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An indirect moisture sensor for some tropical farm produce such as yam

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Summary

Moisture content of agricultural produce is one of the more reliable single indices for indicating storage potential.

This paper reports studies into an indirect method of sensing the moisture content of yam tubers.

This method, which measured a passive property (resistance) of the produce as an index of moisture content, appears to be reliable. The results of a 2^4 factorial experiment to screen for the essential factors are presented. Some results of further experiments to determine the relationship between the important factors, the resistance, and moisture content of the farm produce are also presented.

Introduction

Yam, of the genus *Dioscorea,* is among the more valuable staple food sources for millions of people in tropical and sub-tropical countries. In Nigeria especially, it is one of the most valued sources of food. Unfortunately, however, despite the relatively high production level of this crop, it is in short supply in the market due primarily to the colossal amount of wastage of both the processed and unprocessed produce. Wastage from unprocessed yam tubers results from chemical reactions in the tubers, excessive sprouting, shrinkage and spoilage due to bacterial and fungal attack.

Although considerable efforts by many researchers have been directed toward increasing the storage life of yam tubers, a very practicable technique has not

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emerged. Also, because of bulkiness and the distance between the geographical locations of production and the consuming areas of yam tubers, they are processed into flour forms. The processing of yam tubers into flour involves peeling of the tuber, cutting into pieces (although some are not cut into pieces), parboiling, drying in the sun and grinding. Wastage usually results from development of mould inside and sometimes on the surfaces of the supposedly dried pieces due to moisture migration. During sun drying of the produce, the surface of the whole or cut pieces usually appears very dry whereas the inside might be well above the safe storage moisture level. When the moisture level from such an under-dried product is not high enough to encourage the development of moulds on the surface of the pieces, the unsuspecting customer bears the loss resulting from the development of moulds inside the product.

Losses from these products could be reduced if there were means of indicating moisture levels inside the drying, whole or cut pieces. Also as Nigeria develops her agriculture and industries, there will be a need for effective grading of yam tubers to be processed in the industries. An effective grading system can be based on visual observations, weight and moisture content. Thus there is a need for a means of measuring the moisture content of such bulk pieces. For such a measurement, it appears that the indirect methods are more appropriate, and this report is therefore on an indirect moisture sensoring method. The method involves resistance measurement, and the results obtained appear to be reliable. The results of important parameters affecting the response of such a sensor are presented.

Previous work

Moisture content for both primary and secondary agricultural products is generally accepted as the most reliable single index of their storage potential. Methods that have been employed to determine moisture content can be classified broadly into two: (a) direct methods, and (b) indirect methods.

The direct methods, sometimes described as 'primary methods', involve the determination of moisture present in the product either by loss in weight (oven method) or as condensed moisture (Brown-Duvel moisture tester). These methods have been standardized for grains (ASAE. 1977) but not for tropical crops such as yam. A weakness of these methods is that sometimes loss of volatile components and water released through decomposition by heat are recorded as moisture loss (Fetzer, 1954) but these errors are relatively insignificant in high moisture products such as root crops and fruits.

The indirect methods, sometimes called secondary methods, generally employ a passive property of the material as an index of the moisture content. Passive electrical properties which have been used include resistance and/or dielectric properties. Several meters have been developed for grains and forage using these properties, and such meters are readily available. These meters are usually designed to sense moisture levels not greater than 30% (w.b.) but the moisture

content of yam is far higher than this value. Examples of such meters are Protimeter, Delmhorst, Gallenkamp, and Kappa James, the latter being based on dielectric measurement. The power source is mains or battery. The Kappa James meter is claimed to measure from as low as 0.2% to as high as 60% with an accuracy of from 0.5 to 2% above 40% moisture content. The moisture content of yams, however, can be up to 80% or higher, and thus, the currently available moisture meters appear not to be suitable for the determination of their moisture content. Pos & Ezeike (1976) developed a probe to sense the resistance of root crops. Their probe was essentially two platinum wires which were set at 1.27 cm apart. Their results showed a linear relationship between moisture content and resistance of the samples. They did not , however, report on the basic parameters which influence the resistance of such products. Also they worked at a very small range of moisture content (87.4–88.7% for carrot and 75.9–78.2% for potato). Such sensors developed for other agricultural produce usually specified the volume (shape and size) of specimens but such specification was not mentioned in the studies reported by Pos & Ezeike.

Cox & Filby (1972) reported that for most agricultural materials resistance values may range from few ohms to about 100 megohms. But preliminary investigation showed that a meter that could read up to about 20 megohms was adequate for our studies and hence a Simpson Multimeter was used to read resistance levels.

Theoretical consideration

Generally a yam tuber can be considered to be non-homogeneous in structure and water content. Laboratory experiments by the authors showed moisture content variation along the longitudinal axis of the tuber – 69.8, 66.0 and 62.3% (w.b.) at the distal, middle and proximal sections of the tuber respectively. Also moisture content variation along the transverse section of the yam can be expected, and because resistance is expected to vary with varying moisture content levels, the resistance would vary along the transverse section of the yam.

Variation of resistance with depth

Probes into a yam tuber at varying depths, will be exposed to varying resistance levels along the yam's length. These resistors of varying magnitude arranged in parallel are shown in Fig. 1.

Where R is the equivalent resistance of the system and R_1 is resistance at the outer layer

$$\frac{1}{R} = \frac{1}{R_1} + \frac{1}{R_2} + \frac{1}{R_3} \cdots \frac{1}{R_{n-1}} + \frac{1}{R_n} \cdots \tag{1}$$

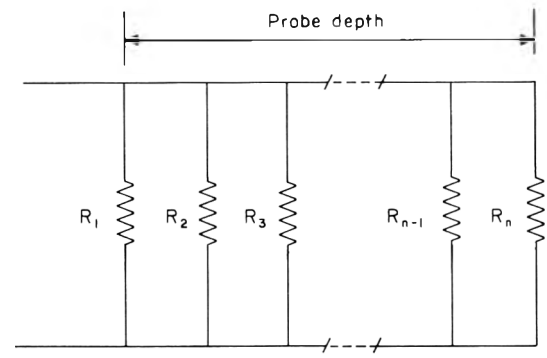

Figure 1(a). Schematic representation of resistances along the depth of the probe into the yam piece.

Figure 1(b). Schematic representation of the resistances between probe distances.

R_1 is usually highest as the outer layer is driest.

Hence eqn (1) becomes

$$\frac{1}{R} = \frac{1 + \frac{R_1}{R_2} + \frac{R_1}{R_3} + \ldots \frac{R_1}{R_{n-1}} + \frac{R_1}{R_{n-1}} \ldots}{R_1} \tag{2}$$

For drying yam pieces, it is possible to identify three drying layer zones:

(i) The dry layer zone which is in equilibrium with the hot air.

(ii) The drying layer zone which is between the dry layer zone and drying front.

(iii) The wet layer zone which is the layer after the drying front where relatively no drying has taken place.

(i) For the dry layer zone, it can be assumed that there is no resistance variation with depth of probe i.e. $R_1 = R_2 = R_3 = \ldots R_n$

Hence eqn 2 becomes

$$\frac{1}{R} = \frac{n}{R_1} \ldots \tag{3}$$

$$R = \frac{R_1}{n} \ldots \tag{4}$$

Where $n > 1$ is a function of depth $R \propto \frac{1}{\text{depth}}$. This shows that the relationship between the equivalent resistance and depth of probe is inverse.

(ii) For the drying layer zone

$$R_{n-1} > R_{n-2} > R_{n-3} > \cdots R_{2n-1} > R_{2n} \cdots \tag{5}$$

But the resistances would be of the same order.

Equation (2) can be written as

$$\frac{1}{R} = \frac{1 + k_1 + k_2 + \cdots k_{n-1}}{R_{n-1}} \cdots \tag{6}$$

which can be written as

$$R = \frac{R_{n-1}}{kn} \cdots \tag{7}$$

Where $k > 1$

Equation (4) can be combined with (7) i.e.

$$\frac{1}{R} = \frac{n}{R_1} + \frac{k_n}{R_{n-1}} \cdots \tag{8}$$

which can be written as

$$R = \frac{R_1}{n(1+k)} = \frac{R_1}{An} \cdots \tag{9}$$

where $A = 1 + k$.

Equation (9) is that of an inverse function with definite change in slope compared to eqn (4).

(iii) At the wet layer zone, the resistance

R_{2n-1} is very small compared with R_1

$$\frac{1}{R} = \frac{An}{R_1} + \frac{1}{R_{2n-1}} + \frac{1}{R_{2n+2}} \cdots + \frac{1}{R_{3n}} \cdots \tag{10}$$

$$= \frac{An}{R_1} + \frac{n}{R_{2n+1}} \cdots \tag{11}$$

$$An + = \frac{R_1}{\frac{n}{R_{2n+1}}} \cdots \tag{12}$$

$$\frac{}{R_1}$$

$$R = \frac{R_1}{An + \dfrac{nR_1}{R_{2n+1}}} \quad \cdots \tag{13}$$

Where R_1 is very large compared with

R_{2n+1}, eqn (13) approximates to

$$R = \frac{R_{2n+1}}{n} \tag{14}$$

This shows that the resistance will suddenly become low when the probe gets to the wet zone.

Variation of resistance with distance between probe

For a given depth, the overall resistance sensed by the probe can be taken as the summation of resistances in series i.e.

$$R = nR_1 \quad \cdots \tag{15}$$

where n is positive.
Equation (15) is that of a straight line.

Experimental procedure

Direct moisture content determination

All indirect methods of moisture determination are calibrated by a direct method such as the oven method. Unfortunately there is no standard yet for determining moisture content of yam. Hence experiments were designed to determine the drying profile of yam at 60, 80, 100, 110 and 120°C. Each drying experiment was replicated three times. About 100 g of samples were dried in each drying moisture can, and the weight of each can in the oven was monitored until consistent constant weights were observed or when differences in weights were negligible.

General observations showed that experimental yam samples dried at temperatures of 110 and 120°C were caked brown before constant weights were reached. Samples dried at temperatures of 60 to 100°C did not become caked nor turn brown. Samples dried at 110°C and above reached constant weights before 16 hr of drying, while samples dried at 60 to 90°C took up to 30 hr or longer to reach constant weights. Samples dried at 100°C reached constant

weight at about 24 hr and did not turn brown nor become caked. Hence an oven temperature of 100°C was selected for determining the moisture content of yam by direct method.

Indirect moisture content determination using resistance

A Simpson Multimeter Model 250 capable of reading up to 20 megohms was used for the measurements. Steel cylindrical probes of about 1.5 mm diameter were used to probe into the tubers. Four factors were first selected for study based on preliminary observations and knowledge of the parameters affecting the resistance of a piece of metal. The factors are: (i) thickness of the material; (ii) depth of probes in the material; (iii) mass of the materials; and (iv) distance between the probes.

A 2^4 factorial experiment was designed to determine the relative effects of the above factors on the resistance of a given piece of yam. Three sets of the 2^4 factorial experiment were carried out using different masses for each set of experiments because of the problem of maintaining a consistent mass while keeping the other three factors constant. The yam pieces had been partially dried naturally and the average moisture content was about 61% w.b. Table 1 gives the 2^4 factorial experiment and the results.

The results of these experiments were analysed using Yates (Davies, 1971) analysis; three of the factors, depth of probe, distance between terminals and mass of the yam piece, were found to be significant at 95% confidence level.

Table 1. 2^4 Factorial experiment and results

Experiment no.	Depth of probe (cm)	Distance between probes (cm)	Mass (g)			Thickness of yam piece (cm)	Resistance (Kilohms)		
			Set 1	Set 2	Set 3		Set 1	Set 2	Set 3
1	1.	2	57.85	50.81	57.98	2.25	5.6	6.0	5.6
2.	2	2	57.85	50.81	57.98	2.25	3.5	3.7	3.8
3.	1	4	57.85	50.81	57.98	2.25	12.0	8.0	7.5
4.	2	4	57.85	50.81	57.98	2.25	5.0	5.0	5.7
5.	1	2	31.95	28.29	28.52	2.25	6.3	6.5	8.0
6.	2	2	31.95	28.29	28.52	2.25	4.0	3.8	5.8
7.	1	4	31.95	28.29	28.52	2.25	13.0	13.0	12.0
8.	2	4	31.95	28.29	28.52	2.25	6.5	6.5	9.0
9.	1	2	57.15	51.10	56.74	3.17	6.0	5.8	5.5
10.	2	2	57.15	51.10	56.74	3.17	3.8	3.5	3.4
11.	1	4	57.15	51.10	56.74	3.17	7.5	8.0	9.5
12.	2	4	57.15	51.10	56.74	3.17	5.5	5.5	5.7
13.	1	2	31.32	28.77	28.74	3.17	7.2	7.5	6.5
14.	2	2	31.32	28.77	28.74	3.17	4.5	5.0	4.0
15.	1	4	31.32	28.77	28.74	3.17	10.5	10.5	9.5
16.	2	4	31.32	28.77	28.74	3.17	7.0	8.0	6.8

More experiments were then carried out varying these three factors. The moisture content of all the pieces of yam used for the experiments was determined by the oven method, as described above, setting the oven temperature at 100°C and drying for 24 hr. The effects of temperature on resistance of the yam were not investigated.

Results and discussions

Table 2 gives the results of the analysis of the experimental results shown in Table 1. The −3.537 shown in first line of second column in Table 2 indicates that the resistance of the yam piece decreased by 3537 ohms when the probe depth was increased from 1 to 2 cm for the conditions of the experiments in set 1. The results show that the probe depth, distance between probes and mass of yam appeared consistently significant at 95% confidence level, while the thickness of the yam pieces appeared not to be an important factor. The interactions between the factors appeared not to be significant except the interaction between the depth of probe and the distance between probes for the first set of experiments; it appeared unimportant in the other sets of experiments.

Figures 2 to 4 show representative curves plotted from several data obtained from the experiments. The correlation coefficients between mass of yam tubers and resistance in ohms range between − 0.969 and 0.978. This shows that as the

Table 2. Effects of factors on the resistance of yam in ohms

		Results divided by 10^3		
Factor		Set 1	Set 2	Set 3
Probe depth (1)		−3.537†	−3.037†	−2.510†
Distance between probes (2)		2.262†	2.830†	2.910†
Mass of yam (3)		1.262†	1.912†	1.710†
Thickness (4)		−0.487	0.125	−0.660
Interactions*	1,2	−0.212†	−0.587	−0.310
,,	1,3	−0.212	−0.512	−0.140
,,	1,4	0.937	0.587	−0.260
,,	2,3	−0.487	0.962	0.390
,,	3,4	−0.012	−0.287	0.130
,,	3,4	0.337	0.137	−0.140
,,	1,2,3	−0.037	−0.362	0.130
,,	1,2,4	1.062	0.537	0.160
,,	1,3,4	−0.287	0.462	0.310
,,	2,3,4	0.162	−0.512	−0.310
,,	1,2,3,4	−0.212	0.412	0.260

*Signifies interactions between probe depth (1) and distance between probes (2).

†Significant at 95% confidence level.

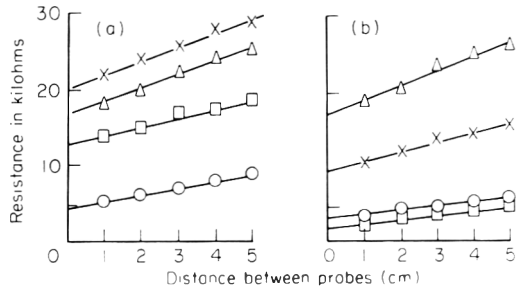

Figure 2. Relationship between resistance of yam pieces and distance between probes at different moisture contents. (a) At 1.5 cm probe depth, moisture contents (w.b.): ○, 61.9%; □, 50.3%; △, 41.8%; ×, 16.1%. (b) ○, probe depth 1.5 cm, m.c. 63.4%; □, probe depth 2.4 cm, m.c. 63.4%; △, probe depth 1.5 cm, m.c. 36.9%; ×, probe depth 2.4 cm, m.c. 36.9%.

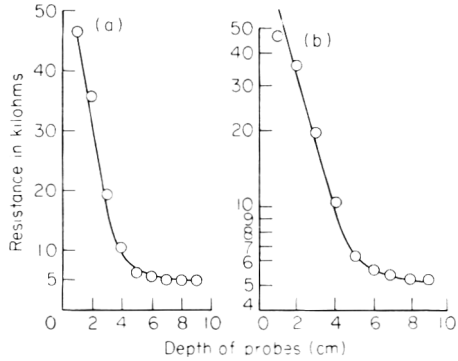

Figure 3. Relationship between resistance of yam pieces and depth of probe.

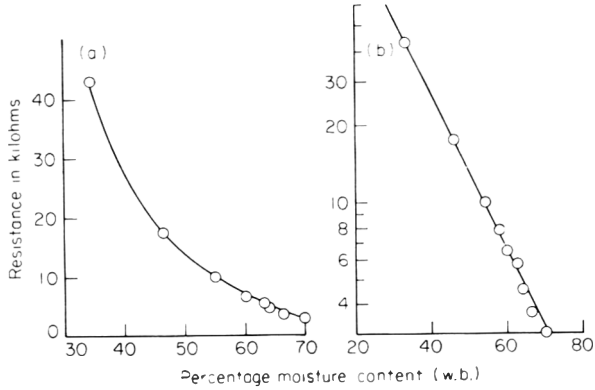

Figure 4. Relationship between moisture content and resistance of yam pieces, at 1.5 cm probe depth, 1 cm probe distance.

mass of a yam piece increases the resistance decreases. This result can also be derived from the results of the 2^4 factorial experiment. The value of the effect of decreasing the mass of yam was positive. The correlation coefficient between the distance between probes and resistance ranged between 0.977 to 0.991. This shows high correlation and also that the relationship is linear as derived from eqn (15). This is shown in Fig. 2 for yam pieces of different moisture contents and also at different probe depths.

The correlation coefficient between probe depth and resistance was -0.992. This shows that resistance decreases with increased depth of penetration as shown by the 2^4 factorial experiment. Curves to show this relationship are plotted both on a linear and on a semi-log scale (Fig. 3). From both curves it appears that the relationship between resistance and depth of probe is not linear and the three zones of drying, considered earlier in this paper, can be identified. The dry zone extends to a depth of about 4 cm in this experiment. The drying zone appears between 4 and 6 cm and the wet zone appears to be after 6 cm depth. Figure 4 shows the curves of the relationship between moisture content and resistance of yam pieces on a linear and semi-log scale. The relationship is a curve on the linear scale and appears linear on the semi-log scale; this indicates an exponential relationship.

The practical implication of these results is that it will be essential to select: (i) a given size of yam piece; (ii) a given distance between probes which may not be more than 5 cm to save on material (yam); (iii) the probes' length should be such that it is capable of penetrating to at least half the depth of the yam pieces so as to be able to detect whether (1) the yam pieces is uniformly dried, (2) there is still a drying zone in the yam piece, (3) there are drying and wet zones in the piece.

Conclusions

An oven drying method suitable for determination of the moisture content of yam pieces has been developed by experiment. Using this method for reference, the relationship between moisture content and a passive property (resistance) of yam tubers has been studied. From the results, the following conclusions can be reached:

(1) The temperature of the oven to be used for direct moisture content determination to calibrate the indirect method should be set at about 100°C and the samples should be dried for about 24 hr.

(2) Indirect moisture content determination using the passive property (resistance) of yam appears reliable. Results of 2^4 factorial experiments show that three factors affect the response of the probe. These are the distance between the probe terminals, the depth of the probe in the produce and the mass of the produce.

(3) The resistance sensed by the probe appears to vary linearly with the distance between the probes terminals as derived in eqn (15). This result

appears consistent with the relationship of resistance of metals and their lengths. However, experience has shown that the overall mass of a material affects the resistance indicated by a probe placed at very small sections of the large mass. This might affect the relationship between the resistance and the distance between the probe. In this study, the high conductivity ($\frac{1}{R}$) of the wet zone in the yam pieces might have limited the effect of the large mass.

(4) The resistance of the yam appears to have an inverse relationship with the depth of probe. As the probes penetrate into the yam piece it is possible to detect whether the whole yam piece is dry, is drying or if there is a wet zone within the yam piece. The probe length must be able to reach to half the depth of the yam piece.

(5) The moisture content of the yam tubers appears to have an exponential relationship with the resistance indicated by the probe at a given probe distance, depth and mass of yam. This exponential relationship can be used to calibrate the dial of the meter to read moisture content directly.

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Chitosan globules

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Summary

We prepared chitosan globules to use as a structural unit or as a means of controlled ingredient release in food products. Globules were fabricated by coagulating drops of chitosan solutions (1–2% w/w chitosan in 3.8–5.6% w/w citric acid solution) in 1 N NaOH. The chitosan solutions, dropped from a 2.9 mm diameter tube, resulted in ellipsoidal globules with diameters of 3.7–5.1 mm and an opaque, whitish appearance similar to that of cooked white rice. The globules contained more than 98% water, about half of which could be squeezed out under compression.

The force-deformation test indicated an increase in the strength, elastic moduli, and relaxation time of fabricated globules when the chitosan concentration was higher in the casting solution. When the citric acid concentration was higher, elastic moduli generally increased, but the longest relaxation time decreased. The globules sustained up to approximately 0.8 linear strain without fracture. Stress relaxation tests showed that the viscoelastic properties of globules may be represented by a one-element Maxwell model with a relaxation time on the order of 1000 sec and an elastic modulus of 0.05–0.27 g/mm^2.

Introduction

In many foods, the characteristic textural properties result from a combination of two or more types of structural units. The structural units are liquid plasma surrounded by cell membrane, and interstitial material in plant and animal tissues. The chewy but tender and juicy characteristic of meat is due to the

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protein filament (muscle cell) enclosed in the protein matrix (connective tissue). The water holding capacity of muscle cell and the rigid and elastic property of connective tissue characterize the texture of meat. In fruits and vegetables, the crispy and juicy texture is due to compartmentalization of watery cellular material with a rigid cell wall.

Our objective was to fabricate a structural unit that could compartmentalize and simulate cellular structure which at the same time would have a broad application in food. The structural unit of particular interest in this study was granules (or globules) which could be used individually or aggregated to form a cellular structural matrix. A structural matrix with varying degrees of rigidity, which contains water that could be freely released upon compression, would have wide use in production and development of fabricated food. Currently, for example, the best fruit structure simulation, prepared with alginate-calcium gel, lacks the crispiness and juiciness of natural fruit. Although alginate is one of the most rigid gel forming food ingredients known, the gel obviously does not provide for a free release of desirable quantities of water. In the structure fabrication of meat analogue, the most difficult problem is again to simulate the juiciness of natural meat. Present technology does not provide a product with the juiciness of meat; instead, most liquid is released quickly in the early stage of mastication leaving dry, unpalatable residue or a mushy texture usually not encountered in natural meat.

Chitosan [(1-4)-2-amino-2-deoxy-β-D-glucan], deacetylated chitins in various stages of deacetylation and depolymerization, was used to make the globules for this study. Chitosan is a novel biopolymer with great potential for industrial use due to its amine content or polycationic nature, and because chitin, from which chitosan is derived, is the second most abundant organic compound on earth. Since chitin can also be extracted relatively easily from crab, shrimp and lobster shells, the seafood processing wastes currently thought of as a nuisance could be considered for specific functional use in food products.

Chitosan has been used to form water insoluble precipitates such as polyelectrolyte complex with anionic polysaccharide (Fakuda & Kikuchi, 1978; Bomstein, 1974; Yamaguchi *et al.*, 1978a); for making gels (Yamaguchi *et al.*, 1978b; Hirano & Ohe, 1975; Hirano & Yamaguchi, 1976; Hirano *et al.*, 1977), as well as films (Averbach, 1978). Recently, the nature and mechanism of the network formed by chitosan have been studied (Kienzle-Sterzer, 1980; Kienzle-Sterzer, Rodriguez & Rha, 1980) and shown to have a negative deviation from neo-Hookean behaviour. The studies also indicated that cross-linking varied with the degree of dilution and spatial configurations of chitosan chains during the film forming process involving removal of solvent from dilute acetic or propionic acid.

Our study demonstrates an example of the approach in structural synthesis, generally applicable in a wide variety of foods, utilizing the unusual nature of a novel biopolymer. We fabricated a suitable structural unit, globules, with mechanical characteristics in the organoleptically acceptable ranges. The processing conditions which affected the mechanical characteristics were then

studied. The globules were prepared using a process similar to that developed earlier (Tran & Rha, 1979; Tran, 1980). Processing conditions were selected based on chitosan's chemical and physical characteristics (Muzzarelli, 1973; Filar & Wirick, 1978; Muzzarelli, 1977; Averbach, 1978; Kienzle-Sterzer, 1980).

This paper reports mechanical characterization of a formed chitosan structure, chitosan globules, as a first step in synthesizing the structure units that can be further fabricated systematically into various food products.

We conducted compression tests on the chitosan globules to determine the strength of the resultant structures fabricated under different conditions; specifically, the concentrations of chitosan and citric acid and the ratios of chitosan to citric acid.

Materials and methods

Preparation of chitosan solution

Chitosan (FLONAC-N, Velsicol, Hampton, VA) was dispersed in citric acid (0.19–0.28 M) to make 1.0, 1.5, and 2.0% w/w chitosan solutions. The solutions were stirred for 1 hr, allowed to stand at room temperature for approximately 24 hr, and filtered through a sintered glass filter to remove insoluble materials. The casting solution thus obtained was clear and homogeneous.

Globule fabrication

The chitosan solution was allowed to flow through a 2.9 mm diameter capillary tube by applying 15 psi. The drops at the end of the tube were allowed to fall into a coagulation bath of 1 N NaOH. Chitosan drops remained in the coagulating bath for 10–15 min to complete the coagulation in order to develop a structure capable of maintaining the integrity of the globules. The residence time in the coagulation bath was selected based on the coagulation rate determined in a preliminary study. The coagulation rate was 0.19–0.15 mm/min for 1.0–2.0 w/w% chitosan in citric acid when the precipitation medium was 1 N NaOH. The coagulated globules were collected, washed several times with distilled and deionized water, and kept in water at room temperature for further experimentation.

Mechanical characterization

Force-deformation relationships at deformation rates of 1, 2, 5, 10 and 20 mm/min and stress relaxation at 20% deformation for chitosan globules were determined under uniaxial compression using an Instron Universal Testing Machine Model 1122 (Instron, Inc., Canton, MA) at room temperature.

Results and discussion

When coagulated, the chitosan droplets assumed an ellipsoidal shape rather than a perfect spheroid. These somewhat elongated globules had diameters of 3.7–5.1 mm (Table 1). These globules, although much larger, resembled cooked rice in colour, size and textural appearance, and sustained more than 80% deformation without rupturing. The chitosan globules contained more than 98%

Table 1. Dimensions of ellipsoidal chitosan globules*

Casting solution		Axis	
Chitosan (% w/w)	Citric acid (% w/w)	Long (mm)	Short (mm)
1.1	2.8	5.1 ± 0.1	4.2 ± 0.2
1.5	4.2	5.1 ± 0.1	4.3 ± 0.2
2.0	5.6	4.7 ± 0.1	4.4 ± 0.2
1.0	3.8	4.7 ± 0.2	3.7 ± 0.2
1.5	3.8	4.9 ± 0.1	4.0 ± 0.2
2.0	3.8	4.9 ± 0.1	4.4 ± 0.2

*Chitosan solution dropped through a 2.9 mm diameter capillary tube and coagulated in 1 N NaOH.

