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J. Fd Technol. (1978) **13**, 81–86

Observations of the relationship between the surface area and weight of eviscerated carcases of chickens, ducks and turkeys

N. L. THOMAS

Summary

A method of measuring the approximate internal, external and total surface area of eviscerated poultry carcases is described.

The results of a series of measurements derived from commercially produced carcasses are expressed as linear equations from which the approximate external and total surface area of eviscerated carcases can be calculated. The surface area of the body cavities can be determined by difference.

The need to supply separate equations to the calculation of the surface areas of turkeys above and below 7 kg is discussed.

Introduction

With the development of improved poultry processing techniques information on the relationship between the surface area, both external and internal, and the eviscerated weight of the carcase is of interest to refrigeration engineers concerned with the design of rapid chilling and freezing systems, to process engineers currently developing improved carcase washing equipment in accordance with EEC requirements and microbiologists concerned with the evaluation of the cleansing efficiency of washing processes and the assessment of microbial levels on processed carcases.

Simonson (1971) suggested that an approximately linear relationship existed between the total surface area of chicken carcases and the eviscerated weight. For the limited weight range of carcases of Danish origin which he examined the total surface area in cm^2 approximated to the weight in grams + 500. This paper reports the results of applying a similar method of measurement to carcases of commercial origin selected from the principle weight ranges of chickens, ducks and turkeys produced in the United Kingdom.

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Materials and methods

Chickens. The carcases from which measurements were taken were obtained from commercial crops of Ross I broilers ranging in age from 40 to 70 days.

Ducks. Carcases were obtained from commercial strains of White Peking duckling from 45 to 55 days of age.

Turkeys. Carcases up to 12 kg eviscerated weight were obtained from commercial crops of Ross Super Midi and Ross Maxi strains killed at 10–22 weeks of age. The heavier carcases were obtained from Ross Super Midi breeder stags killed at 52 weeks.

Measurement of surface area

Fifty eviscerated carcases of each species were selected as being of average carcase conformation in relation to age and weight. The neck skin (neck flap) was left attached to the carcase when the neck was removed. Carcases with machine damage or showing evidence of atypical development were rejected.

The selected carcases were air chilled to dry the surface thus facilitating measurement and removal of the skin. Carcases were weighed individuallly and the overall length of the carcase from the anterior edge of the neck flap over the breast to the tip of the uropygium noted. The transverse circumference of the carcase was measured at approximately the mid point of the sternum.

The intact wings were removed from the carcase at the junction between the humerus and coracoid. A median longitudinal dorsal incision was made through the skin. Lateral incisions were made to the points at which the wings had been removed and over the outside of the thighs of the carcase continuing down the leg to the joint between the tibia and metatarsus. The entire skin was then removed, care being taken to avoid stretching or distortion.

The longitudinal and transverse measurements were transferred to a sheet of waxed paper and the skin adjusted as closely as possible to these reference marks. The skin was then outlined using a felt tip pen and further reference marks made to indicate the limits of the neck flap. The wings were held in a flexed position on waxed paper and similarly outlined.

Each carcase was then halved longitudinally and tracings made of the outline of the exposed thoracic and abdominal cavities. The prepared tracings were then roughly cleaned and air dried.

The outlines were then traced onto calibrated sheets of waxed paper (4.21 g/1000 cm^2) using a cellophane interlay to prevent the transfer of fat to the copies. The wing and neck flap tracings were duplicated to represent each side. The clean tracings were then cut out and weighed in sets made up to represent the external surface area and the total surface area of each carcase. From these weights the corresponding surface areas were calculated.

Results and discussion

Chickens and ducks

The results of the surface area measurements for chickens and ducks are represented in Figs 1–4. Applying linear regression analysis to the results

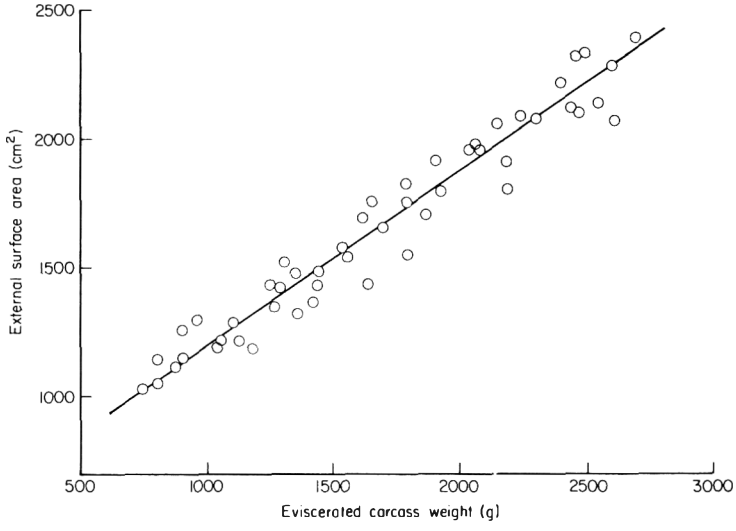

Figure 1. External surface of eviscerated chicken carcases as a function of the weight.

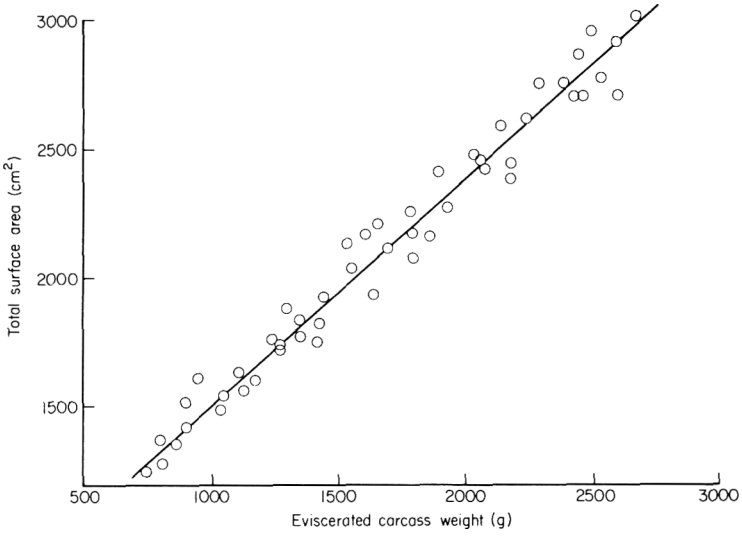

Figure 2. Total surface area of eviscerated chicken carcases as a function of the weight.

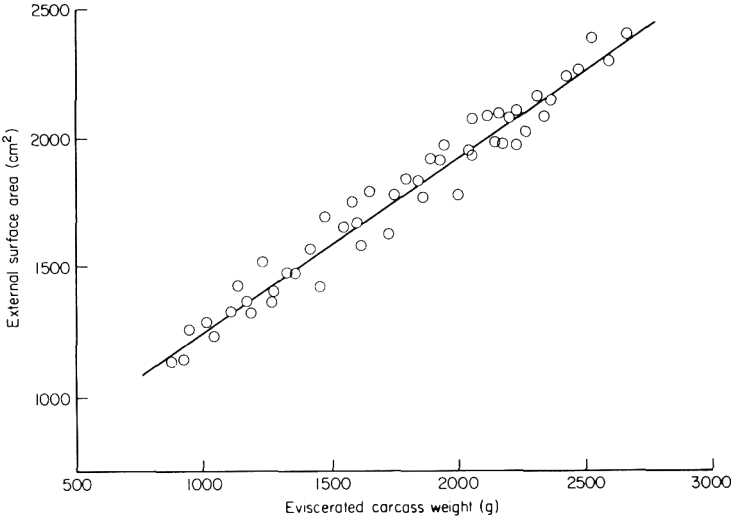

Figure 3. External surface area of eviscerated duck carcases as a function of the weight.

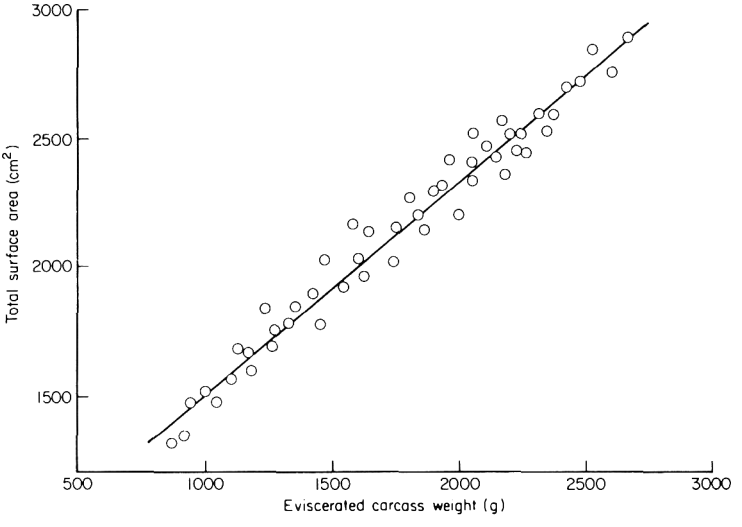

Figure 4. Total surface area of eviscerated duck carcases as a function of the weight.

obtained the 'best estimate' of the external and total surface areas can be expressed as:

Chickens

External surface area $cm^2 = 0.67x + 536$
(standard error of estimate = 95.5. $r = 0.97$)

Total surface area $cm^2 = 0.87x + 635$
(standard error of estimate = 173.3. $r = 0.94$)

Ducks

External surface area $cm^2 = 0.66x + 583$
(standard error of estimate = 68.4. $r = 0.98$)

Total surface area $cm^2 = 0.81x + 696$
(standard error of estimate = 74.2. $r = 0.98$)

where x is the eviscerated carcase weight expressed in grams.

Turkeys

The measurements obtained from eviscerated turkey carcases are expressed graphically in Figs 5 and 6 from which it is evident that a single linear relationship cannot be derived to cover the entire commercial weight range. However

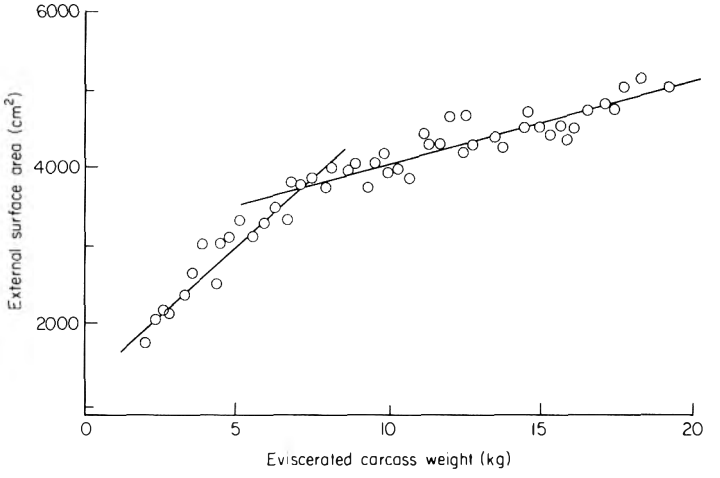

Figure 5. External surface area of eviscerated turkey carcases of two weight ranges expressed as a function of the weight.

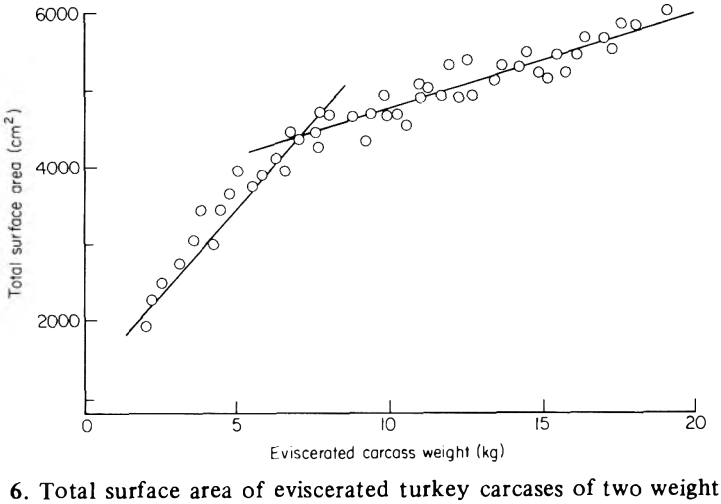

Figure 6. Total surface area of eviscerated turkey carcases of two weight ranges expressed as a function of the weight.

application of linear regression analysis to the measurements taken from eviscerated carcases below and above 7 kg separately showed that satisfactory linear relationships could be obtained. In practice this division accords well with the usual commercial distinction between carcasses processed for retail and catering outlets as 'oven-ready' birds and the larger carcases, including mature breeder birds, which are used for other manufacturing purposes and which are often processed separately.

The regression equations derived are as follows:

Turkeys less than 7 kg

External surface area $cm^2 = 0.36x + 1219$
(standard error of estimate = 156.6. $r = 0.96$)

Total surface area $cm^2 = 0.45x + 1293$
(standard error of estimate = 201.9. $r = 0.96$)

Turkeys over 7 kg

External surface area $cm^2 = 0.10x + 3025$
(standard error of estimate = 170.7. $r = 0.90$)

Total surface area $cm^2 = 0.13x + 3480$
(standard error of estimate = 155.3. $r = 0.94$)

where x is the eviscerated carcase weight expressed in grams.

The regression equation for the total surface area of chickens ranging from approximately 750–2700 g now reported shows good agreement with the formula proposed by Simonsen (area in cm^2 = wt in grams + 500) when applied to chickens up to about 1500 g. However as the eviscerated carcase weights increase beyond 1500 g the Simonsen formula tends to over estimate the total surface area.

The measurements obtained from turkeys covering an extended weight range and including birds at physical maturity show that both linear and interspecies extrapolation can lead to significant error and that in the case of turkeys separate equations are necessary for birds in lower and higher weight ranges.

Acknowledgments

The author is indebted to Mrs Dianne Harmer and Scott Thomas for valued technical assistance.

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β-Carotene loss in palm oil used for frying Nigerian snacks

M. V. RAJAGOPAL AND SUMATI R. MUDAMBI*

Summary

The loss of β-carotene from palm oil used by vendors for the frying of Nigerian snacks was investigated. Samples were collected from a vendor frying 'akara balls' and plantains for sale. Temperature, and duration time of frying each snack was noted. The destruction of β-carotene was found to be greater when used oil was employed for frying than when fresh oil was used.

Introduction

Crude palm oil is used extensively in Southern Nigeria for seasoning food and deep fat frying. Palm oil is usually a thick liquid at ordinary temperatures though some solids separate out when the oil is exposed to low temperatures. It is used as a cooking medium because it has two desirable characteristics, viz. (a) the red colouration which gives the food a colour appeal to the consumer and (b) the flavour which is specific to the taste of the Nigerian consumer. Further, it is locally produced and not as expensive as other fats and oils. The pigments in palm oil are carotenoids, the most important one being β-carotene. (Hunter, Scott & Williams, 1944). Mudambi & Rajagopal (1977) have investigated the effect of heating at different temperatures in the laboratory on the β-carotene content of palm oil. However, no observation has been made so far on the retention of β-carotene in palm oil when foods are fried under commercial conditions. Fried foods such as 'akara balls' (a savoury snack made from bean flour) and plantains which are prepared by vendors and sold as snacks, are very popular in Nigeria. This work was therefore undertaken to study the loss of β-carotene in palm oil when it is used to fry Nigerian snacks for vending in public places such as a market or bus stand.

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Materials and methods

'Akara balls' and fried plantains were the two fried foods made for sale. Palm oil was used for deep fat frying and both plantains and 'akara balls' were fried in the same oil. The order in which these are fried depends on the demand. Thus, sometimes vendors make 'akara balls' first and at other times, plantains.

A seller of such savoury snacks who had a stall in the Nsukka market for preparing these snacks co-operated in obtaining data for this study by allowing us to collect samples of oil used for frying at various stages of the operation. The temperature of the oil was noted accurately from the beginning when the oil was poured into the frying pan till the last item was fried, at hourly intervals. Temperatures were also recorded when any new food was introduced for frying. Samples of palm oil were collected in duplicate at each recording of the temperature. These were cooled and brought to the laboratory for analysis of β-carotene. Whenever any fresh oil was mixed with the used oil from a previous day's frying samples were collected in duplicate of both the fresh oil and the mixture of the fresh and used oil from the frying pan. The item being fried and the length of time it was fried was also noted.

Analysis of β-carotene

β-carotene was determined in each of the samples as outlined in the A.V.C. methods (1966). The carotenoids were extracted completely with several fractions of petroleum ether (b.p. 60–80°C) after saponification with 12% KOH. Solutions of crystalline β-carotene (E. Merck.) were used as standards for each estimation. The carotene was estimated by determining the intensity of colour at 450 μm in a Bausch and Lomb 'Spectronic 20' colourimeter.

Results and discussion

It was observed that vendors re-used the oil left from previous days frying by topping it up with fresh oil to improve the colour of the oil. Normally, the residual oil gets too dark to be used after 3 days frying. Thus there are very few days when the vendors start with a fresh batch of oil. Most of the days the frying is done in a mixture of fresh and used oil and the amount of fresh oil added depends on the quantity of oil left over. Once in the course of the week it was observed that no fresh oil was added at all.

Frying is usually carried out in large iron containers placed on tripods. The fuel used is firewood. Temperature control is achieved by reducing the heat by removing part of the flaming wood or extinguishing part of the burning wood.

Table 1 gives the β-carotene content of palm oil when used for frying plantains in a commercial operation. Here fresh oil was mixed with the oil left over from the frying done the day before. The β-carotene content of the fresh oil and that of the mixture of the fresh and used oil is given in the table. It is

Table 1. β-carotene content of (a mixture of fresh and used) palm oil during frying plantain

Period of heating (min)	Temperature (°C)	Snack made	β-carotene* content (μg/100 g)
0	25	Plantain	57 368†
15	145	Plantain	239
75	168	Plantain	120
135	165	Plantain	0

* β-carotene content of fresh oil was 129 475 μg/100 g.
† β-carotene content of mixture of fresh and used palm oil.

Table 2. β-carotene content of (mixture of fresh and used) palm oil during frying of snacks

Period of heating (min)	Temperature (°C)	Snack made	β-carotene* content (μg/100 g)
0	25	—	88 865†
15	155	Plantain	410
75	147	Akara balls	370
135	151	Akara balls	0

* β-carotene content of fresh oil was 148 400 μg/100 g.
† β-carotene content of mixture of fresh and used palm oil.

Table 3. β-carotene content of used palm oil during frying of plantain

Period of heating (min)	Temperature (°C)	Snack made	β-carotene* content (μg/100 g)
0	25	Plantain	495
15	125	Plantain	0
135	129	Plantain	0
255	140	Plantain	0
345	160	Plantain	0

* No fresh oil was added. All products were fried in used oil.

seen that with an exposure of only 15 min at a temperature of 145°C for frying plantains, the β-carotene content comes down by 99.5%.

Table 2 gives the β-carotene content of palm oil when used for frying plantains and akara balls. Here also a mixture of fresh oil and some oil remaining overnight from a previous days frying was used. There is again almost complete destruction of β-carotene once the temperature is raised to 155°C and held at

Table 4. β-carotene content of fresh palm oil during frying of snacks

Period of heating (min)	Temperature (°C)	Snack made	β-carotene* content (μg/100 g)
0	25	—	115 080
15	150	Akara balls	73 110
60	155	Akara balls	12 960
105	140	Plantain	813
150	155	Akara balls	189
360	150	Akara balls	0

* Only fresh oil was used.

that temperature for frying plantain. By the time the 'akara balls' were taken for frying, the oil contained only 410 μg of carotene. At the end of the frying operation the amount of β-carotene was negligible.

