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Temperature prediction in air drying of food materials: a simple model

P. E. VIOLLAZ, C. SUAREZ AND STELLA ALZAMORA

Summary

The problem of one-dimensional heat and mass transfer in infinite slabs during drying of porous solids is considered. Assuming a known distribution of moisture and temperature, it is possible to estimate effective thermal and mass diffusion coefficients in solids. In this paper it is demonstrated that from these coefficients the equations describing heat and mass transfer processes in the system are easily integrated to obtain average sample temperature and moisture during drying. These theoretical results may be used to evaluate the effects of the degradation reactions during food drying, which depend on the foodstuff moisture content and temperature.

Introduction

Any improvement in the methods of food dehydration would require a better understanding of the drying mechanism and how this is affected by different variables. With this object, we are concerned to develop a simple mathematical model, in an attempt to predict the moisture content and temperature evolution in the sample during drying.

The transport of water in food products may be described in some cases by a mechanism of diffusion and Fick's law has provided a very useful relation in order to describe the process of drying. Many authors have used Fick's law as a way to predict average drying times of food materials (Becker & Sallans, 1955; Jason, 1958). In a more recent paper Vaccarezza, Lombardi & Chirife (1974a) used Fick's law solution in order to correlate moisture content data of sugar-beet root and good agreement was found.

This paper demonstrates that, if a certain initial moisture and temperature distribution is assumed in the solid, it is possible to show a simple relation

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between average temperature and moisture values. Even more, assuming constant kinetic parameters, the temperature and moisture profiles are particularly useful in the evaluation of fictitious film thickness in solids and consequently the corresponding effective conductivities and diffusivities of heat and mass transfer.

A mathematical model is developed using the conservation of energy and mass to solve the problem of one-dimensional heat and mass transfer in infinite slabs, heated from both sides. The resulting coupled heat and mass transfer equations are solved to predict the relative influence of parameter changes on the drying rate.

Theory

Previous work on the drying of sugar-beet root by Vaccarezza *et al.* (1974a) indicated that near parabolic distribution of moisture is developed. Assuming the same for temperature it is possible to define fictitious film thickness in both cases and then to evaluate effective coefficients for heat and mass transfer. The details for the calculations of these coefficients are shown in the Appendix. In developing the model the following assumptions are made:

(1) one-dimensional heat and mass flow;
(2) parabolic initial moisture and temperature distributions;
(3) constant kinetic parameters;
(4) constant physical properties, i.e., specific heat of the sample is assumed to be constant during the process and equal to the specific heat of dry matter;
(5) constant interface moisture content;
(6) transfer processes are in pseudo-steady state.

Performing a mass balance yields the following expression

$$-\frac{V}{S}\frac{d}{d\theta}(\gamma \bar{u}) = k_m \gamma (\bar{u} - u_i) \tag{1}$$

where, if geometry changes are not taken into consideration V/S is constant and equal to R.

Equation (1) may be integrated assuming the initial condition:

$$(u - u_i)_{\theta = 0} = u_0 - u_i$$

$$\frac{u - u_i}{u_0 - u_i} = \exp\left(-\frac{k_m \theta}{R}\right) \tag{2}$$

where the supposition of u_i = constant was used.

Equation (2) written in dimensionless form is

$$\bar{u}^* = \exp(-3\mathrm{Fo}_m) \tag{3}$$

This equation represents the drying rate during the falling-rate period and the plot of log $\bar{u}^*$ versus θ is a straight line with slope proportional to D/R^2.
Performing an energy balance for a slab of thickness $2R$ we get

$$h\ (T_\infty - T_i) = \frac{Rd}{d\theta}\ (\gamma c_p \overline{T}) + k_m \gamma \lambda (\bar{u} - u_i) \tag{4}$$

Now, if we assumed a thermal effective film thickness in the solid it is possible to write

$$R \frac{d}{d\theta} (\gamma c_p \overline{T}) = k_h (T_i - \overline{T}) \tag{5}$$