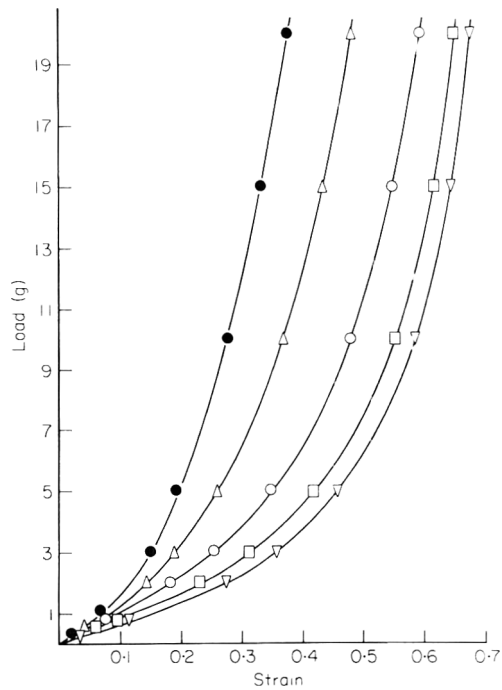

Figure 1. Load versus strain of chitosan globules at 2.0% w/w in 3.8% w/w citric acid. ●, △, ○, □, ▽ correspond to 20, 10, 5, 2, and 1 mm/min compression rate, respectively.

w/w of water; approximately 50% of the water could be squeezed out when the globules were compressed up to 80%.

Mechanical tests on the chitosan globules indicated that under compression, the force required to deform chitosan globules increased as compression ratio increased (Fig. 1). This increase, and the nonlinear relationships between load and strain was a clear indication of the viscoelastic nature of chitosan globules.

The stiffness of the globules could be controlled with the concentration of chitosan, as well as with the amount of acid used in the solution from which the globules were fabricated (Figs 2 and 3). Figure 2 shows that globules fabricated from solutions containing higher percentages of chitosan were harder and required higher loads for the same degree of compression. On the other hand, with the concentration of chitosan held constant, an increase in the citric acid content decreased the stiffness of the resultant structure. This result was probably due to the relatively higher concentration of acid, which would increase the net charge on the chitosan backbone and cause the chain to become more expanded, which in turn would produce a more amorphous structure. As expected, higher concentrations of chitosan gave harder globules when the ratio of chitosan to citric acid was constant (Fig. 3). A linear relationship existed between the required force for a given deformation and the chitosan concentration (Fig. 4).

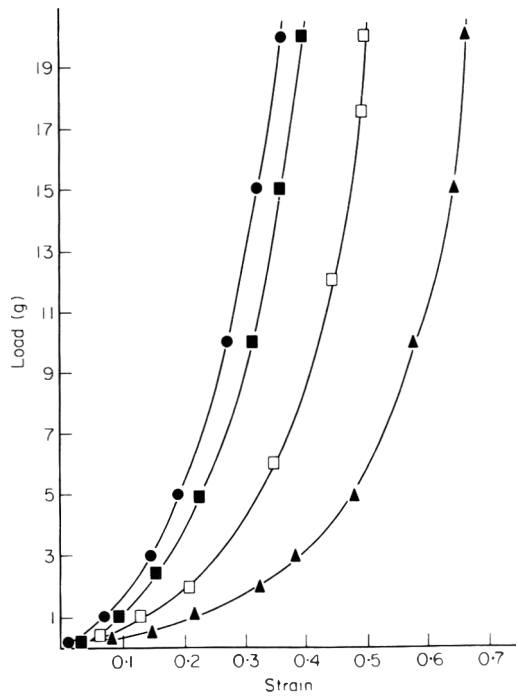

Figure 2. Load versus strain of chitosan globules at 20 mm/min compression rate. ●, 2% w/w chitosan in 3.8% w/w citric acid; ■, 1.5% w/w chitosan in 3.8% w/w citric acid; □, 1.5% w/w chitosan in 4.2% w/w citric acid; ▲, 1.0% w/w chitosan in 3.8% w/w citric acid.

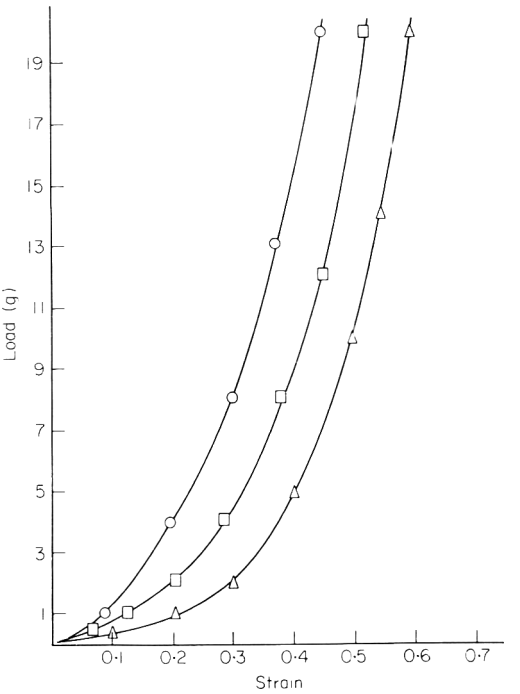

Figure 3. Load versus strain of chitosan globules at 20 mm/min compression rate. ○, □, △ correspond to 2.0, 1.5, and 1.0% w/w of chitosan in 5.6, 4.2, and 2.8% w/w citric acid, respectively.

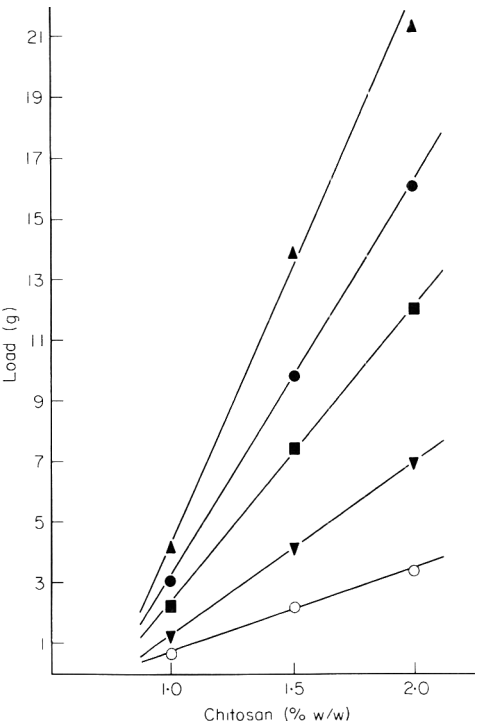

Figure 4. Load versus chitosan concentration of chitosan globules fabricated from 0.19 M citric acid. At a compression rate of 10 mm/min, ▲, ●, ▼, ○ correspond to 0.5, 0.45, 0.3, 0.2 linear strain, respectively.

Stress relaxation experiments were carried out at 0.20 linear strain at a 1000 mm/min compression rate. The globules were suddenly deformed to a given constant strain and the stress decay over time was determined to generate the data. The data was applied to a generalized Maxwell model to characterize the viscoelastic nature of the globules. A generalized Maxwell model is composed of a number of parallel elements with a spring to represent the elastic contribution and a dashpot to indicate the viscous part (Fig. 5). The rheological equation for

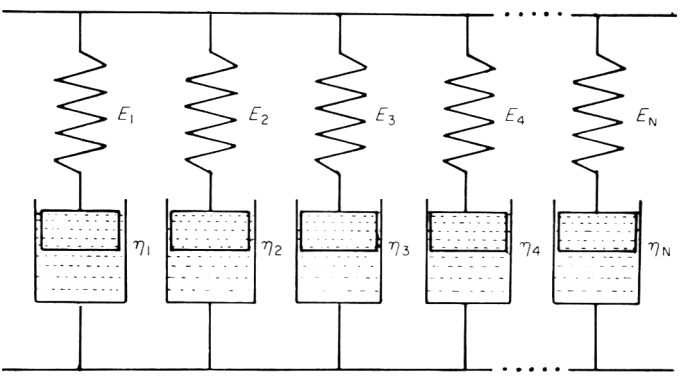

Figure 5. Generalized Maxwell model representing stress relaxation behaviour and containing a number of parallel Maxwell units each composed of elastic and viscous elements in series.

a simple Maxwell model can be derived from assuming Hooke's law for the spring and Newtonian law of viscosity for the dashpot:

$$\sigma(t) = \sigma_0 \exp(-Et/\eta)$$

where

$\sigma(t)$ = stress at any time,
σ_0 = decay stress,
E = Stiffness or modulus of the spring,
η = viscosity coefficient of the liquid in the dashpot, and
t = time;

and replacing the ratio η/E by T_{REL} (relaxation time),
$\sigma(t) = \sigma_0 \exp(-t/T_{REL})$.
The relaxation time is the time required for the stress to decay to $1/e$ times or 36.8% of its original value.

However, in practice, the single Maxwell unit often is not sufficient to describe stress relaxation response of a complex viscoelastic network, rather a combination of a number of Maxwell units, generalized Maxwell model (Fig. 5) is likely to represent the stress relaxation behaviour better. The generalized

Maxwell model can be described by:

$$\sigma(t) = \sum_{i=1}^{n} \sigma_i \exp(-t/T_{REL}) \text{ or}$$

$$E(t) = \sum_{i=1}^{n} E_i \exp(-t/T_{REL})$$

being $E = \sigma/\epsilon$ where:
E = elastic moduli, and
ϵ = strain.

We used the method of successive residuals (Mohsenin, 1970) to calculate the first three contributions to the elastic moduli and the relaxation times. The logarithm of the stress against time was plotted (Fig. 6). The straight portion of this original curve for larger values of time gives the exponential term for the longest relaxation time. The slope of this straight portion gives the time-constant T_{REL}. The intercept obtained by extending the straight line to the ordinate provides the coefficient of the first term, thus defining this segment of the curve (see Fig. 6). Next, the difference between this straight line and the original curve is plotted on the same semi-log paper and the first residual curve is obtained. Similarly, the second exponential (T_{REL}) and the coefficient of the second exponential term is found using the slope and intercept method. By subtracting the corresponding ordinates of the second exponential line from the first residual curve, the second residual curve is obtained. This procedure can be repeated several times until the true curve is represented by a sufficient number of exponential terms.

Table 2 shows that the relaxation times of chitosan globules were of the order of 10^3 sec in all cases. Stress relaxation on polymeric materials may be caused by

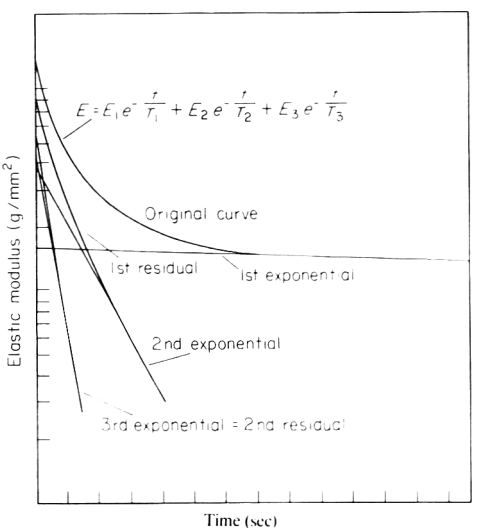

Figure 6. Analysis of stress relaxation by the method of successive residuals to determine relaxation times.

Table 2. Elastic moduli and relaxation times of chitosan globules

Chitosan (%w/w)	Citric acid (%w/w)	E_1 (g/mm^2)	T_1 (sec)	E_2 (g/mm^2)	T_2 (sec)	E_3 (g/mm^2)	T_3 (sec)
1.0	3.8	0.05	4350	0.13	90	0.03	50
1.5	3.8	0.08	5200	0.17	60	0.20	25
2.0	3.8	0.17	6950	0.42	35	0.15	16
1.0	2.8	0.05	4400	0.10	180	0.16	30
1.5	4.2	0.10	3500	0.20	250	0.23	100
2.0	5.6	0.27	2600	0.35	50	0.41	40

orientation or packing of free molecular chain segments, in which case the relaxation times would range from 10^{-9} to 10^{-3} sec. In addition, stress relaxation may involve physical rearrangement and slippage of segments included in the structure, corresponding to relaxation times of 10^{2}–10^{4} sec at room temperature (Bartenev & Kuznetzova, 1974; Bartenev & Zelenev, 1975). Therefore, the relaxation time of 10^{3} sec may indicate that rearrangement of structural elements—such as secondary types of bonds (electrostatic interactions, hydrogen bonds, etc.), entanglements, or free segments—may be responsible for relaxation of chitosan structure in the globules. This agrees with results of stress relaxation experiments reported for many hydrogels commonly used in the food industry (e.g., agar-agar, agarose, curdlan gels). These hydrogels have relaxation times of 10^{2}–10^{5} sec when secondary types of bonds are mainly responsible for the network structure (Kondo, Azechi & Kimura, 1979; Watase & Arakawa, 1968; Miller *et al.*, 1951; Arakawa, 1961). We expected these relaxation times because the chitosan was alkali-precipitated during the fabrication process and the precipitation probably resulted in non-specific inter- and intra-molecular interactions. The longest relaxation time, representative of the main relaxation process, increased with an increase in chitosan concentration and with a decrease in citric acid concentration.

We tried a three-element Maxwell model; however, for all practical purposes, a one-element model could represent relaxation behaviour of the globules since the second and third relaxation times were insignificant compared with the primary relaxation time (Table 2). This implies that the network structure in chitosan globules is based primarily on one mechanism.

Plots of the logarithms of Young's moduli versus time (Fig. 7) showed a relatively fast decrease of the moduli with time, suggesting the existence of free chain segments. The decay of Young's moduli were nearly parallel regardless of chitosan concentration, confirming that the mechanism responsible for the relaxation process and the type of bond remained the same.

In conclusion, we found that:

(1) The chitosan solution in citric acid formed a white, opaque precipitate. When coagulated dropwise in NaOH, the precipitate formed ellipsoidal globules that contained up to 98% w/w of water.

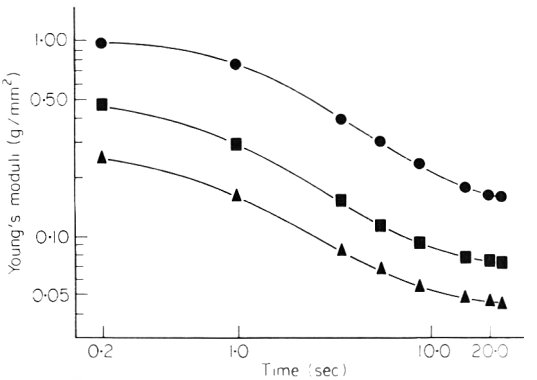

Figure 7. Young's moduli versus time of chitosan globules. ●, ■, ▲ correspond to 2.0, 1.5, and 1.0% w/w of chitosan in 0.19 M citric acid, respectively.

(2) The chitosan globules were viscoelastic; the force-deformation relationship was highly strain-rate dependent.

(3) Chitosan globules were more stiff when concentrations of chitosan were increased or acid concentrations were decreased.

(4) Chitosan globules responded to an applied stress in a similar manner, regardless of chitosan or citric acid concentrations, molar ratio of chitosan to citric acid, or strain rate.

(5) The elastic moduli and relaxation time values indicated that physical junctions were responsible for the integrity of the chitosan globules.

(6) The mechanical responses of the chitosan globules could be represented by a single-element Maxwell model.

Acknowledgments

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Application of multiple linear regression to analysis of data from factory energy surveys

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Summary

When energy surveys are carried out in factories, the usual method is to install expensive metering equipment and to measure energy flows to various parts of the plant. The costs involved in conducting surveys by this method are often prohibitively high. This paper describes an alternative survey method which is simple and inexpensive to use. An estimate of energy use for each product is obtained by the use of multiple linear regression to relate total energy consumption data from existing meters to production of the various products. The accuracy of the estimates depends on the form of the data set collected, but in most factories accurate values can be obtained for the major products. In all seventy-three factories where the technique was applied, it led to a useful analysis of total energy consumption. A summary of data collected in the New Zealand food manufacturing industry by application of the method is presented.

Introduction

Concern over energy prices, and the possibility of limited supply have led to an increasing interest in the way energy is used and to what extent conservation is possible. Energy use in the food chain is of great importance if the world food supply system is to be sustainable in the future. Within the food chain, the processing factory has been recognized as one of the major energy consumers (Leach, 1975). If significant savings are to be made in the food chain, they are most likely to be achieved by improving the energy utilization efficiency of the processing factories. Therefore the New Zealand food manufacturing industry

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was surveyed to quantify the energy use and to determine the potential for energy conservation within factories.

The survey methods used in the past in the food industry can be grouped into two broad categories:

(1) General surveys across a group of factories, or a whole industry where the average use in each factory was found from annual production and energy consumption records.
(2) Specific surveys in individual factories where measurements were taken within various parts of the factory to establish the energy flows. Data from surveys of this type are often incorporated within general surveys to illustrate how the average energy use can be broken down into components.

Within the New Zealand food processing industry, general surveys were previously conducted in the meat industry (Pearson & Pilling, 1975) and the dairy industry (Vickers & Shannon, 1977). In each case some specific surveys were carried out.

The traditional method used to carry out specific surveys is to meter various parts of the processing factory so that the energy use in each can be established, and related to production. Surveying energy use in this manner has a number of disadvantages.

(1) The cost of purchasing, installing and calibrating metering equipment is substantial.
(2) The installation of metering equipment may require a shut-down of production.
(3) Skilled personnel are required, and the time involved in planning and conducting a survey of this nature is substantial.
(4) Such surveys are normally of short duration because of their complexity. Data collected over a short time period may not be representative of the average energy consumption over a longer time period.

Nevertheless, such detailed surveys produce a breakdown of the total energy used directly in processing between different parts of the process, and by subtracting the energy used in processing from the total energy use, the baseload or non-productive energy can be found.

Within the New Zealand food industry, very few specific surveys of this nature have been carried out because few companies consider that the substantial cost involved in a survey will be justified by the opportunities for conservation that may be uncovered. An alternative survey method that is cheap and simple to use is more likely to be adopted. This paper describes a method developed to meet these criteria, and its application in seventy-three factories within the New Zealand food manufacturing industry.

Survey methodology

As the cost of metering equipment is one of the major problems in initiating detailed surveys, it was considered important that the new method should use only existing methods of recording energy use and production. Most companies approached recorded production of their various products on a daily basis, but few recorded energy use at all. However, energy metering equipment always existed to enable the supplier of each energy form to measure the consumption in the factory. In the case of electricity, total consumption could be read from the meters installed by the supplier and there was sometimes a division between day and night rates, and types of use. Occasionally some areas of factories were sub-metered. Similarly total gas use could be read from the supplier's meter. Deliveries of fuel oil were normally made to bulk storage tanks for which the tank manufacturer supplied a calibrated level measurement system by which the volume of oil in the tank could be measured. If oil was not metered in any other manner, total use could be measured by reading the change in oil volume in the storage tank. Only one factory using coal was surveyed by the authors, and in this the number of 'barrowloads' of coal was used as the measure.

It was therefore possible to measure use of all energy forms using existing equipment. As production was normally recorded on a daily basis, the extra effort involved in recording daily energy use was minimal and raw survey data giving both daily production and energy use were easily collected in any factory over significant time periods. Data collected in this way cannot be analysed by the same methods as are used to analyse data from surveys using comprehensive metering. The analysis method developed by the authors uses multiple linear regression to model the energy consumption as a function of production.

The total daily use of any energy form in a factory can be considered to be the sum of several components. The first of these is the use in non-productive activities which is a more or less constant amount each day and is referred to as the baseload. For electricity, it includes use in lighting, in staff amenities, in offices and laboratories, and the electricity used to maintain cold stores and cool stores within the factory. Baseload boiler fuel consumption includes the fuel used in start-up of boilers, in preheating the steam reticulation system, and in the supply of hot water for cleaning. For direct-fired equipment, baseload fuel consumption includes the energy used in start-up, and that used to overcome heat losses during the day.

The other components of the daily energy use are those of direct use in processing the various products. This is the amount of energy consumed by the processing equipment in transforming the raw material into the finished product. Where detailed measurements are taken, the direct use for each product and any variation in it from day to day are measured. If sufficiently detailed data are collected, the direct use can be partitioned between the parts of

the process. The baseload is determined by subtracting the measured direct use components from the total energy use in these detailed surveys.

Mathematically the model of energy consumption and its relationship to production is given by:

$$y = b_0 + b_1x_1 + b_2x_2 \ldots + b_nx_n \quad (1)$$

where y = total energy use per day (or whatever time period is used) (MJ/day)
b_0 = baseload energy use per day (MJ/day)
x_n = production rate of product n (kg/day)
b_n = direct energy use per unit production of product n (MJ/kg).

This model assumes that baseload energy use is independent of the rate of production, and does not vary from day to day. In practice, it has been found that this is true for days on which production is occurring (working days). The baseload energy consumption therefore represents the energy cost of having processing facilities available for use.

The other assumption in the model is that the energy used for any one product is independent of the energy used for all others. Unless heat recovery systems exist which transfer energy recovered from one process to another process for a different product, this assumption is valid. In the factories surveyed by the authors, no heat recovery systems of this nature existed.

If daily energy use data, and daily production data are collected over a number of days then the relationship between the energy consumption pattern and the production pattern will be explained by eqn (1). The 'best fit' relationship can be determined mathematically by application of multiple linear regression to find the values of $b_0, b_1 \ldots b_n$ that give a minimum for the sum of $(y_i - \hat{y}_i)^2$ where y_i is the actual energy consumption on the ith day, and $\hat{y}_i$ is the corresponding predicted value from eqn (1). The arithmetic involved is too tedious for hand calculation but regression routines are available on most computers.

The data generated by the application of regression are the average daily baseload, and the average direct use in processing for the various products. The accuracy with which these values are determined depends on three factors: the size of the data set, the random error and the range of production rates over which measurements were made.

Regression is an averaging procedure for which the accuracy of a mean value depends on the size of the data set. For such procedures, adding more data will increase the accuracy of a mean. In the situation where the accuracy of the means are limited by random error as well as the size of the data set, there is no advantage in increasing the data set beyond the point where the random error becomes the limiting factor. The authors have found by experience that the optimum size data set is usually 5 to 10 times the number of products, with a minimum of 15 to 20 days data. For example for factories producing less than three different products, satisfactory data can usually be obtained from 20 working days, but for ten products 50–100 days are required.

The term 'random error' is used to describe the deviations in energy use on any day that apparently do not depend on the production pattern and are therefore not explained by the model. The energy used to process a particular product is not constant from day to day, but varies around the mean value. In surveys where detailed measurements are taken, the day to day variations are actually measured, and an average can be calculated. In the simple surveys using data analysis by regression, only the average is calculated and the net effect of all the day to day variations in energy usage rate for the various products is seen as the random error, or the energy use not explained by the model.

The random error is important because it 'masks' the changes in energy consumption that can be related to production. An equivalent situation occurs where a best-fit line is to be constructed through a scattered set of data on a graph. The more scattered the data the less certain one can be that the best-fit line (however determined) is the correct line describing the relationship between the two variables. Most regression routines, as well as calculating the coefficients ($b_0 \ldots b_n$) also calculate the standard deviation of these coefficients which represents the uncertainty in them. If the data set includes a sufficient number of daily readings a 95% confidence interval for each coefficient can be established as ± 2 standard deviations. This confidence interval takes account of both the variation in energy use from day to day for that product, and the random error introduced by fluctuations in energy use in other parts of the factory.

The third factor affecting the accuracy of the estimates of average energy use for the various products is the range of production rates over which measurements were made. In any situation where it is required to establish the relationship between two variables the testing should be carried out over as great a range of the independent variable as possible. Similarly, if the relationship between energy consumption and the rate of production for a particular product is to be established the energy use per unit production will be most clearly differentiated from the random error if data are collected over a wide range of production rates. The best data sets for analysis by regression are therefore those with the maximum variation in the production pattern for each of the products over the survey period. The standard deviations of the coefficients calculated from data sets of this nature will be smaller than for those in which production rates do not vary greatly. This is because the change in energy use is proportional to the change in production rate, so large changes in production rate will lead to large changes in energy use. These will be more easily distinguished from the random error than small changes in energy use due to small changes in production rate.

Results and discussion

Examples of the method

The survey method was applied by the authors in seventy-three New Zealand food factories. A typical data set is shown in Table 1. Because some of the

readings represented more than one day's production, the regression model was slightly changed to allow for this.

$$y = b_0x_0 + b_1x_1 + \ldots b_5x_5 \quad (2)$$

where x_0 = number of days represented by each set of readings. The resulting equation was:

$$y = 1260x_0 + 510x_1 + 220x_2 + 5800x_3 + 220x_4 + 400x_5 \quad (3)$$

Table 1. Electricity consumption (MJ) and production (tonnes) in a five product factory

Number of days covered by reading	Electricity use (MJ)	Production (tonnes)				
		x_1	x_2	x_3	x_4	x_5
1	8593	11.8	0	0.2	0	0
1	8114	10.4	0	0	0	0
1	8784	10.3	7.9	0	1.7	0
1	8136	9.4	6.1	0	0	0
2	5976	3.9	5.2	0	0	0
2	9907	11.8	10.8	0	0	0
1	8914	8.7	10.7	0	0	1.9
1	7272	6.0	8.6	0	1.0	0
1	7272	9.9	5.2	0	0.5	0
3	7675	6.3	3.2	0	0	0
1	8417	12.6	6.1	0	0.3	0
1	7783	10.8	4.8	0	0	0
1	8845	9.5	12.1	0	0	2.3
3	7096	5.4	5.6	0	0	0
1	8831	11.0	10.2	0	0	0
1	6847	10.8	3.9	0	2.1	0
1	6228	10.5	5.7	0	0	0
1	7416	9.7	0	0.3	0	0

Table 2. Estimated electricity use and 95% confidence bounds for the data set shown in Table 1. All data in MJ/kg except baseload data which are in MJ/day

Product	Estimated energy use	Lower 95% confidence bound	Upper 95% confidence bound
1	510	400	620
2	220	80	360
3	5800	−2200	13700
4	220	−460	900
5	400	−290	1080
Baseload	1260	770	1750

The standard deviations for the coefficients are given in Table 2. Both the regression coefficients and the 95% confidence interval, were examined in terms of the form of the data set, and in relation to what was known about the energy using equipment on the processing line.

Product 1 was the major product manufactured in the factory and accounted for the largest component of the total electricity use. The random variations in the electricity used for other products did not mask the change in electricity consumption when the production rate of this product changed. The value of 510 MJ/tonne was consistent with the size of the motors, heaters etc. used in the process.

Product 2 followed a much less energy-intensive pathway than product 1, and although produced in substantial quantities, its contribution to total electricity consumption was smaller than that of product 1. The change in electricity use with change in production rate of 2 was therefore masked by the random variation in the data far more than was the case for 1. This is why the confidence interval is much wider. However, when the actual calculated value of 220 MJ/tonne was examined in the light of what was known about the process, and compared to the consumption rate for product 1 it was considered that the true energy consumption rate was probably close to the estimate of 220 MJ/tonne.