In Table 3 is presented the β-carotene content of used palm oil in which frying of plantain was carried out. No fresh oil was added here and it is seen that there is hardly any β-carotene present in the oil to start with.

The vendor was provided with fresh palm oil and asked to use only fresh oil for frying so as to study the effect of frying 'akara balls' and plantain using fresh oil. The results of this experiment are presented in Table 4. The loss of β-carotene is much less and follows almost the same pattern as was observed by Mudambi & Rajagopal (1977) when palm oil was heated under controlled laboratory conditions. While they observed retention of 53% of β-carotene when heating for 30 min at 150°C, here retention of 63.5% was observed when frying was carried out at the same temperature for 15 min. Further frying of 'akara balls' at 155°C for a total period of 60 min resulted in a loss of 88%. By the time the oil was heated for 105 min there was only a negligible quantity of β-carotene left. From Tables 1 and 2 it can be seen that when fresh oil was mixed with used oil in which food had been fried, the destruction of β-carotene was much faster than when fresh oil alone was used as seen in Table 4.

Thus the heating of oil in the presence of food results in accelerating the loss of β-carotene. It may be interesting to determine the absorption of β-carotene by the food being fried.

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A semi-empirical correlation for prediction of hydration characteristics of paddy during parboiling

S. BANDYOPADHYAY AND N. C. ROY*

Summary

A semi-empirical correlation based on the modified diffusion equation proposed by earlier workers is shown to fit the soaking data of a large number of paddy varieties. The correlation gives a unified approach to the diffusional process and can be used for prediction of moisture content as a function of time and temperature of soaking.

The results confirm the authors' previous hypothesis about the mechanism of soaking paddy.

Introduction

The previous study (Bandyopadhyay & Roy, 1976) dealt with the absorption of water by paddy grains during parboiling at different temperatures and times of soaking and showed that the diffusion equations proposed by earlier workers (Becker, 1960; Bandopadhyay & Ghose, 1965; Biswas & Ghose, 1973) can be used to describe the soaking process in a batch operation.

The simplified diffusion model of Becker (1960) is

$$\bar{x} - x_0 = 2/\sqrt{\pi}\,(x_S - x_0)\,(S/V)\,\sqrt{D\theta} \tag{1}$$

or

$$\bar{x} - x_0 = k_m\sqrt{\theta} \tag{2}$$

where: x_0 = initial, uniform moisture content (g/g dry basis),
$\bar{x}$ = average moisture content for a given absorption period (g/g dry basis),
x_S = effective moisture content at the bounding surface at times greater than zero (g/g dry basis),
S = exposed surface area of a solid (paddy grain) (cm^2),
V = volume of a solid (paddy grain) (cm^3),

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D = diffusion coefficient (cm^2/sec),
θ = absorption time (sec),

and

$$k_m = 2/\sqrt{\pi}\,(x_s - x_0)\,(S/V)\,\sqrt{D}. \quad (3)$$

Becker (1960) proved the validity of eqn (1) with soaking data of a particular variety of wheat.

Ghose *et al.* (1965, 1973) modified eqn (1) by considering an Arrhenius-type of relationship for the diffusional process and proposed the following equations:

$$\bar{x} - x_0 = 2/\sqrt{\pi}\,(x_s - x_0)\,(S/V)\,\sqrt{D_0 \exp(-E/RT)\,\theta} \quad (4)$$

and

$$k_m = K\sqrt{\exp(-E/RT)} \quad (5)$$

where

$$K = 2/\sqrt{\pi}\,(x_s - x_0)\,(S/V)\,\sqrt{D_0}$$

and

D_0= diffusion constant (in Arrhenius equation) (cm^2/sec),
E = activation energy (cal/mole),
T = absolute temperature (°K),
R = gas constant (cal/mole °K).

Bandyopadhyay & Ghose (1965) and Biswas & Ghose (1973) tested the applicability of eqn (4) in case of soaking of a few local varieties of Indian paddy. Although the equation was confirmed by Ali (1974) in case of soaking of paddy, the values of the constants obtained by him were not comparable to those of Ghose *et al.* The present authors (1976) have confirmed the applicability of eqns (2) and (4) with three high yielding varieties of Indian paddy with a different approach to the diffusional process and tested their validity under some limiting conditions. While this approach provided a practical basis to the diffusional process with an entirely new set of results, it lacks in generalization of data for a large number of paddy varieties studied by various workers.

The present report incorporates the results of the analysis of soaking data of a large number of varieties studied by the present authors and earlier workers (Bandyopadhyay & Ghose, 1965; Biswas & Ghose, 1973; Ali, 1974). The objective is to provide a generalized and unified approach to the soaking process in parboiling and to use the generalized correlation for prediction of hydration characteristics of paddy.

Correlation and discussion of results

For correlation of the soaking data collected by various investigators, the eqn (1) was modified in the form,

$$\bar{x} - x_0 = 2/\sqrt{\pi}\,k'_m\sqrt{\theta} \quad (6)$$

where

$k'_m = (x_s - x_0)\,(S/V)\,\sqrt{D}$.

Considering the moisture gain due to initial hydration, Δx_i g/g dry basis.

$$\bar{x} - x_0 - \Delta x_i = 2/\sqrt{\pi}\; k'_m \sqrt{\theta}. \tag{7}$$

The experimental data of moisture gain by paddy at different times and temperatures of soaking have been found to fit eqn (2) for a short duration of soaking and up to a limiting moisture content in the range of 0.5–0.6 g/g dry basis. It has been observed by the present authors (1977) that below a temperature of about 65°C this moisture content was insufficient for considerable 'degree parboiling', but sufficient for achieving about 90% 'degree of parboiling' without excessive swelling of grains at a temperature around 65°C and above. This temperature is in the vicinity of gelatinization temperature of rice starch. Soaking above gelatinization temperature ensues rapid hydration due to splitting of husk. These observations have substantiated the earlier one on splitting of husk during soaking at about 65°C which was the limiting condition (Bandyopadhyay & Roy, 1976) for analysis of data.

The extrapolated values of initial moisture gain, Δx_i, obtained from the linear regression of $\bar{x} - x_0$ on $\sqrt{\theta}$, according to eqn (2), have been found to be strongly dependent on temperature of soaking for all the varieties. With increase of temperature it increases up to a temperature of 60–65°C and then decreases. The increasing trend could not be emphasized in the earlier study (Bandyopadhyay & Roy, 1976) for want of data at some intermediate temperature such as 40°C in the lower range. Figure 1 shows that the data on initial moisture gain can be expressed as linear function of temperature as

$$\Delta x_l = a + bt, \tag{8}$$

and

$$\Delta x_l = a - bt \tag{9}$$

in the lower and higher temperature regions respectively, where t is the temperature in °C and a and b are constants. These relations apply in all the varieties except in Bala at temperatures below 65°C. The reasons for the dependence of Δx_i on temperature put forward by the earlier workers (Becker, 1960; Bandyopadhyay & Ghose, 1965; Biswas & Ghose, 1973; Ali, 1974) were not convincing in the context of different trends noted. While re-analysing the data reported by Ali (1974) for the purpose of fitting his data to eqn (7), it was found that the re-calculated values of Δx_i have shown the trends very much similar to that noted by the present authors. This is shown in Fig. 2 for the varieties Patnai–23 and IR–8 studied by Ali (1974). It appears that initial moisture absorption presents a different picture in case of soaking of paddy and reasons put forward by Becker (1960) may not hold good. This may be due to the presence of porous husk and void space in between the husk and pericarp in paddy which has changed the external structure of paddy grains from that of wheat.

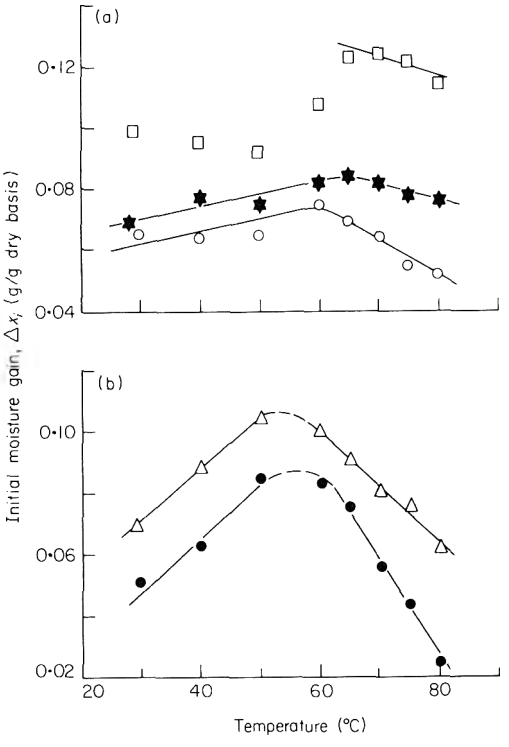

Figure 1. Initial moisture gain as a function of temperature for different varieties of paddy. (a) ○, Padma; □, Bala; ★, Pankaj; (b) ●. Jaya; △, Ratna.

To correlate the soaking data of the varieties Jaya, Padma, Ratna studied earlier (Bandyopadhyay & Roy, 1976) and of the two varieties more viz., Bala and Pankaj, according to eqn (7) k'_m values have been calculated from k_m evaluated by the Arrhenius type of equation, eqn (5). Δx_i values have been calculated from the empirical relations, eqns (8) and (9). Figure 3 shows

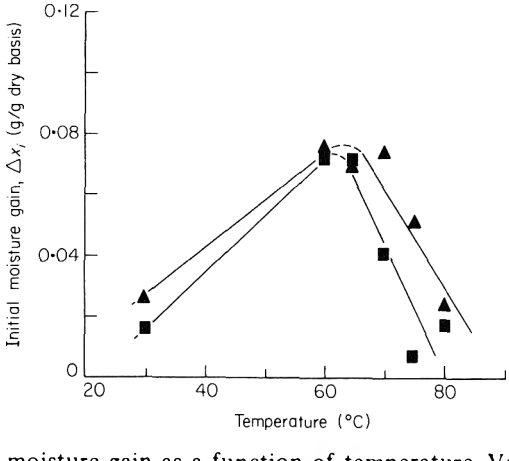

Figure 2. Initial moisture gain as a function of temperature. Values of Δx_i have been re-calculated from the data of Ali (1974). ▲, Patnai-23; ■, IR-8.

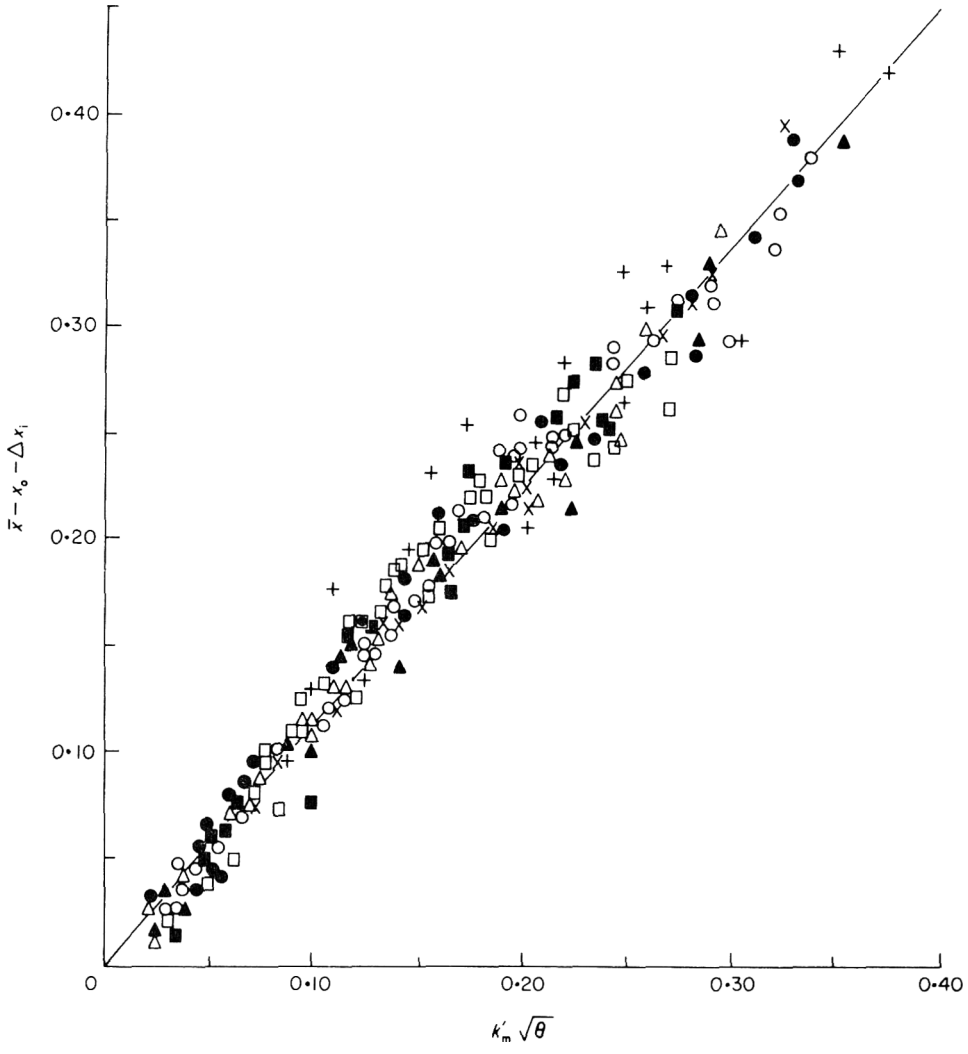

Figure 3. Correlation of present data and re-calculated data obtained from different sources, using the semi-empirical eqn (7). Present data: ○ Padma, ● Jaya, △ Ratna, □ Bala (Bandyopadhyay & Ghose, 1965; Biswas & Ghose, 1973): + Jhingasal, X Sitasal; Ali (1974): ▲ Patnai-23, ■ IR-8.

such correlation embracing the data of the above varieties except Pankaj in the temperature range 28–80°C. The experimental data are in excellent agreement with eqn (7). The coefficient of correlation between the variables has been calculated to be 0.98 which shows that these are highly correlated. Figure 3 also shows the accuracy with which prediction of moisture content can be made as a function of time and temperature of soaking. Prediction was done for the whole range of temperature with the help of eqn (7) for the variety Jaya and is shown in Fig. 4 along with the experimental points, except at temperatures 40 and 65°C.

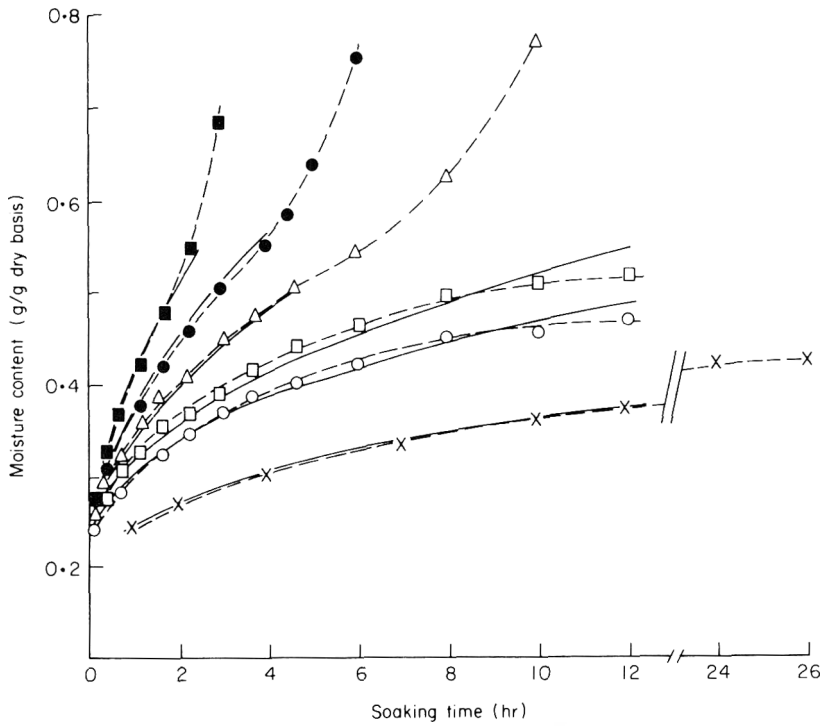

Figure 4. Prediction of moisture content as a function of time and temperature of soaking; variety: Jaya. X, 30°C; ○, 50°C; □, 60°C; △, 70°C, ●, 75°C, ■, 80°C. —— predicted curve, – – – experimental curve.

Data of Bandyopadhyay & Ghose (1965) and Biswas & Ghose (1973). For fitting the data into eqn (7), the values of moisture content at different periods of soaking were read from the plots reported for the varieties Sitasal and Jhingasal. Δx_i values were taken also as reported. The values of k_m were calculated using accurate values of activation energy and constant K from the earlier report (Bandyopadhyay & Roy, 1975). The results are shown in Fig. 3. All the data of Sitasal variety show good fit. The only data which have deviated considerably are those of Jhingasal at 75 and 85°C. Since the data at other temperatures are in excellent agreement with eqn (7) it is not unlikely that those at 75 and 85°C are in error, owing to some causes unknown to the authors.

Data of Ali (1974). Since Ali took an entirely different approach for evaluation of the constants of eqn (5), re-analysis of his data are necessary. Regression analysis of moisture gain on the square-root of absorption time up to the limiting moisture content range for the varieties Patnai–23 and IR–8 gave the fresh values of k_m and Δx_i. The empirical relation of Δx_i with temperature could not be arrived at for scanty data. The fresh plots of $\log k_m$ *v.* $1/T$, as shown in Fig. 5 for Patnai–23, show distinct breaks identical in nature with those observed by Bandyopadhyay & Roy (1975, 1976) with their data and data of Bandyopadhyay & Ghose (1965) and Biswas & Ghose (1973). This observation once again confirms the break-point which takes place in the

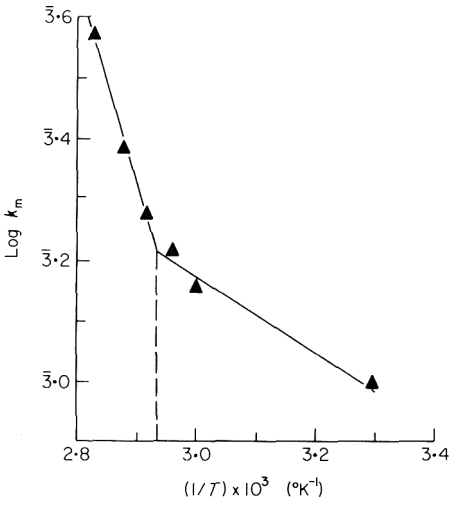

Figure 5. Log k_m as a function of the reciprocal of the absolute temperature for Patnai-23 variety. k_m values have been recalculated from the data of Ali (1974).

vicinity of gelatinization temperature of rice starch. The comparative values of activation energy and temperature of break obtained from different soaking data are presented in Table 1. The experimental data of Ali (1974) were fitted to eqn (7) with the help of re-calculated values of Δx_i, E and K, and plotted in Fig. 3. Almost all the data are in excellent agreement with the correlation.

Table 1. Comparative values of activation energy and temperature of break

Variety of paddy	Source of data*	Activation energy (cal/mole)		Temperature of break (°C)
		Lower temperature region	Higher temperature region	
Jaya	Bandyopadhyay	7654	25420	63
Ratna	&	4400	25500	62
Padma	Roy, 1976	7510	31700	68
Bala	Present	5738	23080	69
Pankaj	data	5540	25000	69
Patnai-23	Ali,	5754	30670	67.5
IR-8	1974	2575	28700	60.0

* Re-calculated values of activation energy from Bandyopadhyay & Ghose (1965) and Biswas & Ghose (1973) could not be compared due to scanty data.

