The pH and weight of each muscle were taken and the length was measured. The muscles were then cut across into three approximately equal sections and these were allocated to ageing periods of two, seven or fourteen days. Within a group of three animals, assigned to any one chilling procedure, the randomization of sections was arranged so that each of the three ageing periods had an anterior, centre and posterior section (assigned to it), i.e. a Latin square arrangement with animals, positions on or in muscle and ageing period as rows, columns and letters. A small sample (1–2 g) was removed from the cut face on the surface of each section for sarcomere length determination. The 'two day' sections were subjected to quality tests at once.

Sarcomere length

Measurement of sarcomere length was made by the method of Hegarty & Naudé (1970). Five to 10 mg of muscle was homogenized in 1 ml of ¼ strength Ringer's solution. The fibres were examined under direct light and an image projected onto a screen. The number of sarcomeres in 10 cm of fibre in the field was counted and the mean sarcomere length calculated. Four replicates were counted in each sample.

Ageing procedures

The sections assigned to seven and fourteen days total ageing were packed in polythene nylon laminate pouches and held at 2°C for five and twelve days respectively. At the end of ageing the bags were opened, the muscles were

examined for evidence of microbial attack and were then mopped dry, weighed and subjected to quality tests.

Quality tests – colour

A steak 2.5 cm thick was cut off one end of a section and four samples of about 3 × 4 cm, with a freshly cut surface, were prepared under PVC. After 1 hr on ice, during which time they 'bloomed', the colour of the samples, expressed as the parameters L (lightness), a (green-redness) and b (blue-yellowness) was measured on a Hunter Colour Difference Meter. The a and b scores were converted into saturation S, a measure of purity or lack of greyness, and hue H, a measure of the attribute of colour denoted by purple to red to orange, etc.

$$S = (a^2 + b^2)^{1/2}, H = \tan^{-1}(b/a)$$

Quality tests – cooking

A second steak 2.5 cm thick was cut from one end of a section. A piece of approximately 150 g was cleared of all fat and gristle, weighed, and sealed in a circular lidded tin in a vacuum pouch. This was immersed in water at 80°C for 40 min then cooled at 10°C for 1 hr (Davey & Gilbert, 1969; Joseph & Connolly, 1974). This meat was removed, mopped dry and weighed to determine cooking loss.

Objective tenderness

Five strips of meat were cut from the cooked blocks to dimensions of approximately 1 × 1 × 3 cm. Fibre bundles were parallel to the long dimension. The shorter dimensions, i.e. thickness and width of each strip were measured with a micrometer gauge. The strips were sheared in half by a Volodkevich Bite Tenderometer and the maximum shear force (VF) in newtons (N) and total work done (VW) in joules (J) were recorded (Joseph & Connolly, 1974).

Subjective quality assessment

The cubes of meat produced by cutting the strips in the Volodkevich were presented to a panel of six judges. Each judge received a cube from the tenderstretch side of the carcase and a cube from the same muscle, at the same ageing period, from the normal side of the carcase. Thus the only contrast presented

to the judges was between 'normal' and 'tenderstretch' meat. At any one session meat from only one animal was presented. Animals were assigned to sessions in the order in which they had been slaughtered. The judges were invited to score the meat on a nine point structured scale from 'extremely tough' to 'extremely tender'.

The judges were also invited to score for juiciness on a similar scale from 'extremely dry' to 'extremely juicy' and for flavour on a hedonic scale from 'dislike extremely' to 'like extremely'. (Fig. 1). The scores were converted to numerical values for analysis.

NAME JUDGE DATE

Here are two samples of cubed cooked beef. They may differ from each other. We would like you to evaluate their tenderness, juiciness and flavour. If there are differences and you can detect them, then your scoring of the samples will show this.

Numerical Value	TENDERNESS	JUICINESS	FLAVOUR
8	Extremely tender	Extremely juicy	Like extremely
7	Very tender	Very juicy	Like very much
6	Tender	Juicy	Like
5	Slightly tender	Slightly juicy	Like slightly
4	Intermediate	Intermediate	Intermediate
3	Slightly tough	Slightly dry	Dislike slightly
2	Tough	Dry	Dislike
1	Very tough	Very dry	Dislike very much
0	Extremely tough	Extremely dry	Dislike extremely

Figure 1. Taste panel score form, with numerical values indicated.

Results*

Chilling rates and weight loss

The average cooling curves obtained are given in Fig. 2.

Average weight losses from sides for the three chilling rates were 1.49% ('slow'), 1.74% ('medium') and 1.29% ('fast') with S.E. of difference 0.144% (12 d.f.). Enclosure in the polystyrene box reduced weight loss by maintaining 100% R.H. around the sides.

Meat quality

Tenderness

M. longissimus dorsi (Table 1). Tenderstretch suspension was associated with an increase in the tenderness score and a reduction in *VF* in every treat-

* N.B. Significance, where mentioned, is $P < 0.05$.

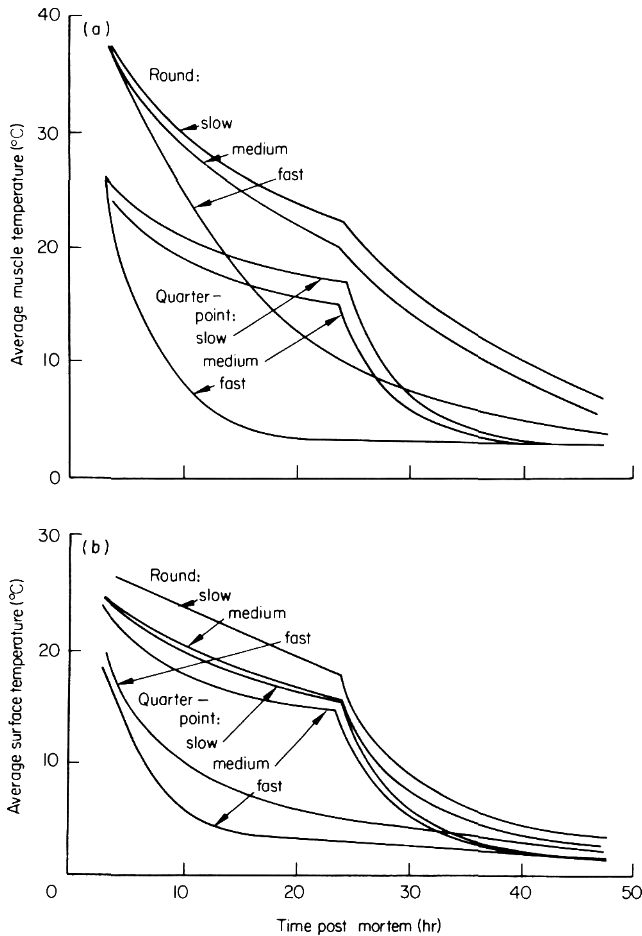

Figure 2. (a) Mean cooling curves at the centre of the round and the centre of the sirloin at the 11/12 rib, 'quarter-point'. L.S.D. at 3 hr = 1.16, at 24 hr = 1.9, at 48 hr = 1.34 in round; at 3 hr = 1.84, at 24 hr = 1.30, at 48 hr = 0.89 in sirloin. (b) Mean cooling curves on the surface of the round, and the surface of the sirloin at 11/12 rib. L.S.D. at 3 hr = 1.84, at 24 hr = 1.57, at 48 hr = 1.25 on round; at 3 hr = 1.80, at 24 hr = 1.75, at 48 hr = 1.06 on sirloin.

ment. *VW* was reduced in all but two treatments. Ageing from two to seven days was associated with increased tenderness, reduced *VF*, and reduced *VW* in all but one of the eighteen comparisons. Between seven and fourteen days the effect of ageing was smaller. Slow and medium chilling comparisons showed small differences in score and *VF*. The differences when compared with fast chilling results, were larger especially at two days post mortem.

At each ageing period the toughest LD was from normally suspended fast chilled sides. This treatment was associated with the lowest tenderness score and the highest *VF*, and at seven and fourteen days it produced the highest *VW*. By contrast 'tenderstretched' slow/medium chilled meat at seven and fourteen days was the most tender meat as assessed by panel or *VF*.

Table 1. LD: mean tenderness scores, *VF* and *VW*

Chilling rate	suspension	Scores 2	7	14	*VF* (N) 2	7	14	*VW* (J) 2	7	14
		(days ageing)			(days ageing)			(days ageing)		
Slow	Tenderstretch	5.17	5.43	6.04	72.89	62.56	58.84	0.345	0.352	0.352
	Normal	4.51	5.17	5.25	96.43	76.73	75.18	0.444	0.359	0.354
Medium	Tenderstretch	4.76	5.84	5.68	77.36	60.75	51.75	0.425	0.313	0.279
	Normal	4.65	5.19	5.40	92.39	70.82	58.53	0.406	0.369	0.326
Fast	Tenderstretch	3.65	5.38	5.59	97.73	66.14	60.47	0.449	0.328	0.342
	Normal	2.40	4.43	4.52	140.66	92.31	82.04	0.442	0.432	0.394

S.E. of difference	Score	(d.f.)	*VF*	(d.f.)	*VW*	(d.f.)
(1) Chilling rate, suspension constant	0.40	(36)	7.33	(36)	0.043	(36)
(2) Chilling rate, alone constant	0.50	(30)	7.66	(26)	0.041	(19)
(3) All other comparisons	0.37	(17)	11.13	(15)	0.049	(28)

Table 2. PS: mean tenderness scores, *VF* and *VW*

Effect of suspension and muscle region:

	Score Anterior	Centre	Posterior (section)
Tenderstretch	6.68	6.42	6.33
Normal	6.66	6.91	6.70

S.E. of difference	Score (d.f.)
1. Suspension constant	0.14 (36)
2. All other comparisons	0.15 (30)

Effect of ageing:

VF (N) 2	7	14	*VW* (J) 2	7	14
(days ageing)			(days ageing)		
58.69	49.18	46.77	0.296	0.270	0.275

S.E. of difference	*VF* (d.f.)	*VW* (d.f.)
	1.37 (36)	0.0105 (36)

M. psoas major (Table 2). *VF* and *VW* decreased with ageing for this muscle but showed the effect of no other factor (Table 2). The panel detected a slight toughening in the centre and posterior regions of the PS under tenderstretch.

M. biceps femoris (Table 3). Tenderstretch suspension improved tenderness score and decreased *VF* and *VW* (Table 3). *VW* also showed small complex interactions with chilling rate and ageing for this muscle.

Table 3. BF: mean tenderness scores, *VF* and *VW*

	Score	*VF* (N)	*VW* (J)
Tenderstretch	3.13	75.58	0.369
Normal	2.81	82.76	0.379
S.E. of difference (d.f.)	0.11 (18)	1.71 (18)	0.007 (18)

M. semitendinosus (Table 4). The taste panel did not detect any tenderness changes as a result of tenderstretching, slow chilling or ageing and the mean score was 3.64±0.86 (S.D.). *VF* and *VW* were decreased slightly but significantly by tenderstretching and independently by ageing.

Table 4. ST: mean *VF* and *VW*

	VF (N)	*VW* (J)
Effect of suspension:		
Tenderstretch	80.13	0.396
Normal	84.87	0.435
S.E. of difference (d.f.)	1.52 (18)	0.0108 (18)
Effect of ageing:		
2 days	86.96	0.438
7 days	82.05	0.412
14 days	78.49	0.396
S.E. of difference (d.f.)	2.02 (36)	0.016 (36)

M. semimembranosus (Table 5). Chilling rate variation did not affect any parameter of tenderness, score, *VF* or *VW* (Table 5). Tenderstretching increased tenderness scores, and decreased *VF* and *VW*. Ageing caused a significant increase in score and decrease in *VF*, over two to seven days. *VW* was reduced in normally suspended meat.

M. gluteus medius (Table 6). Chilling rate variation had no effect on tenderness. Tenderstretching increased tenderness scores, and reduced *VF* and *VW*. Fourteen day ageing reduced *VF* and *VW* and increased score, over two day aged meat. The effect on *VF* was greater in normally suspended meat.

Table 5. SM: mean tenderness scores, *VF* and *VW*

Suspension	Scores			*VF* (N)			*VW* (J)		
	2	7	14	2	7	14	2	7	14
	(days ageing)			(days ageing)			(days ageing)		
Tenderstretch	3.87	4.17	4.24	81.30	73.07	70.86	0.389	0.383	0.396
Normal	2.57	3.28	3.09	114.62	93.22	92.69	0.549	0.472	0.455

S.E. of differences	Score	d.f.	*VF*	d.f.	*VW*	d.f.
(1) Suspension constant	0.22	36	3.72	36	0.023	36
(2) All other comparisons	0.22	28	4.39	24	0.025	25

Table 6. GM: mean tenderness scores, *VF* and *VW*

Suspension	Scores			*VF* (N)			*VW* (J)		
	2	7	14	2	7	14	2	7	14
	(days ageing)			(days ageing)			(days ageing)		
Tenderstretch	4.98	5.08	5.44	67.63	66.03	60.36	0.339	0.328	0.326
Normal	4.67	4.48	4.96	81.64	67.31	67.99	0.381	0.355	0.340

S.E. of difference	Score	d.f.	*VF*	d.f.	*VW*	d.f.
(1) Suspension constant	0.23	36	3.55	36	0.016	36
(2) All other comparisons	0.22	30	3.40	28	0.017	26

Juiciness and flavour

Tenderstretching promoted a slight dryness in LD and GM and juiciness in ST. Ageing made LD, PS, GM and medium and fast chilled ST more dry but made slow chilled ST more juicy.

Tenderstretching improved the flavour very slightly in LD, as did ageing in PS (Tables 7 and 8).

pH values

The mean pH values in the round (SM) and quartering point (LD) are in Table 9. The mean ultimate pH values in the six excised muscles are in Table 10. No effect of chilling rate or suspension on the rate of pH fall or the ultimate pH was recorded.

Table 7. Mean juiciness scores

Muscle	Treatments	Score	S.E. of difference	d.f.
LD	Tenderstretch	4.37		
	Normal	4.55	0.070	18
	Aged 2 days	4.73		
	Aged 7 days	4.38		
	Aged 14 days	4.26	0.188	36
PS	Aged 2 days	4.93		
	Aged 7 days	4.34		
	Aged 14 days	4.08	0.177	36
BF	Overall mean	3.64± 1.00 (S.D.)		
ST	Tenderstretch	3.34		
	Normal	3.21	0.061	36
SM	Overall mean	3.34±0.80 (S.D.)		
GM	Tenderstretch	3.86		
	Normal	4.24	0.064	18
	Aged 2 days	4.43		
	Aged 7 days	3.87		
	Aged 14 days	3.85	0.147	36

Table 8. Mean flavour scores

Muscle	Treatments	Score	S.E. of difference	d.f.
LD	Tenderstretch	4.86		
	Normal	4.72	0.046	18
PS	Aged 2 days	5.60		
	Aged 7 days	5.01		
	Aged 14 days	5.19	0.130	36
BF	Overall mean	3.93±0.74 (S.D.)		
ST	Overall mean	3.99±0.65 (S.D.)		
SM	Overall mean	3.98±0.83 (S.D.)		
GM	Overall mean	4.67±0.69 (S.D.)		

Table 9. pH level in two muscles

Time post mortem (hr)	Mean ± S.D.	
	SM	LD
2	6.50±0.34	6.43±0.30
5	5.78±0.38	5.88±0.37
24	5.49±0.17	5.54±0.21
48	5.49±0.27	5.56±0.26

Table 10. Mean pH ultimate in six muscles

Muscle	Mean ± S.D.
LD	5.52±0.15
PS	5.55±0.11
BF	5.48±0.14
ST	5.49±0.09
SM	5.48±0.12
GM	5.46±0.10

Colour

Results from the first nine and last six animals only are recorded here. A different model of Hunter meter was brought into use while the middle group was being recorded and difficulties were encountered.

Saturation was higher in meat from slow and medium chilled carcases, but hue and lightness were not affected by chilling rate (see Table 11).

Table 11. Effect of chilling rate on saturation

Muscle	Chilling rate			S.E. of difference d.f. = 8
	Slow	Medium	Fast	
LD	21.99	22.50	20.60	0.72
PS	20.27	20.52	20.01	0.44
BF	22.33	22.16	19.86	0.65
ST	22.49	22.49	20.24	0.70
SM	23.30	22.76	20.73	0.57
GM	21.95	21.43	19.97	0.65

Table 12. Effect of ageing on lightness and saturation (d.f. = 24)

Muscle	Ageing (days)						S.E. of difference	
	2		7		14			
	L	S	L	S	L	S	L	S
LD	30.90	18.83	33.61	22.63	33.56	23.02	0.37	0 39
PS	34.14	18.03	35.03	21.06	35.40	21.71	0.48	0 44
BF	31.50	18.62	33.36	22.80	33.78	22.92	0.35	0 32
ST	35.73	19.67	37.42	22.41	38.14	23.13	0.34	0 36
SM	30.23	20.01	32.61	23.16	33.17	23.63	0.38	0.45
GM	30.86	18.99	32.80	21.78	32.68	22.57	0.40	0.41

Saturation and lightness were increased by ageing the meat, but hue was unaffected. The effect of ageing is attributed to increased retention of oxygen in the surface layers of freshly cut meat since the oxygen utilizing enzymes in the deeper tissue became progressively inactivated with the passing of time (see Table 12). No other consistent effects were recorded for any of the three parameters.

Table 13. Mean hue values

	Muscle					
	LD	PS	BF	ST	SM	GM
Mean hue°	31.36	24.87	25.10	29.23	25.54	24.94
S.D.	2.37	2.83	2.45	2.55	3.89	2.61

Cooking loss

No effects attributable to any of the experimental procedures were found. Table 14 gives the mean losses with standard deviations for each muscle.

Table 14. Percentage cooking losses (±S.D.)

	Muscle					
	LD	PS	BF	ST	SM	GM
Cooking loss	31.2±4.1	30.6±3.8	35.2±3.6	36.3±3.3	36.1±3.7	33.2±2.9

Drip loss

Muscle sections aged for twelve days in vacuum bags lost more drip than those aged for five days, though in PS, ST and SM the increase was not significant; Tenderstretch suspension significantly decreased drip loss in SM, and non-significantly in LD, BF, ST and GM. In PS the sections from normal suspension, where the muscle is actually more stretched, lost significantly less drip (see Table 15).

Sarcomere length

With the exception of PS, tenderstretching elongated the sarcomeres in every muscle examined. The sarcomeres in the PS were shortened under tenderstretch suspension since the muscle is on the inside of the hip joint (see Table 16).

Table 15. Percent drip loss from muscle sections in vacuum packs

Muscle	Time in pack (days)			Suspension method		
	5	12	S.E.*	Tenderstretch	Normal	S.E.†
LD	1.64	2.42	0.32	1.93	2.13	0.38
PS	3.09	3.32	0.37	3.98	2.43	0.29
BF	1.21	1.79	0.29	1.31	1.69	0.30
ST	2.20	2.67	0.28	2.25	2.62	0.27
SM	2.31	2.77	0.41	1.87	3.21	0.41
GM	2.70	3.92	0.35	3.02	3.60	0.31

* d.f. 36, S.E. of difference.
† d.f. 18, S.E. of difference.