Products 3, 4 and 5 were all subject to major uncertainties, but examination of the raw data in Table 1 shows the reason. All these products were produced in small quantities, and on a small number of days. Their influence on the overall energy consumption pattern was therefore small, and almost completely masked by the random variation. For example, product 3 was produced on only 2 days during the survey period. If the energy used to produce product 1 on these days was more than the average predicted by the model, the nature of the model is such that it would assume that this increase in use was due to product 3. Conversely, if product 1 required less energy on these two days than average, the model would assume that the shortfall in use was due to product 3 and give a negative coefficient. This illustrates clearly the three effects discussed earlier — size of the data set, random error, and range of production rates. The size of the data set is too small (2 points for product 3), the range of production rates is too narrow (0 to 0.3 tonnes), and the random error resulting from variation in energy use in other parts of the factory swamps the change in energy use due to production of 3.

For product 5, the range of production rates is greater (although the number of data points is the same), and a narrower interval has been calculated. The data set for product 4 is larger and the bounds are narrower than for 3. Product 5 is similar to product 1, and in fact follows about 80% of the same processing path so the value of 400 MJ/tonne is probably close to the true consumption rate for this production in spite of the wide confidence interval. The processes for products 3 and 4 are similar, and on the basis of the equipment used would be expected to be less energy intensive than product 1.

Another problem that limits the application of regression is correlation between the production rates of various products. If two (or more) variables

always appear in a fixed pattern e.g. production of A is always double that of B then it is not possible to identify their separate contributions. Any attempt to do so will produce meaningless results. In this case, the correct procedure is to drop one of these inputs out of the equation and recognize that the other term is now estimating the combined energy consumption of the two products. When this happens, the two variables concerned are said to be aliased or confounded. Similarly if the production rate of A never changes then its energy consumption cannot be separated from the baseload. The correlation does not have to be 100% perfect to cause problems – where there are two variables with similar trends in their production patterns putting both of them into the equation will not give good estimates of their coefficients.

Thus it can be seen that there is no guarantee that the method will accurately estimate the average direct use in processing for all products. However, it gave the most accurate data for products with processes using the major amount of the factory energy, and the least accurate information for minor products with low levels of energy use. Where data from detailed measurements did exist these agreed with the coefficients derived by regression.

Improving estimates

There are three ways to improve the estimates of energy use for minor products. Firstly, more data could be collected at times when the minor products are produced in greater quantities. The second option is to state that as the procedure cannot distinguish the energy used by the problem product this energy must have been added into the baseload. The production data for this product would therefore be deleted from the equation and the regression repeated. The third option is to group minor products to calculate an average estimate for the group as a whole. The combined data will be more extensive, and there is a higher probability that energy use for the group will be distinguishable from the random error than was the case with individual products.

For the data set in Table 1, the similarity between products 3 and 4 was noted and the regression repeated with these products grouped together. The resulting coefficients and 95% confidence intervals are shown in Table 3. The new

Table 3. Estimated electricity use and 95% confidence bounds for the data set shown in Table 1 after regrouping of minor products. All data in MJ/day except baseload data which are in MJ/day

Product	Estimated energy use	Lower 95% confidence bound	Upper 95% confidence bound
1	550	460	640
2	160	50	270
3 + 4	200	−500	900
5	500	−200	1200
Baseload	1280	780	1780

average estimate made physical sense and was more accurate than either of the previous individual estimates. However, the estimated energy use rates for products 1 and 2, and the baseload were altered.

This is an important result as it illustrates one of the problems in applying regression. Because the data set is non-orthogonal any change made in the production pattern by regrouping will lead to some change in the regression coefficients.

The recommended method

In situations where a linear regression model is being applied to a non-orthogonal data set the best model can only be found by trial and error. The best method is to start with all possible products included, and then work down, by grouping products until the final model is reached. The best model has as few coefficients as possible and all the coefficients in this model contribute significantly to the reduction in the sum of squares of the residuals (the difference between the actual energy use on a particular day, and that predicted by the regression model is termed the residual). No other coefficients that could be introduced would reduce the sum of squares of the residuals. Statistical methods for testing the significance of individual coefficients can be found in most statistics textbooks, but the ultimate yardstick in energy surveys is whether the estimate of the energy consumption rate is consistent with the known or estimated energy use by the processing equipment.

In summary, the method meets the criterion of a simple and inexpensive data collection system that uses only existing metering. In some situations the precision with which the energy consumption rate can be determined is less than that obtainable with detailed measurements. However, as the processes and products with high usage are shown, detailed measurements if made, can be concentrated in the important areas, and therefore used more effectively. Further, the method provides a simple method for monitoring energy use on a continuing basis to ensure that the utilization efficiency does not fall. Follow-up from detailed surveys after metering equipment has been removed cannot be carried out in any other way.

Application of the method

Over a period of 1 year, the authors applied the method in seventy-three factories, and in each case were able to achieve a useful subdivision of energy use. Such a wide coverage would not have been possible in any other way. The data from these surveys together with data from a postal survey of annual production and energy use for other factories enabled the authors to determine the average energy use for some sixty product types within the food manufacturing industry. The more important of these data are shown in Table 4, and the detailed data are available elsewhere (Cleland & Earle, 1980).

Table 4. Energy use in factories of the New Zealand food manufacturing industry excluding intra-factory transportation. All data expressed as MJ of purchased energy per kg of finished product. No distinction is made between boiler fuel, and fuel used in direct-fired equipment

Product type	Fuels		Electricity		Total
	Direct use in processing	Allocated baseload	Direct use in processing	Allocated baseload	
Ham and bacon*	6.7†		1.8†		8.5
Processed meats*	3.5†		1.0†		4.5
Frozen poultry	0.61	0.22	0.61	0.70	2.1
Fresh poultry	0.61	0.18	0.11	0.65	1.6
Ice cream‡	0.26	0.38	0.36	0.94§	1.9
Frozen vegetables	1.70	1.61	0.81	1.83§	6.0
Canned fruit and vegetables	2.70	1.06	0.22	0.40	4.4
Other canned products	2.58	1.06	0.28	0.40	4.3
Dehydrated vegetables	38	9	3.4	1.1	51.5
Frozen whole fish and fillets	0	0.8	0.63	2.60§	4.0
Processed fish products	2.8	2.0	1.1	2.1§	8.0
Fishmeal	12.1	1.2	1.3	0.2	14.8
Grain milling (wheat only)	0	0	0.17	0.02	0.2
Breakfast cereals	5.3†		0.7†		6.0
Bread*	1.65	0.80	0.13	0.09	2.7
Chocolate, sugar and gum confectionery	7.8†		1.9†		9.7
Potato crisps	15.7†		1.9†		17.6
Dehydrated lucerne pellets	13.0†		0.5†		13.5
Other pelletized animal feeds**	0.13	0.07	0.08	0.04	0.3
Canned pet foods	2.33	1.24	0.16	0.47	4.2
Distilled spirits††	20†		0.8†		20.8
Wine‡	1.39†		0.53†		1.9
Soft drinks‡	0.19	0.40	0.07	0.12	0.8
Refined sugar	3.83†		0.17†		4.0
Processed fats and oils	7.0†		1.5†		8.5
Pasta products	3.3†		1.0†		4.3
Instant coffee	45†		2.7†		47.7

*Data represent the major part of the industry, but are not representative of the industry as a whole.

†Although the division of energy consumption into baseload and direct use in processing could be made in some factories, insufficient data existed to accurately estimate the division for the New Zealand industry as a whole.

‡Data in MJ/litre.

§Includes energy used in refrigerated storage at the factory after processing but prior to distribution.

‖Average for canned fish, smoked fish fillets, and battered, reformed fish products.

**From dry raw materials.

††Data in MJ/proof litre.

For the data in Table 4 the allocation of baseload or non-productive energy use was obtained by dividing the total baseload energy use over the survey period by the total production of all products in the factory over the same period. Where a division into direct use and baseload components is not given, this is because the number of factories surveyed in detail was too few for the results from these surveys to accurately represent the industry as a whole. For example, for breakfast cereals detailed data were collected from only two of the five major factories in New Zealand whereas the other three provided data from plant records.

The suitability of the method for obtaining large amounts of useful information in a short time using only existing metering equipment has been recognized by the New Zealand Energy Research and Development Committee on whose behalf a report for companies to use in planning and running their own surveys has been prepared (Cleland & Boag, 1979).

Other applications of regression

Data analysis by regression is not limited to energy surveys, the method can be used to assess the individual contributing components to any total. The authors have used it in factory water surveys to sub-divide water use between the various product lines. It was also used to determine the relationship between the yield of an animal feed meal produced in a meat slaughter plant and the various sources of offal passed to the feed meal processing line. Doubtless, other applications exist.

Conclusions

The survey method developed is a simple and cheap alternative to surveys carried out by detailed measurements. It provides estimates of the direct use in processing for all products and an estimate of baseload energy consumption using only data from existing metering. It gives most accurate estimates of energy consumption rate for products using major amounts of energy. The accuracy of data for minor products can be improved in some cases by grouping of production data. The method led to a useful analysis of the total energy consumption in all factories where it was used.

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Effect of pH and prefreezing treatment on the texture of yellowtail rockfish *(Sebastes flavidus)* as measured by the Ottawa Texture Measuring System

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Summary

Texture is very important to the organoleptic quality of fish products. Poor frozen storage conditions or improper prefreezing treatment can result in unacceptably tough fillets. With some species, undesirable soft, mushy texture develops during chill storage.

This study is concerned with the influence of treatment prior to freezing (samples were frozen pre-rigor, in-rigor, post-rigor and after 6 days' chill storage in ice or refrigerated sea water) on the texture of yellowtail rockfish stored frozen as whole gutted fish or as fillets. The effect of pH was also studied. Texture was measured objectively using the Ottawa Texture Measuring System on samples stored for 6 months at −28°C.

A very good negative correlation was found between pH level and toughness as measured using a Kramer shear-compression cell in the Ottawa Texture Measuring System. Fish stored in refrigerated sea water prior to freezing were appreciably more tender. There was no statistical difference in texture (shear press force) values between samples stored as whole fish versus samples stored as fillets.

Introduction

The influence of pH on the texture of fish muscle was first discussed by Little (1965). He found that for fish frozen quickly (after 3 days on ice) and stored frozen at −29°C, lower pH fish had greater drip and a tougher texture. Work by

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Cowie & Little (1966) showed that for cod fillets stored frozen at −28°C, protein solubility is not a good measure of texture changes as the influence of pH is more important. A later report (Cowie & Little, 1967) on cod fillets stored at −7 and −14°C showed that protein solubility decreases faster and toughness increases faster at −7°C than at −14°C. They concluded that both protein solubility and pH measurements are needed to assess toughness.

The relationships of fish muscle texture, pH levels and values obtained from the cell fragility test were studied by Kelly *et al.* (1966). High muscle pH correlates with soft texture and low cell fragility values, while low muscle pH correlates with firm texture and high cell fragility values.

Connell & Howgate (1968, 1969) used both sensory and objective tests to measure the quality of frozen stored North Sea cod and North Sea haddock. From analysis of their data, they obtained equations for the relationship between firmness as measured by a sensory panel and pH. In order to determine the effect of frozen storage on firmness, they used these equations to correct the firmness scores to the same nominal pH.

K.O. Kelly (1969) investigated the influence of pH on cold storage deterioration during frozen storage of cod fillets (at three different temperatures) and concluded that lower pH fillets deteriorate more rapidly. T.R. Kelly (1969), using cod fillets stored at −7°C, reported that for high pH cod, shelf life of the fish was limited by flavour changes whereas for low pH fish, the fillets became unacceptably tough before flavour changes limited shelf life.

The influence of muscle pH, moisture content, protein content and size of fish on texture of the cooked flesh (of Atlantic cod) has been studied by Love *et al.* (1974). They found pH correlated best with cooked texture and concluded that it is important to know the annual variation of the ultimate post-mortem pH of fish from each ground in order to select good ones for freezing. Work by Bremner, Laslett & Olley (1978) showed that the dividing line of Cowie & Little (1967) on a plot relating protein extractability to pH, could be used to separate tough from soft samples for minced flesh of sixteen species of Australian fish.

A comprehensive discussion of the definition and measurement of food texture has been given by Kramer (1973). Szczesniak (1973) reviewed instrumental methods of measuring texture. As mentioned by Gould & Peters (1971), no single objective test for texture of fish muscle has proved completely reliable. However, Bosund & Beckeman (1972) found good correlation between shear press values and panel evaluation of toughness in cod stored at −10°C.

The process of rigor mortis in fish and its effect on keeping quality has been reviewed by Amlacher (1961). In previous work (Kramer & Peters, 1979) on comparison of the frozen storage characteristics of two species of Pacific rockfish, we found that cooked samples of muscle from fish filleted and frozen immediately after catching (pre-rigor) were much tougher than those from fish filleted after chill storage in either ice or refrigerated sea water (RSW). The use of a tripolyphosphate dip improved the texture but did not completely alleviate this toughness.

The present investigation is concerned with the effect of pH and stage of rigor when frozen on the texture of cooked muscle from the yellowtail rockfish, *Sebastes flavidus*. Whole, gutted fish as well as fillets were studied to determine if texture is affected by freezing and thawing of the fillet as opposed to freezing and thawing while it is attached to the skeleton of the fish. An objective measurement of texture, the Ottawa Texture Measuring System (OTMS) using a Kramer shear-compression cell, was used for this work.

Materials and methods

Material

The samples of yellowtail rockfish, *Sebastes flavidus* were caught in April, 1978 in Queen Charlotte Sound using a bottom trawl. Depths of the tows were: start at 70 fathoms to finish at 59 fathoms and start at 69 fathoms to finish at 82 fathoms.

Preparation of fish samples

Fish samples (fillets and whole gutted fish) were prepared from fish at five different stages of chill storage: (1) pre-rigor fish, (2) in-rigor fish, (3) post-rigor fish, (4) fish held 6 days in ice, (5) fish held 6 days in refrigerated sea water (RSW). The samples consisted of ten pairs of fillets and ten whole, gutted fish (head on) for each of these treatments.

The fillets were cut from ten fish, skinned, washed with fresh water and placed into polyethylene bags (the two fillets from each fish were put together in one bag). The remaining ten fish were gutted (head on), washed with fresh water and placed individually in polyethylene bags. The fillets and gutted fish were then frozen in a vertical plate freezer. The gutted fish were then glazed with fresh water (kept at 0°C with ice) and wrapped in two polyethylene bags. Both fillets and gutted fish were stored in covered plastic tubs at −28°C.

pH measurement

Fish fillets were placed inside two plastic bags and thawed in a 20°C water bath for 2 hr. Whole fish were thawed in the same manner except that thawing time of 3 hr was used. After thawing, the whole fish were filleted and the fillets skinned. All fillets were washed in fresh water and patted dry with paper towelling prior to measurement of the pH.

A Metrohm Herisau model E488 pH meter equipped with a Metrohm EA 152 combined electrode assembly was used for all pH measurements. The electrode was inserted directly into the muscle at approximately the centre of each fillet.

Texture measurement

Texture of the cooked muscle was measured objectively using the Ottawa Texture Measuring System (Voisey, 1971). This instrument is very versatile and has been used for many research applications (Voisey, 1977). Exploratory work on measurement of the texture of canned herring indicated that the Kramer shear-compression cell and the OTMS 15 cm^2 4-wire grid were useful for showing texture differences (Voisey, 1972). Bilinski, Lau & Jonas (1977) used the OTMS (with Kramer shear-compression cell) to determine the firmness of various commercial canned herring products. Tomlinson *et al.* (1976) used this instrument to compare the tenderness of the cooked flesh of five species of Pacific rockfish.

We used a Kramer shear-compression cell modified to a four-blade size rather than the full ten-blade size. Texture was recorded using a Daytronic Model 300D Transducer Amplifier-Indicator in combination with a Riken Denshi Speedex Recorder.

Six pieces, approximately $1'' \times 2'' \times \frac{1}{2}''$, were cut from each fish (three pieces from the centre of each fillet). The pieces were wrapped individually in aluminium foil and steam cooked for 10 min, then cooled for 30 min. The cooked muscle for each fish (six pieces) was flaked by hand to uniform pieces approximately $\frac{1}{4}''$ square and mixed thoroughly. A 15-g sample of this mixture was packed in a uniform layer in the cell and the texture measured. Duplicate analyses were done for each fish (triplicate if the results of the duplicates were not in good agreement).

Statistical treatment of the data

All statistics were calculated using a Hewlett-Packard Model 9100B Calculator. Student's *t*-test and analysis of variance (both single factor and double factor) were used to compare texure values obtained for fillets with those obtained for whole fish. Treatments prior to freezing (time and method of holding) were compared by analysis of variance.

Linear regression lines and correlation coefficients (r) for the relationship of texture to pH were calculated using Hewlett-Packard STAT-PAC Volume 1 Program IV–1.

Results

The effect of treatment prior to freezing and storage for 6 months at −28°C of whole gutted *S. flavidus* is shown in Fig. 1 (the solid bars). The fish is slightly tougher if frozen in-rigor compared to freezing before or after rigor. Chill storage in ice or in refrigerated sea water results in a more tender fish, with the fish held in refrigerated sea water having the lowest shear press force values. Table 1 gives the range of texture values for each group of fish and shows the large variation among individual fish.

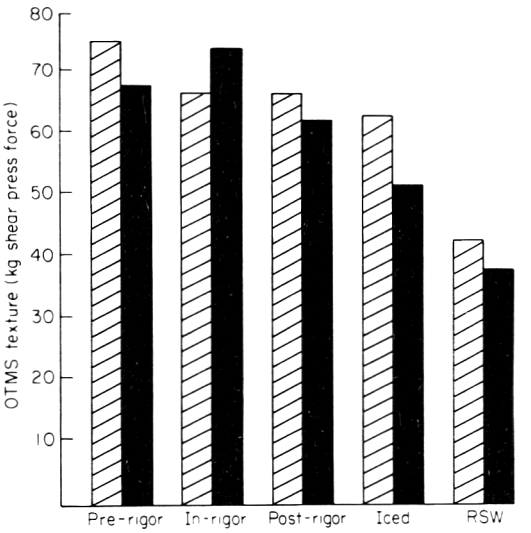

Figure 1. A comparison of the texture (as measured by the Ottawa Texture Measuring System using a Kramer shear-compression cell) of *S. flavidus* frozen for 6 months at −28°C as whole gutted fish or as fillets after various periods of chill storage. The crosshatched bars are for fillets and the solid bars are for whole gutted fish.

The effect of prefreezing treatment of *S. flavidus* on the frozen storage of skinned fillets is shown by the cross-hatched bars in Fig. 1. Fillets from pre-rigor fish were toughest. As was the case for fish stored frozen as whole gutted fish, fillets from fish held in chill storage were more tender than the other samples. Again, muscle from fish held for 6 days in refrigerated sea water had the lowest shear press force values. The range of values for each group of fillets is given in Table 1.

In four of the five groups (excepting only the samples prepared from in-rigor fish), the muscle frozen as fillets was on the average tougher than the muscle frozen as whole gutted fish (see Fig. 1). However, statistical analysis using the Student's *t*-test and analysis of variance showed that the only statistically significant difference was for the samples from iced fish.

Both the highest mean shear press force value and the highest individual shear press force value came from the pre-rigor fillet group (Table 1). The statistical significance of the differences due to treatment prior to freezing were determined using Duncan's multiple range test and the results are indicated in the last two footnotes given for Table 1. For both frozen fillets and frozen whole gutted fish, *S. flavidus* held in RSW were significantly softer than the other samples. For the samples frozen as whole fish, those gutted and frozen in-rigor were significantly tougher than those gutted and frozen post-rigor or after being held in ice or RSW.

Figures 2 and 3 show the relationship of texture to pH for each chill storage period. Each value plotted in these graphs represents one fish. Correlation coefficients for the linear regression lines plotted in these graphs indicate very

Table 1. Ottawa Texture Measuring System (OTMS) values for *S. flavidus* stored frozen as fillets and as whole gutted fish for 6 months at −28°C after various periods of chill storage

Holding method	Texture			
	Fillets		Whole fish	
	Mean*	Range	Mean*	Range
Pre-rigor	75.39 (16.68)[a]	47.2—103.4	68.27 (14.15)[c]	42.0—91.4
In-rigor	66.95 (11.03)[a]	56.3—92.3	72.17 (11.75)[d]	58.3—94.4
Post-rigor	66.83 (16.35)[a]	41.3—87.9	62.49 (9.56)[e]	47.5—81.9
Iced 6 days	63.28 (8.48)[a]	41.9—70.7	51.86 (12.98)[f]	36.3—75.3
RSW-held 6 days	42.90 (5.08)[b]	35.2—51.8	38.24 (8.97)[g]	25.5—54.1

*Each value represents ten fish. Standard deviations are given in parentheses.

[a, b] The mean values having the superscript [a] are statistically not significantly different from each other. The mean value having the superscript [b] is statistically significantly different from each of the values given the superscript [a].

[c, d, e, f, g] The mean values having these superscripts are statistically significantly different from each other as follows: [c] not significantly different from [d] or [e], [d] significantly different from [e], [f] significantly different from all other values and [g] significantly different from all other values.

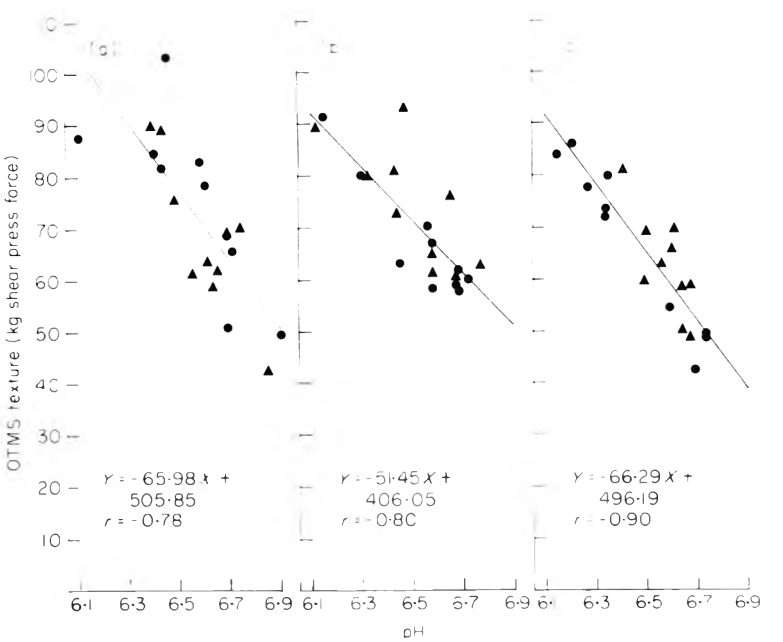

Figure 2. The relationship between texture (as measured by the Ottawa Texture Measuring System using a Kramer shear-compression cell) and pH for *S. flavidus* frozen (a) pre-rigor, (b) in-rigor and (c) post-rigor and held for 6 months at −28°C. ▲, whole fish; ●, fillets.

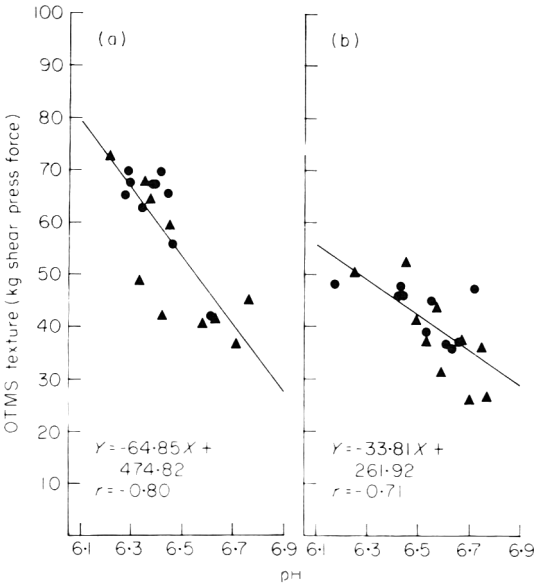

Figure 3. The relationship between texture (as measured by the Ottawa Texture Measuring System using a Kramer shear-compression cell) and pH for *S. flavidus* held 6 days in ice (a), or 6 days in refrigerated sea water (b), prior to freezing and storage for 6 months at −28°C. ▲, whole fish; ●, fillets.

good correlation between shear press force values and pH for each of the different chill storage periods.

The linear regression equations and correlation coefficients shown in Figs 2 and 3 are calculated using data for both whole gutted fish and fillets. Equations and r values calculated for each of these chill storage groups using data based on samples stored as whole gutted fish only or stored as fillets only is given in Table 3. For each group, the equation obtained for whole fish was compared statistically to the equation obtained for fillets. Comparison of slopes and elevations of these equations, using methods described by Zar (1974), resulted in the conclusion that in each case one line can be used to describe the relationship of shear press force values to pH (hence the single line and equation given for each plot in Figs 2 and 3). Significance levels shown in Table 3 for b ($Y = bx + a$) of the linear regression equations were obtained using Student's t-test. These levels correspond to significance levels obtained from the table given by Snedecor & Cochran (1967) for the correlation coefficients of the equations.

There is very good linear correlation between pH and shear press force values for fillets frozen from fish at all chill storage periods; for fillets from fish in-rigor and post-rigor, there is excellent correlation (r values are -0.92 and -0.97 respectively). There is also good linear correlation for the pH—shear press force relationship for muscle from frozen whole fish at all chill storage periods except those held in refrigerated sea water for 6 days. The RSW-held fish had an r value for the linear regression line of -0.57 which indicates some correlation.

Table 2. pH values for *S. flavidus* stored frozen as fillets and as whole gutted fish for 6 months at −28°C after various periods of chill storage

Holding method	pH values			
	Fillets		Whole fish	
	Mean*	Range	Mean*	Range
Pre-rigor	6.55 (0.22)	6.09–6.93	6.60 (0.15)	6.31–6.87
In-rigor	6.53 (0.19)	6.13–6.73	6.50 (0.19)	6.10–6.78
Post-rigor	6.44 (0.22)	6.11–6.73	6.58 (0.09)	6.39–6.69
Iced 6 days	6.38 (0.11)	6.24–6.63	6.48 (0.18)	6.18–6.78
RSW-held 6 days	6.51 (0.16)	6.16–6.76	6.58 (0.16)	6.22–6.82

*Each value represents ten fish. Standard deviations are given in parentheses.

Table 3. Linear regression equations and correlation coefficients for the relationship of shear press force values to pH for *S. flavidus* stored frozen as whole gutted fish and as fillets

Holding method		Linear regression equation	Standard error of estimate* (^{S}Y–X)	Correlation coefficient (r)
Pre-rigor	Whole fish	$Y = -83.04x^{b} - 616.49$	8.19	-0.84^{b}
	Fillets	$Y = -56.71x^{a} - 447.11$	11.67	-0.75^{a}
In-rigor	Whole fish	$Y = -45.58x^{a} + 370.63$	8.50	-0.73^{a}
	Fillets	$Y = -54.57x^{b} + 423.66$	4.64	-0.92^{b}
Post-rigor	Whole fish	$Y = -83.07x^{a} - 609.03$	6.62	-0.76^{a}
	Fillets	$Y = -71.30x^{b} + 525.97$	4.06	-0.97^{b}
Iced 6 days	Whole fish	$Y = -55.74x^{b} + 413.14$	8.70	-0.77^{b}
	Fillets	$Y = -66.55x^{b} + 488.36$	5.31	-0.81^{b}
RSW-held 6 days	Whole fish	$Y = -46.17x^{b} + 341.93$	5.55	-0.81^{b}
	Fillets	$Y = -18.16x + 161.20$	4.43	−0.57

*As defined in Zar (1974), p. 207.
[a] Indicates a value significant at $P < 0.05$.
[b] Indicates a value significant at $P < 0.01$.