Conclusion

It was found that soaking of paddy, irrespective of variety, can be described by a semi-empirical diffusion equation

$$\bar{x} - x_0 - \Delta x_i = 2/\sqrt{\pi}\, k'_m \sqrt{\theta}$$

which unifies the different approaches taken by earlier workers on moisture absorption by paddy. The correlation can be used to predict reasonably well moisture gain as a function of time and temperature of soaking.

The results of a large number of paddy varieties confirm the hypothesis propounded by Bandyopadhyay & Roy (1976) that soaking of paddy is influenced by two different mechanisms, one below and the other above the gelatinization temperature of rice starch.

Acknowledgment

The authors acknowledge Professor T. P. Ojha of Agricultural Engineering Department and the Rice Process Engineering Centre, I. I. T., Kharagpur for providing certain facilities for the work and Dr A. N. Roy, Head of Chemical Engineering Department for his interest in the work.

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On the meaning of the degree of milling of rice*

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Summary

A sample of brown rice was milled to different degrees with four different laboratory millers – two metal rollers, one emery roller and one emery cone. The loss of kernel weight during milling as well as the contents of residual bran pigment, the total fat and the surface fat of each of the milled samples were determined. It was observed that the emery mills had to remove a greater weight of kernel (as 'bran') to yield a milled rice having a given content of pigment or fat, as compared to the metal mills. The degree of milling of rice as conventionally defined by the percentage loss of kernel weight during milling and that as generally understood by product quality (colour or content of a specified constituent of milled rice) are therefore not identical entities. Hence to define degree of milling of rice for product quality needs the use of a different index – such as the percentage retention of bran pigment or the percentage kernel surface area covered with bran – than the conventional criterion of weight loss.

Introduction

Milling of rice consists essentially of two steps: (i) shelling (or dehusking) of paddy, followed by (ii) milling (or whitening, pearling) of the resulting brown rice. The object of the second step is to remove, to a greater or a lesser extent, the outer layers of brown rice, viz. the pericarp, tegmen and aleurone as well as the germ – collectively called 'bran' – which are rich in pigments, fat, fibre and ash. The extent to which these layers are removed is expressed as the 'degree of milling' (d.m.) of rice.

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This term however has not been always uniquely understood or defined. In specifying the d.m. for grading and marketing, the emphasis has been on how much of the *bran* layers has been removed. For example, for grading of rice in the U.S.A. (Webb & Stermer, 1972) and in a proposed international model (F.A.O., 1972), the degree of milling of rice is expressed in such terms as 'well milled', 'reasonably well milled', 'lightly milled', 'undermilled', etc., each of which term is again defined by expressions such as 'paddy from which ... the outer bran layers and the greater part of the inner bran layers have been removed...'. The grades too are usually determined by visual comparison with standard check samples.

However, for various studies on rice in the laboratory, a more objective and quantitative scale was obviously needed. For this, researchers all along intuitively used the percentage loss in *weight* of the brown rice during milling as the criterion of d.m. But even here the concept has evolved over time. In very old literature the weight loss was usually calculated from the simple difference between the weights of the unmilled and milled rice – which meant that the small pieces of broken rice passing through the mill screen were also erroneously included in the 'bran'. For example, it is common in such literature to come across expressions as 'rice was milled to 20%', which would be physically impossible with the archaic equipment then in use unless the fine brokens were disregarded in weighing the milled rice. This error was gradually rectified until now the d.m. for research purposes is always referred to as the percentage loss in weight of brown rice reckoned either from the weight of 18-mesh powder ('bran') produced during milling (Bhattacharya & Sowbhagya, 1972) or from the difference in the 1000-grain weight before and after milling (Barber & Benedito de Barber, 1976).

Meanwhile, a great deal of effort has been devoted during the last four decades to devising a simple laboratory method for determining the d.m. of rice for trade as well as research purposes, as reviewed by Hogan & Deobald (1965) and Barber & Benedito de Barber (1976). The suggested methods are all based on determining the loss (fat, pigments, minerals, vitamins) or gain (starch, whiteness) of a specified constituent or character of rice that accompanies the physical removal of its bran. The criterion of weight loss of the kernel has again usually been used as the reference scale in these studies.

Now defining the d.m. by loss of weight as above and determining it on the basis of product quality (colour, content of a constituent) – either by laboratory analysis or by visual inspection – presupposes, however, that the loss in weight is synchronous with the change in a specified constituent or character irrespective of the mill type. The object of the following study was to test this hypothesis.

Experimental

About 12 kg of a lot of a local nonwaxy tall *indica* variety of paddy (Bangara Sanna), available from laboratory stock, was shelled in a rubber roller sheller.

The resulting brown rice (freed from broken grains and residual paddy) was milled in the following four types of laboratory milling equipment:

(a) A modified (Bhattacharya & Sowbhagya, 1972) McGill miller No. 1, a mill fitted with a horizontal metal roller (hereinafter called McGill metal),

(b) Satake Testing Pearler (type OM-2B), a horizontal metal roller mill of continuous operation (Satake metal),

(c) Satake Grain Testing mill, a horizontal emery roller mill (Satake emery), and

(d) Automatic Patent Minghetti, an emery cone polisher mill (Minghetti emery).

These mills are prototypes of the commerical rice whiteners in use. All the mills except mill (b) were of batch type handling 100–200 g of rice at a time. The d.m. (always referred to by weight in this and the next section) was varied, by varying the feed rate and/or time and pressure of milling, in the range of 0–10.5% for mills (a) and (b) (the limit of milling in these mills) and 0–14% in mills (c) and (d).

The d.m. of each sample was determined by several replicate determinations of the 1000-grain weight (checked with the weight of separated bran in mills (a) and (c) where the bran could be quantitatively recovered). The moisture content of all the samples lay in the range of 11.5–11.9% and hence no correction for moisture difference was applied to any of the results. Grinding for analysis was done in a Buhler disc mill (model MLI-204) to pass a 30-mesh screen.

Results and discussion

The residual bran pigment (Bhattacharya & Sowbhagya, 1972), and the total and surface fat contents (Bhattacharya, Sowbhagya & Swamy, 1972) of the various milled rice samples were determined and plotted against the respective d.m. It was observed that the curves were not identical for all the mills (Figs 1–3). The curves for the two metal mills (a) and (b) were fairly close to each other. Those for Satake emery (mill (c)) diverged from the above especially from 6% d.m. onwards, and those of Minghetti emery (mill (d)) diverged considerably throughout. The interpolated loss in grain weight (d.m.) corresponding to a given loss in bran pigment or fat for the different mills are shown in Table 1. Clearly the emery mills, the Minghetti cone in particular, had to remove a greater weight of grain substance (i.e. they had to mill to a greater d.m.) to remove an equivalent amount of bran pigment or fat compared to the metal mills.

The alcoholic alkali bran-staining test and the alkali degradation test (Bhattacharya & Sowbhagya, 1976) – two approximate visual tests of d.m. – were also carried out. These results too were broadly similar to the above (Table 2). It should be noted in particular that the 9–10% d.m. samples from the metal

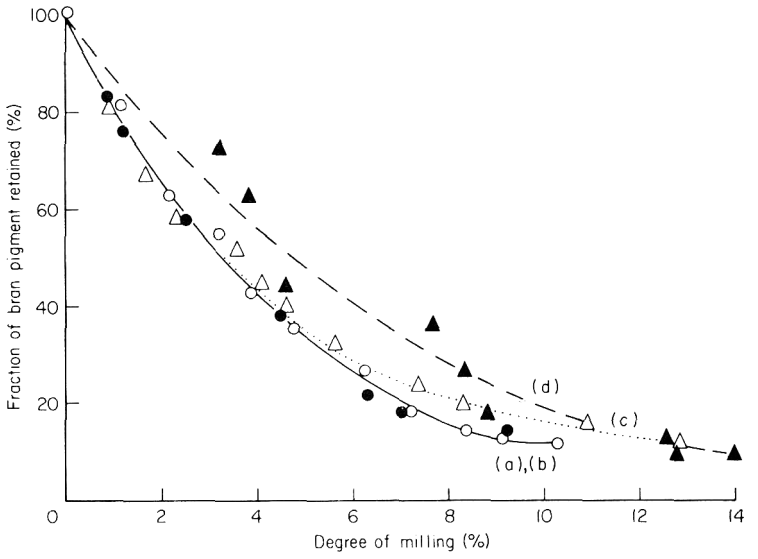

Figure 1. Bran pigment-*v.*-d.m. calibration curves of rice for different milling equipments. ○ (a) McGill metal; ● (b) Satake metal; △ (c) Satake emery; ▲ (d) Minghetti emery.

mills showed practically no trace of bran, while similar samples from the emery mills showed clear evidence of some residual bran.

Clearly there is a difference in the manner of bran removal between different mill types. It would seem that in the metal roller mills, because of only grain-to-grain abrasion, milling was apparently confined only to the relatively soft 'true

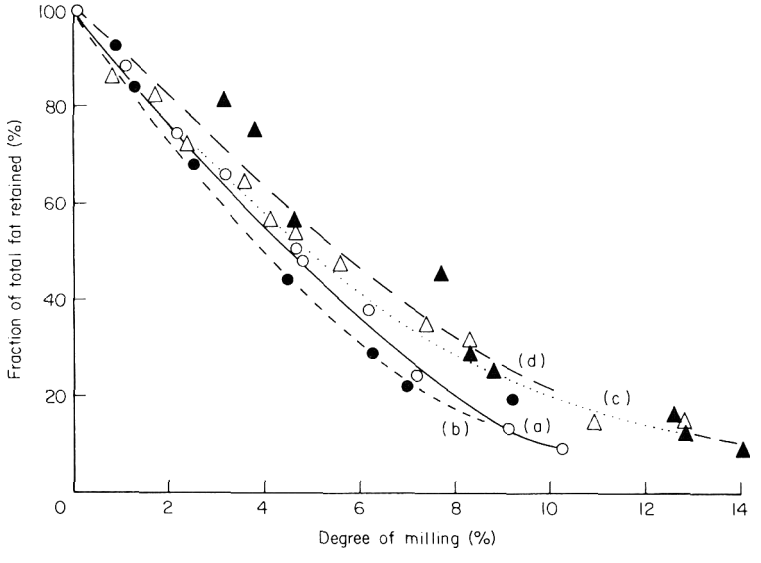

Figure 2. Total fat-*v.*-d.m. calibration curves of rice for different milling equipments. Symbols as in Fig. 1.

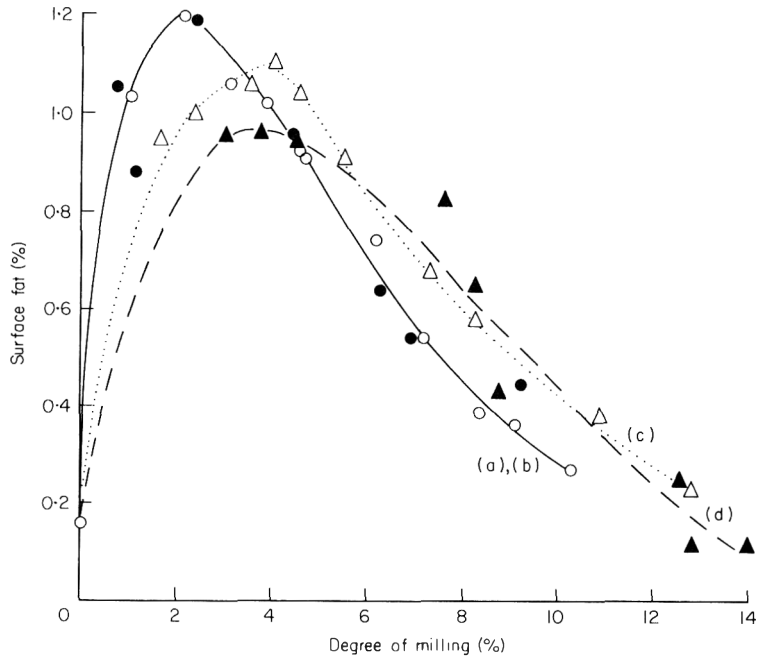

Figure 3. Surface fat contents of rice milled to different degrees in different milling equipments. Symbols as in Fig. 1.

Table 1. Relation of loss of weight (degree of milling) to the loss of specified constituents during milling of rice

Fraction of the constituent removed (%)		Loss of grain weight, d.m. (%)			
Bran pigment	Fat	(a) McGill metal	(b) Satake metal	(c) Satake emery	(d) Minghetti emery
30	—	1.6	1.6	1.6	2.6
40	—	2.4	2.4	2.4	3.5
50	—	3.3	3.3	3.4	4.8
60	—	4.3	4.3	4.4	6.0
70	—	5.5	5.5	5.8	7.6
80	—	7.1	7.1	8.2	9.7
85	—	8.3	8.3	10.6	11.0
90	—	—	—	13.8	13.8
	30	2.6	2.3	2.7	3.3
	40	3.5	3.1	3.7	4.4
	50	4.5	4.0	4.9	5.6
	60	5.6	5.0	6.2	6.8
	70	6.7	6.1	7.7	8.3
	80	8.0	7.5	10.0	10.3
	85	8.7	8.5	11.8	11.8
	90	9.9	9.9	14.0	14.0

Table 2. Grouping rice samples from different milling equipments according to visually perceived equivalence of residual bran

Test	Equipment	d.m. (%) of samples giving equivalent test response					
Alcoholic alkali	(a) McGill metal	10.3	9.1	8.4	7.2	6.2	—
	(b) Satake metal	—	9.2	7.0	—	6.3	—
	(c) Satake emery	12.8	10.9	>8.3	>7.4	>5.6	—
	(d) Minghetti emery	14.0	12.8	12.6	8.8	8.3	—
Alkali degradation	(a) McGill metal	10.3	9.1	8.4	7.2	6.2	4.8
	(b) Satake metal	—	—	9.2	7.0	6.3	4.5
	(c) Satake emery	12.8	10.9	8.3	7.4	5.6	4.6
	(d) Minghetti emery	14.0	12.8	12.6	8.8	8.3	7.7

bran' region without extending to the relatively hard endosperm. This is supported by the fact that milling in these equipments could not go beyond 10–11% d.m. regardless of the time and pressure of milling. The emery mills on the other hand, due to the additional direct abrasive action of the emery surface, seemed to remove the grain substance more randomly from the outer as well as the inner layers (including the endosperm) simultaneously. Indeed rice could be milled to 14% d.m. or more in these mills, indicating that the grain endosperm was not immune from being scratched away in these mills. These would explain why the emery mills by and large had to remove a greater weight of grain substance to remove an equivalent amount of a constituent of its outer layers (pigment, fat). That the surface fat–d.m. curves (Fig. 3) too were shifted to the right in the emery mills as compared to those from the metal mills also seem to point to the same conclusion.

The additional difference observed between the Satake and Minghetti emery mills suggested an emery mill-to-emery mill difference as well. It could be that the emery particles in the former were finer in size: hence the initial removal of bran from the soft outermost layers occurred in fairly successive layers; but once the bulk of the bran had been removed (5–6% d.m.), simultaneous scraping of inner layers (endosperm) began.

Conclusion

It follows from the above results that the degree of milling of rice, as conventionally defined by weight loss, and as analytically (or visually) determined by product quality are not necessarily the same. A given weight loss (conventional d.m.) corresponds, even in a single lot of rice, to different product qualities depending on the mill type used to produce it.

That being the case, there is need to redefine the meaning of the term degree of milling of rice. To know the precise extent of milling in terms of weight loss may or may not have its use. But the main purpose of knowing or determining

the d.m. of rice is to know its quality: in terms of acceptability, storability, nutrient content, or chemical reactivity. Clearly weight loss cannot unambiguously express this concept of d.m., i.e. d.m. as an index of product quality, even for a given variety. Two options are then open for this purpose. (a) One is to discard the criterion of weight loss during milling altogether and to express the d.m. entirely in terms of: either (i) the percentage retention of some specified constituent, say bran pigment (Bhattacharya & Sowbhagya, 1972), or (ii) the CBB (Coloured Bran Balance) index, i.e. the percentage of the kernel surface area still covered by bran (Barber & Benedito de Barber, 1976), or some other similar visual index (Bhattacharya & Sowbhagya, 1976). (b) Alternatively a weight loss scale – which is undoubtedly convenient – can still be retained provided one constructs an idealized weight loss-*v.*-quality scale and expresses the d.m. on the basis of this scale rather than the actual weight loss that occurred with the particular mill under use. The scale given by a metal roller mill, such as the McGill, would be most appropriate for this purpose, for milling in it would remain confined to the 'true bran' region.

Another incidental result of these findings is the light they throw on the efficiency of whitening equipments. If the emery mills needed to remove more kernel matter to yield a rice having a given degree of whiteness or a given content of some constituent, then surely they would yield a lower milling outturn compared to metal roller mills to produce rice having any given criterion of acceptability. In other words, other things being equal, it would be profitable to use metal rather than emery roller units for whitening of rice, particularly if it were to be highly milled.

Perhaps a more extensive study involving different types of rice and other possible mill types, including commercial units, might be desirable in view of the far-reaching conclusions above.

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Moisture determination in starch-rich food products by pulsed nuclear magnetic resonance

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Summary

The possibility of applying the low resolution pulsed NMR technique to moisture determination in starch-rich low-fat food products is described. The water content of the samples is measured from the liquid to solid ratio. The evaluation of the ratio depends upon the way of extrapolating the free induction decay (FID). The values of the extrapolation coefficients for the liquid and solid phases are measured and their dependence upon the sample water content is given. The method of standard addition for converting the percentage of moisture obtained in terms of hydrogen content to percentage in weight is described. The error statistical analysis is also reported.

Introduction

The technique of pulsed low-resolution nuclear magnetic resonance (NMR) can be used conveniently to distinguish between liquid and solid phases in a variety of samples (Van Putte & Van den Enden, 1973, 1974; Hester & Quine, 1976). The main advantages of the technique are the rapidity of the determination, the possibility of automation and, mainly, the fact of being non-destructive.

The purpose of the present paper is to show the applicability of the method to moisture determination in a variety of foodstuffs, rich in starch but very low in fat content. In particular, the following samples were studied: wheat flour, durum wheat semolina, corn flour, rice flour, ground whole rice, ground barley, potato starch, ground beans, ground and whole lentils, pasta made from 100% semolina and from 100% flour, gluten enriched pasta, and different types of bread.

For detailed information about the technique of pulsed NMR, the reader is

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referred to specialized literature, e.g. Farrar & Becker (1971); only its most relevant aspects will be summarized below.

