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Engineering factors in the production of concentrated fruit juices. II. Fluid physical properties of grape juices

M. MORESI AND M. SPINOSI

Summary

In this article the experimentally measured physical properties (*viz.* rise in boiling point, density, specific heat and rheological properties) of grape juices are empirically correlated to their corresponding sugar content so as to make it easier for food process engineers to design and optimize concentrated grape juice handling and processing units. For example, the rise in boiling point of grape juice concentrates, which is a complex function of sugar concentration and pressure, was found to vary non-linearly up to 9.9 K at atmospheric pressure as sugar concentration increases from 0 to 73.1°Brix. Within the same range of sugar concentration and over the temperature range 20–50°C, a power law model was found to be successful in reconstructing the experimental flow curves with an average error less than 6.5%. Moreover, as sugar concentration increases, the flow behaviour index becomes proportionally smaller than one, thus enhancing the pseudoplasticity, that is the time-independent shear thinning behaviour, of the product under study.

Introduction

Although most grape crops are primarily utilized in fermented liquors, there is a positive trend toward the production of single-strength and concentrated grape juices to be utilized not only as natural sources of nutritional value, but also as thirst quenchers.

In order to increase the average grape juice consumption *per capita* (for instance, in the Mediterranean countries and Italy in particular, grape juices, being traditionally associated with wine, have a low degree of acceptability), the grape juice industry tends to produce either cloudy and low Brix/acid ratio red grape juices (so as to maintain their peculiar 'natural' character in the average consumer's mind and enhance their palatability) or soft drinks based on grape juice diluted with water or other fruit juices (such as strawberry, blackcurrant, pineapple, orange, etc.). For further information on the present trend and innovation of grape juice industry see Cantarelli (1977, 1982).

In order to apply classic chemical engineering criteria to design fruit juice-processing units, knowledge of the physical properties of the fluid concerned is required for a number of engineering calculations ranging from prediction of pressure drops in transfer lines and process equipment to estimation of power requirements for pumping, agitation, heating and evaporation. To this end, a series of investigations has been planned to characterize the essential physical properties of a few fruit and vegetable juice concentrates. In the first part of this investigation (Moresi & Spinosi, 1980) the physical properties of orange juices were determined and correlated by means of simple

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empirical models, which were then used to simulate the operation of a few industrial double-effect falling-film evaporators (Angeletti & Moresi, 1983).

In this paper some of the physical properties and engineering data for concentration of grape juices are reported as a function of sugar concentration and temperature.

Materials and methods

Materials

Grape juice concentrates used in this work were reconstituted by diluting a concentrated grape must, prepared from the Italian type ('Trebbiano bianco') grape variety and supplied by Gravinol S.r.l. (Lucera, Italy), with tap water. Its mean composition is shown in Table 1.

Table 1. Mean composition of one lot of the concentrated grape must supplied by Gravinol S.r.l. (Lucera, Italy)

Component	Quantity
Total nitrogen (Kjeldhal method)	0.24% w/w
Ammoniacal nitrogen (Kjeldhal method)	0.13% w/w
Titrable acidity (as tartaric acid)	1.03% w/w
Volatile acidity (as acetic acid)	0.07% w/w
Reducing sugars (Fehling method)	52.84% w/w
Ash	1.07% w/w
Mg	84 ppm
Ca	700 ppm
Na	4800 ppm
K_2	1300 ppm
P_2O_5	Trace
SO_2 (Wartha method)	498 ppm

The natural soluble solid content of each concentrated grape juice under study was measured by refractometer at 20°C and expressed as degrees Brix (percentage by weight of equivalent sucrose in the solution), as described by Tateo (1978). Since the acidity of the concentrated grape must (*circa* 54°Brix) expressed as tartaric acid was equal to $1.03 \pm 0.05\%$ by weight, Stevens & Baier's (1939) correction of degree Brix was neglected.

Vacuum concentration was used to obtain grape juice concentrates with degrees Brix greater than 54 up to *circa* 74.

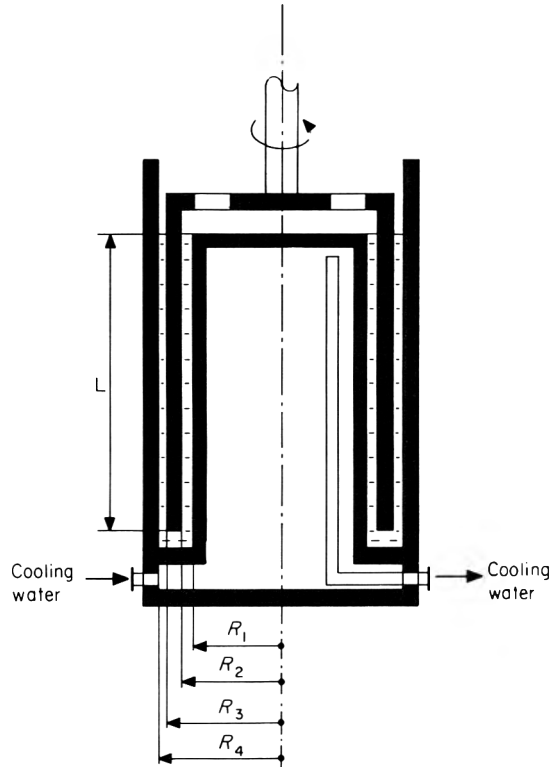
Experimental apparatus and procedures

Measurement of boiling point rise of each concentrated grape juice was carried out by using the experimental apparatus described in the first part of this paper (Moresi & Spinosi, 1980).

The viscosity of each solution was determined with a Haake rotational viscosimeter, type RV12, equipped with: a measuring drive unit, type M150, which can transmit a maximum torque of 1.47 N.cm; a coaxial cylinder sensor system, type NV (see Table 2); and a jacketed vessel which connects the sensor system to the measuring drive unit, centres the bell-shaped rotor ('bob') and the stator ('cup') and allows the temperature of the sample examined to be kept constant during the measurement. By varying the test speed from 0.01 to 256/min, the flow curves (shear stress, τ *versus* shear rate, γ) were constructed.

Table 2. Geometric and characteristic parameters and schematic drawing of the sensor system type NV used in this work

Parameter	Value	Unit
Rotor (bob)		
radius R_2	17.85	mm
radius R_3	20.1	mm
height L	60	mm
Stator (cup)		
radius R_1	17.5	mm
radius R_4	20.5	mm
Sample volume	9	cm^3
ratio $S = R_2/R_1$	1.02	—
ratio $S = R_4/R_3$	1.02	—
α	0.533	Pa
δ	324.6	—



The density was measured by filling a calibrated volumetric flask with the grape juice under study to a marked volume and determining the weight at constant temperature (20°C).

The specific heat was measured with a 'Thermal Analyzer', Dupont 990 (Delaware, USA). The experimental procedures was previously described in Part I (Moresi & Spinosi, 1980).

Results and discussion

Boiling point rise

The boiling point rise, ΔT_b, of grape juice concentrates was determined by carrying out a series of experiments consisting in recording the equilibrium values of the boiling point, T_s, of the solution examined and the associated pressure, p, for different sugar concentrations ranging from 0 to 73°Brix.

Such data are shown in Fig. 1 where the logarithm of the pressure is plotted against the reciprocal of the absolute temperature, T, according to the following expression:

$$\ln p = A - B/T \qquad (1)$$

where A and B are characteristic constants of each solution examined.

Although equation (1) is particularly suitable for describing the vapour pressure of a pure substance, it appears from the data plotted in Fig. 1 that such an equation is able also to reconstruct fairly well the equilibrium values of p and T for all the concentrated grape juices under study. This, of course, does not contrast with theory, since at the boiling point grape juice, being essentially a mixture of water and various non-volatile

constituents, is in equilibrium with a vapour phase consisting of steam only, the fraction of volatile aromatic compounds being practically negligible.

From Fig. 1 it was derived that both constants A and B of equation (1) showed a power dependence on the weight fraction (y) of equivalent 'sucrose' as determined by refractometer, such as:

$$A(y) = \sum_{0}^{m}{}_{j} A_j y^j \tag{2}$$

$$B(y) = \sum_{0}^{p}{}_{j} B_j y^j \tag{3}$$

where A_j and B_j are empirical parameters.

By substituting equations (2) and (3) into equation (1), it was possible to fit all the equilibrium data by means of linear multi-variable regression technique. By varying the degrees m and p of the polynomials (2) and (3) in the ranges 2–6 and 1–4, respectively, it was possible to reduce the average error between the experimental and calculated values of T_s from 15.3 to 0.33%.

Among the several correlations developed, the regression with the minimum number of empirical parameters ($m = 3$ and $p = 1$) statistically significant at the 95% confidence level was chosen by means of an F-test.

The best values of parameters A_j and B_j are listed in Table 3. In this way, the overall error between the calculated and experimental values of T_s was 0.35% and the deviation about the empirical regression line was 19.50 with 243 degrees of freedom.

It can be pointed out that the value of parameter B, showing a slight variation from 5212 K to 5202 K as y ranges from 0 to 73°Brix, is practically in line with that of the corresponding parameter of the Clausius-Clapeyron law. In fact, at 373.16 K the value of this parameter, being equal to the latent heat of vapourization of water, ΔH_v, divided by the gas constant, R, is about 4900 K.

Since the boiling point rise, ΔT_b, is the difference between the boiling point of the solution, T_s, and that of pure water, T_w, at the same pressure, the following expression for ΔT_b can be obtained by combining equations (1–3):

$$\Delta T_b = \frac{\left(\sum_{1}^{p}{}_{j} B_j y^j\right) - \left(\sum_{1}^{m}{}_{j} A_j y^j\right) T_w}{B_0 + \left(\sum_{1}^{m}{}_{j} A_j y^j\right) T_w} T_w \tag{4}$$

where ΔT_b and T_w are expressed as degrees Kelvin.

It is worth noting that for $y \to 0$, equation (4) reduces to:

$$\Delta T_b = \frac{\left(-\sum_{1}^{m}{}_{j} A_j y^j\right)}{B_0} T_w^2 \tag{5}$$

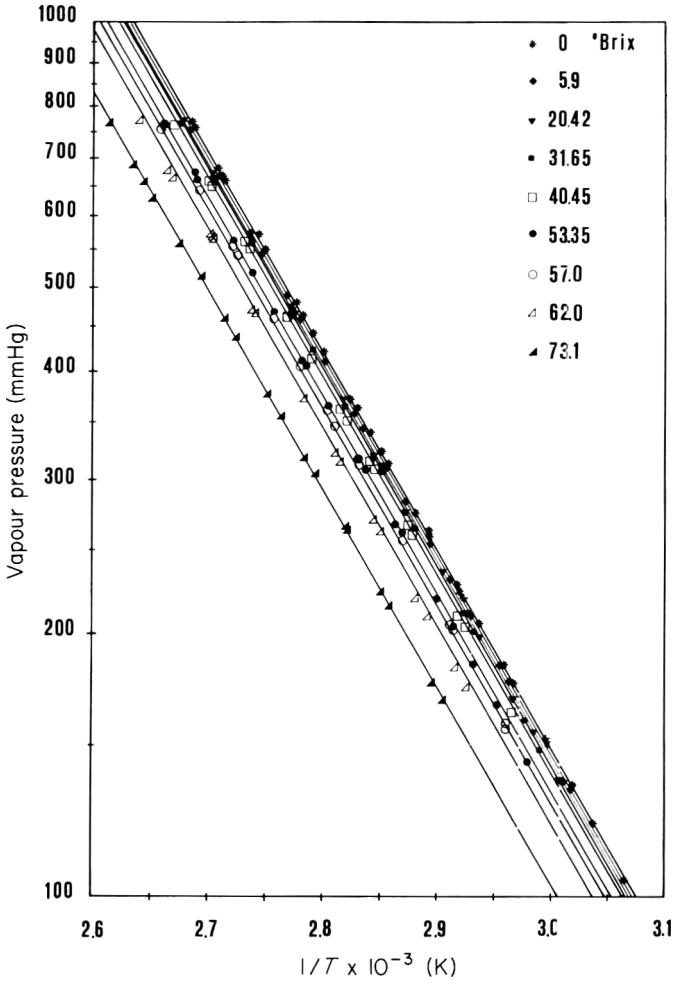


Figure 1. Vapour pressure of steam over various concentrated grape juices as a function of the reciprocal of the absolute equilibrium temperature: the continuous lines were calculated by using equations (1–3).

By replacing B_0 with the approximated expression mentioned above ($\Delta H_v/R$), equation (5) yields:

$$\Delta T_b = \frac{R T_w^2}{\Delta H_v} \left(- \sum_1^m {}_j A_j y^j \right). \tag{6}$$

This equation resembles the expression of the boiling point rise of a solvent caused by the addition of small amounts of a generic non-volatile solute as derived from first principles (Moore, 1962):

$$\Delta T_b = \frac{R T_w^2}{\Delta H_v} x \tag{7}$$

where x is the molar fraction of the solute concerned.

As an example, Fig. 2 shows the experimental values of ΔT_b (closed symbols) *versus* concentration at $T_w = 373.16$ K (i.e., at atmospheric pressure).

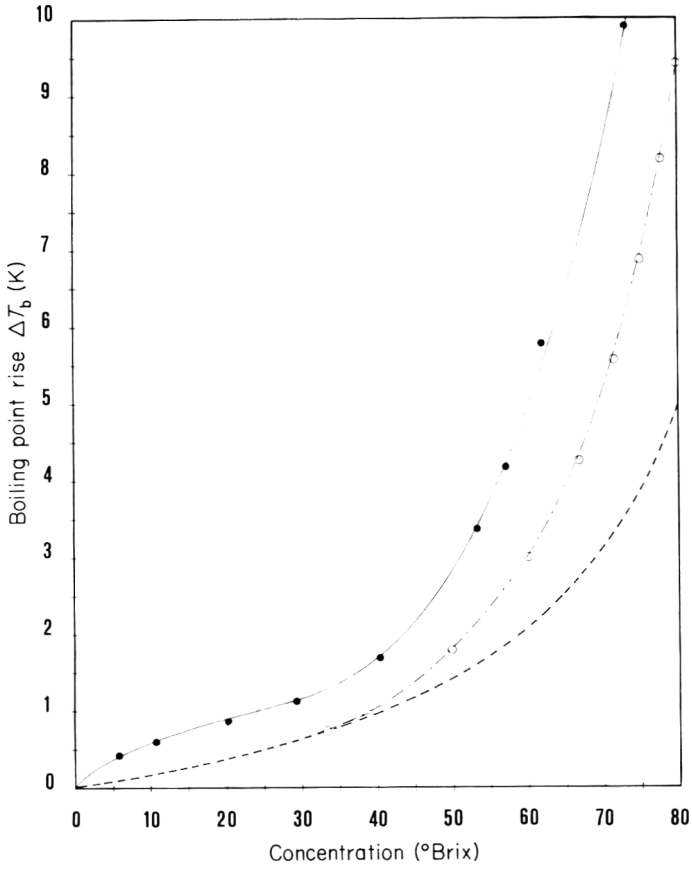


Figure 2. Variation of the boiling point rise of grape juice and sucrose solutions with concentration at atmospheric pressure. ●, grape juice (this work); O, sucrose solutions (Honig, 1953); ———, calculated by using equations (2–4); — — —, calculated by using equation (7).

Table 3. Regression equations of the parameters necessary to predict the physical properties of concentrated grape juices for weight fractions of equivalent sucrose (y) varying from 0 to 0.731

Physical property	Regression	Unit
Boiling point rise	$A(y) = 20.64 - 0.384\,y + 1.297\,y^2 - 2.045\,y^3$	—
	$B(y) = 5212 - 13.75\,y$	K
	$C(y) = -13.38 + 16.93\,y - 95.74\,y^2 + 248.5\,y^3 - 217.6\,y^4$	—
Rheological behaviour	$D(y) = 1903 - 1802\,y + 22\,960\,y^2 - 68\,310\,y^3 + 66\,330\,y^4$	K
	$n(y) = 1 - 0.252\,y$	—
Density	$\rho_{GS} = 1.608$	g/cm^3
Specific heat	$c_{pS} = 1.21$	J/(g K)

By using equation (4) with the values of A_j and B_j listed in Table 3, it was possible to draw the continuous line in Fig. 2 in order to show the fairly good reconstruction of the experimental boiling point rises. Moreover, in Fig. 2 the boiling point rises of sucrose solutions (open symbols) at atmospheric pressure as extracted from Honig (1953) and those calculated by means of equation (7) (broken line) were reported so as to give the

order of magnitude of the error in the estimation of ΔT_b when these data are used instead of the real boiling point rises of concentrated grape juices.

Finally, to further check the correlation developed here, two other trials were carried out by determining the boiling points, T_s, of two commercial red and white grape juices manufactured by Confruit S.p.A. (Faenza, Italy). These data were then plotted *versus* the corresponding boiling point of water, T_w, at the same pressure in Figs 3 and 4, respectively.

Once determined the sucrose-equivalent concentration of each juice, its boiling point rise was predicted for T_w ranging from 323.16 to 373.16 K by means of the model mentioned above. In this way, it was possible to estimate the boiling points ($T_s = T_w + \Delta T_b$) and then construct the Düring's diagrams for both grape juices under study.

Since the continuous lines in Figs 3 and 4 show a remarkable agreement between the calculated and experimental values of T_s, it is possible to stress the reliability of the correlation proposed for the engineering calculations associated with grape juice concentration.

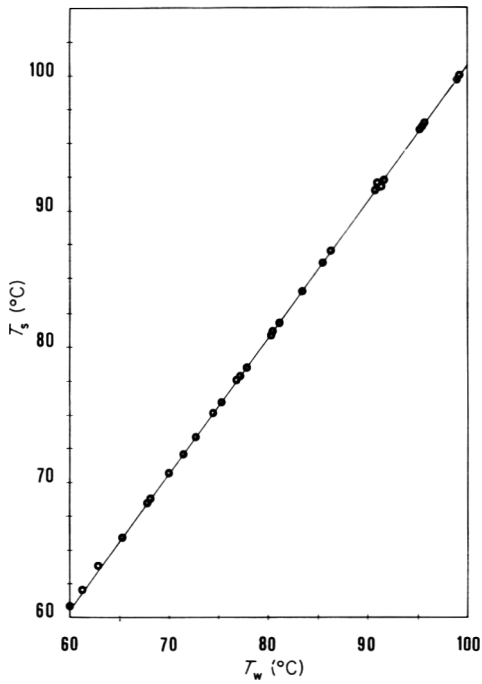


Figure 3. Dühring's plot for a commercial red grape juice manufactured by Confruit S.p.A. (Faenza, Italy) with sugar concentration of 16.6°Brix: boiling point of grape juice (T_s) *versus* boiling point of water (T_w). The continuous line was calculated by using equations (2–4).

Rheological properties

To determine the rheological behaviour of concentrated grape juices, the well-known Moore–Davies double viscosimeter was used, since this instrument is particularly suitable for minimizing end and edge effects on viscosity determination (Whorlow, 1980). Table 2 shows a schematic drawing of the sensor system and its main geometric dimensions. By using a stator (cup) and a rotor ('bob') with internal and external radii so that their ratios, R_2/R_1 and R_4/R_3, are equal, the shear stress at the two

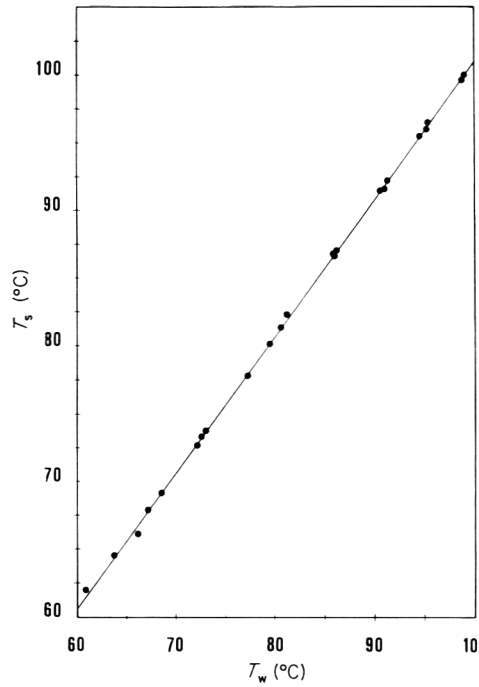


Figure 4. Dühring's plot for a commercial white grape juice manufactured by Confruit S.p.A. (Faenza, Italy) with sugar concentration of 18.3°Brix: boiling point of grape juice (T_s) *versus* boiling point of water (T_w). The continuous line was calculated by using equations (2–4).

'inner' surfaces, τ_1 and τ_3, are equal as well as those at the two 'outer' surfaces, τ_2 and τ_4, thus involving shearing conditions approximately uniform throughout the liquid, especially if the annular gaps are very small (Moore & Davies, 1956).

A torque balance at any one of the stationary surfaces, while the bob is rotating at steady angular velocity, yields:

$$\tau(2\pi RL)R = M = k\Theta \tag{8}$$

where M is the measured torque, k is a suitable spring constant, Θ is the viscosimeter dial reading, R is the radial position of the stationary surface, and L is the rotor length. From equation (8), it follows:

$$\tau = \frac{k}{2\pi R^2 L}\Theta = \alpha\Theta \tag{9}$$

where α is a characteristic constant of the instrument, which depends on the position of the stationary surface. The corresponding shear rate, γ, at the 'cup' surface can be calculated as follows:

$$\gamma = \frac{4\pi N}{1-S^{-2}} C_R \tag{10}$$

where N is the rotational speed of the cup, S the ratio of cup to bob radius, and C_R is a Kreiger & Maroon correction factor (Kreiger & Maroon, 1954). This factor is a complex function of S, the slope of the logarithmic plot of torque *versus* N, and M.

However, it reduces to Calderbank & Moo-Young's (1959) simplified expression for $S < 1.75$ and to 1 for $S \rightarrow 1$. In the latter case, the shear rate approaches a constant value across the two annular gaps (Skelland, 1976).

$$\gamma = \frac{4\pi N}{1 - S^{-2}} = \delta N \tag{11}$$

By using three standard liquids with viscosities of about 5, 100, and 1000 mPas, which were supplied by the manufacturer, it was possible to calibrate the torque and shear rate scales of the viscosimeter, that is to determine the values of α and δ listed in Table 2. These parameters were subsequently used to construct the flow curves for concentrated grape juices.

Figure 5 shows the apparent viscosity, μ_a, of different grape juice concentrates as a function of shear rate, γ, on logarithmic co-ordinates at temperatures ranging from 293.16 to 323.16 K. It can be observed that μ_a depends not only on sugar concentration and temperature, but also on shear rate.

Since μ_a decreses with increasing shear rate and is independent of time, grape juice concentrates can be classified as non-Newtonian fluids of the pseudoplastic type. Therefore, the observations shown in Fig. 5 were described by means of the power law or Ostwald-de-Waele model:

$$\tau = K\gamma^n \tag{12}$$

where K is the 'consistency index' and n the 'flow behaviour index'. For Newtonian fluids K coincides with the viscosity and n is equal to 1.

For a given fluid, the effect of temperature on K has been usually described by using the Arrhenius relationship, that is:

$$K = \exp(C + D/T) \tag{13}$$

where C is the frequency factor, D is a constant proportional to the activation energy flow, and T is the absolute temperature.

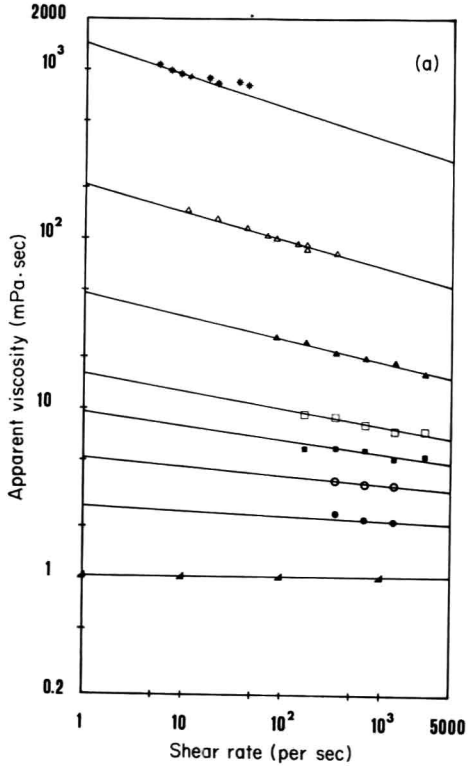
Both constants C and D, being complex functions of the weight fraction (y) of equivalent 'sucrose' in each solution examined, were expressed by means of polynomials of y of the q-th and r-th degrees:

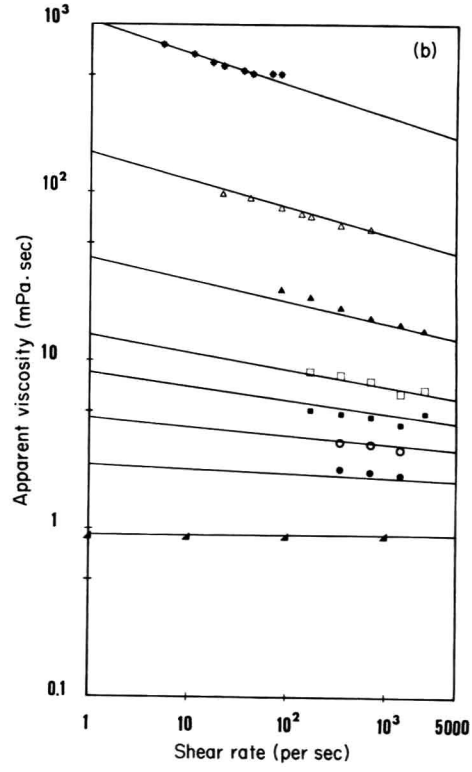
$$C(y) = \sum_{0}^{q} C_j y^j \tag{14}$$

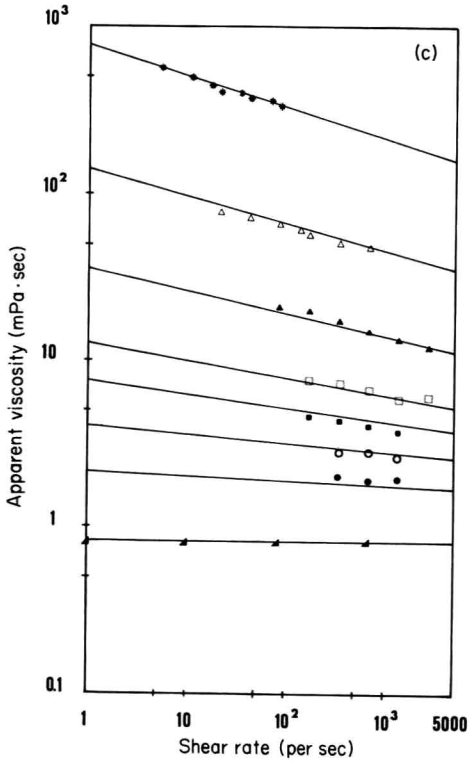
$$D(y) = \sum_{0}^{r} D_j y^j \tag{15}$$

where C_j and D_j are empirical constants.

In general, the changes in n with temperature for non-Newtonian fluids are found to be an order of magnitude smaller than those in K and are usually neglected (Metzer, 1956). In our specific case, such a conclusion was drawn by simply superimposing the flow curves determined for T ranging from 293.16 to 323.16 K (see Fig. 5a–g). Moreover, the more concentrated the grape juice, the smaller the slope of the


(a)
2000
10^3
10^2
10
1
0.2
Apparent viscosity (mPa·sec)
1
10
10^2
10^3
5000
Shear rate (per sec)


(b)
10^3
10^2
10
1
0.1
Apparent viscosity (mPa·sec)
1
10
10^2
10^3
5000
Shear rate (per sec)


(c)
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10
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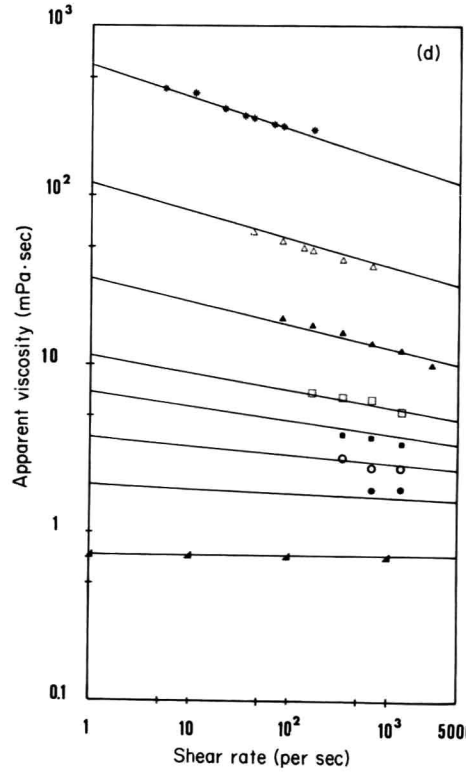
0.1
Apparent viscosity (mPa·sec)
1
10
10^2
10^3
5000
Shear rate (per sec)


(d)
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Apparent viscosity (mPa·sec)
1
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5000
Shear rate (per sec)

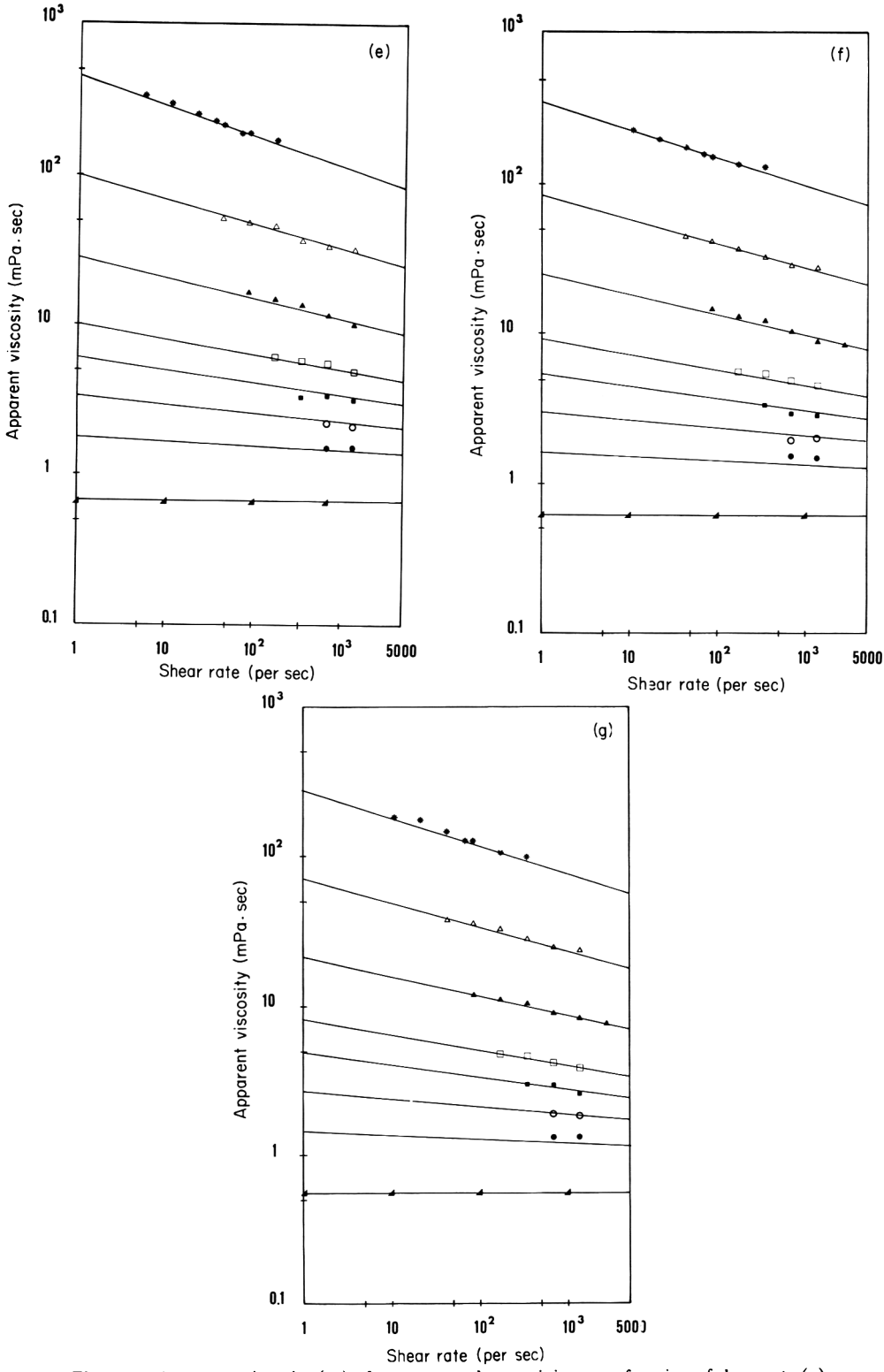

Figure 5. Apparent viscosity (ν_a) of concentrated grape juices as a function of shear rate (γ). sugar concentration expressed as degrees Brix: *, 73.7°Brix; △, 64.3°Brix; ▲, 53.35°Brix; □, 40.45°Brix; ■, 31.65°Brix; O, 20.4°Brix; ●, 10.4°Brix; , 0°Brix (derived from Perry *et al.*, 1963); and temperature: (a) T = 293.16 K; (b) T = 298.16 K; (c) T = 303.16 K; (d) T = 308.16 K; (e) T = 313.16 K; (f) T = 318.16 K; (g) T = 323.16 K. The continuous lines were calculated by using equations (13–17).

logarithmic plot becomes, thus allowing the flow behaviour index to be expressed as follows:

$$n = 1 + \sum_{1}^{v} n_j y^j \tag{16}$$

where n_j is a generic empiric constant and v is the degree of the polynomial concerned.

By substituting equations (13–16) into equation (12), it was possible to fit the shear stress and shear rate observations by means of a linear multiple regression technique. As an attempt to obtain the best reconstruction of the experimental data, the degrees p, q and v of the polynomials (14–16) were varied in the range 1–5. Then the statistically significant regression with the minimum number of empirical constants was chosen by means of an F-test.

The best values of C_j, D_j and n_j shown in Table 3 allowed the experimental values of τ to be reconstructed with an overall error less than 6.5%.

The continuous lines in Fig. 5 were then calculated as follows:

$$\mu_a = K\dot{\gamma}^{n-1} \tag{17}$$

being K and n expressed by means of equations (13–16).

Such lines show that for grape juice concentrates the power law model is notably successful in correlating the data at different concentrations and temperatures. Moreover, it is worthwhile underlining that n increases linearly up to 1 as sugar concentration reduces to zero, indicating that the fluid becomes more Newtonian. In particular, for $y = 0$ (i.e., pure water) the empirical constants of the model proposed reconstruct the viscosity of water with an average error of 4%.

In conclusion, grape juice concentrates show pseudoplastic non-Newtonian behaviour, which is consistent with the existence of highly solvated molecules (sugars) in the dispersions. Progressive shearing away of solvated layers with increasing shear rate results in decreasing interaction between the molecules (because of their smaller effective size) and, consequently, reduction in apparent viscosity.

Density

The experimental values of grape juice density (ρ) at 20°C are plotted against concentration in Fig. 6.

Generally speaking, the reciprocal of the density of a multicomponent mixture can be predicted by combining the weight fraction of each component multiplied by the reciprocal of its density.

Although the main constituents of the soluble solid content of grape juices are glucose and fructose with small amounts of sucrose and smaller quantities of acids (mainly tartaric and malic acids and, to a minor extent, citric, succinic and lactic acids), flavour substances, such as methylanthranilate, tannins and colour substances (Pederson, 1961), in industrial practice concentrated grape juices are usually characterized by means of their overall sugar content expressed as degrees Brix. For this reason, the density of grape juice (ρ) was expressed as follows:

$$1/\rho = (y/\rho_{GS}) + [(1-y)/\rho_W] \tag{18}$$

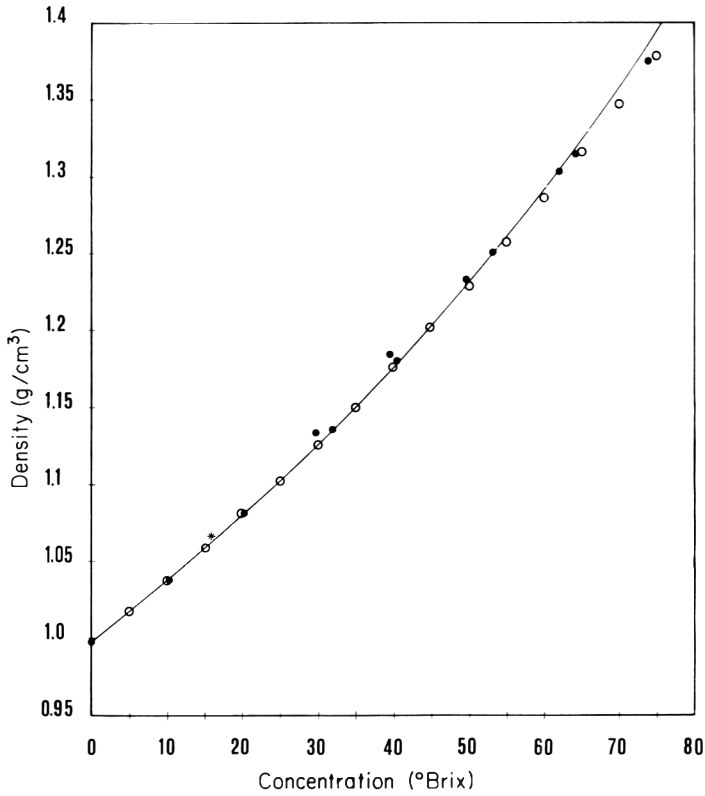

Figure 6. Density of concentrated grape juices and sucrose solutions at 20°C as a function of their concentration expressed as degrees Brix: ●, grape juice supplied by Gravinol S.r.l. (Lucera, Italy); *, commercial white grape juice manufactured by Confruit S.p.A. (Faenza, Italy); O, sucrose solutions (Honig, 1953).

where ρ_w and ρ_{GS} are the densities of pure water and pseudo-sucrose component ('grape sugar'), the weight fraction, y, of which is determined by refractometer.

By using the method of the least squares, it was possible to estimate the best value of ρ_{GS} (1.6079 g/cm^{-3}), which allowed the experimental densities of grape juice to be reconstructed with an average error of 0.4% and a deviation (s_1^2) about the empirical regression line of 2.628×10^{-4} with 11 degrees of freedom.

By determining the density (1.0675 g/cm^{-3}) and concentration (16.6°Brix) of a commercial white grape juice manufactured by Confruit S.p.A. (Faenza, Italy) and applying equation (18) with the value of ρ_{GS} given above, it was possible to predict the experimental density with an error of about 0.2%, thus confirming the reliability of the correlation proposed.