The mean and range of muscle pH values for each group of fish frozen as whole gutted fish or as fillets are shown in Table 2. Except for the in-rigor fish, samples frozen as whole fish have slightly higher pH levels compared to those frozen as fillets. Fish held in ice had slightly lower pH levels compared to the other chill storage groups. However, the differences are too small to be of significance.

Discussion

The mean OTMS values for cooked muscle of *S. flavidus* which were stored frozen for 6 months, ranged from a shear force value of 38.24 for RSW-held fish stored frozen as whole fish to a value of 75.39 for fillets taken from pre-rigor fish and held frozen for 6 months. The shear force values obtained in this study for cooked muscle from fish which were held in ice for 6 days prior to freezing are in good agreement with the data reported by Tomlinson *et al.* (1976) for cooked flesh of five other species of *Sebastes* which were stored for 5 to 11 days in ice prior to freezing.

In a study on Atlantic cod, MacCallum *et al.* (1968) found that the overall texture scores of a taste panel gave clear preference for cooked muscle from pre-rigor compared to in-rigor fish and that fillets frozen pre-rigor gave the best product. Previous work at this laboratory on the chill and frozen storage characteristics of the rockfish species *Sebastes alutus* and *Sebastes flavidus* (Kramer & Peters, 1979) shows fillets removed and frozen pre-rigor to be very tough from both taste panel assessment and instrumental analysis using the OTMS. Fillet blocks were used for that study and the one-inch cross sections cut from the blocks for quality determination gave pieces too small to permit both taste panel and OTMS work on the same piece. Although no direct comparison of shear press force value with taste panel score for fillets which were undesirably tough can be made in that study, it appears from the data on the number of organoleptic scores which were undesirable and the shear press force value ranges for each different treatment that an OTMS value of about 70 is the cut-off. In other words, fillets which have a cooked muscle shear press force value of about 70 or more will be undesirably tough (have a taste panel score of 5 or below).

It seemed likely to us that the reason fillets from pre-rigor rockfish are so tough after frozen storage was related to contraction of the muscle fibres during thaw rigor. We expected this process would be greatly reduced if whole fish were frozen rather than taking a fillet from the frame (skeleton) and freezing the fillet. While texture is improved, i.e. the cooked muscle is more tender, for frozen whole fish compared to frozen fillets in four of the five chill storage periods, the differences are not very large. Only in the case of the fish iced for 6 days is the difference statistically significant.

When the mean OTMS values are corrected for the effect of pH on texture (Table 4), the cooked muscle is more tender for frozen whole fish compared to frozen fillets in three of the five different chill storage periods. However, in every case, the difference between frozen whole fish and frozen fillets is less than before the values were corrected. In all cases, the difference is not statistically significant. That there is no significant difference in texture between frozen whole fish and frozen fillets is confirmed by statistical comparison of the regression equations (discussed in the results section of this report).

Table 4. Mean Ottawa Texture Measuring System (OTMS) values for *S. flavidus* stored frozen as fillets and as whole gutted fish for 6 months at −28°C after various periods of chill storage. These values have been corrected* for the effect of pH on texture by adjusting all values to pH 6.50

Holding method	Texture	
	Fillets	Whole fish
Pre-rigor	78.20	73.88
In-rigor	68.63	74.17
Post-rigor	63.46	66.98
Iced 6 days	56.55	50.74
RSW-held 6 days	43.46	42.73

*The correction factor was obtained using a pooled estimate of the regression coefficient (Zar, 1974). The value found using the ten equations given in Table 3 is $b_c = -56.12$.

Both the fillet and whole gutted samples from fish which were held 6 days in refrigerated sea water had more tender texture than the other samples. All groups of samples, frozen both as fillets and as whole gutted fish, from pre-rigor, in-rigor, post-rigor, and 6-day iced fish had one or more shear press force value above 70, which is the approximate point at which they are unacceptably tough to a taste panel. For the samples held 6 days in refrigerated sea water, all OTMS values were below 55.

The mean OTMS values given in Table 4, which have been corrected for the effect of pH on texture, show more clearly that storage conditions prior to freezing have an effect on texture. Statistical analysis of the linear regression equations given in Figs 2 and 3 (comparison of slopes and elevations as detailed by Zar, 1974) shows that the slopes of the five equations are common but the elevations are not common. The common slopes describe the dependence of texture as measured by the OTMS on pH and show that this relationship is independent of treatment prior to freezing. The different elevations confirm that chill storage has an effect on instrumentally measured texture of the cooked muscle and indicate that both method and time of chill storage are involved.

The extremely good linear correlation coefficients for the relationship of pH to shear press force values indicate that for *S. flavidus* held under the conditions of chill and frozen storage used in this work, pH is by far the most important parameter associated with texture of the cooked muscle. Bosund & Beckeman (1972) found good correlations for the relationships of pH, shear press force values and taste panel evaluation of toughness for frozen cod.

Work with minced flesh of several Australian species (Bremner, Laslett & Olley, 1978), did not give a good correlationof pH with taste panel texture as indicated by correlation coefficients of −0.16 and −0.21. They indicate that both water holding capacity of the muscle and saline extractable protein may be needed to provide an adequate prediction of texture.

The prefreezing treatments used in this work had no significant effect on muscle pH. We measured pH by inserting an electrode directly into the fillet rather than making a suspension of the muscle as described by Cowie & Little (1966). Bosund & Beckeman used the method of Cowie & Little to measure pH of the flesh. Whether the high correlation of pH with OTMS-measured texture depends on taking a direct measurement of flesh pH as was done in the work reported here is not certain and should be investigated.

Conclusions

For the yellowtail rockfish, *S. flavidus*, there is a strong correlation between pH level and cooked muscle shear press force values for samples stored frozen at −28°C both as fillets and as whole gutted fish. Low pH indicates higher shear press force values and tougher texture. Stored fillets are not significantly tougher than stored whole fish.

For species like cod which can become soft and mushy during chill storage, the recommended procedure to obtain the best quality fish has been to fillet and freeze pre-rigor. This work shows that for species which tend to become tough during frozen storage, holding in ice or refrigerated sea water for several days may overcome this problem.

Acknowledgments

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Occurrence of *Exophiala werneckii* on salted freshwater fish *Osteoglossum bicirrhosum*

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Summary

Fungal contamination occurred on the salted Amazonian fish *Osteoglossum bicirrhosum* which were air dried in the shade. The human pathogenic fungus *Exophiala werneckii* was recovered as the sole contaminant from all salted fish. It was implicated for the first time as a halophilic saprophyte causing food spoilage.

Introduction

Curing is a time-honoured preservation procedure. However, the salted food products are by no means completely free from microbial contamination. Known bacterial and fungal contaminants isolated from cured material include *Micrococcus morrhuae*, Halobacterium sp., Flavobacterium sp., *Sporendonema expizoum*, *Aspergillus glaucus* and *A. echinulatus* (Leitão, 1979). Recently, we tested drying salted fish in the shade as an alternative preservation procedure in view of the annual 6-month long rainy season in the Amazon Basin. The freshwater fish *Osteoglossum bicirrhosum* Vandelli 1829, locally known as aruanã, was cured and air dried in the shade. However, microbial contamination occurred. *Exophiala werneckii* was recovered from all contaminated salted fish. This was the first time that the pathogenic fungus *E. werneckii* was implicated as a halophilic saprophyte responsible for food spoilage.

Material and methods

Salting process

Twenty chilled aruanã fish were purchased from the local market. The fish were weighed, eviscerated, split and salted. Fish in groups of five received one of

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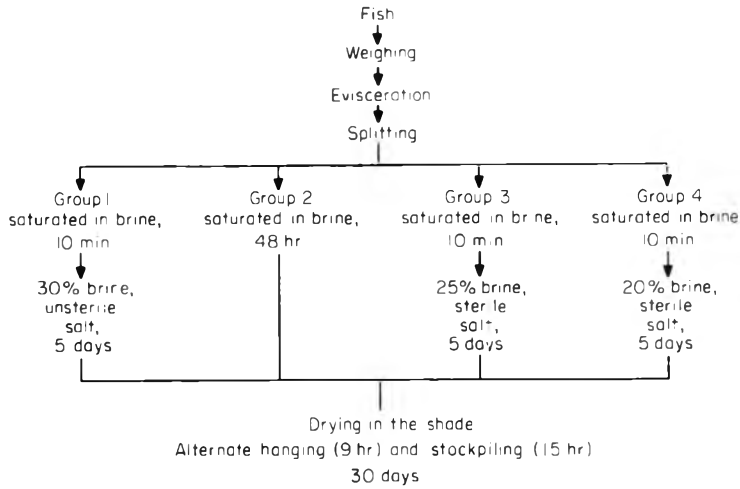

Figure 1. Flow chart of salting process.

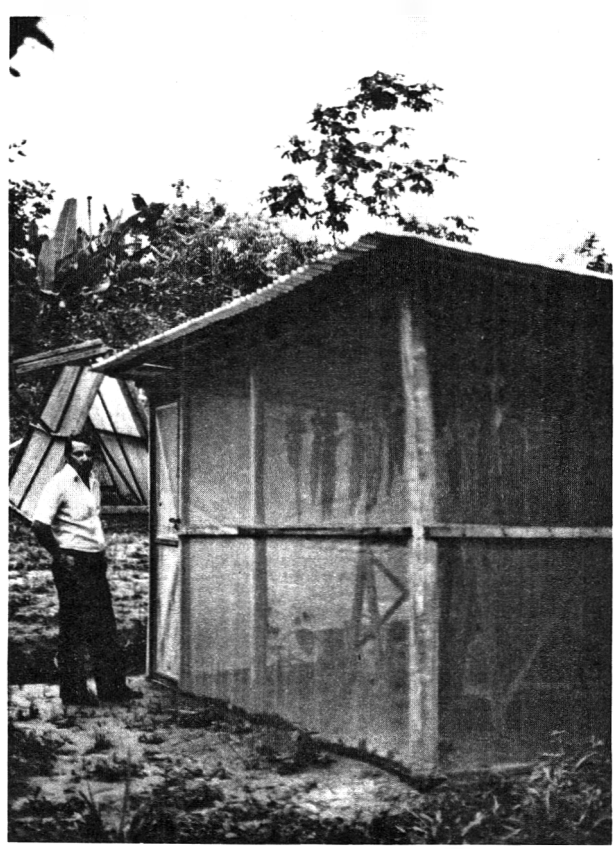

Figure 2. Naturally ventilated screen-walled shed where fish were dried.

the four treatments (Fig. 1). The fish were soaked in saturated brine for 10 min, with the exception of group 2 fish which were left immersed for 48 hr without further curing. Group 1 fish were cured for 5 days in 30% brine prepared from unsterile salt. Groups 3 and 4 fish were also cured for 5 days, but in 25 and 20% brine respectively. The salt in these brine preparations had been previously sterilized at 150°C for 4 hr in a drying oven. After curing, all fish were air dried in a naturally ventilated screen-walled shed (Fig. 2). They were kept alternately hanging for 9 hr and stockpiled for the next 15 hr. The preservation schedule called for drying for a period of 30 days.

Results

Fungal colonization

Group 2 fish showed signs of deterioration shortly after they were left to be air dried. By the 9th day, large quantities of ammonium composites were detected in the fish flesh by the Eber test. Accordingly these fish were discarded.

Fish in the remaining three groups were drying up slowly. By day 14, small round white superficial mouldy plaques were seen on all fish (Fig. 3). They were easily removed by scraping. There was no apparent tissue involvement. Preliminary microscopic examination of the plaques taken from one fish in each group showed the presence of numerous hyphae and conidia. The drying period was extended for observation of fungal colonization. During the next 30 days the number and size of the plaques increased gradually. A plaque count on day 43 showed significant differences in number and size among the three groups (Table 1).

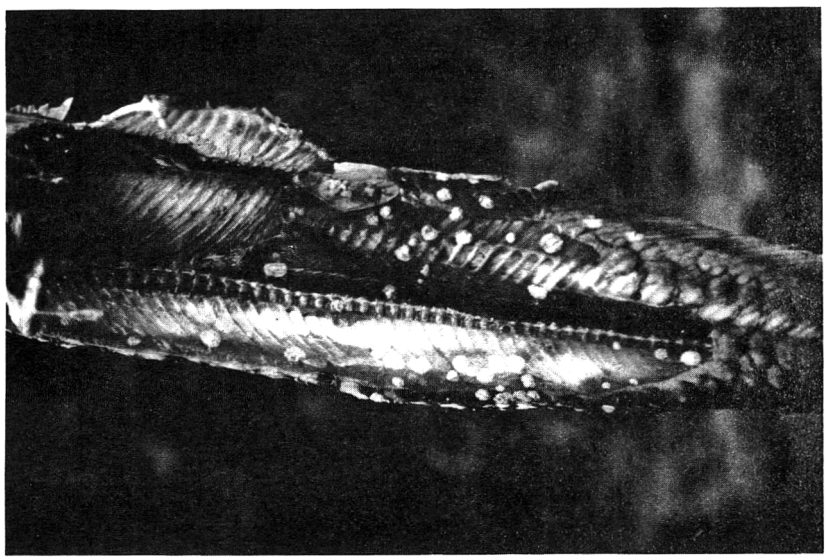

Figure 3. Round, white mouldy plaques on fish surface; 30 days after drying in the shade.

Table 1. Number and size of fungal plaques on fish

	Plaques					
	Number			Size (cm)		
fish	$\bar{x}$	S	sig.	$\bar{y}$	S_y	sig.
Group 1 (n = 4)	95	23.4	+3,4	4.55	1.09	+4
Group 3 (n = 4)	8	9.9	+1	4.90	1.02	—
Group 4 (n = 4)	27	11.7	+1	6.45	1.11	+1

sig. = significant at the 95% confidence level.
+3 = significant difference with group 3.
— = no difference with any other group.

Mycological studies

Four fish from each of the groups 1, 3 and 4 were examined. Ten plaques were chosen randomly from each fish, except for two fish in group 3 which totalled only three plaques. Scrapings were taken from each plaque. Material from one scraping was mounted on a microscopic slide for direct examination with lactophenol cotton blue. Material from a second scraping was inoculated onto mycosel agar (BBL) which was kept at 25°C. Four scrapings were taken from each of the three plaques of the two group 3 fish. Microscopic examination revealed the presence of both hyaline and pigmented septate hyphae, abundant single and budding conidia, occasional arthrospores and two-celled conidia with dark septa (Figs 4 and 5). Fungal structures were observed on all slides.

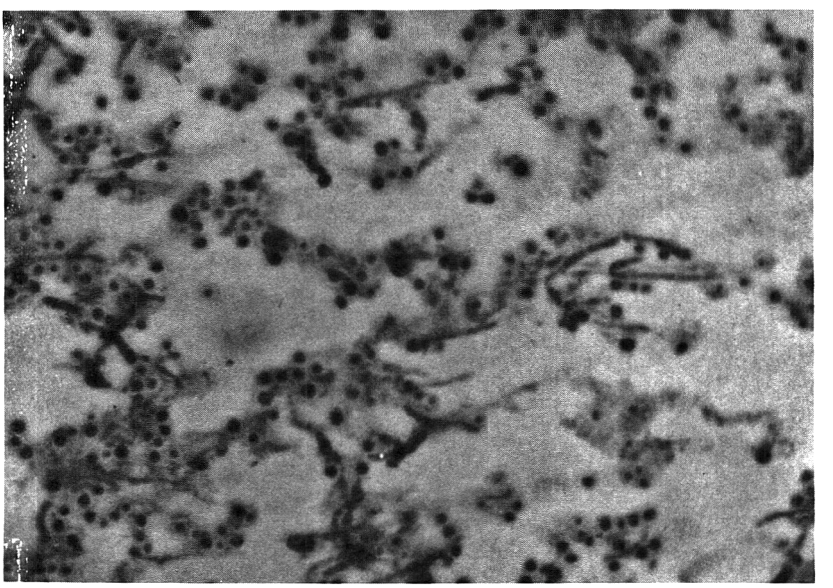

Figure 4. Direct microscopic examination of plaque scraping: conidia and hyphae (240×).

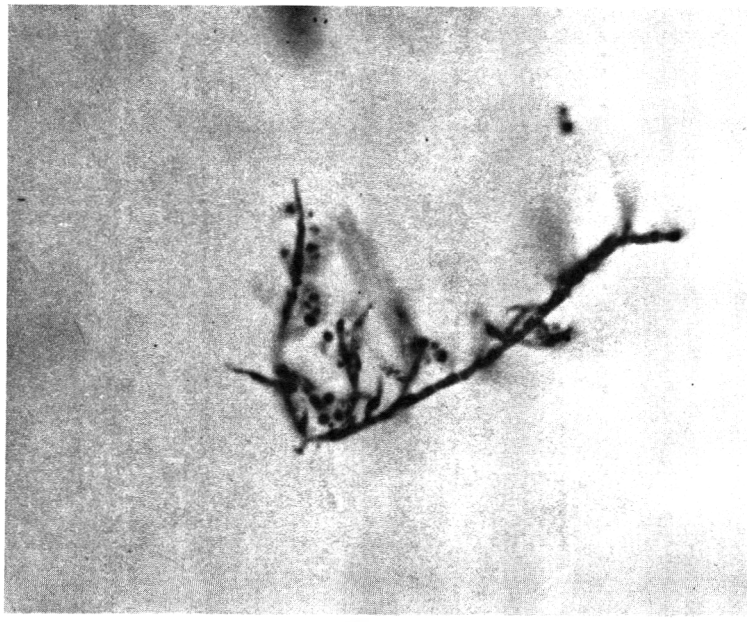

Figure 5. Dematiaceous septate hyphae and conidia (1920×).

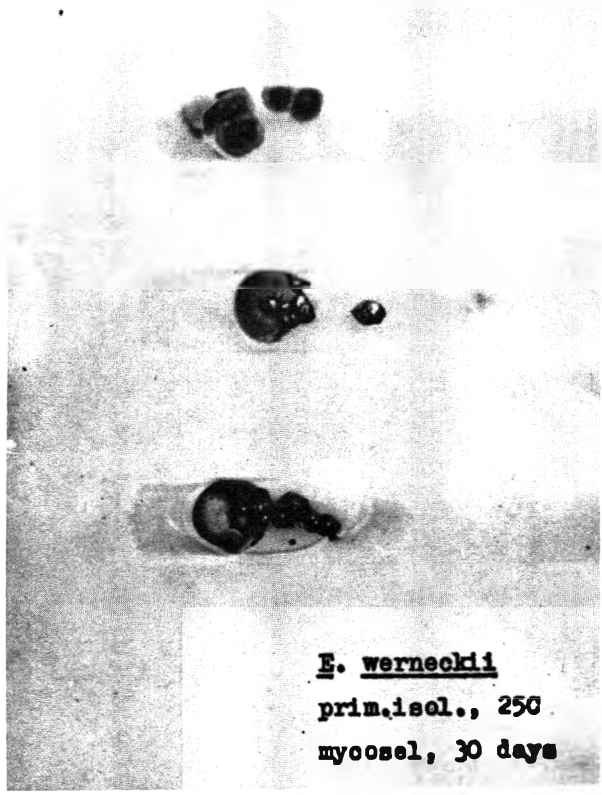

Figure 6. Primary isolation of dimorphic *E. werneckii* on Mycosal agar: yeast and mycelial growth.

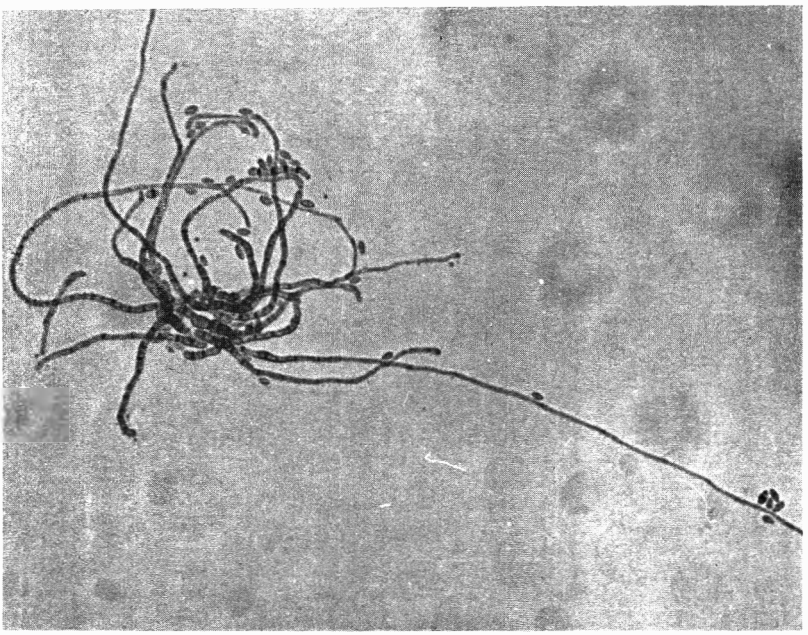

Figure 7. Slide culture of *E. werneckii* on corn-meal agar, 5 days; agglomeration of hyaline hyphae with pigmented septa and conidiogenous cells (480×).

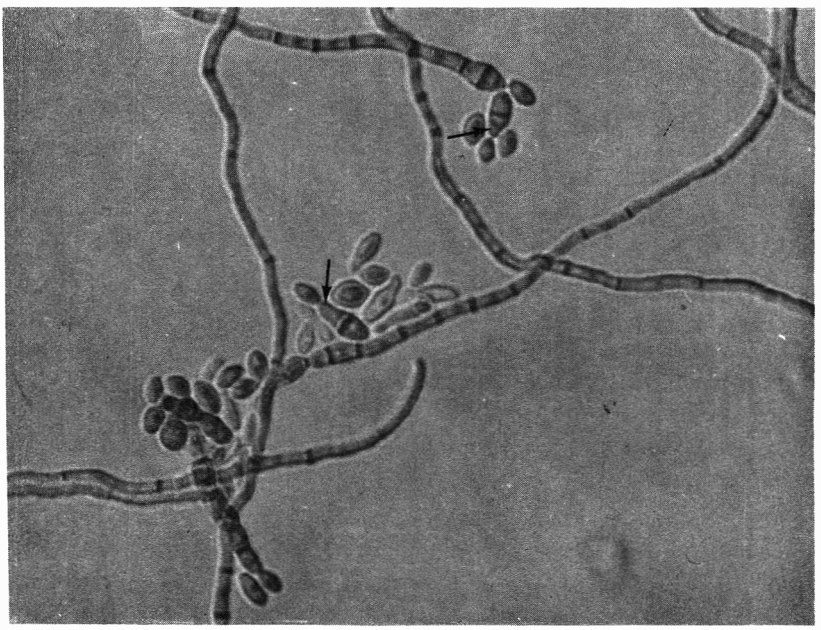

Figure 8. Annelloconidia (arrows indicate annellides) and hyphae with dark septa (960×).

Fungal isolates were recovered from 65, 77 and 65% of the cultures originating from the group 1, 3 and 4 fish respectively. The fungus appeared as early as 7 days after inoculation of the mouldy plaque onto mycosel agar. The colonies were initially shiny, yeast-like and greyish black. On aging they turned olivaceous or light grey with abundant aerial hyphae (Fig. 6). On transfer to Sabouraud dextrose (2%) agar (Merck) at 37°C and to Czapek-Dox agar (Merck) at 28°C, the yeast colonies lost their lustre and showed submerged hyphal growth at the periphery. Slide cultures of the isolates in corn-meal agar (Oxoid) at 25°C showed plentiful annelloconidia with prominent dark septa, hyaline and dematiaceous hyphae, terminal and intercalary fission cells (Figs 7 and 8). Based on their macro- and micro-morphology at 25 and 37°C, the isolates were identified as *E. werneckii* Arx 1970.

Five isolates were chosen randomly for biochemical studies. All hydrolysed casein, utilized sodium nitrate, did not liquefy gelatin, nor utilize paraffin, starch or protein. Since the isolates were recovered from salted fish, their tolerance to salt was examined. All five isolates showed normal growth on Sabouraud agar with NaCl concentrations varying from 10% (w/v) to saturation.

Attempts at isolating *E. werneckii* from the salts used in the brine preparations were made. One-gram granules of the sterile and unsterile salt were inoculated into quintuplicate tubes of mycosel agar, mycosel broth (BBL), Sabouraud dextrose (2%) agar and Sabouraud dextrose (2%) broth (Merck) at 25°C. There was no fungal growth in any of the media at the end of two months.

Discussion and conclusions

The black fungus *E. werneckii* is the causative agent of the superficial human mycosis tinea nigra which is most prevalent in the tropics. The fungus infects the stratum corneum in the form of septate, dematiaceous mycelium (Ajello, 1978). Its saprophytic nature has never been confirmed (Rippon, 1974), nor its tolerance to salinity suspected. The occurrence of *E. werneckii* on air-dried salted fish attests to the ability of the fungus to thrive on saline substrate. Our subsequent experiments confirmed its tolerance to medium with salt concentrations up to saturation. *E. werneckii* was implicated for the first time as a halophilic saprophyte responsible for food spoilage.

The curing process by 20–30% brine for 5 days was apparently sufficient to prevent general bacterial and fungal growth. The experimental drying condition i.e. drying in the shade, most probably predisposed the salted fish to *E. werneckii* colonization. Sterility of the salt exerted a difference only on the magnitude, but not the nature of contamination, since the fungus was recovered from all fish regardless of the difference in brine preparation. The recovery of *E. werneckii* as the sole colonizer must be attributed to both the halophilic and ubiquitous nature of the fungus.

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Effect of refining on the physical and chemical properties of cashewkernel oil

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Summary

Crude cashewkernel oil was subjected to refining by degumming, alkali-refining, bleaching and deodorization. The crude as well as the oil at every stage of refining was characterized. Results indicate an improved quality oil on refining mainly in terms of increased stability. The polyunsaturated fatty acid level and some of the identity characteristics of the oil were not significantly altered by the refining process.

Introduction

The major commercial product of the cashew tree is the cashewnut which is mainly exploited for its kernel. The kernel which constitutes 20–25% of the nut (Wilson, 1975) is the main product of the Nigerian cashew industry where it is processed (roasted and salted) for direct consumption. Being an oilseed, attempts made at exploiting the kernel for vegetable oil resulted in the isolation of an attractive golden-yellow oil (Ojeh, 1980) which would go some way in supplementing the few exploited sources of locally available vegetable oil.

The process of refining edible oils comprises degumming, alkali-refining (or neutralization), bleaching and deodorization. Lall & Slinger (1974) reported that refining affected the nutritional value of rapeseed oils whereas Wilding, Rice & Mattil (1963) found that the overall quality of soyabean and cottonseed oil was not affected by alkali-refining, bleaching and deodorization.