The NMR signal is due to the magnetic moments of the protons present in sample. The protons are excited by a very short pulse of radiofrequency (RF). After the end of the pulse, the protons give rise to a decaying signal, called the free-induction decay (FID), the duration of which varies from a few tens of microseconds for solids to a few milliseconds (limited by the homogeneity of the magnetic field) for liquids. The total signal, just at the end of the pulse, is proportional to the number of protons in the measured volume. As an example, Fig. 1 shows the FID of a sample of flour. At the time $t = 0$, a trigger starts the experiment. After a certain instrumentally-fixed delay, δ, the RF pulse, of an adjustable duration p, begins. Typically, p is 4–5 μsec. After the pulse, the receiver is always inhibited for a certain period of time d, usually called dead-time. Therefore, the signal can be registered only subsequent to a time $t = \delta + p + d$. Since it is rather rare and impractical to digitize the whole FID, given the enormous difference in the decay rates between the solid and liquid signal, it is customary to sample the signal only at two points, placed

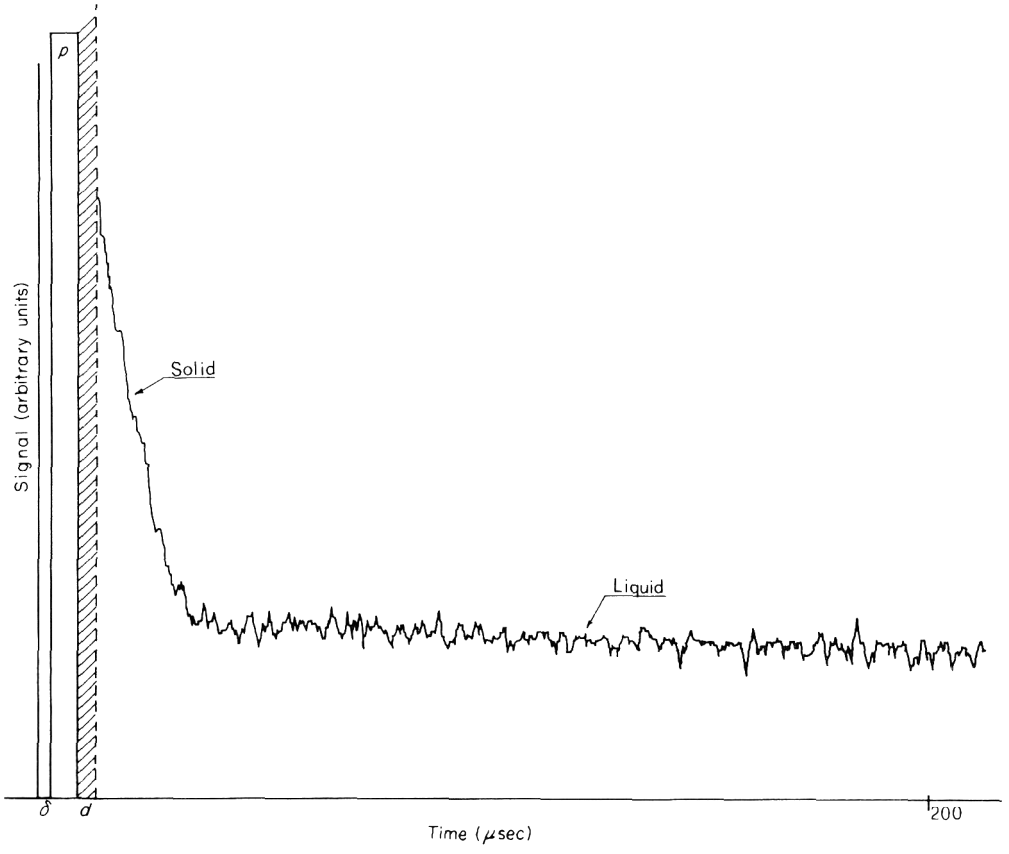

Figure 1. Free induction decay of (FID) of a flour sample.

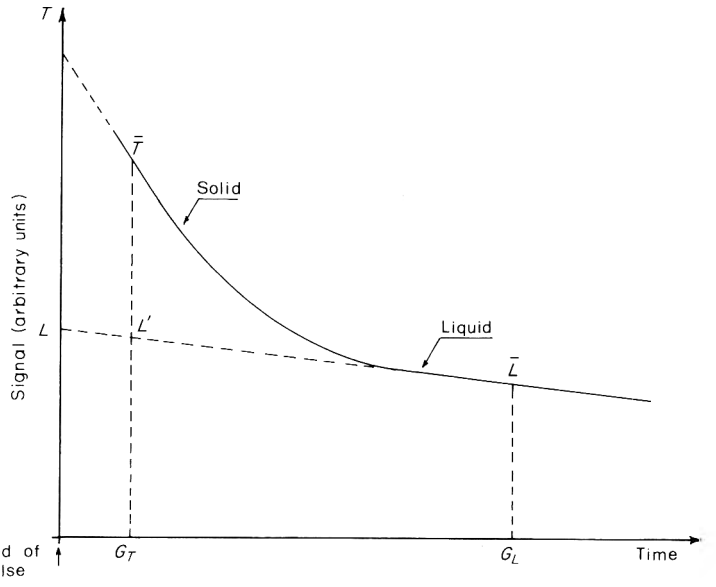

Figure 2. Schematic representation of the FID due to a solid phase and a liquid phase rapidly decaying.

at times G_T and G_L. This is accomplished by electronic sample-and-hold circuits piloted by DC pulses positioned at the desired times.

The signal $\bar{T}$, sampled at time G_T, (see Fig. 2) however does not coincide with the total signal T at the end of the pulse. The value of T can be obtained either by extrapolation based on the two digitized FID or by a semi-empirical extrapolation based on the two values L and $\bar{T}$, as described below. Once T is known, the relative amount of the liquid phase, measured in terms of hydrogen content, is simply L/T. Since the differences $\bar{S} = \bar{T} - L$ and $S = T - L$ are both proportional to the content of the solid phase, it is possible to write

$$S = f_s \bar{S} \tag{1}$$

where f_s is an empirical coefficient. Obviously, f_s depends upon the nature of the solid phase (i.e. the rate at which the solid phase signal decays) and on the position of the gate G_T. Taking into account eqn (1), the percentage of the liquid in the sample is

$$X = L/\,[L + f_s(\bar{T} - L)]\,. \tag{2}$$

This formula has been used for the determination of solid to liquid ratios in fats (Van Putte & Van den Enden, 1973, 1974).

In the present work the situation encountered is somewhat different. In starch-rich foods, the signal due to water decays unusually fast, indicating a dramatically reduced mobility of the water bound to the carbohydrates. In fact, in our case the decay is determined by the transverse relaxation time T_2 rather than by magnetic field inhomogeneity. It is therefore advisable to extrapolate also the signal due to the liquid phase. Denoting $\bar{L}$ the signal at

the time G_L, consequently we have $L = f_1 \bar{L}$, where f_1 is an extrapolation coefficient. Denoting L' the signal of the liquid phase extrapolated to the time G_T, we have (see Fig. 2)

$$L' = \bar{L} + (L - \bar{L})(G_L - G_T)/G_L . \tag{3}$$

Notice that the quantity $(G_L - G_T)/G_L$ is a readily and accurately measureable instrumental constant. The quantity proportional to the solid phase content is then $\bar{S} = \bar{T} - L'$, with eqn (1) remaining valid. Combining all these equations, we have

$$X = f_1 \bar{L} / [f_1 \bar{L} + f_s(\bar{T} - L')] . \tag{4}$$

Experimental

FID values were measured on a pulsed low-resolution NMR spectrometer (20 MHz, model Minispec P20, Bruker Spectrospin). The temperature was 25 or 40°C; no significant difference was observed in relation to the temperature. When needed, the FIDs were digitized by a Bruker fast digitizer (model B-C104) and plotted on a Hewlett-Packard X–Y recorder. Measurements of $\bar{T}$ and $\bar{L}$ were carried out by means of a Bruker double-channel digitizer and analog computer B-AC5. In all cases, 90° pulse (pulse width about 5 μsec) and the phase detection mode were used. The centres of the gates G_T and G_L were placed at 9 μsec and 90 μsec from the end of the RF pulse, respectively. The width of the gates was in both cases 2 μsec. The relative gain in the two channels was made equal by adjusting the apparent solid content to 0% for a completely liquid, slowly decaying sample (oil). The samples were prepared and measured in 10 mm tubes (external diameter). The repetition rate between successive pulses was 1 sec; averages of ten measurements were taken for all digital values.

Results

Extrapolation coefficients

The coefficients f_s and f_l were obtained by a graphical extrapolation of digitized FIDs. In fact, this is the most reliable method. The results for a variety of food products of varying moisture content are reported in Fig. 3. There seems to be no significant difference between the products examined. It turns out that both coefficients depend somewhat on the water content. This dependence has been fitted by a second degree polynomial using the least squares technique. It is important to keep in mind that the extrapolation coefficients f_s and f_l depend on the distance between the end of the pulse and the gates G_T and G_L. The interpolation polynomials for different values of G_T and G_L are listed in Table 1.

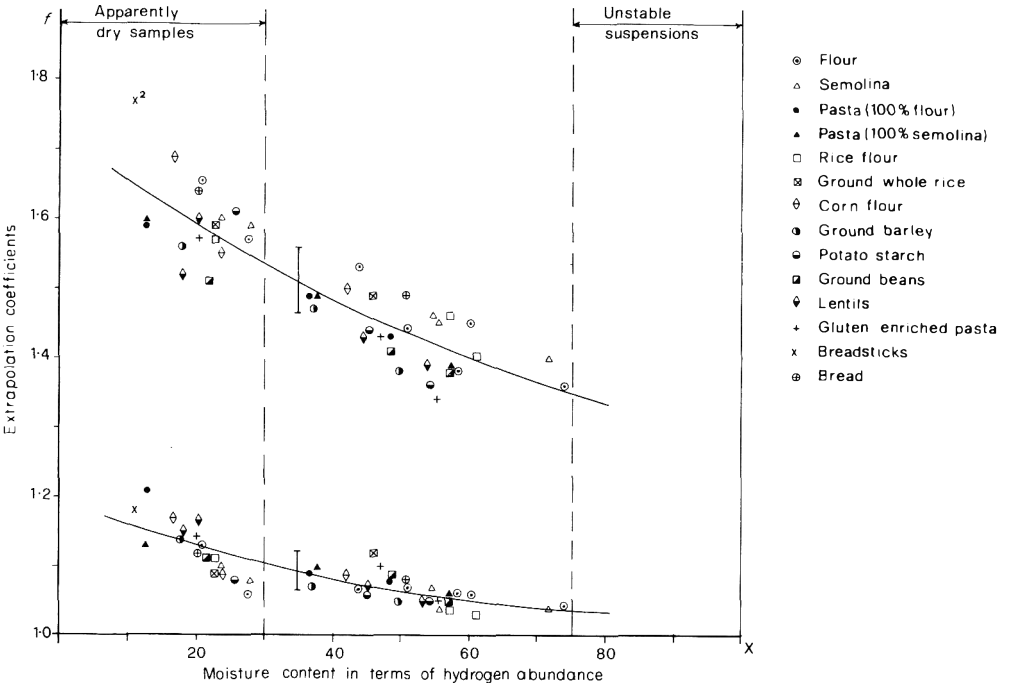

Figure 3. Dependency of f_s and f_l coefficients on moisture content in a variety of samples. Solid lines represent the fitting of the experimental points by a second degree polynomial $f = a + bx + cx^2$ for G_T = 9 μsec and G_L = 90 μsec; bars represent mean square deviations.

Table 1. Least squares fits of the extrapolation coefficients f_s and f_l by a quadratic function for different gate times G_T and G_L (measured from the end of the pulse)

G_T (μsec)	$f_s = a + bx + cx^2 \pm e$			
	a	b	c	e
5	1.335	− 0.447	0.340	0.0271*
7	1.487	− 0.445	0.165	0.0052
9	1.725	− 0.710	0.283	0.0068
11	2.086	− 1.411	0.806	0.0114
G_L (μsec)	$f_l = a + bx + cx^2 \pm e$			
	a	b	c	e
50	1.066	0.032	− 0.178	0.0005†
70	1.159	− 0.352	0.261	0.0023
90	1.196	− 0.363	0.202	0.0035
110	1.233	− 0.352	0.098	0.0064

* Measurements impaired because of dead-time limitations.

† Anomaly due to the (sin At)/t beat observable in the solid phase part of FID.

Using Table 1, the practical determination of X can be carried out as follows: the values $\bar{T}$ and $\bar{L}$ are measured by a two-channel digitizer. Assuming any initial value of X, the coefficients f_s and f_l are calculated and a new value of X is determined from eqn (3) and (4). The coefficients f_s and f_l are then recalculated and the whole process is repeated until self-consistency is achieved. In practice, one or two repetitions are fully sufficient.

At $G_T = 9$ μsec and $G_L = 90$ μsec, the mean square deviations were 0.046 and 0.026 for f_s and f_l, respectively. These values, which are relatively large, are due partially to the low signal-to-noise ratios, typical for powdered samples, and partially to the variations in composition of the samples. Especially in the case of moistened samples, it is very difficult to ensure complete homogeneity. In order to reduce these problems, it would be preferable to use larger sample holders. This usually means lower homogeneity of the magnetic field and, therefore, faster decay of the liquid phase signal. In the particular case of moisture determination in starch-rich products, the latter disadvantage is of less importance, since the water signal decays rather fast anyway, while the advantages, due to the higher signal-to-noise ratio and to the better averaging of sample inhomogeneity, might be quite pronounced.

Determination of the liquid phase content by weight

So far, we have been considering only the X values, representing the relative content of the liquid phase in terms of hydrogen abundance. The quantity of more general interest is however the relative content of the liquid phase by weight, denoted as W.

Suppose two phases A (liquid) and B (solid) are present in the sample (the symbols A and B will be used also to indicate the respective absolute quantities in grams). Further suppose that the relative abundance of hydrogen in A and B is α and β, respectively. Then

$$X = \alpha A/(\alpha A + \beta B) \text{ and } W = A/(A + B). \tag{5}$$

A simple calculation shows that

$$W = X/[X + \mathrm{k}\,(1 - X)] \tag{6}$$

where $\mathrm{k} = \alpha/\beta$ is a constant. Equation (6) can be rewritten also in the form

$$1/W = \mathrm{k}(1/X) + (1 - \mathrm{k}) \tag{7}$$

showing that there is a linear relationship between $1/W$ and $1/X$. This can be used for calibration purposes if the values of W are available by means of another independent method.

Notice that it is common in the literature to apply linear regression to the $W(X)$ relationship, which is obviously incorrect.

When W cannot be determined independently, indirect methods for determining k must be found. One of the possibilities is the use of the 'standard

addition' method. The only condition of its applicability is that it must be possible to homogenize the two phases after addition of the liquid phase A.

Suppose that a quantity a of phase A has been added to the total quantity $(A + B)$ of the sample and that the new value of X, called X', has been measured. We have

$$W' = X'/[X' + k(1-X')] \tag{8a}$$

and

$$W' = (A + a)/(A + B + a). \tag{8b}$$

A somewhat complicated but straightforward calculation based on the above equation shows that

$$k = \frac{1}{m}\left[\frac{1}{1-X'} - \frac{1+mX}{1-X}\right] \tag{9}$$

where $m = a/(A + B)$.

Determination of k

A theoretical estimate of k can be made considering the chemical structure of starch. Assuming that the liquid phase consists of pure water, we have $\alpha = 2/18 = 0.111$. The monomeric unit of starch has a molecular weight of 162, of which 10 is due to hydrogen. Hence, $\beta = 10/162 = 0.062$ and $k = \alpha/\beta$ is 1.79. However, three of the ten hydrogens may be undergoing a rapid exchange with the hydrogens in the liquid phase. In this case, these hydrogens would contribute to the liquid phase signal better than to the solid phase, so that $\beta = 7/162 = 0.043$ and $k = 2.58$.

An empirical determination of k, based on the method of standard addition as described above, should be guided by an estimate of the error involved. Assuming realistically that m can be set very accurately (only weighing and pipetting is involved), eqn (9) leads to the approximate formula

$$\sigma_k^2 = \left(\frac{\delta k}{\delta x}\right)^2 \sigma_x^2 + \left(\frac{\delta k}{\delta x'}\right)^2 \sigma_{x'}^2 = m^{-2}\left[(1+m)^2(1-x)^{-4}\sigma_x^2 + (1-x')^{-4}\sigma_{x'}^2\right] \tag{10}$$

where δ_x, $\delta_{x'}$, and δ_k are the variances of x, x' and k, respectively.

Considering that a typical value of x in 'dry' substances is 0.2, assuming that $k \approx 2.5$, and expressing x' in terms of x, k and m, σ_k can be calculated as a function of m, σ_x and $\sigma_{x'}$. Numerical analysis shows that σ_k increases very fast when m becomes smaller than 0.2, while for higher values of m it varies very little and has a shallow minimum around $m = 0.5$. Its value is almost entirely contributed by $\sigma_{x'}$, while the variance of x is of much less importance; for $0.3 \leqslant m \leqslant 1.0$ the ratio $\sigma_k/\sigma_{x'}$ lies between 13 and 16, regardless of σ_x.

Table 2. Determination of k and true water content in 'dry' starch-rich substances

Sample	§	$\bar{T}$*	$\bar{L}$†	X	k	W‡
Barley flour						
	0.0	483	196	0.200		0.092
	0.5	589	394	0.625	2.60	
Beans						
	0.0	518	157	0.245		0.118
	0.5	454	311	0.643	2.63	
	0.0	469	138	0.237		0.114
	0.7	333	244	0.698	2.55	
Corn flour						
	0.0	604	178	0.238		0.114
	0.5	619	416	0.628	2.44	
	0.0	415	122	0.237		0.114
	0.7	414	279	0.630	1.68	
	0.0	427	123	0.232		0.111
	0.7	472	328	0.654	1.97	
Lentils						
	0.0	569	144	0.200		0.094
	0.5	574	386	0.629	2.64	
	0.0	427	104	0.192		0.089
	0.7	375	268	0.677	2.42	
	0.0	407	83	0.157		0.071
	0.7	388	282	0.691	2.74	
Pasta						
100% flour						
	0.0	421	108	0.203		0.095
	0.7	404	293	0.690	2.56	
	0.0	405	74	0.140		0.063
	1.0	386	304	0.764	2.91	
100% semolina						
	0.0	393	69	0.134		0.060
	0.7	359	249	0.653	2.31	
	0.0	404	76	0.144		0.065
	1.0	308	246	0.777	3.15	
Gluten enriched						
	0.0	416	112	0.215		0.102
	0.7	377	271	0.682	2.40	
	0.0	583	153	0.208		0.098
	0.5	623	445	0.677	3.40	
Potato starch						
	0.0	639	234	0.305		0.154
	0.5	707	476	0.629	2.07	
Rice flour						
	0.0	653	188	0.231		0.110
	0.5	724	428	0.615	2.29	
	0.0	557	163	0.236		0.113
	0.7	519	356	0.644	1.83	
Wheat flour						
	0.0	418	82	0.151		0.068
	0.5	489	369	0.724	2.27	
	0.0	293	58	0.152		0.069
	0.7	453	314	0.652	2.24	
	0.0	349	68	0.150		0.068
	0.7	381	259	0.637	2.08	

Table 2 – *continued*

Sample	§	$\bar{T}$ *	$\bar{L}$ †	X	k	W‡
Wheat semolina						
	0.0	334	67	0.155		0.070
	0.7	478	333	0.656	2.30	
	0.0	405	82	0.156		0.071
	0.7	465	321	0.649	2.19	
	0.0	564	169	0.242		0.117
	1.0	454	360	0.770	2.71	
	0.0	389	77	0.152		0.069
	0.7	495	342	0.650	2.22	

Mean value of k = 2.42 ± 0.08; variance σ_k = 0.388.

* Measured 9 μsec after the end of the pulse.

† Measured 90 μsec after the end of the pulse.

‡ Calculated with the mean value of k = 2.42.

§ Represents the value of the 'standard addition' of water in grams.

A number of measurements aiming at the determination of k is reported in Table 2. The resulting mean value k = 2.42 ± 0.08, corresponds very well to the theoretical estimate under the assumption that most of the exchangeable hydrogens in starch contribute to the liquid phase signal (fast exchange model). The fact that k is somewhat smaller than the theoretical value of 2.58 corresponding to this situation may be due to two factors: (i) the water phase containing a certain amount of dissolved organic matter, such as saccharides, which lowers its hydrogen content, and/or (ii) some exchangeable hydrogens are bound so strongly in hydrogen bonds within the solid matrix that they are not available for the exchange process with protons in the liquid phase.