Substituting eqn (5) into eqn (4)

$$h(T_\infty - T_i) = k_h (T_i - \overline{T}) + k_m \gamma \lambda (\bar{u} - u_i) \tag{6}$$

The left term of the equality corresponds to the quantity of heat transfered to the surface of the body by the surrounding fluid; the first term of the right corresponds to the quantity of heat which passes from the surface inwards to the body, and the second term to the quantity of heat expended in the evaporation of the liquid.
Operating with eqn (6) it becomes

$$T_i = \frac{h}{h + k_h} T_\infty + \frac{k_h \overline{T}}{h + k_h} - \frac{k_m \gamma \lambda}{h + k_h} (\bar{u} - u_i) \tag{7}$$

If eqn (2) is combined with eqn (7) and subtracting $\overline{T}$ from both terms of the equality we have

$$T_i - \overline{T} = \frac{h}{h + k_h} (T_\infty - \overline{T}) - \frac{k_m \gamma = (u_o - u_i)}{h + k_h} e^{-\frac{k_m \theta}{R}} \tag{8}$$

Equations (5) and (8) may be combined to give

$$\frac{d}{d\theta} (\overline{T} - T\infty) = \frac{k_h h (T_\infty - \overline{T})}{R \gamma c_p (h + k_h)} - \frac{k_m \lambda k_h (u_o - u_i)}{R c_p (h + k_h)} e^{-\frac{k_m \theta}{R}} \tag{9}$$

which may be written in the form

$$\frac{d\overline{T}^*}{d\theta} + C\overline{T}^* = A\, e^{-\frac{k_m \theta}{R}} \tag{10}$$

where

$$A = \frac{k_m k_h \lambda (u_o - u_i)}{R c_o (h + k_h) (T_\infty - T_o)} \tag{11}$$

and

$$C = \frac{k_h h}{R \gamma c_p (h + k_h)} \tag{12}$$

The average temperature of the slab is obtained by integrating eqn (10) to give

$$\overline{T} = \frac{A e^{-\frac{k_m \theta}{R}}}{C - k_m/R} + C_1 e^{-C\theta} \tag{13}$$

Equation (13) may be put into dimensionless form

$$\bar{T}^* = \frac{\mathrm{Fo_m\, Ko}\, e^{-3\mathrm{Fo_m}}}{\mathrm{Fo_q\, Bi_q} - \mathrm{Fo_m}\,(1 + \mathrm{Bi_q})} + \mathrm{C_1}\, e^{-\frac{3\mathrm{Fo_q\, Bi_q}}{1 + \mathrm{Bi_q}}} \tag{14}$$

The constants A and C expressed in terms of dimensionless numbers take the form

$$\mathrm{A} = \frac{3\mathrm{Fo_m\, Ko}}{(1 + \mathrm{Bi_q})\theta} \tag{15}$$

$$\mathrm{C} = \frac{3\mathrm{Fo_q\, Bi_q}}{(1 + \mathrm{Bi_q})\,\theta} \tag{16}$$

The Kosovich number (Ko) that appeared in eqn (14) is defined as

$$\mathrm{Ko} = \frac{\lambda\,(u_0 - u_1)}{c_p\,(T_\infty - T_0)} \tag{17}$$

and determines the relations between the heat expended in evaporation and the heat necessary for the heating of a moist body (Luikov, 1966). In order to arrange eqn (14) in a more compact form we use the Luikov number, defined as $\mathrm{Lu} = \mathrm{Fo_m}/\mathrm{Fo_q}$ in this equation and get

$$\bar{T}^* = \frac{\mathrm{Ko}\, e^{-3\mathrm{Fo_m}}}{\mathrm{Bi_q}\left(\frac{1}{\mathrm{Lu}} - 1\right) - 1} + \mathrm{C_1}\, e^{-\frac{3\mathrm{Fo_m\, Bi_q}}{\mathrm{Lu}\,(1 + \mathrm{Bi_q})}} \tag{18}$$

Substituting eqn (3) into eqn (18)

$$\bar{T}^* = \frac{\mathrm{Ko}}{\mathrm{Bi_q}\left(\frac{1}{\mathrm{Lu}} - 1\right) - 1}\, \bar{u}^* + \mathrm{C_1}\, e^{-\frac{3\mathrm{Fo_m\, Bi_q}}{\mathrm{Lu}\,(1 + \mathrm{Bi_q})}} \tag{19}$$

which gives a relation between $\bar{T}^*$ and $\bar{u}^*$.