The objective of this work therefore was to determine to what extent refining will affect the quality of cashewkernel oil with reference to its physical and chemical characteristics. In this paper the term 'refined' refers to the complete

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series of treatments, i.e. degumming, alkali-refining, bleaching and deodorization.

Materials and methods

Supply of raw cashewnuts was from Eleiyele Cashew Factory, Ibadan and Agronomy Division, Cocoa Research Institute of Nigeria, (CRIN), Ibadan.

The manual isolation of the kernel from the nut and petroleum ether extraction of oil from the kernel have been described (Ojeh, 1980).

The crude cashewkernel oil (CCO) was refined by four laboratory scale refining processes. (i) It was degummed by treatment with water and centrifugal separation to effect removal of the dirty white gelatinous precipitate from the oil. (ii) Alkali-refining was carried out with 0.1 N aqueous sodium hydroxide. The mixture was allowed to separate overnight in a funnel, the lower soap layer separated and the oil washed with water to eliminate the last traces of soap. (iii) The oil was bleached by treatment with 1% of its weight of activated charcoal at 80–100°C. (iv) It was deodorized with steam under high vacuum for 2 hr. The refined oil was stored under nitrogen in the refrigerator.

Analysis

The crude oil as well as the oil at every stage of refining (degummed oil, DCO; alkali-refined oil, ACO; bleached oil, BCO; and refined oil, RCO) were analysed.

The refractive index (RI) of the oil samples were determined at 20°C with a Carl-Zeiss refractometer. The ash content, saponification value (SV), unsaponifiable matter (UM), free fatty acid (FFA), and Wij's iodine value (IV) of the oil samples were determined by the British Standard Institution (BSI) methods (British Standard, 1958). The American Oil Chemists Society's (AOCS) method (as in Osisanya, 1976) was used for the peroxide value (PV) determinations while anisidine value (AV) determination was by the IUPAC draft method (as in Osisanya, 1976). Moisture and volatile matter content and melting point were determined by the methods of the AOAC (1970). Copper and iron content were determined by the boiling acid method (Analytical Methods Committee, 1971). The fatty acid composition of the oil samples was also determined. Esterification was by a modified AOAC method using petroleum ether (Analar, BP 80–100°C) for extraction of the acids instead of heptane (Harborne, 1973). An aliquot of the methyl esters in petroleum ether was injected into the inlet port of a Pye series 104 model 4 gas chromatograph with the following operating conditions and characteristics:

Column (glass, coiled)	1.5 mm × 4 mm i.d.
Packing	10% diethylene glycol succinate on diatomite CAW.

Carrier gas	Nitrogen
Inlet pressure	15 psi
Flow rate	25 ml min^{-1}
Oven temperature	170°C isothermal
Detector temperature	210°C
Detector (type)	Flame ionization
Attenuation	2×10^{4}

Peak areas were calculated by multiplying height of peak by width at half height (Christie, 1973) and percentage of fatty acids according to Gurr & James (1971). Methyl esters were identified by comparison with standards.

Results and discussion

In considering the quality of oils and fats, major factors concerned are (a) standards for oils and fats in terms of identity characteristics (e.g. RI, IV, SV, UM) (b) stability (c) nutritional aspects concerning e.g. level of polyunsaturates and (d) toxicological status.

It is known that in oil refining the goal is the removal of all undesirable substances that may have adverse effects on the oil, such as gums, pro-oxidants (e.g. FFA and trace metals) sterols, carotenoid pigments, tocopherols, oxidized materials and breakdown products. Other factors such as odours and colours which are sometimes desirable to Nigerians (e.g. the orange colour of palm oil) are also usually lost in refining especially during deodorization and bleaching respectively.

From Table 1, refining does not appear to have any significant effect on the RI, melting point and IV of the oil samples since there is no definite trend in

Table 1. Some physical and chemical characteristics of crude (CCO), degummed (DCO), alkali-refined (ACO), bleached (BCO) and refined (RCO) cashewkernel oil

Characteristics	Oil samples				
	CCO	DCO	ACO	BCO	RCO
Refractive Index at 20°C	1.4683	1.4645	1.4651	1.4665	1.4681
Ash content (% dry weight)	0.0712	0.0087	0.0086	0.0080	0.0074
Saponification value (mg KOH/g sample)	188.9	188.7	188.4	188.0	187.1
Unsaponifiable matter (% by weight)	0.61	0.60	0.57	0.56	0.51
Free fatty acid (% oleic acid)	1.19	1.02	0.32	0.27	0.23
Iodine value (Wij's, 30 min)	81.98	79.40	82.19	81.63	82.18
Peroxide value (mEq/kg sample)	0.49	0.45	0.39	0.31	0.14
Anisidine value	5.84	3.35	3.14	3.13	2.82
Melting point (°C)	16.0–17.0	16.2–17.0	16.4–17.05	16.25–17.10	16.23–17.00
Moisture and volatile matter (% dry weight)	0.49	0.49	0.45	0.40	0.20
Copper content (ppm)	3.00	1.10	1.05	1.05	1.05
Iron content (ppm)	8.25	5.50	5.50	5.50	4.00

variation and also no significant differences between the values for CCO and RCO. The ash content and AV of the samples decrease with refining mainly after degumming. The decrease in FFA is most significant after alkali-refining as expected, and that of PV is most significant after deodorization. The moisture and volatile matter content of the samples was not altered by degumming but was significantly decreased after deodorization probably because volatile impurities are mostly eliminated during deodorization. The SV and UM only show slight decrease with refining, this is likely due to their being indirectly affected by refining as such. The copper and iron contents decrease with refining especially after degumming (and deodorization for the iron only). There is no constant general trend in the variation of the percentage of individual fatty acid methyl esters (FAME) between the CCO and RCO samples since, whereas there is a decrease in most cases, with oleic there is an increase, but in all cases the variation tends to be minimal; hence it is not significant (Table 2). Likewise during refining, there is no consistent trend in variation of FAME. With the percentage total saturated and unsaturated there tends to be a general decreasing trend with the former while the latter increased; but the changes are minimal and hence, insignificant. The unsaturated:saturated ratio bears this out since it is constant between DCO and ACO and also between BCO and RCO. Even the seeming increase between that of CCO and RCO is insignificant in view of the wide fluctuation among FAME contents of samples.

Table 2. Fatty acid composition of CCO, DCO, ACO, BCO and RCO

Fatty acid (%)	Oil samples				
	CCO	DCO	ACO	BCO	RCO
Palmitic (16.0)	10.40	9.18	9.94	9.32	10.33
Palmitoleic (16.1)	0.56	0.20	0.62	0.40	0.47
Stearic (18.0)	8.35	9.34	8.53	8.90	7.84
Oleic (18.1)	62.64	63.32	64.40	64.56	64.01
Linoleic (18.2)	18.05	17.97	16.51	16.82	17.35
Total saturated	18.75	18.52	18.47	18.22	18.17
Total unsaturated	81.25	81.49	81.53	81.78	81.83
Unsaturated:saturated ratio	4.3	4.4	4.4	4.5	4.5

The refining process, therefore, may be said to have affected the indices of stability mainly as reflected in the decrease in values for the trace metals (copper and iron), FFA, PV, AV and moisture and volatile matter content. In effect a more stable oil was obtained upon refining as shown by these indices, since the lower they are the less readily susceptible an oil is to oxidative deterioration and hence more stable.

The decreases in SV, ash and UM are as a result of the changes in the aforementioned indices of stability, and they therefore indicate the nature of the refined oil. Refining does not appear to have any significant effect on the level of

polyunsaturates in the cashewkernel oil, since no general trend is observed in the variation in linoleic acid (the main polyunsaturated fatty acid) content of the oil samples. This is desirable in view of the nutritional significance of polyunsaturates in the diet (Swoboda, 1974).

The non-significant effect on the RI, melting point and IV is due mainly to the fact that refining did not affect the unsaturated fatty acid content and other components these characteristics may depict.

Conclusively, refining confers a notable improvement in quality on cashewkernel oil mainly in terms of increased stability.

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Influence of temperature and pressure on hydrogenation of low erucic acid rapeseed oil (Zephyr)

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Summary

Low erucic acid rapeseed oil (Zephyr) was hydrogenated at 200, 170 and 140°C and pressure of 48 and 303 kPa. The hydrogenated oils were characterized by determination of the iodine value, trans-isomer content, fatty acid composition, dropping point and solid fat content. From the change in iodine value and trans-isomer content, the specific isomerization index (SII) was determined. The SII was generally lower at high pressure and low temperature. The hydrogenation reaction rate increased with temperature as well as pressure. Selectivity ratios for the hydrogenation reaction were more influenced by pressure than by temperature, the highest selectivity ratios were observed at the lower pressure.

Introduction

Rapeseed has become the major oilseed crop in Canada and through extensive plant breeding research several low-erucic acid varieties have been developed (Downey, 1978). It has been shown (deMan, 1978) that hydrogenation conditions may have an effect on the polymorphic stability of the hydrogenated oil and the margarine manufactured from it. It was shown that the trans-isomer content of the hydrogenated oil was related to polymorphic stability. It is well known that high temperature, high catalyst concentration, low pressure and low rate of agitation favour the formation of high levels of trans-isomers (Coenen, 1976). Much of the research on this subject has been conducted with soybean or cottonseed oil (Swern, 1964). Since the development of the low-erucic acid rapeseed varieties effectively resulted in a new type of oil, and virtually no

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published information is available on the hydrogenation of this oil, it was decided to study the effect of temperature and pressure on the composition and properties of the oil.

Experimental

The oil used in this study was commercially refined and bleached and obtained from *Brassica napus* var. Zephyr. Hydrogenation was carried out in a Parr Pressure Reaction Apparatus Series 4500 using a 2-l bomb and a charge of 600 ml of oil. The catalyst used was Harshaw NI-3609, a dry reduced nickel catalyst with approximately 25% nickel content. The catalyst was used at a level of 0.2% by weight of the oil corresponding to about 0.05% nickel. Samples were withdrawn from the reactor at regular intervals through the sampling valve. Fatty acid composition was determined by the method of Shehata & deMan (1970) using a Varian model 1400 gas-liquid chromatograph equipped with hydrogen flame ionization detector. Column length was 125 cm, diameter 2 mm, carrier gas flow 30 ml/min and column packing was 15% DEGS on Chromosorb RZ 60–80 mesh. Fatty acids reported in this paper are those with 16 or 18 carbon atoms. The oil also contains small amounts (1–2%) of eicosenoic and decosenoic acid. Iodine value was determined by the Wijs method, AOCS official method Cd 1–25. Trans-isomer content was determined by infra-red spectrophotometry, AOCS tentative method Cd 14–61, using a Beckman model 4300 infra-red spectrophotometer. Dropping point of those samples which were solid at room temperature was determined with the Mettler method as described by Mertens & deMan (1972a). Solid fat content was determined by continuous wave wide-line nuclear magnetic resonance using a Newport Quantity Analyzer as described by Mertens & deMan (1972b).

Results and discussion

Hydrogenation conditions selected included pressures of 48 and 303 kPa and temperatures of 140, 170 and 200°C. Iodine values, dropping points and trans-isomer contents of the samples hydrogenated at 200°C are presented in Tables 1 and 2. The iodine value of the oil before hydrogenation was 111.1. Hydrogenation at 200°C and 48 kPa for 60 min resulted in an iodine value of 74.9 whereas only 30 min at 200°C and 303 kPa lowered the iodine value to 61.2. An iodine value of 75.3 was obtained after only 15 min of hydrogenation at 200°C and 303 kPa. At these similar iodine values, the dropping point obtained at 303 kPa was lower and the trans-isomer content was also considerably lower.

Results of hydrogenation at 170°C are presented in Tables 3 and 4. At a pressure of 48 kPa, it took 150 min to reach an iodine value of 71.1 and trans-content of 42.2%. At 303 kPa an iodine value of 70.1 was reached in 45

Table 1. Iodine value, dropping point, trans-isomer content and specific isomerization index (SII) of rapeseed oil (Zephyr), hydrogenated at 200°C and hydrogen pressure of 48 kPa

Time (min)	Iodine value	Dropping point (°C)	Trans %	SII
8	99.2	—	10.0	84
15	93.3	—	14.8	83
21	91.9	—	20.0	104
27	80.9	—	27.1	90
33	80.5	—	30.3	99
39	80.2	—	32.0	104
45	79.7	24.0	33.9	108
52	76.3	28.0	36.2	104
60	74.9	30.3	41.1	114

Table 2. Iodine value, dropping point, trans-isomer content and specific isomerization index (SII) of rapeseed oil (Zephyr), hydrogenated at 200°C and hydrogen pressure of 303 kPa

Time (min)	Iodine value	Dropping point (°C)	Trans %	SII
3	104.2	—	9.1	132
6	95.3	—	14.2	90
8	92.2	—	19.3	102
10	82.2	—	23.9	83
13	78.6	24.5	31.0	95
15	75.3	29.0	34.8	97
17	71.2	33.6	35.7	89
20	68.3	37.2	37.6	88
23	66.4	39.0	38.0	85
26	65.3	41.5	38.1	92
30	61.2	45.1	38.5	77

Table 3. Iodine value, dropping point, trans-isomer content and specific isomerization index (SII) of rapeseed oil (Zephyr), hydrogenated at 170°C and hydrogen pressure of 48 kPa

Time (min)	Iodine value	Dropping point (°C)	Trans %	SII
20	100.0	—	12.7	114
35	93.1	—	19.5	108
50	87.1	—	24.7	103
65	83.0	—	29.5	105
80	80.9	—	32.3	107
100	76.3	29.3	36.4	105
125	74.9	29.8	40.4	112
150	71.1	33.7	42.2	106

Table 4. Iodine value, dropping point, trans-isomer content and specific isomerization index (SII) of rapeseed oil (Zephyr), hydrogenated at 170°C and hydrogen pressure of 303 kPa

Time (min)	Iodine value	Dropping point (°C)	Trans %	SII
4	101.4	—	4.5	46
7	100.5	—	8.6	81
10	96.2	—	14.9	100
13	88.2	—	22.7	99
16	87.5	—	26.1	111
19	86.5	—	29.4	120
22	86.1	—	31.5	126
25	82.2	—	32.3	112
29	79.4	24.8	33.7	106
34	76.5	31.7	35.7	103
39	72.6	34.3	36.6	94
45	70.1	35.8	38.0	93

min. The trans-content was lower at 303 kPa pressure but the dropping point was higher.

Hydrogenation at 140°C resulted in the figures in Tables 5 and 6. At 48 kPa the reaction rate was extremely low, an iodine value of 83.4 was reached after 240 min of hydrogenation. None of these samples was sufficiently solid at room temperature to determine the dropping point. At 303 kPa and 140°C the iodine value was lowered to 73.6 after 110 min of hydrogenation. After 83 min an iodine value of 82.9 was obtained. At this iodine value the trans-content was

Table 5. Iodine value, trans-isomer content and specific isomerization index (SII) of rapeseed oil (Zephyr), hydrogenated at 140°C and hydrogen pressure of 48 kPa

Time (min)	Iodine value	Trans %	SII
30	106.1	4.2	84
60	105.5	6.1	109
90	102.2	7.2	81
105	100.5	8.4	79
130	96.0	11.3	75
150	93.9	13.8	80
180	90.3	17.1	82
210	88.4	20.3	89
240	83.4	23.5	85

Table 6. Iodine value, dropping point, trans-isomer content and specific isomerization index (SII) of rapeseed oil (Zephyr), hydrogenated at 140° and hydrogen pressure of 303 kPa

Time (min)	Iodine value	Dropping point (°C)	Trans %	SII
17	105.7	—	4.6	85
24	102.5	—	6.5	76
30	99.4	—	8.1	69
35	98.6	—	9.0	90
41	97.4	—	11.2	82
46	93.4	—	13.7	77
55	89.1	—	15.9	72
63	88.2	—	18.0	79
72	87.0	—	19.3	80
83	82.9	—	21.3	76
95	80.7	21.4	24.4	80
110	73.6	30.6	26.6	71

slightly lower than that obtained at 48 kPa. Puri (1978) has described the use of the specific isomerization index (SII) to characterize the hydrogenation process. The SII is defined as the number of trans-bonds formed per unit reduction of iodine value. The SII was calculated from the relationship

$$\text{SII} = \% \text{ trans} \times 100/\Delta\text{IV}$$

where ΔIV = drop in iodine value

The SII values are included in Tables 1–6. At 200°C the SII values were generally lower at 303 kPa pressure than at 48 kPa. At 170°C the pattern is not consistent the SII value was more variable at the higher pressure. At 140°C the SII values were generally lower than at the higher temperatures and with slightly lower values at the higher pressure. These observations follow the general rules described by Coenen (1976).

The fatty acid composition of selected samples hydrogenated at 200, 170 and 140°C and 48 and 303 kPa is presented in Table 7. From the changes in fatty acid composition during hydrogenation, the selectivity ratio can be determined (El-Shattory & deMan, 1980). The selectivity ratios for the hydrogenation of the oil at different temperatures and pressures are listed in Table 8. Selectivity increases with temperature and decreases with pressure (Coenen, 1976). The results indicate higher selectivity ratios at the lower pressure especially at 200 and 140°C. The effect of temperature was much less apparent.

The influence of temperature and pressure on the rate of the hydrogenation reaction is presented in Table 9. The reaction rates were greatly influenced by these factors and increased with temperature as well as with pressure. The

Table 7. Fatty acid composition of rapeseed oil (Zephyr) hydro... different conditions of temperature and pressure

Temp (°C)	Pressure (kPa)	Time (min)	Iodine value	Fatty acids (wt %)				
				16:0	18:0	18:1	18:2	18:3
—	—	0	111.1	5.0	2.9	58.3	22.4	10.2
200	48	21	91.9	4.5	3.1	74.4	13.5	2.9
200	48	39	80.2	4.5	5.2	79.4	7.7	1.9
200	48	60	74.9	4.5	10.0	77.5	4.7	1.3
200	303	10	82.2	4.6	3.8	80.2	8.0	2.0
200	303	20	68.3	4.5	16.0	70.5	5.1	2.2
200	303	30	61.2	4.2	20.7	63.6	5.0	1.7
170	48	50	87.1	4.7	4.4	72.4	13.5	3.0
170	48	100	76.3	4.7	8.0	76.5	7.5	2.0
170	48	150	71.1	4.6	13.5	73.8	5.5	1.6
170	303	13	88.2	4.9	5.0	71.6	12.8	3.4
170	303	29	79.4	4.6	11.4	74.2	6.7	1.9
170	303	45	70.1	4.6	15.1	70.9	6.0	1.7
140	48	90	102.2	5.1	4.2	59.6	21.0	8.1
140	48	180	90.3	4.4	5.0	68.4	16.0	4.3
140	48	240	83.4	4.8	6.8	68.8	13.7	3.6
140	303	35	98.6	5.1	4.4	66.2	19.3	5.1
140	303	72	87.0	4.5	7.0	70.6	13.8	2.4
140	303	110	73.6	4.6	14.8	71.5	7.2	0.9

Table 8. Effect of hydrogenation conditions on the selectivity ratio of rapeseed oil (Zephyr)

Temp (°C)	Pressure (kPa)	Time (min)	S–S_0	L/L_0	Selectivity ratio
200	48	60	6.76	.066	13.8
200	303	30	17.81	.113	5.0
170	48	150	10.60	.093	8.6
170	303	45	12.19	.119	7.2
140	48	240	3.91	.459	13.9
140	303	110	11.85	.179	6.9

Table 9. Effect of temperature and pressure on the reaction rate of the hydrogenation of rapeseed oil (Zephyr)

Temp. (°C)	Pressure (kPa)	Final reaction rate IV/min	Reaction rate at IV = 75 IV/min
140	48	0.12	—
170	48	0.27	0.29
200	48	0.60	0.60
140	303	0.34	0.34
170	303	0.91	1.00
200	303	1.60	2.41

rates were calculated for the end of each experiment as well as for reaching a common iodine value of 75. The general pattern is similar but the rate becomes much higher for the highest temperature and pressure conditions.

Table 10. Solid fat content of rapeseed oil (Zephyr), hydrogenated at different temperatures and pressures and 0.2% catalyst concentration

Temp. (°C)	Pressure (kPa)	Time (min)	Iodine value	Solid fat (%) at temp (°C)					
				0	5	10	15	20	25
200	48	39	80.2	30.6	28.6	20.6	10.8	4.3	2.2
200	48	60	74.9	40.5	38.3	37.5	28.5	18.1	9.8
200	303	20	68.3	57.6	54.5	46.3	36.9	26.1	20.5
200	303	30	61.2	71.2	68.0	65.5	58.9	49.1	40.7
170	48	100	76.3	39.2	36.5	28.9	19.0	10.6	6.4
170	48	150	71.1	56.3	54.2	49.8	42.6	33.2	22.9
170	303	29	79.4	44.4	41.2	33.5	24.0	15.1	10.3
170	303	45	70.1	53.8	52.6	49.8	42.1	32.3	23.0
140	48	240	83.4	11.3	9.8	8.5	6.8	4.9	3.7
140	303	110	73.6	29.8	28.1	26.0	19.6	16.7	12.8

The solid fat contents of the hydrogenated oils as determined by continuous wave wide-line NMR is presented in Table 10. These data together with the dropping point values included in Tables 1–6 provide information about the physical characteristics of the hydrogenated oils. The level of stearic acid increased sharply when an iodine value of about 70 was reached and this is reflected in the increased solid fat content at 25°C. The increase in solid fat content during hydrogenation represents the combined effect of increasing saturation as well as increasing trans-isomer levels. Some limited data on formation of trans-isomers during hydrogenation of rapeseed oil were provided by Kurucz-Lusztig, Prepostefy & Jaranek *et al.* (1978). Hydrogenation was performed at 180–200°C, but the oils used contained much higher erucic acid levels than the Zephy oil used in the present study.

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Hydrogenation of low erucic acid rapeseed oil (Zephyr) under selective conditions

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Summary

Low erucic acid rapeseed oil (Zephyr) was hydrogenated under selective conditions, 200°C, 48 kPa pressure, with 0.2% of five different commercial nickel catalysts and the standard catalyst of the American Oil Chemists' Society. From the change in iodine value, the relative activities of the catalysts were calculated. The trans-isomer levels were determined by infra-red spectroscopy and the specific isomerization index calculated. Catalysts with the lowest activity resulted in the highest level of trans-isomers. From the change in fatty composition, the selectivity ratio was calculated. Physical properties were measured by determination of dropping point and solid fat content. The commercial catalysts differed widely in their action on the hydrogenation of Zephyr oil.

Introduction

In a previous report (El-Shattory, deMan & deMan, 1981a), the effect of temperature and pressure on the hydrogenation of low erucic acid rapeseed oil (Zephyr) has been described. The development in Canada of low erucic acid rapeseed cultivars has resulted in oils with erucic acid levels in the 1–2% range. In addition to breeding cultivars of low erucic acid content, attention is being focussed on lowering the level of glucosinolates in the seed. The hydrogenation behaviour of the oils obtained from these cultivars is undoubtedly influenced by the fatty acid composition of the oil as well as by

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the presence of sulphur compounds which may act as catalyst poisons. The newer cultivars of low erucic acid rapeseed oil are also lower in total sulphur content and catalyst poisoning by sulphur compounds is no longer an important problem (El-Shattory, deMan & deMan, 1981b). There is little published information on the hydrogenation behaviour of low erucic acid rapeseed oils. One recent paper (Kurusz-Lusztig, Prepostefy & Jaranek, 1978) deals with the industrial hydrogenation of three rapeseed oils with erucic acid contents of 51.8, 23.7 and 8.7%. These oils are not similar to the ones now produced in Canada. The present study deals with the effect of a number of different commercial catalysts on the hydrogenation of low erucic acid rapeseed oil using selective hydrogenation conditions.

Experimental

The oil was commercially refined and bleached and obtained from *Brassica napus* var. Zephyr. Hydrogenation was carried out in a Parr Pressure Reaction Apparatus Series 4500 using a 2-l bomb and a charge of 600 ml of oil. The catalysts used were five different commercial nickel hydrogenation catalysts (coded A–E) including four of the dry reduced type and one of the wet reduced type and the standard catalyst supplied by the American Oil Chemists' Society (coded S). Catalysts were used at a level of 0.2% of the weight of the oil, corresponding to about 0.05% nickel. Selective hydrogenation conditions were used, temperature 200°C and hydrogen pressure 48 kPa.

Methods of analysis for the hydrogenated oils were as described previously (El-Shattory, deMan & deMan, 1980).

Results and discussion

The change in iodine value as a function of time and using six different catalysts is indicated in Table 1. From these figures, the catalyst activity can be calculated (Table 2). The activity of catalysts A, B and E was similar, that of catalysts C and D somewhat higher and the standard catalyst was the least active under the conditions employed. The trans-isomer levels formed during the hydrogenation with different catalysts are listed in Table 3. The proportion of trans-isomers formed per unit of iodine value reduction has been used to characterize hydrogenation reactions (Puri, 1978) and is termed specific isomerization index (SII). The SII for the selective hydrogenation of Zephyr oil with the different catalysts is presented in Table 4. The standard catalyst had the highest SII. Low activity catalysts appear to result in high levels of trans-isomers in the selective hydrogenation of Zephyr oil.