The variance of k was σ_k = 0.39, which would correspond to $\sigma_{x'}$ = 0.026. In reality, the experimental error in determining x' is smaller (definitely less than ± 0.01). The discrepancy arises from the difficulties in sample preparation (homogenization) which have been mentioned before.

Conclusions

The possibility of determining the moisture content of a large group of nutritionally important substances by pulsed NMR has been demonstrated. The determination is based on two values, $\bar{T}$ and $\bar{L}$, of the FID signal measured at two suitably chosen times, G_T and G_L, after the pulse. It is very fast; one determination takes about 10 sec to collect the data and 20 sec to evaluate them, provided a programmed pocket calculator is available. The evaluation is done in steps. First, the moisture is determined in terms of relative hydrogen content using eqn (4) and the extrapolation coefficients reported in Table 1; the iterative procedure reported in the third section is of interest in this step.

Secondly, eqn (6) is applied in order to obtain the liquid phase content by weight. The value of k is 2.42 ± 0.08. The overall error of a single measurement is about ± 0.01.

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Colour changes in tuna during canning and colour improvement by chemical modification of haeme proteins

ALI KHAYAT

Summary

A significant loss in tristimulus colour values of precooked tuna during canning was observed and the loss was greatest in albacore followed by skipjack and then yellowfin. In terms of CIE chromaticity values, precooked albacore showed a large gain in y and, in particular, x after canning; yellowfin showed a large increase in x, slight increase in y, and large decrease in z after canning; skipjack showed a large increase in y and x, and a large decrease in z. The greater the amount of reducing sugar in the raw fish, the darker the colour of the canned product. Addition of cysteine and homocysteine before cooking caused lightening of the colour of a heated aqueous extract of soluble proteins; cysteine and trimethylamine oxide (TMAO) caused greening, which was prevented by the addition of anti-oxidants; homocysteine and TMAO not only improved the colour but also prevented greening. Similar effects were observed in intact tuna loin muscle. Economically, it is valuable to be able to forecast the colour of a canned product before it is packed.

Introduction

The chemistry of the colour of meat products is the chemistry of the pigments and, more specifically, of myoglobin, the predominant pigment. In a well bled piece of beef skeletal muscle the predominant haeme protein is myoglobin which may account for 95% or more of the total iron (Clydesdale & Francis, 1971). Studies on yellowfin tuna fish have indicated that myoglobin constitutes 69–85% of the total haeme pigments in light muscle and 81–95% in dark muscle (Brown, 1961).

When meat is heated a haeme containing complex composed mainly of denatured myoglobin bound to other available proteins is formed. This complex has the typical pinkish brown appearance of cooked meat (Ledward, 1971).

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Studies on the reflectance of cooked meat, heat denatured muscle extract, and heat denatured mixtures of horse heart metmyoglobin and bovine serum albumin resulted in similar spectra. It was therefore, suggested that the complexes present in cooked meat are haemeproteins where the protein moiety may be any of several heat denatured proteins present in meat (Fox, 1966).

Precooked tuna has a pinkish appearance which is maintained during the canning process although slight browning and darkening may take place (Brown, Tappel & Olcott, 1958). While the pigment concentration was shown to be the major factor in determining the level of reflectance, other factors, such as browning and degradative breakdown of the haeme pigments during processing, should be taken into consideration.

Occasionally, some batches of fish after precooking develop a tan or tannish-green colour instead of the pinkish colour, shown to be the result of ferro-haemochrome oxidation to ferrihaemochrome (Brown *et al.*, 1958; Grosjean *et al.*, 1969). This type of discolouration is known as 'greening' and the green fish represents a loss to the industry.

The purpose of the investigation was to study the relationship between the colour of precooked fish and canned product and enhancement of the quality of the canned product by improving the colour through chemical modification of haeme proteins prior to cooking.

Materials and methods

Materials

High quality frozen tuna fish (albacore, yellowfin and skipjack) were obtained commercially. All chemicals were reagent grade or better and included trimethylamine oxide (K & K Laboratories, Hollywood, California). Butylated hydroxy anisole (BHA), propyl gallate (PG), ascorbic acid, and cysteine hydrochloride (Eastman Organic Chemicals, Rochester, New York). Homocysteine-γ-thiolactone hydrochloride and 95% ethanol (J. T. Baker Chemical Co., Phillipsburgh, New Jersey).

Colour measurement

Reflectance characteristics of canned samples were measured relative to a standard MgO plate. Samples were prepared for colour measurement as described earlier (Khayat, 1973). The proportionate distribution of the 'green, Y', 'red, X' and 'blue, Z', reflectance characteristics of canned tuna can be examined by CIE chromaticity coordinates x, y and z; [$x = X/(X + Y + Z)$, $y = Y/(X + Y + Z)$, $z = Z/(X + Y + Z)$]. The reflectance characteristics of samples relative to the MgO plate were measured with a tristimulus colorimeter (Photovolt, Model 670).

Model system experiments

The colour of heat denatured soluble proteins of tuna was examined in a series of test tubes containing 10 ml of aqueous extract of raw tuna with a 2% protein concentration. Different chemicals were added (see Table 3); the pH was adjusted to 5.70, and the tube placed in a water bath and heated to 75°C for 15 min followed by 15 min in boiling water. The colour of the coagulated protein was examined visually.

Experiments on fish loins

Tuna loins (1–3 kg) were injected manually with 20 ml of various solutions (Table 4). Two to three ml injections were made at about 5 cm intervals. The treated loins were cooked in steam, canned with soyabean oil, and retorted for 80 min at 116.5°C.

Analysis for reducing sugars

Ten grams of tuna fish were homogenized in 30 ml of water, 30 ml of 95% ethanol was added and mixed for 15 min; it was then filtered through a Whatman No. 1 filter paper. Two ml of sodium tungstate solution (12%) was added to the filtrate. The mixture was filtered and the filtrate diluted to 500 ml. Aliquots of this solution were used for reducing sugar analysis by the method of Folin & Malmros (1929).

Results and discussion

Chromaticity coordinates and chromaticity shift of precooked fish during canning

The results of tristimulus colour measurements on precooked tuna loins and corresponding cans are presented in Table 1. These findings indicated that there was a significant loss in tristimulus colour values of the samples during the canning process, and this was greatest in albacore samples followed by skipjack and then yellowfin. These results indicated that during the retorting process the green luminous reflectance decreased in all three species of tuna. This is, perhaps, a direct result of breakdown of haeme pigments and subsequent reactions involving reducing sugars which results in condensation reactions and non-enzymatic browning.

Figures 1 and 2 represent the relationship of the green reflectance values of canned skipjack and yellowfin tuna to the percentage of reducing sugars present in raw fish. The higher the amount of sugar in raw fish, the darker the colour of the canned product. Similar results were obtained by Barret *et al.* (1965).

The chromaticity coordinates and the chromaticity shift of precooked tuna loins after canning are presented in Table 2. The results show that there is a

Table 1. Tristimulus colour values of cooked tuna fish loins before and after canning. The data reported here are the averages and standard deviations of twenty separate runs. An average of five readings was obtained for each run

Variety	Before canning			After canning			Tristimulus colour reflectance loss		
	Y	X	Z	Y	X	Z	ΔY	ΔX	ΔZ
Albacore	53.38 ± 1.03	51.97 ± 1.20	43.78 ± 2.17	47.69 ± 2.46	47.05 ± 2.34	36.24 ± 2.41	5.69	4.92	7.54
Yellowfin	29.78 ± 1.66	30.69 ± 1.58	24.96 ± 1.68	27.18 ± 1.06	28.55 ± 0.95	22.15 ± 0.67	2.60	2.14	2.81
Skipjack	32.59 ± 1.95	33.37 ± 1.94	28.87 ± 1.78	29.25 ± 0.77	30.22 ± 0.83	23.45 ± 0.99	3.34	3.15	5.42

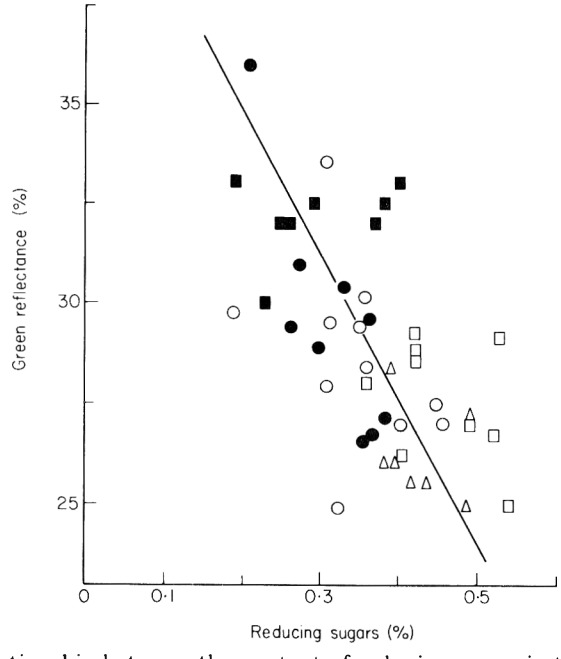

Figure 1. Relationship between the content of reducing sugars in the raw flesh of skipjack tuna and green reflectance of canned product. Each symbol represents a different lot of fish and each point represents one determination within that lot.

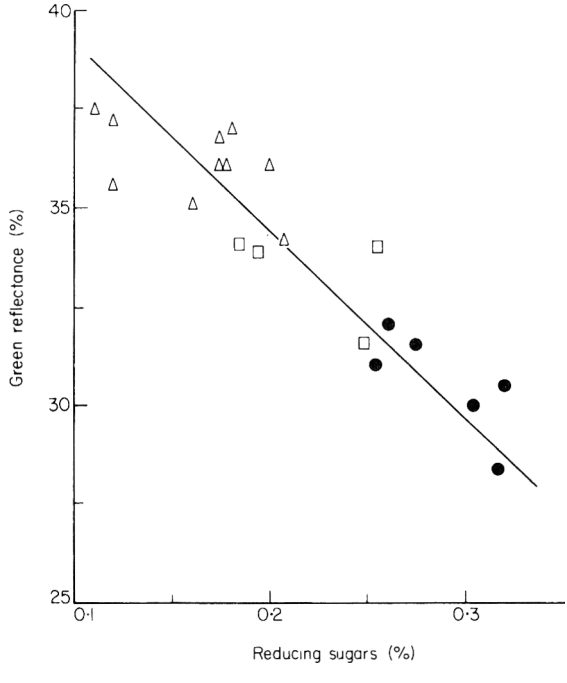

Figure 2. Relationship between the content of reducing sugars in raw flesh of yellowfin tuna and the green reflectance of canned product, Each symbol represents a different lot of fish and each point represents one determination within that lot.

Table 2. Chromaticity coordinates and chromaticity shift of precooked tuna loins before and after canning*

Variety	Chromaticity coordinates						Chromaticity shift $\times 10^3$		
	Before canning			After canning					
	y	x	z	y	x	z	Δy	Δx	Δz
Albacore	0.3580 ± 0.0069	0.3485 ± 0.0080	0.2936 ± 0.0145	0.3641 ± 0.0187	0.3592 ± 0.0178	0.2766 ± 0.0183	+ 6.1	+10.7	−17.0
Yellowfin	0.3485 ± 0.0194	0.3592 ± 0.0184	0.2921 ± 0.0196	0.3489 ± 0.0136	0.3665 ± 0.0121	0.2844 ± 0.0086	+ 0.4	+ 7.3	− 7.7
Skipjack	0.3436 ± 0.0205	0.3518 ± 0.0204	0.3044 ± 0.0187	0.3528 ± 0.0093	0.3644 ± 0.0100	0.2828 ± 0.0119	+ 9.2	+12.6	−21.6

* See Table 1.

Table 3. The effect of added chemicals (in mg/10 ml) on the colour of heat denatured soluble proteins of tuna homogenate*

Samples	Homo-cysteine	Cysteine	TMAO†	BHA†	Ascorbic acid	PG†	pH	Visual colour test
Control			No treatment				5.70	Light colour protein coagulate
1	—	20	—	—	—	—	5.62	Lighter than control
2	—	40	—	—	—	—	5.82	Lighter than control
3	—	20	40	—	—	—	5.76	Slight greening, G‡+
4	—	40	40	—	—	—	5.63	Greening+++
5	—	40	60	—	—	—	5.76	Greening+++
6	—	20	40	10	10	—	5.70	Slightly lighter than control
7	—	40	40	—	10	10	5.68	Lighter than control with a shade of pink+‡
8	—	20	40	—	10	10	5.65	Lighter than control with a shade of pink++
9	—	40	40	—	20	20	5.77	Lighter than control with a noticeable pinkness+++
10	—	20	40	10	10	10	5.88	Lighter than control with slight pinkness++
11	—	20	40	10	—	10	5.85	Lighter than control with a shade of pink+
12	20	—	—	—	—	—	5.80	Lighter than control
13	40	—	—	—	—	—	5.76	Lighter than control
14	40	—	40	—	—	—	5.71	Lighter than control with no detectable greening
15	40	—	60	—	—	—	5.76	Lighter than control with no detectable greening

* Protein concentration in each tube was 2%.

† TMAO is trimethylamine-*n*-oxide, BHA is butylated hydroxy anisole, PG is propyl gallate.

‡ The positive signs designate the degree of colour, one plus sign shows a slight change and three plus signs represent a marked change.

large gain in y and, in particular, x chromaticity values of albacore tuna during the retorting process. This indicates that the colour of canned albacore is much richer in red than the starting precooked fish. Precooked yellowfin results showed a large increase in x chromaticity which is accompanied by a slight increase in y and a large decrease in z chromaticity during the retorting process. Precooked skipjack tuna also gave similar results to albacore except with a greater gain in y and x, and a larger loss in z chromaticity.

Studies on oxidation and reduction of haeme pigments of canned tuna by Little (1969) indicated that when chemically reduced albacore and skipjack canned tuna are oxidized by air there is a large gain in y and a significant loss in x chromaticities. This kind of chromaticity shift is related to the oxidation of 'available' pigments which result in partial darkening.

The relatively large gain in y chromaticity is partially related to the loss of z chromaticity, darkening of the proteins during retorting, brown colour formation, and a shift of colour hue.

The high gain in x chromaticity in albacore during retorting is primarily due to 'available' pigments. These can undergo oxidation or reduction which are accompanied by a colour change (being pink in the reduced state). Since albacore is relatively low in haeme pigment content it is reasonable to assume that the major change in colour during retorting, which is shown to be a high gain in x chromaticity, is due to the reduced state of these available pigments and not a result of breakdown or non-enzymatic browning.

Precooked yellowfin after canning and retorting shows a significant increase in x chromaticity and a slight gain in y chromaticity. In this case discoloration of precooked fish during processing is a result of a colour change by 'available' pigments, which are in the reduced state in the cans, and the colour change contributed by degraded haeme pigments and some condensation type reactions with available reducing sugars during retorting process.

When precooked skipjack tuna, a highly pigmented fish, was canned and retorted, a significant gain in x chromaticity was observed which was accompanied by a marked increase in y chromaticity. These shifts in chromaticity indicate a distinct darkening which were caused mainly by haeme pigment degradation and sugar condensation type reactions.

The effect of added chemicals on the colour

Studies performed in test tubes. The effect of several compounds known to stabilize the haeme pigments against oxidation, either alone or in combinations, was tested on water soluble proteins extracted from tuna homogenates. Table 3 presents the results of such experiments. As was expected, anti-oxidants, especially ascorbic acid in combination with propyl gallate, had a pronounced effect in improving the colour of the heat denatured tuna soluble proteins.

It has been postulated by Grosjean *et al.* (1969) that the free sulfhydryl group of denatured metmyoglobin forms a disulphide bond in the presence of free cysteine by the action of an oxidizing agent, such as TMAO, or air and this

Table 4. Tristimulus reflectance values, chromaticity values, and chromaticity shifts of chemically treated raw albacore tuna loins after canning and retorting*

Samples	Injected chemicals (20 ml each)	Reflectance values of canned product			Chromaticity values of canned product			Chromaticity shift $\times 10^3$		
		Y	X	Z	y	x	z	Δy	Δx	Δz
Control	No treatment	49.55	49.49	40.94	0.3539	0.3535	0.2924	—	—	—
1	250 mg cysteine/kg fish	51.55	51.00	42.48	0.3553	0.3516	0.2929	+1.5	−1.9	+0.5
2	250 mg homocysteine/kg fish	51.30	50.87	42.46	0.3546	0.3517	0.2935	+0.7	−1.8	+1.1
3†	125 mg TMAO/kg fish	51.15	51.20	42.24	0.3537	0.3541	0.2921	−0.2	+0.6	−0.3
4‡	125 mg TMAO and 250 mg cysteine/kg fish	52.35	51.98	42.89	0.3555	0.3530	0.2913	+1.6	−0.5	−1.1
5§	125 mg TMAO and 250 mg homocysteine/kg fish	52.00	51.36	42.48	0.3565	0.3521	0.2912	+2.6	−1.4	−1.2

* Data represent the mean values of nine separate experiments. The raw fish loins were 1000–3000 g.

† Visual observation indicated that TMAO caused slight lightening of colour of canned product which was accompanied by very faint shade of green colour formation.

‡ Treatment of the raw flesh prior to canning and retorting with a solution containing cysteine and TMAO caused a definite greening which was observed visually.

§ Injection of the raw flesh with a solution containing homocysteine and TMAO resulted in lightening of colour which was not accompanied by greening, as observed visually.

complex has a green colour. The results indicated that addition of cysteine alone to the homogenate had no greening effect; and, in fact, it caused a slight enhancement in the lightness of the colour of heat denatured proteins. However, when it was accompanied by TMAO there was a definite greening of the colour of coagulated proteins. This greening was increased when the amount of cysteine was increased to that of the TMAO level. These findings are in agreement with those of other investigators.

The addition of ascorbic acid in combination with propyl gallate to the above mixture not only prevented the greening but also changed the colour to a desirable pinkish shade. A significant improvement on the colour of coagulated proteins was observed when the level of ascorbic acid and propyl gallate was doubled. Addition of BHA combined with ascorbic acid or propyl gallate to the tubes containing cysteine, TMAO, and tuna extracts prevented greening and showed an observed change in colour towards a pinkish tone.

The addition of homocysteine to aqueous extrates of tuna homogenate increased the lightness of the coagulated proteins. When TMAO was added to the above mixture in various amounts, slight lightening of the colour was observed with no detectable greening.

Studies performed on chemical treatment of fish loins. Table 4 presents tristimulus colour values, and Fig. 3 presents chromaticity values of canned tuna packed from chemically treated tuna loins. The results of Table 4 and Fig. 3 indicate that, in general, high tristimulus reflectance values were obtained when the tuna flesh was chemically treated. However, the absolute reflectance values do not indicate the actual shift in colour. In order to observe the colour shift, the chromaticity values should be taken into consideration, because these parameters indicate the shift and change in colour towards any specific direction of the spectrum.

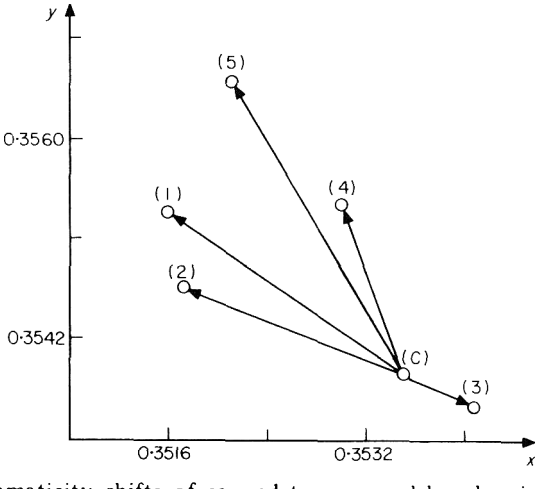

Figure 3. Chromaticity shifts of canned tuna caused by chemical treatment of raw flesh. Tuna flesh were treated with: (1) cysteine, (2) homocysteine, (3) TMAO, (4) cysteine + TMAO, (5) Homocysteine + TMAO, (C) is the control. x and y represent chromaticity coordinates.