If we now use the initial condition

$\theta = 0$; $\bar{T}^* = \bar{u}^* = 1$

the value of $\mathrm{C_1}$ in eqn (19) results

$\mathrm{C_1} = 1 - \mathrm{Q}$

where Q is the factor affecting the variable u in eqn (19) defined as

$$\mathrm{Q} = \frac{\mathrm{Ko}}{\mathrm{Bi_q}\left(\frac{1}{\mathrm{Lu}} - 1\right) - 1} \tag{20}$$

Expressing this result in eqn (19) yields

$$\bar{T}^* = \mathrm{Q}\,\bar{u}^* + (1 - \mathrm{Q})\; e^{-\frac{3\mathrm{Fo_m\, Bi_q}}{\mathrm{Lu}\,(1 + \mathrm{Bi_q})}} \tag{21}$$

Equations (21) and (3) may be combined to obtain

$$\bar{T}^* = \mathrm{Q}\, e^{-3\mathrm{Fo_m}} + (1 - \mathrm{Q})\, e^{-\frac{3\mathrm{Fo_m\, Bi_q}}{\mathrm{Lu}\,(1 + \mathrm{Bi_q})}} \tag{22}$$

or an equivalent expression

$$\bar{T}^* = \mathrm{Q}\, e^{-3\mathrm{Fo_m}} + (1 - \mathrm{Q})\, e^{-\frac{3\mathrm{Fo_q\, Bi_q}}{(1 + \mathrm{Bi_q})}} \tag{23}$$

During drying of food products it was found that $Fo_q \gg Fo_m$, which makes it possible to neglect the second term of eqn (23) and concluding an asymtotic solution given by

$$\overline{T}^* = Q\, e^{-3Fo_m} \tag{24}$$

or

$$\overline{T}^* = Q\, \overline{u}^* \tag{25}$$

From eqns (24) and (25) the value of Q may be found from the distance between the straight lines representing log $\overline{T}^*$ and log $\overline{u}^*$ versus Fo_m, or by plotting $\overline{T}^*$ versus $\overline{u}^*$ and measuring the slope of the straight line.

Discussion

Equations (22) or (23) predict the temperature of a slab being dried in air flow during the falling rate period. The asymptotic solution given by eqn (24) or (25) agrees satisfactorily with the solution reported by Vaccarezza *et al.* (1974b) who have found during the drying of sugar-beet root that the Luikov number was approximately 0.01. If we assume this value as a characteristic one for food products it seems reasonable to account for the first term of eqn (21) almost from the beginning of the dehydration.

It is important to remark here that some of the assumptions made for the development of the model, are only rough approximations, i.e., the specific heat of the sample to be equal to the specific heat of dry matter; the diffusion coefficient to be independent of the moisture content and temperature. However, for practical analysis of drying data it is important to have a simple equation, which, even if it is only an approximation, is readily manipulated.

Conclusions

An expression to predict the variation in time of the temperature and moisture in infinite slabs is obtained by means of a simple model. The equations so obtained may be used to evaluate the damage in biological materials, because degradation reactions, in general depend on moisture content and temperature. In this way we can evaluate the advance of the degradation reaction as a function of time.