Table 16. Sarcomere lengths in normal and tenderstretch muscle (μm) (d.f. = 18)

Suspension	Muscle					
	LD	PS	BF	ST	SM	GM
Tenderstretch	2.31	2.57	2.73	2.34	2.64	2.48
Normal	1.80	3.18	1.83	2.05	1.80	1.73
S.E. of difference	0.058	0.076	0.063	0.050	0.080	0.077

No effect of chilling rate was detected in any muscle. This is surprising especially in LD where rapid chilling produced an internal temperature of 8°C at 10 hr post mortem and a high degree of toughness was subsequently recorded.

Table 17. Total viable counts on the carcases

	$\log_{10}$ total viable organisms cm^{-2}		
	Neck	Quarter-point	Round
Time			
2 hr (average ± S.D.)	2.15±0.64	1.96±0.62	2.03±0.70
48 hr (average ± S.D.)	2.71±1.18	2.71±1.14	2.36±1.24
Effect of chilling rates:			
Slow	3.09	2.74	3.39
Medium	2.89	2.92	1.85
Fast	2.15	2.48	1.84
S.E. of difference (d.f. = 12)	0.40	0.30	0.24
Effect of suspension method:			
Tenderstretch	2.99	2.74	2.41
Normal	2.43	2.69	2.32
S.E. of difference (d.f. = 18)	0.23	0.28	0.27

Microbiological counts

At all sites 2 hr post mortem there was an even distribution of organisms (see Table 17). At 48 hr their numbers had increased, though not dramatically. Only the round showed a very highly significant effect ($P < 0.001$) associated with the slow chilling rate. This may be due to the unique combination of 100% humidity and high surface temperature for a relatively long period in this treatment (see e.g. Fig. 1(b)). Fast chilling gave the lowest counts. We found one example of 'bone taint' (Lawrie, 1974) in a slowly chilled carcase at the femur on one side.

No evidence of deleterious bacterial growth such as slimes or bad smells was found in the vacuum packs of muscle sections aged for five or twelve days.

Discussion and conclusions

In LD, tenderstretching, chilling rate and ageing period all interested in their effects on tenderness. By two days post mortem tenderstretching had produced a bigger improvement in tenderness of LD from fast chilled carcases than of LD from medium and slowly chilled carcases. However, slow and medium chilled carcases gave more tender LD than fast chilled carcases whether tender-stretching or normal suspension was used.

The tenderness of slowly chilled LD has been demonstrated by other authors (Buchter, 1972; Smith, Arango & Carpenter, 1971; Smith *et al.* 1973). However, no development of 'cold shortening' in rapidly chilled beef LD on the carcase has been recorded. We did not detect cold-shortened sarcomeres (see Table 16). The enhanced tenderness of slowly chilled meat has been attributed to the earlier onset of proteolytic breakdown of sarcomere structure. (Smith *et al.* 1971; Parrish *et al.* 1973; Hostetler *et al.* 1975). By seven days post mortem, the tenderness of LD from fast chilled carcases had improved markedly, by about two score points, whereas that from slow and medium chilled carcases had improved by only half as much.

The tenderstretch improvement was still evident at seven days. The ageing effect was additive. Tenderstretched, fast chilled LD was not significantly different in tenderness from LD from slow and medium chilled carcases, but normally suspended, fast chilled LD was tougher.

This difference persisted to fourteen days and no significant improvement occurred in any category between seven and fourteen days.

The maximum level of tenderness was not reached by LD from normally suspended rapidly chilled carcases. Tenderness appeared to stabilize at a lower panel score. Locker *et al.* (1975) have shown that meat toughened by rapid post mortem chilling never became as tender with ageing as that which had been 'conditioned' by slow chilling.

It appears that rapid chilling, under normal suspension, even without producing cold shortening, affects LD in some way, that will stop full development of potential tenderness. This 'cold-toughening' needs further investigation.

Tenderstretching would be commercially useful in overcoming the toughness associated with rapid chilling in the LD. As faster factory chilling is introduced this toughening will become a serious marketing problem. The unconventional shape of the carcase may make shipping difficult and factory cutting followed by vacuum packaging of cuts is probably essential to exploit the technique commercially.

Slow/medium chilling improved tenderness (in the LD alone) and though it has no deleterious effect on colour, drip losses or cooking losses in the meat, it may be unsuitable for factory use owing to the risk of higher bacterial counts, the hazard of bone taint and greater carcase weight loss. The dramatic difference in tenderness between fast chilled and slow/medium chilled LD at two days, emphasizes the necessity of careful control over post mortem chilling and ageing in the interests of eating quality. Ageing does not appear to effect significant tenderizing beyond seven days, and procedures for extended ageing would seem on the present results to be unjustified.

In PS there was no interaction of treatments. The shortening of sarcomeres associated with tenderstretching (since the muscle is on the inside of the hip) was reflected in a slight toughening in the thicker, central posterior section of the muscle, but this was detected only by the panel, not by the shear meter. Hostetler *et al.* (1972) found this effect with the Warner Bratzler shear meter. Ageing produced an effect detected by the shear meter, but not by the panel. All the meat from this muscle was tender and the slight toughening produced by tenderstretching would not be important commercially.

In BF the effect of tenderstretching though significant was small and unlikely to be of any commercial value. Hostetler *et al.* (1972) recorded a significant rise in shear force as a result of tenderstretching, though the increase in sarcomere length was even larger than that detected by us. The increase in sarcomere length induced by tenderstretching in this muscle was the largest in the six muscles examined.

In ST only the shear meter detected slight improvements in tenderness due to tenderstretch or ageing. There was no interaction between the treatments. Hostetler *et al.* (1972, 1975) recorded small non-significant changes due to tenderstretch suspension, and Parrish *et al.* (1973) found that slower chilling produced slightly more tender meat in this muscle. However the changes produced in this muscle by tenderstretching, slow chilling, or ageing do not appear to be large enough to be commercially useful.

In SM tenderstretched muscle is more tender at two days post mortem than normally suspended muscle after fourteen days. Hostetler *et al.* (1975) and Baxter *et al.* (1972) reported the effectiveness of tenderstretching in this muscle. The muscle is normally sold for roasting, for example, as 'topside'. Tenderstretching could be used to produce meat suitable for grilling.

The tenderness of SM from normally suspended carcases appears to stabilize

significantly with ageing before that of SM from tenderstretched carcases. A comparison with LD, that had been chilled at a similar rate to SM, i.e. 'slow' or 'medium'. reveals a much smaller difference between the tenderstretch and normal meat, in LD. This may be related to the smaller change in sarcomere length in LD compared with SM.

In GM, tenderstretch suspension was associated with greater tenderness, confirming the results of Hostetler *et al.* (1975), and Baxter *et al.* (1972). This effect persisted through ageing which increased tenderness significantly between two and fourteen days. There was no evidence in the muscle of any stabilizing of tenderness between seven and fourteen days. The sarcomere length changes due to tenderstretching were larger than those in the LD, though not as large as those in the SM. The relative magnitude of the improvements effected by tenderstretch in these three muscles does not appear to have a simple relationship with sarcomere length changes.

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The effect of several gaseous environments on the multiplication of organisms isolated from vacuum-packaged beef

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Summary

Cubes of sterile beef were inoculated with pure cultures of Gram positive and Gram negative organisms isolated from vacuum-packaged beef and were stored in gaseous atmospheres containing different proportions of air, CO_2 and O_2 at 0 and 5°C. Generally the rate of multiplication decreased and the lag phase increased in increasing levels of CO_2, but Gram positive species were more resistant to the effect of CO_2 than were Gram negative organisms.

Introduction

Haines (1933) observed that concentrations of 10 and 20% CO_2 in the atmosphere at 0 and 4°C suppressed the growth of *Pseudomonas* and *Achromobacter* spp. in nutrient broth. Further work by Scott (1938) showed inhibition of such organisms on ox-muscle at −1°C in an atmosphere of 10% CO_2. Taylor (1971) has reviewed the literature on the uses of various gaseous environments in the packaging of fresh meat and indicated that modifications of the atmosphere together with a temperature just above freezing, is the approach most likely to result in the extension of storage life in vacuum-packages and also of display life for this product.

The aim of this work was to study the effect of different gas atmospheres on the multiplication of pure cultures of organisms (originally isolated from vacuum packaged beef) inoculated on to sterile pieces of beef and stored at 0 and 5°C and also to determine any changes in the composition of the atmospheres during storage. Thus the degree of inhibition exercised by the various gases was assessed with a view to finding a gaseous balance which (i) is bacteriostatic and (ii) maintains the red colour (oxymyoglobin) of the meat.

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Methods

Preparation of sterile meat

Cubes of sterile beef (eighty per organism) of *c.* 1 cm^3 were excised from beef *longissimus dorsi* muscle, using the method of Gardner & Carson (1967), into sterile, preweighed 'Universal' bottles and the bottles reweighed. Apertures of 2 mm diameter had been previously drilled in the metal cap of each bottle to admit a hypodermic needle and the (entire) black rubber liner sealed to the inside of the cap using 'Araldite' (Ciba-Geigy, U.K. Ltd) before sterilization.

Inoculations of organisms

The following isolates from vacuum-packaged beef (Sutherland, Patterson & Murray, 1975) were tested for their ability to grow at 0 and 5°C.

(i) *Pseudomonas fluorescens*, (ii) a non-pigmented *Pseudomonas* sp., (iii) *Alcaligenes* sp. (iv) an '*Acinetobacter*-like' organism, (v) *Microbacterium thermosphactum* and (vi) a homofermentative *Lactobacillus* sp. Each culture was inoculated on to fifty-six of the eighty cubes of sterile meat, leaving the remainder as uninoculated controls.

Preparation of inoculum

A 1/10 dilution of a 24 hr TGY (tryptone glucose yeast extract) broth culture incubated at 25°C was prepared in sterile 0.5% (w/v) peptone water (Oxoid L37) and the meat was inoculated with 0.02 ml. For the *Lactobacillus* sp., MRS broth (de Man, Rogosa & Sharpe, 1960) incubated at 30°C for five days replaced the TGY broth. Duplicate samples of meat were examined microbiologically immediately after inoculation to determine the initial recovery.

Gassing and sealing of bottles

Before gassing, the bottles were divided into four groups such that each group contained twelve bottles of meat inoculated with one of the organisms and six bottles of uninoculated meat. Each of the four groups was treated with one of the following gas mixtures: (i) 10% CO_2 in air,* (ii) 20% CO_2 in O_2,* (iii) 100% CO_2,† (iv) air; the latter were plugged with sterile cotton wool to permit free gaseous interchange with the air and were stored in a moist atmosphere. To act as controls for possible gaseous diffusion, a number of empty

* Supplied by Special Gases Division, B.O.C., Deer Park Road, London.

† Supplied by B.O.C., Prince Regent Road, Belfast.

bottles were filled with gas and stored in the same way as the bottles of meat. Gas was passed into the bottles via a flowmeter (Rotameter Manufacturing Co., Ltd) through a sterile cotton wool filter and a sterile hypodermic needle which penetrated the exposed part of the cap liner of the bottle. After flushing the bottle with the cap loose for 1 min, at a flow rate of 175–200 ml/min, the cap was tightened and gas passed into the bottle until a zero flow rate was recorded. After withdrawal of the needle, the aperture was sealed with a piece of gas-impermeable rubber using 'Araldite'.

Incubation and analysis of samples

Six bottles of inoculated meat and three bottles of sterile meat which had been flushed with each gas were stored at 0°C and the remainder at 5°C. Each week, for three weeks, samples of gas from two inoculated, one uninoculated and two bottles of gas alone were analysed by gas phase chromatography. A gas tight 50 μl 'Terumo' syringe (Shandon Southern Instruments Ltd, Camberley, Surrey) was used to withdraw 20 μl of the atmosphere for injection into the instrument. Samples from each bottle were injected at least in triplicate.

Operating specifications of gas chromatograph

The following conditions were used:

Chromatograph: Philips PV 4000.

Column dimensions: 3 m x 2 mm (I.D.) stainless steel coil.

Column packing: (i) 5A molecular sieve (30/60 mesh size; Phase-Sep Ltd, Queensferry, Flintshire) for separation of O_2 and N_2 at 80°C, (ii) Silica gel for separation of CO_2 from $O_2 + N_2$ (combined peak) at 120°C.

Carrier gas: Helium, flow rate 30 ml/min. Inlet pressure 4.2 kg cm^{-2}, outlet pressure atmospheric.

Detector: Katharometer detector. Katharometer supply 200 mA, attenuation x10.

Recorder: Leeds and Northrup Speedomax W, $-0.1 \rightarrow +1.0$ mV (f.s.d.)

Sample size: 20 μl.

Analysis time: (i) 5A molecular sieve, O_2, 0.38 min; N_2, 0.8 min. (ii) Silica gel, O_2/N_2, 0.25 min; CO_2, 1.2 min.

All of the atmospheres to be analysed were first injected into the silica gel column at 120°C to determine the CO_2 content. The oven was then switched off and the unit cooled to *c.* 80°C. The temperature was reset to 80°C and after equilibration, samples of the gas atmospheres were injected on to the molecular sieve column for analysis of O_2 and N_2.

Microbiological sampling

After gas analysis, 10 ml sterile 0.5% (w/v) peptone water (Oxoid L37) were added to the meat in the bottles, and the bottles shaken for 5 min at high speed on a flask shaker. Suitable decimal dilutions (Murray, 1956) were dropped (three drops of 0.02 ml each and not spread) on TGY agar plates (MRS agar plates were substituted for recovery of the *Lactobacillus* sp.) and the plates incubated at the appropriate temperature, i.e. 0°C (twenty-one days) or 5°C (fourteen days) before enumeration of the colonies. All counts were adjusted to a standard 1 g of meat.

Results and discussion

The results (Table 1) demonstrate different effects of increasing CO_2 concentrations on the rate of multiplication of the organisms studied throughout storage at 0 and 5°C. In most cases the rate of multiplication was diminished and the lag phase extended by increasing levels of CO_2. The *Alcaligenes* and *Lactobacillus* isolates were less affected by increased levels of CO_2 than were the other organisms tested.

Despite the precautions described in 'Methods' there was a small amount of leakage of air into bottles (those in which gross contamination by air was detected were discarded) but the amount was considered not likely to materially affect the results. In addition there was, in some bottles of inoculated meat originally containing 10% CO_2 in air, evidence of accumulation of CO_2 with concomitant depletion of O_2. This was not detected in uninoculated meat controls. It occurred particularly in bottles of meat inoculated with non-pigmented *Pseudomonas* sp., *Alcaligenes* sp. and *M. thermosphactum*. These increased the CO_2 content by *c*. 10% after three weeks at 5°C. The trend at 0°C was similar but changes occurred more slowly.

In an atmosphere of 20% CO_2 in O_2 the *Alcaligenes* and *Lactobacillus* isolates were capable of growth at 5°C, but 100% CO_2 permitted only the *Lactobacillus* sp. to multiply. This level of CO_2 appeared to be bacteriostatic or slightly bactericidal to the other isolates. Incubation at 0°C increased the duration of the lag phase and decreased the rate of multiplication for most isolates. The '*Acinetobacter*-like' organism (the nearest description which can be accorded using Bergey's Manual, 8th edition, 1974) did not grow well on the meat at either temperature even in air and is not considered to be a likely spoilage organism under practical conditions.

In an original atmosphere of 20% CO_2 in O_2, only *M. thermosphactum* showed accumulation of CO_2 (increasing from 20 to 28% over three weeks) with corresponding depletion of O_2. The accumulation of CO_2 and depletion of O_2 by the respiration of *c*. 1 g meat in a 25 ml bottle would be expected to change the CO_2 and O_2 composition of the atmosphere by less than 1% (Urbin & Wilson, 1961; De Vore & Solberg, 1974).

Table 1. Relative changes in numbers of organisms on inoculated meat samples

		Increase (+) or decrease (–) in colony count* of											
		Pseudomonas sp.		*Ps. fluorescens*		*Alcaligenes* sp.		'*Acinetobacter*-like'		*M. thermosphactum*		*Lactobacillus* sp.	
		0°C	5°C	0°C	5°C	0°C	5°C	0°C	5°C	0°C	5°C	0°C	5°C
Initial recovery of inoculum (log_{10})		5.95		5.48		5.30		4.00		5.30		5.70	
Storage condition	Time (weeks)												
Air	1	+	+	+	++	+++	+++	++	+	+++	+++	+	++
	2	+++	+++	+++	++	+++	+++	0	0	++	–	++	++
	3	+++	+++	+++	++	+++	+++	0	>—	–	0	++	++
10% CO_2 in air	1	+	+	0	+	++	+++	+	+	+++	+++	+	++
	2	+++	+++	0	++	+++	+++	0	0	++	–	++	+++
	3	+++	+++	+	++	+++	+++	0	0	–	0	+++	+++
20% CO_2 in O_2	1	0	0	0	0	+	++	0	+	++	++	+	+
	2	+	0	0	0	+	+++	0	>—	++	–	0	+++
	3	0	0	+	0	+	+++	0	>—	>–	>–	+	++
100% CO_2	1	0	0	–	0	–	0	0	0	>—	0	+	0
	2	0	0	0	0	0	–	0	–	0	–	+	0
	3	0	0	–	–	0	>–	0	0	–	–	+	+

*Change indicated; 0, $<\pm 0.5 \log_{10}$ cycle increase or decrease; +, 0.5–1.5 $\log_{10}$ cycle increase; ++, 1.5–2.5 $\log_{10}$ increase; +++, $>2.5 \log_{10}$ cycle increase; –, 0.5–1.5 $\log_{10}$ cycle decrease; —, 1.5–2.5 $\log_{10}$ cycle decrease.

The pattern of multiplication of *M. thermosphactum* was significant in that at both 0 and 5°C a phase of rapid increase in numbers in all except the 100% CO_2 atmosphere was succeeded by an abrupt decline of the population. The highest concentration of CO_2 may have been slightly bactericidal rather than bacteriostatic for this isolate. In contrast, the *Lactobacillus* isolate multiplied in all concentrations of CO_2, although the rate of multiplication was slower during weeks 1 and 2 than for several of the other organisms. This pattern of microbial multiplication correlates to some extent with the changes in the microbial flora of vacuum-packaged beef recorded by Sutherland *et al.* (1975). These authors reported that *Pseudomonas* and *Alcaligenes* spp. were isolated at all stages of storage, '*Acinetobacter*-like' organisms infrequently and the proportion of *M. thermosphactum* in the microflora rose to a maximum after two to three weeks, while lactobacilli constituted a large proportion of the microflora only in the later stages of storage. However, in the naturally occurring context of a mixed flora, ecological interactions will occur between components of the flora, e.g. Roth & Clarke (1975) have suggested that the presence of lactobacilli inhibited the multiplication of *M. thermosphactum* on vacuum packaged beef, while an atmosphere containing 75% CO_2 merely extended the lag phase. From the results herein reported, it would appear that in a practical situation, a minimum of 20% CO_2 in the atmosphere would be necessary to limit the multiplication of the Gram negative organisms tested, though others may be more sensitive, provided that a storage temperature of *c.* 0°C could be maintained. This concentration of CO_2 and temperature of storage would appear however, to permit multiplication of lactobacilli, often isolated from this type of product. The aim of gas flushing vacuum-packaged beef would be to restrict the development of Gram negative organisms sufficiently to give an extended shelf life to retail portions cut from the primal joints and repackaged in oxygen-permeable film. It has been reported (Sutherland *et al.*, 1975) that a low initial contamination of primal joints prolongs the shelf life. Although an atmosphere of 100% CO_2 gave better control of microbial multiplication, noticeable discoloration of the meat occurred, whereas 20% CO_2 in O_2 did not cause discoloration. Nevertheless such discoloration may be tolerated in vacuum-packaged primal joints since it would be eliminated during butchery into retail cuts when the freshly cut surfaces of the meat are exposed. Huffman *et al.* (1975) found that an atmosphere of 100% CO_2 and also one of 25% CO_2, 70% N_2 and 5% O_2 controlled the aerobic and anaerobic counts of beef 'ribeye' samples held at 1.1°C in the dark for twenty-seven days although the colour was adversely affected. Taylor (1971) reported that CO_2 accumulates in vacuum packaged beef to a pressure of 70–150 mm Hg and remains at this level throughout storage. Consequently, flushing the package with a CO_2 containing atmosphere before sealing will be mainly of benefit during the early stages of storage, while the gradual accumulation of CO_2 from bacterial and possibly muscular respiration is taking place.