Finally, in order to establish whether the density of grape juice concentrates significantly differs from that of sucrose solutions at constant concentration, the experimental densities of sucrose solutions as derived from Honig (1953) were simulated by means of equation (18) and the aforementioned value of ρ_{GS}, thus yielding a deviation $s_2^2 = 3.904 \times 10^{-4}$ with 11 degrees of freedom. Being the ratio $s_2^2/s_1^2 = 1.49 < F_{0.95}(11, 11) = 2.82$, no statistically significant difference between the two sets of data examined was assessed at the 95% confidence level, thus pointing out that in engineering calculations the density of sucrose solutions would cause no significant error when used instead of the real density of grape juices.

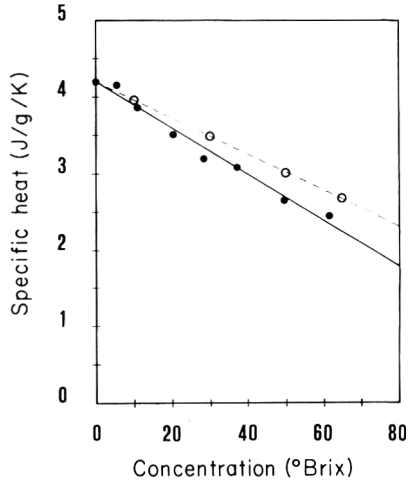

Figure 7. Specific heat of grape juices and sucrose solutions at 25°C as a function of their concentration: ●, grape juice (this work); O, sucrose solutions (Honig, 1953).

Specific heat

Figure 7 shows the experimental values of the specific heat (c_p) at 25°C *versus* concentration of juice. Since the specific heat of a mixture can be predicted by combining the specific heat of each component multiplied by its weight fraction, the specific heat of grape juice, having been previously assimilated to a binary mixture of water and grape sugar, was calculated as follows:

$$c_p = y\, c_{pS} + (1-y)c_p \tag{19}$$

where c_{pw} and c_{pS} are the specific heat values of water and grape sugar, respectively.

By using the method of the least squares, it was possible to estimate the value of c_{pS} listed in Table 3. Such a value allows the experimental values of c_p to be predicted with an average error of 2.8%, while the deviation about the empirical regression line plotted in Fig. 7 was 0.0032 with 7 degrees of freedom.

Finally, it is worth underlining that the difference between the values of c_{pS} for grape sugar and sucrose [1.857 J/g/K as derived from Honig's data (1953)] relies upon the fact that grape juice is a complex mixture of many organic compounds, which reduce the value of c_p of the juice.

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Studies on the development of texturized vegetable products by the extrusion process. I. Effect of processing variables on protein properties*

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Summary

The effect of process temperature, screw speed, moisture content and pH on the protein properties of cowpea (CP), mung bean (MB), defatted soybean (DSB) and air classified mung bean (ACMB) were studied. At high process temperature and low moisture content, increased screw speed resulted in decreased nitrogen solubility index (NSI). Water absorption capacity (WAC) measurement reflected differences in the composition of the raw materials. WAC values increased with pH, screw speed and process temperature and with decreased moisture content for high protein containing ACMB and DSB products, while with low protein containing CP and MB products, WAC values decreased at high moisture content. The insolubilized protein of the extruded products was resolubilized by sodium dodecyl sulphate (SDS) and dithiothreitol (DTT) reagents in a pH 7.6 buffer indicating the presence of both non-covalent interactions and disulphide bonds. During the extrusion process, the decrease in solubilities of extruded products can either be due to the formation of non-covalent interactions and of new disulphide bonds.

Introduction

In recent years, the extrusion process has become a popular processing operation in the production of pre-cooked starches, texturized vegetable proteins and other products. In the Philippines, cowpea and mung bean are major legume crops. They have high protein (22–30%) and low lipid contents (1–4%) (Coffman & Garcia, 1977; Okaka & Potter, 1977). The potential quality of cowpea, mung bean and air classified mung bean flours could be utilized in the production of snack foods, instant baby foods, texturized vegetable protein and other food formulations by the extrusion process.

The methodology of texturized vegetable products has been well developed and established. The functional properties of these products are determined by the operating variables of the extrusion process. The solubility behaviour and water uptake provide a good index for the potential application of products and give information useful in the optimization of the process (Wu & Inglett, 1974).

The effect of extrusion variables on nitrogen solubility index (NSI) has been studied in full fat soybean by Mustakas *et al.* (1970) and in corn-soy blend by Maga & Lorenz (1978). The effect of extrusion variables on water absorption capacity (WAC) has also

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been investigated in corn, waxy corn and amylomaize by Anderson *et al.* (1969) and Mercier & Feillet (1975), in defatted glandless cotton seed meal by Taranto *et al.* (1975), defatted soybean by Aguilera & Kosikowski (1976) and corn-soy blend by Maga & Lorenz (1978).

Values of NSI decreased with increased process temperature and moisture content, and decreased screw speed (Mustakas *et al.*, 1970; Maga & Lorenz, 1978). Values of WAC increased with increased process temperature (Maga & Lorenz, 1978; Mercier & Feillet, 1975; Anderson *et al.*, 1969; Taranto *et al.*, 1975; Aguilera & Kosikowski, 1976), screw speed (Maga & Lorenz, 1978; Taranto *et al.*, 1975; Aguilera & Kosikowski, 1976) and moisture content (Mercier & Feillet, 1975).

This study was conducted to gain an understanding of the effect of the extrusion variables on the properties of protein flour from cowpea, mung bean and air classified mung bean flours. Defatted soy bean flour was also used for comparison.

Materials and methods

Raw materials

Legume seeds used in this study were cowpea [*Vigna sinensis* (L.), Wilczek], mung bean [*Vigna radiata* (L.) Wilczek] and soybean [*Glycine max* (L.) Merr.]. Seed samples were obtained from the open market at Divisoria in Manila. These dried samples were contained in rice bags and stored in a cold room (10°C) prior to use.

Preparation of sample

Before dehulling, samples were dried at 65°C overnight. Cowpea (CP) and soybean (SB) were dehulled using the Bean Splitting and Dehulling machine (Cecoco). The dehulled samples were ground into a 60 mesh flour using a Bauer Bros Mill. CP meal contained 23.47% protein (d.b.), 66.74% carbohydrate (d.b.) and 8.24% moisture.

Hexane (food grade) was used and extraction of lipid from SB flour was carried out for 8 hr. SB meal contained 50.62% protein (d.b.), 31.35% carbohydrate (d.b.) and 8.18% moisture.

Mung bean (MB) was dehulled using a Hill Thresher and cleaned using a Vac-A-Way seed cleaner. The coarse flour of MB was ground using a Bauer Bros Mill. MB meal contained 28.83% protein (d.b.), 63.25% carbohydrate (d.b.) and 8.12% moisture.

The fine MB flour sample was milled further on an Alpine Augsburg Pin Mill and the flour sieved through a 120 mesh before air classification. MB flour was separated into two fractions, namely, a high protein fraction and low protein fraction. This was accomplished by setting the cut off point of the air classifier at 12 μ which allowed the separation of protein bodies from starch granules. Air classified mung bean (ACMB) meal contained 58.11% protein (d.b.), 35.79% carbohydrate (d.b.) and 7.89% moisture.

The feed mixture was prepared by dry blending each material and mixing with a minimum volume of 0.1 M acetate buffer to bring the pH and moisture content to the desired level. Sodium bicarbonate and acetic acid solutions were used to adjust the pH

to 7.4 and 6.2, respectively. The feed moistures were 30, 35 and 45%. The rehydrated meal was placed in plastic bags and stored at 0–4°C overnight to allow the moisture to equilibrate. The rehydrated meal was brought to room temperature prior to extrusion.

Experimental design

A four-variable, three-level fractional factorial design (Davies, 1963) with two replicates at the centre point was used. The centre point of the design was chosen based on a Bonnot 5.715 cm extruder for the production of texturized vegetable products. Process temperature, feed moisture, screw speed and pH were designated as independent variables of the process, which satisfactorily fits a quadratic polynomial equation of the type:

$$y = x_0 + b_1x_1 + b_2x_2 + b_3x_3 + b_4x_4 + b_{11}x_1^2 + b_{22}x_2^2 + b_{33}x_3^2 + b_{44}x_4^2 + b_{12}x_1x_2 + b_{13}x_1x_3 + b_{14}x_1x_4$$

where y = response function, x_0 = constant, x_1 = moisture content (%), x_2 = pH buffer, x_3 = screw speed (rev/min), x_4 = process temperature (°C).

Extrusion processing

Experimental runs were performed randomly in a pilot plant extruder (The Bonnot Co., Kent, Ohio, USA; Model 21/4; serial 2442). The extruder barrel was divided into four jacketed sections which can be individually heated or cooled and a head plate containing a round die orifice (0.635-cm diameter) equipped with a thermocouple for recording process temperatures. The barrel was 115 cm in length with a 5.715-cm internal diameter. The extrusion screw (designated a texturized soybean products screw by the Bonnot Co.) was a compression-type screw. The process temperatures were controlled at 93, 112.5 and 132°C for CP and MB samples; 149, 154.5 and 160°C for DSB samples and 154.5, 160 and 165.5°C for ACMB samples. Screw speeds were 100,

Table 1. Experimental design and coded transformation applied for cowpea, mung bean, defatted soybean and air classified mung bean flours

Moisture content (%)		Screw speed (rev/min)									
			−1			−0.20*/0†			+1		
		Process temperature (°C)									
			−1	0	+1	−1	0	+1	−1	0	+1
		−1	×		×		×		×		×
−1		0		×		×	×	×		×	
		+1	×		×		×		×		×
		−1		×		×		×		×	
−0.3	pH	0	×	×	×		×		×	×	×
		+1		×		×		×		×	
		−1	×		×		×		×		×
+1		0		×		×	×	×		×	
		+1	×		×		×		×		×

* Applied for mung bean and cowpea flours.

† Applied for defatted soybean and air classified mung bean flours.

× Sample collection.

140 and 200 rev/min for CP and MB samples; 10, 20 and 30 rev/min for DSB and ACMB samples. The temperature and screw speed were chosen to permit optimum interaction of feed materials based on preliminary runs. Extruded samples were cut into small cylindrical particles by a cutter and collected as shown in Table 1 and then placed in the drying tray. The extruded products were dried at 65°C overnight. The dried samples were then placed in plastic bags, sealed and stored at −18°C prior to analysis.

Nigrogen solubility index

Nitrogen solubility indexes (NSI) of the extruded products were determined by the procedure of Inklaar & Fortuin (1969) with some modifications.

A 1 g extruded sample was preweighed and placed in a 50 ml centrifuge tube. Twenty-five millilitres of dispersion medium were added and the sample was dispersed for 1 hr with a magnetic stirrer. The dispersion medium was water adjusted to pH 7.2 using NaOH solution. After removal of the tubes from the magnetic stirrer and centrifugation for 15 min at 15 000 rev/min, the supernatant liquids were decanted into 100 ml volumetric flasks and the residues were re-extracted for 1 hr with 25 ml of the dispersion medium. Supernatants were combined with the corresponding extracts and the whole made up to 100 ml. Extracts were filtered and analysed for nitrogen by the microkjeldahl method (AOAC, 1980). The unextracted extruded samples were also analysed for nitrogen content and the NSI of the experimental samples was calculated by the following equation:

$$\text{NSI} = \frac{\text{N in water extract}}{\text{N in unextracted extruded sample}} \times 100$$

Water absorption capacity

Percentage water absorption capacity (WAC) was determined for the same sample by a modification of the procedure of Sosulski (1962). The centrifuge tubes containing the residue were placed with their mouths at an angle of 15–20° on paper towel in an air oven at 50°C and allowed to drain and dry for 25 min. The samples were cooled in a desiccator for 30 min and then weighed. The WAC was calculated as the difference between hydrated weight and original weight expressed as a percentage of the original dry weight of the sample.

Determination of protein solubility

Protein solubility was determined by the method of Jeunink & Cheftel (1979), with pH buffer modification. The samples of raw or extruded ACBM (with extrusion variables: MC 35%, pH 7.4, screw speed 20 rev/min, temperature 165.5°C) were extracted with sodium dodecyl sulphate (SDS) (0.01 M), an agent known to disrupt non-covalent interactions and with dithiothreitol (DTT) (0.001 M), an agent known to reduce disulphide bonds. Both reagents were dissolved in a 0.035 M phosphate buffer, pH 7.6.

Statistical analysis

The data were statistically analysed by means of a stepwise multiple regression to determine the process variables significant in the NSI and WAC. The method of least squares was used to obtain the best estimate of the dependent variables. All terms not significant at the 0.05 probabiliy level were combined with the residual. Random error was estimated from replicate extrusion. Lack of fit was calculated as the difference

Table 2. Nitrogen solubility index of extruded cowpea (ECP), mung bean (EMB), defatted soybean (EDSB) and air classified mung bean flours (EACMB) as affected by processing variables (%)

Source of variation	Coefficient				Mean square				Degree of freedom			
	ECP	EMB	EDSB	EACMB	ECP	EMB	EDSB	EACMB	ECP	EMB	EDSB	EACMB
Model					90.10*	171.65*	56.47*	68.53*				
x_0	16.43*	15.56*	11.49*	9.37*					10	7	6	4
x_1	2.44*	3.13*	0.90*	0.72*	358.44	588.56	169.23	135.39	1	1	1	1
x_2	1.09*	1.57*	0.58*	0.56*	65.50	136.47	50.42	48.68	1	1	1	1
x_3	− 1.02*	− 0.89*	− 0.32†	− 0.81*	64.82	48.81	9.53	40.60	1	1	1	1
x_4	− 1.66*	− 2.52*	− 1.32*	− 0.94*	152.67	353.80	96.34	49.22	1	1	1	1
x_3x_3	0.96*	0.95*			16.62	16.31			1	1		
x_4x_4	− 1.20*		0.59†		27.75		6.75		1		1	
x_1x_2	0.95†		0.39†		35.85		6.15		1		1	
x_1x_3	− 0.47†	− 0.39†			8.92	5.93			1	1		
x_1x_4	− 1.96*	− 0.14*			153.31	51.67			1	1		
x_3x_4	0.63*				16.56				1			
Residual					1.26	1.59	1.32	1.48	79	82	83	85
Lack of fit					2.11	2.33	2.06	2.06	34	37	38	40
Error					0.63	0.93	0.70	0.91	45	45	45	45
Corrected total					11.24	14.98	5.04	4.49	89	89	89	89

*Significant at $P = 0.01$.

†Significant at $P = 0.05$.

$R^2_{ECP} = 0.87$; $R^2_{EMB} = 0.91$; $R^2_{EDSB} = 0.91$; $R^2_{EACMB} = 0.88$.

between the residual sum of squares and the pure error sum of squares. The regression equations from each type of product fit a response surface type equation including first order effects, squared terms and first order interactions. terms were allowed to enter the equation which corresponds to the F-test value with a confidence level of approximately 95%.

Results and discussion

Nitrogen solubility index

CP flour has 89.94% NSI, but after the extrusion process, NSI was reduced to only 16.43%. The decrease of 74.51 NSI was caused by heat during the thermoplastic extrusion process. Similar reduction in NSI values were observed (Table 2) in extruded MB (EMB), DSB (EDSB), and ACMB (EACMB) products. Statistical analysis of the data indicated a strong dependence of the NSI values on process temperature and moisture content. With the increase in process temperature or decrease in moisture content, the extent of denaturation subsequent aggregation of the protein increased (Table 2). Low moisture content presumably induced heat generation and

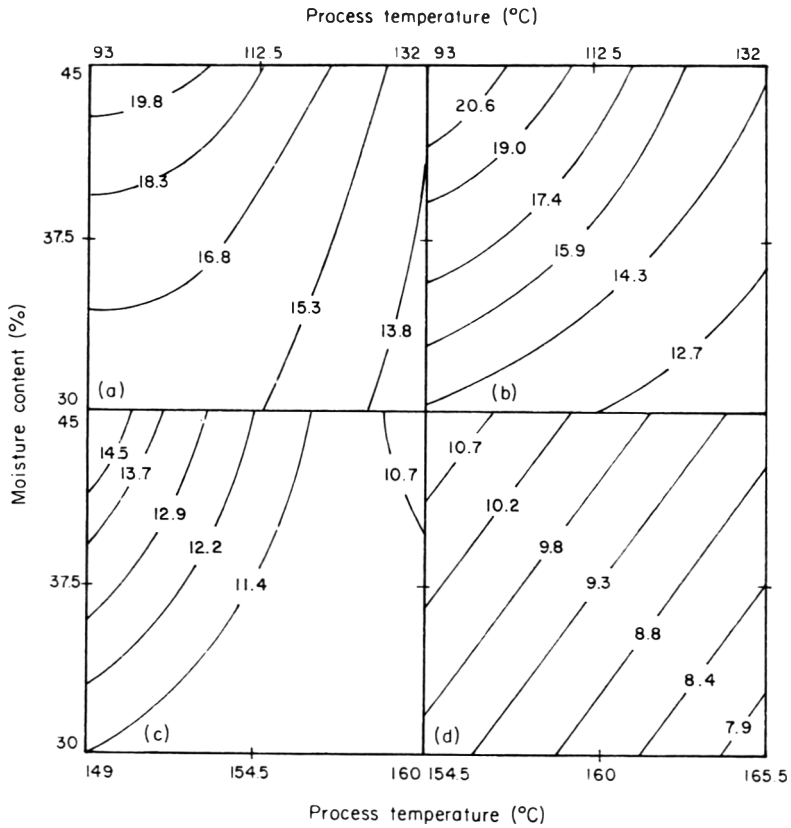

Figure 1. Nitrogen solubility index contour maps as function of moisture content and process temperature in extruded: (a) cowpea (150 rev/min, pH 6.8); (b) mung bean (150 rev/min, pH 6.8); (c) defatted soybean (20 rev/min, pH 6.8); (d) air classified mung bean (20 rev/min, pH 6.8).

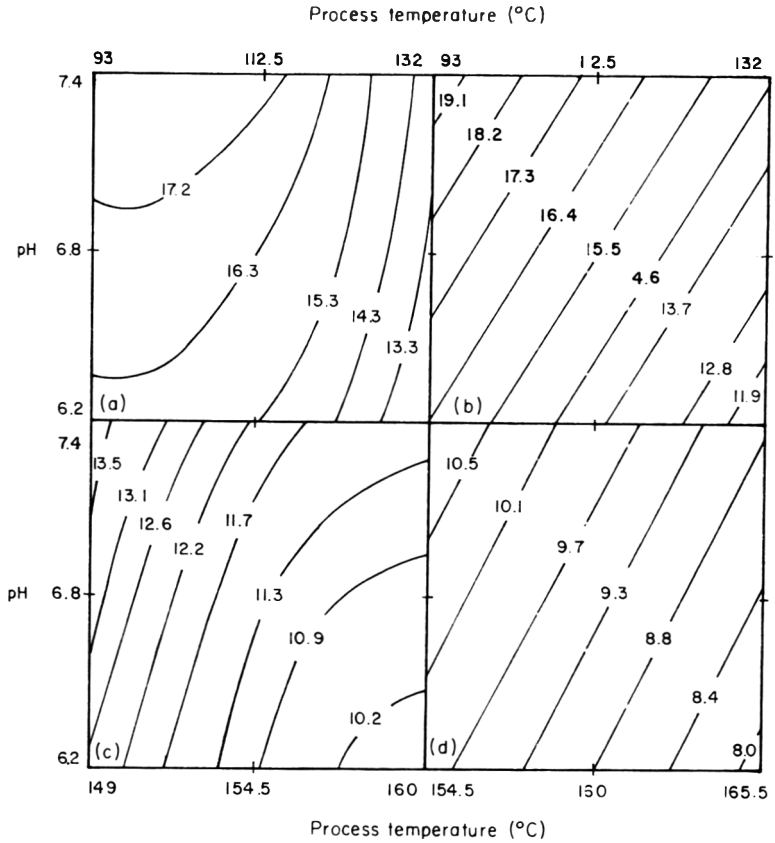

Figure 2. Nitrogen solubility index contour maps as function of pH and process temperature in extruded: (a) cowpea (150 rev/min, MC 37.5%); (b) mung bean (150 rev/min, MC 37.5%); (c) defatted soybean (20 rev/min, MC 37.5%); (d) air classified mung bean (20 rev/min, MC 37.5%).

consequently decreased NSI. In the case of EACMB, NSI had a minimum value of 7.92% at about 164°C and 30% moisture content (Fig. 1).

The effect of pH was temperature dependent. At a given process temperature, NSI increased with increasing pH (Fig. 2). At pH 6.2, the low solubility of extruded product compared with that extruded at pH 7.4, reflected the well known response to pH near the vicinity of the isoelectric point. Baker and Mustakas (1973) had reported that the effect of alkali (1% NaOH) on the NSI of DSB was considerably less than the effect of acid (1% HCl). The decreased protein insolubilization with increased pH was more pronounced with CP and MB than with DSB and ACMB proteins. This difference could be due to the effect of pH and the process temperature. CP and MB products had lower process temperatures than the DSB and ACMB products. Contour maps in Fig. 2 show that at pH 7.0 and process temperature of 100°C, ECP would have the maximum NSI. The highest NSI values for EMB required a lower temperature of about 95°C and pH greater than 7.25. At high screw speed and at a given temperature and moisture content, the protein insolubilization was higher in ECP and EMB than in EDSB and EACMB. These results indicate that the difference in the carbohydrate contents of the feed materials may influence the protein denaturation.

Table 3. Water absorption capacity of extruded cowpea (ECP), mung bean (EMB), defatted soybean (EDSB) and air classified mung bean flours (EACMB) as affected by processing variables (%)

Source of variation	Coefficient				Mean square				*Degree of freedom*			
	ECP	EMB	EDSB	EACMB	ECP	EMB	EDSB	EACMB	ECP	EMB	EDSB	EACMB
Model					16779*	31305*	4502*	1320*	9	6	7	8
x_0	277.05*	241.27*	270.41*	299.40*								
x_1	−20.23*	−17.50*	−7.96*	−3.96*	24394	13252	3744	933	1	1	1	1
x_2	12.56*	13.50*	8.88*	7.28*	8619	9965	4304	2897	1	1	1	1
x_3	35.06*	41.21*	8.99*	13.66*	74315	102700	4893	11279	1	1	1	1
x_4	26.93*	30.23*	17.65*	12.13*	39631	49932	16616	8033	1	1	1	1
x_1x_1	+8.89*		+4.65*		1217		334		1		1	
x_3x_3	−15.16*			−9.90*	3752			1603	1			1
x_4x_4		11.36*	7.04*	4.61*		2483	955	409		1	1	1
x_1x_3	6.66*			2.16†	1856			195	1			1
x_1x_4	−6.89*			−2.72†	1949			308	1			1
x_2x_3		−5.23*				1144				1		
x_2x_4			2.57†				262				1	
x_3x_4	−7.33*				2246				1			
Residual					143	172	43	49	80	83	82	81
Lack of fit					268	260	65	58	35	38	37	36
Error					46	190	31	41	45	45	45	45
Corrected total					1826	2271	397	325	89	89	89	89

*Significant at $P = 0.01$.
†Significant at $P = 0.05$.
$R^2_{ECP} = 0.94$; $R^2_{EMB} = 0.94$; $R^2_{EDSB} = 0.90$; $R^2_{EACMB} = 0.87$.

Water absorption capacity

The average WAC of EACMB (299.50%) compared favourably with that of EDSB (270.41%). The higher WAC of EACMB products is a consequence of its higher protein content (58.11%) compared to that of EDSB (50.62%). The WAC of EMB (241.27%) is lower than that of ECP (277.05%) (Table 3). The presence of carbohydrate (CP 66.74%; MB 63.25%) could account for the difference in the WAC values since hydrophilic polysaccharides absorb more water than the protein alone (Kinsella, 1976). On the other hand, Mercier *et al.* (1979) found that the available lipids and amylose interacted to form an amylose butanol complex which absorbed less water. MB flour (2.26%) has a higher lipid content than CP flour (1.08%). This would explain why the WAC of ECP is higher than that of EMB.

As the moisture content of the feed materials increased, the WAC of the products increased as the process temperature increased (Fig. 3). The products with high carbohydrate content, such as ECP and EMB, at high moisture content expanded more with high water vaporization upon extrusion from the die, thus becoming more porous. Therefore, greater penetration of water occurs during rehydration. This fact agrees with the findings of Mercier & Feillet (1975) for corn, rice and wheat products.

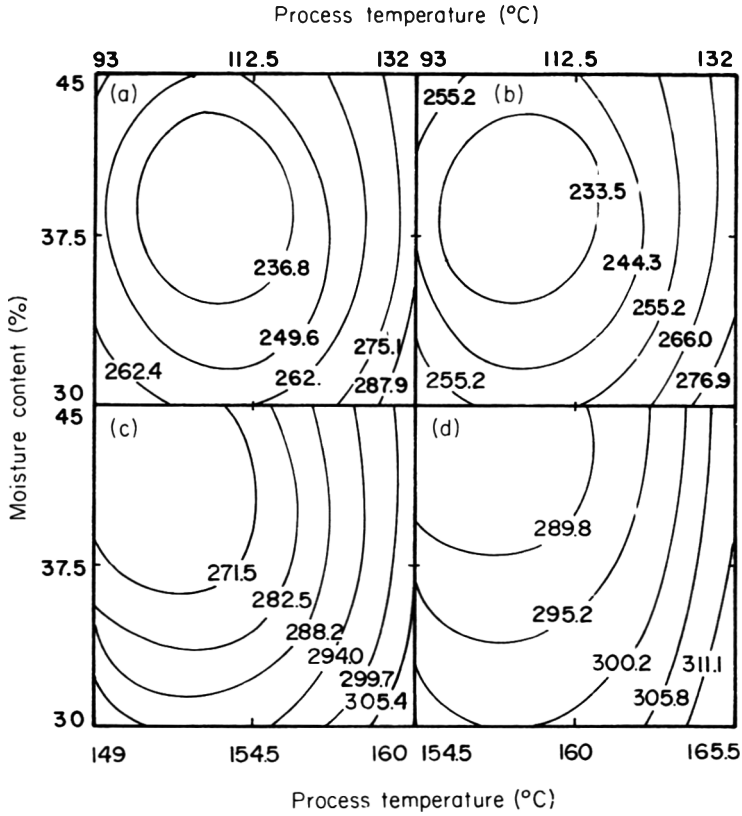

Figure 3. Water absorption capacity contour maps as function of moisture content and process temperature in extruded: (a) cowpea (150 rev/min, pH 6.8); (b) mung bean (150 rev/min, pH 6.8); (c) defatted soybean (20 rev/min, pH 6.8); (d) air classified mung bean (20 rev/min, pH 6.8).

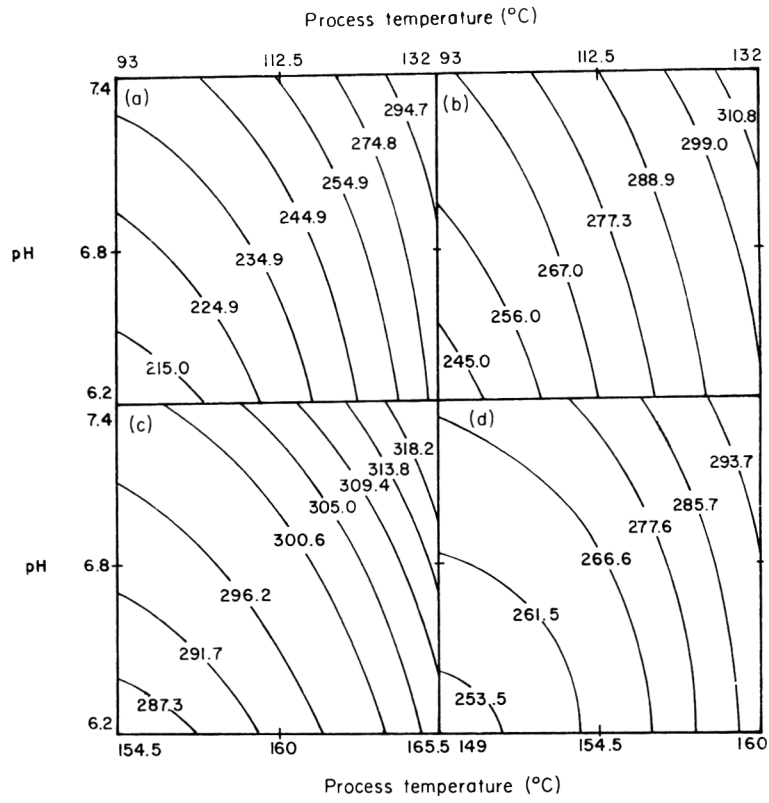

Figure 4. Water absorption capacity contour maps as function of pH and process temperature in extruded: (a) mung bean (MC 37.5%, 150 rev/min); (b) cowpea (MC 37.5%, 150 rev/min); (c) air classified mung bean (MC 37.5%, 20 rev/min); (d) defatted soybean (MC 37.5%, 20 rev/min).

The extent of WAC was also determined by screw speed and pH. As pH increased from 6.2 to 7.4, the WAC of the extruded products increased. The effect of pH was increased as the process temperature was increased (Fig. 4). At a given pH the absorption capacity of water in the extruded products depended strongly on the screw speed. The WAC increased when the screw speed was increased under a given process temperature (Fig. 5). The effect of both pH and temperature may be viewed in terms of its effect on the nature of the various components of the systems which in turn may effect its water holding capacity.

Protein solubility

Protein solubility as a percentage of the total protein present in the sample is shown to be 74.15% in ACMB in 7.6 pH buffer, whereas after extrusion, it was only 18.5%. The insolubilized protein of the extruded products was resolubilized to some extent by sodium dodecyl sulphate (SDS) and dithiothreitol (DTT) reagents in a 7.6 pH buffer. SDS is capable of disrupting the non-covalent interactions and DTT ruptures any SS bonds present (Table 4). The reduced solubilities could therefore be due to non-covalent interactions between polypeptide chains, the formation of new SS bonds or the non-peptide bonds involving carboxyl and amino groups. The studies of Sternberg, Kim & Plumkelt (1975) and Aymard, Cuq & Cheftel (1978) have shown that the formation

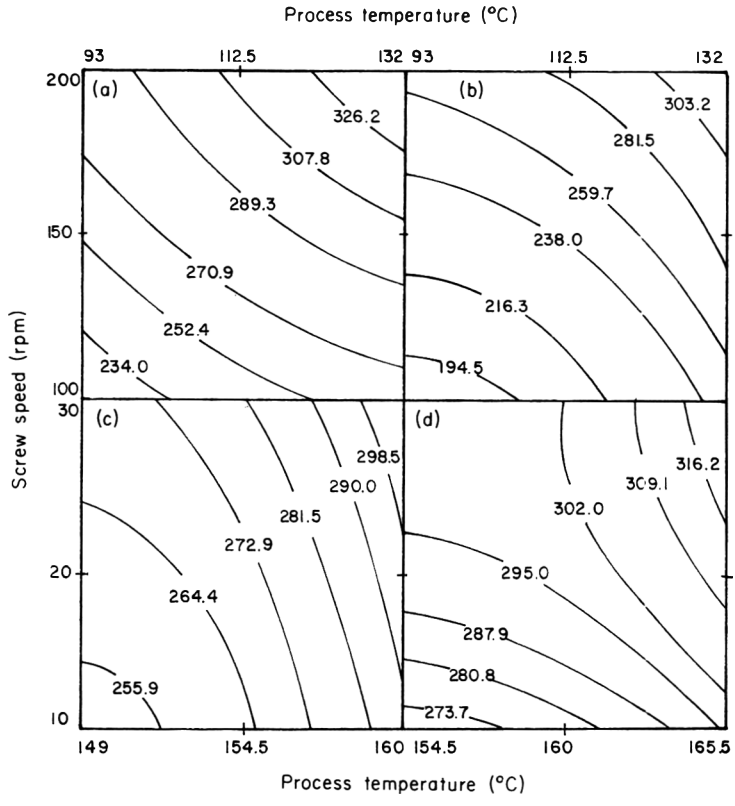

Figure 5. Water absorption capacity contour maps as function of screw speed and process temperature in extruded: (a) cowpea (MC 37.5%, pH 6.8); (b) mung bean (MC 37.5%, pH 6.8; (c) defatted soybean (MC 37.5%, pH 6.8); (d) air classified mung bean (MC 37.5%, pH 6.8).

of lysinoalanine (LAL) and lanthionine (LAT) crosslinks occurred in both heat and/or alkali treated proteins. The insolubilization of the EACMB protein may be attributed to the formation of unusual amino acids such as the LAL ands LAT acids. However, Jeunink & Cheftel (1979) found that these reactions do not occur to any significant extent in field bean and soybean proteins during the extrusion process. Another

Table 4. Protein solubility of the air classified mung bean (ACMB) and extruded ACMB (EACMB) in 0.035 M phosphate buffer pH 7.6 with sodium dodecyl sulphate (SDS) and/or dithiothreitol (DTT)*

	Average % protein solubility (% of total protein)	
Reagents	ACMB	EACMB
Buffer pH 7.6	74.15	18.52
Buffer pH 7.6 + 0.01 M SDS	75.16	32.25
Buffer pH 7.6 + 0.001 M DTT	75.85	33.84
Buffer pH 7.6 + 0.01 M SDS + 0.001 M DTT	79.82	34.71

*Average of two replicates.

possibility is that the polymerization amino acids rich in di-amino and di-carboxyl groups plays a role in the texturization of protein by the extrusion process. The possibility of such a reaction occurring during the extrusion process is being studied in this laboratory.

Conclusions

Texturized CP, MB, DSB and ACMB products were developed by extrusion. The decrease in NSI values depended on the protein content of products. The higher the protein content, the greater is the protein denaturation and subsequent aggregation which presumably resulted in the decreased NSI and better texture.

The average WAC of EACMB compared favourably with that of the EDSB products and exceeded 200%. The differences in WAC reflect the differences in composition of ACMB and DSB proteins. The WAC of EMB (241%) is lower than ECP (277%). It is possible that the presence of carbohydrate might be responsible for the observed effect of absorbed water rather than protein alone.

The insolubilized protein of the extruded products was resolubilized to some extent by SDS and DTT reagents in a pH 7.6 buffer. These reagents are capable of disrupting the non-covalent interactions (SDS) between polypeptide chains and disulphide bonds (DTT). The formation of new disulphide bonds which decreased the solubility of the protein may play an important role in texturization of protein.

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Studies on the development of texturized vegetable products by the extrusion process. II. Effects of extrusion variables on the available lysine, total and reducing sugars*

C. B. PHAM AND R. R. DEL ROSARIO

Summary

Available lysine (AL) losses in extruded cowpea (ECP) and in extruded mung bean (MB) proteins from commercial legumes, were predicted by a least squares regression equation using process temperature, screw speed, buffer pH and moisture content as the major independent variables. The destruction of AL depended strongly on the process temperature, moisture content, screw speed and pH. Total sugars released increased with increased process temperature and moisture content of the products and when screw speed and pH were decreased. The average total sugars was 16.25 mg/g of sample in ECP and EMB products. Reducing sugars were released in a quadratic function wih total sugars. Contour-maps show that the AL retention value could be improved if either the concentration of total or reducing sugars in the products is lowered.

Introduction

Legume seeds contain a considerable amount of the essential amino acid lysine which determines their nutritive value after thermal processing. A characteristic that restricts the availability of dietary lysine is that its ϵ-amino group under the influence of light, heat, alkali and other factors, interacts with other food constituents to become nutritionally unavailable (Tanaka, Lee & Chichester, 1975).

There is no available information on the non-enzymic browning reaction which usually occurs during the extrusion process. The browning reaction depends upon process temperature, duration of heating, moisture content and pH. The presence of reducing sugars markedly accelerates these reactions. Knowledge of the reducing sugar changes in extruded legume products is essential to better understand the role of raw material composition in extrusion cooking.

The mathematical method for the prediction of available lysine (AL) in model food systems during thermal processing was studied by Jokinen, Reineccius & Thompson (1976); Thompson, Wolf & Reineccius (1976) and Wolf & Thompson (1977). However, these model systems do not resemble the real food processes for the production of snack or baby foods in the local food industry. Therefore, this study was carried out to determine the relationship of AL, total and reducing sugars with the processing variables in the extrusion of mung bean and cowpea flours.

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*Part of the doctoral dissertation of the senior author.

Materials and methods

Raw materials

Cowpea (CP) [*Vigna sinensis* (L.) Wilczek] and mung bean (MB) [*Vigna radiata* (L.) Wilczek] were obtained from the open market at Divisioria in Manila. These dried samples were contained in rice bags and stored in a cold room (10°C) until used.

Methodology

The preparation of samples, experimental design, extrusion processing and statistical analysis were described in part I of this series (Pham & del Rosario, 1984).

Determination of available lysine (AL)

The freeze dried samples were analysed for AL and moisture content. AL was determined using the fluorodinitrobenzene (FDNB) procedure of Carpenter (1960) with the modifications proposed by Booth (1971). This involved reaction of the samples with FDNB to form the dinitrophenyl (DNP)-lysine derivative and hydrolysing the sample with 6.1 M HCl for 16 hr. After refluxing, the samples were filtered and two aliquots were taken from each sample. One aliquot was extracted with ether to remove any excess FDNB or DNP and used as a control. The methyl chloroformate derivative of lysine was formed in the second aliquot and the compound extracted with ether. The absorbance of both extracts was read at 435 nm against water. No correction factor was applied in the calculation of AL.

Determination of total sugars

Total sugars were extracted from the extruded samples by the procedure of Chiang & Johnson (1977). A 5 g sample was extracted with 50 ml of 80% hot ethanol and agitated for 15 min with a magnetic stirrer at room temperature. The suspension was then centrifuged at 10000 rev/min for 10 min. The supernatant was decanted and the residue was re-extracted under the same conditions to insure complete extraction of soluble carbohydrates. The total sugar content in the combined supernatant was determined by the anthrone method (McReady *et al.*, 1950).

Determination of reducing sugars

Reducing sugars were determined from the extracted solution of total sugars by reaction with 3,5-dinitrosalycyclic acid and phenol (Borel, Hosteller & Deuel, 1952).

Results and discussion

Available lysine

The ϵ-amino group of lysine is free to react like any free amino group in a system. Thus, it could be used as an indicator for the reaction undergone by the protein. AL is 6.18 g amino acid/16 g of nitrogen in CP and 6.46 g amino acid/16 g of nitrogen in MB. After the extrusion process, AL retained was 4.43 g amino acid/16 g of nitrogen in extruded CP (ECP) and 5.06 g amino acid/16 g of nitrogen in extruded MB (EMB). This accounted for 77.42 and 75.25% retention in ECP and EMB products, respectively. The *F*-test values obtained, 56.23 and 66.30, exceed the 99% probability *F* value of 2.29 for ECP ad EMB, respectively (Tables 1 and 2). These significant differences imply that the treatment variables modify the extent of AL loss.