Appendix

Estimation of effective film thickness in solids

Assuming a parabolic moisture profile in the solid during dehydration we may obtain an analytical expression for the film thickness. Then, for a slab of thickness $2R$ the moisture content distribution is as follows

$$u = az^2 + bz + c \tag{1}$$

where u is the local moisture content and the parameters a,b,c are evaluated with the following boundary conditions

$z = 0; u = u_{max}$
$z = R; u = u_i$
$z = -R; u = u_i$

Operating, eqn (1) becomes

$$u = (u_i - u_{max})(z/R)^2 + u_{max} \tag{2}$$

If an expression of the form

$$W = k_m \gamma (\bar{u} - u_i) \tag{3}$$

is accepted for mass transfer into the solid where the coefficient $k_m = D/\delta$, the problem is to know the effective film thickness where the moisture change defined by eqn (3) takes place expressing the mass flow as

$$W = -D\gamma \, (du/dz)_{z=R}$$

and deriving eqn (2) we obtain

$$W = \frac{D\gamma}{R/2}(u_{max} - u_i) \tag{4}$$

From eqn (4) we see that if the driving force is $(u_{max} - u_i)$, $\delta = R/2$. In general it is more convenient to define the driving force in terms of average moisture content.

We may calculate the value of u which results

$$\bar{u} = \frac{1}{3}(u_i + 2u_{max}) \tag{5}$$

Replacing u_{max} from eqn (5) into eqn (4) and rearranging, it follows

$$W = \frac{D\gamma}{R/3}(\bar{u} - u_i) \tag{6}$$

and the effective thickness for mass transfer that results in this case is $R/3$.

Correspondingly the same value for the effective thickness for heat transfer is obtained assuming a parabolic distribution of temperature in the solid and operating analogously.

Nomenclature

a : thermal diffusivity $= k/c_p\gamma$, cm^2 sec^{-1}
A : constant, defined by eqn (11), sec^{-1}
Bi_q : Biot number for heat transfer $= hR/3k$, dimensionless
c_p : specific heat of dry matter, cal gr^{-1} (°C)$^{-1}$

C : constant, defined by eqn (12), sec^{-1}
D : moisture diffusivity, $cm^2\ sec^{-1}$
Fo_m : Fourier number for mass transfer $= D\theta/R^2$, dimensionless
Fo_q : Fourier number for heat transfer $= a\theta/R^2$, dimensionless
h : heat transfer coefficient, cal $cm^{-2}\ sec^{-1}\ (°C)^{-1}$
k_h : effective coefficient for heat transfer $= 3k/R$, cal $cm^{-2}\ sec^{-1}\ (°C)^{-1}$
k_m : effective coefficient for mass transfer $= 3D/R$, cm sec^{-1}
k : thermal conductivity of dry matter, cal $cm^{-1}\ sec^{-1}\ (°C)^{-1}$
Ko : Kosovich number $= \lambda\ (u_o - u_i)/(T_\infty - T_o)\ c_p$, dimensionless
Lu : Luikov number $= Fo_m/Fo_q$, dimensionless
Q : constant, defined by eqn (20), dimensionless
R : half thickness of the slab, cm
S : area of the slab, cm^2
$\overline{T}$: average temperature of the slab, °C
T^* : $(T - T_\infty)/(T_o - T_\infty)$, dimensionless
T_i : surface temperature of the solid, °C
T_o : initial average temperature, °C
T_∞ : air dry bulb temperature, °C
$\overline{u}$: average moisture content, g water/g dry solid
$\overline{u}^*$: $(\overline{u} - u_i)/(u_o - u_i)$, dimensionless
u_i : equilibrium moisture content, g water/g dry solid
u : local moisture content, g water/g dry solid
u_o : initial average moisture, g water/g dry solid
u_{max} : local moisture content in $z = 0$, g water/g dry solid
V : solid volume, cm^3
W : water flux at the surface of the solid, g water $cm^{-2}\ sec^{-1}$
z : length, cm

Greek letters

λ : latent heat of vaporization, cal g^{-1}
γ : density of the dry solid, g cm^{-3}
δ : effective thickness for heat and mass transfer $= R/3$, with $(\overline{u} - u_i)$ as driving force, cm
θ : time, seconds

References

Becker, H.A. & Sallans, H.R. (1955) *Cereal Chem.* **32,** 212.
Jason, A. (1958) In: *Fundamental Aspects of the Dehydration of Foodstuffs*, p. 103. Society for Chemical Industry, Colchester.
Luikov, A.V. (1966) *Heat and Mass Transfer in Capillary-porous Bodies*, 1st edn. Pergamon Press, Oxford.
Vaccarezza, L.M., Lombardi, J.L. & Chirife, J. (1974a) *J. Fd Technol.* **9,** 317.
Vaccarezza, L.M., Lombardi, J.L. & Chirife, J. (1974b) *Can. J. Chem. Engng.* **52,** 576.