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Effect of post harvest factors on quality attributes of dehydrated banana products

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Summary

Unripe Cavendish banana fruits were stored at 7°C and normal or 0.14 normal atmospheric pressure for two weeks prior to ripening at 20°C. On ripening, the fruits were either cut into discs and air dried into chips or puréed and drum dried into flakes. Dried samples were stored at 20°C for two months prior to rheological, colorimetric and sensory quality evaluations.

Flavour and colour of the banana chips and flakes respectively were affected significantly by the preripening chilling treatments, as were the rheological properties of banana flake purées. End product quality from chilled fruits was acceptable but inferior to those made from non-chilled fruits. The practical and technological implications of the findings are discussed.

Introduction

Bananas, like many tropical crops, suffer from chilling injury when exposed to temperatures in the range of 10–12°C or lower (Fidler, 1968; Fidler & Coursey, 1969; Palmer, 1971). Depending on the severity of the exposure to chilling temperature, the green peel develops extensive subepidermal browning or blackening and on ripening the peel may become entirely black (Palmer, 1971).

The effect of chilling on ripening behaviour is variable. In general, where peel discoloration is slight to moderate, ripening processes in the pulp are little affected. However, such fruits are generally downgraded on their appearance with subsequent economic loss to the wholesalers and retailers. In international trade, annual losses of bananas worth over £10 million are, in part, the result of chilling injury (Moy, 1976).

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Dehydrated banana products are now gaining popularity in many countries, particularly in the developing tropical countries, where dehydration is an appropriate technology (Ngoddy, 1975). The dehydrated products are usually prepared by air drying sliced ripe banana pulp to about 20% moisture, or drum drying banana purée into flakes.

Since the adverse effects of chilling are generally confined to the peel, processing chilled banana fruits into dehydrated products may be an economic way of reducing chilling injury losses.

The object of this research was to compare selected quality attributes of dehydrated banana products prepared from fruit ripened at 20°C and fruit which had been held chilled at atmospheric or subatmospheric pressure prior to room temperature ripening.

Materials and methods

Full, green Cavendish banana fruits obtained from a wholesale outlet were used. At the laboratory the banana hands were reduced to hands with fewer fruits and then randomly divided into three treatments of: (1) 20°C ripening; (2) preripening storage at 7°C for two weeks, followed by 20°C ripening; (3) preripening storage at 7°C under 0.14 atmospheric pressure for two weeks, followed by 20°C ripening. Humidity in all the storage treatments was 95% R.H.

After ripening, the pulp was either sliced into 2 mm thick discs which were dipped in a solution containing 400 ppm SO_2 for 2 min at 20°C and then air dried at 65°C to about 20% moisture, or puréed, sulphited to 250 ppm and drum dried into flakes.

The dehydrated banana chips were packed in small pouches of 170 μm thick polyethylene while the flakes were nitrogen-flushed and sealed in small two-piece aluminium cans. The flakes and the chips were stored at 20°C for two months prior to evaluation.

The banana chips were compared with a commercial product by a sensory method (Larmond, 1970). Nine panelists evaluated the different banana chips twice with another commercial product serving as a reference. A multiple comparison difference analysis was used with a rating scale where 1 and 9 were equated to 'like extremely' and 'dislike extremely' respectively.

Rheological behaviour of the reconstituted banana tissue is an important attribute affecting the use of these products in formulations and processing operations, thus viscometric measurements were made on reconstituted, puréed banana flakes. The flakes were reconstituted to their original moisture content by macerating with distilled water in a Waring blendor for 30 sec to obtain a homogeneous purée. After standing at room temperature for 30 min, portions of the purée (about 90 ml) were then rheologically evaluated using a Haake Rotovisko Model RV1 coaxial cylinder viscometer described by Van

Wazer *et al.* (1963), and equipped with the MV1 spindle. This provided a maximum shear rate of 1370/sec with a gap width of 0.96 mm.

The sample was kept at 20°C using a Kryomat constant temperature bath connected to the water jacket that surrounded the sample holder. During each determination the spindle rotation speed was varied stepwise from maximum to minimum, then back to maximum. The torque, due to viscous drag in the fluid at known shear rates, was sensed by a 25 cm strip chart recorder.

Flow behaviour curves were constructed using data derived from the viscometric tests. The power law flow model was fitted to these data.

$$\sigma = m\dot{\gamma}^n$$

where σ = shear stress, dynes cm^{-2}; $\dot{\gamma}$ = shear rate, sec^{-1}; m = consistency coefficient, dyne $sec^{\bar{n}}$ cm^{-2}; n = flow behaviour index.

The flow parameters m and n were evaluated by a computer using the method of least squares and a nonlinear curve fitting technique. This procedure included evaluation of statistical parameters that would indicate the accuracy with which the flow model fitted the data. Flow behaviour of purées from different treatments was compared by a covariance method (Snedecor, 1965).

Colour of the banana flakes was measured with a Hunterlab Model D25 colour difference meter using the Rd scale.

Results and discussion

The mean values for the multiple comparison analysis for flavour and crispness of banana chips are presented in Table 1. Flavour was affected significantly by the preprocessing storage treatments. Chips made from fruits held at 7°C prior to ripening at 20°C were significantly poorer in flavour than normally ripened banana chips. Chips made from fruit held at 7°C and 0.14 atmospheric pressure had intermediate flavour scores. Low pressure seemed to reduce the deleterious effect of chilling on flavour development although this result was not statistically significant. Unexpectedly, the commercial product evaluated along with the experimental samples was poorly rated for flavour. The sensory panel found its salty flavour unacceptable.

Chips from the fruits pretreated by chilling prior to ripening were said to lack natural banana flavour. It is possible that these fruits failed to develop some of their natural flavour components during ripening as a result of damage to enzyme systems responsible for flavour development during the preripening chilling treatment.

However, as chilling did not seem to affect crispness significantly (Table 1), it should be possible to improve the acceptibility of such chips by the addition of artificial or natural banana flavour. This aspect is now being considered.

Viscometric data fitted accurately the power law flow model with a mean coefficient of determination of 0.995 (Table 2). These data confirm that

Table 1. Sensory scores for flavour and crispness in banana chips*

Treatments	Flavour†	Crispness†
Control, (ripening at 20°C)	4.5^{b}	5.7^{x}
Preripening storage at 7°C and 0.14 atmospheric pressure for two weeks, ripening at 20°C	5.0^{ab}	5.7^{x}
Preripening storage at 7°C for two weeks, ripening at 20°C	6.2^{a}	6.3^{x}
A commercial product	6.1^{a}	5.5^{x}

* Sensory scores are the mean of eighteen evaluations. Lower values indicate higher preference (1 = like extremely, 9 = dislike extremely).

† Values with the same superscript are not significantly different ($P \leqslant 0.05$) by Duncan's multiple range test.

Table 2. Effect of preripening storage conditions on power law flow model parameters for dispersions of reconstituted banana flakes

Treatments	Consistency coefficient (m), (dynes sec^{n} cm^{-2})*	Flow behaviour index (n)*	Coefficient of determination (r^2)
Control, ripening at 20°C	135.0^{a}	0.326^{x}	0.995
Preripening storate at 7°C and 0.14 atmospheric pressure for two weeks, ripening at 20°C	18.6^{b}	0.451^{y}	0.994
Preripening storage at 7°C for two weeks, ripening at 20°C	17.7^{b}	0.456^{y}	0.997

* Values with the same superscript are not significantly different ($P \leqslant 0.05$).

banana purée is pseudoplastic and non-Newtonian ($n < 1$) as was reported by Charles (1972).

Consistency coefficients (m) of the reconstituted purées from control samples were generally higher than those from banana fruits pretreated by chilling. On the other hand, flow behaviour index (n) was higher for purées prepared from samples obtained from chilled fruits; that is, purées from chilled samples were slightly more Newtonian than those from control samples.

Chilling bananas prior to ripening appeared to affect the rehydration properties of the flakes. Flakes from chilled fruits were observed visually not to rehydrate as completely as those from control samples, presumably as a result of the effects of chilling on the cell wall properties or the starch/sugar conversion characteristics in the ripening banana pulp (Palmer, 1971). Introduction of low pressure at chilling storage did not significantly improve the rehydration properties of the flakes, as reflected by the flow parameters, m and n, in Table 2.

Colour of the banana flakes was affected slightly by chilling (Table 3). Flakes from fruits stored at 20°C were a lighter yellow as reflected in their

Table 3. Effect of preripening storage conditions on colour of banana flakes*

Treatments	Hunterlab scale		
	Rd	+a	+b
Control, ripening at 20°C	29.6	7.9	26.4
Preripening storage at 7°C and 0.14 atmospheric pressure for two weeks, ripening at 20°C	28.0	9.0	26.0
Preripening storage at 7°C for two weeks, ripening at 20°C	28.8	9.4	25.0

* Flakes were stored under nitrogen in cans at 20°C for two months prior to colour evaluation.

higher Rd and +b values compared to flakes from chilled fruits. The colour was essentially the same in flakes made from fruits of the two chilling treatments; both had slightly higher +a (red scale) value which indicated some browning, but overall differences in comparison to the control were small.

In summary, the possibility of making dehydrated products from banana fruits chilled prior to ripening was demonstrated. Quality of the flakes and chips produced, when evaluated in terms of flavour and colour, was inferior to similar products made from non-chilled fruits. Rheological properties of reconstituted, puréed banana flakes were also affected by chilling treatments. It is suggested that future work on the production of dehydrated banana products from chilled tissues should be directed towards the problem of improving quality.

Acknowledgment

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Biochemical changes in experimental soy sauce Moromi

F. M. YONG* AND B. J. B. WOOD

Summary

Biochemical changes occurring during the mash or Moromi stage of experimental soy sauce fermentations are described. Despite the high temperature (40°C) and salinity (18% salt w/v) employed, and the low pH (4.5) which developed, amylase and proteinase activity from fungal enzymes present at the beginning of the fermentation, remained detectable throughout the thirty-day incubation period. Considerable increases in reducing sugar, total dissolved nitrogen and amino nitrogen levels occurred, despite active growth of yeast and *Lactobacillus*. In some cases the increases were greater in mashes containing one or both organisms, than in uninoculated controls.

Introduction

In previous papers (Yong & Wood, 1976, 1977) we have described microbial changes in the course of laboratory soy sauce fermentation, and biochemical changes in the course of the mould-growth of Koji stage. In the present paper we describe biochemical events in the Moromi or soy-mash stage.

Materials and methods

All the techniques employed have been described in our two previous papers (Yong & Wood, 1976, 1977). The Moromis, were prepared from 72 hr old Koji.

Results

In the work on Koji we examined and compared three different mould strains. It would have been interesting to compare the sequence of events in Moromis

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prepared from the three varieties of Koji, but we wished to compare the behaviour of the yeast and bacterium found in the Moromi, both alone and in combination with each other. We therefore had to select one mould Koji in order to keep the work-load to a reasonable level, and chose to use Koji made with *Aspergillus oryzae* strain NRRL 1989, since this organism gave the highest level of proteinase, total soluble organic nitrogen and amino-nitrogen, of the strains being examined.

As described in our paper on microbiological changes (Yong & Wood, 1976), we set up different Moromis as follows:

(i) Inoculated with both yeast and bacterium
(ii) Inoculated with bacterium only
(iii) Inoculated with yeast only
(iv) Inoculated with yeast only, but the pH was corrected to 4.5 with D–L-lactic acid.
(v) Neither yeast nor bacterium added.

The yeast cannot grow in the very salty (18%) mash until the pH drops to below 5.0. Even in the mash inoculated with yeast only, the pH decreased until it reached a value at which the yeast could grow, but the decline was accelerated by the action of lactic acid bacteria where these were present in the mash. In all mashes containing yeast, except the one where the pH had been artificially corrected with lactic acid, the yeast cell viable count declined from its initial value until the pH dropped below 5.0, whereupon it increased rapidly in numbers. In the case where the pH had been artificially corrected to 4.5, yeast grew rapidly after a short lag period.

Before proceeding to analyse the moromis, we needed to determine if extraction with 18% salt solution gave a different measure of enzyme activity

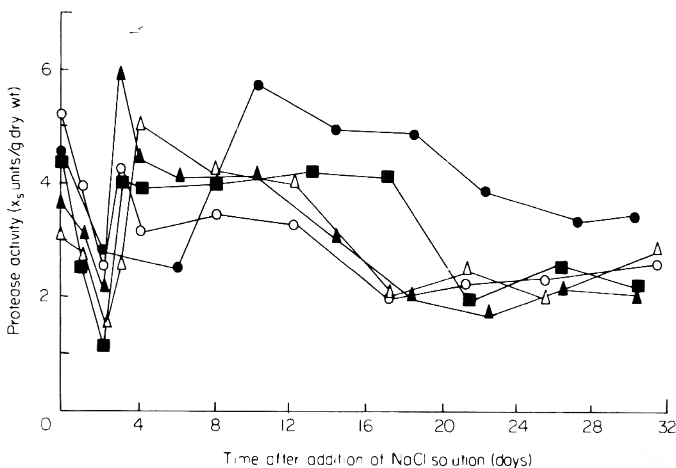

Figure 1. Proteinase in Moromi. ▲, Moromi with *Sacch. rouxii* and *L. delbrueckii*; ●, Moromi with neither yeast nor bacterium added (control); ■, Moromi with *Sacch. rouxii* only; ○, Moromi with *Sacch. rouxii* only, but the pH was adjusted to 4.5 with D-L-lactic acid; △, Moromi with *L. delbrueckii* only.

from that found if extraction was with distilled water, since the former solution corresponded to the condition found in the moromi, but caused problems with subsequent steps in the assay of enzyme activity. No significant difference in the yield of active enzyme was detected, between extracts made with either fluid. This was true with both of the enzymes tested for.

In reporting enzyme levels in the Moromi, it is important to stress that the figures reported represent the amount of enzyme *active under the conditions employed in the assay* present in the Moromi. We have not examined the activity of the enzymes at the pH and salinity found in the Moromi, and therefore a figure cannot be placed upon the extent of the enzyme's activity in the Moromi, although the analytical changes observed in the Moromis containing neither yeast nor lactobacillus must represent changes brought about by the mould enzymes, since no organisms were detectable on microbiological examination of such moromis.

Proteinase

Results (Fig. 1) showed that for the first two days after mixing, the level of proteinase which could be detected declined sharply, much more so than was the case with α-amylase. (Fig. 2). The results with total nitrogen, amino and ammonia nitrogen and reducing sugars analyses (Figs 3–5) show that this initial decrease is not a simple function of the extraction processes going on in the brine. It may well be that there is some initial denaturation or precipitation of the enzyme, followed by re-solubilization and/or the release of fresh enzyme upon lysis of mould hyphae. *De novo* synthesis of enzyme in this phase of increasing enzyme concentration, by the mould, seems unlikely to make a significant contribution, since it is generally accepted that the levels of salt used in successful soy-sauce production are sufficient to totally inhibit the mould, and that if reduced levels are used, permitting mould growth, the resulting mash and the soy sauce made from it have rancid off-flavours (Yong & Wood, 1974).

Whatever the cause, the events in Moromis inoculated with either or both organisms follows a similar path, an initial decrease in proteinase, and equally rapid increase to near or beyond the original level extractable from the freshly prepared Moromi, then a gradual decline in enzyme level as the fermentation progressed, with small fluctuations probably accountable for by the problems encountered in sampling from the very inhomogeneous Moromi. The uninoculated control mash took nearly twice as long to recover as did any of the other preparations, although it is difficult to see how the yeast and/or bacterium could occasion this difference. Thereafter, the uninoculated Moromi behaved much like the others, showing a gradual decline in enzyme level.

Overall, the rather high level of enzyme present in the mash after thirty days' incubation at 40°C in 18% brine at a final pH around 4.5 is the most striking result. Unpublished work (Goel, 1974) seems to rule out the possibility of

significant enzyme release from the yeast or bacterium. It appears therefore that the fungal proteinase is rather stable under these conditions.

α-Amylase

On all samples but one, the pattern of initial decline followed by recovery in enzyme level, as seen in the examination of proteinase, was repeated (Fig. 2). However the decrease was smaller, and both decrease and recovery were slower in the case of α-amylase. The exception noted above was the yeast-only Moromi with pH initially adjusted to 4.5 with lactic acid, where the enzyme showed an initial increase, followed by an overall decline, finally to an undetectably low level.

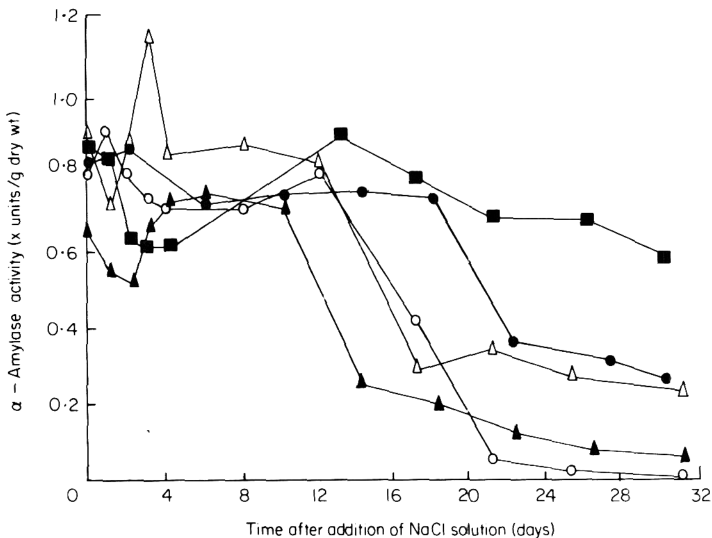

Figure 2. α-amylase in Moromi. Key as in Fig. 1.

Overall, this enzyme seems to have been rather less stable than was the proteinase activity. The shape of all the plots seems to suggest that there was more than one enzyme contributiing to the α-amylase activity, for there was a distinct and sharp loss of activity between the twelfth and twentieth days of incubation, with only a slow decrease in activity thereafter. This decrease in enzyme level was comparatively delayed in the uninoculated control Moromi, and somewhat reduced in extent. The extent of loss of activity was very much reduced in the Moromi which was inoculated with only the yeast, and was not artificially acidified. The decrease in detectable α-amylase was not simply related to either the increasing acidity of the Moromi or to the growth of either the yeast or the lactic acid bacterium, therefore.