As the process temperature was increased, the destruction of AL increased. The

Table 1. Available lysine retained in cowpea during the extrusion process. Analysis of variance for regression

Source of variation	Coefficient	MS*	df†
Regression		6.75‡	8
Constant, x_0	4.43		
Moisture content, x_1	−0.56‡	19.14	1
pH, x_2	0.28‡	4.40	
Screw speed, x_3	0.29‡	5.05	
Temperature, x_4	−0.66‡	24.00	
Screw speed × screw speed, x_3x_3	−0.18‡	0.56	
Temperature × temperature, x_4x_4	−0.10§	0.19	
Moisture content × screw speed, x_1x_3	−0.07§	0.20	1
Moisture content × temperature, x_1x_4	−0.18‡	1.34	1
Residual		0.07	81
Lack of fit		0.12	36
Error		0.02	45
Corrected total		0.67	89
R^2 = 0.91			

*MS = mean square.
†df = degree of freedom.
‡Significant at P = 0.01.
§Significant at P = 0.05.

Table 2. Available lysine retained in mung bean during the extrusion process. Analysis of variance for regression

Source of variation	Coefficient	MS*	df†
Regression		6.47‡	9
Constant, x_0	5.06‡		
Moisture content, x_1	−0.28‡	4.83	1
pH, x_2	0.33‡	5.89	1
Screw speed, x_3	0.24‡	3.40	1
Temperature, x_4	−0.82‡	37.48	1
pH × pH, x_2x_2	−0.15‡	0.41	1
Temperature × temperature, x_4x_4	−0.28‡	1.52	1
Moisture content × screw speed, x_1x_3	−0.09§	0.29	1
Moisture content × temperature, x_1x_4	0.21‡	1.84	1
pH × screw speed, x_2x_3	−0.19‡	1.57	1
Residual		0.07	80
Lack of fit		0.11	35
Error		0.03	45
Corrected total		0.72	89
R^2 = 0.93			

*MS = mean square.
†df = degree of freedom.
‡Significant at P = 0.01.
§Significant at P = 0.06.

effect of process temperature was greater on the destruction of AL from EMB (25.43%) than from ECP (21.27%). The protein of MB (28.83%) and CP (23.47%) therefore, is proportional to the effect of the destruction of AL. At a given process temperature, the destruction of AL increased with increasing moisture content. The effect of moisture content on the AL destruction was less than the effect of process

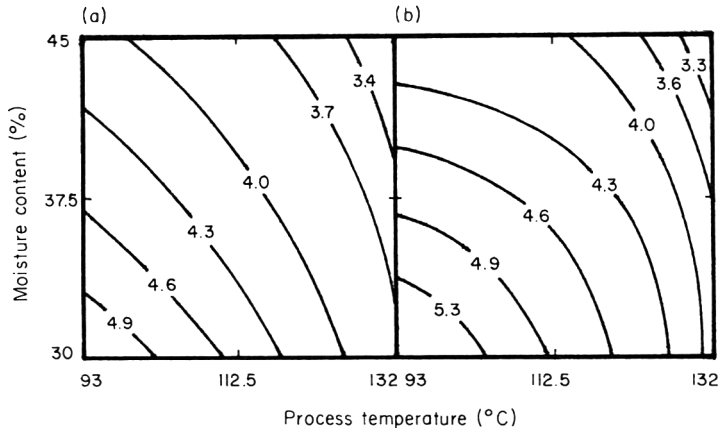

Figure 1. Available lysine contour maps as function of moisture content and process temperature in extruded products with screw speed 150 rev/min and pH 6.8. (a) Cowpea. (b) Mung bean.

temperature and AL decrease was greater in ECP (18.28%) than in EMB (8.79%) (Fig. 1). This difference may be due to the presence of different levels of carbohydrate in CP (66.74%) and MB (63.25%).

The extent of destruction of AL also depends on the effect of screw speed and pH. At a given process temperature and moisture content, the destruction in the products decreased as pH or screw speed increased (Tables 1 and 2). The increase of screw speed results in decreased residence time of the products in the extruder, so that AL destruction is decreased. The formation of reducing sugars from hydrolysis of complex carbohydrate and heat through the effect of pH presumably resulted in further AL losses (Jokinen *et al.*, 1976). In mung bean flour, the available sugars are sucrose, raffinose, stachyose, fructose and glucose (Takeuchi, Kitahara & Inaba, 1963). The available reducing sugars are formed during heating, therefore greater AL loss at pH 6.2 was observed (Fig. 2).

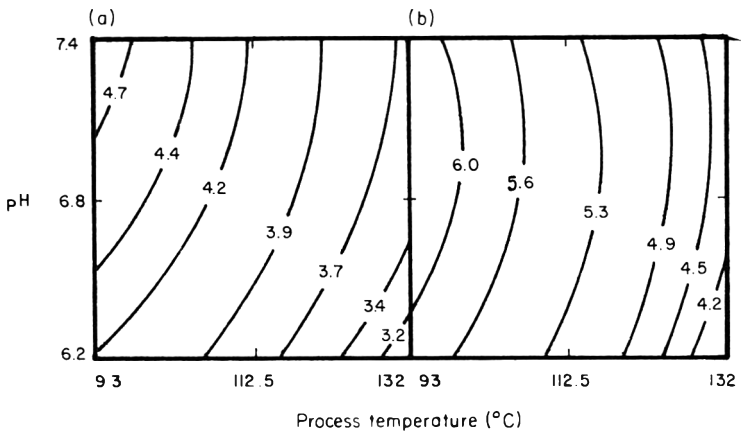

Figure 2. Available lysine contour maps as function of pH and process temperature in extruded products with screw speed 150 rev/min and moisture content 37.5%. (a) Cowpea. (b) Mung bean.

The degradation of lysine may be due to the formation of isopeptide crosslinks, i.e. ϵ-(γ-glutamyl)-lysine or ϵ-(β-asparty)-lysine (Hurrell *et al.*, 1976) or lysinoalanine and lanthionine (Sternberg, Kim & Schwende, 1975; Aymard. Cuq & Cheftel, 1978; Friedman, 1977) or to the Maillard condensation reaction with reducing sugars (Hannan & Lea, 1952; Hodge, 1967; Carpenter, 1973; Wolfrom, Kashimura & Horton, 1976). However, Jeunink & Cheftel (1979) have stated that there was no significant formation of lysinoalanine or lanthionine, so that the Maillard browning reaction may be the major factor involved in the decrease of the amount of lysine in ECP and EMB products during the extrusion process.

Total sugars

The average total sugar content after the extrusion process, was 16.25 mg/g of sample in ECP and EMB products (Tables 3 and 4). Total sugars released from carbohydrate decreased with increasing pH of the products (Fig. 3). This would indicate the influence of pH in the hydrolysis of carbohydrates. The extent of formation of total sugars is also a function of the process temperature. As the process temperature was increased, total sugars also increased. At a given process temperature and pH, total sugar released from the carbohydrate degradation is also determined by the moisture content and screw speed. High moisture content as well as high process temperature increases the rate of heat penetration of gelatinized starch and this aids sugar release. In addition, the ECP and EMB products which have high protein contents may also have high free amino acids. Through the Maillard type reactions these may react with considerable amount of total sugars.

As screw speed was increased, the total sugars decreased apparently as a result of the reduction in the residence time of sample in the extruder. The reduction in

Table 3. Available total sugars in cowpea during the extrusion process. Analysis of variance for regression

Source of variation	Coefficient	MS*	df†
Regression		23.46‡	11
Constant, x_0	16.25‡		
Moisture content, x_1	0.71‡	30.53	1
pH, x_2	−0.55‡	16.96	1
Screw speed, x_3	−1.37‡	108.16	1
Temperature, x_4	0.79‡	34.46	1
Moisture content × moisture content, x_1x_1	0.73‡	10.29	1
pH × pH, x_2x_2	−0.56‡	5.94	1
Screw speed × screw speed, x_3x_3	−0.55‡	6.06	1
Temperature × temperature, x_4x_4	−0.54‡	5.80	1
Moisture content × pH, x_1x_2	−0.64‡	16.49	1
pH × screw speed, x_2x_3	−0.40‡	6.27	1
pH × temperature, x_2x_4	−0.46‡	8.63	1
Residual		0.91	78
Lack of fit		1.77	33
Error		0.28	45
Corrected total		3.70	83
$R^2 = 0.80$			

*MS = mean square.
†df = degree of freedom.
‡Significant at $P = 0.01$.

Table 4. Available total sugars in mung bean during the extrusion process. Analysis of variance for regression

Source of variance	Coefficient	MS*	df†
Regression		10.50‡	9
Constant, x_0	16.25‡		
pH, x_2	−0.48‡	12.69	1
Screw speed, x_3	0.72‡	12.69	1
Temperature, x_4	0.73‡	29.73	1
Moisture content × moisture content, x_1x_1	0.38§	2.82	1
pH × pH, x_2x_2	−0.44§	3.77	1
Moisture content × screw speed, x_1x_3	−0.32§	4.19	1
pH × screw speed, x_2x_3	−0.25§	2.54	1
pH × temperature, x_2x_4	−0.24§	2.35	1
Screw speed × temperature, x_3x_4	0.38‡	5.28	1
Residual		0.89	80
Lack of fit		1.62	35
Error		0.23	45
Corrected total		1.82	89
$R^2 = 0.71$			

*MS = mean square.
†df = degree of freedom.
‡Significant at $P = 0.01$.
§Significant at $P = 0.05$.

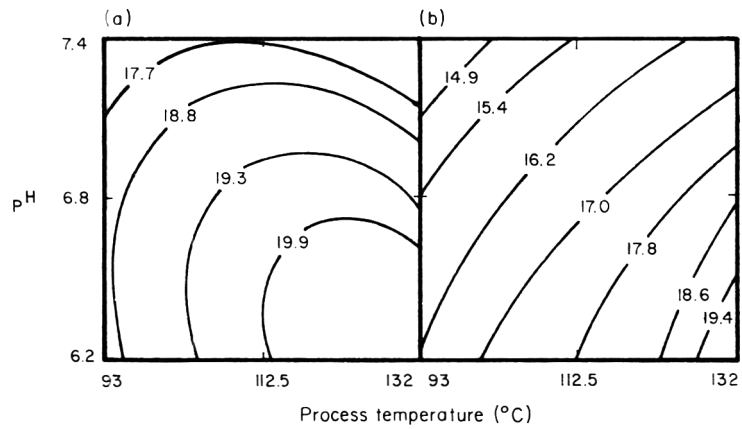

Figure 3. Total sugar contour maps as function of pH and process temperature in extruded products with screw speed 150 rev/min and moisture content 37.5%. (a) Cowpea. (b) Mung bean.

residence time would result in gelatinization of starch but with little breakdown of the (1 → 4) glycosidic bonds (Chiang & Johnson, 1977). The decrease in total sugar could also be associated with the occurrence of Maillard browning reactions which takes up a considerable amount of reducing sugars. This finding was reported by Mercier & Feillet (1975) in their study of the carbohydrate components of cereal products by extrusion cooking where in the ethanol soluble carbohydrate fractions decreased after extrusion cooking.

Reducing sugars

After the thermal extrusion process, the average reducing sugar values were 5.50 mg/g of sample in ECP and 8.32 mg/g of sample in EMB (Tables 5 and 6).

The reducing sugar values relate to the total sugars present as shown in Fig. 4. The formation of reducing sugars from total sugars is a quadratic function and depends upon the effect of variables in the extrusion process. One important factor is the increase of

Table 5. Available reducing sugars in cowpea during the extrusion process. Analysis of variance for regression

Source of variance	Coefficient	MS*	df†
Regression		6.40‡	7
Constant, x_0	5.45‡		
Moisture content, x_1	0.19§	2.15	1
pH, x_2	0.32‡	5.59	1
Temperature, x_4	0.40‡	8.69	1
Moisture content × moisture content, x_1x_1	0.54‡	5.09	1
pH × pH, x_2x_2	0.48‡	4.31	1
Moisture content × screw speed, x_1x_3	0.63‡	15.98	1
Screw speed × temperature, x_3x_4	0.28§	2.91	1
Residual		0.72	82
Lack of fit		0.58	37
Error		0.02	45
Corrected total		2.17	89
$R^2 = 0.86$			

*MS = mean square.
†df = degree of freedom.
‡Significant at $P = 0.01$.
§Significant at $P = 0.05$.

Table 6. Available reducing sugars in mung bean during the extrusion process. Analysis of variance for regression

Source of variation	Coefficient	MS*	df†
Regression		17.06‡	7
Constant, x_0	8.32‡		1
Screw speed, x_3	0.45‡	11.46	1
Temperature, x_4	0.53‡	15.63	1
Moisture content × moisture content, x_1x_1	− 1.15‡	25.72	1
pH × pH, x_2x_2	− 0.56§	5.05	1
Temperature × temperature, x_4x_4	0.55§	5.03	1
Moisture content × screw speed, x_1x_3	0.17‡	54.66	1
Screw speed × temperature, x_3x_4	0.45§	7.71	1
Residual		0.78	82
Lack of fit		1.68	37
Error		0.04	45
Corrected total		2.06	89
$R^2 = 0.85$			

*MS = mean square.
†df = degree of freedom.
‡Significant at $P = 0.01$.
§Significant at $P = 0.05$.

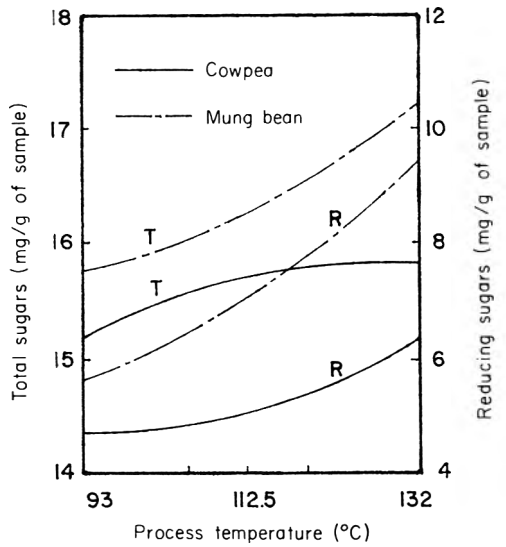

Figure 4. Effect of process temperature on total (T) and reducing (R) sugars in extruded cowpea products.

process temperature which increases the rate of starch degradation as well as that of other sugars. The starch and sugars of ECP exhibited more resistance to increasing temperature than these of EMB products. Similarly, at a given process temperature, as the moisture content increased, the degradation also increased. At high moisture content and process temperature, the reducing sugars reached 6.9 mg/g of sample in ECP (Fig. 5). In the case of EMB, the rate of degradation slightly increased when the moisture content was increased from 30 to 37.5% content at 93 or 132°C. The rate of degradation decreased at a higher moisture content.

With moisture content at 37.5%, increasing the pH from 6.2 to 7.0, increased the levels of reducing sugars, while at pH 7.0 to 7.4, the reaction was constant at low process temperature (Fig. 6). However, at pH 7.2, with increased process temperature, the

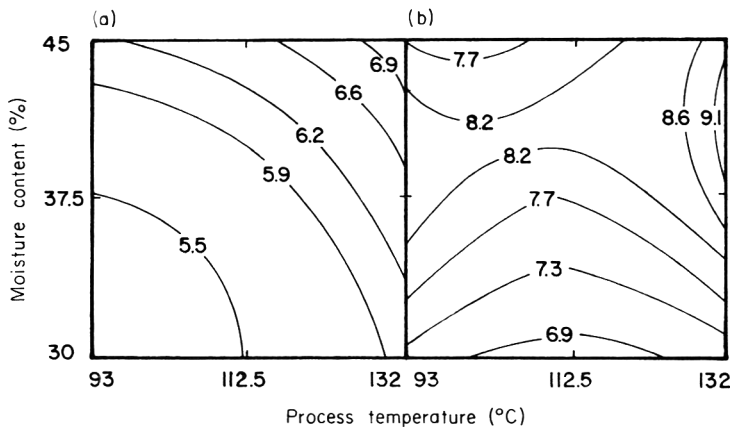

Figure 5. Reducing sugar contour maps as function of moisture content and process temperature in extruded products with screw speed 150 rev/min and pH 6.8. (a) Cowpea. (b) Mung bean.

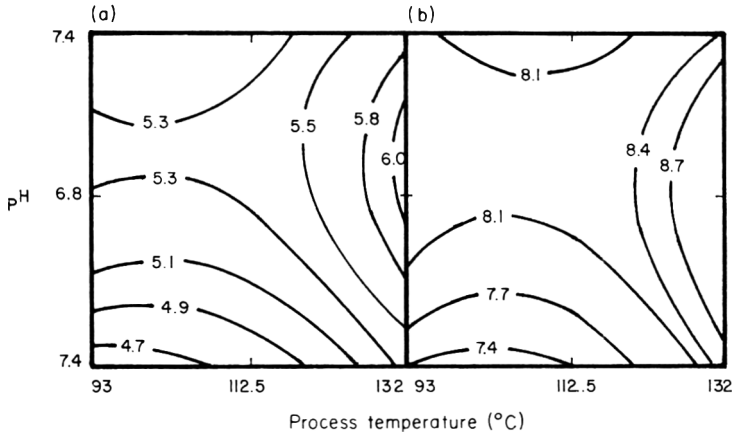

Figure 6. Reducing sugar contour maps as function of pH and process temperature in extruded products with screw speed 150 rev/min and pH 6.8. (a) Cowpea. (b) Mung bean.

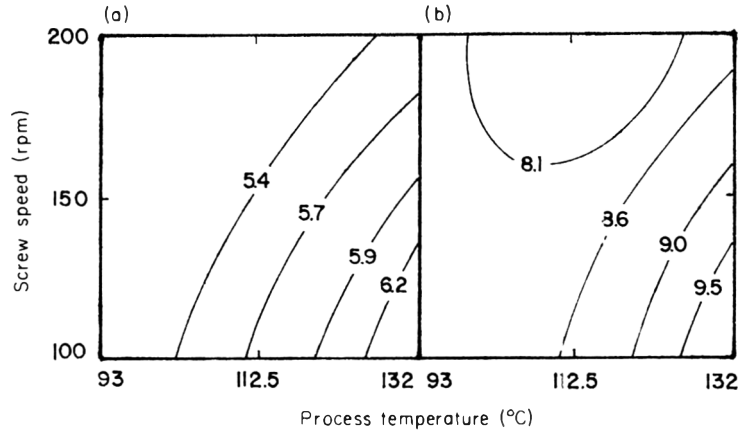

Figure 7. Reducing sugar contour maps as function of screw speed and process temperature in extruded products with moisture content 37.5% and pH 6.8. (a) Cowpea. (b) Mung bean.

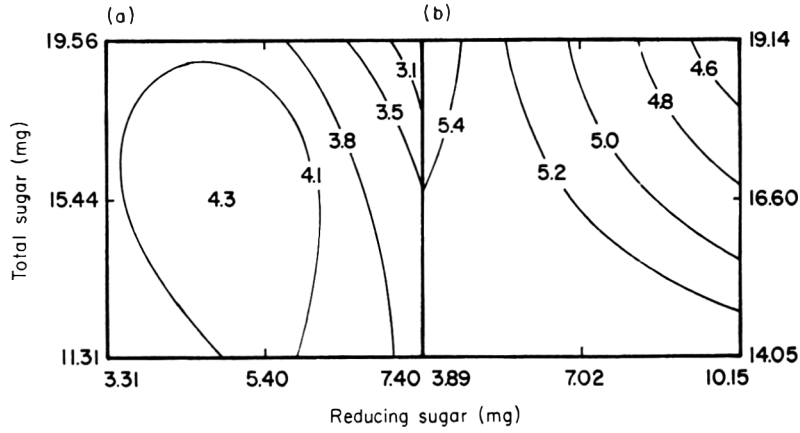

Figure 8. Available lysine contour maps as function of total sugars and reducing sugars in extruded products. (a) Cowpea. (b) Mung bean.

amount of reducing sugars released, decreased. The rate of release for reducing sugars was accelerated as the screw speed and the process temperature increased (Fig. 7). Chiang & Johnson (1977) studied the gelatinization of starch in extruded products and found a significant increase in fructose and glucose levels. The increase in the amount of reducing sugars was brought about by he breakdown of (2 → 1) glycosidic bonds of sucrose and raffinose and the (1 → 4) glycosidic bonds of the malto-oligosaccharides when the cereal products were extruded. In this study, at a low pH 6.2, 30% moisture content, 200 rev/min screw speed, and at the highest process temperature, reducing sugars showed minimum values. This may be due to the interaction between the reducing sugars and the ϵ-amino acids which reduce the amount of reducing sugars. As the pH was increased, the Maillard reaction was accelerated. This agrees with the result of Jokinen *et al.* (1976) who reported that sucrose would be hydrolysed into reducing sugars making available glucose which could be consumed through Maillard reaction in the thermal process of the model food system used.

Correlation beteen total and reducing sugars and available lysine

The contour maps show that maximum retention would follow if the amount of total sugar and reducing sugar is kept to a minimum. This will result in minimum production of reducing sugar from the breakdown of more complex carbohydrates and therefore less reactant available to react with lysine.

Acknowledgments

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Nutritional significance of trypsin inhibitors from edible dry beans (*Phaseolus vulgaris* L.)

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Summary

Specific and non-specific trypsin inhibitors were isolated by means of affinity chromatography from three edible dry bean cultivars, for determination of their sulpho-amino acid content, and evaluation of these protein fractions as potential sources of nutritional improvement.

Qualitative differences were found among these protein fractions by gel electrophoresis and amino acid analyses. Resistance to heat varied significantly.

Introduction

Trypsin inhibitors constitute 2–5% of the total seed protein of the edible dry bean (Liener, 1979; Kakade, 1974; Dechary, 1970). Generally they are considered to be antinutritional factors. When experimental animals were fed with raw bean flour (Jaffe, 1973; Liener, 1979) trypsin inhibitors accounted for about 60% of the pancreatic hypertrophic effect; they also reduced protein digestibility and availability of amino acids, vitamins and minerals (Liener, 1979; Rackis, 1974). Trypsin inhibitors are on the other hand good sources of cystine (Kakade, 1974). High trypsin inhibitor content may be related to high sulpho-amino acid levels in beans and therefore may be desirable. Trypsin inhibitors with high methionine content have been found in soybeans (Hwang *et al.*, 1977). A protein fraction rich in both cystine and methionine would be of great interest from point of view of genetic improvement of nutritional quality in beans. The purpose of this study was to examine if trypsin inhibitors rich in sulpho-amino acids are present in beans. Two protein fractions, one with specificity against trypsin, which was isolated by means of affinity chromatography, and another with general proteinase activity are described.

Materials and methods

Reagents

Sepharose 4B was obtained from Pharmacia Fine Chemicals. Reagents for the gas chromatographic analysis of methionine and 6-aminohexanoic acid (AHA) were obtained from Aldrich Chemical Company. Reagents for gel electrophoresis, 1-ethyl 3-(3-dimethyl-1-aminopropyl)-carbodiimide hydrochloride (EDC), hydrazine hydrate (64% hydrazine), bismuth nitrate pentahydrate, potassium sodium tartrate, Na-benzoyl-L-arginine ethyl ester (BAEE) and trypsin (bovine, type III, lyophilized, salt free) were obtained from Sigma Chemical Company. 'Spectrapor' cellulose casings with 3500 molecular weight cut off, were obtained from Fisher. Edible dry bean seeds,

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were ground to pass through an 80-mesh sieve, defatted by five volumes of petroleum ether (three times) and dried under vacuum.

Preparation of the affinity gel

Activated Sepharose 4B was prepared by cyanogen bromide activation of Sepharose 4B, followed by reaction with AHA (Pharmacia Fine Chemicals, 1974). Fifty millilitres of activated gel were mixed with an equal volume of a solution containing 200 mg EDC and 800 mg of trypsin in water adjusted to pH 6.0. The mixture was agitated for 15 hr at 6°C while the pH was maintained at 6.0 by the addition of 100 mM NaOH. After this treatment excess ligand was washed away under mild suction and excess remaining reactive groups were blocked by 1 M ethanolamine, pH 8.0. The gel was successively washed with borate buffer (0.1 M pH 8.5) and acetate buffer (0.1 M, pH 4.1) and finally stored in 0.05 M acetate buffer pH 4.1 containing 0.02% (w/v) sodium azide as preservative.

Protein fractionation

Ten grams of defatted seed meal were extracted twice for 30 min at 6°C, in 100 ml of 0.1 M sodium phosphate buffer pH 7.5, containing 0.2 M NaCl and 0.05 M β-mercaptoethanol. The combined supernatant liquids were centrifuged at 25000 **g** for 30 min and the clarified supernatant liquid passed through an affinity column [1.6 (i.d.) × 20 cm] with a flow rate of 20 ml/hr. Fractions of 3 ml were collected in a fraction collector equipped with a u.v. monitor. The eluted protein was monitored at 280 nm and the trypsin inhibitor activity of each fraction was determined. The protein fraction which was not absorbed on the affinity column, was exhaustively dialysed against 0.1 M sodium acetate buffer pH 4.1, containing 0.2 M NaCl, to yield two subfractions, one soluble and the other insoluble in the pH 4.1 acetate buffer. These fractions were dialysed against distilled water, freeze dried and stored at −18°C. The affinity column was washed with the pH 7.5 phosphate buffer until the absorbance of the eluate reached a value less than 0.05 (A_{280}). The protein absorbed on the affinity column was eluted with acetic acid 0.1 M, pH 3.0, and subsequently freeze dried and stored at −18°C. Glycoprotein II was isolated according to Pusztai & Watt (1970).

Determination of inhibitory activity

Trypsin inhibitor activity was measured as residual tryptic esterolytic activity, using BAPA as substrate (Kakade *et al.*, 1974). Trypsin inhibitor activity is expressed as trypsin units inhibited per mg of sample (Kakade, Simons & Liener, 1969).

Disc electrophoresis

Native proteins were analysed in a Tris-glycine, pH 8.3, buffer system in 7.5 and 12% polyacrylamide gels. One half of slab gel was stained specifically for detection of trypsin inhibitors (Uriel & Berges, 1968). while the other half containing similar samples was stained for protein (Mauer, 1971). Denatured protein fractions were analysed in 12% SDS polyacrylamide slab gels (Mauer, 1971).

Determination of sulpho-amino acids

Methionine content was determined by means of cyanogen bromide digestion of the sample and quantitation of released methylthiocyanate by gas liquid chromatography (Apostolatos & Hoff, 1981). Cystine plus cysteine were converted to H_2S by

hydrogenolysis and subsequently reacted with bismuth tartate: the resulting bismuth sulphide was monitored in a spectrophotometer at 400 nm (Herrick, Lawrence & Coahran, 1972). Nitrogen in the seed meal was determined by a modified Berthelot assay following sulphuric acid digestion (Apostolatos, 1980), while protein content in isolated fractions was determined by a modified Lowry method (Hartree, 1972).

Results and discussion

Three cultivars of edible dry bean, 'Swedish Brown', 'Sutter Pinto' and 'Bonus', selected for their relatively high methionine content were used in this study. Their protein content was 28, 22.5 and 22%, respectively, on dry meal basis.

While direct coupling of trypsin to CNBr activated Sepharose 4B resulted in a non-specific and low in capacity affinity matrix, the coupling at pH 6.0 through carbodiimide resulted in an affinity matrix which has much greater capacity for trypsin inhibitors than the one prepared by performing the coupling at alkaline pH (10.0–11.0). Since the affinity chromatography profile was identical for the three cultivars, only the profile of cv. Cuva appears in Fig. 1. Approximately 2.5 g of crude protein passed through the column.

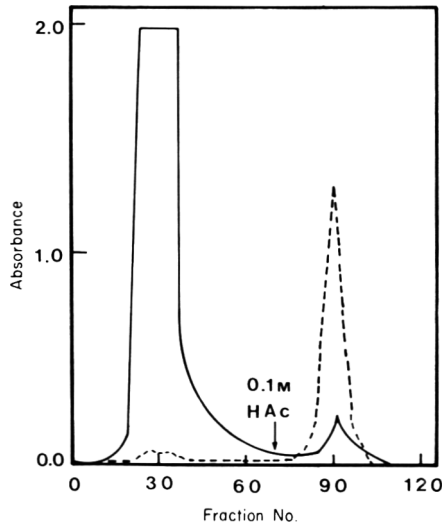

Figure 1. Affinity chromatography of a crude edible dry bean protein extract, through a Sepharose 4B-Trypsin column. The retarded proteins were eluted with 0.1 M acetic acid (HAc) solution. Solid line shows absorbance at 280 nm; dotted line shows trypsin inhibitor activity expressed as units/mg of protein.

More than 85% of the total trypsin inhibitor activity (TTIA) present in the crude protein extract, was associated with the protein fraction retarded by the affinity column (Fraction II), which accounts for less than 5% of the total extracted protein (w/w). Approximately 10% of the TTIA was associated with the unretarded protein fraction (Fraction I), while preparative losses were restricted to less than 5% of the TTIA. When Fraction I was extensively dialysed against 0.1 M acetate buffer pH 4.1, containing 0.5 M NaCl, it was found that the trypsin inhibitor activity (TIA) was associated with the protein fraction soluble in the pH 4.1 buffer (Fraction I_a), while no activity was found in the precipitate (Fraction I_b). Autoclaved Fraction I_a and Glycoprotein II retained approximately 50% of their original TIA, while TIA of

Fraction II was reduced by 90% (Table 1). The inhibition ratio (i.r.) varied significantly among the isolated protein fractions within the cultivar, but it was rather indifferent for a given protein fraction among the cultivars with exception that of Fraction II which is an heterogeneous mixture of TI's.

Table 1. Trypsin inhibitor activities in edible dry bean protein fractions

	'Swedish Brown'			'Sutter Pino'			'Bonus'		
	TIU*		i.r.†	TIU*		i.r.†	TIU*		i.r.†
	I	II		I	II		I	II	
Total protein extract	260	35	55:1	225	23	59:1	208	20	60:1
Fraction I_a	25	18	19:1	20	16	23:1	20	18	20:1
Fraction I_b	—	—	—	—	—	—	—	—	—
Fraction II	220	10	1:2.3	195	6	1:2.1	180	0	1:2.0

*TIU: trypsin inhibitor units. I: native proteins; II: autoclaved proteins (10 min, 15 psi).
† i.r.: inhibition ratio, weight of inhibitor per weight of enzyme.

Gel electrophoresis patterns of Fraction I_a proteins are given in Fig. 2. Although this heterogeneous fraction exhibits TIA when tested by the enzyme assay (Table 1), none of the protein bands remained unstained when the gel was treated according to Uriel & Berges' technique (1968), a positive indication of absence of specific trypsin

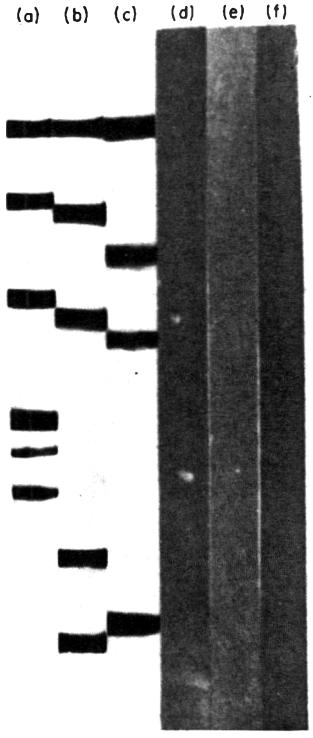

Figure 2. Polyacrylamide gel electrophoresis of Fraction I_a proteins in 7.5% gels; a,d: 'Swedish Brown'; b,e: 'Sutter Pinto'; c,f: 'Bonus'. Gel portion, a,b,c was stained for protein (Mauer, 1971); gel portion d,e,f was stained specifically for TI's (Uriel & Berges, 1968).

inhibitors. Two protein bands of this fraction showed similar electrophoretic mobility to Glycoprotein I and II fractions (Fig. 3). The presence of the major glycoproteins in Fraction I_a was expected because of the isolation scheme followed. The refractory nature of Glycoprotein II is well known (Jaffé, 1973; Liener, 1979; Seidl, Jaffé & Jaffé, 1969) and this explains the remaining TIA present in the autoclaved Fraction I_a.

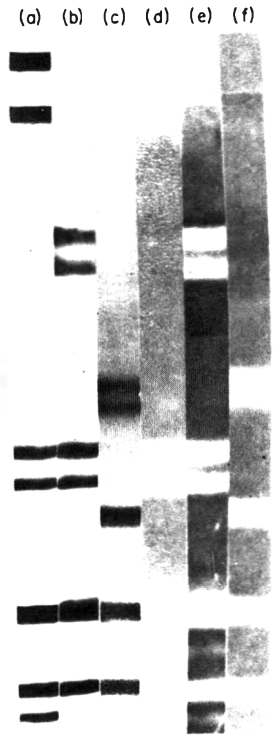

Figure 3. Polyacrylamide gel electrophoresis of Fraction II proteins in 12% gels: a,d: 'Swedish Brown'; b,d: 'Sutter Pinto'; c,f: 'Bonus'. Staining as in Fig. 2.

Gel electrophoresis patterns of Fraction II proteins varied among the cultivars tested (Fig. 4). Some of these protein bands showed similar electrophoretic mobilities, while the slow moving components may represent polymeric forms of trypsin inhibitors. The latter is sustained by SDS polyacrylamide gel electrophoresis of Fraction II proteins, which revealed major subunits with molecular weights of 21000, 13000 and 8500 daltons, and some minor of 18000, 16000 and 10000 daltons. In contrast to Fraction I, Fraction II proteins exhibited specific TIA and remained unstained in the polyacrylamide gel (Fig. 4).

Methionine and half cystine contents of isolated protein fractions are presented in Table 2. Fraction I_a constitutes approximately one-third of the edible dry bean protein. This fraction contains less than the average seed methionine and it is low in half cystine. Fraction II proteins have a relatively high half cystine content but its methionine content was found to be too low to justify further characterization of individual trypsin inhibitors.

While quantitative intervarietal variation of TI's are within the experimental error, qualitative differences are apparent. In agreement with other investigators (Liener, 1979; Rackis, 1974), it is confirmed that the protein fraction which is responsible for

Figure 4. Comparative polyacrylamide gel electrophoresis opatterns of Fraction I_a proteins (b: 'Swedish Brown': c: 'Sutter Pinto': d: 'Bonus' and of glycoproteins I and II (a_1, e_1 = G I; a_2, e_2 = G II).

Table 2. Protein and s-amino acid content of selected edible dry bean protein fractions

	'Swedish Brown'			'Sutter Pinto'			'Bonus'		
	% P*	% Met‡	% half Cys‡	% P	% Met	% half Cys	% P	% Met	% half Cys
Total protein extract	100	1.8	1.3	100	1.7	0.9	100	1.7	1.1
Fraction I_a	28	1.1	0.15	30	0.9	0.2	26	1.3	0.18
Fraction II	4.2	0.2	6.8	3.9	0.2	6.5	3.4	0.3	7.1

* % P: percentage of protein based on 100 g extracted seed protein from edible dry bean.
Percentages of ‡methionine and ‡half cystine are given on protein basis (w/w).

most of the trypsin inhibitor activity in the raw bean meal can be inactivated by heat treatment, in contrast to the non-specific proteinase inhibitors (Fraction I_a), which retains half of their inhibitory action even following autoclaving. In conclusion, the nutritional significance of denatured trypsin inhibitors remain high because of their half cystine content, but their low methionine content restricts the use of this protein fraction as a potential source of genetic material for the nutritional improvement of edible dry beans.

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Fractionation of proteins from limabeans (*Phaseolus lunatus*)

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Summary

Proteins extracted from the seeds of limabeans have been separated by ammonium sulphate fractionation and paper electrophoresis into fractions: two soluble in salt solution and five water-soluble. Four of these fractions had haemagglutinating activity and inhibited trypsin activity while three were active as chymotrypsin inhibitors. Amino acids most abundant in the different fractions included aspartic and glutamic acids. Methionine and cystine were the most limiting amino acids while tryptophan was border line in fractions A, C, D, E and F-11. All the fractions contained high amounts of lysine, making limabean proteins a good source of this amino acid.

Introduction

The leguminous seeds, particularly limabean (*Phaseolus lunatus* L. Walp), are very important sources of protein in the diets of West African populations of low economic status. In Nigeria, limabeans provide from 25 to 30% of the protein in the diet and are second only to maize and cowpea as a staple food (Luse & Okwuraiwe, 1975). Studies with these beans have shown that they are good sources of some essential nutrients but deficient in methionine and cystine (Luse, 1979; Ologhobo & Fetuga, 1983b) and contain high levels of antinutritional factors including the trypsin inhibitors (Haynes, Osuga & Feeny, 1967), phytohaemagglutinins (De Muclenaere, 1965; Manage, Joshi & Sohonie, 1972) and cyanogenic glycosides (Ologhobo & Fetuga, 1983a). However, very little is known about the nature of limabean proteins or their chemical composition.

This paper describes an investigation into the number of proteins present in limabeans and their propensity as sources of antinutritional factors.

Materials and methods

White limabeans (*Phaseolus lunatus*) acquired from the International Institute of Tropical Agriculture, Ibadan, Nigeria, were dried and finely ground. The soluble proteins were fractionated as summarized in Fig. 1. One-kilogram lots of the meal were extracted overnight at 4°C with 5 litres each of 1% NaCl solution filtered through cloth and centrifuged; the clear solutions were dialysed against tap water. The precipitated globulins were separated by centrifugation and the soluble proteins were precipitated by saturation with ammonium sulphate. The globulins were separated into two fractions, using the technique of Goa & Strid (1959). After repeating this procedure three times, both fractions were dried by lyophilization; they were called fraction D (insoluble in 0.2 M NaCl, pH 4.5) and fraction E (soluble).

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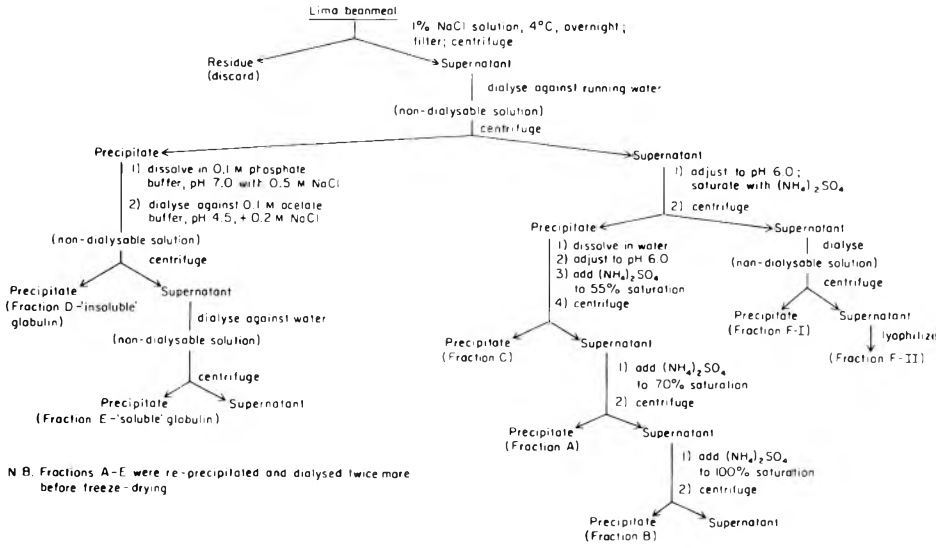

Figure 1. Steps involved in the fractionation of bean proteins (1 kg ground beans suspended in 5 litres 1% NaCl solution).