Treatment of the raw tuna flesh prior to cooking and canning with cysteine or homocysteine alone decreased the x chromaticity which was accompanied by a relatively significant increase in y and some increase in z chromaticity. Comparison of chromaticity values of Plochere colour system standards indicated that when a pink colour, the colour of normal albacore tuna, was changed to a very light pink the chromaticity shift towards a gain in y and some z but loss of x. This is in accord with the above finding which indicates slight lightening of colour.

The injection of TMAO into raw flesh resulted in a slight greening which was detectable visually. However, it was difficult to arrive at any conclusion based on the data obtained, due to insignificant shifts in chromaticity in this case.

When TMAO was injected with cysteine or with homocysteine, there was a significant gain in y and loss in x and z chromaticity. However, TMAO combined with cysteine resulted in a large increase in y which was mainly a result of loss of z; and the x chromaticity was essentially maintained at the same level. Therefore, it is reasonable to conclude that the increase in y was the result of greening and not the loss of x; which was confirmed by visual observations.

In contrast to the above findings when the flesh was treated with TMAO and homocysteine, there was a definite lightening of the flesh which was not accompanied by greening, as was observed visually. Chromaticity shift data indicated that the large gain in y is mainly the result of significant loss of x, i.e. loss of redness, which resulted in the lightening of the colour, and not greening (Khayat, Courtney & Dunn, 1974).

This lightening of colour might be due to two separate chemical reactions: the disulphide bond formation between added homocysteine and free cysteine present in tuna flesh, and disulphide bond formation between homocysteine and free sulfhydryl groups of denatured tuna metmyoglobin.

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Procedure for the isolation of lipases produced by *Pseudomonas* species and *Achromobacter lipolyticum*

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Summary

The extracellular lipase systems from three *Pseudomonas* species and a strain of *Achromobacter lipolyticum* were concentrated and partially purified using a D.D.S. plate-and-frame ultrafiltration unit. Recoveries ranging from 75–97% were reported for the pseudomonad enzyme systems with increases in specific activity of 18–40 times. The low yields obtained for *Achromobacter lipolyticum* preparations were concluded to be due to the loss of a loosely bound ion or low molecular weight compound necessary for optimum activity.

Introduction

Membrane-moderated procedures, in particular ultrafiltration (UF), have emerged as practical methods of enzyme isolation and purification. The availability of a range of synthetic, non-fouling, high flux, anisotropic membranes has made possible the concentration and purification of virtually any enzyme (Porter & Michaels, 1971). Unlike salt and solvent precipitation methods, UF involves no phase change (Porter, 1972), is gentle and non-destructive and there is maintenance of constant ionic strength and pH of the concentrate thereby avoiding inactivation of the enzyme.

Isolation of microbial lipases

Blatt *et al.* (1965) demonstrated that UF could be used to concentrate protein solutions without denaturation and with recovery efficiencies greater than 90%. They also showed that the rate of UF was much greater than that of conventional cellulose acetate dialysis. Wang, Sonoyama & Mateles (1968)

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reported somewhat lower recoveries for the concentration of penicillinase, β-galactosidase and trypsin which they postulated might be due to enzyme-membrane interaction. Ultrafiltration of other enzymes including cellulase, mannase, β-galactosidase and ary-β-glucosidase without loss of activity have been reported by Eriksson & Rzedowski (1969). Other researchers (Griffin & Wu, 1968; McDonald *et al.*, 1969; Rechler, 1969) have observed the effectiveness with which membrane UF can isolate, concentrate and purify enzyme solutions. Richmond (1963) observed that maximum enzyme throughput, or retention, during UF processing could be influenced by varying the pH of the initial solution. Similar findings with respect of salt concentration have been reported by Melling & Scott (1972)

This study examines the applicability of UF to the recovery of the extracellular lipase enzyme systems produced by psychrophilic bacteria grown in liquid culture.

Materials and methods

Organisms

The three strains examined were *Pseudomonas fluorescens* (NCTC 10038), *Pseudomonas fragi* (NCIB 8542), and *Achromobacter lipolyticum* (NCDD 1003). The strains were maintained in Plate Count Agar (Oxoid) at 4°C and transferred monthly. Cultures to serve as inocula were prepared as suspensions of 0.3 × 100 units optical density (E.E.L. Nephelometer, Evans Electroselenium, Essex) in glass distilled water.

Media

The organisms were grown in Pope and Skerman's mineral salts medium (Skerman, 1967) supplemented with 0.5% (w/v) of commercial media shown to exhibit high levels of lipase production for each of the test strains (O'Donnell, 1975):

Ps. fluorescens – Oxoid Bact. Peptone
Ps. fragi – Evans Bact. Peptone
A. lipolyticum – Difco Bact. Casamino Acids.

For static growth conditions the media were dispensed in 1200 ml volumes in 5 litre Erlenmeyer flasks. The flasks were autoclaved at 641 kN/m² for 20 min. For aerated growth conditions the media were dispensed in 35 litre volumes in a 45 litre sealed tank which was sterilized at 100°C for 5 hr. Sterility was checked by incubating samples at 30°C for 2 days.

Lipase production

Each culture was prepared by inoculating 1 ml of standard culture into 1200 ml medium incubating at 25 or 5°C. Aerated media were incubated only at 5°C with a sterile air flow rate of 10 l/min. At intervals cell growth was estimated by nephelometry. Cell growth was stopped towards the end of the logarithmic growth phase by the addition of 0.1% (w/v) merthiolate (Koch-Light). The cells were then removed from the medium by centrifugation at 12 000 *g* using a Griffin–Christ continuous centrifuge. Throughput was limited to 75 ml/min for efficient cell removal. The supernatant fluid was used as the enzyme source.

Lipase concentration

Enzyme concentration was achieved using a De Danske Sukkerfabrikker ((D.D.S.) plate-and-frame UF module with an effective membrane area of 1.8 m^2. The membranes used (D.D.S., type 600) were constructed of cellulose acetate, giving 100% retention of compounds > 20 000 daltons mol wt.

The centrifuged medium to be concentrated was cooled to 4°C to minimize enzyme activity losses during processing. The medium was continuously re-cycled through the module by a variable speed positive displacement pump. Operating pressures were 7×10^3 N/m^2 inlet pressure and 5×10^5 N/m^2 outlet pressure. Concentrate flow rate was 3.5 litre/min with an average permeate flow rate of 1 litre/min. The material was concentrated to a final volume of 2.5 litre. Concentrate temperatures increased to approximately 14°C. Desalting was carried out by the slow addition of ten successive 2.5 litre volumes of distilled water at 4°C. The total volume was reduced to 2.5 litre after each addition. Salt concentration was effectively reduced by a factor of at least 4000 and concentrate temperature reduced to 4–6°C. The desalted concentrate was flushed from the membranes with cold distilled water.

Before discarding the permeate it was checked for lipase activity.

Lipase assay

Lipase production was measured using a modification of the extraction/titration method of Alford & Elliot (1960). A preliminary emulsion consisting of 20% (v/v) butterfat, 0.3% (w/v) alginate emulsifier (Manucol Ester M, Alginate Industries Ltd, Ayrshire, Scotland) was made using a Silverson mixer. The emulsion was homogenized in a Rannie homogenizer with re-cycling at 275×10^5 N/m^2 for 20 min at a temperature not exceeding 55–60°C. After homogenization 0.1% (w/v) merthiolate was added and the emulsion stored under nitrogen in screw-cap bottles at 4°C. The emulsion had a fat globule diameter of < 0.5 μm, peroxide value of < 8 and remained stable for up to 6 months.

For lipase assay, 30 ml of emulsion was added to 40 ml of 0.25 M phosphate buffer (0.25 M KH_2PO_4, adjusted to pH 7 with 0.25 M Na_2HPO_4). The

merthiolate level was adjusted to 0.1% (w/v) and 10 ml volumes of mixture dispensed in 50 ml Erlenmeyer flasks. To the mixture was added 5 ml of centrifuged supernatant and the whole incubated at 35°C for 24 h. Control flasks containing supernatant autoclaved at 103×10^3 N/m^2 for 15 min were prepared in a similar manner. Where high lipase activities were encountered assay samples were diluted with distilled water to avoid inhibition of hydrolysis by high free fatty acid development in the emulsion.

After hydrolysis, a 10 ml aliquot of the reaction mixture was measured into a Majonnier fat-extraction flask and acidified with 25% H_2SO_4 to approx pH 2. Ten ml of ethyl alcohol was added and the sample extracted three times with 20 ml aliquots of petroleum ether.

The combined extracts were titrated with 0.02 M alcoholic NaOH using phenolphthalein as indicator and the results regarded as a measure of lipase activity.

Protein determination

Protein determinations were carried out using Miller's (1959) modification of the method of Lowry *et al.* (1951). Protein was measured by an absorbancy of 650 nm and quantified by reference to a standard graph using bovine serum albumin (Koch-Light).

Results and discussion

Isolation of the extracellular lipases was performed by the sequence of operations shown in Fig. 1.

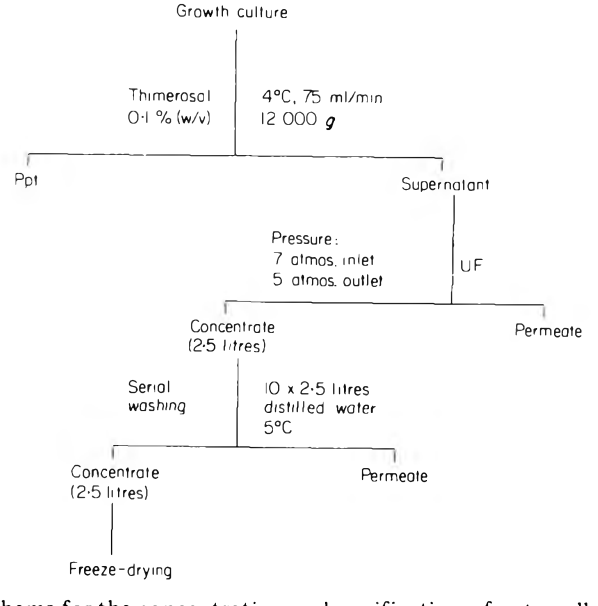

Figure 1. Scheme for the concentration and purification of extracellular bacterial enzymes.

Table 1. Efficiency of purification of lipase activity of *Ps. fluorescens*

Growth conditions	Procedure	Total protein (mg)	Total activity (ml 0.02 M NaOH)	Specific activity (units/mg protein)	Yield (% of original activity)	Purification*
25°C static	Centrifuged broth	240	300	1.25	100	–
	UF concentrate	10	284	28.4	94.6	22.72
5°C static	Centrifuged broth	200	280	1.4	100	–
	UF concentrate	8.5	253	29.76	90.4	21.25
5°C aerated	Centrifuged broth	296	560	1.89	100	
	UF concentrate	7	527	75.28	94	39.8

*Specific activity of UF concentrate divided by specific activity of centrifuged broth.

Table 2. Efficiency of purification of lipase activity of *Ps. fragi*

Growth conditions	Procedure	Total protein (mg)	Total activity (ml 0.02 M NaOH)	Specific activity (units/mg protein)	Yield (% of original activity)	Purification
25°C static	Centrifuged broth	290	140	0.484	100	–
	UF concentrate	8.3	108	13.01	77.14	26.87
5°C static	Centrifuged broth	247	236	0.955	100	–
	UF concentrate	11.5	202	17.57	85.61	18.39
5°C aerated	Centrifuged broth	263	164	0.623	100	–
	UF concentrate	12.3	137	11.14	83.54	17.87

Table 3. Efficiency of purification of lipase activity of *A. lipolyticum*

Growth conditions	Procedure	Total protein (mg)	Total activity (ml 0.02 M NaOH)	Specific activity (units/mg protein)	Yield (% of original activity)	Purification
25°C static	Centrifuged broth	185	187	1.034	100	—
	UF concentrate	14	83	5.929	44.39	5.733
5°C static	Centrifuged broth	168	221	1.315	100	—
	UF concentrate	9	93	10.34	42.08	7.856
5°C aerated	Centrifuged broth	152	603	3.968	100	—
	UF concentrate	5.2	201	38.66	38.27	9.742

Table 4. Effect of protein precipitation methods on the purification of lipase activity of *Ps. fluorescens*

Growth conditions	Procedure	Total protein (mg)	Total activity (ml 0.02 M NaOH)	Specific activity (units/mg protein)	Yield (% of original activity)	Purification
25°C static	Centrifuged broth	275	300	1.09	100	—
	Ammonium sulphate	4.2	82.4	19.62	27.47	18.0
	Acetone	7.1	60.3	8.49	20.1	7.78
5°C static	Centrifuged broth	240	255	1.06	100	—
	Ammonium sulphate	3.7	75.8	20.5	29.73	19.34
	Acetone	6.8	68.0	10.0	26.67	9.4
5°C aerated	Centrifuged broth	314	540	1.72	100	—
	Ammonium sulphate	5.0	152.0	30.4	28.15	17.67
	Acetone	10.3	112.2	10.89	20.78	6.33

Tables 1–3 illustrate the degree of purification of each enzyme system produced by the test strains under the various growth conditions. All calculations are based on a nominal quantity of 100 ml of starting material. With the exception of *A. lipolyticum* preparations, recoveries varied from 75% to approx. 95%, with increases in specific activity ranging from 18–40 times. Lipase activity was not detected in the permeates thus signifying that the lipolytic systems had molecular weights > 20 000 daltons.

Table 4 illustrates the degree of purification obtained for *Ps. fluorescens* lipase systems using the classical acetone and ammonium sulphate precipitation methods. Although effective purification was achieved, the percentage of the original activity recovered was low compared with the UF method. Similar low yields of extracellular enzyme activity associated with precipitation methods have been reported by other workers (Chorvath & Fried, 1970; Fallon, 1972; Lu & Liska, 1969).

The degree of purification obtained by UF is related to the proportion of low molecular weight material, < 20 000 daltons in this case, which is present in the culture fluid. Since the commercial media used in the formulation of the growth media are composed of material of lower molecular weight than the membrane cut-off, most of this inert proteinaceous material was removed during ultrafiltration. In addition to desalting the concentrate, the serial washing procedure aided in the removal of low molecular weight material, thereby enhancing the degree of purification achieved.

As few enzyme purifications using semi-industrial UF units have been reported it is not possible to make a comparative assessment with the method used in this study. The majority of reports in the literature have been based on the use of laboratory-scale UF units which do not permit the continuous circulation through the membranes and serial washing procedures used with the D.D.S. module. Thus the degree of enzyme purification reported has been limited although effective concentration was achieved (Gabay & Valcourt, 1968; Griffin & Wu, 1968).

The protein contents, < 0.02% (w/v), of the concentrates removed from the module were considerably lower than the performance potential of the unit, which is capable of producing a final product containing *c.* 20% (w/v) of protein. This would allow the effective concentration of considerably larger quantities of crude culture fluid to a final volume of 2.5 litres, this being equivalent to the total volume entrained in the equipment. Whether increasing the process volume would lead to increased specific gravity and purification of the recovered enzyme systems would depend on achieving an increased efficiency of removal of compounds of molecular weight < 20 000 daltons. No data were collected to show the molecular size distribution of the inactive impurities contained in the concentrates.

It is possible that marked loss in activity exhibited by the *A. lipolyticum* preparations is due to the loss of a cofactor or activator necessary for optimum activity. This is supported by the observation of increased enzyme activity on recombination of concentrate and permeate. Similar loss of activity was noted

after conventional cellulose acetate dialysis of crude media preparations, or acetone or ammonium sulphate preparations prior to lyophilization.

This work indicates the applicability of UF in the concentration and partial purification of extracellular lipolytic enzymes. The results show that greater enzyme recovery is to be expected compared to acetone and ammonium sulphate precipitation techniques. However, the results also indicate that the effectiveness of UF is influenced by whether or not the enzyme relies on the presence of dialysable moieties necessary for optimum activity.

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Delayed crystallization of amorphous sucrose in humidified freeze dried model systems

HÉCTOR A. ISLESIAS AND JORGE CHIRIFE

Summary

Freeze dried model systems containing sucrose were humidified and moisture uptake measured as a function of time. Systems studied included combinations of sucrose with carboxymethylcellulose, guar gum, garrofin gum, sodium alginate, starch and microcrystalline cellulose. In all systems studied sucrose crystallization occurred as indicated by the loss of moisture after an initial uptake period. Apparent rates of crystallization of amorphous sucrose were evaluated from changes in the moisture content. Sucrose crystallization was delayed in all systems studied as compared with pure sucrose.

Introduction

It is well known that the hygroscopic character and caking ability of dried food products with a high sugar content are ascribed to the amorphous state of sugars (Makower & Dye, 1956; White & Cakebread, 1966). Prevention of caking and stickiness depends upon the knowledge of the environmental conditions that govern the transformation from the amorphous to the crystalline state. For this reason, during storage of dried sugar-containing products, adequate moisture and temperature conditions must be maintained to avoid sugar crystallization with the resultant loss of certain desirable product properties. Flink & Karel (1972) and Chirife & Karel (1974) showed that sugar crystallization during storage promotes the loss of volatile compounds entrapped in freeze-dried carbohydrate solutions. Recently, Tsourouflis, Flink & Karel (1976) studied the loss of structure in different freeze dried carbohydrate solutions in response to environmental stresses.

The effect of relative humidity on the crystallization of amorphous sugars have been studied by a number of workers (Makower & Dye, 1956; Guilbot & Dapron, 1969; Iglesias, Chirife & Lombardi, 1975; Simatos & Blond, 1975).

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These studies, however, have been carried out using pure sugars. When other food components are present it may be expected that they may influence the physical state of the dried sugar and also the kinetics of crystallization. Consequently, the purpose of the present work is to study some aspects of sucrose crystallization in humidified freeze dried systems, when other food components are also present.

Methods

Preparation of amorphous sucrose and model systems

Systems included sucrose and sucrose with the addition of various food polymers. The composition of the systems studied are shown in Table 1. The different food polymers, in powder form, were added to solutions of sucrose in water at room temperature, thoroughly mixed, poured in weighing bottles with outside ground stoppers, frozen and freeze dried. It is noteworthy that starch was not gelatinized because the mixes were always prepared at room temperature. Freeze drying was carried out at room temperature and a pressure less than 100 μm. It is known that this procedure mostly yields amorphous cakes (White & Cakebread, 1966).

Measurement of moisture sorption and rate of crystallization

Water uptake by the initially dry sucrose and model systems was determined periodically from changes in weight of samples kept at 35°C in a vacuum dessicator with a saturated salt solution to maintain a constant relative humidity of 54%. It has been previously observed (Iglesias *et al.*, 1975) that at this relative humidity the sorbed water started crystallization of the amorphous sucrose with subsequent release of moisture. The rate of crystallization of sucrose was thus evaluated from changes in the moisture content (Makower & Dye, 1956). Equilibrium moisture contents of the individual different food

Table 1. Composition of systems studied

System	Composition (% dry basis)
A	Sucrose
B	95.2% Sucrose + 4.8% Carboxymethylcellulose (CMC)
C	90.9% Sucrose + 9.1% Carboxymethylcellulose
D	76.9% Sucrose + 23.1% Guar gum
E	76.9% Sucrose + 23.1% Garrofin gum
F	76.9% Sucrose + 23.1% Carboxymethylcellulose
G	76.9% Sucrose + 23.1% Sodium alginate
H	43.5% Sucrose + 43.5% Starch + 13% CMC
I	43.5% Sucrose + 43.5% Microcrystalline cellulose + 13% CMC

Table 2. Equilibrium moisture content at 54% relative humidity and 35°C, of the different polymers utilized

Component	Equilibrium moisture content (% dry basis)
Carboxymethylcellulose	13.6
Guar gum	15.0
Sodium alginate	17.0
Garrofin gum	10.5
Starch	12.5
Microcrystalline cellulose	6.6

polymers utilized, at 54% relative humidity and 35°C, were also determined and are shown in Table 2.