Total soluble nitrogen

Initially the total soluble nitrogen (T.S.N.) level increased rapidly in all but the control Moromi (Fig. 3); why the control (uninoculated) Moromi should behave so differently from the others is not clear. Thereafter, all the inoculated Moromis showed a small but distinct decline in T.S.N., followed by a clear division into two groups. The mashes containing lactic acid bacteria both settled down at a T.S.N. level close to that of the control Moromi; the two containing only yeast, finished at a markedly higher level.

In mashes inoculated with a single organism, the phase of decrease in T.S.N. corresponded exactly with the period of rapid increase in cell numbers (see Yong & Wood, 1976), as would be expected. The curve from the culture with both microorganisms is more complex; there was a small decrease between

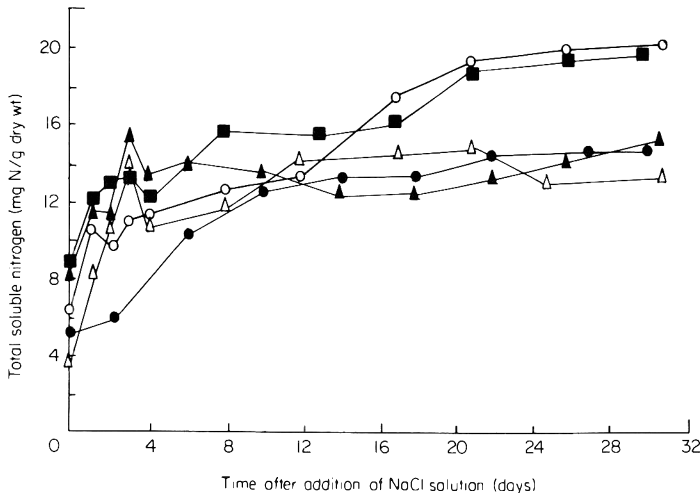

Figure 3. Total soluble nitrogen in Moromi. Key as in Fig. 1.

days 1 and 2, when the lactic acid bacteria grew, a second decrease between days 3 and 4 when no change in viable numbers of either organism was observed (a decrease also seen in the mash inoculated with yeast alone, and with the pH not adjusted to 4.5), and a further decrease between days 10 and 14 when the yeast grew rapidly.

The reason for the clear separation between moromis only inoculated with yeast and all other Moromis, in the final level of T.S.N., is less easy to explain. The differences are less striking when evaluated in terms of the increase in T.S.N. between the first and last determinations of this value, but the overall effect is much the same, see Table 1.

These results suggest that either the yeast can in some way protect, and/or promote the action of, the mould enzymes present in the mash, or that the initial shift to pH 4.5 in one case benefits the mould's acid proteinase. The higher level of T.S.N. reached in the Moromis fermented by yeast alone is of

possible practical interest, since the total soluble nitrogen is a factor of considerable value in determining the quality of the final product.

Amino-nitrogen

As with the T.S.N., there was a rapid initial increase in amino-nitrogen (A.N.) for the first few days (Fig. 4). Although not so marked as with the T.S.N., there is a suggestion of decreases in the A.N. during periods of rapid increase in microbial numbers. Also, there is a suggestion of a grouping into mashes with yeast only and those with bacteria, although the segregation is likewise not so clear-cut as with the T.S.N. results, indeed the mash with bacteria alone shows a sudden increase right at the end of the fermentation

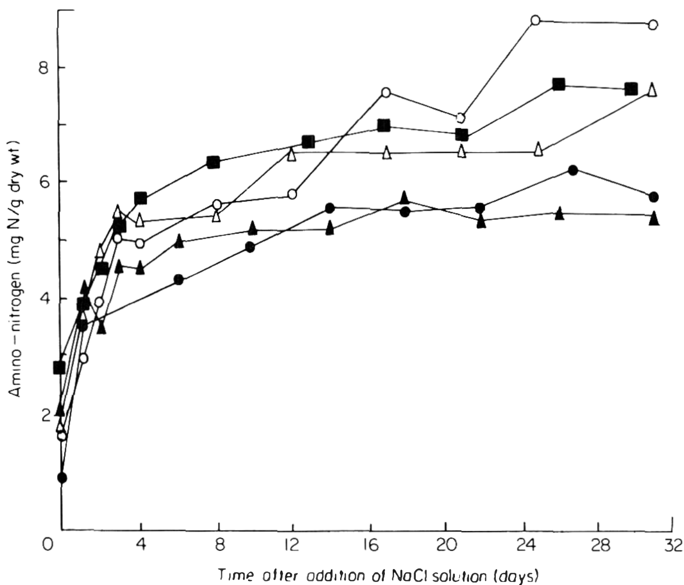

Figure 4. Amino-nitrogen in Moromi. Key as in Fig. 1.

which could possibly relate to lysis of the moribund cells, although the mixed culture shows no such increase in A.N. Although the absolute increase in A.N. was smaller than the absolute increase in T.S.N., in percentage terms, the A.N. analyses always showed a bigger increase than did the corresponding results for T.S.N. (Table 1). The distinctly better performance by the Moromi inoculated with yeast alone and with its pH adjusted to 4.5 – this time in both absolute and percentage terms – suggests once more that this might be a better method for producing the sauce, than is the usual mixed-culture approach.

Ammonia nitrogen

Increases in this (undesirable) component of soy sauce were comparatively small, averaging about a doubling of it during the thirty-one-day fermentation

Table 1. Analysis of total soluble nitrogen and amino-nitrogen in Moromi

Inoculum	Total soluble nitrogen			Amino-nitrogen			Reducing sugars as glucose			Increase in soluble protein (N x 6.25)
	Original	Final	Increase (%)	Original	Final	Increase (%)	Original	Final	Increase (%)	
Yeast and bacterium	8.1	14.6	80	2.0	4.1	105	6.5	59.6	817	40.6
Bacterium	3.4	12.8	277	1.8	7.6	322	5.5	96.0	1645	58.8
Yeast	8.5	19.0	124	2.7	7.2	167	2.3	87.5	3704	65.6
Yeast pH 4.5	6.2	19.6	216	1.6	8.8	450	1.9	93.7	4832	83.8
Control	4.0	14.0	250	0.9	5.3	489	8.3	82.3	892	62.5

All results except percentage changes or in $mg\,g^{-1}$ dry weight of Moromi.

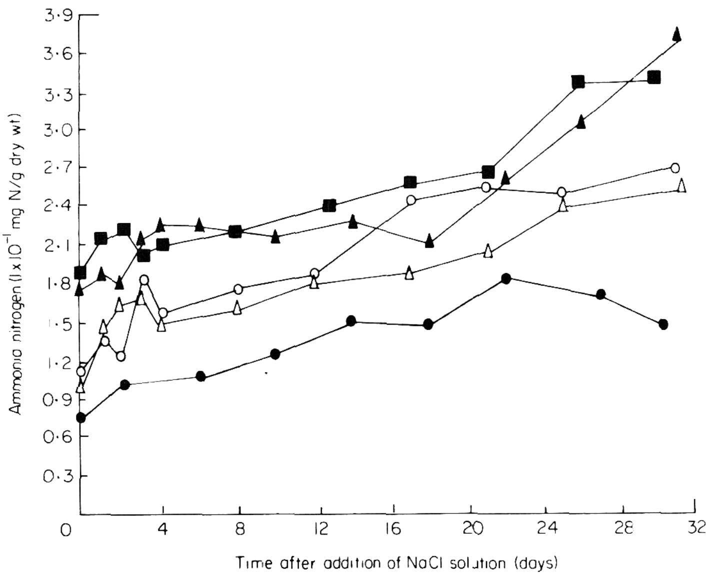

Figure 5. Ammonia nitrogen in Moromi. Key as in Fig. 1.

period. No particular trends were evident, although the mixed culture showed a steady and sustained increase during the final two weeks' incubation. The small net increase found in the mash without added yeasts or bacteria suggests that some at least of the ammonia in the other mashes was due to metabolic activities of the added microorganisms, although at the pH found in the Moromi, decarboxylation of amino acids would seem to be a more likely activity of the lactic acid bacteria (Blood, 1975) than would deamination.

Reducing sugars

Reducing sugar levels showed the biggest percentage increase of any of the biochemical changes examined in this study, although if the increases in total dissolved organic nitrogen are converted to protein (N × 6.25), the absolute increases are seen to be of the same order as each other (Table 1).

The general pattern of change is one of very rapid increase for the first five or six days and of a slower increase thereafter (Fig. 6). The mash containing both yeast and bacteria gave the lowest final level of reducing sugar, but the single organism cultures all gave similar final levels, rather higher than those found in the control Moromi. The mash inoculated with yeast alone gave a pattern of increase clearly divided into two phases of rapid increase with a marked pause between them. It may be significant that this mash alone did not show a sharp decrease in available α-amylase around the middle of the incubation period, although the similar mash which had its pH artificially corrected to pH 4.5 gave the greatest fall in α-amylase of any of the experimental Moromis, without adverse effect on the rate of increase in reducing sugars.

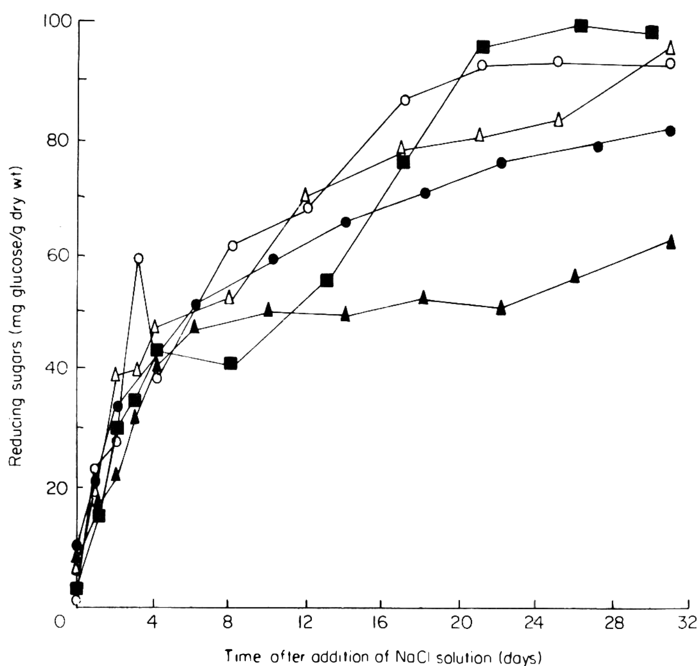

Figure 6. Reducing sugars in Moromi. Key as in Fig. 1.

These results suggest a need for a more detailed examination of the carbohydrates present in the various Moromis and of the pattern of oligomer hydrolysis therein.

Quality of the soy sauces obtained from the five experimental Moromis

The final extracts from all five types of mash were dark brown in colour and of generally similar appearance. Flavour and aroma of the soy sauce made with both yeast and bacteria were good, comparable with a fully brewed product made by traditional fermentation. The sauce from the yeast-only mashes with pH adjusted to 4.5 was of very similar quality, perhaps just slightly inferior. The sauce from the unacidified yeast-only mashes was acceptable, but distinctly inferior to the previous sauces; in particular its aroma was less intense. The mashes from runs with only lactic acid bacteria, or with no organism at all, had no aroma or true soy sauce flavour and were judged to be unacceptable.

Discussion

The results presented in this paper answer very few questions, but serve to make clear the need for detailed study of the changes occurring in the Moromi. The fact that we were able to produce soy sauce of acceptable organoleptic

quality suggests that the system gives a reasonable simulation of events occurring in commercial soy sauce brewing, and that it can be employed as a convenient means for detailed examination of the complex changes which occur during this process, and for studying the questions suggested by the investigation reported in these papers.

One interesting aspect of this work which is not fully discussed elsewhere in these papers, is the nature of the controls restricting microbial growth in the mash. The high salt level and lack of oxygen must control the fungus, and the lactic acid bacteria are killed by the acid products of their own metabolism. The reason why the yeast stops growing is less clear. The cells remain viable until the end of the fermentation, ample reducing sugars and organic nitrogen seem to be present, the acid conditions are a prerequisite for yeast growth in the salty mash, yet growth ceases. The obvious candidate for an inhibitor is ethyl alcohol, but the alcohol concentration is low; Yokotsuka (1972) quotes 1–2%. The inability of yeast to effect under strictly anaerobic conditions desaturation steps necessary in the production of polyunsaturated fatty acids, and biosynthetic steps necessary in the production of sterols, would be a restriction on growth. The fact that soya is an oilseed, containing 15–20% lipid, and that lipases are produced by the mould during the Koji stage of fermentation would suggest that this constraint might be less severe than is the case in beer or wine making. In the latter case, the restriction on growth does not limit alcoholic fermentation, which may continue up to 14% or more of ethanol by volume, whereas soy sauce only contains 1–2% by volume. In some Japanese reports (cited by Yong & Wood, 1974) there is reference to using compressed air for stirring the mash. The frequency of such aeration is not specified, although other workers suggest that too much stirring may hinder the fermentation. On the other hand, Onishi (1971) reports that browning of the mash on exposure to air was accompanied by the development of off-flavours. It would be interesting to examine the effect of controlled aeration during the phase of active yeast growth. This is another interesting problem in the physiology of the organisms involved in the fermentation which needs resolution. It is a matter of practical interest since the extent of yeast fermentation is an important factor in the flavour and quality of the finished soy sauce.

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The influence of endosperm structure, protein content and grain moisture on the rate of water penetration into wheat during conditioning

R. MOSS

Summary

The effect of protein content, endosperm structure and grain moisture on the rate of water penetration into wheat grains was studied. Other factors which are known to alter the rate of water penetration were kept constant. An increase in the amount of endosperm protein was found to increase the time required to establish an equilibrium distribution of water regardless of whether the additional protein was present as discrete masses in the sub-aleurone cells or throughout the entire endosperm. Grain moisture also influenced the rate at which any subsequent water passed through the endosperm. Moisture equilibrium was achieved more rapidly in those grains with a higher initial moisture content.

Introduction

During previous investigations into the conditioning behaviour of six Australian wheat cultivars (Butcher & Stenvert, 1973) an attempt was made to relate the differences in water penetration to the observed differences in morphology (Moss, 1973). It was suggested that the morphological components of major importance in this regard were first, the outer cuticle and testa due to the presence of waxy, hydrophobic cutins; secondly, the degree of compression of the outer epidermal and inner parenchymal cells; and thirdly, the number and size of the sub-aleurone protein masses which can bind or otherwise impede the penetration of water into the centre of the grain. Recent work by Stenvert & Kingswood (1977) indicates that the disruption of endosperm structure associated with the presence of air spaces is also a factor that affects the rate of water penetration. These air spaces are not readily observed using the light microscope, probably because of swelling of the starch granules during fixation. It was therefore decided also to examine the endosperm structure using a scanning electron microscope as this does not require fixation of the tissue. A

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possible additional factor influencing moisture penetration is the initial moisture content of the wheat. This aspect would also be expected to be pertinent to a better understanding of the significance of the two-stage conditioning process.

In the previous investigation (Butcher & Stenvert, 1973) all the above factors tended to vary independently and it was therefore difficult to assess the relative importance of the individual morphological characteristics which might influence water penetration. It was thus decided to design two series of experiments to investigate, and hopefully clarify, the relative importance and interaction of these factors during conditioning.

Materials and method

Effect of protein content on water penetration

Many single cultivar samples of wheat contain mixed populations of both vitreous and mealy (starchy) grains, even when grown under indentical conditions and at the same site. When sections of these wheats were examined under the microscope there was rarely any observable difference in the morphology of any of the bran layers, but simply a difference in the distribution of protein within the grains. Therefore by selecting such samples and separating the vitreous and mealy grains, the effect of variations in protein distribution on water penetration rates could be studied independently of variations in bran morphology.

Samples of three wheat cultivars (Timgalen, Eagle and Gamenya) were selected that contained both vitreous and mealy grains. For each cultivar these grains were hand picked and separated, the mottled grains being dis-

Table 1. Protein contents for some wheat varieties

Cultivar	Protein (%)	Protein difference (%)	Frequency of occurrence of protein masses*
Timgalen			
Vitreous	12.6	1.8	3
Starchy	10.8		2
Eagle			
Vitreous	11.7	1.3	2
Starchy	10.4		1
Gamenya			
Vitreous	11.6	1.6	1
Starchy	9.8		1

* 3 = 75% or more of the sub-aleurone cells contain protein masses; 2 = 25–75% of the sub-aleurone cells contain protein masses; 1 = less than 25% of the sub-aleurone cells contain protein masses.

carded. Kjeldahl nitrogen determinations were carried out on the six samples. The protein contents are listed in Table 1. It was apparent that, in each case, the vitreous grains had a higher protein content than the mealy grains. Insufficient material was available for moisture determinations but the samples were allowed to equilibrate for several days under ambient conditions (55% R.H., 21°C). From previous experience this would be expected to result in a common moisture content for all samples of approximately 12%.

Samples (10 g) of vitreous and mealy grains of each cultivar, were conditioned by the addition of 2.5% of tritiated water (100 mCi), in the manner previously described (Butcher & Stenvert, 1973). Twenty to thirty wheat grains were quickly removed from the sealed flasks after lying times of 3, 6, 12 and 24 hr. The grains were immediately placed in 100 ml flasks surrounded by dry ice and the flasks sealed. The frozen grains were stored over dry ice until ready for *block-autoradiography* (Moss, 1977).

Effect of initial wheat moisture content on rate of water penetration

Three wheat samples were selected for study, two (cv. Falcon and cv. Heron) had a relatively high moisture content and the third sample (cv. Eagle) was selected because of its naturally low moisture content. Sound grains were selected for investigation and each sample divided into two groups which were stored in air-tight tins prior to adjustment of moisture.

The moisture content of the wheat was increased by the addition of the appropriate amount of distilled water, and lowered by storage in a dessicator over concentrated sulphuric acid for seven days. All wheats whose moisture contents had been adjusted were allowed to equilibrate for at least seven days. Protein content and initial and final moisture contents of the grains are given in Table 2.

Table 2. Protein content, initial and final moisture content of the three wheat samples selected.

Sample	Original moisture (%)	Original protein (%)	Modified moisture (%)
Eagle	9.6	10.5	12.0
Heron	12.4	13.4	6.1
Falcon	12.4	14.0	8.4

Tritiated conditioning water (2.5%) was added to 5 g-aliquots of wheat for selected lying times. Trial experiments conducted on the Eagle sample indicated that a lying time of 12 hr was suitable for detecting differences in moisture penetration rates. This time interval was also satisfactory for the other two cultivars.

Light and electron microscopy

Several grains from each of the vitreous and starchy wheat samples were taken from the first part of the experiment for light microscopy, and sections from four grains were stained and examined as previously described (Moss, 1973).

Samples of the wheats used in the second part of the experiment were not examined under the light microscope since this technique would not be expected to reveal any structural differences between the dry and more moist samples.