The ammonium sulphate precipitated material was dissolved in water, dialysed, adjusted to pH 6.0 and precipitated with ammonium sulphate at 55% and later at 70 and 100% saturation. All three fractions thus obtained were reprecipitated twice more, dialysed against running water, then against distilled water and lyophilized. The fraction precipitated with $(NH_4)_2SO_4$ at 55% saturation was called fraction C; the next, fraction A and the one precipitated last, fraction B.

A small amount of additional material had been obtained from the supernatant of the first ammonium sulphate precipitation by dialysis against distilled water; this was called fraction F.

Free-flow continuous electrophoresis of Hanning (1960) was used for further purification of the different fractions. The soluble fractions were submitted to paper electrophoresis (Elphor apparatus; Bender and Hobein, Munich, Germany) in pH 8.6 veronal buffer. Four paper strips were used for each fraction; one was coloured with amido black, the other was cut in 1-cm wide strips which were put separately into test tubes containing 1 ml of a 2.0% erythrocyte suspension, in order to study the haemagglutinating activity of the different proteins. Other strips were stained by the periodate-fuchsin-sulphate (PAS) method for the detection of glycoproteins (Block, Durrum & Zweig, 1958) or with sudan black for lipoproteins (Block *et al.*, 1958).

Trypsin inhibitor activity was evaluated in terms of the extent to which portions of the different proteins inhibited the action of benzoyl DL-arginine-p-nitroanilide (Kakade, Simmons & Liener, 1969a). Chymotrypsin inhibitor activity was measured on the basis of the ability of the protein extract to inhibit the digestion of casein by chymotrypsin (Kakade *et al.*, 1972). Haemagglutination was studied with 0.2% washed trypsinized rabbit erythrocytes, employing the two-fold serial dilution technique of Kabat & Mayer (1961).

Nitrogen content was assayed using the micro-Kjedhal method of the AOAC (1970). Amino acid contents were determined in protein samples of about 2 mg. They were hydrolysed with redistilled HCl in ampoules sealed under nitrogen at 100°C for 24

hr and the amino acids were analysed with the automatic Technicon Model TSM Sequential Analyzer (Bidmead & Ley, 1958). Cystine was determined in duplicate as cysteic acid by the method of Moore (1953) while tryptophan was chemically determined by the method of Miller (1967).

Results

At the commencement of protein fractionation, discarded residue, obtained by soaking overnight in 1% NaCl solution, contained 0.15 (g%) nitrogen. The yield, nitrogen content, trypsin inhibitor, chymotrypsin and haemagglutinating activities in the extracted protein fractions are presented in Table 1. The yield of the water-insoluble proteins (fractions D and E) formed about 75% of the total extractable proteins. Fractions A, B and C (water-soluble) were obtained in about equal amounts with some differences between different lots of seeds while fractions F-1 and F-11 gave the lowest total protein yields. Nitrogen contents in all fractions varied between 0.20 and 19.00%.

The water-soluble protein most easily isolated in a pure form was fraction A. This fraction also exhibited the highest haemagglutinating activity, closely followed by fractions C and B, respectively. Fraction E showed a very slight haemagglutinating activity while fraction D was negative. Trypsin inhibitor activity ranged between 30 and 85 TUI/mg protein while chymotrypsin inhibitor activity, was highest in fraction E (22 CUI/mg protein) and lowest in fraction C (10 CUI/mg protein).

Paper electrophoresis of the total bean extract would not permit recognition of all the different components. The bulk of the water-insoluble proteins migrated as a broad band at a velocity similar to serum globulin (Table 1). Electrophoretic mobility of fractions A and C were so similar that this method alone would not permit complete separation. All the protein fractions gave identical pattern of zones when stained with amido black and with the exception of D, these fractions were identified as glycoproteins by the PAS staining technique. However, only the slow moving fractions A, B and C, which gave positive haemagglutinin reaction could be stained with sudan black under the experimental conditions.

The amino acid composition of the protein fractions, expressed as moles of each amino acid per 100 mol of all amino acids (Mol %), are presented in Table 2. The most abundant amino acids in the different fractions were aspartic acid, glutamic acid and

Table 1. Yield, total nitrogen and some antinutritional components in limabean protein fractions

Fraction	Yield (g/kg)	Nitrogen content (g %)	Electrophoretic mobility as % human serum albumin	Trypsin inhibitor activity (TUI/g protein)*	Chymotrypsin inhibitor activity (CUI/g protein)†	Haemaglutination, highest dilution
A	16.00	2.50	0.56	35	—	1:142.00
B	18.50	2.80	0.39	—	—	1: 86.00
C	16.88	2.66	0.54	84	10	1:138.00
D	112.50	19.00	0.76	72	18	—
E	75.46	11.75	0.84	54	22	1: 16.00
F-1	4.55	0.76	—	—	—	—
F-11	1.80	0.12	—	—	—	—

*Expressed as trypsin units inhibited (TUI) per mg protein as defined by Kakade *et al.* (1969a).

†CUI, chymotrypsin units inhibited as defined by Kakade *et al.* (1972).

Table 2. Amino acid content of different limabean protein fractions (expressed as Mol %)

	A	B	C	D	E	F-11
Aspartic acid	14.80	10.55	11.25	10.13	11.00	4.32
Glutamic acid	13.60	17.56	15.00	16.60	8.75	6.64
Threonine	3.84	2.25	3.76	2.80	2.55	1.83
Serine	5.08	6.22	6.80	3.56	3.70	3.31
Proline	5.04	4.35	5.30	4.62	4.48	0
Glycine	5.66	3.17	3.02	3.11	2.95	7.88
Alanine	6.85	7.20	5.80	7.12	5.33	7.04
Cystine	0.35	0.28	4.05	2.00	0.20	0.10
Valine	6.77	5.36	6.22	6.60	5.72	6.13
Methionine	0.22	0.66	0.40	1.05	0.86	0.98
Isoleucine	6.33	4.20	4.83	5.31	4.86	7.02
Leucine	4.66	8.34	7.73	9.05	9.01	7.60
Tyrosine	3.18	5.66	3.94	4.76	4.88	2.85
Phenylalanine	6.90	8.20	6.14	6.73	7.90	3.11
Histidine	1.86	2.42	2.88	3.86	3.99	1.06
Lysine	6.86	7.21	6.32	6.88	7.81	6.40
Arginine	5.81	4.78	6.90	5.77	6.55	5.00
Tryptophan	0.40	1.56	0.46	0.31	0.38	0.16

glycine. Fractions A and C were particularly low in methionine compared to fractions D and E while tryptophan appeared to be deficient in fractions A, C, D, E and F but adequate in B. Lysine levels varied between 6.40 and 7.81 Mol %, and in fraction A, in contrast to all the other fractions, the quantity of leucine (4.66 Mol %) was smaller than that of isoleucine (6.33 Mol %).

Discussion

Seven protein fractions have been separated from crude limabean extracts; three were soluble in water and two were insoluble. The two fractions F-1 and F-11, which were not precipitated by saturated ammonium sulphate and which had low nitrogen contents, are not typical proteins and their detailed study has not been included in the present work. Fractions D and E are globulins according to their insolubility in water, the electrophoretic mobility of the latter was greater than that of all the others and was similar to human serum albumin. Of the three water-soluble fractions, A and C were most similar in electrophoretic mobility and it is more likely that they are identical proteins especially, when they are also viewed in respect of their similar nitrogen contents, amino acid compositions and haemagglutinating activity. Fractions A and B, both active haemagglutinins, differed significantly in respect of electrophoretic mobility, trypsin inhibitor activity and composition of amino acids; they are most probably different proteins.

Four protein fractions were active as trypsin inhibitors and three as chymotrypsin inhibitors. These activities were more concentrated in the water-insoluble protein fractions which made up over 70% of the total limabean proteins. The presence of antinutritional factors has long been known to adversely affect protein utilization (Phadke & Sohonie, 1962; Frost & Mann, 1966) and depress growth through interference with the absorption of certain essential amino acids (Flux *et al.*, 1956). The detection of trypsin inhibitors in up to four different fractions also confirms previous

observations (Tauber, Kershaw & Wright, 1949; Frattah & Steiner, 1968) that more than one type of trypsin inhibitors was present in limabeans.

The adequacy of a protein for man depends upon the content of essential amino acids and the availability of the amino acids. One of the principal drawbacks in the utilization of the limabean protein, aside from the presence of a number of antinutritional factors, is an amino acid profile that is very deficient in methionine and cystine (Liener, 1969; Bressani, 1972). In the present study, all protein fractions showed marked deficiency in methionine and cystine and were borderline with respect to tryptophan. The exceptions were fraction C for cystine and fraction B for tryptophan. It is perhaps important to note here that the value obtained for cystine in fraction C is higher than in most other plant sources reported to be high in cystine: (cotton seed meal, 3.26 Mol %; bambara nut, 3.80 Mol %; ground nut, 3.55 Mol %; cowpea, 2.57 Mol %). One would therefore expect that this amino acid would not constitute a dietary problem in limabean proteins. But because fraction C represents a very small percentage (1.68%) of the limabean proteins, its amino acid contents do not have any significant effect on the total amino acid contents of the whole limabean seed protein. However, fraction C, in addition to the high cystine level, also contained the highest amounts of haemagglutinin and trypsin inhibitors. It is probable therefore, that the high cystine content of fraction C is not a true representation of the amount that is nutritionally available. Kakade *et al.* (1969b) have shown that the trypsin inhibitors of such legumes as navy bean and kidney bean are quite rich in cystine and, although they comprise only 2.5% of the total bean protein, they account for about 30–40% of the total cystine content. An earlier report (Tauber *et al.*, 1949) had also indicated that the most characteristic feature of the limabean trypsin inhibitor was its very high cystine and absence of tryptophan. The values obtained in this study for cystine in fraction C and other trypsin inhibitor-active fractions, are therefore an exaggeration of the levels present. It is plausible to suggest that the trypsin inhibitors in limabeans not only reduce protein digestibility by resisting enzymic digestion, they also 'lock in' a significant fraction of the total cystine of the bean protein which is already in short supply.

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Lipid oxidation in chicken breast and leg meat after sequential treatments of frozen storage, cooking, refrigerated storage and reheating

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Summary

Oxidative rancidity in fresh and processed chicken breast and leg meat was evaluated by measuring malondialdehyde (MDA) in fat from meat with an improved TBA assay with antioxidant protection, and by measuring the relative fluorescent products of organic and aqueous layers from Folch extracted meat. Fresh meat was treated sequentially with common household meat processing techniques, including: frozen storage, cooking (both microwave and convection), refrigerated storage, and reheating. Fat from fresh breast meat contained double the concentrations of free MDA and fluorescent products than fat from leg meat; these differences were due to a larger phospholipid fraction in fat from breast which contained more polyunsaturated fatty acids. Frozen storage for 3 and 6 months before cooking and refrigerated storage after convection or microwave cooking substantially increased MDA concentrations in fat from meat; whereas cooking and reheating treatments were more effective in generating fluorescent products. Generally, it was concluded that the initial levels of either MDA or fluorescent products in meat are of primary importance in determining the final MDA and fluorescence levels after processing.

Introduction

Many methods of meat processing such as mechanical deboning (Dawson & Gartner, 1983), frozen storage (Igene *et al.*, 1979; Igene *et al.*, 1980), cooking (Shamberger, Shamberger & Willis, 1977; Siu & Draper, 1978; Newburg & Concon, 1980; Yamauchi, Nagai & Ohashi, 1982), refrigerated storage after cooking (Dawson & Schierholz, 1976; Wilson, Pearson & Shorland, 1976; Yamauchi *et al.*, 1982) are known to enhance oxidative rancidity in meat as measured by thiobarbituric acid (TBA) test for malondialdehyde (MDA). Chicken meat is especially susceptible to oxidative rancidity because of the highly polyunsaturated nature of chicken fat (Marion & Woodroof, 1965; Edwards *et al.*, 1973; Fristrom & Weihrauch, 1976). Although the effects of certain individual meat processing methods have been investigated, it is reasonable to examine the cumulative effects of several methods of meat processing which commonly occur in households. These processes include in sequence: frozen storage, cooking, refrigerated storage of cooked 'leftovers', and reheating 'leftovers'. A sequential

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analysis is especially appropriate in view of the extreme variability in TBA test results from different laboratories, due mainly to sample autoxidation, which makes comparisons between studies impossible (Melton, 1983; Pikul, Leszczynski & Kummerow, 1983).

In our report we investigate the cumulative effects of common household meat processing techniques on oxidative change in chicken meat by using an improved TBA assay with antioxidant protection (Pikul *et al.*, 1983). In addition, the relative concentrations of fluorescent products in organic and aqueous extracts of all meat samples were recorded as a measure of MDA crosslinking with protein and lipid components of meat (Chio & Tappel, 1969; MacDonald *et al.*, 1980). These two parameters were investigated in meat after cooking both by microwave oven and convection oven, after refrigerated storage, and after reheating in order to determine if there is a difference in these basic cooking techniques.

Materials and methods

Preparation of meat samples

Meat samples were prepared from sexually mature White Leghorn hens which were beyond their peak egg-laying period. Birds were killed by decapitation and inverted carcasses were suspended until bleeding was completed. After removal of skin, whole breast and leg meat was manually deboned, wrapped in aluminium foil and stored at −18°C. After 3 and 6 months of frozen storage, meat samples were cooked, then refrigerated at 4°C, and finally reheated after 4 days refrigerated storage. Portions of meat samples were analysed after each experimental treatment (frozen storage, cooking, refrigerated storage and reheating).

Cooking conditions

For experimental cooking, breast and leg meat from one side of each bird was cooked in a 0.27 m^3 convection oven (South Bend Range, Model V-15; South Bend, Indiana), while meat from the other side was cooked in a 0.042 m^3 700 W microwave oven (Litton model 1550). Before cooking, breast and leg meat was trimmed to give homogeneous size and weight; breast samples averaged 69.8 g and leg samples averaged 62.8 g. All samples were cooked until the average temperature in the centre of the meat was 76.7°C as measured by a recording thermometer in the convection oven (Zero Centurion Elite, Campbell Scientific, Inc.) or a temperature probe sensor supplied with the microwave oven. Both temperature recorders were monitored by a mercury bulb thermometer for accuracy. Convection cooking in a preheated oven at 177°C required an average of 14 min 18 sec while microwave cooking (at 60% of full power) required an average of 2 min 48 sec. For reheating, breast meat averaged 27.8 g and leg meat averaged 22.8 g; these samples were heated to an average temperature of 71°C. Reheating times averaged 10 min 30 sec in the convection oven and 1 min 12 sec in the microwave oven. All samples were allowed to cool to 40°C, then transferred to 4°C until extraction.

Analytical methods

For chemical analysis, meat samples were finely minced and then 5-g portions were blended and homogenized with chloroform-methanol solvents for total fat extraction according to the basic procedures of Kates (1972) with modifications previously presented in detail (Pikul *et al.*, 1983). Total fat content of each individual meat sample

was determined gravimetrically, from the organic layer after extraction. This same organic layer was used for the determination of total cholesterol by modified methods of Glick, Fell & Sjolin (1964); triglycerides by the methods of Foster & Dunn (1973); and total phosphorus by the methods of Eng & Noble (1968) and multiplied by 25.5 to estimate total phospholipids. Aliquots of the lipid extract were also used for determining the fatty acid compositions. Fatty acid methyl esters from purified phospholipids and triglycerides were prepared according to Cho (1981). Analysis of fatty acid methyl esters was accomplished in a Hewlett-Packard model 5890A gas chromatography machine equipped with a Supelco 30 m × 0.24 mm fused silica capillary column packed with 20% SP2540 at an oven temperature of 185°C with injection port and detector temperatures set at 200°C. For thiobarbituric acid analysis of malondialdehyde (MDA) and the determination of TBA numbers, 4–6 mg of dried fat samples were prepared by evaporating appropriate volumes of the organic extract with nitrogen and these samples were then analysed with protection against autoxidation according to the detailed methods of Pikul *et al.* (1983).

Secondary products of lipid oxidation were determined by the measurement of fluorescence excitation (360 nm) and emission (440 nm) spectra in both the organic and aqueous layers obtained from chloroform-methanol (2 : 1) extraction, where 1 g meat samples were extracted with 20 ml Folch reagent (Folch, Lees & Sloane-Stanley, 1957). Both the clear aqueous and organic layers of the Folch extract were appropriately diluted to avoid sample quenching and then read in a Perkin-Elmer model 650–10 s fluorescence spectrophotometer with 10^{-8} M quinine sulphate (DAB standard, Fluka, Switzerland) as the standard, which was set equal to 50 fluorescence units (FU) as described by Goldstein, Rozen & Amoruso (1979). Total lipid in the organic layer of Folch extract was determined gravimetrically and total protein in the aqueous layer was determined by the methods of Lowry *et al.* (1951).

Results

Fat composition of breast and leg meat

After slaughter and 2 days frozen storage, portions of breast and leg meat were analysed for fat content and composition. Breast meat contained 1.38% total fat, which was composed of 50.1% triglycerides, 45.5% phospholipids and 4.2% total cholesterol; leg meat contained 3.67% total fat, composed of 74.5% triglycerides, 21.7% phospholipids and 3.8% total cholesterol. Gas chromatography analysis of the fatty acids from the triglyceride and phospholipid fractions separated by thin layer chromatography did not show much difference between breast and leg meat; however, the fatty acid composition of the triglyceride fraction was considerably different from the composition of phospholipids in several respects. Total fatty acids from meat triglycerides were 33.5% saturated (14:0, 16:0, 18:0), 51.8% monoenoic (14:1, 16:1, 18 : 1, and 14.6% polyunsaturated (18 : 2, 18 : 3, 20 : 4); whereas fatty acids from meat phospholipids were 45.2% saturated, 23.4% monoenoic and 32.3% polyunsaturated. A very large difference in arachidonic acid (20:4) content was observed; total fatty acids from meat phospholipids contained above 15% arachidonic acid, while only a small trace, below 0.5%, was present in triglyceride fatty acids.

Malondialdehyde content of meat

The initial concentrations of MDA in fat from breast and leg meat were 21.4 and 10.4 μg MDA/g fat, respectively. These values may be multiplied by the total amount of

fat in breast and leg meat and expressed as mg MDA per kg tissue in order to calculate the TBA numbers which were 0.31 and 0.38 for breast and leg meat, respectively.

After 3 months of frozen storage, the concentration of MDA in fat from breast and leg meat rose to 27.3 and 13.0 μg MDA/g fat, respectively. These values were all significantly larger ($P < 0.05$) than the comparable values obtained from fresh meat. When portions of these samples were cooked in convection and microwave ovens, and then refrigerated for 1 and 4 days, and finally reheated after 4 days refrigerated storage, the concentrations of MDA in fat and the TBA numbers of meat increased to levels indicated in Table 1. Compared to values of raw meat, the concentrations of MDA in fat from breast and leg meat increased after cooking, but there was a significant difference ($P < 0.05$) only in the MDA content in fat from leg meat. TBA numbers were significantly higher in both breast and leg meat after cooking, but this result was partially due to an increase in fat content caused by the loss of juices which occurred during cooking. Neither MDA concentrations in fat nor TBA numbers of meat increased significantly after 1 day of refrigerated storage compared to values obtained immediately after cooking; however, after 4 days refrigerated storage both TBA and MDA values were significantly higher. Reheating had no significant effect on MDA concentrations or TBA numbers. There were no significant differences between convection and microwave cooking in any of the parameters measured, as indicated in Table 1.

Table 1. Malondialdehyde concentration after cooking, refrigerator storage and reheating in meat samples which had been frozen for 3 months

Meat sample	μg MDA/g fat	% fat	TBA number
Convection oven			
Breast			
after cooking	32.1 ± 2.43^{cd}	1.70 ± 0.10^{a}	0.54 ± 0.04^{a}
1-day storage	38.6 ± 3.16^{d}	1.72 ± 0.10^{a}	0.66 ± 0.04^{a}
4-days storage	50.3 ± 3.52^{e}	1.73 ± 0.11^{a}	0.90 ± 0.08^{bc}
after reheating	52.0 ± 3.42^{e}	1.82 ± 0.10^{a}	0.95 ± 0.09^{bc}
Leg			
after cooking	18.9 ± 1.16^{a}	3.97 ± 0.08^{b}	0.74 ± 0.06^{ab}
1-day storage	21.6 ± 1.84^{ab}	3.92 ± 0.12^{b}	0.89 ± 0.06^{bc}
4-days storage	28.6 ± 2.06^{bc}	3.91 ± 0.13^{b}	1.12 ± 0.08^{c}
after reheating	28.7 ± 1.96^{bc}	4.03 ± 0.13^{b}	1.14 ± 0.08^{c}
Microwave oven			
Breast			
after cooking	33.0 ± 2.40^{cd}	1.67 ± 0.07^{a}	0.53 ± 0.05^{a}
1-day storage	42.6 ± 2.67^{d}	1.62 ± 0.09^{a}	0.72 ± 0.07^{ab}
4-days storage	54.9 ± 3.08^{e}	1.67 ± 0.08^{a}	0.91 ± 0.07^{bc}
after reheating	55.5 ± 3.49^{e}	1.73 ± 0.08^{a}	0.95 ± 0.07^{bc}
Leg			
after cooking	19.6 ± 2.55^{a}	3.82 ± 0.05^{b}	0.75 ± 0.05^{ab}
1-day storage	22.1 ± 2.24^{ab}	3.84 ± 0.07^{b}	0.85 ± 0.07^{bc}
4-days storage	27.9 ± 1.92^{bc}	3.86 ± 0.07^{b}	1.07 ± 0.08^{c}
after reheating	27.6 ± 1.86^{bc}	3.92 ± 0.08^{b}	1.08 ± 0.09^{c}

Whole data are presented as means ± standard deviations of four samples. Mean values within each column which do not have the same superscript letter are significantly different ($P < 0.05$, two tailed t-test).

Portions of meat which were kept in frozen storage for 6 months were also analysed for MDA concentration in fat and TBA number as indicated in Table 2. After 6 months storage, fat from raw breast and leg fat contained 37.1 and 16.6 μg MDA/g fat; and calculated TBA numbers for breast and leg meat were 0.57 and 0.68, respectively. After either convection or microwave oven cooking, both the MDA concentrations and the TBA numbers of breast and leg meat were significantly higher than samples assayed before cooking. It can be seen from the statistical comparisons in Table 2, that the increases in MDA concentrations in fat and TBA numbers in meat after refrigerated storage and reheating followed the same general pattern observed in meat stored for 3 months but with higher absolute values. No significant differences in MDA concentrations in fat or TBA numbers in meat were observed between convection oven cooking and microwave oven cooking. These results demonstrate that the magnitude of MDA concentrations in fat from meat and the TBA numbers of meat generated by cooking and refrigerated storage are strongly dependent on the initial MDA and TBA values of the samples before those treatments.

Table 2. Malondialdehyde concentration after cooking, refrigerator storage and reheating in meat samples which had been frozen for 6 months

Meat sample	μg MDA/g fat	% fat	TBA number
Convection oven			
Breast			
after cooking	53.6 ± 3.91^{cd}	1.73 ± 0.10^{a}	0.93 ± 0.08^{ab}
1-day storage	65.5 ± 4.91^{de}	1.74 ± 0.09^{a}	1.13 ± 0.10^{ab}
4-days storage	85.8 ± 4.84^{f}	1.75 ± 0.08^{a}	1.49 ± 0.11^{c}
after reheating	85.3 ± 5.25^{f}	1.83 ± 0.08^{a}	1.59 ± 0.10^{c}
Leg			
after cooking	22.0 ± 2.08^{a}	3.91 ± 0.10^{b}	0.86 ± 0.08^{a}
1-day storage	26.5 ± 2.12^{ab}	3.93 ± 0.13^{b}	1.04 ± 0.10^{ab}
4-days storage	35.2 ± 2.51^{b}	3.97 ± 0.11^{b}	1.40 ± 0.10^{c}
after reheating	34.9 ± 2.67^{b}	4.12 ± 0.13^{b}	1.44 ± 0.11^{c}
Microwave oven			
Breast			
after cooking	49.8 ± 3.09^{c}	1.60 ± 0.08^{a}	0.80 ± 0.08^{a}
1-day storage	64.4 ± 5.03^{cde}	1.59 ± 0.07^{a}	1.03 ± 0.19^{ab}
4-days storage	83.1 ± 5.95^{ef}	1.60 ± 0.07^{a}	1.33 ± 0.13^{bc}
after reheating	82.5 ± 5.35^{ef}	1.67 ± 0.10^{a}	1.38 ± 0.15^{c}
Leg			
after cooking	23.4 ± 2.39^{a}	3.79 ± 0.08^{b}	0.89 ± 0.09^{ab}
1-day storage	26.7 ± 2.27^{ab}	3.80 ± 0.09^{b}	1.01 ± 0.09^{ab}
4-days storage	35.7 ± 2.66^{b}	3.83 ± 0.09^{b}	1.37 ± 0.16^{c}
after reheating	36.7 ± 2.75^{b}	3.94 ± 0.10^{b}	1.42 ± 0.16^{c}

Whole data are presented as means ± standard deviations of four samples. Mean values within each column which do not have the same superscript letter are significantly different ($P < 0.05$, two tailed t-test).

Fluorescent lipid oxidation products of meat

Fluorescent oxidation products in the organic (total fat extract) and aqueous (soluble protein) layers obtained from Folch extraction of breast and leg meat were

determined by fluorescence excitation and emission spectroscopy. The relative levels of fluorescent products in the organic and aqueous layers from fresh breast meat were 76.3 FU/mg fat and 1.01 FU/μg protein, respectively; and for leg meat were 38.4 FU/mg fat and 1.39 FU/μg protein. After 3 months of frozen storage, values from breast meat extractions increased to 91.2 FU/mg fat and 1.13 FU/μg protein and from leg meat extractions increased to 42.7 FU/mg fat and 1.55 FU/μg protein. After 6 months of frozen storage, values from breast meat extractions were 140.4 FU/mg fat and 1.25 FU/μg protein and values from leg meat extractions were 59.1 FU/mg fat and 1.83 FU/μg protein.

The relative levels of fluorescent products in organic and aqueous layers obtained from Folch extractions of 3- and 6-month stored breast and leg meat samples after convection and microwave oven cooking, refrigerated storage, and reheating are presented in Tables 3 and 4. The fluorescent products in the organic layers obtained from Folch extracted 3- and 6-month frozen meat always increased after cooking, refrigerated storage for 1 and 4 days and reheating. There were no significant differences before and after reheating.

Table 3. Relative levels of fluorescent products present in the organic and aqueous layers from Folch extracted meat samples which had been frozen for 3 months

	Fluorescence units (FU)	
Meat sample	FU/mg fat	FU/μg protein
Convection oven		
Breast		
after cooking	103.1 ± 5.04^{c}	1.39 ± 0.08^{abc}
1-day storage	133.4 ± 5.18^{d}	1.56 ± 0.08^{cd}
4-days storage	173.6 ± 5.92^{e}	1.90 ± 0.09^{ef}
after reheating	184.2 ± 5.46^{e}	2.07 ± 0.08^{f}
Leg		
after cooking	46.7 ± 2.16^{a}	1.84 ± 0.09^{def}
1-day storage	56.6 ± 2.31^{b}	2.05 ± 0.09^{ef}
4-days storage	94.2 ± 3.42^{c}	2.46 ± 0.10^{g}
after reheating	103.3 ± 4.42^{c}	2.75 ± 0.10^{g}
Microwave oven		
Breast		
after cooking	105.3 ± 5.34^{c}	1.17 ± 0.06^{a}
1-day storage	137.1 ± 5.48^{d}	1.25 ± 0.08^{ab}
4-days storage	166.2 ± 5.73^{e}	1.38 ± 0.10^{abc}
after reheating	173.8 ± 6.09^{e}	1.49 ± 0.10^{abcd}
Leg		
after cooking	51.5 ± 2.30^{ab}	1.60 ± 0.05^{cd}
1-day storage	59.1 ± 1.79^{b}	1.71 ± 0.09^{cde}
4-days storage	96.0 ± 3.07^{c}	1.90 ± 0.08^{ef}
after reheating	104.6 ± 3.45^{c}	2.16 ± 0.07^{f}

Whole data are presented as means $\pm$ standard deviations of four samples. Mean values within each column which do not have the same superscript letter are significantly different ($P < 0.05$, two tailed t-test).

Table 4. Relative levels of fluorescent products present in the organic and aqueous layers from Folch extracted meat samples which had been frozen for 6 months

	Fluorescence units (FU)	
Meat sample	FU/mg fat	FU/μg protein
Convection oven		
Breast		
after cooking	154.7 ± 5.43^{c}	1.69 ± 0.08^{bc}
1-day storage	185.1 ± 5.91^{f}	1.90 ± 0.10^{cd}
4-days storage	233.1 ± 5.36^{g}	2.18 ± 0.11^{def}
after reheating	233.7 ± 5.11^{g}	2.38 ± 0.10^{fg}
Leg		
after cooking	66.5 ± 2.67^{a}	2.30 ± 0.09^{ef}
1-day storage	74.8 ± 2.18^{ab}	2.56 ± 0.10^{fgh}
4-days storage	115.8 ± 3.83^{c}	2.76 ± 0.12^{gh}
after reheating	125.0 ± 3.87^{cd}	3.02 ± 0.14^{h}
Microwave oven		
Breast		
after cooking	158.1 ± 5.07^{e}	1.30 ± 0.06^{a}
1-day storage	191.8 ± 5.06^{f}	1.45 ± 0.06^{ab}
4-days storage	221.2 ± 5.36^{g}	1.60 ± 0.08^{bc}
after reheating	223.8 ± 5.29^{g}	1.74 ± 0.10^{bc}
Leg		
after cooking	69.1 ± 1.89^{a}	1.95 ± 0.10^{cde}
1-day storage	80.5 ± 2.31^{b}	2.32 ± 0.12^{efg}
4-days storage	128.6 ± 2.86^{cd}	2.49 ± 0.15^{fgh}
after reheating	136.8 ± 3.18^{d}	2.71 ± 0.10^{gh}

Whole data are presented as means $\pm$ standard deviations of four samples. Mean values within each column which do not have the same superscript letter are significantly different ($P < 0.05$, two tailed t-test).

Fluorescent products in the aqueous layers from breast or leg meat were significantly higher ($P < 0.05$) after convection cooking but not after microwave cooking compared to raw meat. This difference in cooking effects between convection and microwave oven continued throughout refrigerated storage and reheating for both leg and breast meat as indicated in statistical comparisons in Table 3 where convection oven cooked samples always had significantly higher fluorescent products (FU/μg protein) compared to corresponding microwave oven cooked samples. This same trend was also apparent in Table 4 where 6-month frozen breast meat samples which were initially cooked by convection oven always have significantly higher fluorescent products compared to corresponding samples which were initially cooked by microwave oven.

Discussion

Meat rancidity is due largely to lipid oxidation and it is the polyunsaturated fatty acids (PUFA) of lipid which are particularly susceptible. Chemical oxidation of PUFA generates various oxidation products, but especially MDA, a compound which has

been associated with human cancer and heart disease (Pearson *et al.*, 1983). The TBA assay is the most popular method for measuring MDA in meat, but it is unreliable due to variable degrees of sample autoxidation during homogenization, distillation or extraction, and boiling steps of the assay (Pikul *et al.*, 1983; Melton, 1983). In our study, an improved TBA assay with antioxidant protection (Pikul *et al.*, 1983) was used to measure the concentration of free MDA in fat extracted from meat which was treated with a series of food processes common in most households. In our study, the TBA numbers of both raw and cooked chicken meat samples were similar to values obtained by Yamauchi *et al.* (1982), who protected their samples with antioxidants during TBA analysis by a steam distillation method. We found no significant differences in free MDA concentrations or TBA numbers in chicken meat between microwave and convection oven cooking methods.

Our analyses of free MDA in chicken meat have revealed two important points. First, the concentration of MDA in fat from breast is more than double that in fat from leg. This difference can be explained by fat composition. Fat from breast meat contains twice the proportion of phospholipid fraction than fat from leg meat; and the fatty acids in phospholipids are much more polyunsaturated than those in triglycerides, and therefore more susceptible to oxidation. The calculated TBA number of chicken leg meat is higher than that of breast meat mainly because leg meat contained more than twice as much fat as breast meat. Second, the absolute level of MDA generated by meat processing is strongly dependent on the initial MDA levels of meat before processing. The higher initial MDA levels in fat from fresh breast meat compared to leg meat results in proportionately higher MDA in processed breast compared to processed leg meat. The higher initial MDA levels in meat stored frozen 6 months compared to meat stored 3 months also resulted in proportionately higher MDA content in 6-month frozen meat after further processing. Therefore, it is concluded that the initial malonaldehyde levels in meat, which strongly depend on fat content, composition, and fatty acid composition, determine the absolute MDA levels in that meat after cooking, refrigerated storage and reheating.

Free MDA reacts with amino acids, proteins, amino-containing phospholipids and itself to form fluorescent products (Chio & Tappel, 1969; Pearson *et al.*, 1983). The measurement of these fluorescent products which are soluble in chloroform/methanol solvents has been successfully used as an analytical method for quantitation of peroxidation damage to biological tissue (Pearson *et al.*, 1983). In our study, cooking had a greater effect on the generation of fluorescent products in the aqueous layer from Folch extraction compared to the organic layer; however, refrigerated storage after cooking and frozen storage before cooking, generated a relatively greater increase of fluorescence in the organic compared to the aqueous extract. For instance, meat which was frozen for 6 months had an 11% increase of fluorescence per g fat, but a 31% increase of fluorescence per μg protein. After 4 days of refrigerated storage, the fluorescence of cooked meat increased 62% per g fat, but only 25% per μg protein. We found significantly more fluorescent products, especially in the aqueous layers of extractions, in meat cooked by convection oven compared to microwave oven. These data may relate to our observation of visibly more browning reaction on the surface of convection cooked chicken meat. Generally, we conclude that primarily the initial levels of fluorescent products in meat determine the absolute levels of fluorescent products after processing.

Acknowledgments

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Moisture and temperature dependence of thermal diffusivity of cod minces

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Summary

The moisture content M, of minced cod (*Gadus morhua*) was varied between 33 and 90% by drying and water addition. The thermal diffusivity, a, of the minces was measured in the temperature range from −40 to +38°C and found to increase with increasing M at all temperatures. In the unfrozen state the relationship between a and M is linear, $a(M) = A + BM$, B decreasing with increasing temperature. In the frozen state the relationship is non-linear but can be approximated by $\log a(M) = C + DM$ with D positive and increasing with increasing temperature.

Introduction

The rate at which heat diffuses through a material depends on the thermal diffusivity, defined as:

$$a = \frac{k}{\rho c} \tag{1}$$

where ρ is the density, k the thermal conductivity and c the specific heat capacity of the material.

Data on the thermal properties of foodstuffs are needed to understand their thermal behaviour and to control heat transfer processes. The existing data are rather scattered and not systematically related to the composition of the foodstuffs. This hinders the use of the data and the development of models for predicting the thermal properties of foodstuffs from their composition (Lamb, 1976; Miles, van Beek & Veerkamp, 1983).

The component which most strongly influences the thermal properties of water-containing materials is the moisture content, M. This is because the thermal properties of water are markedly different from those of the other components (proteins, fat, carbohydrates and air). The presence of water also causes a strong temperature dependence of the thermal properties, especially in the freezing range (i.e. between approximately −40°C and the initial freezing point, T_f).

Riedel (1969) measured the thermal diffusivity of fifteen different foodstuffs with M ranging from 30 to 100% (wet basis). Hermans (1979) measured the thermal properties of a model food (a mixture of water, oil and starch with M ranging from 50 to 95%) and also carried out a regression analysis of 160 observations taken from the literature. Both Riedel and Hermans confirmed the strong moisture and temperature dependence of thermal diffusivity and expressed the dependence mathematically. However, these authors considered only the non-freezing range.

The object of this paper is to report the results of thermal diffusivity measurements in both the freezing and non-freezing ranges (from −40 to +38°C) for minces prepared from cod (*Gadus morhua*). The cod was minced because in this state it could be dried

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easily and uniformly. Also, additional water could be introduced to the mince to increase its moisture content above the natural level. This enabled the moisture content of a single type of material to be varied from 33 to 90% (wet basis) and the thermal diffusivity to be measured as a function of moisture content over this range.

Materials and methods

Sample preparation and characterization

In each experiment fresh cod fillets were skinned, deboned and passed through a mincer with 4-mm diameter perforations.

Quantities of 3–4 kg of mince were spread out in approximately 1-cm thick layers on several trays and dried in a wind tunnel at an air temperature 24–25°C, wind speed 1.2 m/s and ambient humidity. The mince was stirred at intervals to ensure uniform drying. After drying to the required moisture content (monitored by weighing the trays) the mince was either used immediately or sealed in polythene bags and frozen for later use. Before the thermal diffusivity measurement the frozen mince was broken up by a bowl chopper and thawed by air at room temperature.

To achieve moisture contents higher than 80%, water and ice were added to the mince, the mixture being equilibrated overnight. Before commencing the measurement, excess water was drained off (the remainder being held by capillary forces).

The moisture contents of the minces were determined gravimetrically using vacuum oven drying at 80°C for 26 hr. For each mince six representative samples of 30–50 g each were analysed, yielding the mean and standard deviation of the moisture content, given in Table 1.

The densities of the minces (Table 1) were determined from the masses (ranging from 1.09 to 2.42 kg) and the volume (2.3×10^{-3} m^3) of the samples contained in the thermal diffusivity measuring apparatus.

Table 1. A summary of sample characteristics

Sample code	Treatment	Moisture content M (% wet basis)	Density, ρ (kg/m^3)
1	water added	90.4 ± 0.2	1050
2	water added	87.6 ± 1.8	1040
3	none	81.2 ± 0.2	866
4	1 h drying	78.8 ± 0.4	887
5	11 h drying	73.9 ± 0.2	902
6	21 h drying	62.1 ± 0.4	574
7	25 h drying	53.2 ± 0.9	702
8	26 h drying	50.5 ± 4.0	626
9	29 h drying	33.7 ± 4.0	473

Method of thermal diffusivity measurement

The apparatus and the method used was essentially that described by Nesvadba (1982).

The two major surfaces of a slab sample ($20 \times 17 \times 7$ cm) were simultaneously cooled or heated by temperature controlled metal plates to generate approximately symmetrical temperature profiles across the sample. Examples of the profiles are shown

in Fig. 1. The profiles were measured at intervals of $\Delta t = 60$ sec by twenty thermocouples embedded in the sample. The thermocouple junctions were arranged in a line passing through the centre of the slab. The thermocouple spacing was $\Delta x = 2.54$ mm.