Results and discussion

The course of the moisture uptake with time for sucrose and the different model systems is shown in Figs 1–4. It can be seen that at 54% relative humidity and 35°C all types of sample gained moisture. After a maximum was reached the cake lost moisture indicating that crystallization of sucrose occurred

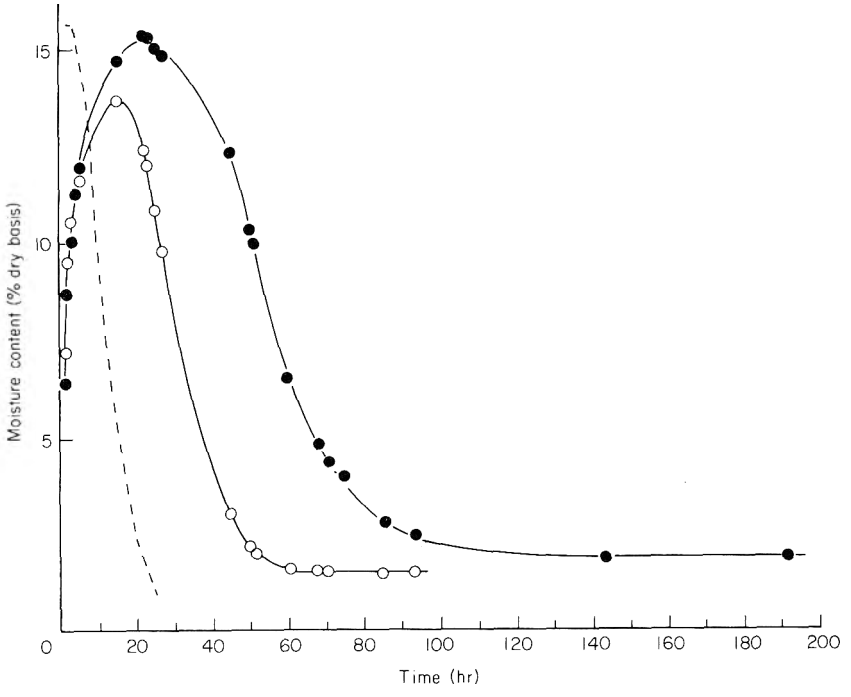

Figure 1. Water uptake by amorphous sucrose and model systems at 54% relative humidity and 35°C. ○ 95.2% sucrose + 4.8% CMC; ● 90.9% sucrose + 9.1% CMC; – – – sucrose control.

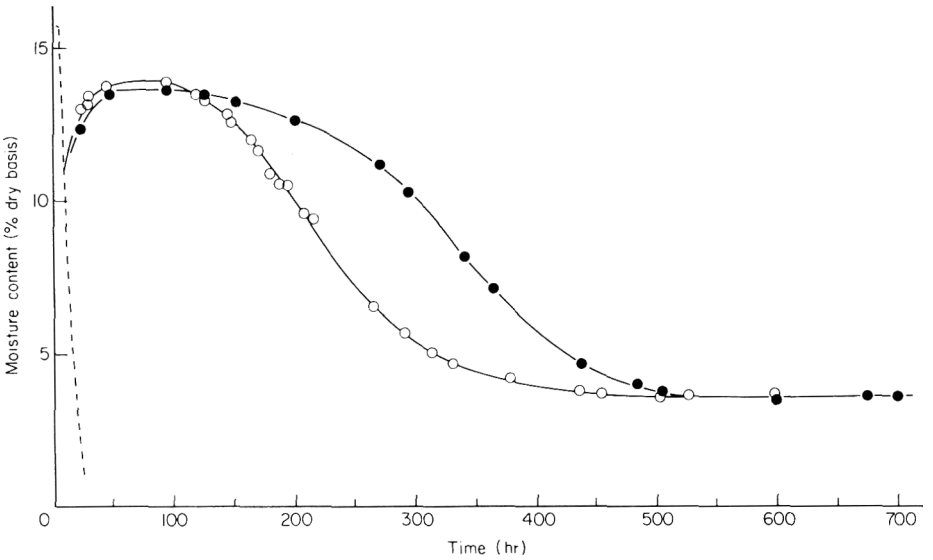

Figure 2. Water uptake by amorphous sucrose and model systems at 54% relative humidity and 35°C. ○ 76.9% sucrose + 23.1% CMC; ● 76.9% sucrose + 23.1% sodium alginate; – – – sucrose control.

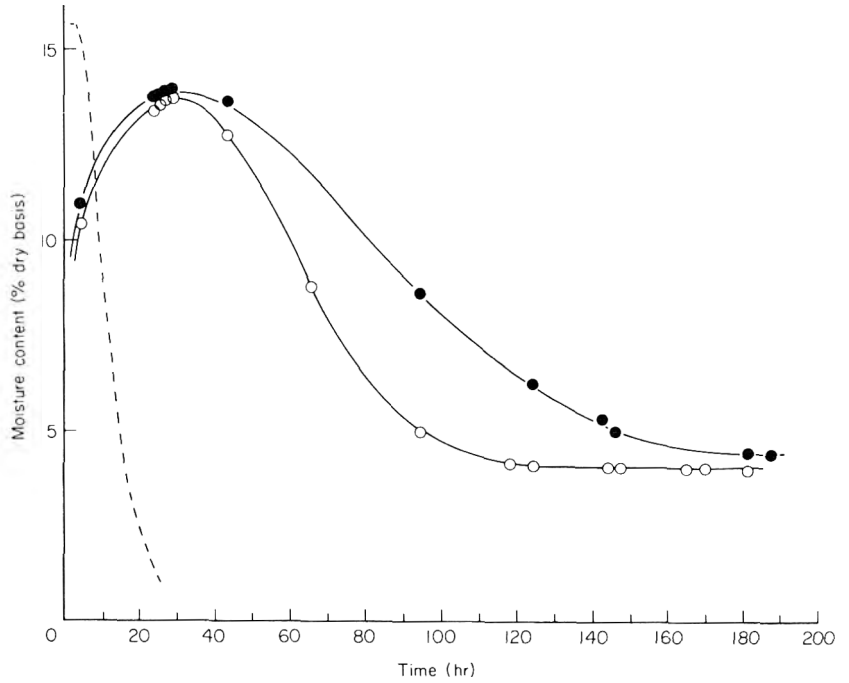

Figure 3. Water uptake by amorphous sucrose and model systems at 54% relative humidity and 35°C. ○ 76.9% sucrose + 23.1% garrofin gum; ● 76.9% sucrose + 23.1% guar gum; – – – sucrose control.

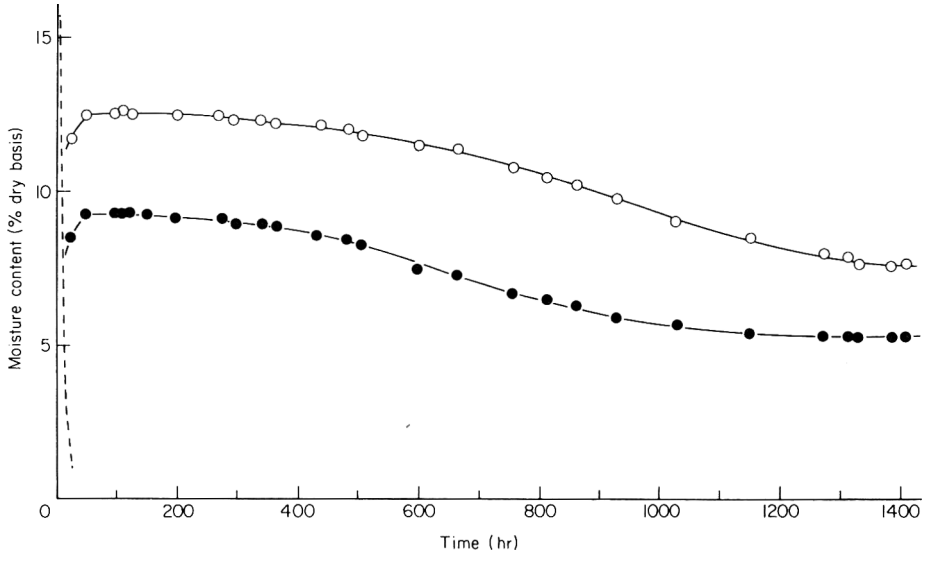

Figure 4. Water uptake by amorphous sucrose and model systems at 54% relative humidity and 35°C. ○ 43.5% sucrose + 43.5% starch + 13% CMC; ● 43.5% sucrose + 43.5% avicel + 13% CMC; – – – sucrose control.

(Makower & Dye, 1956; Karel, 1973); the process of crystallization was always delayed in the model systems as compared to pure sucrose. It is observed that the values of the maximum moisture content and the final or near equilibrium ones, are different for the various systems examined. This is due to the different sorptive capacity of the polymers studied, as shown in Table 2. The kinetics of water uptake by each polymer separately was also measured and is shown in Fig. 5 for guar gum and sodium alginate; similar curves were obtained for the other polymers. In all cases it was found that in a time period shorter than

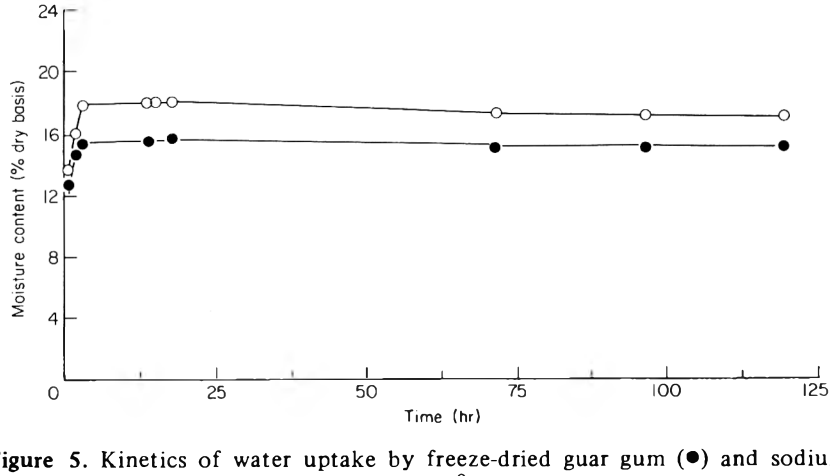

Figure 5. Kinetics of water uptake by freeze-dried guar gum (●) and sodium alginate (○) at 54% relative humidity and 35°C.

that corresponding to the beginning of sucrose crystallization (peaks in curves of Figs 1–4), equilibrium was essentially reached.

Amorphous sucrose is theoretically unstable but the very high viscosity of the medium prevents a molecular rearrangement and stabilizes the amorphous state for a relatively long time (White & Cakebread, 1966; Chevally, Rostagno & Egli, 1970). The sorption of water imparts mobility to the sucrose molecules and this results in the transformation of sucrose from the metastable amorphous state to the more stable crystalline state (Karel, 1973). In this process the sucrose loses water as indicated by the experiments shown in Figs 1–4. Makower & Dye (1956) showed that the rate of crystallization of amorphous sucrose, evaluated from changes in moisture content, followed an exponential law with respect to time after an initial induction period. This period was interpreted to be the time for a build-up of sufficient nuclei to initiate an appreciable rate of crystallization. In order to test this behaviour with the present experiments, the data shown in Figs 1–4 were plotted as log moisture content versus time. A non-dimensional moisture content, X/X_{max}, was utilized which was calculated by dividing the moisture content at each time by the moisture content corresponding to the observed maximum in the water uptake curves (Figs 1–4). This was done in order to facilitate comparison between the different model systems. The time axis corresponds to time after the maximum moisture content (X_{max}) was reached. The results are shown in Fig. 6; it can be seen that straight lines were obtained in all cases after an initial induction period. It can be seen that the induction period is significantly increased by the presence of other components, as compared to pure sucrose. A linear regression analysis was used to obtain the slopes of the straight lines which are referred as 'first order rate constants', and are shown in Table 3. It can be seen that the presence

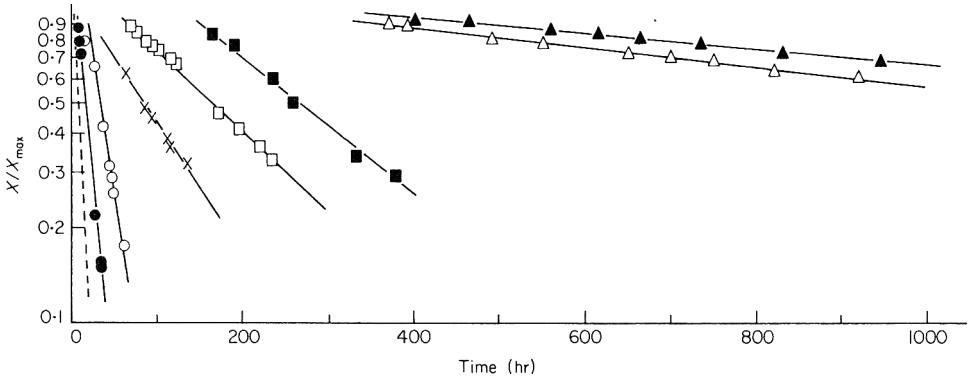

Figure 6. Logarithm of non-dimensional moisture content (X/X_{max}) versus time for sucrose and model systems at 54% relative humidity and 35°C. ● 95.2% sucrose + 4.8% CMC; ○ 90.9% sucrose + 9.1% CMC; × 76.9% sucrose + 23.1% guar gum; □ 76.9% sucrose + 23.1% sodium alginate; ■ 76.9% sucrose + 23.1% CMC; △ 43.5% sucrose + 43.5% avicel + 13% CMC; ▲ 43.5% sucrose + 43.5% starch + 13% CMC; – – – sucrose control.

Table 3. First order rate constants (k) obtained from the linear plots shown in Fig. 6

System	k (hr^{-1})
A (Control)	1.1×10^{-1}
B	6.3×10^{-2}
C	4.0×10^{-2}
D	9.7×10^{-3}
G	5.9×10^{-3}
F	5.0×10^{-3}
I	7.5×10^{-4}
H	5.7×10^{-4}

of other components greatly reduced the apparent rate of crystallization of sucrose.

It is to be expected that the physical state of sucrose is influenced by the presence of other components of the system. Gal (1969) showed that after freeze drying of a casein, sodium chloride solution, sodium chloride can exist in three different states (a) bound to the protein, (b) free and amorphous, and (c) free and crystalline. The observed delayed crystallization of amorphous sucrose may be attributed to interactions with the other components, as well as to the increased viscosity of the medium which reduces the mobility of sucrose molecules leading to its transformation to the crystalline state. A purely steric effect should also be considered because it has been shown (Iglesias *et al.*, 1975) that an inert system also delays sucrose crystallization upon humidification.

Acknowledgments

The authors acknowledge the financial support from the Consejo Nacional de Investigaciones Científicas y Técnicas de la República Argentina (Legajo No. 7809/76-2).

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J. Fd Technol. (1978) **13**, 145–148

Technical note: The effect of curing and maturing of bacon on muscle pH

W. J. GALLWEY AND P. V. TARRANT

The ultimate pH (pH_u) of pig muscle is normally reached within 24 hr of slaughter. Its value is determined by the glycogen concentration in the muscles at death. Post-rigor muscle has a considerable buffering capacity (Hamm, 1977) and during extended ageing of fresh meat under aseptic conditions only small changes in pH occur (Lawrie *et al.*, 1961). The present investigation concerns the changes which occur in muscle pH during curing and maturing of sides of Wiltshire bacon. A procedure for estimating carcase pH_u values from bacon pH could be of practical use in certain circumstances. Thus, when bacon is of unsatisfactory quality, bacon pH measurements could be used to determine whether carcase pH_u values were abnormal, resulting in quality defects. It is well established that high carcase pH_u values adversely affect the bacteriological keeping quality and colour of raw, cured meat products (Bem, Hechelmann & Leistner, 1976; Barton, 1971).

The pH_u was measured in thirty-eight pork sides at about 24 hr *post mortem*. The muscles used for pH measurement were *M. semimembranosus* (*SM*) and *M. adductor* (*AD*) in the ham, *M. longissimus dorsi* (*LD*) in the loin (at the level of the last rib) and *M. semispinalis capitis* (*SC*) in the shoulder. A radiometer 29 pH meter equipped with a combined glass electrode (GK 2320 C) and calibrated using buffers pH 4.0 and 6.5 was used. For all measurements of muscle pH, the electrode was inserted into the carcase to a depth of 2 to 3 cm below the surface and the pH recorded. The results relate to pH changes in the interior of muscles; changes on the muscle surface were not investigated.

The sides were cured using a multineedle injection machine, with brine of the following composition (% w/v): sodium chloride 20.0; sodium nitrate 0.1; potassium nitrate 0.1. They were tanked for 4 days in a mature immersion brine of similar composition. Soluble muscle constituents may leach into the brine at this point, contributing to changes in muscle pH. The sides were withdrawn and matured in the bacon cellar at 4°C. Bacon pH was measured 4 days after removal from the tank. Eight of the bacon sides were then held at 4°C until definite signs of bacteriological spoilage were (subjectively) observed by

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0022-1163/78/0400-0145 $02.00

Table 1. Effect of curing and ageing on pig muscle pH*

	No. of sides	*SM*	*AD*	*LD*	*SC*
Pork pH_u	38	5.85 (0.04)	6.30 (0.06)	5.63 (0.03)	6.38 (0.06)
Bacon pH (4 days *ex* tank)	38	5.89 (0.06)	6.15 (0.05)	5.73 (0.03)	6.18 (0.04)
Significance		NS	$P < 0.001$	$P < 0.001$	$P < 0.001$
Bacon pH (4 days *ex* tank)	8	5.88 (0.12)	6.05 (0.13)	5.73 (0.07)	6.14 (0.13)
Bacon pH at spoilage	8	5.88 (0.10)	6.05 (0.10)	5.72 (0.06)	6.14 (0.12)
Significance		NS	NS	NS	NS

* Results as mean pH values and standard errors.

Table 2. Regression of bacon pH on pork pH_u

Muscle	Regression equation	Correlation	Significance $P <$
AD	Bacon pH = 0.7226 Pork pH_u + 1.6020	0.857	0.001
SM	Bacon pH = 0.6757 Pork pH_u + 1.8747	0.938	0.001
LD	Bacon pH = 0.7033 Pork pH_u + 1.7740	0.783	0.001
SC	Bacon pH = 0.6699 Pork pH_u + 1.9080	0.902	0.001
Combined	Bacon pH = 0.6586 Pork pH_u + 1.9968	0.928	0.001

an experienced panel of three individuals. Onset of spoilage occurred at about 4 weeks after slaughter and was indicated by development of slime, discolouration and off-odours on the surface of the Wiltshire sides. The pH of the aged bacon was then recorded.