Samples of the wheat grains were also prepared for scanning electron microscopy* by fracturing the grains transversely in the median region. The half grains were then mounted onto electron microscope stubs, fractured surface uppermost, and coated with carbon and gold before examining in a J.S.M.-U3 scanning electron microscope.

Development of autoradiographs and examination of the cut surface of the grains

After 48 hr exposure over dry ice the wheat grains were washed off the emulsion covered slides and the slides placed in Kodak Dektol developer (1 : 1) for 4 min, rinsed in water, fixed in Ilford Hypam and rinsed in running water for 1 hr before air drying. The wheat grains were placed in numbered, perforated containers and washed for 2–3 hr in fast running water to remove tissue-tek and reduce radioactivity. The grains were then fixed for at least 18 hr in buffered glutaraldehyde and stained with Ponceau 2R. The stained grains were then examined using a stereomicroscope to give an indication of the gross morphology of the grain and the distribution of the protein within the endosperm.

Results and discussion

Effect of protein

The observed patterns of moisture distribution are illustrated in Fig. 1. It was immediately obvious that the rate of water penetration into the vitreous grains of each cultivar was slower than that into the corresponding mealy grains notwithstanding the relatively small differences in protein (1.3–1.8%). Details of protein distribution are listed in Table 1 and those of bran morphology in Table 3. Timgalen had the most open bran structure and Eagle had the most compressed, but these differences are not as marked as have been observed in several other cultivars.

* The sample of starchy Eagle was insufficient to enable scanning electron micrographs to be prepared.

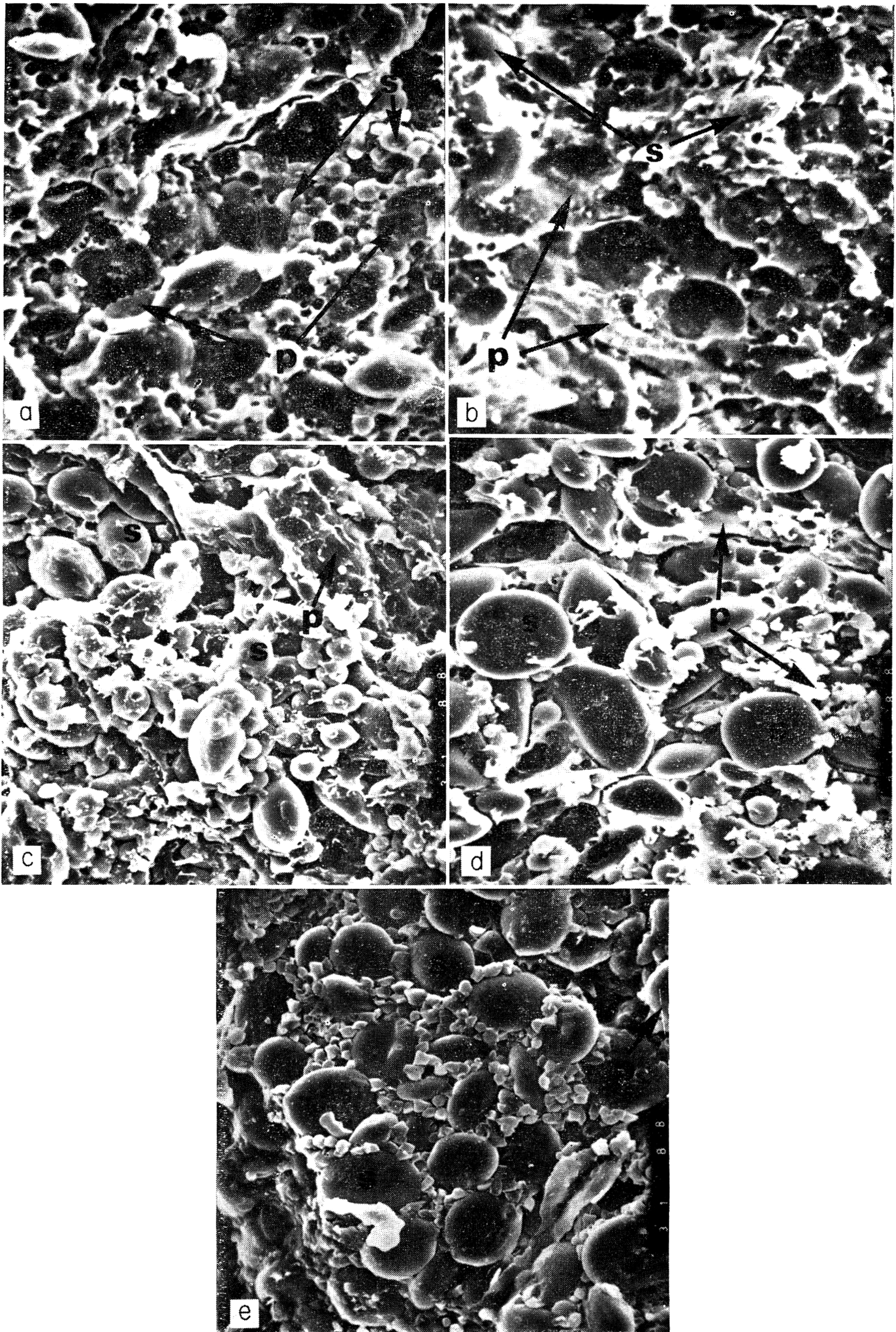

PLATE 1. Scanning electron micrographs of vitreous and mealy grains of three wheat cultivars. (a) vitreous Eagle; (b) vitreous Timgalen; (c) mealy Timgalen; (d) vitreous Gamenya; (e) mealy Gamenya. (The mealy sample of Eagle was not examined in the S.E.M. due to the limited number of grains available.) p—Protein, s—Starch granules. ⊢—⊣ 10μm

(*Facing p. 278*)

Table 3. Details of bran morphology

	Timgalen	Eagle	Gamenya
Outer epidermal cells (A)	Slightly compressed to slit-like lumina	Slightly compressed to compressed lumina	Open to compressed lumina
Inner parenchymal cells (B)	Slightly compressed to compressed lumina	Slit-like to compressed lumina	Slightly compressed to compressed lumina
Combined depth of A and B (μm)	30–45	20–40	30–40
Testa colour	Orange	Orange	Dark orange
Thickness of testa (μm)	6	8	6–8

The two samples of Gamenya had no clearly discernible differences in either the number or the distribution of sub-aleurone protein masses, whereas the differences between the vitreous and mealy samples of the other two cultivars were marked. Nevertheless, the rates of water penetration in both samples of Gamenya were dissimilar indicating that, in order to reduce the rate of water penetration, the extra protein in the vitreous samples does not have to be in the form of an increase in either the number or size of protein masses. However protein content, *per se,* is not the sole factor governing water penetration. If the six samples are listed in order of decreasing protein content this sequence is not the same as that of the increasing rate of water penetration.

Differences in the bran morphology of the three samples might also influence the water penetration rates. However, provious work has suggested that the rate of water penetration through bran is very rapid (Moss, 1977). This conclusion is based on many observations but on only one wheat cultivar, but similar observations have been reported by Stenvert & Kingswood (1976) on English wheats. Nevertheless bran morphology could exert an influence on both the initial absorption of water and the early stages of conditioning.

The vitreous sample of Timgalen had the highest protein content of all the wheats yet the water tended to penetrate into these grains at a faster rate than into either of the vitreous samples of Gamenya or Eagle. The bran from this sample of Timgalen had a typically open cellular structure, which previous work had indicated was associated with a rapid uptake of water. The bran structure of Eagle was also the most compressed of the three cultivars examined and the vitreous sample of this wheat had the slowest rate of water penetration.

The samples of Timgalen and Gamenya had similar bran structures and the protein difference between the vitreous Gamenya (11.6% protein) and the mealy Timgalen (10.8% protein) would suggest that the water would move at a more rapid rate through the latter. However both samples show very similar

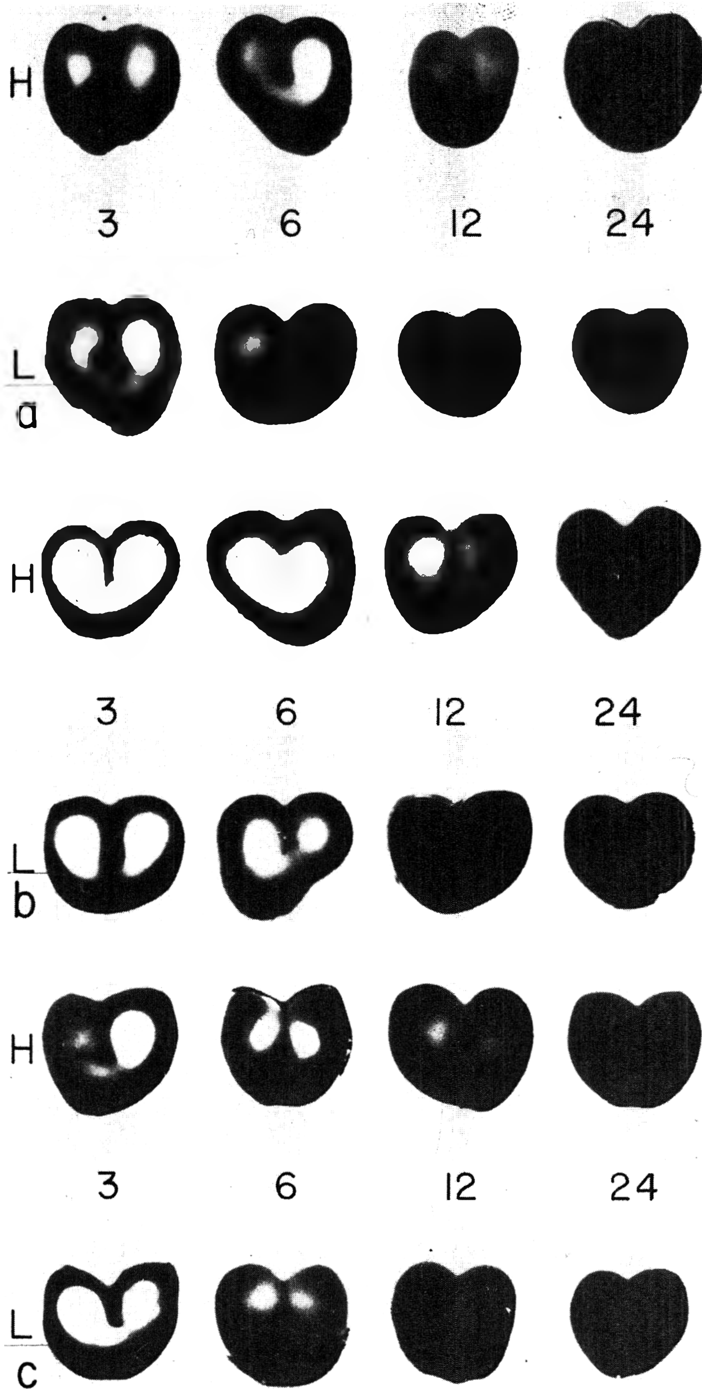

Figure 1. Autoradiographs of wheat grains to show the effect of protein content on water penetration rates. (a), cv. Timgalen; (b), cv. Eagle; (c), cv. Gamenya. H = higher protein level (see Table 1) (vitreous grains), L = lower protein level (see Table 1) (mealy grains); numbers indicated lying times in hours.

patterns of moisture distribution at each lying time. Concepts other than protein content and bran structure must therefore be invoked to explain some of these differences in water uptake.

The autoradiographs shown in Fig. 1 represent the most common rate of water penetration observed at the selected lying time, but for every sample variations in water penetration were observed. The crude, low power, stereo-microscopic examination of the wheats provided evidence to help account for these variations. Slight differences in the amount of sub-aleurone protein were noted for each sample and those wheats which permitted a slightly faster rate of water penetration had less protein than those that delayed the rate of water penetration. It was also noted that due to the difficulties associated with working under safe-light conditions, some grains were sectioned near to the germ. It would be expected that this would also lead to an apparently increased rate of water penetration.

A study of the scanning electron micrographs provided additional information that helped to explain some of the differences that were un-accounted for by the evidence provided by the light photomicrographs. The vitreous sample of Eagle was extremely compact in both the sub-aleurone, and the central endosperm (Plate 1(a)).

Examination of the fractured surface indicates that cleavage had taken place near the intercellular boundary and not through the centre of cells. This suggests that the cellular contents were very hard and compact. Both Timgalen samples appeared to be compact in the sub-aleurone region, but the starchy samples (Plate 1(c)) appeared to be more open than the vitreous sample in the central endosperm (Plate 1(b)). In the vitreous sample of Gamenya some voids can be seen in the protein matrix. These are particularly apparent in the central endosperm, but they can also be detected in random areas of the sub-aleurone. Plate 1(d) and (e) show clearly the open appearance of the central endosperm of both the vitreous and starchy Gamenya samples. It seems reasonable to suggest that, at the same protein content, water would penetrate more rapidly through an open endosperm structure than through a hard compact structure. However, the protein level and degree of endosperm com-paction can vary independently, which makes the precise prediction of water penetration rates difficult. This is compounded by the difficulty in differentiat-ing degrees of compaction at higher protein levels.

Effect of wheat moisture

Water penetration through the moist samples was faster than through the corresponding drier samples in each case (Fig. 2). This conclusion is equally valid for wheats that are received at a higher moisture content and dried and for the drier samples that have been predamped. The latter has particular relevance when considering the two-stage conditioning process.

Comparison of the scanning electron micrographs of each pair of wheats

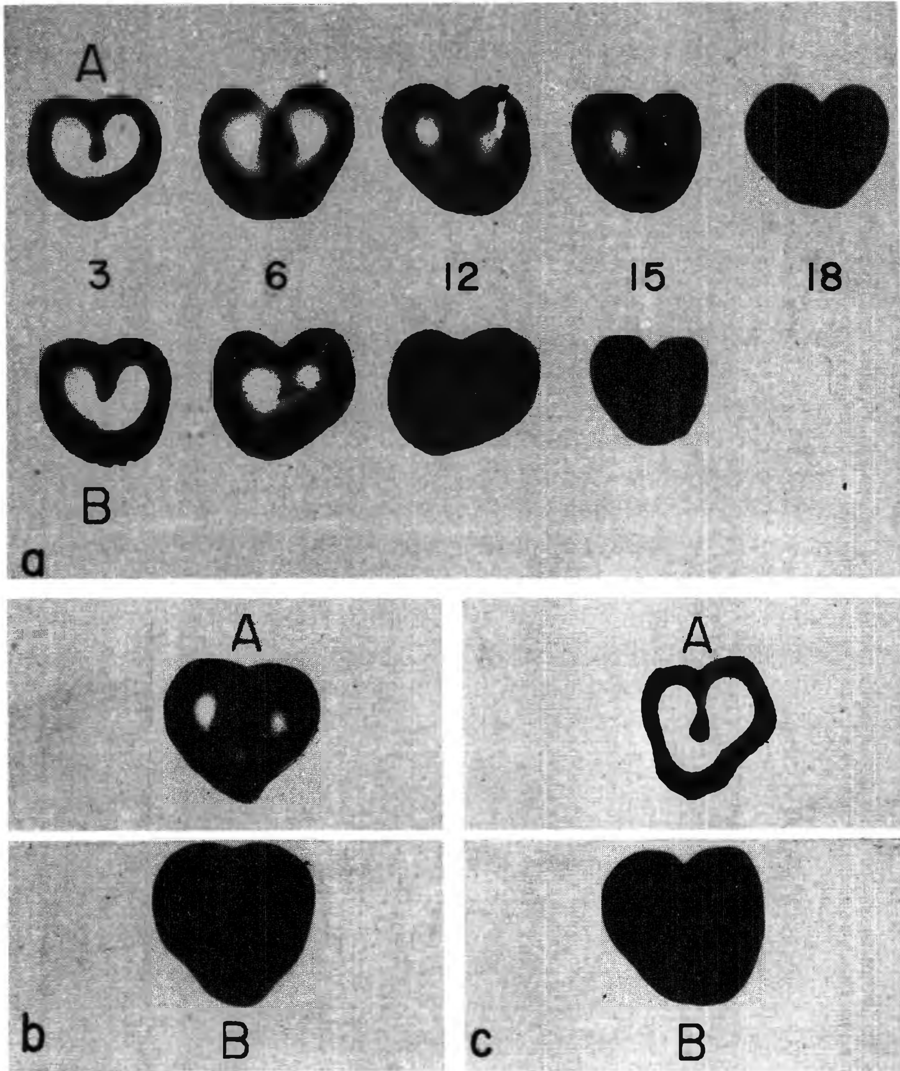

Figure 2. Autoradiographs of three wheat cultivars at different moisture levels. (a), cv. Eagle; (b), cv. Heron; (c), cv Falcon. A = lower moisture level (see Table 2); B = higher moisture level (see Table 2); numbers indicate lying times in hours.

revealed little difference in the endosperm structure. In all three cultivars the protein matrix was continuous. Frequently the amount of protein was such that it tended to mask the starch granules and reduced contrast in the S.E.M. No increase was detected in either the number or extent of cracks within the endosperm of the more moist samples.

An explanation for the difference in water penetration rates can be found in either the swelling that takes place on hydration of grain or the corresponding contraction that occurs on dehydration (Campbell & Jones, 1955a, 1955b). The swollen and therefore more open nature of the more moist grains would be

expected to facilitate a more rapid movement of water through the endosperm. When conditioning predamped wheats this factor is of greater significance than the reduced water differential across the grain.

Conclusion

Although bran morphology appears to affect the initial uptake of water, and its movement through the grain during the early stages, it has been shown that endosperm structure is of paramount importance in governing water penetration in the later stages. It is known that after a lying time of 1 hr water has reached the aleurone/sub-aleurone interface (Moss, 1977; Stenvert & Kingswood, 1976). The nature of these two components must therefore have a profound influence on the subsequent rate of water penetration.

This study has shown that although protein alone can retard water penetration the degree of compaction of the endosperm also has a significant influence. Even at high protein levels the endosperm of certain cultivars is porous and hence allows a more rapid water penetration.

Increasing the water content of wheat grains also affects the nature of the endosperm. The grains swell resulting in a less compact endosperm without necessarily resulting in the formation of cracks within the endosperm. It can therefore be seen that the process of predamping dry wheat enables a more rapid uptake of the subsequent conditioning water.

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Effect of processing variables on the quality of dehydrated carrot

I. Leaching losses and carotenoid content

A. K. BALOCH, K. A. BUCKLE AND R. A. EDWARDS

Summary

Enzymic destruction of carotenoids in unblanched carrot tissue, incomplete extraction of pigments from raw carrot, thermal destruction of carotenoids by blanching and cooking of carrot, and leaching of soluble solids during processing of carrot were examined as possible explanations for apparent increases in carotenoid content during processing.

The leaching of soluble solids was found to be the major factor responsible for apparent increases in carotenoid when results were expressed on a water insoluble solids basis.

β-Carotene, the most biologically active carotene and the major pigment of carrot, was found to be about 1.9 times more susceptible to heat damage than α-carotene during normal blanching and cooking processes.

Introduction

Dehydrated vegetables have long maintained a prominent role in commerce, hence it is important that knowledge be available for the leaching of soluble constituents during processing, and of the storage stability of the leached product, in order to achieve a maximum yield and long storage life of the product. Processing operations such as blanching, cooking, post blanching treatments and dehydration, though essential to the process, are all responsible for nutrient losses and the initiation of undesirable changes.