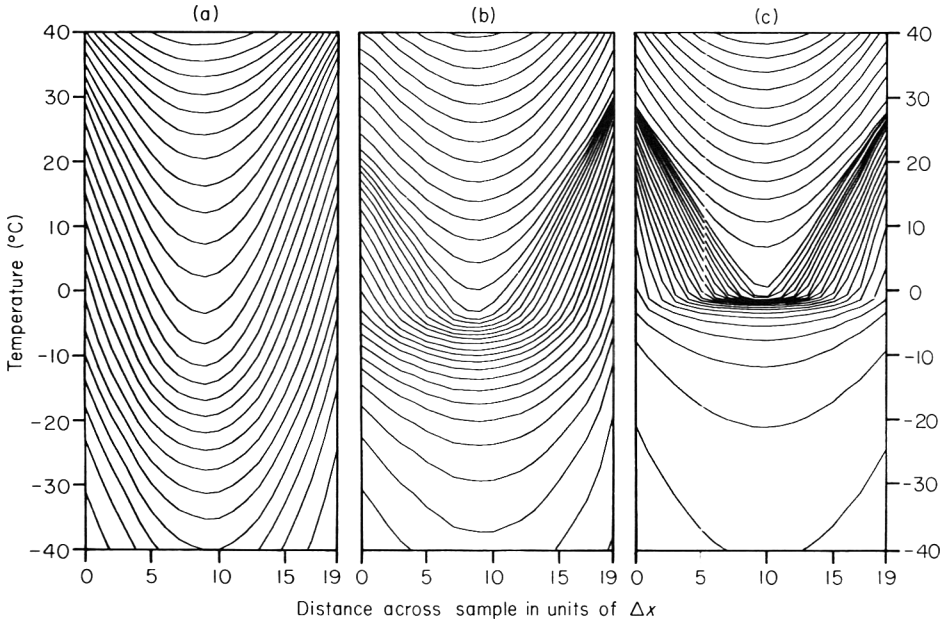

Figure 1. Temperature profiles in samples of cod mince during thawing: $\Delta t = 360$ sec (only every sixth profile plotted), $\Delta x = 2.54$ mm. Moisture contents: (a) 33.7%, (b) 53.2%, (c) 87.6%.

The peripheral surfaces of the sample were thermally insulated so that the heat flow could be regarded as one-dimensional, normal to the plates. For each profile, the first and second partial derivatives with respect to time and co-ordinate, respectively, were obtained by numerical differentiation at the local extremum of the profile (i.e., at approximately the mid-plane of the sample). The thermal diffusivity was calculated as the ratio of the derivatives.

The precision of thermal diffusivity measurement was 3–10% for temperatures outside the range −10 to +5°C. At temperatures near T_f the measurements were much less precise because the time derivative was small and comparable with the uncertainty of its estimation (Nesvadba, 1982).

During the measurement the mid-plane temperature varied between −40 and +38°C and thus the thermal diffusivity was obtained at a large number of temperatures in this range (one data point for each temperature profile). Since these temperatures did not necessarily coincide with the specific temperatures required for data presentation in this paper (−40, −30, −20, −10, 10, 25, 35°C) the thermal diffusivity values were calculated using points neighbouring the specific temperatures.

Results

Regime dependence of thermal diffusivity

At temperatures below T_f the thermal diffusivity depended on whether the measurement was made during freezing or thawing. The values obtained during

thawing, a_t, were almost always higher than those obtained during freezing, a_f. The average difference expressed as a percentage, $2\times(a_t-a_f)/(a_t+a_f)\times 100\%$, was 19.5%.

In the following presentation average values, $(a_t+a_f)/2$, are given.

No significant regime dependence was observed at temperatures above T_f.

Temperature dependence of thermal diffusivity

Plots of thermal diffusivity against temperature for each moisture content are shown in Fig. 2. Each of the curves approximates a large number of data points obtained during freezing and thawing in the range from −40 to 38°C. Only the data points for specific temperatures are shown for clarity. A logarithmic scale is used to accommodate the large range of thermal diffusivity values.

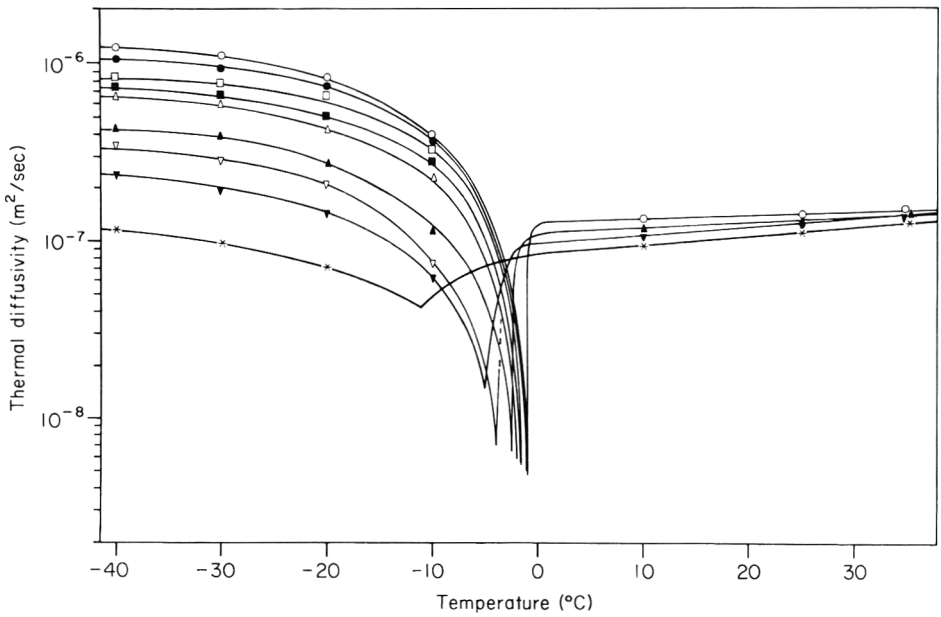

Figure 2. Thermal diffusivity of nine cod minces with varying moisture contents. Data points for samples 1–9 (see Table 1) denoted by: ○, ●, □, ■, △, ▲, ▽, ▼ and ★, respectively. Only four curves shown above freezing for clarity.

Above T_f the thermal diffusivity increases with temperature. The dependence can be approximated by straight lines; the slope of the lines increases with decreasing moisture content from 5.2×10^{-10} m²/sec per °C at 90.4% (for sample 1) to 1.2×10^{-9} m²/sec per °C at 33.7% (sample 9).

At temperatures below T_f the thermal diffusivity increases markedly as the temperature decreases, the rate of increase falling as the temperature approaches −40°C.

The thermal diffusivities are lowest at the initial freezing temperatures. These temperatures decrease with decreasing moisture content from −1°C at 90.4% (for sample 1) to −11°C at 33.7% (sample 9).

Moisture dependence of thermal diffusivity

Non-freezing range. Thermal diffusivity data for 10, 25 and 35°C are plotted against

moisture content in Fig. 3. The data are approximated by straight lines, the slope of which decreases with increasing temperatures. A linear regression model $a(M) = A + BM$ gives the results shown in Table 2. The last column in Table 2 shows the standard deviation of the measured thermal diffusivity values about the regression line (the standard error of prediction).

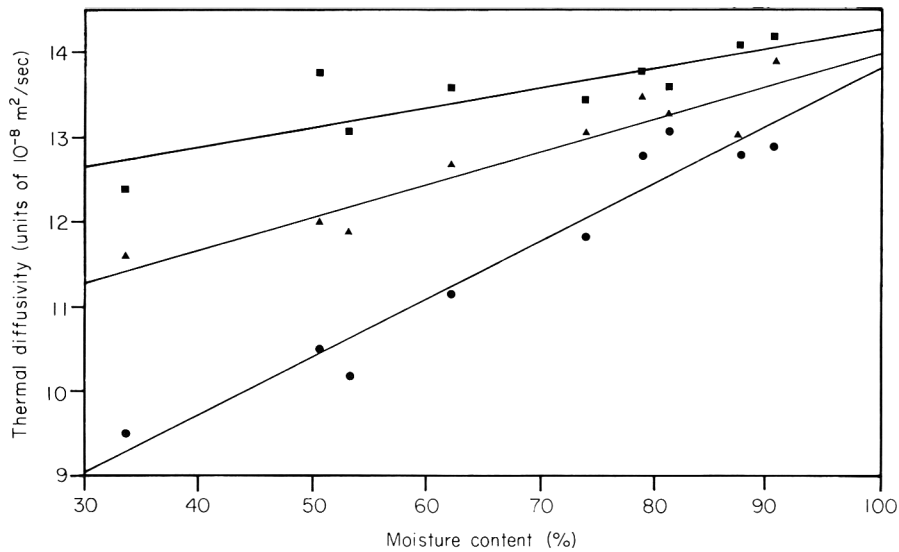

Figure 3. Thermal diffusivity values at 10 (●), 25 (▲) and 35°C (■) (non-freezing range) plotted against moisture content.

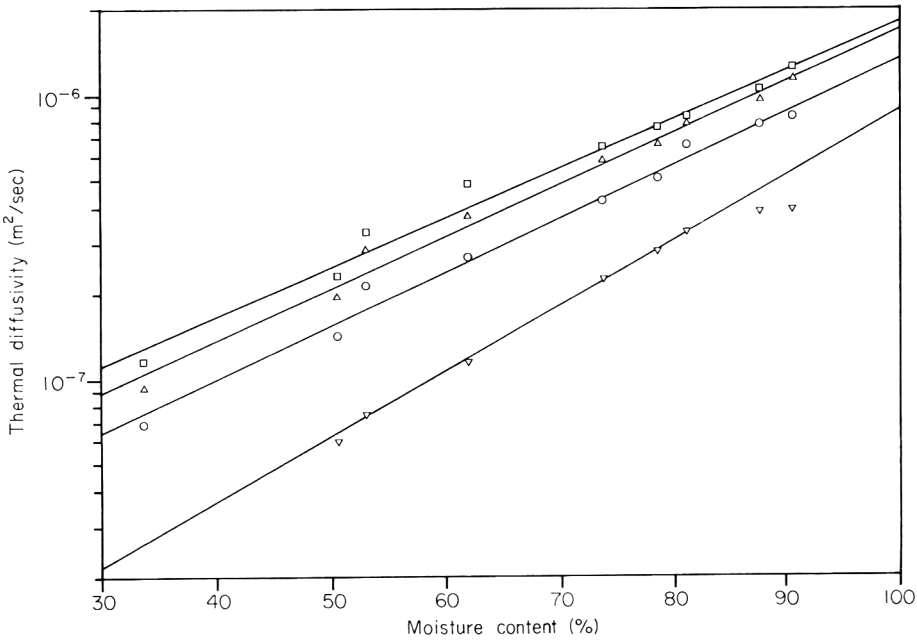

Figure 4. Thermal diffusivity values at −40 (□), −30 (○), −20 (O) and −10°C (△) (freezing range) plotted against moisture content.

Freezing range. Thermal diffusivity values for −40, −30, −20 and −10°C are plotted against M on a semi-logarithmic scale in Fig. 4. On this scale the data can be approximated by straight lines, the slope of the lines decreasing with decreasing temperature. A linear regression model $\log_{10} a(M) = C + DM$ gives the results shown in Table 2. The standard error of prediction refers to the scatter of $\log_{10} a(M)$ and is typically 0.045 (at −30°C). The corresponding relative standard error of $a(M)$ is thus approximately 11%, higher than in the non-freezing range where it is 2–4%. The standard errors of prediction are comparable with the estimated experimental errors of thermal diffusivity measurement (Nesvadba, 1982).

Table 2. Regression coefficients for moisture dependence of thermal diffusivity of cod minces

Non-freezing range, model: $a(M) = A + BM$ (10^{-7} m²/sec, %)			
Temperature (°C)	A (10^{-7} m²/sec)	B (10^{-7} m²/sec/%)	Standard error of prediction (10^{-7} m²/sec)
10	0.70 ± 0.05	0.00683 ± 0.00065	0.035
25	1.01 ± 0.04	0.00385 ± 0.00054	0.029
35	1.19 ± 0.04	0.00236 ± 0.00060	0.032
Freezing range, model: $\log_{10} a(M) = C + DM$ (10^{-7} m²/sec, %)			
Temperature (°C)	C (−)	D (%$^{-1}$)	Standard error of prediction (−)
−40	−0.475 ± 0.051	0.0173 ± 0.0002	0.040
−30	−0.594 ± 0.058	0.0183 ± 0.0008	0.045
−20	−0.755 ± 0.057	0.0188 ± 0.0008	0.044
−10	−1.341 ± 0.093	0.0228 ± 0.0013	0.051

Discussion

Regime dependence of thermal diffusivity

The reason for the difference between data obtained during thawing and freezing is not known at present. Tests will be made to ascertain whether imperfect thermal insulation on the periphery of the slab sample and heat leaking into the sample are causing the rate of freezing to be slower (and the rate of thawing faster) than the rate with perfect insulation. It is intended to use a long cylindrical sample subjected to radial heat flow. In this arrangement the possibility of heat leak will be minimized.

If the difference between the thawing and freezing data is still observed then it will be necessary to consider other possible explanations. Those connected with the method could be slipping of thermocouples relative to the sample (caused by thermal expansion or contraction) or thermal contact problems.

The regime dependence could also be caused by rate dependent processes altering the state of the sample in different ways during freezing and thawing. The possible processes include:

(i) moisture migration induced by thermal gradients;
(ii) ice recrystallization on thawing;

(iii) diffusion limited crystallization of ice (causing the ice content during rapid freezing to be below the equilibrium content); and

(iv) large internal stresses developing in the sample during freezing; Miles & Morley (1978) showed that these stresses decrease the temperature in the centre of meat samples.

Temperature and moisture dependence of thermal diffusivity

Non-freezing range. The linear increase of thermal diffusivity with temperature above T_f is in agreement with observations of Hermans (1979) and Riedel (1969). However, in the present work the slope of the lines $a(T)$ decreases with increasing moisture content (Fig. 2) while Hermans gives a moisture independent slope (3×10^{-10} m^2/sec per °C) and Riedel finds the slope increasing with increasing moisture content.

Similarly, the slope of the isotherms $a(M)$ for cod minces decreased with increasing temperature (Fig. 3) while both Hermans and Riedel found the slope increased with increasing temperature.

This apparent disagreement can be explained in terms of the difference in the structure of the measured materials. Riedel and Hermans analysed materials which were all non-porous. The dried minces used in the present work are extremely porous and therefore contained appreciable amounts of air and water vapour. Heat transfer in the minces therefore involves not only conduction through the matrix (liquid water/protein fibres) but also heat transfer across the air and vapour filled spaces in the matrix by radiation, convection and possibly by evaporation-condensation. The rate of these processes increases strongly with temperature whilst the temperature dependence of conduction through the matrix is relatively unimportant. Thus, as the porosity of minces increases (and M decreases) the heat transfer is activated to a greater extent by temperature which explains why the slope of the lines $a(T)$ in Fig. 2 increases with decreasing moisture content.

Freezing range. As temperature decreases below T_f, the water in the sample freezes out of a progressively more saturated salt and protein solution. With the increasing amount of ice the thermal conductivity of the sample increases and the quantity ρc in the expression (1) decreases, the combined effect being a marked increase in thermal diffusivity (Fig. 2). The thermal diffusivity increases with decreasing temperature at all moisture contents and therefore the isotherms $a(M)$ for lower temperatures are above those for higher temperatures (Fig. 4).

At any given temperature the ice content increases with increasing moisture content and therefore $a(M)$ is an increasing function, as in the unfrozen state. However, owing to T_f increasing with increasing M, the amount of ice increases with increasing M faster at lower temperatures than at higher temperatures and therefore the slope of $a(M)$ isotherms increases with increasing temperature (Fig. 4).

To the authors' knowledge no previous work has been published with which to compare these results.

Density dependence of thermal diffusivity

The density of cod minces shown in Table 1 does not decrease monotonically with the moisture content. This means that the density depends not only on the moisture content but also to some extent on the way in which the cod was minced and dehydrated and on the degree of compression of the mince in the measuring apparatus.

Since thermal diffusivity depends on density some of the scatter in the data (Figs 3 and 4) could be removed by taking the density into account not only implicitly (via the moisture content) but explicitly as a second independent regression variable. This was confirmed by employing a bivariate regression model $a(M,\rho) = P+QM+R\rho$. However, at none of the selected temperatures was the reduction in the residual sum of squares significant (at 5% level). This means that the simple regression model using moisture content only explains adequately the observed variation in thermal diffusivity of cod minces.

Conclusions

The thermal diffusivity of cod minces increases linearly with temperature above freezing, the slope of the graphs $a(T)$ decreasing with increasing moisture content. For temperatures below freezing, thermal diffusivity decreases non-linearly with increasing temperature, the rate of decrease being highest near the initial freezing point.

The thermal diffusivity of cod minces increases with increasing moisture content for temperatures both above and below freezing. Above freezing $a(M)$ is approximately linear with slope decreasing with increasing temperature. In the frozen state $\log a(M)$ is approximately linear with slope increasing with increasing temperature.

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A laboratory-scale technique for controlled production of Cheddar cheese

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Summary

A laboratory technique for the production of Cheddar cheese has been developed. It has been shown that high-quality Cheddar cheese, identical in chemical and organoleptic properties to commercial Cheddar cheese can be produced using the techniques described. The system is particularly effective for studies on cheese yield.

Introduction

The yield and quality of Cheddar cheese produced from a given batch of milk has been of prime interest to the cheesemaker for over a century and the pioneering work of Van Slyke (Van Slyke & Price, 1927) laid down principles which remain valid. However, despite a substantial literature on cheese yield (e.g., Davis, 1965) and the major advances which have been made in the fundamental understanding of the composition of milk, control of the yield of Cheddar cheese in the factory still depends on the traditional skills of individual cheesemakers. Unfortunately, the scope for intervention by the cheesemaker has been restricted by the advent of modern continuous cheesemaking systems (see Crawford, 1976, for a review). As a result an overall deterioration in cheese yield has been observed in some factories. Against this background of change, a re-examination of factors influencing cheese yield and quality has been instigated.

In a preliminary investigation, the relationship between seasonal variation in milk composition and cheese yield at two factories in southwest Scotland was studied over a 2-year period (Banks *et al.*, 1981). The results of this survey indicated that there was a significant difference in efficiency of cheese production between factories and also between years at the same factory. The variations in yield were attributed to differences both in raw-milk composition and in manufacturing technique. As a result, it was decided that to obtain a clearer understanding of the effects of milk composition on cheese yield and quality the method of cheese production would have to be standardized. Further work in the factory was considered impractical because of the difficulties of interrupting normal production schedules and it was equally difficult to scale down the automated curd handling equipment used in industry. Therefore, a laboratory-scale Cheddar cheesemaking system, which was based on traditional methodology, but allowed precise control of processing variables, was developed. This paper describes the apparatus and methodology suitable for production of Cheddar cheese identical in composition and organoleptic properties to the equivalent commercial cheese. The procedures used to obtain an overall assessment of mass, total solids, nitrogen and fat balances are also described.

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Materials and Methods

Cheese production

Milk samples. Bulk milk from the Hannah Research Institute Farm tank was collected on the morning of cheesemaking and pasteurized at 72°C for 16 sec.

Starter cultures. A combination of two single strain starters, AM_2 (NCDO 1986) and ML_8 (NCDO 1994) were used in the ratio of 2:1. A culture programme, shown schematically in Fig. 1, was devized to eliminate the need for continuous subculture, thereby ensuring a continuous supply of reproducible starter activity.

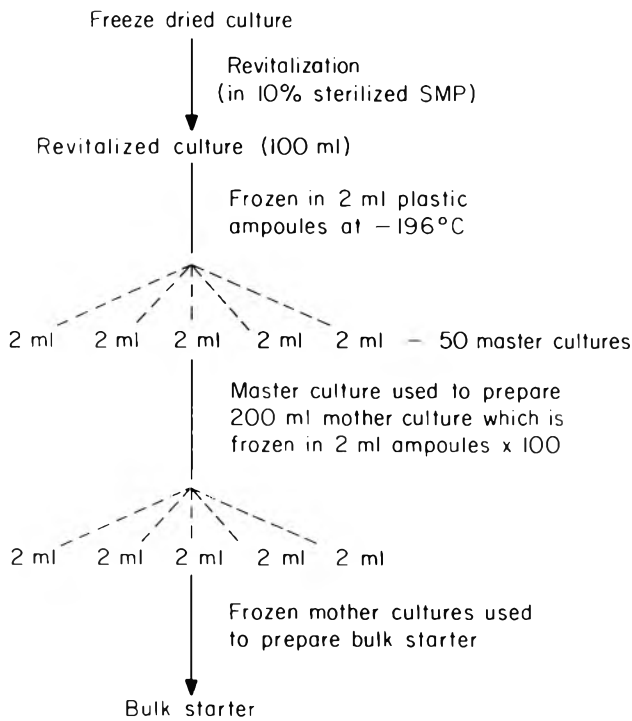

Figure 1. Starter culture programme.

Freeze-dried cultures were obtained from the National Collection of Dairy Organisms (NCDO), Shinfield, Reading. Culture activity was revived by propagation in a 10% (w/w) sterilized skim milk powder (SMP) solution. One per cent (v/v) of active culture was inoculated into 10% (w/w) sterilized SMP solution and incubated at 22°C for 16 hr. The culture was then transferred to 2-ml sterile ampoules (Union Carbide) which were heat sealed, rapidly frozen and stored in a liquid nitrogen refrigerator at −196°C.

Bulk starters for cheesemaking were prepared by thawing the liquid nitrogen cultures at room temperature for 2 hr, inoculating into 10% (w/w) sterilized SMP solution at a level of 1% (v/v) and incubating at 22°C for 16 hr. Starter activity was then assessed by determination of pH. Cultures of AM_2 and ML_8 were prepared separately and were added to the milk for cheesemaking at levels of 1 and 0.5% (w/w), respectively.

The growth medium used in all culture preparation was an antibiotic free heat treated dried skim milk. To prevent deterioration in powder quality over the extensive experimental period, the powder was canned, under nitrogen, in batch quantities and stored at −40°C. For preparation of growth media, the powder was reconstituted to 10% (w/w) total solids, sterilized at 110°C for 10 min, and cooled to 10°C prior to inoculation with starter.

Rennet. A standard preparation of calf rennet (Chr. Hansen's Laboratory Ltd) was used at a level of 0.03% (w/w) in cheesemaking.

Salt. Pure vacuum dried salt (Imperial Chemical Industries Ltd) was used in salting the milled curd.

Cheesemaking apparatus

The cheese was made entirely within rectangular vats (305×200×200 mm, capacity 10 litres) fabricated from 16 gauge 316 grade stainless steel with radiused corners and a centrally sloping floor (*circa* 5 slope) (Figs 2a, b and 3. An engineering drawing of the

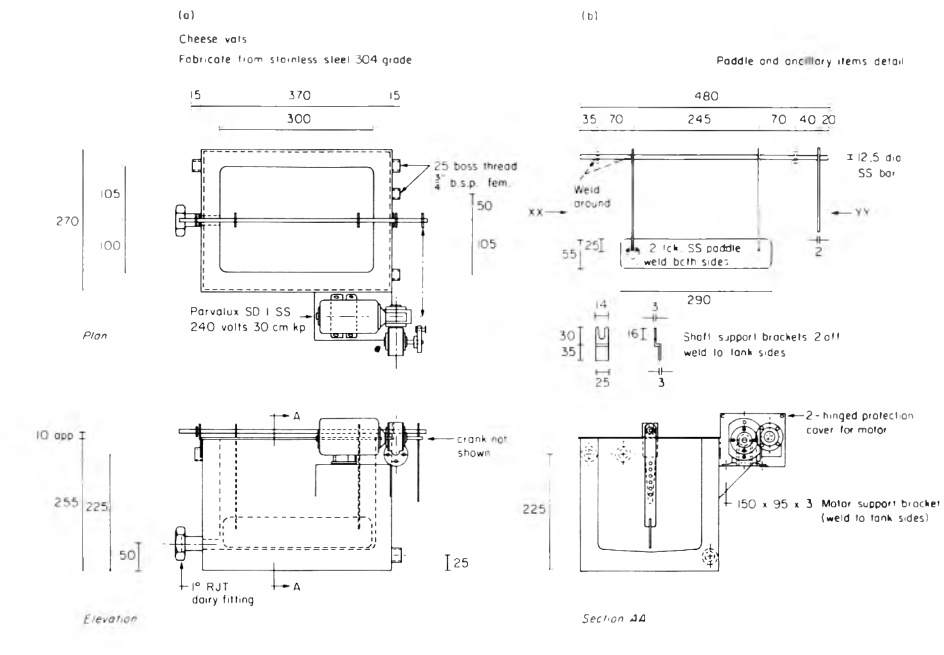

Figure 2. Apparatus for cheese manufacture; dimensions mm.

apparatus is available on request). Each vat was fitted at one end with a 25.4-mm bottom outlet to which a stainless steel sieve could be attached. A paddle (290×60-mm blade) was centrally mounted in the vat with a blade clearance of 12 mm. The paddle was driven by an eccentric arm to describe an arc which transversed the breadth of the tank. The electric motor (Parvalux 250 VAC) driving the arm was fitted with gearing and a thyristor speed controller (SEA Electronic Power Controller) to allow stirring to be controlled in the range of 10–25 excursions per min. In routine processing of cheese a stir speed of 15 rev/min was used. The vat was completely enclosed by a stainless steel

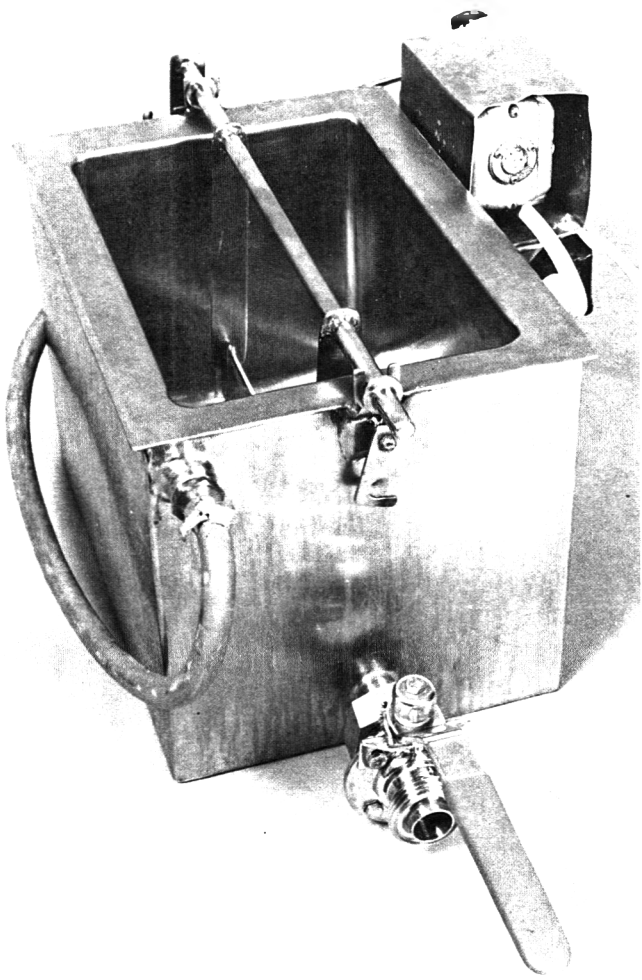

Figure 3. Model (10 litre) cheese vat.

jacket with a uniform 25-mm space all round through which the water was circulated by an electronically controlled flow heater (Grant Instruments, Cambridge, Model FH15).

The coagulum, when formed, was cut using knives scaled down from the traditional horizontal and vertical cheese knives. The knives were 90 mm wide by 300 mm long with 1-cm blades spaced at 11 mm separation (Fig. 4).

To allow pressure to be applied to curd during the cheddaring process in the vat, a cheddaring box was fabricated from 16 gauge 316 stainless steel (205 × 150 × 360 mm) and the outside of the box, which comes into contact with the cheese curd, was polished (Fig. 5). Pressure on the curd was adjusted by placing multiples of 2-kg weights inside the box.

The finished curd was packed into stainless steel cylindrical (130 mm diameter) perforated moulds (Perfora Ltd) which were lined with strips of synthetic cheese cloth and after filling were pressed in a gang press constructed as shown in Fig. 6.

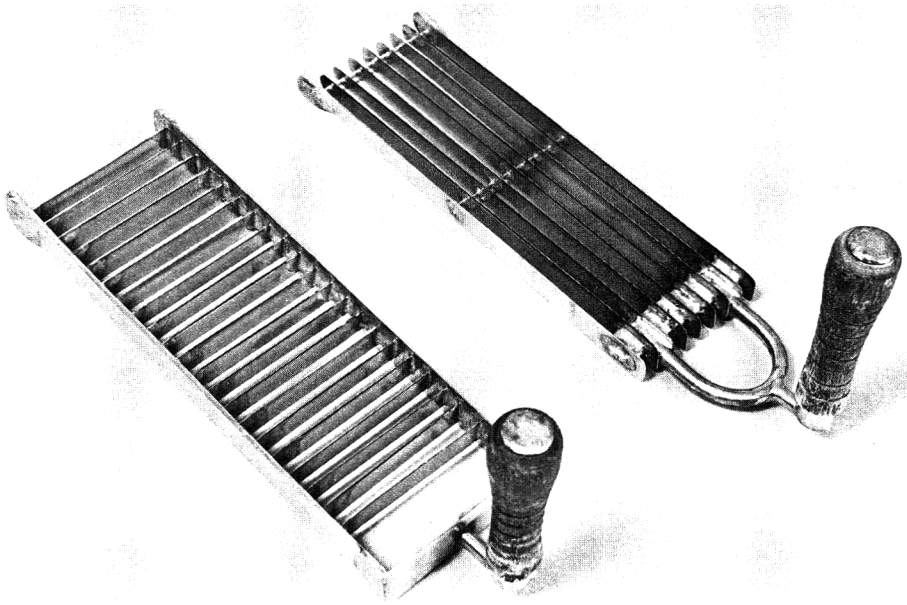

Figure 4. Model cheese knives.

Throughout the cheesemaking process, acidity development was estimated by pH measurement using a Philips PW9409 digital pH meter fitted with a cheese electrode (406 M6 E7) and temperature compensating probe (PT100).

Standard process of cheese manufacture

Pasteurized milk (10 450 g) at 30°C was weighed into the vat and, after mixing, three aliquots (150 g) were removed into sterile sample jars for routine chemical and microbiological analysis. Starters (100 g AM_2, 50 g ML_8) were weighed into the vat and the milk was then ripened for 15 min prior to addition of rennet (3.0 g). Stirring was continued for 5 min after rennet addition and the central paddle was then removed from the vat. Coagulum formation was allowed to proceed for 45 min, after which the pH and temperature of the curd prior to cutting were recorded. A standard procedure of cutting was devized to ensure uniformity in the size of the curd particles produced. The vertical knife was first drawn lengthwise along the vat and then across it. This procedure was then repeated with the horizontal knife. This cutting operation was completed in approximately 3 min, after which the curd was allowed to 'heal' for a further 7 min. The central paddle was replaced in the vat and stirring was continued at 15 rev/min, while the temperature control unit was used to raise the temperature of the curd-whey mixture from 30 to 39°C in a linear manner over a period of 1 hr. The mixture was stirred isothermally (at 39°C) for a further hour, after which the central paddle was removed and the curd allowed to settle (10 min). The vat sieve was placed in the vat outlet during settling, and the pH and temperature of the whey were recorded. The vat outlet valve was then opened and the whey was removed. A domestic flour sieve was used to collect curd particles which were trapped in the vat outlet valve on the external side of the main vat sieve, and consequently were removed with the whey. These particles were returned to the curd in the vat. Whey removal proceeded over 10 min, after which the granular

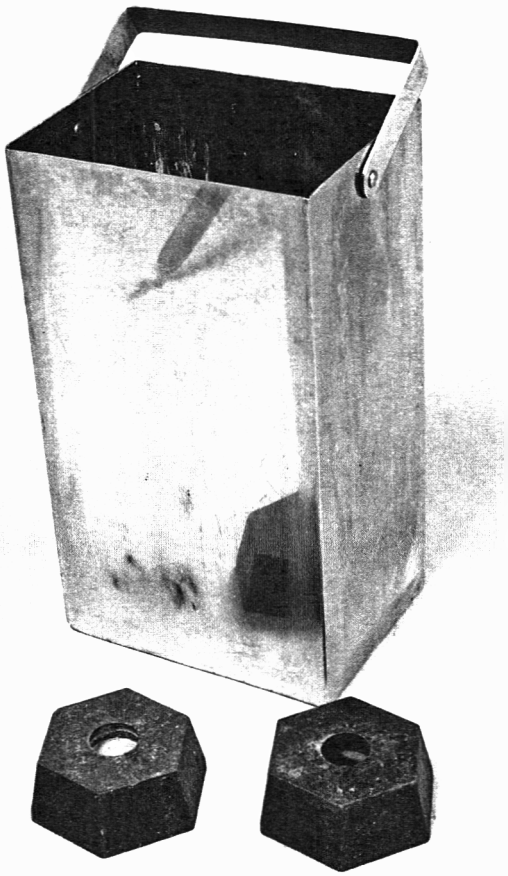

Figure 5. Cheddaring box.

curd particles consolidated on the base of the vat under the influence of heat and acidity. The curd mass was then cut in two, crosswise, and the lower half stacked on top of the other portion. The process of cheddaring in which the fibrous curd structure typical of Cheddar develops, was thus initiated.

The development of the fibrous curd structure is dependent on curd flow and the manual operations of cutting and stacking curd undertaken by the traditional cheesemaker are designed to encourage this. The essential requirements for curd flow are the availability of space and the application of mechanical pressure.

In small-scale cheesemaking, the relatively small quantity of curd (~1 kg) available prevents the traditional process of curd piling from being carried out effectively. Therefore, we simulated the traditional cheddaring process using a system in which pressure is applied to the curd in a controlled and incremental manner (Table 1) and this

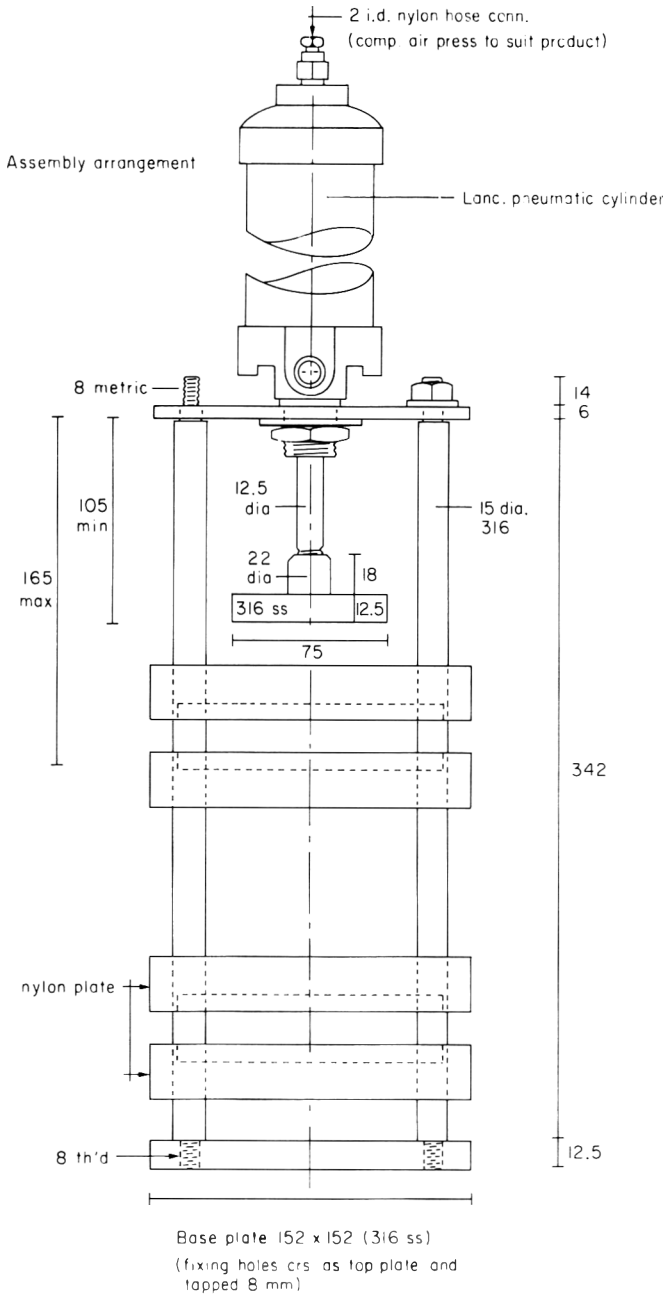

Figure 6. Model gang press.

is combined with curd cutting to facilitate curd flow in the base of the vat. A maximum pressure of 5.05 kPa was used since any increase beyond this value gave rise to too fast a flow and uneven pressure on the curd.

At the end of the cheddaring period (1.5 hr), the 'chicken-breast' type structure typical of traditional Cheddar cheese was obtained. The cheddared curd was then milled by hand, using a sharp knife, into cubes of $30 \times 20 \times 20$ mm. Salt (2.5% w/w) was

Table 1. The cheddaring process

Cheddaring time (min)	Mass in cheddaring box (kg)	Approximate pressure on curd (KPa)
0–15	0	—
15–30	2	0.63
30–45	4	1.26
45–60	8	2.53
60–75	16	5.05
75–90	16	5.05

applied to the milled curd which was then left to drain for 30 min. The drained, salted curd was divided into two equal portions which were hooped in stainless steel cylindrical moulds (130 mm diameter, Perfora) and pressed at a line pressure of 275.8 kPa for 16 hr. Finally the pressed curd was removed from the moulds, vacuum packed in heat shrinkable film (Cryovac) and left to ripen at 7°C for 7 months. Chemical analysis of the cheese was carried out immediately after removal from the press.

Analytical methods

Chemical analysis

Milk starter, whey and cheese. The total solids and fat content of milk, starter and whey samples were determined using the methods described in British Standard (BS) 1741:1963. To obtain a greater accuracy in fat analysis, the Röse Gottlieb procedure was chosen in preference to the Gerber method, and a Mojonnier-type flask as described in BS 5522:1977 was used in extraction. The protein content of milk, starters, whey and cheese samples was estimated by measurement of the total nitrogen content using the routine macro-Kjeldahl technique developed by Dr J. C. D. White at the Hannah Research Institute. The procedures detailed in BS 770:1976 were used to determine the total solids, fat and sodium chloride content of cheese.

The pH of cheese was measured by direct insertion of a cheese electrode (Pye-Unicam 406-M6-E7) into the sample.