The change in fatty acid composition during selective hydrogenation is presented in Table 5. There was considerable variation in the levels of 18:3

Table 1. Decrease in iodine value during hydrogenation of rapeseed oil (Zephyr). Selective conditions and 0.2% catalyst concentration. Initial iodine value 111.1

Time (min)	Catalyst A	B	C	D	E	S
8	102.6	99.3	99.8	99.2	102.0	109.3
15	97.6	93.2	98.9	91.1	95.9	105.1
21	92.6	89.0	92.8	86.3	91.6	102.2
27	86.9	85.3	89.9	83.9	87.5	100.0
33	84.2	83.7	85.1	81.3	86.5	95.6
39	81.9	82.6	83.6	80.4	85.8	94.0
45	81.4	81.5	81.1	78.2	84.4	91.8
52	79.3	80.6	78.5	76.2	82.7	91.4
60	76.2	77.8	71.8	69.1	79.6	89.5

Table 2. Catalyst activity after 60 min of hydrogenation of rapeseed oil (Zephyr). Selective conditions and 0.2% catalyst concentration

Catalyst	Activity Δ IV/min
A	0.58
B	0.56
C	0.66
D	0.70
E	0.53
S	0.36

Table 3. Trans-isomer content (%) of hydrogenated rapeseed oil (Zephyr). Selective conditions and 0.2% catalyst concentration

Time (min)	Catalyst A	B	C	D	E	S
8	9.3	12.2	12.5	12.8	9.7	7.5
15	11.6	19.0	17.1	17.8	12.2	11.9
21	12.7	23.0	21.0	24.4	21.8	17.9
27	20.2	29.8	23.6	25.5	25.9	21.9
33	23.8	32.5	26.6	32.1	28.0	24.8
39	27.2	36.0	27.9	33.7	29.4	27.6
45	27.5	37.0	31.4	36.8	30.1	28.8
52	29.7	38.9	32.9	38.3	31.7	31.0
60	36.7	42.7	33.2	41.6	35.2	33.3

Table 4. Specific isomerization index (SII) for the hydrogenation of rapeseed oil (Zephyr). Selective conditions and 0.2% catalyst concentration

Time (min)	Catalyst					
	A	B	C	D	E	S
8	1.09	1.03	1.11	1.08	1.07	4.17
15	0.86	1.06	1.40	0.89	0.80	1.98
21	0.69	1.04	1.15	0.98	1.12	2.01
27	0.83	1.16	1.11	0.94	1.10	1.97
33	0.88	1.19	1.02	1.08	1.14	1.60
39	0.93	1.26	1.01	1.10	1.16	1.61
45	0.93	1.25	1.05	1.12	1.13	1.49
52	0.93	1.28	1.01	1.10	1.12	1.57
60	1.05	1.28	0.84	0.99	1.12	1.54

Table 5. Fatty acid composition of hydrogenated rapeseed oil (Zephyr). Selective conditions and 0.2% catalyst concentration

Time (min)	Catalyst	Iodine value	Fatty acids (wt %)				
			16:0	18:0	18:1	18:2	18:3
0	—	111.1	5.1	5.8	60.8	18.0	8.3
21	A	92.6	4.5	5.6	70.8	13.6	2.3
21	B	89.0	4.6	5.5	76.1	8.8	3.0
21	C	92.8	4.6	5.8	74.8	10.1	2.6
21	D	86.3	4.5	5.6	78.6	7.5	1.7
21	E	91.6	5.1	5.6	72.4	12.0	2.3
21	S	102.2	5.3	5.5	68.2	16.3	3.9
39	A	81.9	5.2	7.8	77.3	5.5	1.5
39	B	82.6	5.0	7.8	78.6	4.0	2.3
39	C	83.6	5.6	8.1	76.2	4.8	2.3
39	D	80.4	4.6	11.1	74.2	5.5	1.8
39	E	85.8	4.9	8.3	74.3	8.2	1.8
39	S	94.0	4.7	5.9	73.3	12.1	1.9
60	A	76.2	5.4	10.5	76.9	3.8	0.5
60	B	77.8	4.7	12.2	76.5	2.5	1.5
60	C	71.8	4.7	17.5	71.5	2.4	1.6
60	D	69.1	5.2	19.8	68.2	3.1	1.1
60	E	79.6	5.3	10.3	75.5	5.6	0.6
60	S	89.5	4.9	6.2	76.5	9.8	1.0

Table 6. Selectivity ratio for the hydrogenation of rapeseed oil (Zephyr) with different catalysts after 60 min

Catalyst	$S-S_0$	L/L_0	Selectivity ratio
A	4.70	0.21	16.8
B	6.42	0.14	13.5
C	11.71	0.13	7.4
D	13.98	0.17	5.9
E	4.48	0.31	15.3
S	0.40	0.54	114.0

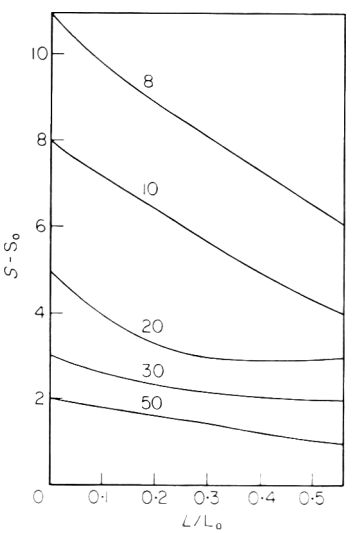

Figure 1. Graph for the determination of selectivity ratio (indicated by numbers on curves) for the selective hydrogenation of low erucic acid rapeseed oil (Zephyr).

fatty acids remaining after 60 min hydrogenation with different catalysts and this was also the case for the levels of 18:2 fatty acids. From the change in fatty acid composition, the selectivity ratio was calculated according to the procedure described earlier (El-Shattory *et al.*, 1980), see Table 6. The selectivity ratio is defined as K_2/K_3, where $K_2 = 1 - L/L_0$ and $K_3 = S - S_0$ (L_0 and S_0 represent the linoleic and stearic acid content of the oil and L and S the levels in the hydrogenated sample). From the data, it is possible to construct a graph as shown in Fig. 1 which enables the value of the selectivity ratio to be determined graphically. The selectivity ratio was highest for the standard catalyst, intermediate for catalysts A, B and E and lowest for catalysts C and D.

The physical characteristics of the hydrogenated oils were characterized by measurement of dropping points and solid fat contents. The dropping points of the solid samples are listed in Table 7. The values obtained for different catalysts show considerable variation. There appears to be some relationship between catalyst activity and dropping point as the most active catalysts resulted in the highest dropping point and the least active catalysts in the lowest dropping points. Similar observations can be made with respect to the solid fat contents (Figs 2–3). Generally, the least active catalysts resulted in the lowest solid fat contents.

Table 7. Dropping point (°C) of rapeseed oil (Zephyr) hydrogenated with 0.2% of different catalysts for 52 and 60 min

Time (min)	Catalyst					
	A	B	C	D	E	S
52	25.2	24.4	28.0	28.3	21.4	21.2
60	28.2	27.8	33.6	33.8	24.1	23.8

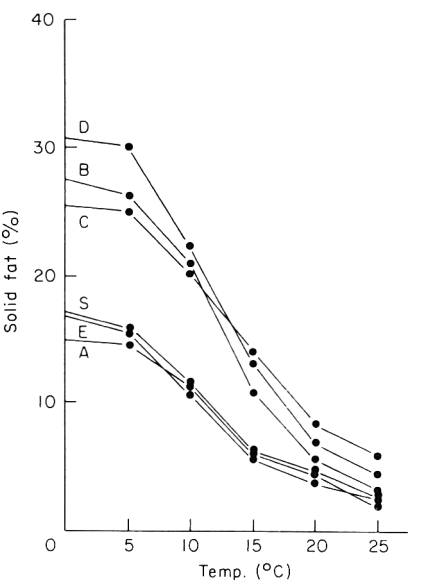

Figure 2. Solid fat content of hydrogenated rapeseed oil (Zephyr). Hydrogenation time 39 min, selective conditions and 0.2% catalyst concentration.

The results presented in this study indicate that various commercial catalysts may yield considerably different results in the hydrogenation of low erucic rapeseed oil (Zephyr).

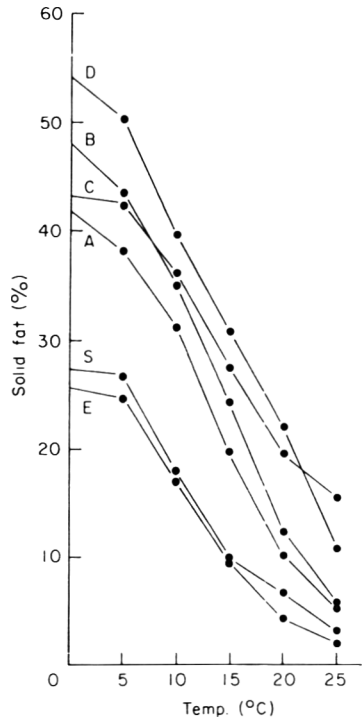

Figure 3. Solid fat content of hydrogenated rapeseed oil (Zephyr). Hydrogenation time 60 min, selective conditions and 0.2% catalyst concentration.

Acknowledgments

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Relationship between colour and brown pigment concentration in orange juices subjected to storage temperature abuse

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Summary

The browning index of orange juices which had been subjected to storage temperature abuse (55°C for up to 40.5 hr) was highly correlated with the CIE tristimulus values as measured on a Neotec Du-Color Colour Difference Meter. The correlation coefficients and the multiple regression equation have been calculated. When the tristimulus values were converted to USDA colour scores, the values obtained were not in the same range as the USDA plastic standards for orange juice. The level of correlation between the colour scores and the browning index was too low to be useful for prediction purposes. When the tristimulus values were converted to Hunter's citrus red and citrus yellow parameters, a significant correlation was found between these parameters and the browning index. However, the level of variation explained by the correlation was again too low to be useful for prediction purposes.

Introduction

One of the most important attributes of citrus juices is their colour. Although traditionally colour assessments of juices have been made subjectively by comparing samples with plastic colour standards (e.g. Anon., 1963), instrumental methods of colour measurement are available (Hidalgo, Rodrigo & Alcedo, 1974). The principles of tristimulus colorimetry were applied in a modified form when the Hunter Citrus Colorimeter was developed, principally for the measurement of citrus juice colour (Hunter, 1967). Recently, tristimulus colour measurement of fruit drinks was made in relation to pasteurization conditions, storage behaviour and type of raw materials used (Van der Heijden *et al.*, 1979).

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Although the applicability of tristimulus colour measurement for pigment concentration is limited because of light scattering by insoluble particles in the juice, a statistically significant relationship could still be of use commercially.

Citrus juices are susceptible to a number of deteriorative reactions during processing and storage, which result in the development of off-flavours and brown pigments. Until recently, methods for the measurement of visual browning changes in juices were not particularly accurate, due mainly to interference from carotenoids (Joslyn, 1957; Karel & Nickerson, 1964). Recently, however, a method for determining browning in citrus products was described (Meydav, Saguy & Kopelman, 1977) which eliminated carotenoid interference and gave good repeatability.

The development of brown pigments is a useful indicator of storage temperature abuse in citrus juices (Nagy & Dinsmore, 1974). Therefore we sought to determine whether or not an instrumental method of colour measurement was sensitive enough to detect changes in orange juice colour as a result of storage temperature abuse, and whether such changes in colour correlated with the development of browning in the juice.

Materials and methods

Orange juices

Six commercial frozen orange juice concentrates (65°Brix) were used, one from Australia (coded A1), three from Brazil (coded B1, B2 and B3) and two from U.S.A. (coded U1 and U2). Although their precise age was unknown, they were all less than 1 year old and had been stored at −18°C since production. The concentrates were diluted with distilled water to 16 ± 0.5°Brix before being subjected to the treatments described below.

Instrumental colour measurements

These were made using a Du-Color Model 220 Colour Difference Meter (Neotec Corporation, Rockville, Maryland, U.S.A.), fitted with an illuminant C light source, the prototype of which has been described by Faulhaber & Witherell (1971). It measures and displays the absolute reflectance of the sample in CIE (Commission Internationale de l'Eclairage) tristimulus values (Y, X, Z). These three values define the colour of the sample. Typically, red, yellow and blue coloured samples have as their largest value X, Y and Z respectively. Orange samples have similar values for X and Y, both of which are much higher than the Z value.

A 30-g sample was used for all measurements. The sample was placed in a covered, optically-clear sample holder, and a white teflon backing block fixed inside the sample cover (Neotec accessories C15 and C16) contacted the upper surface of the sample, thereby giving constant sample geometry. The results are

the mean values of three readings of each parameter taken after thoroughly mixing the sample in the cup each time.

Browning determinations

The method described by Meydav, Saguy & Kopelman (1977) was used: the browning index of the clarified samples was taken as the absorbance at 420 nm as measured in a Spectronic 70 spectrophotometer (Bausch and Lomb). All results are the means of duplicate determinations.

Treatments

Instrumental colour measurements and browning determinations were made on the six orange juices within 2 hr of reconstitution. The juices were then placed in an incubator and held at 55°C for 19 hr, after which time the measurements were again made. The juices were returned to the same incubator for a further 20.5 hr and the measurements repeated.

Results and discussion

The results of the instrumental colour measurements and browning determinations are presented in Table 1. The tristimulus values obtained for the six reconstituted orange juices are similar to those obtained by other workers. For example, Van der Heijden *et al.* (1979) reported average values of 33.9, 34.8 and 6.6 for Y, X and Z respectively for nine commercial orange juice concentrates diluted to 20°Brix. The browning indexes (BI) are greater than those reported by Meydav, Saguy & Kopelman (1977) but this is to be expected since they examined fresh juices whereas the juices used in this study had been concentrated and then reconstituted.

As expected the browning index increased with the time for which the juices were held at 55°C. The readings for BI, Y, X and Z were averaged for the six juices for a given time, and these averages were fitted to zero, half, and first order reaction rate equations. The best fit for all parameters was obtained with the half order equation (Table 2), although because of the limited amount of data, the fit to the other equations was not significantly worse. Furthermore, because of the limited data, only the model for BI was significant at the 5% level. The relationship derived was

$$BI = 0.000157t^{2} + 0.326$$

It has been reported (Van der Heijden *et al.*, 1979) that a decrease in Y is linear with an increase in storage time (i.e. that the reaction is of zero order). It has also been reported (Meffert, 1964) that browning in citrus juices is a half order reaction, and our results support this report.

Table 1. Tristimulus values and browning index of reconstituted orange juices after various treatments

Sample	Tristimulus values Y	X	Z	Browning index ($A_{420\,nm}$)
After reconstitution				
A1	34.9	34.2	8.3	0.300
B1	34.8	33.9	8.4	0.321
B2	31.6	32.1	7.5	0.350
B3	33.4	32.6	8.9	0.295
U1	32.2	33.5	5.9	0.375
U2	35.4	34.6	8.2	0.265
After 19 hr at 55°C				
A1	30.6	30.4	7.8	0.319
B1	30.3	30.0	7.4	0.352
B2	26.4	26.8	6.4	0.485
B3	26.6	26.5	7.3	0.397
U1	28.4	29.4	5.6	0.460
U2	31.8	31.2	8.1	0.348
After 40.5 hr at 55°C				
A1	19.1	19.4	5.9	0.54
B1	19.3	19.8	5.6	0.57
B2	18.1	18.6	5.2	0.60
B3	16.0	16.2	5.2	0.63
U1	19.7	20.5	4.5	0.65
U2	21.0	21.3	6.4	0.495

Table 2. Correlation coefficients for the averages of the tristimulus values and browning index with time of juice treatment, fitted to a half-order reaction rate expression

Tristimulus values Y	X	Z	Browning index ($A_{420\,nm}$)
−0.995	−0.995	−0.994	0.997*

*Significant at $P \leq 0.05$, 1 d.f.

The correlations between the three tristimulus values and the browning index are presented in Table 3. The Y and X values are highly correlated with each other, the regression equation of Y on X being

$$Y = 1.05X - 1.38$$

($r = 0.996$; $P < 0.001$; 16 d.f.). All three tristimulus values were highly

correlated with the browning index, Y being the most highly correlated. The difference between the correlation coefficients is not significant.

Table 3. Correlation coefficients* for tristimulus values and browning index

	Tristimulus values		
	Y	X	Z
X	0.996		
Z	0.839	0.793	
Browning index	−0.956	−0.940	−0.923

*All coefficients significant at $P < 0.001$; 16 d.f.

Multiple regression analysis for the browning index based on the three tristimulus values gave as an estimating equation:

$$BI = 0.0309Y - 0.0402X - 0.065Z + 1.1289 \quad (1)$$

($r = 0.986$; $P < 0.001$; 14 d.f.). However, this was only a marginal improvement in fit over the multiple regression equation based on X and Z:

$$BI = -0.0112X - 0.0450Z + 1.0418 \quad (2)$$

($r = 0.984$; $P < 0.001$; 15 d.f.). In eqn (1) the coefficients of Y and X are not significant at $P = 0.05$, while in eqn (2) the coefficients of X and Z are significant at $P < 0.001$.

Thus there is little advantage in using a colorimeter with three rather than two photodetectors to determine storage temperature abuse in orange juices, since both correlate to a similar extent with the browning index.

The tristimulus data from Table 1 were converted to Hunter's 'citrus red' (CR) and 'citrus yellow' (CY) values using the equations of Hunter (1967):

$$CR = 200\left(\frac{1.277X - 0.213Z}{Y} - 1\right)$$

and $$CY = 100\left(1 - \frac{0.847Z}{Y}\right)$$

The citrus red and yellow values did not correlate as well with the browning index (Table 4) as did the individual Y, X and Z values (Table 3). This could be due to the incorporation of the errors caused by dissimilarity in instrumental geometry or sample background.

Table 4. Correlation coefficients for citrus red, citrus yellow, browning index and tristimulus values

	Y	X	Z	BI
CR	−0.426	−0.346	−0.817*	0.579†
CY	0.665*	0.718*	0.165	−0.462

*Significant at $P < 0.01$; 15 d.f.
†Significant at $P < 0.05$; 15 d.f.

Until the development of suitable instruments, the standard method of colour scoring of orange juice was to compare the colour of the juice with a series of plastic tubes designed specifically for direct visual comparison with test juices. Six plastic tubes were available designated OJ1 to OJ6. The tubes OJ2 to OJ6 respectively were assigned visual colour scores of 40, 39, 37, 36 and 34 (Anon., 1963).

In the instrument developed by Hunter (1976) to measure citrus juice colours, the USDA plastic standards were used for calibration purposes. Using such an instrument, the best single-number measurement of orange juice colour is known as Hunter *a*. It is possible to calculate Hunter *a* values from the Y and X tristimulus values using the expression (Francis and Clydesdale, 1975):

$$a = 1.75\left(0.51\left(\left(\frac{21+0.2Y}{1+0.2Y}\right)\right)(1.02X - Y)\right) \quad (3)$$

The relationship between USDA colour score (CS) and Hunter *a* values has been reported (Edwards *et al.*, 1962) as follows:

$$CS = 39.44 + 0.87a \quad (4)$$

The data in Table 1 were substituted into eqns (3) and (4) to calculate *a* and CS values. The resulting colour scores ranged from a low of 38.8 to a high of 45.1, both of which were for non-heat treated samples. Generally, however, the colour scores increased with an increase in the browning index ($r = 0.653$; $P < 0.01$; 16 d.f.).

Comparing the colour scores obtained by the application of eqns (3) and (4) to our data with published scores for the USDA plastic standards shows that our scores cover a range five points higher. This could be due to the differences in optical geometry and calibration between the Hunter Citrus Colorimeter and the Neotec Du-Color, since the data of Van der Heijden *et al.* (1979) which was also obtained with a Du-Color, converts to give colour scores in the same general range as for our data.

Our results have shown that the storage temperature abuse of orange juices as measured by the browning index can be closely predicted by the CIE tristimulus values as measured on a Neotec Du-Color Colour Difference Meter. Therefore tristimulus values could be used commercially for routine quality control purposes instead of browning index measurements. Although the browning index was significantly correlated with Hunter's citrus red and citrus yellow parameters, the level of variation explained by the correlation was too low to be useful for prediction purposes.

Tristimulus data obtained from a Neotec Du-Color Colour Difference Meter did not convert to colour scores in the same range as the USDA plastic standards for orange juice. While the colour scores correlated significantly with the browning index, for prediction purposes the level was too low to be useful.

Acknowledgments

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Amyloglucosidase and maltase activities in soy sauce fermentations

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Summary

Evidence for the participation of amyloglucosidase and maltase in the koji (mould growth) stage of experimental soy sauce fermentations is presented. Despite the problems associated with distinguishing between these two enzyme activities in a complex mixture, it seems clear that both are present and that maltase increases in concentration throughout the fermentation whereas amyloglucosidase concentrations declined in fermentations conducted for more than 64 hr. TLC showed that maltose quickly disappears from the koji leaving glucose as the only major sugar. Submerged liquid fermentations and incubation of starch solution with crude enzyme extracts from koji both supported the conclusions obtained from experiments with koji.

Introduction

Enzymes involved in experimental soy sauce fermentations have been reported as including sucrase, endo-amylase (α-amylase), proteinases, lipase and phosphatase (Yong & Wood, 1974, 1975, 1976, 1977a,b) and cellulase and exo-amylase (Goel & Wood, 1978); and their activities in hydrolysing the polysaccharides, proteins, fats etc., in the soy bean-wheat mixture have been discussed by these authors.

This report deals with the activities of two other amylolytic enzymes, amyloglucosidase and maltase (α-glucosidase) which have been detected in soy

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sauce fermentations. Amyloglucosidase hydrolyses starch and dextrin to glucose. It breaks down both α-1,4- and α-1,6-linkages and also α-1,3-linkages (Pazur & Ando, 1959: Fogarty, Griffin & Joyce, 1974). Maltase hydrolyses maltose to glucose and also shows some considerable activity towards the α-1,4 linkages in saccharides consisting of several glucose units. This enzyme also acts as a limit dextrinase (i.e. it is able to hydrolyse the α-1,6-linkages of limit dextrin) thus making possible the complete conversion of starch into fermentable sugars (Pazur & French, 1952; Underkofler & Hickey, 1954; de Menezes, 1978). Maltase also splits α-1,2- and α-1,3-linkages (Gutman, 1970).

Pazur & French (1951) described an enzyme of *Aspergillus oryzae* which synthesized a trisaccharide, panose (4-α-isomaltosyl-D-glucose) from maltose and a year later presented evidence for the mechanism of action of this enzyme, a transglucosidase, on maltose. Evidence for the presence of both amyloglucosidase and maltase in an enzyme preparation is given by Pazur & Ando (1959) who studied the action of these enzymes from *A. niger* on starch and malto-oligosaccharides. They showed that purified amyloglucosidase was capable of hydrolysing maltose to glucose but did not effect the synthesis of transglucosylic products. In contrast, the original (non-purified) enzyme preparation was capable of synthesizing transglucosylic products (isomaltose and panose) during the hydrolysis of maltose to glucose. These observations were interpreted as evidence for the presence of a maltase with transfer activity in the non-purified enzyme preparation. The maltase was separated from the amyloglucosidase by chromatography. Although amyloglucosidase itself possessed maltase-type activity, it did not effect transglucosylation reactions.

As far as we know, these two amylolytic enzymes have not been reported in the soy sauce fermentation, although their activity might equate to some or all of the exo-amylase reported by Goel & Wood (1978) but not further characterized as to end product(s) by these authors. In addition to following the production of these enzymes in soy sauce koji fermentation, further studies were carried out on their production in submerged fermentation.

Materials and methods

Koji fermentation was carried out with the mould *Aspergillus oryzae*, strain NRRL 1989. Moromi or soy mash was prepared by mixing koji with 18% sodium chloride solution; lactic acid was added to lower the pH to 4.5 and then the mixture was inoculated with yeast, *Saccharomyces rouxii* strain NRRL, Y-27 and incubated at 40°C for one month (see Yong & Wood, 1976 for details).

Enzyme extracts from koji were prepared by suspending koji samples (approximately 2 g wet weight) in 0.1 M acetate buffer, pH 5.0 and shaking on a Griffin Flask Shaker (Serial No. 9020, Griffin and George Ltd), operating at medium speed for 15 min at 10°C. The suspension was centrifuged at 4000 rpm for 30 min on an MSE 18 Centrifuge at 10°C. Enzyme activities in koji were reported as units per gram dry weight. Determination of dry weights of koji was

based on the method of Jacobs (1951). The soy sauce was diluted five times and used directly as enzyme extract; enzyme activities in moromi fermentations were not assayed.

Submerged fermentation with *A. oryzae* was carried out with Czapek-Dox medium with or without glucose (2.5 g/litre) and starch (1%) as substrate. A wide-neck one-litre conical flask containing 250 ml of medium was inoculated with a spore suspension of *A. oryzae* (1.0×10^8 spores/250 ml medium) and incubated at 30°C for 4 days on an orbital shaker (L.H. Engineering, Buckinghamshire) operating at 200 rpm. A portion of the medium (20 ml) was taken immediately after inoculation and further samples were withdrawn at intervals and immediately centrifuged at 2000 rpm for 10 min on an MSE 18 Centrifuge. Supernatants were used for enzyme assay and reducing sugar determinations.

Amyloglucosidase assay was based on the method of Lineback, Russell & Rasmussen (1969) and determination of glucose was an adaptation of the glucose oxidase method of Barham & Trinder (1972). The enzyme extract was first diluted to give a glucose concentration not exceeding 0.2 mg/ml. The enzyme activity was measured by incubating 1.5 ml of 4% starch solution with 0.5 ml of enzyme extract at 50°C for 30 min. The reaction was terminated by the addition of 8.0 ml of 70% ethyl alcohol and the precipitate formed was removed by centrifugation at 5000 rpm for 15 min on an MSE 18 Centrifuge. A blank was prepared under the same conditions as above except that acetate buffer (0.5 ml) was used instead of enzyme extract. An aliquot (0.2 ml) of the supernatant was used for the determination of glucose. Glucose was determined by incubating 0.2 ml of the sugar solution, 3.0 ml of colour reagent and 1.0 ml of coupler at 37°C for 15 min and absorbance read at 515 nm. The colour reagent was prepared, with modification, from that of Barham & Trinder, by dissolving peroxidase (5 mg), glucose oxidase (50 mg), aminophenazone (35 mg) and sodium azide (50 mg) in phosphate buffer, (100 ml), pH 7.0. This modification of decreasing the amount of peroxidase was necessary to obtain the standard graph for our purpose. The coupler was sulphonated 2,4, dichlorophenol (0.123 M) diluted twenty-five times with distilled water. Glucose present in the enzyme extract was determined separately by treating 0.5 ml of the extract as above and subtracting this value from the enzyme assay. The enzyme unit was that amount of enzyme which produced 1.0 mg glucose in one minute under the above conditions.

Maltase assay was based on determining glucose produced from maltose solution (Pazur & French, 1952; Seetharam, Swaminathan & Radhakrishnan, 1970). Enzyme activity was measured by incubating equal volumes (0.5 ml) of the enzyme extract and maltose (2.0%) at 30°C for 10 min. The reaction was terminated by immersing the reaction tubes in a boiling water bath for 5 min. Distilled water (4.0 ml) was next added to the tubes and 0.2 ml aliquots were removed for determination of glucose. Other assay procedures and definition of the enzyme unit were similar to those described for amyloglucosidase.

Reducing sugar content was determined by the dinitrosalicylic acid (DNS) method of Sumner (1925).

The activities of carbohydrases during the koji and moromi fermentations were followed by thin-layer chromatographic (TLC) analyses. Koji extract for TLC was prepared in the same way as the koji enzyme extract (above) except that water was used instead of buffer. Moromi extract was prepared by filtering the mash through Whatman No. 1 filter paper and diluting the filtrate three times. The soy sauce was also diluted three times. These diluted extracts were applied to TLC plates. The method of Lato *et al.* (1968), was used for chromatographic analyses of low mol. wt carbohydrates with ethyl acetate/acetic acid/methanol/water (60 : 15 : 15 : 10) as solvent system and the spots were detected with naphthoresorcinol reagent (Adachi, 1965), which reacts with sugars to give coloured spots ranging from blue to green-blue against a white background with all the sugars tested except sucrose and fructose which gave crimson spots.

Results and discussion

The levels of amyloglucosidase and maltase activities during koji fermentation are shown in Fig. 1. Amyloglucosidase was detectable after 20 hr of fermentation and started to decline after about the 64th hr, while maltase activity was first detected about 18 hr after inoculation and increased fairly steadily throughout the fermentation. Athough the enzymes rivalled each other during the first two days of fermentation, there was a significant change in the levels after 48 hr of fermentation. The activities of these enzymes were not followed during the moromi fermentation, however the final product, soy sauce, showed only very small amounts of either enzyme (Table 1).