Apparent increases in carotenoid content of processed vegetables compared to the fresh materials from which they are derived have been a matter of interest for some time. Although several studies have been made to explain such increases in carotenoid content of vegetables including carrot (Zscheile, Beadle & Kraybill, 1943; Bailey & Dutton, 1945; Lee, 1945; Booth, 1949; Gooding, Tucker & MacDougall, 1960; Weckel *et al.*, 1962; Della Monica & McDowell, 1965; Nutting, Neuman & Wagner, 1970), no satisfactory

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explanations have yet been suggested. A number of possible causes for the apparent increase in carotenoid content of carrot were, therefore, examined.

Materials and methods

Blanching and treatment of carrot

Carrots (*Daucus carota*) were purchased from the local market, trimmed, hand peeled and washed in running tap water. The carrots were diced (9.4 mm^2 × 3.1 mm), thoroughly mixed and used immediately for further processing.

Diced carrots on trays (7.4 kg/m^2) were steam blanched for 5 min to inactivate peroxidase (Masure & Campbell, 1944). In order to achieve different levels of leaching of soluble solids, the blanched carrot was either dipped in, or washed with water or detergent solution, as explained in Table 1. The treated carrots were drained for 10 min prior to analysis.

Table 1. Processing of carrot for leaching of soluble solids

Treatment	Procedure
Water dip	About 2.3 kg blanched carrot was immersed for 6 min in 2.5 litre of distilled water at ambient temperature in a stainless steel bucket
Detergent dip	About 2.3 kg blanched carrot was dipped in 0.1% (w/v) sodium lauryl sulphate solution (0.9 kg/litre) at room temperature
Water wash	Blanched carrot was immersed in distilled water (0.9 kg/litre) at room temperature, stirred, then drained. The washing was repeated four times
Detergent wash	About 2.3 kg blanched carrot was washed four times with sodium lauryl sulphate solution (0.1% w/v) at ambient temperature
Water dip at 75°C	Blanched carrot (0.9 kg) was dipped for 5 min in 1 litre distilled water at 75°C

Sample preparation

Unblanched or blanched carrot dice (200 g) were ground in a nitrogen atmosphere in the chilled bowl of a Sorvall Omni mixer. The blended sample homogenate was placed into an air tight amber coloured bottle, flushed with nitrogen, and stored at 2°C until used for further tests.

Determination of moisture content and water insoluble solids

Moisture content was estimated as the loss in weight of 10 g of fresh sample on drying in a vacuum oven at 70°C for 6 hr. Water insoluble solids were estimated by the method of the AOAC (1970). Percent leaching loss was

calculated by subtracting the percentage of water insoluble solids of unblanched samples from that of samples given various dipping and washing treatments.

Estimation of carotenoid pigments

Carotenoid pigments from raw and blanched carrots were extracted by the method of Baloch, Buckle & Edwards (1977). An accurately weighed 10 g sample of homogenate from unblanched and blanched processed carrots was extracted with chilled 90% acetone containing 20 ppm hydroquinone to minimize oxidation (Booth, 1960). The residue was ground with methanol and again extracted with acetone until colourless. The pigments were transferred to petroleum ether (b.p. 40–60°C) by means of 5% NaCl solution, and the absorbance measured at 600 and 449 nm in 1 cm matched silica cells on a Unicam SP600 spectrophotometer, and spectra recorded on a Unicam SP800 spectrophotometer. Total pigment concentrations were determined as β-carotene using $E^{1\%}_{1\,cm}$ 2500 (Davies, 1965).

The individual pigments were separated on thin layers of Hyflo Super Cel: magnesium oxide : calcium sulphate (8 : 2 : 0.7) reported by Baloch *et al.* (1977), and the concentration of each pigment calculated using appropriate absorption coefficients (Davies, 1965).

Results and discussion

During preliminary studies on the dehydration of carrot, apparent increases in the carotenoid content of carrots processed in various ways were observed, confirming many reports cited previously. Several possibilities were investigated to explain such apparent increases in carotenoid content using carrot as a raw material.

Carotenoid destruction by enzymic systems

The possibility of enzymic destruction of carotenoids during processing of unblanched carrots was examined. Unblanched and blanched carrot homogenates were incubated in the dark for up to 60 min at 25°C, and analysed for carotenoid content (Table 2). The carotenoid content of the unblanched carrots decreased slightly with increasing incubation time at 25°C, whereas that of the blanched samples remained constant, indicating the possible involvement of a carotenoid-destroying enzymic system in unblanched carrots. Lipoxygenase (lipoxidase) activity in carrot has been reported by Rhee & Watts (1966), although no lipoxidase activity in carrot was found by Pinsky,

Table 2. Carotenoid content of unblanched and blanched carrot homogenates incubated in the dark at 25°C

Incubation time (min)	Carotenoid content* (μg, wet weight basis)		Carotenoid loss (%)	
	Unblanched	Blanched	Unblanched	Blanched
0	122	128	—	—
30	119	126	2.5	1.5
60	114	128	6.6	0

* Mean of two samples.

Grossman & Tropp (1971). However, pigment determinations on raw or unblanched tissues may be in error to an increasing extent if the time before pigment extraction is prolonged. The low pigment loss in carotenoid content of 6.6% which occurred during incubation of unblanched carrot could be responsible for only a small apparent increase in carotenoid content of processed carrots. However, the reported increases (35–85%) in carotenoid content of processed carrots (Bailey & Dutton, 1945; Weckel *et al.*, 1962; Della Monica & McDowell, 1965) cannot be wholly attributed to an avoidance of enzymic reactions.

Inadequate extraction of pigments from raw carrot

To determine whether incomplete extraction of carotenoid pigments from unblanched carrot was contributing to apparent increases in carotenoid content, the residue obtained after pigment extraction from unblanched carrot was refluxed in 50% methanol for 5 min and the residual carotenoid pigment extracted (Table 3).

No further pigment was recovered from unblanched carrot tissues during refluxing with extracting solvents following normal pigment extraction.

When unblanched carrot was cooked for 30 min prior to pigment extraction, the carotenoid content decreased progressively with increased cooking time, with a maximum loss of 10.6% after heating for 15 min at 121°C (Table 4).

Table 3. Extraction of carotenoid pigments from raw carrot

Sample	Carotenoid content of raw carrot (μg/g, wet weight basis)	
	First extraction	Second extraction
1	123	0
2	120	traces
3	122	0

Table 4. Effect of cooking on carotenoid destruction of carrot

Cooking time (min)	Wavelength maximum of extract (nm)	Carotenoid content (μg/g, wet weight basis)	Carotenoid loss (%)
0	449.3	132	—
2	449.3	130	1.5
5	449.0	128	3.1
10	448.5	124	6.1
15	448.3	121	8.2
15*	448.0	118	10.6

* Autoclaved for additional 15 min at 121°C.

Similar losses in carotenoid content of several vegetables processed under various conditions have also been reported (Borenstein & Bunnell, 1966). However, Panalaks & Murray (1970) reported an increase in carotenoid content of 53 and 72% on cooking and canning of carrots respectively. They assumed the increase was due to increased extraction of carotenoid pigments from the carrot tissues ruptured during processing. In the present investigation, however, the extraction technique adopted provided complete removal of the pigments from raw and processed carrots. Since Panalaks & Murray (1970) did not give details of the cooking and canning procedures, other possibilities cannot be ignored.

In the present studies, a gradual decrease in the wavelength of maximum absorption from 449.3 to 448.0 nm was observed on cooking carrot for 30 min (Table 4). During cooking, a loss of 7.0% of total pigment occurred (Table 5), 66.6% of which was loss of β-carotene, the major pigment of carrot (Table 6).

Table 5. Effect of autoclaving for 30 min at 121°C on carotenoid pigments of carrot

Carotenoid pigment	Carotenoid content (μg/g, wet weight basis)		Loss of pigment in relation to total pigment content (%)	Loss of pigment in relation to total pigment loss (%)
	Raw	Autoclaved		
α-Carotene	22.4	17.7	3.7	35.6
β-Carotene	86.0	77.1	7.0	66.6
ζ-Carotene	7.0	7.3	−0.2	−2.3
γ-Carotene	2.9	2.8	0.1	0.8
cis-Neurosporene	0.9	0.9	0.0	0.0
Neurosporene	2.1	2.0	0.1	0.8
Lycopene	0.6	0.6	0.0	0.0
Lutein	3.6	3.8	−0.2	−1.5
Unidentified carotenoids	0.3	0.3	0.0	0.0
Total	125.8	112.6	10.5	—

Table 6. Carotenoid pigments of raw carrot

Carotenoid	Proportion of total pigment (%)
α-Carotene	17.8
β-Carotene	68.4
ζ-Carotene	5.6
γ-Carotene	2.3
cis-Neurosporene	0.7
Neurosporene	1.7
Lycopene	0.5
Lutein	2.9
Unidentified	0.2

In contrast to these findings, Panalaks & Murray (1970) reported a considerable loss in the α-carotene fraction during cooking of carrot. Critical examination of their data for carotene content of carrot indicates that the low concentration of α-carotene obtained after cooking of carrot (4.3%) was not due to cooking alone, since the percentage of carotenes (excluding α-carotene) remaining after each processing treatment, including cooking, is almost constant (72.1–79.5%). This indicates that losses in the α-carotene fraction on cooking were not greater than with the other processing treatments.

No attempts were made to isolate the *cis*-isomers of α- and β-carotene formed during autoclaving of carrots, however, a slight increase in the R_f values and a diffused band of β-carotene were indicative of the formation of pigments with *cis* configurations. This was further substantiated by a shift in the absorption pattern towards shorter wavelengths accompanied by losses of extinction and resolution of the absorption peaks in the visible range, and an increased absorption in the UV region (Table 4). The formation of neocarotene B during cooking of yellow vegetables, including carrot, has also been reported (Sweeney & Marsh, 1970, 1971).

Table 7. Effect of leaching of soluble solids on carotenoid content of processed carrot

Treatment	Loss in soluble solids (% dry weight basis)	Increase in carotenoid content (% dry weight basis on leached material)
Unblanched	—	—
Blanched	2.7	9.1
Water dipped	6.9	26.1
Detergent dipped	7.5	29.1
Water washed	11.9	48.2
Detergent washed	14.5	58.0
Water dipped at 75°C	8.1	27.9

Table 8. Effect of processing variables on the carotenoid content of carrot

Treatment	Moisture content, dry weight basis (%)	Water insoluble solids, dry weight basis (%)	Carotenoid content ($\mu g/g$)			Carotenoid increase, dry weight basis, leached material (%)	Carotenoid loss, water insoluble solids basis (%)
			Wet weight, basis, leached material	Dry weight basis, leached material	Water insoluble solids basis		
Unblanched	89.1	23.1	106	973	4212	—	—
Blanched	88.7	25.8	120	1062	4116	9.1	2.3
Water dipped	91.2	30.0	108	1227	4090	26.1	2.9
Water washed	92.3	35.0	111	1442	4120	48.2	2.2
Water dipped at 75°C	92.2	31.2	97	1244	3987	27.9	5.3

Effect of leaching of soluble solids on carotenoid content

In order to examine the effect of leaching of soluble solids on the apparent increase in carotenoid content, carrots were subjected to various dipping and washing treatments (Table 1) to achieve several levels of leaching loss, and water insoluble solids and carotenoid content were determined (Table 7). Measurable leaching losses (2.7–14.5%) resulted on dipping and washing of blanched carrots, and the apparent increase in carotenoid content was higher in samples with a higher leaching of soluble solids. Several workers have also found leaching losses to be responsible to a great extent for such increases during processing of carrot (Bailey & Dutton, 1945; Lee, 1945; Weckel *et al.*, 1962; Della Monica & McDowell, 1965).

Increases in carotenoid content were obtained when results were calculated on a dry weight basis for the leached material (Table 8), showing a positive relationship between apparent increase in carotenoid content and leaching loss. When carotenoid content was calculated on a water insoluble solid basis, the carotenoid loss (2.2–5.3%) was almost equal to the apparent loss resulting from the cooking of carrot, indicating that a leaching of soluble solids is a major factor responsible for the apparent increase in the carotenoid content of carrot during processing. When the results are calculated on a water insoluble solids basis, no such increase in carotenoid content is apparent.

Lee (1945) demonstrated the use of alcohol insoluble solids as a reference base for the calculation of the carotenoid content of processed carrots. Since various alcohol insoluble materials are likely to be leached out when carrot is treated with hot water, as is normally practised in cooking and canning processes, the alcohol insoluble solids basis is unlikely to provide an accurate estimation of the real carotenoid content of the sample. Hence water insoluble solids were adopted as the reference base for such calculations.

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Effect of processing variables on the quality of dehydrated carrot

II. Leaching losses and stability of carrot during dehydration and storage

A. K. BALOCH, K. A. BUCKLE AND R. A. EDWARDS

Summary

Leaching of soluble solids had a considerable effect on the stability of carrot during drying and storage. Carotenoid destruction and lipid oxidation increased with increased leaching of soluble constituents. Non-enzymic browning and pH changes decreased and rehydration properties improved as the result of leaching of soluble solids. Increased leaching of soluble solids, though favourable for extending the storage life regarding non-enzymic browning, encouraged carotenoid destruction. A maximum storage life of ninety-six days at 37°C was obtained at a leaching loss of 5.7% soluble solids, which is slightly lower than that produced by a water dip treatment after steam blanching of carrot. Optimization of post-blanch treatments is required to maximize storage life and to minimize nutrient losses.

Introduction

Several deteriorative reactions that affect the colour, nutrient properties, texture and flavour of dehydrated products are initiated during processing and dehydration operations, and continue during storage at a rate that is controlled by storage conditions. Processing steps such as blanching, cooking, post-blanching treatments and dehydration, though essential to the process, are responsible for nutrient losses and the initiation of many undesirable changes.

Although the leaching of soluble solids during the pre-dehydration steps has been reported by many workers (Hendel, Legault & Talburt, 1953; Gooding, Tucker & MacDougall, 1960; Della Monica & McDowell, 1965; Baloch, Buckle & Edwards, 1977a), little information is available concerning the effect of leaching on the storage stability of dehydrated products. This paper reports on the effect of leaching of water soluble solids during pre-dehydration processing on changes in carotenoid stability, lipid oxidation, non-enzymic browning (NEB) and rehydration properties during dehydration and subsequent storage.

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Materials and methods

The source of carrot, the procedure for blanching of diced carrot and various post blanching water and detergent dipping and washing treatments designed to achieve several leaching levels of soluble solids, and methods used for the determination of water soluble solids and carotenoid content of carrot have been reported in previous publications (Baloch *et al.*, 1977a, b).

The treated carrots were dehydrated for 9 hr at dry bulb and wet bulb temperatures of 71 and 38°C respectively to a moisture content of 3.5–4.0%. The dehydrated carrots were equilibrated to 4% moisture content by storing over a predetermined quantity of distilled water in a desiccator in the dark at room temperature. The samples (30 g) were hermetically sealed into tinplate cans (74 × 61.5 mm), and stored at 37°C. Samples were removed after 30, 60, 120 and 180 days of storage, ground to pass a 20 mesh sieve and analysed for carotenoid content, lipid oxidation, NEB and pH. Rehydration properties were measured on carrot dice before grinding.

Lipid oxidation

Lipid oxidation was measured by the 2-thiobarbituric acid (TBA) method of Tarladgis *et al.* (1960). Absorbance of the coloured complex was measured at 532 nm after reaction of TBA with malonaldehyde obtained by distillation at pH 1.5 of a slurry made from 5 g powder.

Non-enzymic browning, pH and rehydration ratio

The NEB of dehydrated carrot was estimated by measuring the absorbance at 420 nm of a carrot extract clarified with lead acetate and ethanol (Baloch, Buckle & Edwards, 1973).

The pH of a slurry of 5 g powder in 10 ml freshly boiled and cooled distilled water was measured with a Pye Dynacap pH meter.

Rehydration ratio was measured by the method of Anon. (1944). Dehydrated carrot dice (5 g) in 100 ml distilled water was boiled for 5 min, immediately filtered under a slight vacuum and weighed. Rehydration ratio was calculated from the ratio of the drained weight of rehydrated carrot to the initial weight of the dehydrated sample.

Results and discussion

Carotenoid destruction and lipid oxidation

Increased leaching of soluble solids from carrots before dehydration increased the loss of carotenoid pigments during dehydration (Table 1).

Carotenoid destruction in dehydrated carrot during storage for 180 days at 37°C is shown in Fig. 1. In all treatments studied, the rate of pigment breakdown was rapid up to 50–60% destruction, but then decreased. The results agree with those reported for ground dehydrated carrots (Stocking & Weier,

Table 1. Effect of leaching of soluble solids on destruction of carotenoids during dehydration of leached carrots

Treatment	Loss in soluble solids (g/100 g, dry weight basis)	Carotenoid content (μg/g, dry weight basis)		Carotenoid loss (%)
		Before dehydration	After dehydration	
Blanched	2.7	1062	1007	5.2
Water dipped	6.9	1227	1125	8.3
Detergent dipped	7.5	1259	1143	9.0
Water washed	11.9	1442	1285	10.9
Detergent washed	14.5	1537	1339	12.9

1949; Weier & Stocking, 1949) and for dehydrated carrot flakes (Stephens & McLemore, 1969).

Since gross pigment spectra of extracts from dehydrated treated carrots before and after storage did not differ appreciably, the effect of leaching of soluble solids on the storage stability of individual carotenoid pigments was not studied. However, minor shifts in wavelengths for maximum absorbance to

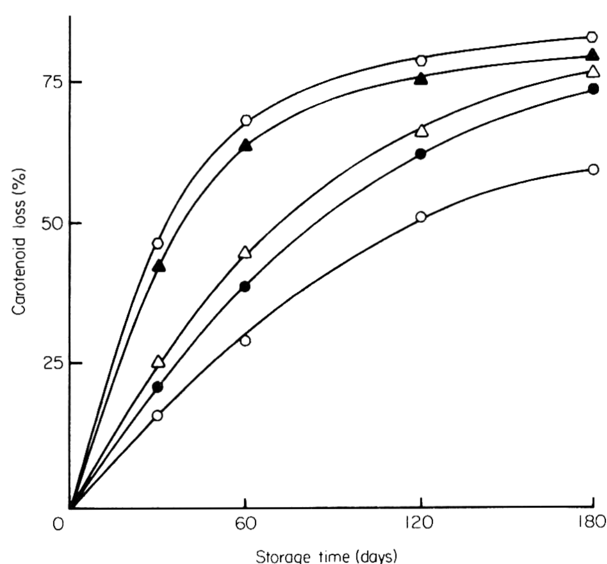

Figure 1. Effect of post-blanching treatments on the loss of carotenoids during storage at 37°C. ○——○ = blanched, ●——● = water dipped, △——△ = detergent dipped, ▲——▲ = water washed, ⬡ —— ⬡ = detergent washed.

shorter wavelengths were observed for all of the dehydrated treated samples during storage. The absorbance of the peak at the shorter wavelength increased during the earlier periods of storage but then decreased to the original absorbance value.