Evaluation of cheese quality

Organoleptic assessment. The flavour and body of cheese are the principal organoleptic factors which influence quality. Experimental cheeses were evaluated by a trained taste panel, with several years' experience in this type of assessment. A scoring system, in which the quality and intensity of Cheddar flavour and the overall acceptability of the cheese were evaluated on a scale which ranged from 0 to 8, was used. An increase in score represented an increase in desirable qualities. Cheese body was assessed on a similar scale, but a score of 4 was designated optimum while respective values of less than or greater than 4 represented weakness or overfirmness in body.

Compositional grading. Compositional grading schemes provide an objective means of detecting a typical cheese. The New Zealand scheme (Gilles & Lawrence, 1973) which utilizes four compositional parameters *viz*, moisture in non-fat solids

(MNFS), fat in dry matter (FDM), salt in moisture (S/M) and pH, to classify cheese into three categories (Premium, First Grade and No Grade), was used in this study.

Results and discussion

Measurement of cheese yield

Recovery of mass, total solids, fat and nitrogen in model cheesemaking. Initial experiments were carried out with a single vat to obtain a measure of the efficiency of the model cheesemaking system in recovery of mass, total solids, fat and nitrogen. Mass balance experiments were carried out in which the starters, milk and rennet used in cheesemaking and the cheese and individual wheys produced, were weighed and sampled for chemical analysis. The output of mass, total solids, fat and nitrogen in cheese and whey was expressed as a percentage of the input of these components in milk, starter rennet and salt. The recoveries of mass and total solids were calculated as follows:

$$\%\ \text{mass recovered} = W(\text{cheese} + \text{first whey} + \text{Cheddar whey} + \text{salt whey} + \text{press whey}) / W(\text{milk} + \text{starter} + \text{rennet} + \text{salt})$$

$$\%\text{Total solids (TS) recovered} = [W\ \textit{cheese} \times \%\text{TS} + (W\ \text{first whey} + \text{Cheddar whey}) \times \%\text{TS} + (W\ \text{salt whey} + \text{press whey}) \times \%\text{TS}] / [(W\ \text{milk} \times \%\text{TS}) + (W\ \text{starter} \times \%\text{TS}) + (W\ \text{rennet} \times \%\text{TS}) + (W\ \text{salt} \times \%\text{TS})]$$

Fat and nitrogen balances were carried out in a manner identical to that described for total solids.

Table 2. Recovery of mass, total solids, nitrogen and fat in laboratory scale cheesemaking

Quantity estimated	Mean recovery*(%)	Standard error
Mass	97.5	0.10
Total solids	99.3	0.18
Nitrogen	100.0	0.36
Fat	98.8	0.48

*Mean of 20 trials.

The mean and standard error in recovery of mass, total solids, fat and nitrogen in twenty trials can be seen in Table 2. The mean recovery of mass (97.5%) was highly reproducible. Recovery of total solids was consistently higher (99.3%) which suggested that the lower recovery of mass was related to loss of moisture during cheese processing. The recovery of fat and nitrogen were equally high but slightly more variable. Since the recovery of total solids was highly reproducible the variation in recovery of fat and nitrogen reflects the greater difficulties of sampling and analysis of these constituents.

Replication of cheese yields and moisture contents between vats. Having established that mass balance experiments would be carried out successfully on the model cheesemaking system, with comparative ease, the system was duplicated. It was then necessary to verify that the individual vats did not differ in their efficiency of conversion

of milk constituents to cheese and that cheese of relatively constant moisture content could be produced in individual vats.

The results of four trials in which duplicate vats of milk were processed can be seen in Table 3. There was little variation in cheese yield or moisture content between vats. The maximum standard deviations in yield, moisture and yield at constant moisture (Y_{35}) were 0.03, 0.33 and 0.04, respectively. The maximum errors in replication of yield at constant moisture between vats in the trials A, B, C and D were 0.40, 0.49, 0.10 and 0.10%, respectively. The results confirm the suitability of the model cheesemaking system as a means of measuring cheese yield and demonstrate a significant improvement in precision when compared with laboratory scale cheesemaking systems previously described in the literature (Freeman & Bucy, 1971; Hicks, O'Leary & Langlois, 1981). Later, when a third vat was added, the same high degree of uniformity was attained.

Table 3. A comparison of cheese yields and moisture contents in processing duplicate vats of cheese

	Yield*				Moisture (%)				$Yield_{35}$†			
Trial	Vat A	Vat B	Mean	s.d.	Vat A	Vat B	Mean	s.d.	Vat A	Vat B	Mean	s.d.
A	10.08	10.12	10.10	0.03	34.92	34.95	34.94	0.02	10.10	10.14	10.12	0.03
B	9.99	10.02	10.01	0.02	33.86	34.32	34.09	0.33	10.18	10.13	10.16	0.04
C	9.84	9.84	9.84	0.00	34.39	34.58	34.49	0.13	9.94	9.91	9.93	0.02
D	10.22	10.25	10.24	0.02	36.56	36.71	36.64	0.11	9.98	9.99	9.99	0.01

*Cheese yield $= \frac{W \text{ cheese}}{W \text{ milk}} \times 100\,(\%)$.

†Cheese yield at 35% moisture $= \frac{W \text{ cheese} \times \%\text{ TS in cheese}}{W \text{ milk}} \times 0.0154 \times 100\,(\%)$.

Quality of cheese produced in model cheesemaking

A comparison of the flavour and body of Cheddar cheese produced on model and pilot scale cheesemaking systems. The organoleptic properties of a sample of Cheddar produced on the model cheesemaking systems were compared with those of a cheese processed, using similar conditions of manufacture, on a pilot-scale system which produces 20-kg commercial-size blocks of cheese.

On four occasions, samples of Cheddar produced on the model system were presented to the taste panel for organoleptic evaluation along with a sample of Cheddar, of the same age, produced on the pilot-scale system. The results of the taste panel's evaluations are shown in Table 4. From these data it can be seen that the overall acceptability of cheese produced on the model system is comparable with that produced on the pilot system. There were slight differences in Cheddar flavour and intensity of Cheddar flavour between samples produced in 20-kg blocks and 500-g rounds both scores being slightly less for the cheese produced on the model system. However, the mean scores for texture indicate that the body of the cheese produced on the model system was closer to the optimum desired by the panel; the pilot scale samples were classified as 'slightly firm'. The mean overall acceptability of the model and pilot-scale samples of cheese were identical.

Compositional quality. The compositional data of cheese produced on the model

Table 4. A comparison of cheese produced on the model cheesemaking system with that produced in a 20-kg block using pilot scale facilities

	Mean score* (s.d.)	
	Pilot	Model
Cheddar flavour	4.5 (0.37)	4.1 (0.68)
Intensity of cheddar flavour	3.9 (0.13)	3.5 (0.65)
Texture, body	5.2 (0.29)	4.3 (0.29)
Overall acceptability	4.4 (0.54)	4.4 (0.88)

*Mean value for four tasting sessions.

Table 5. Compositional quality of cheese produced on the laboratory cheesemaking system over a 30-month period

	MNFS*	FDM†	S/M‡	pH
Total number of samples	274	275	251	130
% of total samples classified as				
Premium	71.17	51.64	47.41	8.33
First grade	15.33	32.73	52.19	34.09
No grade	13.50	15.64	0.40	57.58
Premium + first grade	86.50	84.37	99.60	42.42

*MNFS = moisture in non-fat solids.
†FDM = fat in dry matter.
‡S/M = salt in moisture.

cheesemaking system over a 30-month period were applied to the New Zealand compositional grading scheme. The samples produced in this period were mainly processed from milk showing normal seasonal variation, but some experiments in which the casein to fat ratios of milk were altered have been included. The classification of the cheese on the basis of composition can be seen in Table 5. The values for MNFS, FDM and S/M are within the limits prescribed for Premium and First Grade Cheddar in more than 84% of the samples. Values for pH in model Cheddar were within the required limits in 42% of the samples. Further examination of the pH data on cheese samples produced in three replicate vats over an 18-month period (Table 6) indicated that in the majority of samples the pH was slightly greater than that allowed by the New Zealand grading scheme. However, it should be noted that the pH values of the cheese did not

Table 6. The mean pH values of cheese samples produced on the laboratory cheesemaking system over an 18-month period

	No. of samples	Mean pH	I.S.D.
Vat 1	57	5.20	0.06
Vat 2	54	5.21	0.09
Vat 3	29	5.20	0.04

vary to any great extent over the extensive experimental period. This would verify the success of the starter system, and suggests that a slight modification to the cheese processing technique could be used to produce Cheddar with a lower pH. However, since the relatively high pH values have not been detrimental to the organoleptic properties of model Cheddar, alteration to our processing schedule has not been considered necessary.

Conclusions

The laboratory-scale Cheddar cheesemaking system described in this work has been shown to be an effective system for measuring cheese yield and for producing high quality Cheddar cheese.

Mass balance experiments indicated that high recoveries of total solids, fat and nitrogen can be obtained routinely with the system. On replication of the system it was shown that the variability between duplicate vats was minimal and significantly improved as compared with other laboratory methods of cheesemaking reported in the literature.

The organoleptic properties of the Cheddar cheese produced on the laboratory system compared favourably with the flavour and texture of commercial Cheddar. Compositional grading of the cheese indicated that the MNFS, FDM and S/M were within the limits prescribed for high quality Cheddar. The pH of the cheese was slightly higher than that recommended for quality Cheddar, but this did not have an adverse effect on the organoleptic properties of the cheese.

Acknowledgments

The authors would like to thank Miss Gillian Stewart and Mrs Nancy West for technical assistance. They would also like to express their gratitude to Mr J. Ross for preparing the engineering drawings.

Note. A full set of engineering drawings for the complete system can be supplied on request.

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Batch pasteurization of liquid whole egg

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Summary

The heat sensitivity of α-amylase under batch pasteurization conditions at various temperatures was examined. A scraped surface commercial batch pasteurizer was tested for use with liquid whole egg and found to be satisfactory in bakery trials.

Introduction

The potential health hazard of the *Salmonella* group has been the cause for concern for many years and in this context their incidence in commercial liquid egg and products incorporating egg has also been well documented. The means of contamination of the egg with these organisms is in doubt and although recommendations made by Murdock *et al.* (1960) would assist in the reduction of the infection, the certain assurance of the absence of Salmonella can only be achieved by the application of heat treatment to the 'broken out' liquid egg.

At the temperatures necessary to ensure destruction of the *Salmonella*, denaturation of the egg proteins begins to take place and the desirable characteristics of liquid egg may begin to be adversely affected. The pasteurization of liquid whole egg is essentially a borderline operation.

In view of the necessity for strict temperature control to ensure minimal damage to the properties of the egg, coupled with the destruction of the *Salmonella*, initial work in the United Kingdom was based on a plate type heat exchanger which had been modified to satisfy the heating requirements and also accommodate the physical characteristics of the egg. From this work, reported by Heller *et al.* (1962) the requirements were established for the pasteurization of liquid egg which must be heated to 64.4°C, held at that temperature for 2.5 min, then cooled to 3.3°C. These requirements are laid down in Statutory Instruments (1963) No. 1503.

The temperature of holding has been questioned as being unnecessarily high (Brant, Patterson & Walters, 1968; Lineweaver *et al.*, 1969) but this has been made necessary to ensure the destruction of *Salmonella seftenburg* which is the serotype, which, whilst not being a common infectant of liquid egg, is less sensitive to heat than other serotypes of *Salmonella*. In addition, this time-temperature combination destroys α-amylase, the detection of which provides a simple control test (Brooks, 1962).

Whilst the pasteurization of liquid whole egg is obligatory in the U.K., this is not the pattern in other countries as discussed by Erdtsiek (1973).

In the U.S.A. the pasteurization requirement is for 60°C for 3.5 min and whilst this is claimed to destroy *Salmonella* it is inadequate to permit the use of Brooks' α-amylase control test. A modified test has been proposed by Imai (1979) for use with the U.S. standard.

Whilst the operation of the high temperature short time method of liquid egg pasteurization is now well established the capital investment precludes its use except for

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large egg producers or groups of smaller ones. The latter depends upon the geographical proximity of the participants, which could also increase operating costs. This situation results in the uneconomic disposal of whole egg, at a time when the industry is weak. This situation is therefore unsatisfactory to the producer, the industry and nationally.

The availability of the batch pasteurizer which could give results comparable to the HTST system in microbiological reduction with minimal damage to the other properties of the egg could only be advantageous to the industry.

Considerable work has been carried out at Auchincruive in the development of pasteurizers for small volumes of milk and it was considered that the experience gained could be utilized to develop a batch pasteurizer for liquid egg. The development of a batch pasteurizer of smaller capacity and at a lower capital investment would then permit a greater number of egg producers to pasteurize their egg and this in turn could lead to an extension of the liquid whole egg market into products such as omelette mixes and quick-scrambled egg with further developments of egg-based derived products.

Materials and methods

Eggs

Size 4 or 5 infertile eggs were supplied by the Poultry Department of the West of Scotland Agricultural College. These were broken out by hand on the day previous to processing and held at 4°C overnight. The yolks were ruptured but the liquid egg was not mixed or homogenized prior to pouring into the pasteurizer.

Commercially pasteurized frozen whole egg, which had been previously stored at −20°C, was thawed and used as comparison in the baking trials.

Pasteurization of liquid egg

Laboratory batch procedure. Liquid whole egg was held in a stainless steel beaker (250 cm^3) fitted with an overhead stirrer. The beaker was supported within a thermostatically controlled water bath which was allowed to heat to the desired treatment temperature over a period of 30 min in order to simulate the conditions of a commercial batch pasteurizer. The temperature of the egg and the water bath were monitored continuously and samples of egg withdrawn at regular intervals for the determination of α-amylase activity.

Commercial batch procedure: equipment. In the selection of a suitable batch pasteurizer it was considered that a swept surface heat exchanger would be preferable to reduce problems arising from the deposition of heat denatured egg on the heating surface. The effective time in a continuously operated plate heat exchanger is frequently limited by such deposition. Whilst Brant *et al.* (1968) successfully used both impellor agitator and rotary coil batch pasteurizers for liquid egg, it was felt that the swept wall type would utilize less agitation and thus cause less physical damage to the egg.

The type of batch processor used during the trials had been developed for use with confectionary products (Fig. 1). It consisted of a tank of 26-kg capacity fitted with a centrally mounted agitator to which was attached scraping elements which swept the inner surface of the tank clean from product. The temperature of the process was controlled by a thermo regulator and the time of holding could be either for 30 min or

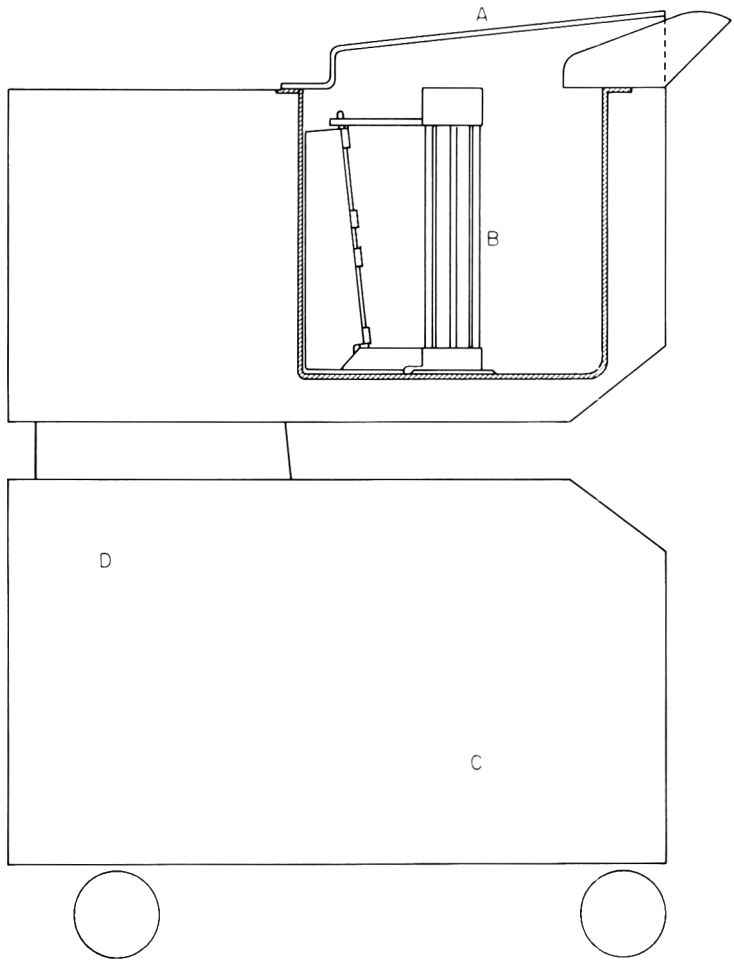

Figure 1. Schematic diagram of swept wall batch pasteurizer. (A) Transparent cover fitted during operation. (B) Processing tank with agitator. (C) Refrigeration system. (D) Mechanical tipping system which permits processed egg to be tipped from tank.

less than 1 min, these being the requirements for the processes for which the equipment had been developed. Once the holding period was over, the tank contents immediately commenced to be cooled by a refrigeration system contained in the base of the unit which circulated chilled refrigerant through the jacket of the tank. During the trials it was necessary to control the plant manually in view of the different time/temperature conditions required for liquid egg.

The average batch weight of whole egg processed was 22.47 kg. Temperatures at several points in the liquid were recorded during processing using an Ellab (a–s) multipoint temperature recorder. Samples were withdrawn for testing before and after the test runs.

α-Amylase test

The method of Shrimpton *et al.* (1962) was used except that a 5-cm^3 sample of liquid whole egg was taken and the starch concentration correspondingly reduced. The iodine colours produced were measured on a Spectronic 20 spectrophotometer at 565 nm.

Standard curve for conversion of spectrophotometer readings to starch

In order to express the readings on the spectrophotometer at 565 nm as mg of residual starch a scheme for calibration was developed. Whole egg was pasteurized to completely inactivate the α-amylase. To each 5.0-cm^3 portion of egg was added varying concentrations of starch solution. The mixtures were then treated as in the Shrimpton assay and the spectrophotometer readings obtained plotted against mg starch added (Fig. 2). This graph was used to calculate the amylase activity with unknown egg samples.

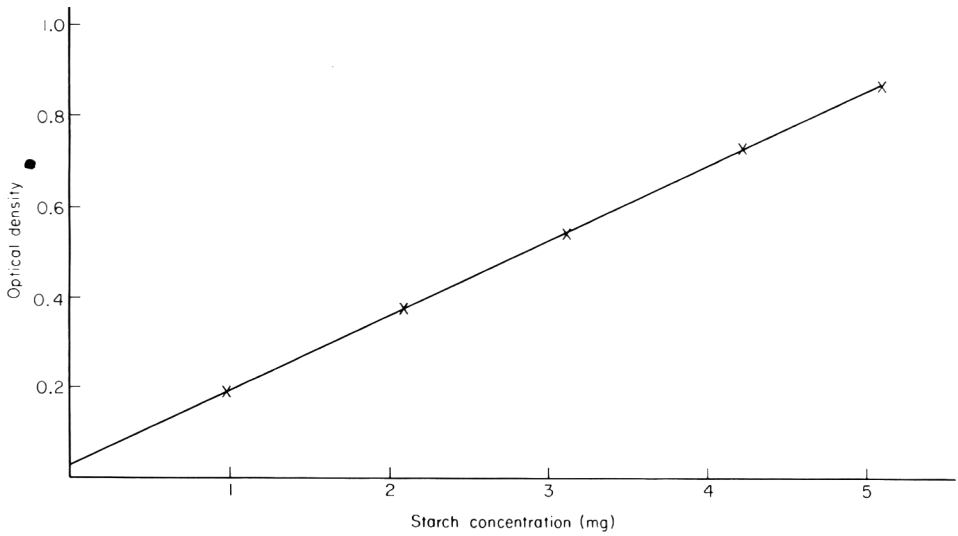

Figure 2. Standard curve for starch-iodine reaction.

Activity of α-amylase

The velocity constant of the destruction of the α-amylase activity for any given condition of heating was calculated by reference to the rates at which starch (0.7%) was hydrolysed by the heated and raw egg.

The velocity constants k_1 and k were calculated for the hydrolysis of 0.7% starch by the heated and raw egg, respectively, using the equation: $k = 2.303 \log_{10} \left(\frac{a}{a-x}\right)$, where a is the initial concentration of the starch (4.55 mg) and x is the amount of starch being hydrolysed.

As in Brooks (1962), the concentration of α-amylase after pasteurization was calculated in relation to an initial concentration arbitrarily taken to be 100 from the expression $\frac{(k-k_1)}{k} \times 100$. This was regarded as the percentage destruction of the enzyme's activity by the heating process. P values were determined as the time in minutes to reach the pasteurization reading of 0.230 at 565 nm on the Spectronic 20, after allowing the temperature of the batch to rise for 30 min from room temperature to the temperature being tested. The value 0.230 at 565 nm corresponds to the standard of Shrimpton *et al.* (1962).

The velocity constant of the destruction of α-amylase during heating, k_p was

calculated assuming that within this range of temperatures the reaction was one of the first order. This would agree with Brooks (1962). The Arrhenius relation between rate and temperature of reaction was confirmed by plotting the $\log_{10}$ of the different values obtained for k_p for different times and temperatures of pasteurization against the reciprocals of the pasteurizing temperatures in degrees absolute.

Baking trials

The effect of heat processing could be reflected in the extent of alterations to the performance, quality or the appearance of the product, since the most sensitive of the attributes of the egg are those which can be utilized in baking. King, Morris & Whiteman (1936), MacDonnell *et al.* (1955) and Parkinson (1973) all use baking trials to evaluate egg quality. Tests were carried out at the local research bakery of a national bakery company. Two recipes were used:

(a) An egg sponge which contains no fat and which was felt to give a true indication of egg performance. (1.132 kg of egg at 21°C was whisked with 0.905 kg of sugar for 20 min at high speed, when 0.905 kg of soft flour was gently folded in. The mix was baked at 199°C).

(b) Yellow, high ratio cake which is sensitive to egg quality. (0.6 kg of cake flour, 0.3 kg of catering margarine and 0.03 kg of baking powder mixed together for 3 min on medium speed. 0.8 kg of sugar and 0.24 kg of water next added, followed by 600 g of egg with continuous mixing. The cake was baked at 182°C).

In both cases, cake volume was measured by seed displacement (King *et al.*, 1936).

Results and discussion

Activity of α-amylase in egg

The initial amylase from college eggs gave rise to a velocity constant for the hydrolysis of starch [0.7% (w/v)] of 3.9. This was compared with egg from other local sources. Egg from source A showed a velocity constant (k) equal to 4.02 and source B, $k = 3.56$. Allowing for the differences in assay procedure these figures agree well with the data of Brooks (1962).

As the batch process was being considered, since 30 min could be anticipated to be required to raise the temperature of the egg to approximately 60°C, samples of egg were withdrawn after this period and the degree of inactivation of α-amylase measured. The velocity constant (k_1) obtained from such samples was 2.19 corresponding to 43.85% inactivation of the enzyme. Murthy (1970) quotes a 'come-up' time from 45 to 133°F and 140°F (7.2 to 56.1°C and 60°C) taking 26 and 81 min, respectively, and causing 16 and 43% inactivation. This was compared with 42–44% inactivation with minimal heating time procedures, suggesting that the time needed to reach 60°C did not alter the amylase activity.

Continuing the heating until a 'pasteurization colour', i.e., a reading of 0.230 at 565 nm on the Spectronic 20 was obtained from the assay, the k_1 value becomes 1.34 and 66.6% destruction is obtained.

The time required to reach 'pasteurization colour' (P value) was observed. Normally the egg was heated in a jacketed water bath to take 30 min from 16°C to the temperature being tested. The P value represents the time in minutes after the initial 30 min heating period. Table 1 shows the time-temperature relationship.

Table 1. Time-temperature relationship to reach P value

Temperature (°C)	P value (min)
58.5	59
59	42
60	24
60.4	23
60.5	22
60.7	16
60.9	15
61.6	10
62.6	4

P value is the time in minutes (beyond 30 min come-up period) required to reach the degree of amylase inactivation equated as 'pasteurization colour' by Shrimpton *et al.* (1962).

Shrimpton *et al.* (1962) have shown that a unique time–temperature relationship exists for the destruction of α-amylase and *S. seftenburg*. They indicated that the amylase pasteurization test would be less satisfactory if different conditions were used, i.e. heating at lower temperature could destroy *Salmonella* but not affect the activity of α-amylase whilst shorter periods of heating at higher temperatures would destroy the enzyme but not the heat resistant *S. seftenburg*.

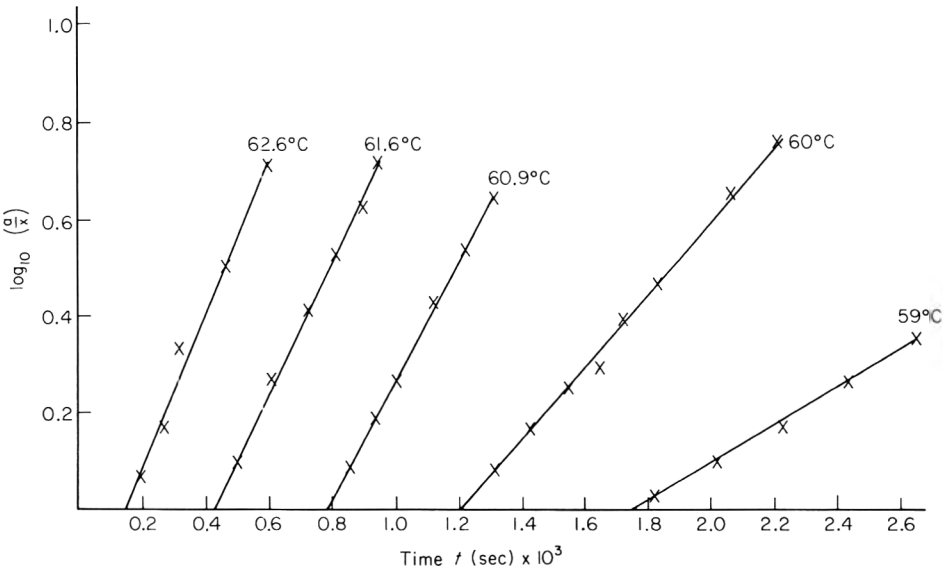

Figure 3. Graph of starch utilization against time for various temperatures where a = initial concentration of starch, x = starch hydrolysed. Only a limited number of results obtained have been plotted to illustrate the range recorded during the trials.

The value of k_p (the velocity constant of the destruction of α-amylase during pasteurizing) was calculated for the temperature range 58.5 to 62.6°C. Figure 3 shows log a/x plotted against t, where a is the initial concentration of starch per assay, x is the

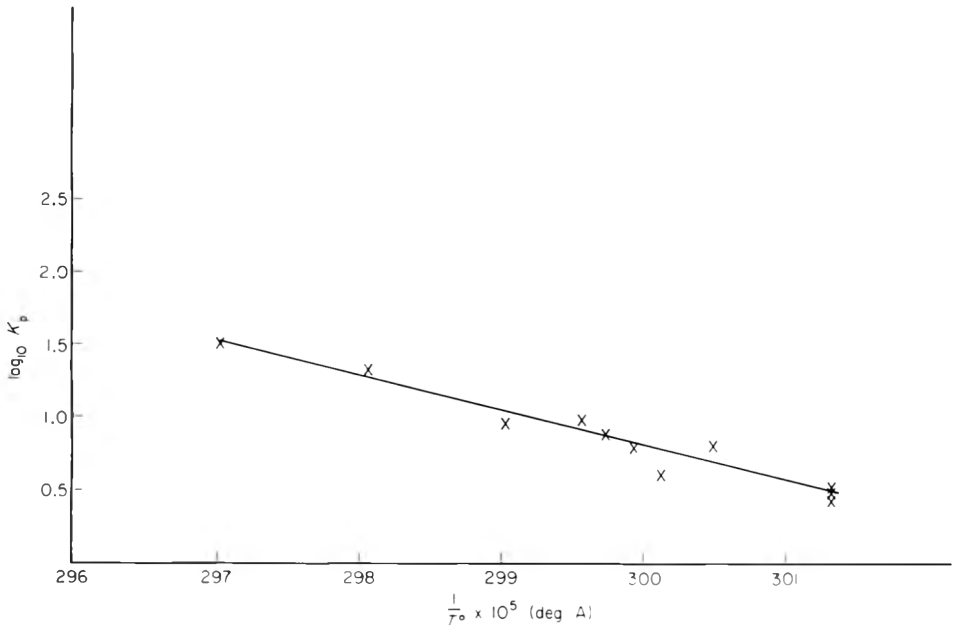

Figure 4. Arrhenius plot of thermal inactivation of α-amylase.

starch utilized in assay and t is time in seconds for each temperature studied. The slope of the line is equal to k_p. When $\log_{10} k_p$ is plotted against $1/T$ (the reciprocal of the pasteurizing temperature in °A) as in Fig. 4, a linear relationship is seen to exist (Arrhenius plot). Murthy (1970) suggested that the slope of this line could be used in the equation $E = 2.303\,RS$, where E is the energy of activation, R is the gas constant (1.987 cal/deg per mol) and S is the slope of the line in the Arrhenius plot. This would seem doubtful as k_p has been measured using the concentration of starch added to the assay tubes in milligrams. Further comment of a physical chemistry nature is also limited by this provision.

Commercial batch treatment of whole egg

Following the study of α-amylase reaction to heat treatment and considering the wide recognition of the Shrimpton *et al.* (1962) pasteurizing test it was decided to base the commercial trials at 64.4°C. A typical experiment using the batch pasteurization involved an approximate 30 min warm-up period, a holding period of 2.5–3 min and a cooling period of about 30 min to 7°C. Temperatures were recorded during the processing and Fig. 5 illustrates the normal pattern. The contents of the process tank appeared to exhibit a uniform (±0.5°C) temperature profile. There were no signs of foaming with this design of machine and cleaning was easily effected manually using a standard proprietary detergent sanitizer.

The percentage inactivation of amylase under these conditions was between 92 and 100% compared with 66.6% on the laboratory method. On closer examination of the temperatures actually attained during pasteurization, maximal temperatures of 66.2°C were noted in the unmodified machine and this would suggest that a more sensitive control on the holding temperature is required. Nevertheless, when such pasteurized egg was used in bakery trials in a sponge mix, the average specific volumes of control and test baked products were 4.62 and 4.67. In the yellow cake mix the average specific

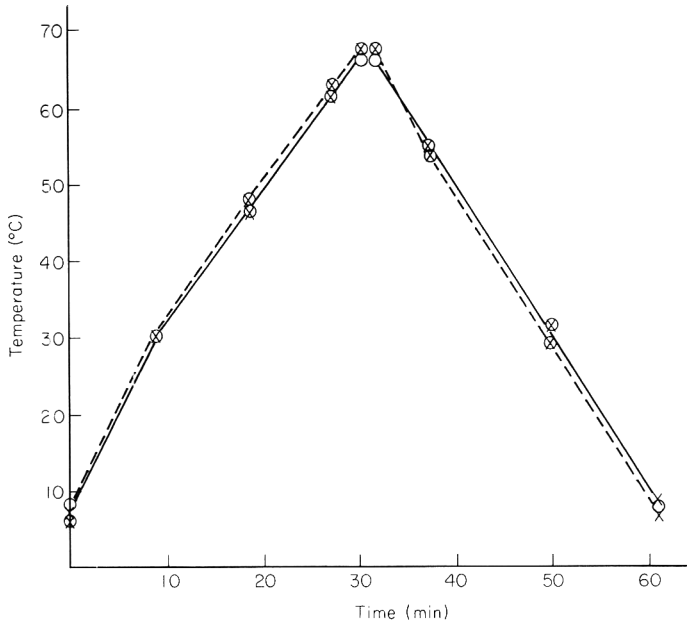

Figure 5. Operating temperatures within drum of batch pasteurizer: x ——— x Centre of the drum, just under egg surface. o ——— o Centre of the drum, just clear of base. x — — — x Outside of the scraping paddle, just under egg surface. o — — — o Outside of the scraping paddle, just clear of base.

volumes of control and test were 2.43 and 2.36. The estimated error of such test baking trials is ±0.4, and ten batches of egg were baked. Hence, the batch pasteurized egg would appear to exhibit similar characteristics to those of the egg pasteurized by the HTST process and more extensive baking trials with egg processed on such a modified batch pasteurizer could be justified.

Conclusions

1. The batch process of heating does not alter the basic physical chemical characteristics of α-amylase in whole egg as established by Brooks (1962) and Murthy (1970).
2. There is approximately 44% inactivation of α-amylase effected when the egg temperature is raised from 16 to 60°C whether 30 or 81 min (Murthy, 1970) is allowed. It would thus appear that batch warming times below 60°C are not critical.
3. Pasteurization of liquid whole egg as defined by Shrimpton *et al.* (1962) equates to a 66.6% α-amylase inactivation.
4. The commercial batch process examined gave 92–100% α-amylase inactivation and so must entail more severe heat treatment than is required by British Statutory Regulations (64.4°C for 2.5 min).
5. Despite the above, the bakery trials conducted indicated a satisfactory product in comparison with commercial HTST processed whole egg.

Acknowledgments

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Solvent dehydration of potato: selection of solvent and processing conditions

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Summary

The technical problems related to the solvent dehydration of food material (potato) were investigated. The effect of different pretreatment methods on the organoleptic quality of the dehydrated product was examined and it was observed that blanching in 0.2% $CaCl_2$ solution for 2 min yielded a product having the desirable quality. Of the eight solvents tested ethyl acetate was found to be best from the point of view of moisture removal capacity, product quality and ease of recovery and reuse of solvent. A method was also developed for estimation of the residual solvent in the final product.

Introduction

Food materials undergo extensive physicochemical changes during conventional air drying and sun drying processes, it is therefore desirable to investigate newer drying techniques and evaluate their commercial applications.

Of the newer techniques, solvent dehydration has considerable commercial potential, but it has not, as yet, been widely practised. The pioneers in the field of solvent drying were Baniel (1961) and Thompson (1965) and the industrial application of solvent dehydration was first attempted by Bohrer (1967). Meat, vegetable and other foodstuffs such as whole strawberries, pieces of banana and potato have been dried successfully by solvent dehydration (Holdsworth, 1969).

The solvent dehydration process is generally carried out in three steps: (a) pretreatment of the food material, (b) dehydration in the presence of solvent and finally (c) removal of residual solvent from the product.

The reasons for pretreatment are to inactivate the enzymes, to lower the bacterial load and also to bring about structural changes so as to facilitate water transfer from the interior of the pieces to the surface. Considerable work has been carried out on the pretreatment of potato prior to air drying (Talburt & Smith, 1967; Van Arsdel, Copley & Morgan, 1973), but the effect of pretreatment on the quality of solvent dried potato has not been reported in detail.

The second step, that is, dehydration with solvent may be carried out in either of the following two ways: (1) extraction of water from the food by a solvent miscible, or partially miscible, with water or (2) simultaneous water extraction and distillation. As the dehydration rate by the second technique is faster it is usually the method of choice.

The characteristics of the dehydrated product such as texture, odour, flavour, rehydration ratio and retention of nutrients are likely to be affected by the solvent used. Previous investigators have used various solvents but their efficiencies have not been reported.

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The efficient recovery and reuse of the solvent is an important factor for the commercial success of the solvent dehydration process, but previous investigators have not reported their findings, if any, regarding solvent loss, recovery and reuse.

Most of the reported work (Blaw-Knox Co., 1969; Levin, 1971; Nakamura, 1966) on solvent dehydration is covered by patents and is mainly related to the feasibility of the application of solvent drying technique to some specific materials.

The final step in the solvent dehydration process is removal of the residual solvent from the dehydrated product. Even trace amounts of solvent may alter the taste of foodstuffs and mask the original flavour. Previous workers (Anon, 1968; Francis, 1971; Heisler *et al.*, 1953; Levin & Finn, 1955; Srivastava, 1971) have tried different methods of solvent removal but did not report any analytical procedure for the detection and/or estimation of residual solvent in the final product. The methods used consisted of heating the material under vacuum, sometimes in the presence of inert gas or steam.

For the present work potato was selected as it could be cut to any desired shape and size prior to dehydration. During the process of dehydration deformation of potato is moderate.

The aim of the present investigations were (1) to find out the effect of the different pretreatment methods on the quality of the solvent dried product, (2) to find the moisture removal capacity of the different solvents and thereby select a suitable solvent, (3) to find suitable means for the recovery and reuse of solvent, and finally (4) to devise a convenient method for estimating the residual solvent in the final product.

Materials and methods

Potatoes (*Solanum tuberosum* Jyoti) were sliced to the desired thickness and diced to a final size of $8\times8\times4$ mm. These pieces were kept in 1% NaCl solution to prevent immediate browning.

Commercially pure solvents were used for carrying out the treatments. In order to select a suitable solvent for dehydration purposes the following categories of solvents were tested: (1) solvents almost immiscible with water at room temperature, e.g., benzene, heptane, toluene and trichloroethylene, (2) solvents parially miscible with water at room temperature, e.g., ethyl acetate, and (3) solvents completely miscible with water at room temperature, e.g., acetone and ethyl alcohol.

Dehydration procedure

The experimental system consisted of a distilling flask fitted with water cooled condenser, distillate receiver, thermometer pocket and pressure measuring tap. The distilling flask was connected to a vacuum pump through a dehydrating tower. Heating was done by keeping the flask partially immersed in a water bath, the temperature of which was controlled. Solvent dehydration was carried out by adding solvent to the sample of potato to be dehydrated and distilling the mixture until only a small volume of the solvent remained in the vessel. The temperature difference between the boiling liquid mixture and the water bath was maintained constant at 8 ± 0.2°C throughout the process. The remaining solvent and the product was taken out and separated. The product was desolventized and analysed.

In order to inactivate the enzymes present, blanching of potato samples was carried out in boiling water, steam and boiling aqueous solution of certain chemicals as shown in Table 1. To test the effectiveness of various blanching methods about 30 g of

Table 1. Effect of pretreatment on solvent dehydrated potato quality

Blanching condition	Drying time (min)	Rehydration ratio	Rehydration time (min)	Residual solvent	Organoleptic score	Organoleptic quality
Unblanched	200	3.21	60	appreciable	4	Acute browning, bitter taste, fair texture.
Steam at atmospheric pressure for 2 min	180	4.85	30	trace	3	Slightly brown, bitter taste, very soft texture, surface disintegration.
Boiling for 2 min in						
a. water	185	4.92	35	small	4	Yellowish, bitter taste, poor texture.
b. 0.2% $Na_4P_2O_7$	180	4.75	20	trace	5	Good colour, fair taste, very poor texture, surface breakage.
c. 0.2% $Na_2S_2O_5$	185	4.52	30	appreciable	5	Fair colour, bitter taste, good texture.
d. 0.2% NaCl	170	4.80	20	small	7	Slightly brown, good taste, fair texture, powdery surface of dry product.
e. 0.2% $CaCl_2$	170	5.20	30	trace	8	Good appearance of product, good colour, very good texture, taste of fresh cooked sample.

blanched samples were solvent dried with 1000 ml of C.P. grade ethyl acetate solvent at 275 mmHg pressure and a temperature of 46–49°C. The samples, after dehydration, were desolventized in a vacuum oven at 38–41°C and 75 mmHg pressure for 45 min prior to organoleptic assessment.