The results showed that muscle pH changed significantly on curing and that ageing of bacon until the onset of spoilage caused no further significant changes in pH to occur (Table 1). It was concluded that major pH changes occurred only during the curing operation. A standard regression programme was used to test the relationship between pork pH_u and bacon pH (4 days after removal from the curing tank). The results for the four individual muscles and for the combined data are shown in Table 2. The regressions are all highly significant, the equations for individual muscles are not significantly different from each

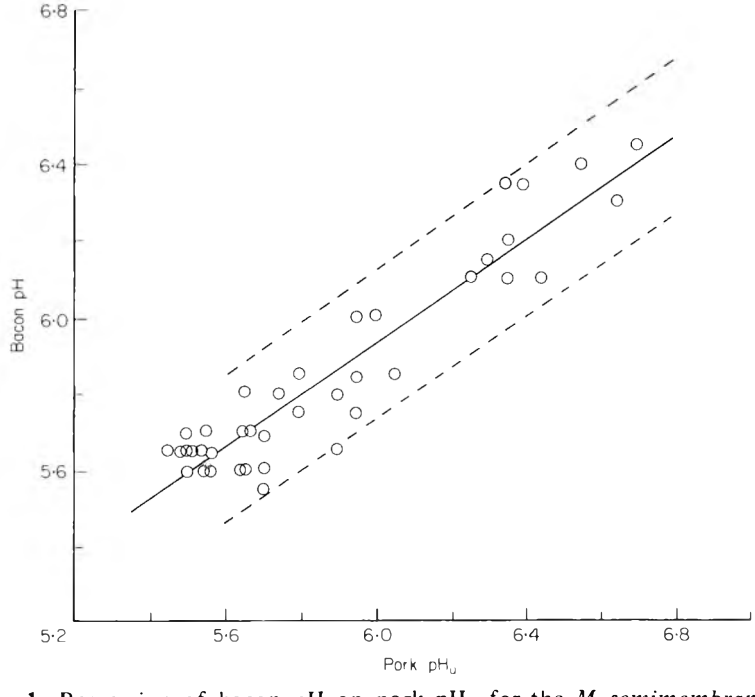

Figure 1. Regression of bacon pH on pork pH_u for the *M. semimembranosis*, the 95% confidence intervals are shown.

other. The correlation between pH_u and bacon pH is highest for the *SM* and the regression line and 95% confidence intervals are shown in Fig. 1. The results indicate that meat pH tends to level out on curing, low values increasing and high values decreasing, in agreement with the observations of Barton (1971).

Acknowledgments

This investigation was partly financed by the bacon industry. The authors wish to thank Mr. A. Kelly, Statistics Department, An Foras Taluntais for assistance.

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Technical note: The incorporation of fresh cassava into bread

JOAN CRABTREE*, E. C. KRAMER* AND JANE BALDRY†

Dendy *et al.* (1975) have reported a number of papers on the use of cassava in breadmaking; these relate specifically to the dried products of cassava, i.e. flour and starch. The production of these is wasteful in terms of the energy required for drying, particularly since the dried products will be rehydrated during the breadmaking process.

Little attention has been paid to the use of fresh cassava in bread, in spite of the potential advantage to the small baker in the less developed countries: a fresh cassava product could easily be prepared using simple equipment.

Although the use of fresh cassava has not been previously reported, fresh potatoes (*Solanum tuberosum*) were being incorporated into bread in the United Kingdom as early as 1795 (McCance & Widdowson, 1956) and, during the Second World War, the bread regulations were amended to allow potatoes as an ingredient of the national loaf, at a time when wheat was scarce (Minister of Food, 1943).

The present note describes the preparation of a product of fresh cassava, its incorporation into bread and the assessment of the finished product.

Preparation of the fresh cassava product

Cassava tubers were obtained through the Kenya Trading Corporation and air-freighted from Nairobi. The cassava roots were washed and partially peeled with a mechanical bench peeler, peeling being completed by hand. The roots were sliced on a small slicing machine at a setting to give slices of 1 cm thickness. The slices were then minced mechanically by passing them successively through 3/8 in and 3/16 in plates. The resultant minced cassava had a moisture content of 63% as measured by the A.A.C.C. method (1969).

Breadmaking

The minced cassava was incorporated into bread at a substitution level of 20% (calculated on a 14% moisture basis). Control loaves were baked using a

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Table 1. Recipe for breadmaking

Dried yeast reconstitution	Control	Test	
Active dried yeast (g)	10	10	Aerobic
Sugar (g)	3	3	respiration
Water at 38°C (ml)	110	110	15 min at 38°C
Bakers' flour (g)	1300	1040	
Fresh cassava pulp (g)	—	604	
Salt (g)	23.4	23.4	
Fat (g)	9.1	9.1	
Sugar (g)	10	10	
Water (ml)	600	355	

bakers' flour (extraction rate about 72%, protein content 12.4%). A Bulk Fermentation method of breadmaking was used, according to the recipe in Table 1.

The ingredients were mixed in a laboratory scale mixer at slow speed for 4 min and the dough was fermented in a large polythene bag at 27°C in a temperature controlled cabinet. After fermentation, the dough was scaled at 450 g, shaped by hand and allowed to recover for 15 min. The dough balls were mechanically moulded in a laboratory scale moulder and allowed to prove to a height of 11.5 cm at 40°C, 75% relative humidity. The dough was then baked for 25 min at 218°C in a Simon Rotary oven. The specific volumes of the baked loaves were determined by seed displacement (Table 2). The loaves were frozen in sealed polythene bags and stored at −10°C prior to flavour evaluation.

Sensory evaluation

Samples were warmed to ambient temperature by microwave thawing shortly before presentation. A panel of twenty-five assessors, working on several occasions, judged hardness or softness, dryness or moistness, doughiness or sponginess, and degrees of freshness and acceptability by marking along a 10 cm line. These assessments were converted into scores out of forty (Table 3).

Determination of hydrocyanic acid (HCN) content

Cassava tubers contain varying amounts of hydrocyanic acid (HCN) which is usually removed during the preparation of traditional cassava products, e.g. gari. To determine the safety of the minced cassava for human consumption when incorporated into bread, its HCN content and that of the crumb of the baked test loaf were determined by an alkaline titration method (Table 4 in Grace, 1971a).

Results

Figures 1 and 2 show the profiles of the control and test loaves, respectively.

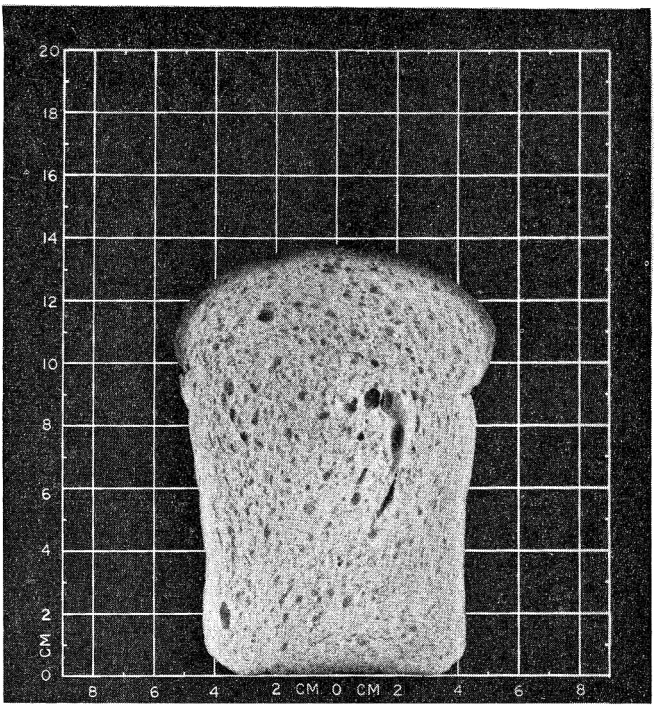

Figure 1. Profile of the control loaf.

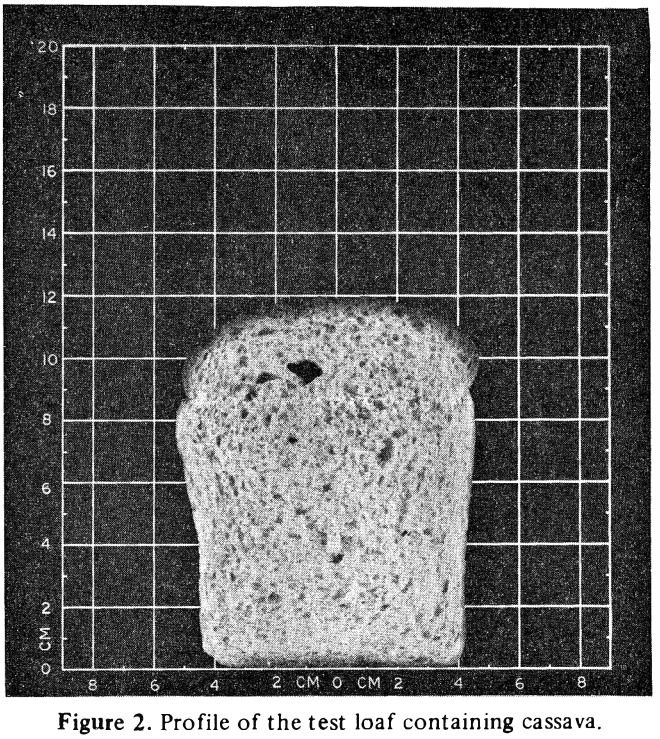

Figure 2. Profile of the test loaf containing cassava.

Table 2. Loaf measurements before and after baking (mean of four loaf measurements)

	Control	Test
Proof height (cm)	11.5	11.5
Height from oven (cm)	14.5	12.2
Oven spring (cm)	3.0	0.7
Specific volume (ml/g)	4.2	3.6
Specific volume as a percentage of control	100	85.1

Table 3. Sensory evaulation of the control and test loaves (mean scores)

	Control	Test	Remarks
Softness	30.5	28.3	Test bread significantly firmer than control, $P = 0.05$
Moistness	23.7	23.8	Differences not significant
Sponginess	23.5	24.4	Differences not significant
Freshness	28.3	26.6	Test bread rates less fresh than control, $P = 0.05$
Acceptability	28.4	27.1	Differences not significant

Table 4. Hydrocyanic acid (HCN) contents in mg HCN/kg material

	Expected	Found
Minced cassava	—	50
Test dough before baking	14	not measured
Test loaf after baking	16	10

Discussion

The minced cassava was readily and easily prepared. Peeling and slicing of the cassava tubers were carried out on essentially modern equipment for the convenience but could be adequately achieved by hand. The mechanical mincer could be replaced by a simple hand mincer or grater. The minced cassava was easily incorporated into the bread dough.

Although the specific volume of the test loaf was lower than that of the control loaf, volume, shape and crumb structure were all acceptable (Table 2). The incorporation of the minced cassava gave the loaf a speckled appearance both internally and externally, and the crumb had a yellow tinge with a faint cassava odour. However, none of these characteristics was objectionable nor did they detract from the overall acceptable appearance of the loaf.

The panel found the test loaf to be firmer than the control loaf. This was reflected in the panel's assessment of freshness. Increased firmness may be attributed to the fibre content of cassava or lower loaf specific volume.

The amount of HCN in fresh cassava tubers varies from harmless to lethal or from a few milligrams to 250 mg or more per kilogram (Grace, 1971b). Tubers containing less than 50 mg HCN/kg are classified as sweet or non-toxic and the tubers used in this study fall within this category. The level of 10 mg HCN/kg found in the baked test loaf corresponds to a reduction of 6 mg HCN/kg – i.e. 37% (Table 4). If cassava tubers containing high levels of HCN were used, the safety aspect of this method of substitution would require further elucidation and it would be necessary to make regulations governing permitted levels of HCN in bread.

This brief study has shown that it is possible to manufacture wholesome, acceptable bread containing up to 20% fresh cassava.

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Book reviews

Food-borne Micro organisms of Public Health Significance (2 Vols).
Ed. by K. A. Buckle, R. A. Edwards, M. J. Eyles, G. H. Fleet and W. G. Morrell.
N.S.W. Branch Food Microbiology Group of the Australian Institute of Food Science and Technology. CSIRO Division of Food Research.
School of Food Technology, University of New South Wales, 1976. Pp. xxxiv + 117. Aus $25.00 plus postage.

This is a revised two volume loose leaf publication which was designed primarily for the use of participants in the second intensive laboratory course devoted to the detection of micro-organisms of public health significance in foods held in the School of Food Technology UNSW in 1976. The text has been prepared by acknowledged experts in their respective fields, names which I am sure are familiar to food microbiologists in this country. It was hoped that the publication would prove of interest to food scientists and microbiologists in the food industry, and to government and teaching laboratories that have responsibilities for the microbiological status of Australian foods.

I am sure that these aims will be achieved and that these books will prove to be almost as useful in the United Kingdom and possibly other countries as well. Every chapter is well documented with references to British and American as well as to Australian standards and methods. In many cases they are, of course, very similar if not identical. We have used these books in our teaching laboratories for a few months and have found them most useful and we are sure that others will find the same. The methods are given in detail, they are easy to follow and many refer to recently adopted techniques.

It would appear that this is only an interim publication because Part 3 of Volume II states that 'Draft methods for this part (examination for Specific Products) have not yet reached sufficient detail to be included in this volume'. It is to be hoped that a complete version will appear before long and that it will then be found possible to include an index.

H. D. G. Roper

Cheese. By J. G. Davis
Volume III. **Manufacturing Methods,** 1976. Pp. 567. £17.
Volume IV. **Annotated Bibliography with Subject Index,** 1975. Pp. 306. £10.
London: Churchill Livingstone.

The earlier volumes in this series on cheese, Volume 1, *Basic Technology* and Volume II, an *Annotated Bibliography with Subject Index,* covering the scientific and technical literature from earliest times up to 1961, were published

in 1965. Volume III on *Manufacturing Methods* has been written as a continuation of Volume I starting at Chapter 20. Likewise Volume IV has been planned on the same lines as Volume II as a further *Annotated Bibliography* covering the period 1962–1970.

Whilst scientific and technical facets of cheesemaking were reviewed in detail in Volume I of the series, the substance of Volume III on *Manufacturing Methods* is the description of the manufacture of the known cheese varieties of each country in the world. After an initial chapter on the general principles of cheesemaking, the next three chapters are devoted to British, U.K. and Irish cheese varieties with a detailed treatment of cheddar and the territorial cheeses. Separate chapters are devoted to the cheese manufactured in the United States, Australasia, France, Italy and Switzerland. All the remaining cheese manufacturing areas are covered in the subsequent chapters including Africa, Asia and Central and South America.

Sufficient detail is included of the manufacturing methods for the reader to appreciate the different processes adopted to produce the characteristic cheese varieties. Supporting references are given for the main varieties and manufacturing methods for each country. The final chapters on equipment, mechanization, packaging and recent installations essentially list equipment and systems recently introduced for factory manufacture. One chapter is devoted to continuous cheesemaking and the attempts which have been made to establish truly continuous systems. The text of Volume III is illustrated with a miscellany of black and white photographs.

There is no doubt that Volume IV together with Volume II of this series provide a valuable annotated bibliography up to 1970 (not 1966 as stated on the fly-leaf) of the extensive literature on cheese. These volumes, whilst providing full references for Volumes I and III are also independent sources of information through the comprehensive subject indexes.

The publishers' claim that these volumes would be expected to become the standard work in the English language for those concerned with the scientific, technological and practical aspects of cheese, could well be justified. In any event, these volumes will be essential reading for those interested in scientific and technical aspects of cheese and its manufacture.

It is perhaps worth noting that an encyclopaedia of cheese was published in German only, in 1974, based on contributions by experts in different countries (*Handbuch der Käse*, by Dr Heinrich Mair-Waldburg, Volkswirtschaftlicher Verlag GmbH, Kempten Allgau).

Certainly Dr Davis has made a further important contribution to the literature on dairy science and technology by collating and publishing in text book form information on cheese collected from worldwide sources.

W. A. Cox

Books received

Fibre Optics. By D. A. Hill.
London: Business Books Ltd, 1977. Pp. x + 176. £10.

An elementary introduction into fibre optics and their use in solving industrial problems. The book contains a brief section on applications in the food industry.

Protein Crosslinking. Ed. by N. Freedman.
I. Biochemical and Molecular Aspects.
II. Nutritional and Medical Consequences.
New York: Plenum Publishing Corporation, 1977. Pp. ix + 760 and pp. xx + 740. US$71.40 each.

A collection of eighty-two papers read at a symposium held in San Francisco in 1976.

World Review of Nutrition and Dietetics, Vol. 28. Ed. by G. H. Bourne.
Basel: S. Karger AG, 1978. Pp. xii + 257. US$71.75.

This volume contains monographs on the following topics: parenteral nutrition, biochemistry and physiology of magnesium, bone growth and development in protein–caloric malnutrition, hepatocarcinogens in Nigerian foodstuffs and carcase evaluation of cattle, sheep and pigs.

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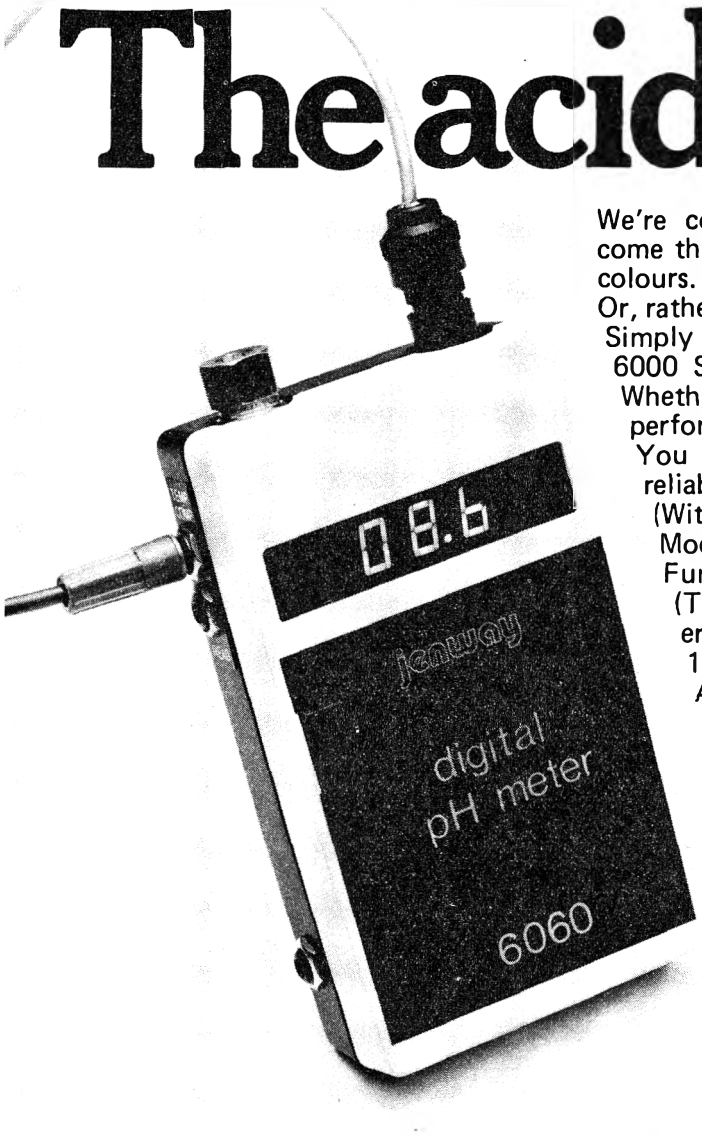

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Arrangement. Papers should normally be divided into: (a) Summary, brief, self-contained and embodying the main conclusions; (b) Introduction; (c) Materials and methods; (d) Results, as concise as possible (both tables and figures illustrating the same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

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

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

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

SI UNITS

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

NON SI UNITS

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

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

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

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