For all treatments the peak height ratio first decreased and then increased, but the extent of variation differed for different treatments. The extent of the decrease was equal within dipped treatments and within washed treatments. Moreover, the decrease was maximum for washed materials and minimum for carrot which was blanched only. These results indicate the production during storage of some pigments which show maximum absorption at wavelengths shorter than that of the major peak (449 nm) of the gross pigment extract. The rate of production of these pigments was possibly higher than that of their destruction during the initial period of storage, while the converse was no doubt true during later storage periods. Similar observations have been reported on the oxidation of carotene in model systems (Budowski & Bondi, 1960) and in freeze-dried carrot (Falconer *et al.*, 1964).

Breakdown products of carotenoid origin could be significant in determining the overall storage life of the product (Purcell & Walter, 1968; Walter, Purcell & Cobb, 1970). Tomkins *et al.* (1944) and Tomkins, Mapson & Wager (1946) reported that odours were noticeable when about 20–30% of the carotenoids present had been destroyed. A significant correlation between the development of off-flavour and carotenoid destruction has been reported by Falconer *et al.* (1964) for oxidizing freeze-dried carrot powder stored at 18°C. Although no quantitative data are available, several attempts that have been made to characterize non-volatile and volatile products arising from oxidizing dehydrated foods and from carotene in model systems indicate the involvement of carotenoid oxidation in production of off-flavour (Cole & Kapur, 1957a, b; Purcell & Walter, 1968; Fishwick, 1969; Land, 1969; Walter *et al.*, 1970).

Carotenoid oxidation in dehydrated leached carrots followed a first order reaction up to about 60% carotenoid destruction, confirming the results reported by Martinez & Labuza (1968) and Chou & Breene (1972). However, Chen & Gutmanis (1968) reported that the extractable carotenoid pigments in dried chilli pepper deteriorated by a second order reaction. Although kinetic studies of carotenoid destruction reveal little about the mechanisms of carotenoid oxidation, they provide a means of comparing the effects of different applied treatments. Consequently, the first order rate constants reported in Table 2 were calculated.

It can be seen that the major effect of leaching of soluble solids is the significant reduction in storage life of the product and a considerable increase in the rate of carotenoid destruction. These results are similar to those of Weier (1944) who found 50% carotenoid loss during 21 hr storage at 60°C in carrot previously steam blanched and washed for 1.5 hr in running cold water.

These results suggest that some substances, which stabilize carotenoid pigments, were leached out during the processing of carrot. The stability of carotenoid pigments in carrot tissue has been considered to be due mainly to

Table 2. Effect of leaching of soluble solids on rates of carotenoid destruction and NEB, and storage life of dehydrated treated carrots, during storage at 37°C

Treatment	Soluble solids loss (%)	Rate of deterioration		Rate ratio		Storage life (days)	
		Carotenoids*	NEB†	Carotenoids (K_t/K_b)‡	NEB (K_b/K_t)‡	Carotenoids§	NEB¶
Blanched	2.7	2.4	43.9	1.00	1.00	121	30
Water dipped	6.9	3.3	16.7	1.36	2.63	87	120
Detergent dipped	7.5	4.0	14.4	1.66	3.04	72	134
Water washed	11.9	7.1	8.8	2.92	5.00	41	> 180
Detergent washed	14.5	8.1	5.2	3.33	8.45	36	> 180

* First order rate constant ($hr^{-1} \times 10^4$); † rate of increase of 0.05 absorbance unit ($day^{-1} \times 10^3$); ‡ K_t = rate for post-blanch dipping and washing treatments; K_b = rate for blanched treatment alone; § days for 50% loss in carotenoids (Falconer *et al.*, 1964); ¶ days for 0.15 unit increase of absorbance at $E_{1\,cm}^{1.25\%}$, 420 nm (Legault *et al.*, 1951).

the presence of natural antioxidants such as tocopherols (Heftmann, 1947). However under the present processing conditions where carrots were treated with aqueous solutions alone, the leaching of significant levels of such fat soluble antioxidants is unlikely. Alternatively, Weier (1944) suggested the liberation of water soluble antioxidants during carrot blanching. The polyphenolic compounds present in carrots (Geissman, 1962; Harborne, 1971) would be expected to be leached out during processing, otherwise they could act as antioxidants (Bate-Smith, 1954; Lea, 1958a; Pratt & Watts, 1964; Ben Aziz *et al.*, 1968; Witt *et al.*, 1971). The rapid oxidation of carotenoids by increased formation of peroxides during drying, resulting from increased diffusion of oxygen through the spongy texture of the tissue, is another possibility (Budowski & Bondi, 1960). The increased rehydration ratios obtained (Table 6) would also tend to support this hypothesis.

Irrespective of leaching loss incurred, lipid oxidation was initially rapid and then gradually decreased (Table 3), and was accelerated by increased leaching of soluble solids, i.e. the extent of lipid oxidation was highest in the sample which suffered the highest leaching loss.

Lipid oxidation corresponded well with carotenoid destruction. In samples which showed a high degree of lipid oxidation, carotenoid oxidation was also high. However, 76% of the initial carotenoids were oxidized before any significant amount of lipid oxidation occurred in carrots washed with a detergent solution. Since no appreciable amount of lipid oxidation was noticed in any of the samples, it can be concluded that carotenoids are oxidized preferentially and act as an antioxidant for lip oxidation, as suggested by Bonner (1950) and Lime (1969).

Objectionable volatiles were produced in increasing concentration to parallel increased oxidation of lipids and carotenoids. The concentration of TBA

Table 3. Effect of post-blanching treatments on lipid oxidation of dehydrated carrot of 4% moisture content during storage at 37°C

Treatment	TBA number (mg malonaldehyde/kg)				
	Storage time (days)				
	0	30	60	120	180
Blanched	0.49	0.76	0.99	1.14	0.86
Water dipped	0.58	0.88	1.10	1.28	1.24
Detergent dipped	0.61	0.89	1.11	1.38	1.44
Water washed	0.64	0.93	1.18	1.50	1.67
Detergent washed	0.74	1.08	1.33	1.68	1.81

reactive substances indicated that lipid oxidation was not a serious problem in dehydrated carrot, since relatively low TBA values were found compared to published values for rancid animal products. Nevertheless, it must still be considered a possibility that peroxide decomposition in plant foods may proceed differently to animal products, with low concentrations of breakdown products causing undesirable changes.

Leaching of soluble and insoluble solids is a potential economic loss to processors and also causes effluent problems. In the present studies a maximum loss of 14.5% soluble solids resulted from washing of steam blanched carrot with detergent solution (Table 1). Gooding *et al.* (1960) found that water blanched carrot lost up to 30% dry solids by leaching, but the losses were reduced to 10–11% if the concentration of solids in the blanching liquor was increased to 4–5%. However, only 8% of the dry matter was lost with steam blanching in those investigations.

In addition to steam blanching, various other blanching methods have been investigated for their ability to reduce leaching losses. Individual quick blanching (Bomben *et al.*, 1973), fluidized-bed blanching (Brown, Farkas & DeMarchena, 1972) and hot gas blanching (Ralls *et al.*, 1973) have been demonstrated to be particularly useful in reducing substantial volumes of wastewater and BOD levels in blancher effluents.

Changes in NEB, pH and rehydration properties

Post-blanching treatments had a significant effect on NEB, pH changes and rehydration ratio of carrot during dehydration and subsequent storage at 37°C. After dehydration, the NEB of blanched carrot was considerably greater than that of samples given a water wash and a detergent wash treatment (Table 4). On the other hand, the reverse was true for pH changes (Table 5) and rehydration ratio (Table 6).

During storage, the rate of NEB remained low in products with high leaching of soluble solids (Table 4, Fig. 2). Dehydrated carrot given a water wash or

detergent wash treatment showed little NEB, whereas the sample that was blanched only was an intense brown colour.

The pH of the dehydrated material decreased during storage, the decrease being directly related to storage time and inversely related to the loss of soluble solids resulting from treatments prior to dehydration (Table 7).

Table 4. Effect of post blanching treatments on NEB of dehydrated carrot of 4% moisture content stored at 37°C

Treatment	NEB (absorbance at 420 nm x 10^3)				
	Storage time (days)				
	0	30	60	120	180
Blanched	88	152	216	345	474
Water dipped	18	39	69	150	231
Detergent dipped	17	32	59	128	197
Water washed	15	16	27	69	114
Detergent washed	13	14	14	30	57

Table 5. Effect of post-blanching treatments on pH of dehydrated carrot of 4% moisture content stored at 37°C

Treatment	pH				
	Storage time (days)				
	0	30	60	120	180
Blanched	5.60	5.54	5.48	5.37	5.24
Water dipped	5.72	5.68	5.65	5.56	5.48
Detergent dipped	5.75	5.71	5.68	5.60	5.54
Water washed	5.80	5.77	5.74	5.69	5.63
Detergent washed	5.84	5.83	5.80	5.78	5.74

Table 6. Effect of post-blanching treatments on rehydration ratio of dehydrated carrot of 4% moisture content stored at 37°C

Treatment	Rehydration ratio				
	Storage time (days)				
	0	30	60	120	180
Blanched	4.1	4.0	3.9	3.7	3.6
Water dipped	4.6	4.5	4.4	4.3	4.2
Detergent dipped	4.7	4.6	4.6	4.5	4.4
Water washed	5.2	5.0	4.9	4.7	4.5
Detergent washed	5.6	5.3	5.2	4.9	4.8

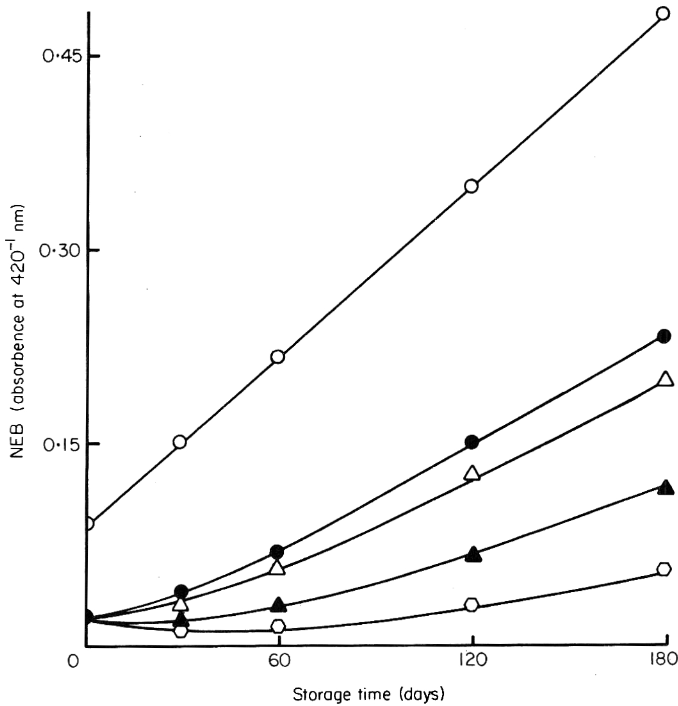

Figure 2. Development of NEB in treated dehydrated carrot during storage at 37°C. ○——○ = blanched, ●——● = water dipped, △——△ = detergent dipped, ▲——▲ = water washed, ⬡ —— ⬡ = detergent washed.

Rehydration ratio also decreased during storage. However, the 5–8% loss in rehydration ratio which occurred during 180 days of storage at 37°C was insignificant compared to the 46–61% loss which occurred during dehydration (Table 8).

Treatments after blanching had a considerable effect on the stability of carrot during dehydration and storage. Deteriorative reactions related to NEB and pH changes were significantly reduced by the leaching of soluble solids. A long induction period was noted for the NEB of samples given only washing treatments, whereas no induction period was present in the sample that was

Table 7. pH changes in dehydrated leached carrots stored at 37°C for 180 days

Treatment	pH change	Rate × 10^4 (units/day)
Blanched	0.36	20.0
Water dipped	0.24	13.3
Detergent dipped	0.21	12.0
Water washed	0.17	9.5
Detergent washed	0.10	4.2

blanched only (Fig. 2). The rate of NEB as calculated from the slope of the straight line after the induction period for the sample given a detergent treatment was nearly 8.5 times slower than for the sample given a blanching treatment only, increasing the storage life (days required to increase NEB index by 0.15 units in absorbance as defined by Legault *et al.*, 1951) from thirty days to more than 180 days (Table 2). Hence storage life, as determined by the

Table 8. Effect of leaching of soluble solids on rehydration ratio during dehydration and storage at 37°C for 180 days

Treatment	Soluble solids loss (%)	Loss in rehydration ratio (%)		
		During dehydration	During storage	Total
Blanched	2.7	60.6	4.8	65.4
Water dipped	6.9	55.5	3.9	59.6
Detergent dipped	7.5	54.9	2.8	57.7
Water washed	11.9	50.0	6.7	56.7
Detergent washed	14.5	46.1	7.8	53.9

NEB of the product, increased with increased leaching of soluble solids, indicating that soluble solids are involved in the development of NEB, and that the rate of development of NEB is determined only by the level of soluble solids still present in the blanched tissue. Similar observations have been reported by Hendel *et al.* (1953), who found little NEB in dehydrated carrot products stored at 38°C in which leaching losses were 54 to 55%.

Leaching of soluble solids also had an effect on the kinetics of NEB reactions. A zero order reaction rate was observed for NEB of dehydrated stored carrot given no post-blanch treatments and hence suffering minimal losses in soluble solids (Fig. 2), whereas an induction period or slow rate period, which increased with increasing leaching losses, followed by a linear rate, appeared in dehydrated samples treated with an aqueous solution. The results for samples without post-blanch treatments are similar to those reported by Legault *et al.* (1947) for dehydrated non-sulphited vegetables, while those for samples given post-blanch treatments are similar to those reported for dehydrated sulphited samples (Legault *et al.*, 1951). The appearance of an induction period in the rate of NEB for carrots given only a post-blanch treatment suggests that the components involved in NEB reactions are leached out during processing prior to dehydration, and that a certain time is required to produce sufficient concentrations of such NEB precursors again for the NEB rate to accelerate. The variable kinetics thus observed indicate that NEB occurs in dehydrated carrots after several reaction stages.

Changes in pH corresponded well with NEB (Tables 2 and 7). The rate of pH change was higher in carrot samples given treatments which produced higher levels of NEB, indicating that pH changes are associated with NEB for de-

hydrated foods as suggested by Hodge (1953) and Lea (1958b). However, the change in pH for all treated carrots followed a zero order reaction without an induction period (Fig. 3), whereas in the case of NEB, for all treated carrots except those which were not treated after blanching, an induction or slow rate period was observed, and the rate increased with increased leaching loss (Fig. 2). These results suggest that brown colour formation and acid production do not occur concurrently, and that pH changes result from the initial stages of NEB reactions that are initiated during dehydration, whereas colour formation occurs only during the final stages of the reaction sequence.

NEB reactions affect the rehydration properties of dehydrated carrot. The rehydration ratio was lowest in the sample with a maximum NEB index, and was improved by factors which inhibit NEB development. Consequently a relatively porous structure resulted from the dehydration of carrot previously leached during post-blanching treatments. However, the effect of NEB reactions on rehydration ratio of dehydrated carrot was of minor importance only, since the maximum loss in rehydration of 61% occurred during dehydration, whereas development of NEB occurred primarily during storage

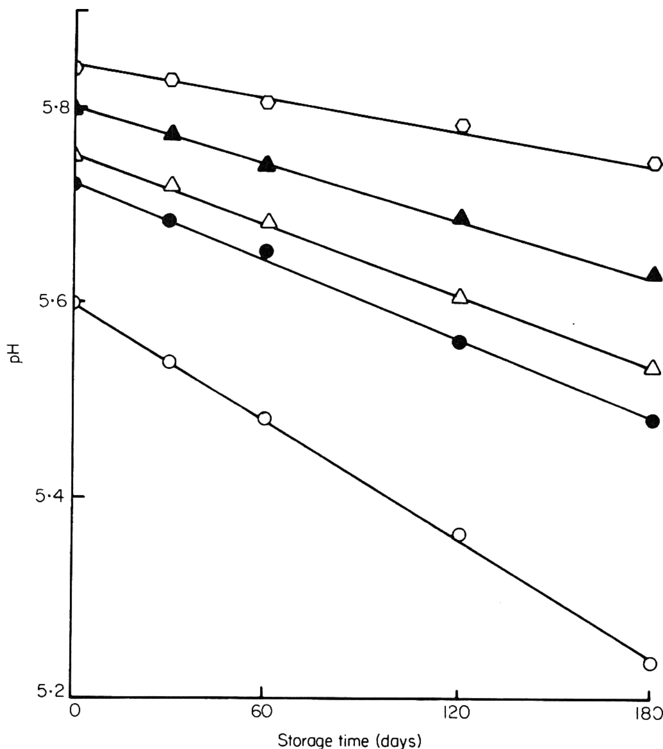

Figure 3. Effect of processing treatments on pH changes in treated dehydrated carrot during storage at 37°C. ○——○ = blanched, ●——● = water dipped, △——△ = detergent dipped, ▲——▲ = water washed, ⬡——⬡ = detergent washed.

at 37°C. Moreover, in samples where little NEB occurred during drying, the loss in rehydration ratio was considerable.

Deteriorative reactions associated with changes in rehydration properties are not well defined, but reactions between amino acids and carbohydrates which are invariably involved in brown colour formation play an insignificant role (Tables 4, 6 and 8), and the likely causes of significant losses in rehydration ratio are irreversible changes in macromolecular and colloidal constituents of carrot. These changes include denaturation of proteins by heat or localized high concentrations of soluble constituents, gelatinization of starch and crystallization of cellulose. Comparing the excellent rehydration ratio of freeze-dried products with that of conventional dehydrated food it appears that the technique by which water is removed from the food has an important effect on these deteriorative reactions.

Maximizing the leaching of soluble solids may not always be the most desirable means of extending the storage life of dehydrated carrots. For example, on increasing the leaching loss of soluble solids from 2.7 to 14.5%, the rates of NEB and pH change decreased by nearly 8.5 and 4.6 times respectively (Tables 2 and 8 respectively), while the rate of carotenoid destruction increased by 3.3 times (Table 2). Thus there is a conflict between the needs to avoid NEB and pH changes as compared with the need to avoid carotenoid destruction. Since NEB and carotenoid breakdown are the two major factors which individually determine the storage life of dehydrated carrot, the overall storage life of dehydrated carrot can be determined best by taking into account simultaneously the storage life regarding both sets of factors. The storage life of blanched dehydrated carrot was thirty days, and was limited by intense brown colour formation (Table 2), whereas the storage life of dehydrated carrot given a detergent wash treatment was thirty-six days, and was limited by carotenoid breakdown.

The maximum overall storage life obtainable before acceptability was limited by carotenoid oxidation and NEB was found to be ninety-six days at a leaching loss of 5.7% soluble solids which is slightly lower than that shown by carrot given a water dip treatment after steam blanching of carrot. Thus a level of leaching of soluble solids, of the order 4–6%, is required to achieve the maximum possible storage life of the product forming the basis of these studies.