In carrying out trials for the selection of a suitable solvent about 30 g of pretreated potato pieces were dehydrated with 900 g of each of the solvents mentioned earlier at an operating pressure such that the distillation temperature was maintained below the critical temperature of potato (65.6°C). After solvent removal the final product characteristics were evaluated organoleptically.

In the present investigation solvent removal was attempted in a vacuum oven at 75 mmHg pressure at the temperatures given in Table 4.

Gas liquid chromatography was used to detect and estimate the quantity of residual solvent in the product after solvent removal. The residual solvent (ethyl acetate) in the samples was extracted with toluene and analysed in a Hewlett Packard Model 5731a Research Chromatograph using 3% SE52 column at 90°C with nitrogen as dynamic phase. About 3 g of both dehydrated and rehydrated samples were crushed, mixed with 6 ml of chromatography grade toluene, with agitation, for 1 hr and then kept overnight at room temperature. The supernatant liquid was decanted off and chromatographed.

Physico-chemical tests

The peroxidase test was used to detect the enzymes responsible for product discoloration (ISI, 1968).

The moisture of the potato samples, prior to dehydration, was estimated by vacuum oven drying for 6 hr at 70°C and a pressure not exceeding 100 mmHg (Horwitz, 1965). The moisture content of the solvent dehydrated product was estimated by immiscible solvent (toluene) distillation (Tate & Warren, 1936).

Rehydration ratio was determined by the ISI method (1968). The weighed sample was cooked in 1% NaCl solution for 30 min and allowed to cool, at room temperature, for 45 min. Excess solution was drained off and the material reweighed. Rehydration ratio is expressed as the ratio of the weight of the reconstituted product to that of the dehydrated sample.

Sensory evaluation

The dehydrated and desolventized product was assessed for its acceptability on the basis of its sensory characteristics by a trained panel using the 9-point hedonic scale of Peryam & Shapiro (1955). Each dehydrated sample was soaked in 1% salt solution for 1 hr, the mixture being kept hot at 60°C during the soaking period. The sample was then boiled in the same solution for 5 min and strained. It was evaluated after removal of surface moisture.

Results

The peroxidase test on pretreated potato revealed that enzyme inactivation could be achieved by blanching in boiling water for 2 min. In Table 1 the effects of different pretreatment methods on the quality of the solvent dehydrated products are summarized. It was found that solvent dehydrated fresh potato (without any pretreatment) had an excessively high rehydration time and was not properly cooked even after 60 min boiling. The dehydrated product after cooking was of very poor quality having appreciable solvent flavour and a bitter taste. On the other hand

Table 2. System performance with various solvents

Solvent (CP grade)	Operating pressure (mmHg)	Solvent loss (%)	Moisture content		Drying time (min)	Rehydration ratio	Organoleptic score
			Dehydrated product (%)	Desolventized product (%)			
Acetone	760	5.00	15.00	12.40	120	4.94	6
Benzene	465	0.77	34.00	14.96	120	4.82	6
Ethyl alcohol	342	0.69	32.20	13.80	120	4.75	7
Ethyl acetate	480	0.90	8.85	8.58	120	4.90	8
Heptane	238	1.22	69.00	57.10	90	2.58	4
Hexane	645	1.92	75.00	69.20	90	1.92	3
Toluene	175	1.33	15.30	13.10	120	5.08	6
Trichloroethylene	360	2.22	57.20	39.70	120	3.38	5

dehydrated pretreated samples rehydrated quickly (in 20–30 min) and the rehydration ratios were much higher. Of the several pretreatment processes tried, calcium chloride was most effective in retarding non-enzymatic browning and preventing sloughing of the reconstituted product.

The results of dehydration trials with various solvents are shown in Table 2. Of the different solvents tested, hexane was found to have the poorest moisture removal capacity and ethyl acetate the highest. The organoleptic quality of the ethyl acetate dried product was found to be the best.

Ethyl acetate solutions containing different initial moisture contents were tested as the dehydrating agents and the results indicated that dried ethyl acetate had the highest moisture removal capacity (Table 3). CP grade ethyl acetate produced a product which on removal of solvent had a moisture content of about 7%, as is desired.

Table 3. Effect of ethyl acetate quality on the dehydration characteristics of potato

Solvent quality	Moisture content: Dehydrated product (g/g dry solid)	Moisture content: Desolventized product (g/g dry solid)	Drying time (min)	Dehydration temperature (°C)
CP grade	0.088	0.075	140	61.3–65.0
Ethyl acetate saturated with water at −10°C	0.093	0.082	175	60.2–64.5
Ethyl acetate saturated with water at room temperature (30°C) (3.5 wt% water)	0.182	0.120	175	59.7–63.0

The ethyl acetate content of the dehydrated potato samples are given in Table 4. It is seen that the residual ethyl acetate content of the sample was not very dependent on the method of solvent removal employed. The rehydrated samples were found to be free from ethyl acetate.

The ethyl acetate content of samples of known ethyl acetate content were in good agreement with the experimentally determined values (Table 4).

Discussion and conclusions

From the experimental data it is evident that blanching prior to solvent dehydration is essential as it not only improves the product quality but also reduces the dehydration time. Calcium chloride added to the blanching solution improved the texture of the products by preventing sloughing of potato. It should be noted here that during solvent dehydration the food materials are kept submerged in solvent, as a result oxidation is prevented.

Of the eight solvents tested ethyl acetate was found to be the best from the points of view of moisture removal capacity and the organoleptic quality of the dehydrated product. In addition ethyl acetate has the following advantages: (1) its use in food is permissible, (2) the rehydrated product (potato) contains no solvent and (3) as the solubility of water in ethyl acetate is low, it can be separated easily from the entrained water in the distillate.

Dry ethyl acetate (CP grade) has a higher moisture removal capacity than water-saturated ethyl acetate. However, to obtain CP grade ethyl acetate from the distillate

Table 4. Estimation of residual ethyl acetate in dehydrated potato and control samples

Sample description	Sample weight (g)	Toluene added (ml)	Toluene obtained after extraction (ml)	Relative amount of ethyl acetate in toluene (%)	Concentration of ethyl acetate in the sample (ppm)	Concentration of ethyl acetate in artificially prepared sample (ppm)
I. Treated at 38–41°C, 75 mmHg for 45 min	2.971	6	2.2	0.372	2750	—
II. Treated at 58–62°C, 75 mmHg for 45 min	2.949	6	2.0	0.361	2450	—
III. Treated at 58–62°C, 75 mmHg for 45 min kept overnight and treated for a further 45 min	2.925	6	2.0	0.324	2210	—
IV. Rehydrated sample of I	3.077	8	3.0	0	0	—
V. Rehydrated sample of II	2.9	8	3.2	0	0	—
VI. Rehydrated sample of III	3.0	8	3.1	0	0	—
VII. Ethyl acetate added to crushed vacuum oven dried product*	5.771	16	10	0.144	2520	2770
VIII. Ethyl acetate and toluene mixture*	—	—	—	0.012	1200	1130

*Control sample.

would require more energy and hence the use of water-saturated ethyl acetate may be more economic.

Solvent loss occurs in three stages: (1) with the non-condensable gases during distillation, (2) during vacuum removal of solvent and (3) with the water separating out of the distillate. Solvent recovery during removal from the potato and from the entrained water was not attempted in the present investigation. The loss of ethyl acetate was found to be 0.9% but by installing an ice trap it was possible to reduce this to 0.5%.

Solvent removal by vacuum did not lead to a solvent-free product. This could have been due to insufficient heat penetration (to meet the latent heat requirement, since latent heat needs to be supplied for evaporation of solvent) to the interior of the pieces under vacuum. However, during rehydration the residual solvent appears to be removed as the rehydrated product was solvent-free.

The method of extraction of residual solvent with toluene and analysis of the extract by GLC yielded good results as indicated by analysis of products containing known amounts of ethyl acetate.

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Uncertainties associated with the estimation of F_0 values in cans which heat by conduction

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Summary

The variations in heating phase lethality between replicate cans of a conduction heating substance subjected to a long heating process (F_0 approximately 30) have been quantified. There were significant differences in heating phase lethality (F_h) between cans subjected to the same nominal thermal processes, with variations ranging from 13 to 26% of the mean F_h value for any run. The fifty-six individual F_h values followed a normal type of frequency distribution. Increasing the headspace in cans significantly increased the heating phase lethality. Because of the between can and between run variation, it was not possible to make any widely applicable recommendation as to the number of test cans per run or the number of runs necessary to establish a minimum safe thermal process.

Introduction

The successful thermal processing of foods in hermetically sealed containers depends on the application of heat for a definite time and at a definite temperature under specific conditions to obtain a commercially sterile product. F values are a measure of the total lethality of a thermal process. In the New Zealand canning industry a procedure commonly used to determine F values relies on data from a small number of cans processed in a pilot plant scale retort. The implicit assumption is made that the F value calculated for a few cans processed in a small retort will be the same as for production run cans in a fully loaded, commercial scale retort.

Calculation of F values requires a knowledge of the time–temperature history at the slowest heating point in the can. This is commonly obtained by using a thermocouple connected to a recording potentiometer. There have been numerous thermocouple systems used in heat penetration studies: the most practical and elegant arrangement utilizes epoxy resin to form a vacuum tight seal in a pierced can (Board, 1977).

Many procedures are available for the calculation of the total lethality of a thermal process (Hayakawa, 1977). These procedures may be classified into two groups: those based on the concentration of survivors at the slowest heating point in the food (e.g., Ball, 1923; Bigelow *et al.*, 1920; Patashnik, 1953; Pflug, 1968; Board, 1977) and those based on the mass average concentration of survivors in the food (e.g. Gillespy, 1951; Hayakawa, 1969; Teixiera *et al.*, 1969).

In order to avoid the seasonal effects of food ingredients, can to can and batch to batch variations in the rate of heating, early studies used aqueous suspensions of bentonite as a food substitute (Jackson & Olson, 1940; Alstrand & Benjamin, 1949). Townsend *et al.* (1949) investigated the nature and stability of bentonite solutions used in heat penetration studies and found that a 1% suspension could be used to give a

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reproducible convection heating system and a $5 \pm 0.5\%$ suspension could be used satisfactorily for a conduction heating system.

Despite the large number of publications which have appeared on thermal process evaluation during the past 60 years, there has been relatively little information reported on the magnitude of the uncertainties and errors involved in thermal process determination. Coefficients of variation (c.v.) of 5–15% in f_h values (the parameter specifying the slope of the heating curve) were reported by Hicks (1961) who observed that it did not seem possible therefore to use a c.v. for f_h that would have any general validity. He argued against the more usual practice of making thermal process calculations using the data obtained from the slowest-heating can of a batch (i.e., the can giving the largest value of f_h) in favour of basing all calculations on the mean values and making use of the calculated standard deviations to estimate the precision of the final result. Powers *et al.* (1962) reported that F_0 values rarely followed the normal frequency distribution, the distributions being positively skewed at short processing times and negatively skewed at long processing times. The c.v. for the F_0 values of six products studied ranged from 16.3 to 57.4%. They used extreme-value analysis to estimate the proportion of F_0 values expected to be below any selected sterilizing value. They also used composite curves to predict the minimum lethality of individual heating–cooling curves determined thereafter; statistical evaluation indicated that even when the composite curve was based on 100 or more heat-penetration determinations, approximately 3% of individual containers might be expected to be below the composite curve in lethality. They cite as a reason for this, the fact that the slowest-heating and fastest-cooling curves may occur in the same container, thus giving tacit support to the suggestion of Hicks who argued against using data from the slowest-heating can.

Lenz & Lund (1977) reported that variations in the rate of destruction of spores and thermal diffusivity of the food (the latter being inversely proportional to f) could result in a standard deviation for the F value of up to 15% of the F value. As the F value increased, the confidence intervals decreased. In a later paper Lund (1978) stressed the need for caution in using any of these estimates of standard deviation for lethality since calculations based on slowest-heating containers have been successful.

The standard reference book for canners in the U.S.A. (National Canners Association, 1968) states that for homogeneous products, one test run of 6–8 cans may provide sufficient data, but because nearly all products vary considerably in composition according to packer, season, method of preparation and other factors, many runs may be necessary to insure that the conditions of slowest heating have been included in the study. No reference is made to the level of uncertainty associated with the calculated F values.

Lund (1978) states that currently most industrial processes in the U.S.A. are based on the slowest-heating container in a sample of twenty.

The equivalent U.K. publication (Anon., 1977), while outlining possible critical factors in establishing thermal processes, does not discuss the magnitude of the uncertainty associated with such calculations. It suggests that a minimum of three separate heat penetration runs should be carried out with a minimum of three replicate cans in each run to obtain nine results in close agreement before a provisional scheduled heat process, based on the lowest value obtained, is set.

There appears to be no conclusive data to establish what is an 'adequate' trial with respect to the number of cans and runs necessary. Variations between cans may occur in the come-up period, the cooling period, and during the 'stable' processing period. This

study was carried out to expand the data base and quantify the variations which occur in F values determined for replicate cans of aqueous bentonite suspension subjected to the same nominal thermal processes in a pilot plant retort. To minimize the between cans' variation, it was decided to examine only the 'stable' processing conditions. As a consequence, long thermal processing times giving F_0 values of 24–30 min were used and both between cans and between runs variation investigated since both are important with respect to reproducibility.

Materials and methods

Preparation of a 5% bentonite slurry

A 10-kg batch of the test substance was prepared by adding 0.500 ± 0.005 kg calcium bentonite granules to 9.500 ± 0.005 kg water at approximately 65°C. Using a Silverson mixer a vortex was formed in the water and bentonite was slowly sprinkled into it. The suspension was then mixed for approximately 15 min to facilitate hydration and to prevent clumping and settling of bentonite particles. It was then screened to remove any clumps and held for 48 hr to fully rehydrate.

Preparation of test cans

The bentonite slurry was heated to 65°C and filled into cans (74 × 112.5 mm with a sulphur resistant lacquer), seamed under vacuum and retorted twice at 121°C for 2½ hr to stabilize the suspension (Townsend *et al.*, 1949).

Cans were filled so as to leave gross headspaces of either 5, 10 or 20 mm, such values covering the range likely to be encountered in a commercial cannery.

Thermocouple construction and type

Using 26 swg copper and constantan wires, a thermocouple junction was mounted at the geometric centre of each can using the tension method (Board, 1977). For this the copper and constantan wires were separated and threaded through holes on opposite sides of the can, joined and soldered. The holes in the can walls were sealed with epoxy resin and hardened. Although Cowell *et al.* (1959) showed that 26 swg copper wires gave rise to conduction errors, our experience has shown that finer wires are not able to reliably withstand the handling associated with repeated heat treatments such as were used in the work reported here.

Retort operation

The pilot plant scale retort was 900 mm long with a diameter of 560 mm. Cans at 40°C were loaded into baskets and arranged as required. Unless otherwise stated cans were processed in a vertical position. The retort was vented until its temperature reached 121°C. An automatic controller maintained the temperature of the retort at 121 ± 0.5°C. For pressure cooling, compressed air was admitted to the retort through a control valve to give a pressure of 138 kPa. When cans were cooled without a counterbalancing air pressure, the pressure in the retort was reduced to atmospheric over a period of 5 min. Water at 16°C was used to cool the cans.

Treatment of temperature data

The thermocouples were connected to a 12-point recording potentiometer (Phillips Transokomp 250) operating on a 30-sec print cycle. The can temperature was read off the chart (measurement error ±0.25°C) and treated in the following way.

(i) Can centre temperature was plotted against time for the heating and cooling phases of each of the cans.
(ii) The logarithm of the difference between retort temperature and the can centre temperature was plotted against time for the heating phase. An asymptote was fitted to the straight line part of the curve by eye and the slope of the asymptote (f_h) was measured (Lund, 1975).
(iii) The lethality of the heating phase (F_h) was calculated by the method of Board (1977) and by the formula method using the equation given by Lund (1975). The heating phase was considered to end when the steam was turned off.
(iv) The lethality of the delay period (F_d), i.e., the period between 'steam off' and 'water on', was calculated by the graphical method. When cans were cooled under a counterbalancing air pressure, there was effectively no delay period.
(v) The cooling phase was considered to start at 'water on' and the lethality of this part of the process (F_c) was calculated by the graphical method.
(vi) The total lethality of the thermal process (F_o) was calculated using the graphical method of Board (1977) and a z value of 10°C.

Results and discussion

The average F values of thermal processes of 90 min at 121°C in a pilot plant scale retort for seven replicate cans subjected to three consecutive processes are presented in Table 1. It is evident that the F_h values calculated using the graphical method and the formula method agree closely, the mean ratio of the F_h values calculated by the two methods being 1.015. This ratio is not significantly different from unity and agrees closely with the value of 1.0008 reported by Board *et al.* (1960).

Although the average F_c values were similar, there is often considerable variation of cooling rates, particularly for products which cool by conduction. For example, temperature gradients of up to 15°C between cans at the top and bottom of the retort have been reported (Anon., 1977). For this reason the cooling phase is sometimes neglected in calculating safe processes.

However, in conduction heating products at steam-off, large temperature gradients can exist from the outside surface to the thermal centre of the can, leading to a subsequent temperature rise (known as 'overshooting') during the first part of cooling and during the delay period before the cooling water contacts the cans. The results in Table 1 showed that the F value of the delay period was appreciable, being of the order of 13% of the F_o value.

The cooling curves for all the cans processed in these trials without pressure cooling were irregular in shape compared to those cooled under pressure. Irregular cooling curves for cans of products which heat by conduction are mainly attributed to ebulition or internal boiling which causes mixing and hence more rapid cooling of the can contents (Hemler *et al.*, 1952). This makes the estimation of cooling phase lethality (F_c) difficult (Cleland & Gesterkamp, 1983). To avoid buckling of cans when cooling without air over-pressure, it is necessary to release the steam pressure slowly from inside the retort. This process took 5 min in the pilot plant scale retort used in these trials.

With their long heating process, the cans will be substantially at retort temperature throughout during the delay period and the start of cooling, which virtually standardizes the delay and cooling conditions. Shorter processes with greater values of g (the uncompleted temperature rise at the centre) could have produced more significant F_d

Table 1. Mean F values (min) of thermal processes conducted for 90 min at 121°C in pilot plant scale retort for seven replicate cans of bentonite with 10-mm headspace

F_h		F_d	F_c	F_o
graph*	form†	graph*	graph*	graph*
26.5	26.6	4.1	1.3	31.9
25.5	26.2	4.1	1.2	30.8
26.9	26.7	4.3	1.3	32.5

*Calculated using the graphical method of Board (1977).
†Calculated using the formula method (Lung, 1975).
F_h, F_d *and* F_c are, respectively, the lethalities of the heating, delay and cooling periods.

and F_c values, compared with F_h, and values whose distribution might well be different from those observed here due to the larger overshoot with larger g values.

The individual F_h values (calculated graphically) of seven replicate cans over eight runs are presented in Table 2. These results show the variability of the F_h value in replicate cans given the same nominal processes. The 56 individual F_h values were plotted on probability paper and fell on or close to a straight line, thus indicating that in this instance the values followed a normal type of frequency distribution. This is in contrast to the results of Powers *et al.* (1962) who reported that their lethality values generally did not have a normal distribution, but agrees with the results of Toepfer *et al.* (1946) who found that the lethality values for two of the products were normally distributed. However, little emphasis can be placed on these comparisons since different processing conditions were used by these other workers.

Table 2. F_h values (min), calculated graphically for eight runs of seven replicate cans of bentonite with 10-mm headspace processed for 90 min at 121°C

	Runs								Row
Cans	1	2	3	4	5	6	7	8	means
1	25.3	23.5	26.2	25.0	24.4	24.2	21.5	22.1	24.0^{e}
2	24.3	23.6	25.1	25.3	25.3	23.9	21.0	21.7	23.8^{e}
3	26.6	25.0	27.1	27.8	27.4	25.8	23.3	21.7	25.6^{f}
4	25.9	25.1	25.5	26.7	28.0	25.6	23.2	23.6	25.5^{f}
5	28.1	26.9	28.1	27.8	27.6	26.0	23.4	23.2	26.4^{f}
6	27.3	27.3	28.3	29.4	29.7	28.6	24.9	25.3	27.6^{g}
7	28.3	27.0	28.5	30.0	29.8	28.6	26.5	27.1	28.2^{g}
Column means	26.5^{cd}	25.5^{b}	27.0^{cd}	27.4^{d}	27.5^{d}	26.1^{bc}	23.4^{a}	23.5^{a}	25.9

Pooled standard deviation = 0.68.
Means marked with the same letter are not significantly different at the 99% level.

Two-way analysis of variance indicated that there were highly significant ($P = 0.99$) variations both between cans in any particular run and between runs. Calculation of the least significant difference from the residual mean variance showed that the F_h values for cans 1 and 2 were significantly lower and those for cans 6 and 7 significantly higher

than cans 3, 4 and 5. Furthermore, runs 7 and 8 gave significantly lower F_h values than the other runs.

These variations occurred despite operating the retort in a standard controlled manner for each of the eight runs. Bee & Park (1978) list twenty-five factors that have been associated with unreliable heat penetration data. Possible causes of the variations in the present experiments could be due to many of these factors but the most likely would include the position of the thermocouple junctions in relation to the geometric centre of the cans, variation in retort temperature, heat conduction along the thermocouple wires affecting temperature readings, minor differences in headspace and the accuracy of the thermocouples. According to Navankasattusas & Lund (1978) the accuracy of a thermocouple alone is in the range of $\pm 0.1-1°C$, leading to a corresponding error in accomplished lethality of 2.3–26%. Cowell *et al.* (1959) reported that conduction errors are largest in the early stages of heating and cooling and often cause large errors in the evaluation of F_c. Since very long heating periods were used in the present work, conduction errors from thermocouples are likely to be relatively low for F_h values but significant in the case of F_c values.

The results in Table 2 emphasize the importance of carrying out several heat penetration runs on a particular product before deciding if the required thermal process has been delivered. However, because of the between can and between run variation, it is not possible to make any widely applicable recommendation as to the number of test cans per run or the number of runs necessary to establish a thermal process. As Board (1977) pointed out, recommended thermal processes contain large, but ill-defined safety margins and, although the real value of the safety margin is unknown, commercial experience has shown that the procedures currently adopted result in safe processes. That these procedures are relatively vague when specifying the number of cans and heat penetration runs would suggest that the safety factor is considerable, given the variation in process lethality reported in Table 2.

The effect of headspace on mean process lethality is shown in Table 3. The greater

Table 3. Mean F_h values (min), calculated graphically for seven cans of bentonite with varying headspaces processed for 90 min at 121°C (for a given headspace, the same seven cans were used for each run)

Gross headspace (mm)	F_h	
	Mean (n = 7)	Standard deviation
5	23.5	1.8
5	20.4	1.8
5	23.3	1.8
5	20.4	1.5
10	26.5	1.5
10	25.5	1.6
10	27.0	1.4
20	28.4	2.43
20	28.2	a

a = Data incomplete due to buckling of cans.

the headspace in the can, the higher was the F_h value. Using analysis of variance, the differences in process lethality between cans with different headspaces were found to be significant at $P = 0.01$. This confirms the recommendation (Anon., 1977) that cans used for pilot plant scale determination of process lethalities should have the minimum headspace likely to be encountered on a commercial canning line.

Conclusion

The average lethality of seven replicate cans containing a 5% suspension of bentonite heated in a pilot plant scale retort at 121°C for 90 min varied significantly ($P = 0.01$) between runs. The mean values of F_h were not significantly different when calculated using the graphical and formula methods. Within runs the range of F_h values varied from 3.4 to 7.1 min or 13–26% around the mean F_h. Increasing the headspace in cans significantly increased F_h values.

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Effects of gamma irradiation on the lipids, carbohydrates and proteins of Iranian pistachio kernels

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Summary

The effect of two levels of gamma irradiation 0.05 Mrad (for controlling insect pests) and 1 Mrad (for controlling fungi and bacteria) on the lipids, carbohydrates and proteins of the Iranian pistachio kernels var. Badami was studied. Neither 0.05 Mrad, nor 1 Mrad had any significant effect on the various individual simple or complex lipids or their totals or on total available carbohydrates, total free sugars, total starches and dextrins and on the eight individual free sugars identified. There was no significant effect on total proteins, total free amino acids or on the seventeen amino acids either. Of the other parameters investigated, iodine value and malonaldehyde content were not significantly altered, only peroxide value was slightly increased after 1 Mrad irradiation. So, gamma irradiation at such low levels which successfully controls insects, fungi and bacteria has no deleterious effect on the main food constituents of Iranian pistachio kernels.

Introduction

The agriculture of pistachio nuts plays an important role in the economy of a number of countries, especially in those areas where ecological conditions do not favour the development of other agricultural crops, since the pistachio is a dry climate tree and requires little irrigation. The total amount of pistachio nuts exported from Iran has increased from 6388 metric tons in 1969 to 15070 in 1974 (Woodroof, 1977). There are, however, two types of postharvest pests, namely insects and fungi, which may put the entire production at risk. In the spring of 1972 a consignment of pistachio nuts entering the U.S.A. was found to be heavily contaminated with aflatoxins and had to be destroyed. Conventional methods of controlling postharvest diseases by fungicides and insecticides may leave toxic residues in the crop and these compounds cannot penetrate the kernels where insect larvae or fungal spores might be located. Irradiation on the other hand has no such disadvantages and provides a better method of preservation. It is known that physico-chemical properties of a number of nutritional compounds are modified by the radiation treatment. The purpose of this study was therefore to investigate the effect of two levels of gamma irradiation, 50 Krad and 1 Mrad (which control insects, and fungi and bacteria, respectively) on the lipids, the carbohydrates and the proteins of Iranian pistachio kernels.

A thorough investigation of the lipids of six varieties of Iranian pistachio kernels has been carried out by Kashani & Valadon (1983) who found that they contained on average 55% lipids with over 75% of fatty acids made up of 18:1 and 18:2, the latter

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being an essential fatty acid. There are other nutritional compounds in pistachio kernels (Kashani & Valadon, 1984), namely carbohydrates and proteins which may act as protective agents.

The object of the present study was to ascertain whether the effects of radiation at the two levels used were to impart rancidity and off-flavour, as has been observed in other irradiated foods (Rao, Vakil and Sreenvasan, 1978), and also whether there was any hydrolytic effect on the protective agents, as has been shown with model systems.

Materials and methods

The same six varieties of Iranian pistachio nuts (Kashani & Valadon, 1983) kindly provided by the Institute of Modification and Preparation of Seeds, Karadj, Iran, were used. The cultivars were from different regions of Iran and varied somewhat in their sizes from 90.5 to 135.5 g/100 nuts (Kashani & Valadon, 1983). Kashani (1982) has studied these six cultivars in detail but did not observe very wide differences in their nutritive values. The effect of irradiation was studied on all six, but the differences between them were so small that the only one to be discussed will be the variety Badami from Rafsanjan, whose lipid, carbohydrate and protein components before and after salting and roasting were discussed fully by Kashani & Valadon (1983, 1984).

Irradiation of pistachio kernel

Samples of pistachio kernels (500 g) were packed in polyethylene bags, heat sealed and irradiated in ^{60}Co gamma cells (Irradiated Products Ltd, Swindon, Wilts, England). They were irradiated to give 0.05 Mrad (0.5 Mrad/hr for 6 min) and 1 Mrad (1.1 Mrad/hr for 55 min). The purpose of the lower irradiation was for the control of insects (Tilton & Brower, 1973) while the higher dose was for fungal and bacterial control (Urbain, 1978).

Lipid extraction

Ten grams of pistachio kernels were homogenized twice in 200 ml chloroform and 100 ml methanol in a Waring blender for 2 min and then washed with 0.2% of its volume of 0.73% NaCl according to Folch, Lees & Stanley (1957). Total lipids were stored at −20°C in benzene:amyl alcohol:chloroform (1:1:1 v/v) with 0.01% butylated hydroxytoluene added to prevent oxidation (Deven & Manocha, 1975).

Separation and identification of lipid classes

These were carried out as already described by Farhangi & Valadon (1982).

Total fatty acids and free acids

To obtain the fatty acids of total lipids, the latter were hydrolysed for 2 hr under reflux in a solution of 5% KOH in methanol containing 20% benzene and 2% water. The alkaline solution was then acidified with dilute H_2SO_4 and extracted with diethylether to yield total fatty acids (Asselineau & Montrozier, 1976). Free fatty acids were prepared by the method of Draper (1969). Fatty acid methyl esters were prepared by using BF_3-methanol reagent according to Metcalfe & Schmitz (1961).

Gas–liquid chromatography (GLC) and GC–MS of fatty acid methyl esters

These were carried out as described previously (Farhangi & Valadon, 1982).

Iodine value (i.v.), peroxide value and malonaldehyde content

i.v. (Hanus & Wijs) and peroxide value were determined according to the Association of Official Analytical Chemists (1980). The malonaldehyde content was estimated by the method of Shamberger, Shamberger & Wills (1977).

Carbohydrate extraction and estimation

Total available carbohydrates, soluble sugar, starches and dextrins, and individual sugars were extracted and estimated by methods already described (Farhangi & Valadon, 1983; Kashani, 1982; Kashani & Valadon, 1984).

Protein extraction and determination of amino acids

These have already been described (Farhangi & Valadon, 1982; Kashani & Valadon, 1984).

The solvents were from AR stock, redistilled where necessary.

All the experiments were repeated four times and the results are the average ± s.d. of the four determinations.

Results and discussion

It can be seen in Table 1 that simple lipids make up the bulk of the lipids (95%) and therefore complex lipids are only 5% total lipids. The two levels of γ-irradiation (0.05 and 1 Mrad) have no effect on total lipids, on total complex or on total simple lipids. The individual simple lipids identified were sterols and their esters; mono-, di- and tri-glycerides; free fatty acids; while the complex lipids were sterol-glycosides, mono- and di-galactosyl diglycerides; cardiolipin; phophatidyl-choline, -ethanolamine, -serine and -inositol; phytosphingolipid and phosphatidic acid. After irradiation at the two levels (Table 1) there was no significant difference in any of the simple or complex lipids or in any of the individual lipids. Roasting caused an obvious increase in fatty acids and in phosphatidic acid (Kashani & Valadon, 1983), therefore irradiation at the higher level used here (1 Mrad) does not affect lipids as much as roasting does. There is no doubt that even 1 Mrad does not produce any ester compounds such as fatty acid esters or increases in sterol esters in pistachio nuts. This was probably to be expected as Funes (1969) concluded that 3 Mrad did not induce esterification of pure olive oil. Our results are comparable with those of Rao *et al.* (1978) who found no appreciable changes in total lipids in wheat after up to 1 Mrad irradiation. However, they showed a significant increase in free lipids which was not observed in the present study.

Very little is known concerning the fate of complex lipids after irradiation. Bancher, Washoetl & Goller (1972) have reported that phosphatidic acid is formed from the polar lipids of peanuts and walnuts when irradiated at 10 Mrad. Nawar (1977) has reported that volatile compounds are produced from bovine phospholipids by irradiation of unspecified dose under vacuum. However, Rao *et al.* (1978) have shown that 1 Mrad irradiation has no effect on the phospholipids of wheat, as was found in the present study.

Table 2 shows that there are differences between the free fatty acids which contain C16:0, C18:1 and C18:2 and total fatty acids which in addition contain C14:0, C18:3 and C20:0. It can be seen though that even 1 Mrad irradiation has practically no effect on individual free fatty acids or on total fatty acids. So, once again the low irradiation dose of 1 Mrad has no obvious effect on the lipids of pistachio nut (in this case free fatty acid and total fatty acid components).

Table 1. The effect of gamma-irradiation on the lipid constituents of pistachio var. Badami. Unless otherwise stated the results are expressed as mg/g fresh weight ± s.d. (+ indicates a trace)

	Radiation dose (Mrad)		
	0	0.05	1
Simple lipids			
Hydrocarbons	+	+	+
Sterol esters	3 ± 0.2	3 ± 0.1	3 ± 0.2
Triglycerides	438 ± 26.1	440 ± 30.2	427 ± 35.3
Free fatty acids	3 ± 0.2	3 ± 0.3	3 ± 0.2
Unknown	4 ± 0.3	4 ± 0.3	4 ± 0.3
Sterols	11 ± 0.9	10 ± 0.7	9 ± 0.8
Diglycerides	81 ± 6.1	75 ± 5.4	74 ± 7.9
Monoglycerides	+	+	–
Total simple lipids	540 ± 33	535 ± 30	520 ± 41
Complex lipids			
Sterol glycosides	4 ± 0.3	4 ± 0.2	4 ± 0.3
Monogalactosyldiglyceride	3 ± 0.2	3 ± 0.2	3 ± 0.2
Cardiolipin	1 ± 0.1	1 ± 0.1	1 ± 0.1
Phosphatidylethanolamine	2 ± 0.1	2 ± 0.2	2 ± 0.2
Sphingolipid	2 ± 0.2	2 ± 0.2	2 ± 0.2
Digalactosyldiglyceride	1 ± 0.1	1 ± 0.1	1 ± 0.1
Phosphatidylcholine	7 ± 0.4	6 ± 0.4	6 ± 0.4
Lysophosphatidylcholine	6 ± 0.3	5 ± 0.3	5 ± 0.3
Phosphatidylserine	+	+	+
Phosphatidylinositol	+	+	+
Phosphatidic acid	1 ± 0.1	1 ± 0.1	1 ± 0.1
Total complex lipids	27 ± 1.9	25 ± 1.9	25 ± 1.9
Total lipids	567 ± 42	560 ± 37	545 ± 39
Iodine value (cg I_2 absorbed/g fat)	128 ± 2.4	128 ± 1.6	128 ± 3.2
Peroxide value (meq. peroxide/100 g fat)	7 ± 0.5	7 ± 0.3	9 ± 0.4
Malonaldehyde content (mg/100 g)	2 ± 0.1	2 ± 0.4	2 ± 0.2

Table 2. The effect of gamma-irradiation on individual and total free fatty acids (FFA) and on whole oil fatty acids (TFA) of pistachio var. Badami. The results are expressed as percentage total

	Radiation dose (Mrad)					
	% Total FFA			% Total TFA		
Fatty acids	0	0.05	0.1	0	0.05	0.1
C14:0	0	0	0	0.9	0.8	1.0
C16:0	23.1	24.3	24.1	16.8	17.1	17.3
C18:1	50.0	47.6	47.2	46.3	45.6	45.8
C18:2	26.9	28.1	28.7	29.2	29.6	29.3
C18:3	0	0	0	4.0	4.0	3.6
C20:0	0	0	0	2.8	2.9	3.0

To identify other possible effects of irradiation on the quality of pistachio oil, measurements of iodine and peroxide values and of malonaldehyde content were carried out.

The two levels of γ-irradiation have no effect on the iodine value, or on the malonaldehyde content of the pistachios used (Table 1). However, there was a slight but significant increase in peroxide value at 1 Mrad irradiation, representing a slight oxidative change in the fatty acids. Wills & Rotblatt (1964) have studied the effects of low-dose irradiation (between 0.05 and 1 Mrad) on diets containing saturated fats and have shown that peroxide formation increased with increasing irradiation dose. These results are in part similar to those reported here in that there was increase peroxide formation after 1 Mrad irradiation. However, in the present study 0.05 Mrad had no effect at all on the peroxide content. The results obtained have shown clearly that γ-irradiation up to 1 Mrad has very little effect on the lipids and lipid constituents of pistachio kernels. Furthermore, no off-flavour or rancidity was observed after irradiation.

Table 3. The effect of gamma-irradiation on total available carbohydrates, free sugars, total starches and dextrins and on individual sugars of the pistachio var. Badami. The results are expressed as mg/g fresh weight ± s.d.

	Radiation dose (Mrad)		
	0	0.05	0.1
Total available carbohydrates	101.3±6.2	102.9±7.9	96.3±6.5
Total free sugars	27.7±2.0	27.9±1.6	26.4±1.9
Total starches and dextrins	73.6±5.0	75.1±3.4	69.9±4.3
Fructose	1.3±0.1	1.3±0.1	1.3±0.1
Glucose	1.7±0.1	1.7±0.1	1.6±0.2
Sucrose	13.5±0.9	13.6±1.0	13.0±0.8
Maltose	1.4±0.2	1.4±0.1	1.3±0.1
Isomaltose	0.3±0.1	0.3±0.1	0.3±0.1
Cellobiose	0.3±0.1	0.2±0.1	0.2±0.1
Raffinose	6.2±0.7	6.3±0.5	5.7±0.5
Stachyose	1.4±0.1	1.5±0.2	1.4±0.1
Unknown	1.6±0.2	1.5±0.2	1.6±0.2

Total available carbohydrates, total free sugars and total starches and dextrins of the pistachio var. Badami were 101.3, 27.7 and 73.6 mg/g fresh weight, respectively (Table 3). The two levels of irradiation have no significant effect on these three components, neither have they on the eight individual free sugars already identified by Kashani & Valadon (1984). Kashani (1982) has already shown that sugars were not significantly degraded by commercially recommended doses of ionizing radiation for insect disinfestation (max. 50 Krad) and Auda & Khalaf (1978) have shown similar effect (i.e., no changes in reducing sugars) after irradiating three varieties of dates with up to 270 Krad.

So even at 1 Mrad, the carbohydrates of pistachio nuts are not affected but presumably higher doses might do so as was shown by Dauphin & Saint-Lebe (1977). Several reports have indicated that the end products of starch breakdown by irradiation include glucose, maltose, maltotriose, gluconic acid and other low molecular weight organic

Table 4. The effect of gamma-irradiation on total protein, total free amino acids and on individual amino acids of the pistachio var. Badami. The results are expressed as mg/g protein ± s.d.