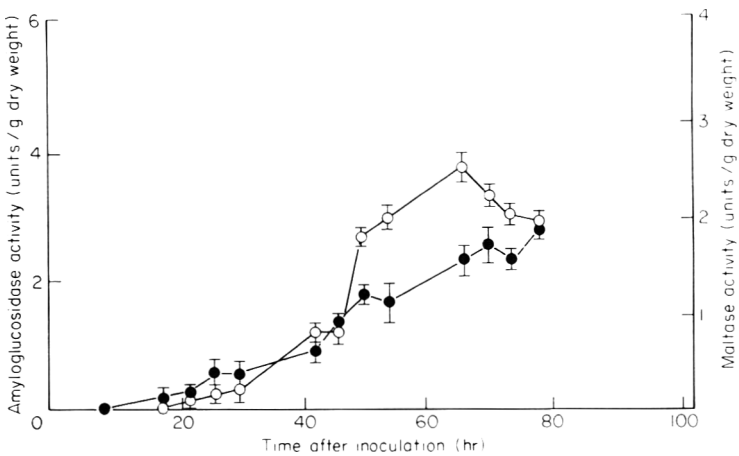

Figure 1. Amyloglucosidase and maltase activities in koji fermentation. ○, Amyloglucosidase; ●, maltase. Each point was derived from three separate experiments and each experiment was determined in duplicate.

Table 1. Amylolytic Enzymes in Soy Sauce

Sample	Amyloglucosidase (units/100 ml)	Maltase (units/100 ml)
Experimental soy sauce	1.00 ± 0.02 (0.03)	1.00 ± 0.01 (0.03)
Tamari soy sauce (commercial)	0	2.00 ± 0.07 (0.06)

These results represent the mean of duplicate determinations for three separate experiments on the experimental soy sauce. Duplicate determinations were performed on a sample of commercially available soy sauce.

Figures in brackets indicate enzyme unit per gram dry weight koji based on addition of 200 ml brine solution to 100 g koji with 35% moisture content.

Chromatographic analysis of koji extract showed that sucrose was converted within 24 hr of fermentation to glucose and fructose, and maltose started to disappear after 24 hr of fermentation; moromi extract and soy sauce both showed glucose as the major sugar present, with only traces of fructose. Other minor spots which appeared in the koji extracts were unidentified.

Submerged fermentation also showed that both amyloglucosidase and maltase were produced by *A. oryzae* and added glucose affected their activities in media with starch as principal substrate (Fig. 2). The presence of glucose in the medium seemed to increase maltase activity in comparison with that in the medium without glucose. Amyloglucosidase activity on the other hand, was higher in the medium without glucose than in that with added glucose. With added glucose in the medium, *A. oryzae* first utilized the glucose and then produced amylolytic enzymes to hydrolyse the starch to reducing sugars.

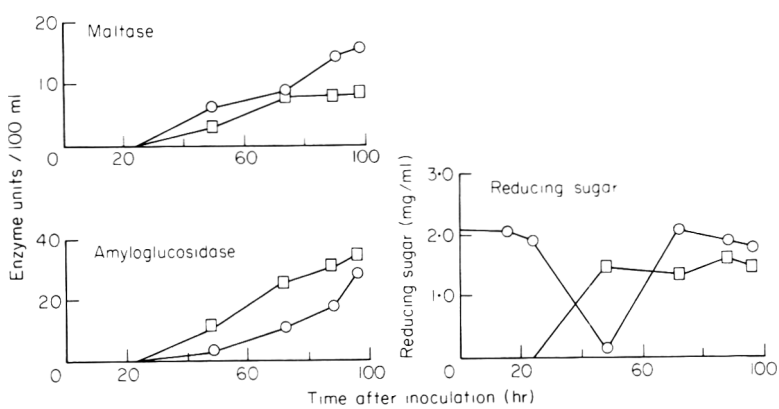

Figure 2. Amyloglucosidase and maltase activities in submerged fermentations with Czapek-Dox medium. ○, Medium with 0.25% glucose and 2% starch; □, medium with 2% starch only. Each point was derived from three separate experiments and each experiment was determined in duplicate.

Crude enzyme extract from koji was incubated with starch. When samples from this incubation were examined by TLC they showed a progressive increase in glucose during a 3-hr incubation and appearance and disappearance of maltose. Maltose level increased for the 90 min of incubation and thereafter started to decrease, which may have been caused by maltase action.

It is difficult to distinguish between maltase and amyloglucosidase activities since the latter has maltase type activity, however in the complex koji fermentation, there is evidence that the fungus, *A. oryzae* produces extracellular amylolytic enzymes which have both amyloglucosidase and maltase activities.

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Gelation property of salt soluble protein of turkey muscle as related to pH*

S. ANGEL AND Z. G. WEINBERG

Summary

The gelation property of the salt soluble proteins of turkey breast and thigh plus drumstick were compared, and the proteins of the latter were found to have greater gelation capacity. The experiments were repeated on three separate occasions during which the pH level of the breast muscle was found to range from 5.8 to 5.95 while that of the leg meat ranged from 6.0 to 6.8.

It is concluded that the different gelation capacities which are apparently related to the initial pH level in the breast and leg meats, could affect palatability and should be taken into account in the development of turkey products.

Introduction

Swift & Berman (1959) reported that the increasing pH values in eight different muscles which ranged from 5.5 to 5.79, were closely correlated with increasing water retention of these muscles. Hamm (1959) found good correlation between the water holding capacity (WHC) and the swelling capacity of cow meat, and between the WHC and the quality of sausage produced from this meat. Shimizu, Simidu & Ikeuchi (1954) obtained increased gel strength in fish muscle by increasing the pH from 5.0 to 7.0.

Trautman (1966b) used the least concentration end-point (LCE) test i.e., determination of the lowest concentration at which a protein will gel, to test pH effects on muscle protein extractability and water binding. When applied to solutions of salt-soluble proteins this test appeared to have greater sensitivity than the WHC or protein extractability tests in evaluating post mortem muscle changes and the accuracy and reproducibility were excellent. Experiments with pre-rigor pH indicated that the initial pH level may determine gelation capacity of the salt-soluble proteins. In post rigor muscles minimum LCEs of 0.7 to 0.8%

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were found in extracts from muscles whose initial pH levels had ranged from 6.25 to 5.8. He concluded that there was a definite relationship between the LCE of the purified extract and the initial muscle pH level.

The object of the present experiments was to determine whether there is a difference in LCE between salt-soluble extracts of breast (white) and leg (dark) meat of turkeys, and whether this is related to post rigor muscle pH levels.

Materials and methods

The gelation capacities of the salt soluble proteins of turkey breast and leg meats were compared. The hand deboned breasts and legs (comprising meat from the thigh and drumstick) were brought from the sales room of a local dressing/packing plant on three occasions and used each time for a repeated experiment designated experiments 1, 2 and 3 respectively.

The meats of the breasts and the legs were each cubed and cooled to 0°C, then minced once through a quarter-inch plate and once through an eighth-inch plate in previously cooled mincers. The pH of the minced meat was determined directly with a glass electrode. Portions of the minced meat, each weighing 650 g were stirred and gently macerated with a rubber spatula, to prevent foaming, for one hour in an ice-cold bath of 1650 ml capacity containing 0.67 M NaCl plus 0.003 M $NaHCO_3$, pH 6.6. This was followed by centrifugation at 10 000 × ***g*** at 2°C for 20 min. The supernatant was diluted 1:10 with double distilled water in large Erlenmeyer flasks, and the myofibrillar proteins allowed to settle overnight in a cold-room at 2°C. The excess liquid was siphoned off the settled protein, which was then further dewatered by centrifugation at 2500 × ***g*** at 2°C. The pellet was suspended in 800 ml of 0.67 M NaCl plus 0.003 M $NaHCO_3$, at pH 6.6 to obtain a protein concentration of approximately 2 to 2.5%. The exact concentration was determined by the method of Lowry *et al.* (1951). Two 0.67 M NaCl solutions were prepared in phosphate buffer at pH 6.2 and 5.8 and 200-ml portions of the suspended pellets were dialyzed against these salt solutions in a cold-room until the pH values inside the dialysis bags had reached those of the surrounding solutions.

The LCE was determined according to Trautman (1966b) using a series of dilutions of the extracted proteins of the breast and leg meat at pH 6.6 and those which had been dialyzed to pH 6.2 and 5.8. In the LCE test one looks for the lowest concentration of protein which will form a gel following heating in conical bottom shaped test tubes placed in water at 80°C for 10 min. The determination of the LCE is carried out by inverting each of the tubes three times in succession. If the material does not flow out of the tube this is evidence of gel formation.

Results and discussion

The results are presented in Tables 1 and 2.

Table 1 gives the pH values of the raw minced meats. The table shows that there were differences in the pH values depending on the source of the meats. The pH values of the leg meat ranged from 6.0 to 6.8 in the three experiments. while the pH values of the breast meat ranged from 5.8 to 5.95.

Table 1. pH Values of minced, chilled turkey breast and leg meat on three separate occasions

Raw meat	Experiment 1	Experiment 2	Experiment 3
Breast	5.85	5.80	5.95
Leg (thigh plus drumstick)	6.05	6.80	6.00

Table 2. Least concentration endpoint (% protein) of protein extracted at pH 6.8 and of extracts dialyzed to pH 6.2 and 5.8 from turkey breast and leg meats

	Experiment 1			Experiment 2			Experiment 3		
pH	6.8	6.2	5.8	6.8	6.2	5.8	6.8	6.2	5.8
Breast	1.1	0.95	0.7	1.2	1.2	0.9	—	—	—
Leg (thigh plus drumstick)	1.1	0.82	0.4	1.2	1.2	0.7	1.4	1.4	0.7

Table 2 shows the minimum protein concentration at which a gel was formed in the breast and leg meat at the three pH values tested. It can be seen that the proteins of the leg meat formed a gel more readily than the breast meat proteins. This was especially evident at pH 5.8; at this pH the LCE ranged from 0.4 to 0.7 for the dark turkey leg meat and from 0.7 to 0.9 for the light breast meat proteins. According to Trautman (1966b), the lowest LCE for the various protein extracts obtained from pre- and post-rigor porcine muscle was found at pH values ranging from 5.7 to 6.0 and from 5.8 to 6.25 respectively. The changes in the proteins at these pH ranges were therefore the most significant. Trautman (1966a) inferred that the initial pH of the meat influenced the LCE of the extracted proteins. In the present work the pH ranges of the turkey thigh and drumstick meat in the three experiments were initially higher than those of the breast meat, and the LCE of the protein extract of the leg meat at pH 5.8 was lower, i.e. it had better gelation properties.

Conclusions

The results of the above experiments appear to indicate a difference in the quality of breast and leg meat with regard to their gelation capacity and therefore their water-holding capacity. This could influence mouth feel of the cooked

meat and should be taken into consideration when choosing processing methods. According to Trautman (1966b) low pH meat in pigs produces a very inferior sausage, and extremely high pH pre-rigor muscle produces a sausage emulsion with very low viscosity.

In the course of our work in turkey dressing plants in Israel, where the meat is made kosher by salting, we have observed that the salt can cause an excessive uptake of water. The koshering process results in a salt concentration of approximately 0.5 M in the outer layers of the muscle. This could cause dissolution of myofibrillar proteins (Haurowitz, 1963), which tend to bind water as a result. The selective binding of salt ions to protein chains adds electric charges and increases the water-holding capacity of the protein (Hamm, 1960). In other words, salt decreases the isoelectric point of the protein, and upon adding salt, it is as if we had started to work with meat with a higher pH level. For example, if we start with meat without salt at pH 6.0 and the pH range between the raw material pH and the isoelectric point is 0.5 pH units, koshering might bring the isoelectric point down to pH 5.1. The pH range then becomes 0.9 pH units, and it is as if we had started to work with meat with a pH of 6.4 (pH $5.5 + 0.9 = 6.4$).

Salting of turkey meat according to the kosher laws could thus affect the functional properties of the proteins and have a beneficial influence on palatability and product texture. These possibilities are being investigated. On the other hand increased water uptake in the raw meat is undesirable from the consumer point of view and presents difficulties in developing products according to standards, due to the relative decrease of other meat components.

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Water activity in multicomponent non-electrolyte solutions

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Summary

An equation has been developed for calculating water activity in multicomponent non-electrolyte solutions. The model is used for predicting the water activity of fruit juice concentrates on the basis of their sugar contents.

Introduction

Water activity (a_w) is the single most important factor affecting the shelf life of intermediate moisture foods (IMF), (a_w between 0.65 and 0.90). Troller & Christian (1978) and Troller (1979) recently reviewed the effect of a_w on the microbial and physico-chemical stability of IMF.

Foods containing a high proportion of soluble solids, such as fruits or fruit juices, may be transformed to IMF by the concentration (i.e. drying or evaporation) of their soluble constituents, which are composed mainly of sugars.

A number of approaches have been taken in order to correlate water activity data in multicomponent non-electrolyte solutions relating to food products. The majority of the equations were developed for use by the confectionery industry in order to predict the a_w (or equilibrium relative humidity) of sugar solutions, syrups and confectionery products (Grover, 1947; Norrish, 1966).

The present study introduces a new and simple equation for correlating water activity data in non-electrolyte mixtures. The model is tested against a_w experimental data of apple juice concentrates and is further used for predicting the a_w lowering behaviour of various fruit juice concentrates.

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Development of the correlating equation

Very recently Ferro Fontán and Chirife (1980a) have shown that the water activity of a non-electrolyte multicomponent solution may be calculated as follows

$$(a_w)_m = \pi\,[a_{w,s}(m)]^{m_s/m} \tag{1}$$

where $(a_w)_m$ is the water activity of the complex solution, $a_{w,s}(m)$ is the water activity of s component evaluated at the total molality (m) of the mixture, and m_s is the molality of the s component.

Assume that the water activity of any single solute may be represented according to Norrish's (1966) model, which may be written (Chirife, Ferro Fontán & Benmergui, 1980)

$$a_{w,s}(m) = X_1 \exp[-K_s X_2^2] \tag{2}$$

where X_1 and X_2 are molar fractions of water and solute, respectively, and K_s is the correlating constant. Further

$$X_2(m) = \frac{m}{m + 55.5}, \quad X_1 = 1 - X_2$$

Substituting eqn (2) in eqn (1) it results in

$$(a_w)_m = X_1 \exp\left[-\left(\sum_s K_s \frac{m_s}{m}\right) X_2^2\right] \tag{3}$$

which is similar to Norrish's (1966) equation for binary solutions but with a correlating constant given by

$$K_m = \sum K_s \frac{m_s}{m} \tag{4}$$

Equation (4) can be transformed in the following way. Let w be the total weight of solutes per litre of water; the weight of solute s is

$$w_s = w C_s$$

where, C_s is the weight ratio of solute s to total solids ($\Sigma C_s = 1$). The molality is given

$$m_s = \frac{w_s}{M_s}$$

where, M_s is the molecular weight of s component. The total molality m is

$$m = \sum_s m_s = \sum_s \frac{w_s}{M_s} = w \sum_s \frac{C_s}{M_s} = \frac{w}{\bar{M}}$$

where $\bar{M}$ is an average molecular weight defined as

$$\bar{M} = \left(\sum_s \frac{C_s}{M_s} \right)^{-1} \tag{5}$$

then

$$\frac{m_s}{m} = \frac{w_s}{M_s} \frac{\bar{M}}{w} = C_s \frac{\bar{M}}{M_s}$$

and by substituting in eqn (3)

$$K_w = \sum_s K_s C_s (\bar{M}/M_s) \tag{6}$$

The final correlating equation is

$$(a_w)_w = X_1 \exp[-K_w X_2^2] \tag{7}$$

Thus, the water activity of a complex solution may be described by an equation identical to that of Norrish (1966) for binary systems, but with a correlating constant K_w defined by eqns (6) and (5).

Prediction of a_w lowering behaviour of fruit juice concentrates

Major components in solution in fruit juices consist essentially of 10 to 20% sugars, ~1% of acids, <1% of inorganics and very small quantities of volatile flavour compounds (Matsuura, Baxter & Sourirajan, 1973). From the point of view of colligative properties, fruit juices are usually considered as a mixture of the monosaccharides fructose and glucose, and a disaccharide sugar, sucrose (Bellows, 1971; Abdel-Monem Omran, 1972).

The predictive value of eqn (7) may be tested by comparison with experimental freezing point (FP) data reported by Gachot (1955) for apple juice

concentrates. Freezing point values may be readily transformed to water activity ones according to Ferro Fontán & Chirife (1980b)

$$-\ln a_w = 9.6934 \cdot 10^{-3}\theta_f + 4.761 \cdot 10^{-6}\theta_f^2 \quad (8)$$

where θ_f is the FP depression. The water activity obtained from eqn (8) is, of course, the activity at the corresponding FP. Ferro Fontán & Chirife (1980b) suggested that for most solutes of interest in the food area, the a_w calculated from FP depression is not likely to differ in more than 0.01 a_w units of its value at 25°C. This applies as long as the water activities considered are above about 0.85.

This can be verified for the solutes of interest to the present situation. Apple sugars are a mixture of fructose, glucose and sucrose (Matsuura *et al.*, 1973), and Bellows (1971) has reported their equilibrium freezing diagrams which may be converted to water activity according to eqn (8). Figure 1 compares the water activity data for glucose and sucrose evaluated from the FP, as compared with literature data of a_w measured at 25°C (Chirife *et al.*, 1980). It can be seen that in spite of the considerable temperature differences involved, the a_w values calculated from FP measurements are in good agreement with those measured at 25°C. It is noteworthy that the a_w lowering curve of fructose may be considered similar to that of glucose (Cakebread, 1973; Rüegg & Blanc, 1981). From these results it may be concluded that the water activity of apple juice concentrates, calculated from FP depression will also correspond to a_w data at 25°C.

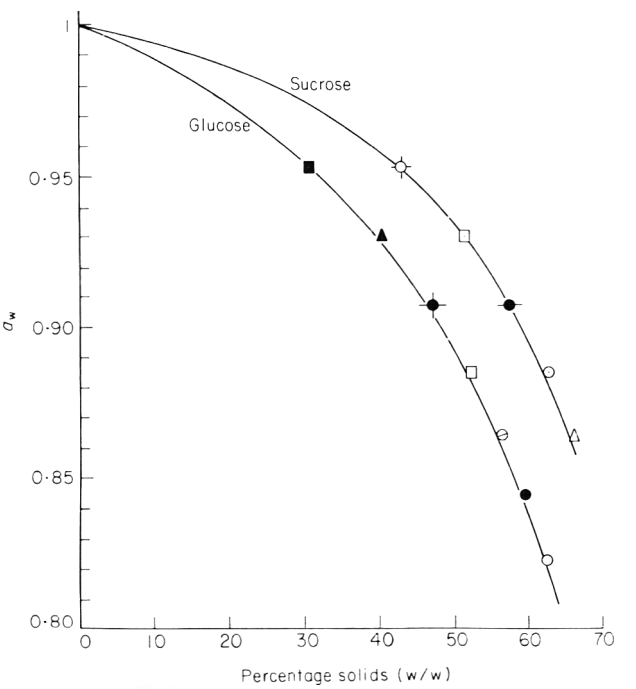

Figure 1. Comparison of a_{w_m} values calculated from freezing point data with values measured at 25°C for sucrose and glucose solutions. —, 25°C; ■ ○, − 5°C; ▲ □, − 7.5°C; ◆ ●, − 10°C; □ ⊙, − 12.5°C; ⊘ △, − 15°C; ●, − 17.5°C; ○, − 20°C.

According to Watt & Merril (1963) and as used by Abdel-Monem Omran (1972), the typical sugar make-up of apple juice is as follows: fructose 62.4%, glucose 14.8% and sucrose 22.7%. The a_w lowering behaviour of sucrose and glucose (or fructose) may be very well expressed according to

$$a_w = X_1 \exp[-KX_2^3]$$

K being 6.47 and 2.25 for sucrose and glucose, respectively (Chirife *et al.*, 1980). The water activity lowering curve of apple juice may be now predicted from the aforementioned composition data and eqn (7), with K_w and $\bar{M}$ calculated through eqns (5) and (6).

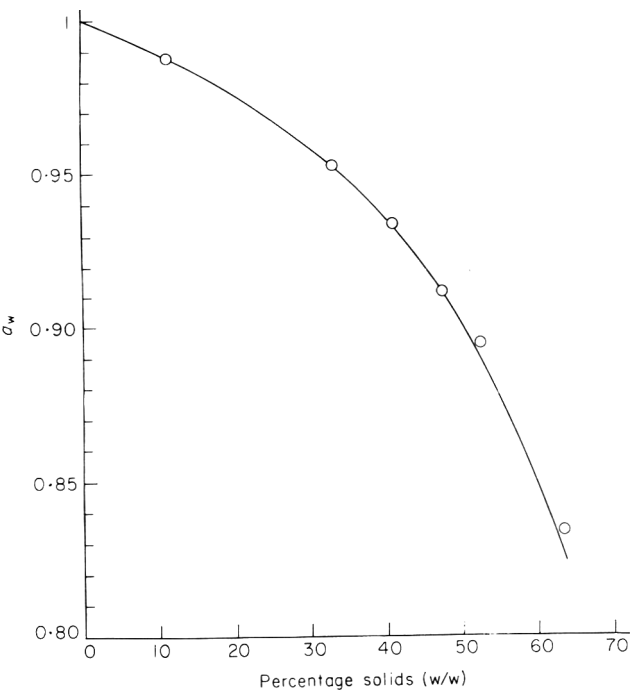

Figure 2. Comparison of predicted and experimental a_w lowering behaviour of apple juice concentrates. ○, Experimental; —, predicted.

Figure 2 compares the predicted and experimental (derived from FP data) a_w lowering curves for apple juice. It can be seen that the agreement is fairly good. Calculated parameters were found to be $K_w = 2.81$ and $\bar{M} = 202$.

On the basis of these results and the knowledge of sugars composition it may be possible to predict the parameters K_w and $\bar{M}$ needed for the a_w lowering curve of various fruit juices. Table 1 shows the typical sugar make-up of various juices (Watt & Merril, 1963) and the calculated values of K_w and $\bar{M}$. Figure 3 shows the predicted a_w curves for the juices.

Table 1. Typical sugar make-up of various fruit juices (after Watt & Merril, 1963) and calculated parameters K_M and $\bar{M}$

	Proportions of major sugars (wt %)				
Fruit juice	Fructose	Glucose	Sucrose	K_M	$\bar{M}$
Lemonade	6.4	6.4	87.1	5.55	307.2
Orange	21.2	23.9	54.8	3.90	243.6
Prune	24.8	75.0	0.1	2.25	180
Pineapple	33.6	52.2	14.1	2.59	193.2
Grape	35.8	64.2	0.0	2.25	180
Apple	62.4	14.8	22.7	2.81	202

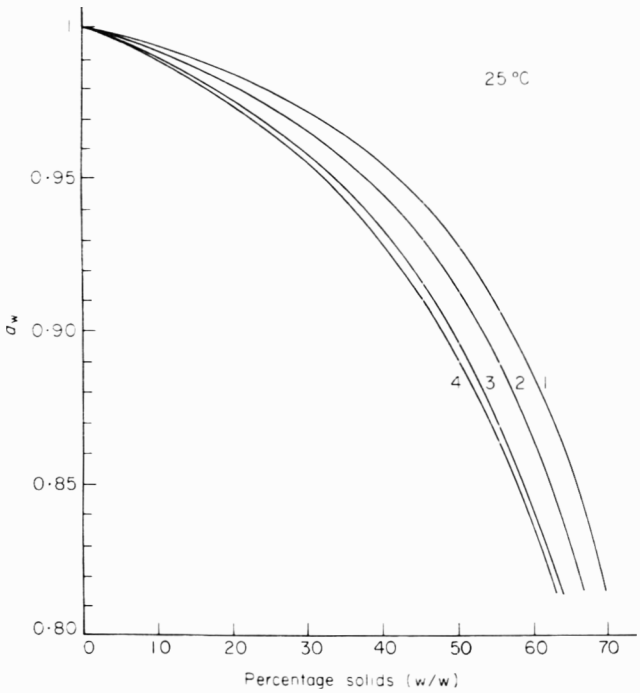

Figure 3. Predicted a_w lowering behaviour of various fruit juice concentrates. 1. Lemonade; 2. orange; 3. pinepple; 4. prune and grape.

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Errata

Chirife, J., Ferro Fontán, C. & Benmergui, E.A. (1980) *J. Fd Technol.* **15**, 59. On page 62 in Fig. 3 the a_w-concentration curve for mannitol has been drawn incorrectly: however, the a_w lowering behaviour of mannitol can be calculated correctly by equation 2 with $K = 0.906$ as indicated in Table 4.

Ferro Fontán, C. & Chirife, J. (1981) *J Fd Technol.* **16**, 21. Equation 6, on page 24, should read:

$$-\ln a_w = 35.127 \cdot 10^{-3} \cdot \theta_B - 1.1195 \cdot 10^{-4} \cdot \theta_B^2$$

J. Fd Technol. (1981) **16**, 561–566

Solubilization of wheat gluten with sodium hydroxide

I. L. BATEY* AND P. W. GRAS

Summary

The solubility of wheat gluten may be increased greatly by treatment with 0.25 M sodium hydroxide. At temperatures of 40°C and above, the solubility of the protein exceeds 100 mg/ml in a reaction time of 6 hr or less. The products from the reaction of 60°C and 80°C were darker than the original gluten and had an unpleasant odour of H_2S on dissolution in water. They also contained traces of lysinoalanine. The 40°C reaction product was about the same colour as gluten, and its solutions in water did not smell of H_2S. Reaction of gluten at 20°C also caused a large increase in solubility, but after 24 hr the solubility was only 77 mg/ml. This product was also of the same colour as gluten, and did not smell of H_2S in solution. Neither the 20°C nor the 40°C products contained detectable traces of lysinoalanine. For reasons of flavour, colour, lysinoalanine content and economy of time, the optimum reaction conditions are 6 hr at 40°C.

Introduction

The use of wheat gluten as a starting material for a soluble protein derivative has attracted a great deal of interest in recent years. The mixture of proteins comprising gluten is characterized by the relatively small number of charged amino acids, and the large proportion of glutamine residues present. The removal by hydrolysis of some of the primary amides from both glutamine and asparagine residues to give the corresponding carboxylic acid side chains, should enhance the solubility of the proteins by increasing their charge at $pH > 5$. In addition, the tendency of these proteins to aggregate will be diminished by decreasing the amount of hydrogen bonding between molecules that occurs through the amide side chains. Gluten deamidated by acid hydrolysis is more water soluble and has been considered as a substitute for egg

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white in meringue desserts and cake icings (McDonald & Pence, 1961), and as an emulsifier in milk type products (Wu, Nakai & Powrie, 1976). The amide side chains of glutamine residues in gluten have also been converted into ester derivatives by treating it with acidic solutions of the appropriate alcohol (Beckwith, Wall & Dimler, 1963). Such esterified gluten derivatives also show increased solubilities in aqueous solutions. In addition, chemical modifications such as acylation with acid anhydrides after alkaline treatment have also been used by other workers to prepare water soluble gluten derivatives (Melnychyn & Pollock, 1972). Although acylation has been carried out in alkali, detailed studies of the deamidation of gluten have only been conducted under a variety of acidic conditions.

During acid treatments, some peptide linkages are hydrolysed. This factor no doubt contributes to the increased solubility of the derivatives, but probably also results in the astringent flavour described by some workers in such deamidated glutens (Finley, 1975). The nutritional quality of the protein is unchanged, as it has been observed that there is no damage to the essential amino acids during the acid reaction (Wu, Nakai & Powrie, 1976).