Losses in soluble solids can be reduced substantially by adopting those techniques for blanching of vegetables which minimize leaching losses as mentioned previously, or by increasing the level of soluble solids in the blanching liquor. However, the latter process encourages deleterious reactions. Gooding *et al.* (1960) found that dehydrated carrot that had been water-blanched had a shorter storage life at 37°C than that which had been steam-blanched, and theorized that precursors of NEB reactions were formed in the blanching liquor, and the carrot became impregnated with these products. In other studies of water blanching (Anon., 1958), a build-up of solids in the blanch liquor was found to be restricted to a maximum of 1% for potatoes,

1.5% for cabbage, and 2.5–3.0% for carrots, before initial off-flavour developed and storage stability of the dehydrated product was impaired.

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Stability of β-carotene in model systems containing sulphite

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Summary

The effect of sulphur dioxide, a_w, storage temperature and storage atmosphere on the stability of β-carotene in non-sulphited and sulphited model systems was examined. Stability of β-carotene was enhanced greatly by SO_2 added either as a sulphite solution to cellulose powder prior to β-carotene adsorption, or as a gas in the headspace of tinplate cans containing β-carotene. β-Carotene stability was much improved with increasing nitrogen levels in the atmosphere. The effect was more significant when N_2 was replaced by SO_2. The stability of β-carotene was also increased when non-sulphited and sulphited samples were stored at a monolayer a_w.

Activation energies for destruction of β-carotene in non-sulphited and sulphited systems (10.3–12.8 kcal/mole) were considerably lower than for autoxidation of linoleic acid or its esters (15.2–17.2 kcal/mole), suggesting that carotenoid oxidation is more favoured than the autoxidation of linoleic acid or its esters.

Introduction

Although carotenoid destruction in vegetables during dehydration and storage is reduced by treatment with sulphur dioxide (Roberts & McWeeny, 1972), literature reports on such effects in dehydrated vegetables, particularly carrot, are confusing (Tomkins, Mapson & Wager, 1946; Weier & Stocking, 1949; Masure *et al.*, 1950; Farine *et al.*, 1965). Recently Baloch (1976) drew attention to the effect of drying and storage conditions on the stability of SO_2 consequently affecting carotenoid stability of dehydrated carrot.

The present studies were undertaken to investigate the effect of SO_2 treatment on the stability of β-carotene in model systems.

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Materials and methods

Preparation of non-sulphited and sulphited carotene-cellulose model systems

Microcrystalline cellulose powder (Merck) was ground in a mortar for 30 min with either distilled water or with sodium metabisulphite solutions (10 : 3 g/ml) for preparation of non-sulphited or sulphited samples respectively and vacuum dried for 1 hr at 50°C. Since 40–60% of the SO_2, in the concentration range studied, was lost during drying of the samples, 140–160% of the calculated amount of sodium metabisulphite required was added to cellulose powder. The dried sample was powdered to pass a 60 mesh sieve, cooled and stored at room temperature in an airtight container flushed with nitrogen. Samples were used within one week.

To the non-sulphited and sulphited cellulose powder was mixed pure β-carotene (Hoffman-La Roche & Co. Ltd, Basle, Switzerland) in chloroform previously neutralized with magnesium carbonate, and the mixture was vacuum dried in the dark for 30 min at 35°C and ground in a mortar until the carotene was uniformly distributed throughout the cellulose. The samples were stored at −12°C in the dark until used for further studies.

Moisture content and SO_2 determination

Duplicate 2 g samples were dried for 6 hr in a vacuum oven at 70°C and 100 mm Hg pressure. During drying a current of dry air scrubbed through sulphuric acid was admitted to increase the drying rate. Moisture was expressed on a dry weight basis.

Sulphur dioxide was estimated by the Monier-Williams method as modified by Shipton (1954).

Measurement of β-carotene concentration

An accurately weighed 1–2 g sample of carotene-cellulose powder was extracted with chloroform. The solvent was vacuum evaporated in the dark at room temperature and the pigment dissolved in petroleum ether (b.p. 40–60°C). The absorbance of the carotene solution was measured at 450 and 600 nm on a Varian Techtron model 635 spectrophotometer, and the concentration of pigment determined as β-carotene using $E^{1\%}_{1\,cm}$ 2500 (Davies, 1965).

Moisture sorption isotherm

Moisture sorption isotherms of non-sulphited and sulphited samples were determined at 37°C using the desiccator method of Stitt (1958). The samples

(2 g) were stored over saturated salt solutions of different a_w (Rockland, 1960) until equilibrium was established. The a_w values at monolayer coverage were calculated using the BET equation (Salwin, 1959).

Headspace analysis

The headspace volume and pressure were determined by the method of Board & Elbourne (1964). The gas composition in the headspace was measured with a dual column Fisher Gas Partitioner model 25V gas analyser. Separation of O_2 and N_2 was made on di-2-ethylhexylsebacate on 60–80 mesh 'Columpak' and molecular sieve (type 5A, 30–60 mesh), whereas SO_2 and CO_2 were separated on butylphthalate on 40–60 mesh 'Columpak T' and hexamethyl phosphoramide on 'Columpak'.

Gas concentration in a mixture was calculated by comparison of peak heights of each component with that of standards of known gas composition.

Storage studies

Cellulose samples were sulphited to various SO_2 levels, equilibrated over a saturated solution of magnesium chloride to a_w 0.31, hermetically sealed in an oxygen atmosphere into lacquered tinplate cans and stored at 37°C for analysis. The samples required for evaluation of the effect of a_w on the stability of β-carotene were equilibrated to a_w 0.11, 0.31 and 0.51 prior to storage. The samples equilibrated at a_w 0.31 were also stored at 44 and 50°C to examine the effect of storage temperature.

The effect of atmospheres of O_2, N_2 and SO_2 on the stability of β-carotene in non-sulphited carotene-cellulose systems was determined on samples of a_w 0.31 hermetically sealed into lacquered tinplate cans containing 25, 75 or 100% N_2 or SO_2 in O_2 and allowed to oxidize at 37°C.

To evaluate the effect of factors on the stability of β-carotene, first order rate constants, activation energies, Q_{10} values and storage life (50% carotene loss) were calculated using the method reported by Labuza (1972).

Results and discussion

Effect of sulphur dioxide concentration

Sulphur dioxide not only increased the induction period for β-carotene oxidation, but also reduced considerably the rate of carotene destruction (Tables 1 and 2). No carotene remained in non-sulphited samples stored for 110 hr at 37°C in air, however, 40.2% of the original carotene content was still present in a sulphited system of 8666 ppm SO_2 stored for up to 480 hr under

Table 1. Stability of β-carotene in model system of a_w 0.31 at various concentrations of SO_2 during storage at 37°C

Storage time (hr)	β-Carotene remaining (%) Initial SO_2 content (ppm)			
	0	890	2050	8666
20	98.1	99.9	99.8	99.9
30	83.5	92.0	99.6	99.8
40	71.0	83.6	97.4	99.9
50	58.3	75.0	96.0	99.0
60	47.9	71.0	94.1	98.6
70	38.2	69.0	92.2	98.0
90	22.0	52.3	86.1	97.0
110	0.0	41.0	85.4	94.8
140	—	30.7	80.9	92.8
480	—	—	—	40.2

Table 2. Effect of SO_2 on rate of destruction of β-carotene in a model system of a_w 0.31 during storage at 37°C

SO_2 content (ppm)	First order rate constant ($hr^{-1} \times 10^3$)	Storage life for 50% carotene loss (hr)
0	17.0	60
890	10.1	91
2050	1.7	435
8666	1.2	617

similar conditions (Table 1). The rate of carotene destruction decreased rapidly with increasing concentration of SO_2 up to about 2000 ppm, but further levels of SO_2 showed little increased protection (Table 2). The storage life of samples containing 8666 ppm SO_2 increased by more than ten times compared with that of non-sulphited samples.

Effect of a_w

The sorption isotherms for non-sulphited and sulphited carotene cellulose model systems are shown in Fig. 1. The a_w values for monolayer coverage for non-sulphited and sulphited systems were found to be 0.30 and 0.31 corresponding to a moisture content of 2.6% and 2.8% respectively. The first order rate constant for carotene oxidation was a minimum in samples that were stored at the monolayer a_w of 0.31 (Table 3). The increase in rate constant was

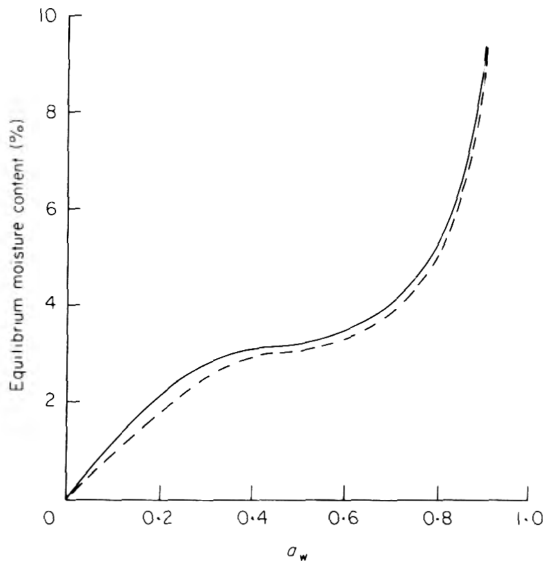

Figure 1. Moisture sorption isotherm of sulphited (———) and non-sulphited (– – – – –) carotene-cellulose model system at 37°C.

greater at lower a_w than at higher a_w, the effect being more pronounced in sulphited samples than in non-sulphited samples. The results agree with the finding of Chou & Breene (1972), who reported a higher rate of carotene oxidation at an a_w lower than that of the BET monolayer value. Various mechanisms have been suggested by which water reduces lipid oxidation. Water attaches to sites on the food surface, thus excluding oxygen from the lipid materials (Salwin, 1959), and hydrates and insolubilizes metal catalysts (Kamiya *et al.*, 1963). Water also bonds with peroxides to make them unavailable for decomposition (Maloney *et al.*, 1966). The increase in oxidation rate with increasing a_w could be due to solubilization and mobilization of catalysts present and the exposing of new sites on the solid matrix as a result of swelling of the matrix. Thus it is suggested that dehydrated foods should be

Table 3. Effect of a_w on the rate of destruction of β-carotene in non-sulphited and sulphited carotene-cellulose model systems during storage at 37°C

a_w	First order rate constant ($hr^{-1} \times 10^3$)	
	Non-sulphited	Sulphited*
0.11	28.7	7.0
0.31†	17.0	1.7
0.51	18.7	2.3

* 2050 ppm SO_2; † from Table 2.

stored at a monolayer a_w to increase carotenoid stability and thus to extend storage life.

Effect of storage temperature

Destruction of β-carotene in non-sulphited and sulphited samples increased with an increased storage temperature from 37 to 50°C (Table 4), the oxidation being more rapid in non-sulphited than in sulphited samples. Activation energies and Q_{10} values for β-carotene oxidation in non-sulphited and sulphited samples (Table 4) are similar to those found in other model system studies (El-Tinay & Chichester, 1970; Chou & Breene, 1972) and in dehydrated carrot (Baloch, 1976), but they are lower than those for autoxidation of linoleic acid (15.2 kcal/mole) and its ethyl ester (17.2 kcal/mole) reported by Bolland & Gee (1946). The higher activation energies for oxidation of these lipids, which are normally present in foods, suggest preferential oxidation of carotene in oxidizing lipid materials, in agreement with the findings of Holman (1949) and Lime (1969).

Table 4. Effect of storage temperature on rate, activation energy and Q_{10} value for destruction of β-carotene in non-sulphited and sulphited model systems at 0.31 a_w

Sample	First order rate constant ($hr^{-1} \times 10^3$) Storage temperature (°C)			E	Q_{10}
	37*	44	50		
Non-sulphited	17.0	23.9	36.6	10.3	1.7
Sulphited†	1.7	2.6	4.1	12.8	1.9

* From Table 2; † 2050 ppm SO_2.

Effect of storage atmosphere

The stability of carotene increased markedly with an increase in concentration of N_2 or SO_2 in the atmosphere (Table 5). The effect of SO_2 was highly significant. An induction period of about 19 hr was observed for samples stored in O_2 only, whereas an induction period of 252 hr was obtained for samples stored in SO_2.

In spite of the presence of O_2, N_2 and CO_2 in all of the cans indicating some contamination with air when the containers were sealed, the headspace volume and pressure inside the cans after storage were similar for all samples (Table 6). Since similar and large amounts of O_2 were present at the end of storage in all non-sulphited and sulphited samples, it is unlikely that the antioxidant effect of SO_2 is due merely to removal of O_2 by oxidation with SO_2 present in the

Table 5. Effect of storage atmosphere on destruction of β-carotene in non-sulphited and sulphited model systems of a_w 0.31 at 37°C

Storage atmosphere in O_2 (%)		Induction period (hr)	First order rate constant (hr^{-1})
N_2	0*	19.2	17.0×10^{-3}
	25	28.8	7.9×10^{-3}
	75	48.0	2.9×10^{-3}
	100	120.0	0.9×10^{-3}
SO_2	25	177.6	2.6×10^{-4}
	75	228.0	1.4×10^{-4}
	100	252.0	1.2×10^{-4}

* From Table 2.

system as was expected. SO_2 is known to be a reactive chemical (Schroeter, 1966; Roberts & McWeeny, 1972), and it may react with carotene through free radical mechanisms (Kharasch, May & Mayo, 1938), it can possibly react with peroxide radicals produced during processing and storage (Anbar, Hefter & Kremer, 1962), or it may react with heavy metals. Most likely SO_2 protects carotenoids in food through all these mechanisms. However, a large amount of potentially active SO_2 is destroyed as a result of reaction with sugars, carbonyl compounds, non-enzymic browning components and several food constituents, and with atmospheric oxygen in the pack, the remaining concentration of free SO_2 effective for carotenoid stability becomes low, thus showing confusing results.

It appears from the present studies that the carotene can be oxidized by molecular oxygen even in the absence of other lipid materials. Moreover, SO_2 present in the system as a compound or as a gas inhibits very effectively carotene breakdown caused by molecular O_2. However, the precise mechanism by which carotene is stabilized by SO_2, besides the well known O_2 scavenging action, remains to be established.

The mechanism of carotenoid destruction appears to be complicated. However, the presence of an induction period found for the rate of carotene destruction in both non-sulphited and sulphited carotene-cellulose model systems and an increased induction period with increasing SO_2 content suggests the reaction to be autoxidative. This is further substantiated by the facts that the reaction followed a first rate during the early period of change, while it deviated towards an increased rate of carotene breakdown during the later period of the reaction, and moreover, was controlled by SO_2 treatment. From these observations and from the complexity of products from carotene destruction reported (Land, 1962, 1969; Fishwick, 1969; El-Tinay & Chichester, 1970; Walter, Purcell & Cobb, 1970; Chichester & McFeeters, 1971), it can be concluded that the reaction proceeds through a free radical mechanism.

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J. Fd Technol. (1977) **12**, 317–318

Book review

Gas Chromatography in Food Analysis. By G. J. Dickes and P. V. Nicholas. London: Butterworths, 1976. Pp. vii+393. £16.

The authors have reviewed the vast literature concerned with the application of gas chromatography to food analysis and have produced a most valuable reference handbook containing recommended methods as well as hints on the practical aspects of the technique and the interpretation of analytical data. Only a small working knowledge of gas chromatography has been assumed. The first seven chapters provide a concise description of GC apparatus including the basic instrument, columns and detectors, together with reaction chromatography, derivative formation and associated techniques for confirmation of identity. The emphasis at all times is on practical considerations; only brief mention being made of the theoretical aspects of column efficiency, overlapping peaks, Kovat's indices and quantitative measurements. Appendices of equivalent stationary phases and support materials are provided, along with a selection of liquid phases to cover the majority of applications in a food laboratory.

Part 2 consists of GC methods for the quality control of foods including the determination of additives and contaminants. The methods selected are those which, from the authors' personal experience, have been found to be the most reliable with respect to simplicity, sensitivity and recovery tests. They are intended for the worker who has an immediate need for a reliable method without the resources to search the literature and evaluate methods for himself.

In the remaining thirteen chapters, various commodities such as dairy products, fats and oils, protein foods, essential oils, fruits, vegetables, beverages, sugars and cereals as well as additives and contaminants are considered in greater detail. A number of alternative GC methods are discussed in each section and the principal GC conditions are tabulated for easy reference. The section is amply illustrated with appropriate chromatograms.

This book will make a valuable addition to the library of the food chemist and is likely to be used on a day to day basis. Since the majority of the references are prior to 1973/4, it provides the reader with a comprehensive compendium of all but the most recent work. No doubt it will be easier to update subsequent editions now that the basic format has been established. The book is well produced and substantially free from even typographical errors.

N. J. Crosby

Books Received

Microbiology in Agriculture, Fisheries and Food. Ed. By F. A. Skinner and J. G. Carr.
London: Academic Press, 1976. Pp. xv+274. £9.00.

Conference papers read at a symposium of the Society of Applied Bacteriology at Aberdeen in 1974.

Microbiological Methods. 4th edition. By C. H. Collins and P. M. Lyne.
London: Butterworth, 1976. Pp. v+521. £9.50.

A revised and updated edition of a well established handbook of laboratory methods.

Living Nutrition. 2nd Edition. By F. J. Store and M. McWilliams.
New York: John Wiley & Sons, 1977. Pp. x+497. £8.70.

An introductory textbook for students emphasizing the sociological aspect of the problems of nutrition.

Toxicological Evaluation of Some Food Colours, Thickening Agents and Certain Other Substances.
Geneva: World Health Organisation, 1975. Pp. 89. £3.00.

Monographs on the toxicological evaluation of amaranth, ferrous gluconate, quinoline yellow, carob bean gum, gum guar, gum tara, microcrystalline cellulose, amidated pectin, dichlorodifluoromethane, tertiary butyl hydroquinone and triacetin.

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INHIBITION AND INACTIVATION OF VEGETATIVE MICROBES

edited by F. A. Skinner and W. B. Hugo

January 1977, xiv+378 pp., £12.00/$26.25 0.12.648065.6

It is surprising that after a century of non-empirical disinfection practice, many problems concerning the inhibition of microbes remain to afflict industry. In the food industry, where the work of destroying microbes is hampered by the need to protect the product, these problems prove especially costly and difficult to solve. Contributions cover methods of inhibition and inactivation including heat, cold, chemical treatment, irradiation, application of high pressures and the modification of water activity as encountered in preservation procedures involving brines and syrups. Specialist areas like the food industries, where microbial growth may be inimical, are given particular attention: separate chapters are devoted to the dairy and beverage industries.
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Food Science and Technology: a series of monographs

The Science and Technology of Gelatin

edited by A. G. Ward and A. Courts

May/June 1977, xvi+564 pp., £18.00/$39.50 0.12.735050.0

The importance of gelatin and its raw material, collagen, to the food industries and the culinary arts, to glue-making, to the photographic industry and to macromolecular and colloid chemistry make the study of this protein based product of great interest to a wide range of specialists. Extensive research concentrated over the last 30 years has resulted in exciting discoveries about the chemical and physical structures and properties of gelatin. These studies have made it possible to produce better quality substances and consumer products, pointing to uses which previously had not been considered. The book commences with a detailed review of this research provided by contributors with first-hand knowledge, including the two editors, former research directors of the British Gelatine and Glue Research Association. The latter part of the book is concerned with specific applications of gelatin ranging from its use as a whipping agent to its employment in printing techniques.

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

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

SI UNITS

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

NON SI UNITS

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

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