	Radiation dose (Mrad)					
	Free amino acids			Protein amino acids		
Amino acids	0	0.05	1.0	0	0.05	1.0
Lysine	23 ± 1.2	21 ± 2.1	23 ± 2.0	86 ± 6.9	90 ± 6.1	86 ± 7.2
Histidine	4 ± 0.2	4 ± 0.3	4 ± 0.3	15 ± 1.1	16 ± 1.0	15 ± 1.4
Arginine	4 ± 0.3	4 ± 0.2	4 ± 0.3	15 ± 1.2	15 ± 1.0	17 ± 1.2
Aspartic acid	20 ± 0.2	18 ± 0.0	20 ± 1.7	75 ± 7.0	77 ± 6.4	78 ± 6.0
Threonine	8 ± 0.5	8 ± 0.6	8 ± 0.6	31 ± 2.4	32 ± 2.8	34 ± 2.2
Serine	8 ± 0.5	8 ± 0.5	8 ± 0.5	29 ± 2.1	30 ± 2.2	28 ± 2.5
Glutamic acid	47 ± 4.1	44 ± 3.2	48 ± 4.0	180 ± 16.2	181 ± 16.1	187 ± 16.2
Proline	10 ± 0.8	9 ± 0.3	10 ± 0.7	37 ± 3.0	40 ± 2.3	38 ± 3.1
Glycine	11 ± 0.9	10 ± 0.7	11 ± 0.8	41 ± 2.9	45 ± 3.1	48 ± 4.0
Alanine	11 ± 0.9	11 ± 0.8	12 ± 1.0	43 ± 3.3	45 ± 3.0	44 ± 3.6
Cysteine	2 ± 0.1	2 ± 0.1	2 ± 0.1	7 ± 0.4	7 ± 0.6	8 ± 0.5
Valine	13 ± 1.0	13 ± 1.0	14 ± 1.1	51 ± 3.3	51 ± 4.3	50 ± 3.7
Methionine	4 ± 0.3	4 ± 0.3	4 ± 0.3	16 ± 1.1	16 ± 1.3	16 ± 1.2
Iso-leucine	10 ± 1.0	10 ± 0.9	11 ± 1.0	38 ± 2.6	37 ± 2.8	40 ± 2.6
Leucine	17 ± 0.1	15 ± 1.1	17 ± 0.4	65 ± 4.7	64 ± 5.1	69 ± 4.9
Tyrosine	6 ± 0.4	5 ± 0.3	6 ± 0.4	22 ± 1.8	22 ± 1.7	23 ± 2.0
Phenylalanine	10 ± 0.7	10 ± 1.1	10 ± 0.9	37 ± 2.0	37 ± 2.4	38 ± 3.1
Total free amino acids	208 ± 16	196 ± 14	212 ± 17			
Total protein amino acids (mg/g protein)				789 ± 59	805 ± 67	817 ± 53

acids (Kashani, 1982). It has to be said that these changes were produced with relatively high dosage levels, higher than the maximal dose used in the present study.

Many substances, among these amino acids and proteins, provide protection against radiation degradation of carbohydrates (Phillips, 1972) and therefore these compounds have also been investigated. It can be seen from Table 4 that the pistachio kernels var. Badami contain 789 and 208 mg/g protein of protein- and of free-amino acids, respectively. The two levels of gamma irradiation used do not have any significant effect on them, neither have they on the seventeen individual amino acids present in the free form or in proteins. There is a report, however, which shows a significant destruction of certain amino acids in leguminous seeds after irradiation (Lotti *et al.*, 1975). Irradiation of protein has resulted in modification of side chains, production of new groups, splitting of peptide bonds, the formation of inter- and intra-molecular cross-links, and the breaking of hydrogen bonds (Schubert, 1969). There have also been reports of degradation and specific or non-specific amino acid damage (Patten & Gordy, 1964). However, all these studies were on isolated protein, rather than on complete food systems, and using relatively high levels of irradiation. The lower levels of irradiation on the other hand (up to 1.5 Mrad), do not affect the protein of wheat and wheat flour (Milner, 1961; Pape, 1972) and of macacar beans (Coelho, de Medeiros & Flores, 1978), although the latter authors found losses of certain amino acids when irradiated

beans were cooked. Even after 3 Mrad, Nene, Vakil & Sreenivasan (1975) did not observe any effect on the total proteins of red gram (*Cajanus cajan*), although they reported an increase of 15% free amino acids after 1 Mrad irradiation. This latter result is in sharp contrast to that of the present study where no increase in free amino acids was observed using the same dose and different but yet complete food systems. Furthermore, Rao *et al.* (1978) have shown the disappearance of some purothionine bands when wheat was irradiated with 1 Mrad; wheat containing only 1.8% total lipids. It may well be that in pistachio nuts the high levels of lipids together with those of carbohydrates may act as a protection against radiation.

Kashani & Valadon (1983, 1984) have already shown that lipids, carbohydrates and proteins of the pistachio kernel var. Badami are affected after salting and roasting. On the other hand, the two levels of gamma-irradiation used (0.05 and 1 Mrad) have no significant effect on the three main constituents of the pistachio nuts: proteins, carbohydrates and lipids.

At the present time a number of foods have been given regulatory approval for consumption by the general public. In the U.S., wheat and wheat products are approved for insect disinfestation by irradiation and in Japan potatoes are irradiated commercially with Government approval (Urbain, 1978). Although in the present study no safety tests were carried out, yet, sufficient evidence has been presented to show that 1 Mrad irradiation which can control fungi and bacteria has practically no effect on the major constituents of Iranian pistachio nuts. In fact, roasting has more obvious effect on lipids as was shown by Kashani & Valadon (1983), and on the carbohydrates and proteins of these nuts (Kashani & Valadon, 1984).

In conclusion then, 0.05 and 1 Mrad irradiations have no significant effect on total lipids, on total complex and simple lipids, on individual lipids, on total available carbohydrates, on total free sugars, on total starches and dextrins, on total protein amino-acids and on free amino-acids of six varieties of Iranian pistachio nuts. Free fatty acids an total fatty acids are not affected either. The other parameters studied, iodine value and malonaldehyde content were not affected by these two levels of irradiation, while peroxide value was only slightly affected at the higher dosage (1 Mrad) used. It can be said then that the two levels of irradiation have practically no effect on the various nutritive compounds studied.

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Technical note: A rapid inexpensive procedure for determination of nitrogen in plant materials

G. APOSTOLATOS

Introduction

The most commonly used and most widely accepted method for the determination of nitrogen and protein content in plant materials, especially in crude samples, is the wet combustion method developed by Johan Kjeldahl in 1883. The nitrogen present in the sample is converted to ammonium sulphate and finally to ammonia which is titrated with a standard acid solution.

However, the number of total nitrogen analyses which can be performed daily is generally limited by a lack of sample digestion facilities, a direct result of the relatively high cost of Kjeldahl digestion units and the considerable laboratory space required for these units. The methodology described in this study includes wet combustion of the sample in a laboratory made block digestor and subsequent spectrophotometric determination of nitrogen.

Materials and methods

Equipment

A block digestor was constructed by fixing an aluminium block (30×30×5 cm, L×W×H) holding 25 graduate Pyrex tubes (2.1 i.d. × 19 cm, 75 ml capacity), on top of a high temperature heating plate, developing 450°C maximal temperature. The whole system was insulated with ceramic slabs which were cemented with heat resistant (up to 1900°C) 'Alundum' refractory cement and was operated under a uniform airflow in a hood with protective shield.

Reagents

The following reagents were used: sodium hydroxide solution (50% w/v), concentrated sulphuric acid (96% w/v), ammonium sulphate primary standard (20.98–21.38% N), phenol crystals and mercuric oxide, obtained from Fisher Chemical Co; sodium nitroprusside and ethelenediamine tetracetic acid disodium salt (EDTU), obtained from Sigma Chemical Co; 1,3-diphenyl guanidine (DPG, 97% w/w) from Aldrich, and commercial CHLOROX solution (5–7% available chlorine).

Stock solutions

The following stock solutions were made.

Solution A. Nine parts of 0.5 M NaOH solution were mixed with 70 parts of 150 ppm EDTA solution in deionized water adjusted to pH 10.0.

Solution B. Five grams of phenol crystals and 25 mg sodium nitroprusside were made to 500 ml with deionized water.

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Solution C. This was prepared just prior to the assay by mixing 2 parts of commercial CHLOROX solution with 98 parts buffer made of 2.5 g NaOH, 1.87 g Na_2HPO_4 and 15.9 g $Na_3PO_4 \times 12\ H_2O$ in 500 ml deionized water.

Nitrogen standards. (a) Ammonium sulphate, 0.472 g per litre; (b) Diphenylguanidine (DPG), 1.257 g in 50 ml warm ethyl alcohol.

Seed meal

Seeds were ground to pass through a 100-mesh sieve, defatted three times in 5 volumes of petroleum ether, then dried under vacuum and stored in stoppered glass vials.

Procedure

One gram of catalyst (K_2SO_4/HgO, 20:1) was placed in the bottom of a digestion tube and well mixed with 50 mg of finely ground seed meal or 10 mg of crude protein (either finely ground or in solution of known concentration); 4 ml of concentrated sulphuric acid were pipetted in, and the mixture was heated at 350°C for 10 min in the digestion block. The tubes were then removed from the heat and allowed to cool for 5 min, after which 2 ml of H_2O_2 solution (27% v/v) were added dropwise on the walls; the tubes were placed back into the digestion block and a small glass funnel (25 mm i.d.) was placed in the mouth of each tube to insure efficient refluxing of the digestion mixture and prevent loss of H_2SO_4. The mixture was digested for another 45 min at 410°C, cooled down, and before the suspension solidified, deionized water was added carefully to 50 ml for vegetable seed samples, and 25 ml for cereal seeds; then it was mixed in a Votex, capped with a rubber stopper and kept at room temperature until further use. For the assay, 7.9 ml of *Solution A* were pipetted in to a 20-ml test tube, followed by 100 μl of diluted digest and immediately, by 1.0 ml each of *Solutions B* and *C*. Triplicate analyses were made for each digest. The colour was allowed to develop for 30 min at 37°C before reading at 630 nm in a 1.0-cm path cuvette A DPG standard (300 μl) and a blank made by digestion of 100 mg ammonia-free filter paper were included in each run.

Results and discussion

Because of the small amount of the sample being used in this method, it is essential that finely ground samples and protein solutions are used, for homogeneous sampling. From the various catalysts tested, the K_2SO_4/HgO mixture was superior in regard to effectiveness of digestion and minimal interference in the development of colour. Other catalysts tested in the same system ($CuSO_4$, SeO_2) required twice as much digestion period and resulted in coloured blanks (Apostolatos, 1980).

The samples should be fully carbonized prior to addition of H_2O_2 which otherwise may oxidize some nitrogeneous compounds irreversibly (Bradstreet, 1965). The major advantage of adding H_2O_2 is the better refluxing one achieves during digestion. Beecher & Whitten (1970) reported that enough time has to be given after the addition of H_2O_2 for its complete destruction, as its presence in the assay mixture restricts the colour development. It has been found that the addition of a few drops of 0.1 M $KMnO_4$ solution approximately 10 min prior to the end of digestion, accelerates the decomposition of H_2O_2 without affecting the colour in the assay.

Problems regarding the stability of solutions (Mitchell, 1972; Weatherburn, 1967)

were eliminated by keeping *Solution B* in an amber bottle, but mainly by mixing the two components of *Solution C* just prior to the assay. The latter precaution is necessary, as *Solution C* rapidly deteriorates even when kept cold (Apostolatos, unpublished results). The storage life of *Solution B* and of the buffer used in *Solution C*, is approximately 2 months when kept at 4°C. In the presence of ammonia and of sodium nitroprusside acting as a catalyst, phenol is oxidized by sodium hypochloride (CHLOROX) to form indophenol (Lubochinsky & Zalta, 1954), with the intensity of colour affected by the final pH value of the reaction mixture. In agreement with other investigators (Weatherburn, 1967), optimal results were obtained in the pH range 11.5–12.3. Standard curves for nitrogen determination were made by using DPG as digested standard, as well as ammonium sulphate (without digestion). Quantitative recovery of nitrogen was obtained in the concentration range from 1.0 to 8.0 μg nitrogen per 100 μl diluted digest (Fig. 1).

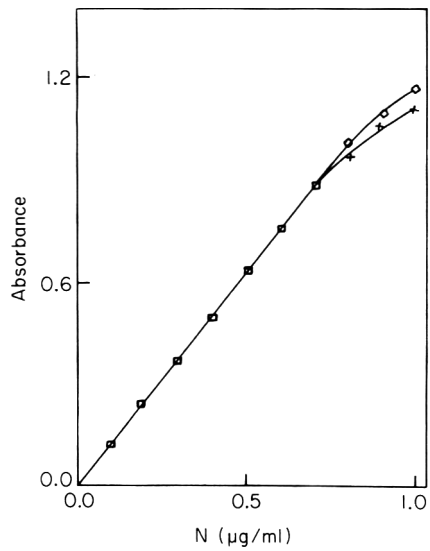

Figure 1. Standard curves for nitrogen determination. (a) DPG standard; (b) ammonium sulphate standard.

Results of nitrogen determination as well as protein content of various seed meals are given in Table 1. In case of cereal seeds the concentration of NaOH in *Solution A* is 0.75 M, instead of 0.5 M used for the vegetable seeds, and the final dilution volume of the digest is adjusted to 25 ml. This is necessary because of the low protein and high carbohydrate content of cereal seeds; the combined effect of carbohydrates and K_2SO_4 causes considerable frothing during digestion with subsequent losses due to overflow. This difficulty can be minimized by adding the K_2SO_4 after the preliminary digestion of the sample with H_2SO_4.

The described method has been used in our laboratory extensively for mass screening of legume, cereal and vegetable seeds for protein content. The facility of the block digestor (easily made, inexpensive, space saver) which makes possible simultaneous treatment of twenty-three samples per run, the direct utilization of the diluted digest, which offers an almost unlimited number of replications, the sensitivity, reproducibility and precision of the method ($\pm 1.5\%$) makes it suitable for screening

Table 1. Protein content of various seed meals

Seed meal	% N	F^*	% Protein
1. Soybean	8.5	5.71	48.5
2. Peanut	4.7	5.46	25.7
3. Navy beans	3.6	6.25	22.5
4. Cowpeas	3.4	6.25	21.3
5. Chickpeas	2.8	6.25	17.5
6. Peas	3.2	6.25	20.0
7. Lentils	4.3	6.25	26.8
8. Tomato	4.7	6.25	29.3
9. Cucumber	3.1	6.25	19.4
10. Squash	4.4	6.25	27.5
11. Watermelon	2.6	6.25	16.3
12. Barley	2.1	5.83	12.2
13. Corn	1.9	6.25	11.9
14. Oat	2.0	5.83	11.7
15. Rice	1.3	5.95	8.1
16. Sorghum	1.7	6.25	10.6
17. Wheat	2.2	5.83	12.8
18. Cotton	3.6	6.26	22.5

F^*: conversion factor from N to protein taken from: 'Amino acid content of foods and biological data on proteins'. FAO. Nutr. Div., Rome. Italy. No. 24. 1970.

tests. More than 160 samples per man-day can be analysed by this method which requires only one-tenth of the size of sample and the reagents used by the automated methods.

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Technical Note: A rapid procedure for determination of phosphorus from a Kjeldahl digest. Application to plant protein isolates

G. APOSTOLATOS

Introduction

Phosphorus is largely present in seeds in the form of phytin, a complex salt of phytic acid with calcium, sodium and potassium. Under favourable pH conditions, proteins extracted from seed meals react with phytin to form complex products of variable composition. How far the phytic acid is 'available' to human nutrition is unclear. Phytin-protein complexes also decrease the bioavailability of minerals and vitamins (Rackis, 1971). Autoclaving, addition of chelating agents and exogeneous phytase have been used to eliminate phytin from vegetable protein isolates.

Phosphorus can be determined spectrophotometrically either by the vanadate (Kitson & Mellon, 1944; Stuffins, 1967) or reduced molybdate (Allen, 1940; Fiske & Subarrow, 1925) procedures, following wet or dry ashing of the sample. The phosphorus determination in plant extracts is frequently complicated by the fact that these extracts exhibit appreciable turbidity and/or colour, due to the presence of protein and carbohydrates. This study presents a procedure for mass screening of phosphorus in seed meals and proteins using a Kjeldahl digest.

Materials and methods

Equipment

A block digestor was constructed by fixing an aluminium block (30×30×5 cm, L×W×H) holding twenty-five graduated pyrex tubes (2.1 i.d. × 19 cm, 75 ml capacity), on top of a high temperature heating plate, developing 450°C maximal temperature. The whole system was insulated with ceramic slabs which were cemented with heat resistant (up to 1900°C) 'Alundum' refractory cement, and was operated under a uniform Airflow in a hood with protective shield.

Reagents

Four grams of ammonium molybdate were dissolved in 40 ml distilled water maintained at 50°C. One hundred milligrams of ammonium vanadate were dissolved in 30 ml boiling distilled water. The two solutions were mixed, cooled down, 20 ml concentrated nitric acid were added and the volume was made up to 100 ml with distilled water. This solution is stable enough for 2 months when kept in amber bottles at room temperature.

Protein isolates

Protein was extracted by stirring 10 g of defatted, finely ground seed meal (to pass through a 100-mesh sieve), in a 100-ml solution of 10 mM sodium hydroxide, for 30 min

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at 4°C. The suspension was centrifuged at 20000 *g* for 15 min. Protein isolates were obtained by adjustment at the clear supernatant liquid at pH 2.0 and 4.5, respectively, by 3 N HCl; the precipitated protein was recovered by centrifugation (5,000 *g*, 10 min), dialysed against distilled water, freeze dried and stored at − 18°C.

Procedure

Fifty milligrams of finely ground seed meal of 10 mg of crude protein isolate were digested for 1 hr at 410°C in 4 ml concentrated sulphuric acid (96% w/v), according to a modified microKjeldahl procedure (Apostolatos, 1980). The digest was made up to 50 ml with deionized water. For the assay, 1 ml of diluted digest and 5 ml of $Na_2S_2O_3 \times H_2O$ solution (2 mg/ml) were pipetted into a 20-ml test tube and were heated either in an open flame for 30 sec, or in a water bath for 7 min at 95°C; the solution was cooled down and partially neutralized with 1 ml of 0.8 M sodiumhydroxide solution. To this solution 2 ml of phosphoreagent solution were added and the mixture was incubated at room temperature for 7 min; the developed colour was determined spectrophotometrically at 436 nm.

Results and discussion

Because of the small amount of the sample being used in this method, it is essential that finely ground samples are used, for homogeneous sampling. The phosphoreagents used by other investigators (Fiske & Subarrow, 1925; Allen, 1940) were interfering with the ammonia present in the digest, forming a white precipitate (ammonium molybdophosphate) during incubation. This interference is eliminated by including vanadate in the phosphoreagent solution. It is fairly well established (Kitson & Mellon, 1944; Pons & Gunthrie, 1946) and confirmed by this investigator, that most metals do not interfere either with the spectrophotometric assay or with the stability of solutions at concentrations up to 100 ppm in the assay solution. Interference above this concentration is induced by ferric and arsenate ions at the maximal wavelength of 436 nm, but it is diminished at 366 nm The presence of heavy metals is rather unusual in seed meals, though pesticide residues cannot be excluded.

The potassium permanganate used in the digestion step for destruction of H_2O_2 excess (Apostolatos, 1980) could be a potential error source (±3%), if it were not converted to inert manganese sulphate in the acid medium. Mercuric catalyst did not cause any interference, the thiosulphate added in the assay solution precipitating the mercury in the form of black mercuric sulphide. Standard curves were constructed at three different wavelengths (436, 405 and 366 nm). The absorptivity of the coloured products decreases from the highest to the lower wavelength values, but metal interference is also diminished in the same direction. Beer's law applies for phosphorus concentration range from 1 to 100 μg phosphorus per ml of diluted digest (Fig 1).

The heat treatment of the assay solution prior to the addition of phosphoreagents, is essential in order to convert any pyro and polyphosphates formed during the digestion of the sample, to reactive orthophosphates. The final acidity of the assay medium should be between 0.2 and 1.4 N; less than 0.2 N acidity results in development of a yellow-orange colour, while at higher than 1.4 N acidity the colour development is inhibited. In solutions of 0.7–0.9 N sulphuric acid containing phosphorus, the development of the colour takes place within 6 min and it remains stable for at least 12 hr.

Average values of total phosphorus content in various seed meals is given in Table 1; these values varied considerably for a given species depending upon the cultivar,

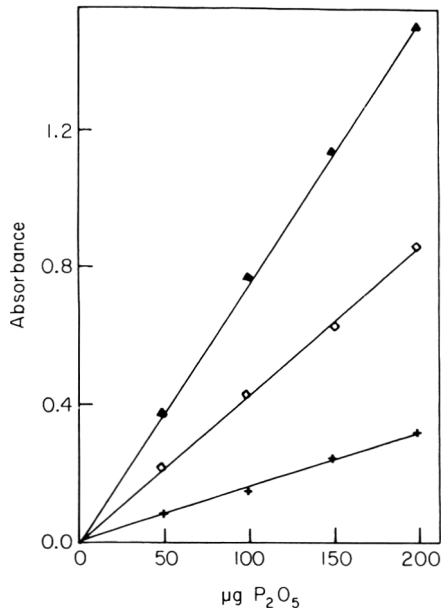

Figure 1. Standard curves for phosphorus determination: (+) A_{366}; (□) A_{405}; (▲) A_{436}

Table 1. Total phosphorus content in selective seed meals

Source	Population *N**	Phosphorus content (g/100 g seed meal) Average	Range
Beans	10	0.460	0.320–0.540
Lentils	6	0.540	0.480–0.610
Soybean	9	0.480	4.400–0.620
Peas	8	0.380	0.340–0.420
Chickpea	7	0.320	0.280–0.370
Lupine	8	0.380	0.320–0.450
Cottonseed	5	0.400	0.350–0.500
Peanut	8	0.560	0.230–0.660

* Population *N*: number of different samples tested.

environmental effects and growth factors. Protein isolates obtained by isoelectric precipitation at pH values of 2.0 and 4.5, contained different amounts of protein and phosphorus. At pH 2.0 the phosphorus to nitrogen ratio in the precipitated isolates ranged from 1.8 to 5.6; at pH 4.5 this ratio approaches one (Table 2). It is significant that for lupine and lentil seed meal a small amount of proteins precipitated at pH 2.0, interacts with most of the available phytin. The latter indicates specific affinity of some proteins to phytin.

The modification described here for the determination of phosphorus in plant materials from a microKjeldahl digest is fast, precise (±1.5%) space and equipment saving, and ten-fold more sensitive than the methods having been published for determination of phosphorus from a Kjeldahl digest (Stuffins, 1967; Thomas, Sheard &

Table 2. Nitrogen and phosphorus content (% of the total) of selective protein isolates obtained by isoelectric precipitation

Protein isolate	% *N*		% P		P/*N* ratio	
pH	2.0	4.5	2.0	4.5	2.0	4.5
Soybean	16	88	42	90	2.6	1.0
Peanut	15	93	84	96	5.6	1.0
Pea	18	75	32	85	1.8	1.1
Lupine	15	64	80	92	5.3	1.4
Lentil	22	82	75	88	3.4	1.1
Cottonseed	42	94	84	96	2.0	1.0

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Book Reviews

Trichothecenes: Chemical, Biological and Toxicological Aspects. Ed. by Y. Ueno. (Developments in Food Science, **4**).
Amsterdam: Elsevier, 1983. Pp. xiii + 313. ISBN 0 444 99661 3. D fl. 200.00.

This is a timely publication on a highly topical subject. The growing concern now being registered in food technology and agriculture on the recognitior of the wide distribution of the trichothecenes in nature will make this book widely appreciated.

The trichothecenes are a family of closely related sesquiterpenoids and were first discovered as antibiotics before even the aflatoxins had been discovered. Next to the aflatoxins the trichothecenes have a major economic impact because of their adverse effects on domestic animals and man. Indeed the number of outbreaks of poisoning in humans due to trichothecenes far exceeds those from other mycotoxins. These toxins show a variety of toxic syptoms in man and animals including skin irritation, haematological disorders, vomiting and feed refusal and inhibition of immune systems. The effect on the immune system and the ability to reduce growth of farm animals are the two main areas of concern.

The book is multiauthored and is made up of six sections. After an interesting and well balanced coverage of the history of the tricothecenes, the chemistry of the trichothecenes is given admirable coverage. The complexity of the taxonomy of the Fusaria explain why there has been some element of confusion in associating toxin production with specific species of *Fusarium*. This is a particularly informative section and offers much valuable information to the reader. Analysis of toxins is clearly described and will be invaluable to all who attempt to work in this field.

Toxicology is more than adequately covered and shows the wide extent of toxic response in man and animals. This section will be of particular interest to veterinary readers. The final section is very large and deals with the worldwide natural occurrence of the trichothecenes. Although in part it is very repetitive it is important to recognize the almost universal nature of the problem.

Professor Ueno must be complimented for bringing this distinguished group of scientists together. The end product is a fascinating presentation that will be a major work of reference for many years to come. The book abounds with simple typographical errors and it is a pity that the subediting had not been more careful. However, this does little to detract from this excellent book which should be well received by microbiologists, biologists, veterinary scientists, agriculturists, biochemists, pharmacists and many others. For all who are interested in mycotoxins this book would be a source of much pleasurable and informative reading.

J. E. Smith

Handbook of Indigenous Fermented Foods. Edited by K. H. Steinkraus. Basel: Marcel Dekker, 1983. Pp. ix+671. ISBN 0 8247 1848 8. SwFr. 211.

This volume is based on the Symposium Workshop on Indigenous Fermented Foods which was held in conjunction with the Fifth International Conference on Global Impacts of Applied Microbiology in Bangkok, Thailand in 1977. Its pedigree is impressive—the conference was sponsored by, *inter alia*, UNEP, UNESCO, and ICRO and the Handbook by the Joint Bioconversion Programme of the World Hunger Programme and the National Resources Programme of United Nations University.

The aim of the Symposium Workshop was to promote 'co-operation among developing countries for increasing their food production and for building a system geared towards food availability and security'. The Handbook is presented with the hope of providing an authoritative and comprehensive text on indigenous fermented foods, which would be useful in 'teaching applied microbiology not only in the developing world but in the developed world as well', and in serving 'the future needs of mankind as a source of information for producing low-cost, protein-rich nutritious foods'. The proposed readership includes also microbiologists, food technologists, foreign aid administrators, nutritionists and manufacturers of fermented foods, and the publishers put forward the view that it is an ideal textbook for courses in food science and food technology. The contents of the book need to be judged against this background.

There are six sections. The first, on 'Indonesian tempe and related fermentations', subtitled 'Protein-rich vegetarian meat substitutes', comprises eighty-four pages of text plus contents and bibliography. The second section on 'Indigenous fermented foods involving an acid fermentation', subtitled 'Preserving and enhancing organoleptic and nutritional qualities of fresh foods', has 180 pages of text, plus contents and bibliography. The products covered include acid-fermentation of vegetables (e.g. sauerkraut), bread and pancakes, cereal gruels, seafood, rice and meat mixtures, milk and milk/cereal foods. Section III covers in 117 pages of text, alcoholic beverages such as wines from honey, sugar cane and palm; pulque; kaffir and other beers; rice wines; tropical vinegars; nata and tea fungus. The fourth section deals with such products as soy sauce, miso, fermented fish-shrimp sauces and pastes, and Chinese red rice (125 pages of text). The fifth section is on the use of the mushrooms *Pleurotus*, *Volvariella* and *Termitomyces* to convert various food wastes and agricultural wastes to food (27 pages). This section is subtitled 'Producing single cell (microbial) protein . . .', but this would seem to be a misnomer since the procedures described are for the production of the typical macrofungal fruiting bodies by traditional means, rather than use of fermentation vessels to culture these fungi in single cell form. The final short section is of miscellaneous contributions including a brief resumé of microbial genetics and the selection of strains for fermentations, and a brief discussion of the problems of mycotoxins in fermented foods.

The unfortunately unbalanced presentation (as judged against the stated objectives of the text) probably result from the book's origins in the Symposium Workshop. As an example, the foreword comments on the potential of fermented foods for enhancing nutritional value and making more wholesome foods available to low- and average-income groups. In spite of this, 117 pages of the book are concerned with alcoholic beverages, because of their ability to act as sources of calories, B-group vitamins and, it is claimed, protein. Yet the forty-four pages on fermented milk and milk/cereal products only briefly deal with yoghurts, kefir, koumiss and other fermented milks, and

do *not* discuss cheeses at all. While it must be admitted that for the Handbook to include a discussion of typical cheese types from around the world, perhaps a doubling or tripling in size would have been required, it is strange that the Preface and Foreword (there is no Introduction) do not give an explanation of the choice of topics; nor do they define the phrase 'indigenous fermented foods'. Indeed, 'indigenous' is used interchangeably with 'traditional', although the meanings and the socioeconomic implications of these two adjectives are very different.

In my opinion it must therefore be seen not as a Handbook, which implies that it is reasonably comprehensive over the full range of topics encompassed in the title, but rather as a useful additional text to place alongside the many other texts on fermented foods. Neither can it be regarded as a suitable text for courses in food science and food technology, since it treats of much fine detail of selected specific fermentation processes, but does not deal with the scientific bases of the production—the biochemistry and physics of the changes induced in, for example, the composition, nutritional value, texture or flavour—nor with the preservative effects. In addition, a UK price of around £68 is not particularly conducive to student purchase!

In summary, the book offered an exciting prospect, but in the event this reviewer was rather disappointed in not finding a reference work capable of acting as the sole source in this field. Nevertheless, it does serve as a useful additional text alongside other books on fermented foods, providing useful information especially on products derived from soybeans (tempe, shoyu, miso, natto) and from fish and shrimps.

W. F. Harrigan

A Modern Introduction to Food Microbiology (Basic Microbiology, Volume 8). By R. G. Board.
Oxford: Blackwell Scientific Publications, 1983. Pp. x+236. Paperback: ISBN 0 632 00165 8. £8.75.

This is a fine book that will be widely used in the teaching of microbiologists as well as food scientists and food technologists. It will be used in study of the principles of microbial ecology in a specific context—the food industry. As such it will serve as an excellent focus within general microbiology. Its main use, though, will be to clearly present and explain to students who plan to join the food industry the range of microbiological processes and problems in that industry. An appreciation of both the ancient art and the modern scientific and technological principles of food processing is conveyed. A knowledge of basic microbiology is assumed.

The book consists of nine chapters: ecology and food microbiology, inhibiting the growth of micro-organisms; control of contamination; appertization, pasteurization, radiation and asepsis; deliberate infection; microbial food spoilage; water; sewage treatment; and food-mediated disease. There are no references in the text, a feature that contributes to the clear, uncluttered style. Guidance to further reading is provided by a well-constructed section at the back of the book organized by chapter topic. The book is well produced in clear type remarkably free from errors. There is a useful index. The treatment of the subject is incisive and very readable. There are many useful tables and diagrams which reward careful study.

A flavour of the book can perhaps be conveyed by the following examples, which show how underlying principles and practical application are constantly related to one another and which demonstrate Dr Board's thorough knowledge of and concern for his subject.

'Preservation can be considered to be a form of enrichment culture, unless sterility is achieved'. 'Indeed a food microbiologist has to attempt the application of fundamental knowledge in an area where at times quite arbitrary restrictions operate'. Treatment of food on aircraft, because of weight restrictions, means that systems 'are designed so that chill temperatures are maintained rather than *attained* during flight' making it imperative that food is at the desired temperature when loaded. Pork pies may become contaminated by moulds growing on crumbs collecting in cooling rooms behind shutters set up to ensure turbulence of cooling air. Some metabolic products (e.g. lactobrevin) 'remain poorly defined because they appear to have attracted the attention of etymologists rather than organic chemists'. In practice in considering control of food poisoning 'the food microbiologist has to take a broad view . . . his role is that of fire prevention officer rather than that of a fireman at the scene of the fire'.

It is my belief that this book will quickly establish itself as a major text for many University and College courses, and for training in the food industry.

R. W. A. Park

High and Low Erucic Acid Rapeseed Oils: Production, Usage, Chemistry and Toxicological Evaluation. Ed. by J. K. G. Kramer, F. D. Sauer and W. J. Pigden. Toronto: Academic Press, 1983. Pp. xx + 582. ISBN 0 12 425080 7. $79.00.

The development of low erucic acid and low glucosinolate varieties of rapeseed has led to a rapid increase in the production and application of rapeseed oil and rapeseed meal in food products and animal feed, respectively. This book describes the background to the development of the new varieties of rapeseed, and it gives a comprehensive account of all aspects of rapeseed oil. The book includes twenty-two chapters which cover production and trade, history and marketing, chemical composition, plant biosynthesis, oil production and processing, consumption and nutritional studies on the effects of high and low erucic acid oils in the diets of humans and animals.

Low erucic acid rapeseed oil has been established by a concentrated research effort in Canada and the editors have collected contributions from many of the research workers who have been at the forefront of the studies on the oil. The standard of the individual contributions is high and the editors have been successful in avoiding significant overlap between the different chapters. Errors appear to be few, although the formula of brassicasterol is erroneous on page 106. The references, given at the end of each chapter, are generally up to date, and the index is useful and comprehensive.

The book will be of great value to all who are interested in an account of the research and development programme required in the change of rapeseed oil from an oil of doubtful nutritional value to a product of similar composition and nutritional quality to other vegetable oils. Users of rapeseed oil, as well as research workers interested in oils and fats generally, will find the book of great value.

M. H. Gordon

Third International Symposium on Preharvest Sprouting in Cereals. Ed. by J. E. Kruger and D. E. LaBerge.
Boulder, Colorado: Westview Press, 1983 (distributed by Bowker Publishing Co., Epping). Pp. xii+312. ISBA 086531 535 3. £16.00.

The problem of preharvest sprouting damage in cereals affects all cereal based food manufacturers where consistency in the quality of raw materials is increasingly required. The growing need for exchange of information among research workers with an interest in preharvest sprouting culminated in the first international symposium in 1975 in Svalov, Sweden. Its success led to further meetings in 1979 in Cambridge, England, and then most recently in June 1982 in Winnipeg, Canada.

The proceedings of this, the 'Third International Symposium on Preharvest Sprouting in Cereals' were ably edited by J. E. Kruger and D. E. LaBerge. The attractive, hard-back, 312-page book comprises some thirty-eight contributions divided among three sections concerned respectively with the physiology, chemistry, and plant breeding and genetic aspects of preharvest sprouting. Certainly the publishers, Westview Press, Inc., are responsible for a presentation much improved in general layout, and freedom from typographical and cross referencing errors which marred the publications of the proceedings of the first and second symposia.

Food scientists and technologists will find several useful and concise review articles by, for example, R. W. King ('The Physiology of Pre-Harvest Sprouting'), C. M. Duffus ('Recent Progress in the Biochemistry of Immature Cereal Grains in Relation to Pre-Harvest Sprouting'), and M. D. Gale ('Alpha-Amylase Genes in Wheat'). Some other contributions were more specialized and will be of most value to plant physiologists, cereal chemists and geneticists.

For the general reader, V. Stoy's Introduction ('Progress and Prospect in Sprouting Research') summarizes well the current problems of pursuing consistent research into preharvest sprouting ('. . . a general interest in the problem flares up for a few years under the influence of a bad weather period only to die off again as soon as the climatic situation improves'). He also summarizes the major results and development of understanding accruing from the 1979 symposium. Thus, one criticism of editorial policy is that for the far wider group of people unable to attend the symposium, and to learn individually from the discussions, a final summary of the more important developments could be included at the end of each section, or at least at the end of the proceedings as a whole.

Seven years, since the first symposium, is a comparatively short time in relation to cereal breeding programmes. Nevertheless, it was disappointing but not surprising to find, particularly among the plant breeders' contributions, little cause for optimism that the problem of preharvest sprouting damage is diminishing. This confirms that the size and complexity of the problem argue for the continuation of these valuable symposia.

J. R. S. Ellis

Sanitation, Safety and Environmental Standards. By L. J. Minor.
Westport, Conn.: AVT, 1983. Pp. xii+245. ISBN 0 87055 428 X. US$22.00.

This volume is the second in a series by Lewis J. Minor and associates. Mr Minor is a member of the staff of the School of Hotel, Restaurant and Institutional Management

of Michigan State University and he is much concerned in his writings with the establishment of standards for nutrition (Volume 1) and standards for sanitation, safety and the environment within the food manufacturing and, more particularly, the catering industries (Volume 2). This book advocates the development of more legal and in-house standards for the catering industry (i.e. foodservice standards), but its aim also appears to be to provide background reading and basic technical and scientific information for non-technical management within the catering trade in the United States. It is clearly intended to be an essential text book and reference manual for both trainee and established management.

Mr Minor accomplishes his task with credit. His style and approach reflects a deep knowledge and experience of the catering industry, a perhaps flamboyant teaching style and dedication to improving hygiene and safety standards within the U.S. foodservice industry. The microbiology and chemistry appear to the scientist to be a little sparse at times and perhaps even suspect on occasions, but this could possibly be attributed to Mr Minor's over-simplification of technical matters in deference to the non-scientific reader. A few passing phrases may also horrify responsible food manufacturers and caterers, e.g. 'chemicals often get into food during processing', 'imitation foods concocted or manipulated in the laboratory', 'although many die every year from eating fugu (a Japanese sea food), it is still very popular', 'the cook, in remorse, committed suicide', etc., but again, they probably result from Mr Minor's honourable attempt to provide an easy-to-read-and-assimilate book for the non-scientist.

The book, of course, is intended for a North American readership and much of it is concerned with the indigenous food industry and the requirements of the U.S. legislation. A great deal of this is inapplicable in the United Kingdom and some of the information is quite misleading, e.g. the use of pantothenates in bread as mould inhibitors, the use of radiation sterilization in meat. The U.K. reader would do well to ignore the legal matters unless he is *au fait* with the current U.K. legislation, when the commentaries on the present U.S. scene become useful and informative background reading.

This volume is easy to read, often enjoyable, excellently set out with a multitude of tables and checklists and with large type headings for easy reference. Illustrations are clear and well annotated. The index is adequate without being elaborate. The American phraseology, terminology and spellings, the constant confusion (to the U.K. reader, at least) between bacteria and moulds and the regular reference to standards, or the need for standards, may jar a little with the U.K. reader, but accepting these generally insubstantial reservations, it is a volume well worthy of being read and retained for reference by a wide spectrum of general and line managers, junior technical and scientific staff, safety officers and personnel executives in all food manufacturing and food handling establishments.

S. Wood

JOURNAL OF FOOD TECHNOLOGY: NOTICE TO CONTRIBUTORS

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Steiner, E. H. (1966). Sequential procedures for triangular and paired comparison tasting tests. *Journal of Food Technology*, **1**, 41–53.

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) place of publication and publisher—e.g.

Lawrie, R. A. (1979). *Meat Science*, 3rd edition. Oxford: Pergamon Press.

In the case of edited multi-author monographs, the editor(s) should be indicated in parentheses after the book title—e.g.

Hawthorn, J. (1980). Scientific basis of food control. In *Food Control in Action* (edited by P. O. Dennis, J. R. Blanchfield & A. G. Ward). Pp. 17–33. Barking, Essex: Applied Science.

Standard usage. The *Concise Oxford English Dictionary* is used as a reference for all spelling and hyphenation. 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 one hundred or greater.

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	$\mu = 10^{-6}$ m	minute	min
nanometre	nm = 10^{-9} m	second	sec
litre	l = 10^{-3} m^3		

Chemical formulae and solutions. The correct molecular formula should be used indicating whether the substance is anhydrous or hydrated. Solutions should be given in terms of molarity. The arithmetic basis of other expressions of concentration must be clearly defined.

Species names. In addition to common or trivial names of plants, animals or microorganisms, the species should normally also be indicated by use of the latin binomial at least once. This should be given in full and italicized (underlined once in typescript). In subsequent use, the generic name may be abbreviated to a single letter provided that this does not result in ambiguity.

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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