Alkali has been neglected as a reagent for the deamidation of gluten, probably because of the degradation of some of the essential amino acids that can occur during the reaction (Masters & Friedman, 1979). Cross-linked amino acids, such as lysinoalanine and lanthionine can also be formed in alkali-treated proteins (Provansal, Cuq & Cheftel, 1975). Although these side reactions must be considered disadvantageous for the preparation of food products, there are some advantages in the use of alkali for solubilizing gluten by the deamidation of glutamine and asparagine residues. In both alkali and acid, unsubstituted amides are hydrolysed more rapidly than N-substituted or N,N-disubstituted amides. In alkali, however, the hydrolysis of amides as compared with N-substituted amides is more selective than it is in acid. It is likely, therefore, that alkali treatment of gluten should result in a high degree of deamidation while causing little or no hydrolysis of the peptide backbone, which consists of N-substituted amides. If the protein chains are retained intact, one of the properties of a more selectively deamidated, soluble gluten should be that its solutions are more viscous than those of acid-solubilized gluten, thus giving it additional and alternative uses.

For this reason it was considered desirable to investigate the solubilization of gluten in alkali. If suitable derivatives could be prepared by hydrolysis in alkali, further work could then be directed towards determining conditions to minimize the amount of unwanted side reactions which might impair the nutritional quality of the products.

Methods and materials

Vital gluten from a number of commercial producers was reacted as a 16% suspension in 0.25 M sodium hydroxide. The mixture was held at 20, 40, 60 or

80°C with vigorous stirring for 24 hr, 6 hr, 2 hr or 20 min respectively. It was then neutralized with 5 M hydrochloric acid to pH 6.8–7.0, and the product was isolated by freeze drying the resulting solution.

The nitrogen content of the dry samples was determined by the Kjeldahl method. The percentage protein was calculated by multiplying the nitrogen content by 5.7, the standard factor for wheat products (American Association of Cereal Chemists, 1976). Because the nitrogen content is reduced by deamidation, the actual factor should be higher but is unlikely to be as high as 6.25, which is used for most proteins. For this reason, the biuret method was used for determining the soluble protein in solutions of the products.

Protein solubilities were determined as follows. Solutions of the dry, modified glutens were prepared at concentrations of 10 and 20% w/v in distilled water. After centrifugation at $6000 \times g$ for 30 min, the supernatant fractions were diluted, 1:10 in the case of the 10% solution and 1:20 in the case of the 20% solution, in order to have solutions with soluble protein within the range of 0–10 mg/ml. The amount of protein dissolved in the solutions was determined by the biuret procedure of Simmonds & Ronalds (1975). A graph of absorbance at 500 nm *vs* protein concentrations was drawn using standard solutions containing 0, 1, 2, 4, 6, 8, and 10 mg/ml respectively of bovine serum albumin (Calbiochem, Carlingford, N.S.W.) and the protein concentrations of the test solutions were read from the graph.

Gel filtration was carried out on Sephadex G-150 and G-200 (Pharmacia South Seas Pty. Ltd., North Ryde, N.S.W.) in AUC, a solvent consisting of 3 M urea – 0.1 M acetic acid – 0.01 M cetyltrimethylammonium bromide in water.

Lysinoalanine was detected qualitatively by the method of Sternberg, Kim & Plunkett (1975). A pure sample of lysinoalanine for reference purposes was prepared by the method of Okuda & Zahn (1965).

Results

The solubilities obtained by treatment of vital gluten with dilute alkali for various reaction times and temperatures are shown in Table 1. The target solubility of approximately 10% (i.e. 100 mg/ml) could be achieved by conducting the reaction in sodium hydroxide at 40° (6 hr), 60° (2 hr) or 80°C (20 min), but not within 24 hr at 20°C under the conditions described. As a result of the need to use hydrochloric acid (or some other acid) for neutralization, the dried products contained approximately 5% of sodium chloride, a quantity small enough to be acceptable for the end-uses envisaged. Gel filtration showed that there was little difference in the molecular weight distribution of the samples (Fig. 1), although the gel filtration media used were incapable of separating the extremely high molecular weight fractions of gluten as these are excluded from the columns and elute in the void volume.

There were some differences observed during the course of the reactions at the different temperatures, and in some of the characteristics of the products.

Table 1. Solubilities of vital wheat gluten after treatment in 0.25 M sodium hydroxide

Reaction conditions		Protein solubility* (mg/ml)	
Temperature (°C)	Time	10% solution	20% solution
20	24 hr	49	77
40	6 hr	66	—†
60	2 hr	62	130
80	20 min	68	139

*The protein content of the lyophilized samples was 67% in each case.

†The 20% solution of the 40°C reaction product was too viscous to measure accurately. A 15% solution of the product, though rather viscous, gave a protein solubility of 114 mg/ml.

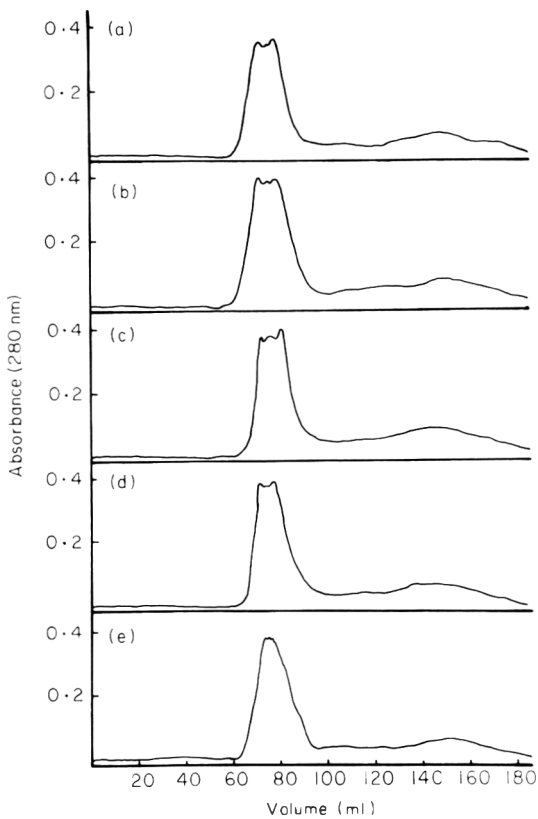

Figure 1. Elution profile from gel chromatography on Sephadex G-150 in AUC of (a) untreated wheat gluten, and gluten treated with 0.25 M sodium hydroxide for (b) 24 hours at 20°C, (c) 6 hours at 40°C, (d) 2 hours at 60°C, (e) 20 minutes at 80°C.

The original dispersion of gluten in sodium hydroxide had a light yellow-brown colour. After treatment at 20° (24 hr) or 40° (6 hr), neutralization and lyophilization, the products had a pale tan colour, approximately the same shade as the original gluten. Some traces of ammonia were emitted during the course of the reactions and subsequent neutralization. Analysis of samples of the products, after hydrolysis in hydrochloric acid, showed no detectable lysinoalanine by a method which has been claimed to have a threshold limit of 12 ppm (Sternberg, Kim & Plunkett, 1975).

At 60 and 80°C, the reaction mixtures turned a much darker colour, and on neutralization the colour changed to greenish-grey, the changes being more noticeable at 80°C. After lyophilization, these products were still an unattractive grey colour. During these reactions, the smell of ammonia was much stronger, probably due to its increased volatility at the higher temperatures. On neutralization a strong smell of hydrogen sulphide accompanied the observed colour changes. When the lyophilized products were redissolved in water, this unpleasant smell was again present, although it was not apparent in the dry product. Detectable traces of lysinoalanine, that is > 12 ppm (Sternberg, Kim & Plunkett, 1975), were present in the 6 M hydrochloric acid hydrolysates of these products, but facilities were not available to determine quantitatively the amount present.

Discussion

The time required for the solubilization of vital gluten with 0.25 M sodium hydroxide, is dependent on the temperature, but a high degree of solubility can be achieved at either 40, 60 or 80°C in acceptable reaction times for commercial purposes. At 20°C, the solubility of the product was lower, and although this could probably be increased with a longer reaction, the reaction time of 24 hr could already be considered too long for commercial use. There are factors which would favour the use of 40°C, although at this temperature the length of the reaction is 18 times that required at 80°C. In non-food uses where visual and flavour characteristics are unimportant, the higher reaction temperature would probably be selected because of the shorter reaction time.

The colour of the products obtained from the reaction at either 20 or 40°C differs little from that of the gluten used, while the colour of the 80°C product is considerably darker and lacks aesthetic appeal for use in foods. The unpleasant sulphurous smell of solutions of the 60 and 80°C products, the presence of lysinoalanine and the darker colours would make these products unsuitable for use in food items.

Although the presence of small amounts of protein-bound lysinoalanine in foods is probably not harmful (O'Donovan, 1976), it is possible that future restrictions may be placed on permissible levels of this substance in foods. With this in mind, the product from the 40°C reaction, in which lysinoalanine was not detected, would be the preferred choice.

All of the products obtained in the described reactions have novel physical properties, markedly different from those of any conventional gluten proteins. Further work on the functional properties of these solubilized proteins needs to be carried out to assess their suitability for a variety of food and non-food uses.

Acknowledgments

We wish to thank Miss Helen Wane for technical assistance in carrying out some of the experimental work described here.

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Book reviews

Fodder Biofactors, Their Methods of Determination. By E. Knobloch and J. Černá-Heyrovská.
Amsterdam: Elsevier Scientific Publishing Co., 1980. Pp. 317. ISBN 0–444–99783–0. $63.00.

The major portion of this book, some 200 pages, is given over to the determination of vitamins in feedstuffs. The remaining chapters cover the analysis of amino acids, antibiotics and chemotherapeutic agents and stabilizers.

The first impression is that 'Dr Knobloch's book gives a complete and objective picture of the present state of the analysis of vitamins and some other biofactors in fodders and . . .', as described in the preface. However, on more careful examination the reader will discover that the majority of the methods, and their associated references, are in fact several years old. This should not be taken to imply that all old methods are obsolete, but it is rather disconcerting to find that some 75% of the references are taken from the literature prior to 1970. The book does indeed provide a comprehensive survey of those methods that are available but there is no emphasis on modern techniques. This may in part be due to a long delay in publication.

The chapter covering methods available is very perfunctory, in particular in the chromatographic methods section high pressure liquid chromatography (HPLC), which is rapidly becoming the method of choice for many vitamin analyses, is only mentioned, without even one reference to the methodology. The general statement that physicochemical methods are not sufficiently reliable, sensitive or specific for B vitamin analyses would be questioned by many practising analysts.

The sections on individual vitamins provide excellent background material concerning biological properties and chemical modifications. The surveys of methods appear complete and the examples at the end of each section provide experimental details for particular determinations. However, as the most recent references are for 1977 there is little mention of modern chromatographic techniques. These comments apply to all the sections on vitamins.

The chapter on amino acids again illustrates the non-selective coverage of methodology. No one would dispute the fact that amino acids are optically active but also no one would suggest that this is a relevant parameter for their determination in feedstuffs. Similar comments could also be made about other methods cited in connection with other non-vitamin components. In the chapter on antibiotics the emphasis is strongly on microbiological methods.

In conclusion this book provides a well presented comprehensive coverage of methodology for fodder biofactors up to 1977. The almost complete absence of modern chromatographic methods detracts seriously from its merits and it is hoped that this will be rectified in a subsequent edition.

Robert Macrae

Spices and Condiments: Chemistry, Microbiology, Technology. By J. S. Pruthi.
(Advances in Food Research, Supplement 4). New York: Academic Press, 1980. Pp xiv + 449, ISBN 0–12–016464–7. $39.50.

This book has been written by an eminent Indian scientist who has specialized in the field of spices. It is ambitious in its scope and provides chapters on properties and uses; analytical techniques; chemical composition; post-harvest technology; products technology; utilization of spice wastes; packaging; storage and transportation; microbiology; insect infestation; marketing, quality control and standards; and research needs. The author's intention is to provide a technical compendium on these subjects, to highlight some of the major findings and to point to avenues for further research.

The book is a veritable mine of information and contains some 1700 references. However, coverage of the literature is by no means comprehensive, as is acknowledged by the author, and a number of important papers published in recent years are not mentioned. A very useful account is given of research and development work on spices in India, but the book is understandably weaker when dealing with developments elsewhere in the world. The heavy reliance on the Indian experience, combined with a tendency to awkward presentation, can give the unwary reader an incorrect perspective on some topics. For example, the section dealing with preparation methods for white pepper commences with what is essentially a review of experimental work in India on this subject, and then proceeds to briefly describe methods in other geographical areas. Since India is not a significant producer of white pepper, its very small production being sold only on the domestic market, a more useful and balanced discussion would have dealt with traditional, commercial practices in Indonesia, Malaysia and Brazil, followed by notes on Indian innovations.

The major disappointment of the book is that for large sections it relapses to an annotated bibliography with no linking comment between items and it fails to provide a critical assessment of the information presented. The sections dealing with the microbiology of spices and gas chromatography analysis of spice oils are unsatisfactory in this respect. The structuring of the book can also present difficulties to the reader. Discussion of the post-harvest technology of individual spices is rarely continuous and the sequence of events in preparation is not easily followed. For example, various aspects of cardamom preparation are covered at intervals on pages 180, 185 and 188. Elsewhere, the positioning of topics is

rather eccentric; the microbiology of dehydrated onion appears in the Technology chapter and aspects of mycotoxin and bacterial contamination in Chapter 11, entitled Insect Infestation and Its Control, rather than in Chapter 10 (Sanitation – Microbiological Aspects).

The text is also flawed by a number of errors which must be assumed to have arisen from haste in compilation and proof checking and from the desire to include all available information. Unaccountably, a sentence noting Eschenmoser's elucidation of the structure of zingiberene, the major sesquiterpene component of the volatile oil of ginger, appears in the middle of a discussion of the non-volatile, pungent principles of ginger (p. 128); a reference to a Madagascan artificial curing process for vanilla occurs in a section entitled 'Dehydration of pimento berries' (p. 190); a note on a study of the utility of irradiation treatment to increase the volatile oil content of basil and anise appears unexplained in a section devoted to sterilization of spices (p. 317); similar anomalies occur elsewhere. A considerable number of typographical errors, particularly of authors' and place names, occur throughout the text, and there are some errors in the references section. The index is generally adequate but there are some notable omissions; for example, ethylene oxide is not listed.

The chapter dealing with research needs is interesting and wide-ranging, but could have been improved by identifying priority areas more clearly. Some questionable proposals are made by the author for a substantial expansion of production of certain spices (nutmeg, cassia, cinnamon, cloves and pimento) without providing supporting evidence. While a number of these spices may be in short supply in particular areas, e.g. India, supply to the international market seems adequate in most years. Similarly, the author's statement that pepper (*Piper nigrum*) is the most important spice in the world is not strictly accurate. Pepper is the most prominent spice in trade with developed countries, but *Capsicum* products (chilies, capsicums and paprika) are the most extensively cultivated and consumed on a global scale.

In conclusion, the author has achieved his primary objective of preparing a technical compendium, but the book fails to live up to expectations and to adequately fill an acknowledged gap on the bookshelf. It is likely to prove of value mainly to those already familiar with the field of spices, requiring a ready source of information which they are able to assess critically, rather than as a guide to those readers new to the subject.

C. L. Green

Handbook of Sugars. Ed. by H. M. Pancoast and W. R. Junk. Westport, Connecticut: AVI Publishing Co., Inc., 1980. Pp. xii + 598. ISBN 0–87055–348–8. $49.50.

The book is a revised and updated second edition incorporating new ideas and research since the original publication in 1973. The book aims to present a

compendium of available information on the physical properties of nutritive sweeteners commonly used by the food processor. By presenting a great deal of information fundamental to discussions on food formulation it does not attempt to give detailed instructions and so enables planning for a variety of circumstances. It is thus presented as a manual of sugars.

The first edition was sub-divided into three sections, namely, Section I; sucrose, invert syrups and related sugars; Section II: corn syrups and sugars; Section III; blends. Section IV, dealing with lactose and fructose, is new to the revised edition. These two nutritive sweeteners are adopting a more important role in food applications, lactose, from the point of its availability, and fructose, now being produced industrially, by its interesting intrinsic and physical properties. Another addition (Section V) is that of food applications. Although fairly short, it succinctly presents economic considerations and functional properties of the nutritive sweeteners discussed earlier.

A considerable amount of material has been published since the first edition on high fructose corn syrups. The authors have expanded this area concurrently with the important role these modified carbohydrates are now playing in the food industry.

Section III on blends has also been enlarged to include multi-component systems and a method for determining the refractive index of these solutions is described.

A constructive and pragmatic view is taken on the question of units. The data tables have been revised to include metric information although the English system is still documented in keeping with the industry's approach to metrication.

Amongst other additions are the Recommended International Standards of the Food and Agricultural Organization of the United Nations for nutritive sweeteners and the United States Food and Drugs Standards for these products. Revised and updated refractive index values have also been included.

As stated by the authors, the purpose of the book has been to familiarize the food processor with the physical properties of sugar and the effects which may be attained by variation in environment, quantity and type of sugar present. In this, the authors have produced an authoritative work.

The book will provide a constant reference for the subject area. Although slightly expensive for the individual, the book is a must for the library shelf.

S. Z. Dziedzic

Tropical and sub-tropical fruits. By Steven Nagy and Philip E. Shaw. Westport Connecticut: AVI, 1980. Pp. x + 570. ISBN 0–87055–350–X. $49.50.

This multi-author book in the established tradition of the Avi texts on food-related subjects is very welcome, although there are naturally variations in the style and quality of the treatments of different authors. It sets out to survey the

current state of knowledge of 21 tropical and sub-tropical fruits as far as their composition, properties and uses are concerned. All the major fruits, except the citrus fruits, and many minor ones are considered. The book opens with a most interesting and informative chapter on the origins, culture, importance and future prospects of each of these fruits and their different cultivars and subsequent chapters deal with the individual crops in greater detail.

As always in a multi-author text, the chapters of individual authors vary considerably in their emphasis and their thoroughness of coverage, in this case regarding maturity, ripening, storage, post harvest problems and utilization and compositional changes associated with these variables, although information on nutritional value and on flavour components follows a much more consistent pattern through the book.

Of the individual chapters, one might start with that on banana and plantain, in which dessert bananas (AAA) are given a compact and informative treatment which is, however, not as comprehensive as their outstanding importance in international trade compared with other tropical fruits would seem to justify. This reviewer would also like to have seen a fuller treatment of work on plantains (AAB) and other cooking bananas (ABB).

Other fruit crops which are both widely and extensively cultivated, to which separate chapters are devoted, are mango, papaya, avocado, guava. These are all treated in a competent and authoritative manner by established specialists, who provide good coverage of the available literature, combined with valuable new sources of information on these crops, especially for avocado and mango for which most information is available. The latter in particular receives a very impressive and detailed treatment.

Another important economic crop, the pineapple, is covered much more sketchily, the chapter being heavily biased towards a discussion of flavour volatiles, which are treated well, while other aspects are rather inadequately treated. That ancient Mediterranean crop, the fig, also deserves a fuller treatment than it gets, although the discussion of its composition is of value.

Many others of the fruits covered, although minor in terms of global production, are locally of very great importance, and this reviewer was especially pleased to see the inclusion of durian and prickly pears (the latter oddly combined in a chapter with sapodilla). These, together with passion fruit, dates and persimmon are well covered, while acerola is described in an exceptionally thorough chapter which brings together a great deal of information on the composition and utilization of this fruit, one of the richest known sources of vitamin C.

Other fruits of lesser importance, such as lychee, loquat, mangosteen, soursop, tamarind and chironja (why the last named should be included when other, vastly more important citrus are not, is not obvious) receive only brief treatment, but this arises more from the lack of information that is available than any deficiency in the coverage of what there is.

The inclusion of macadamia nuts in a book otherwise devoted to fruit is

surprising, but the chapter constitutes a useful review of the crop.

The book is of Avi's usual high standard of production. In spite of some few deficiencies, it will be a most valuable addition to the library of any organization concerned with the subject, though the price puts it beyond the reach of most individual purchasers.

D. G. Coursey

Books received

Developments in Food Packaging, Volume 1. Ed. by S. J. Palling.
London: Applied Science Publishers, 1980. Pp. xv × 190. ISBN 0–85334–917–7. £18.00.

The first in a new series, this volume contains chapters on metal containers and closures (J. D. Malin): rigid plastics packaging (J. H. Briston); flexible plastics packaging (R. R. Goddard); Glass (D. G. Osborne); Paper and board developments (W. A. Jones); Food technology to the year 2000 (M. C. Philip); and International legislation (M. A. Andrews).

The Laboratory Diagnosis of Toxoplasmosis. By D. G. Fleck & W. Kwantes (Public Health Laboratory Service Monograph Series No. 13).
London: HMSO, 1980. Pp. iii + 20. ISBN 0–11–887104–8. £2.00.

Toxoplasmosis is one of the commonest worldwide protozoan parasitic infestations of humans. About 300 cases are diagnosed and notified each year in England and Wales, but there are indications that the infection rate is 1 in 200. It is believed that uncooked meat and unwashed salads are common vehicles of infection. This booklet describes serological tests used to diagnose the disease in humans, but there is only a passing mention of the detection of the parasite in meat.

Benchbook on Brucella. By L. Robertson, I. D. Farrell, P. M. Hinchliffe and R. A. Quaife.
(Public Health Laboratory Service Monograph Series No. 14). London: HMSO, 1980. Pp. vii + 45. ISBN 0–11–887105–6. £3.00.

Brucellosis can be contracted not only through direct contact with infected animals and from airborne droplets, but also by ingestion of infected milk and cream. This booklet describes methods for the isolation of *Brucella* from clinical specimens and also from milk and other dairy products, and provides a guide to identification, with a prefatory caution on the hazard arising from laboratory work with the organism.

Current Food Consumption Practices and Nutrition Sources in the American Diet. By R. L. Rizek & E. M. Jackson.
Hyattsville, Maryland Consumer Nutrition Center, U.S. Department of Agriculture, 1980. Pp. 35.

This booklet describes changes in food habits and diet status which occurred between 1965/66 and 1977/78 based on surveys carried out in those years. Sixteen tables are included which illustrate intake of specific foods, value, intakes in terms of energy, protein, fat and iron.

Experimental Food Chemistry. By N. I. Mondy.
Westport, Connecticut: AVI Publishing Co., 1980. Pp. ix + 269. ISBN 0–87055–343–7. $13.50.

This book, designed for American college use, lists experiments possibly suitable for C.S.E. and 'O' level courses in Home Economics.

Handbook of Legumes of World Economic Importance. Ed. by J. A. Duke.
New York: Plenum Press, 1981. Pp. xi + 345. ISBN 0–306–40406–0. $45.00.

Nearly 150 economically important species of legumes are listed under Latin binomial and a few trivial names. They are then described under the following standard headings: uses; folk medicine; chemistry (particularly nutritional and toxicological aspects); description and line drawings of the plant; germplasm; distribution; ecology; cultivation' harvesting; yields and economies; and pests.

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CEREALS

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We also need a Biochemist to join a team in the field of cereal biochemistry with the emphasis on the proteins and proteinases of sorghum grain and malt. Enzyme assays, protein determinations and the monitoring of the biochemical/structural modifications that occur in sorghum grain when germinated are among the tasks involved.

Both Biochemists will need at least a BSc (Hons) plus, ideally, experience in cereal research.

Research Assistants

To be involved in cereal biochemistry laboratory research and statistical analysis within the Sorghum Beer Unit, you will need at least a BSc in Chemistry, Biochemistry or Agriculture plus experience in one or more of the following: enzyme biochemistry, plant physiology and statistical analysis.

Additionally we seek a BSc graduate (or equivalent) to assist in the development of nutritious cereal foodstuffs.

Electron Microscopist

To join a team working on the structural and physiological/biochemical functions of the cereal grain, sorghum. So, in addition to being trained and qualified in electron microscopy (up to PhD level), you should possess some experience in Botany and/or Plant Biochemistry.

BREWING

Brewing Engineer/Technologist

Beer brewed with the cereal, sorghum, is the traditional alcoholic beverage of southern Africa's black peoples. It is different from barley beer in that it is not hopped; contains significant amounts of particulate material; is more acidic and sold in an active fermentation state.

The Brewing Technology Division within our Sorghum Beer Unit seeks an Engineer/Technologist to be involved in all the Division's activities including brewing processes and plant R&D; trouble-shooting throughout the industry; the development of new packaging forms/systems, lecturing and the training of professional brewery staff – all necessitating a considerable amount of travel. So you should possess a recognised formal qualification in the engineering aspects of brewing together with sound relevant post-training experience and fluency in English, both verbal and written.

All salaries are negotiable and these will be augmented by South Africa's high standard of living at relatively low cost and the following benefits: ● free air passages (return for contract appointees) ● assistance with removal costs ● settling-in allowance ● generous house purchase scheme, where applicable ● annual bonus (13th cheque) ● medical aid, pension/life assurance schemes ● outstanding schooling, sports and recreational facilities, etc.

For an application form, either ring London 242 1766 during normal office hours or send brief personal and career details (including phone number) to The Scientific Counsellor, Chichester House, 278 High Holborn, London WC1V 7HE, England, **quoting reference 11/1/645 clearly on your envelope.** All interviews will be conducted in the UK.

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COUNCIL FOR SCIENTIFIC AND INDUSTRIAL RESEARCH

World Review of Nutrition and Dietetics, Vol. 36

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Human Nutrition and Diet

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Fields of Interest
Nutrition, Metabolic Diseases; Biochemistry, Dietetics, Genetics, Gynecology, Laboratory, Neuropathology, Pharmacology, Toxicology

In this volume, nutrition scientists from Europe, America, Egypt, and Africa review accumulated knowledge on six topics important to the future of human health. The first review compares human and bovine milk, offering extensive information on the complex differences in biochemical composition and physicochemical characteristics. Drawing upon personal studies as well as the world literature, the author examines differences in protein, lactose and ash contents, as well as in the particular milk components. His conclusions strongly support the nutritional advantages of breast-feeding.

In a second review, the consequences of malnutrition during pregnancy and lactation are discussed through summaries of research on the histological, metabolic and socioeconomic aspects of perinatal nutrition. The volume's third contribution evaluates human experimental work testing the effects of dietary fibre on both the level of serum lipids and colonic function. Suggested mechanisms for the action of dietary fibres are considered together with the possibility that Western diets would benefit from a higher fibre content. Other contributions include an analysis of dietary factors in the pathogenesis of tropical neuropathy and a report on current efforts to improve the protein content of barley, corn and oats through genetic means. The final review evaluates the uses of laboratory animals in nutrition research.

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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	μ = 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^2
cubic foot	ft^3	= 0·028317 m^3
gallon	gal	= 4·54611
pound	lb	= 0·453592 kg
pound/cubic inch	lb in^{-3}	= 2·76799 × 10^4 kg m^{-3}
dyne		= 10^{-5} N
calorie (15°C)	cal	= 4·1855 J
British Thermal Unit	BTU	= 1055·06 J
Horsepower	HP	= 745·700 W
Fahrenheit	°F	= 9/5 T°C + 32

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

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