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Aqueous extraction of black leaf tea

IV. Pilot-scale batch-simulated continuous counter-current extraction

M. J. BLOGG AND V. D. LONG*

Summary

Four-stage countercurrent aqueous extraction of black tea at 80°C in about 27 litre batches with a stage contact time of around 3 min and having centrifugal separation between stages was programmed to simulate continuous operation. Final extract concentrations in the range 5–12 mass % tea solids were obtained at yields of 35–29% of the as-received leaf when operating with overall water-to-tea ratios in the range 10:1–5:1. Trends in the results are discussed in relation to a simple three-component model of the tea-water system and an underlying generalization of stage performance proposed. Comparison with earlier small-scale equilibrium extractions indicates an average stage efficiency of around 60%.

Introduction

In work aimed at developing commercial extraction processes for black leaf tea, bench-scale experiments (Long, 1977, 1978, 1979) yielded basic information regarding phase equilibria and rates of solution. Application of the former to the design of continuous extraction processes required a knowledge of likely stage efficiencies or their equivalent which, although calculable from solution kinetics and postulated stage flow behaviour, it seemed preferable to measure directly under conditions of practical interest in pilot-scale experiments. Such experiments were in any case required for other reasons, e.g. to gain operational experience, resolve materials handling problems and obtain adequate amounts of extract for large-scale product evaluation.

The experiments reported below relate to stage-wise extraction in four

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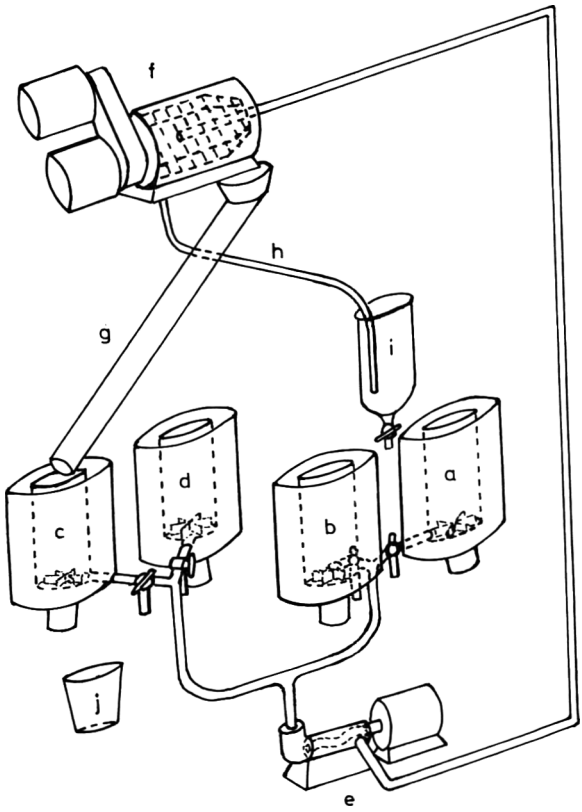

Figure 1. Diagram of apparatus

physically separate stages, each stage comprising a mixer and a separator. Because of the high cost of separating equipment, the same separator had to be used for all stages thereby restricting operation to batch processing which was programmed to simulate continuous operation.

Experimental

The leaf extracted was the same blend of large-leaf orthodox teas used in the earlier small-scale work but came from five different batches. The size range was essentially 10–40 mesh BS sieve, the average moisture content was 5.9 mass %, standard deviation 0.4 mass % and the insolubles content measured by extraction at high dilution in sealed bottles at 80°C by the method described in part I (Long, 1977) averaged 52.7 mass % of the as-received tea with a standard deviation 2.1 mass %.

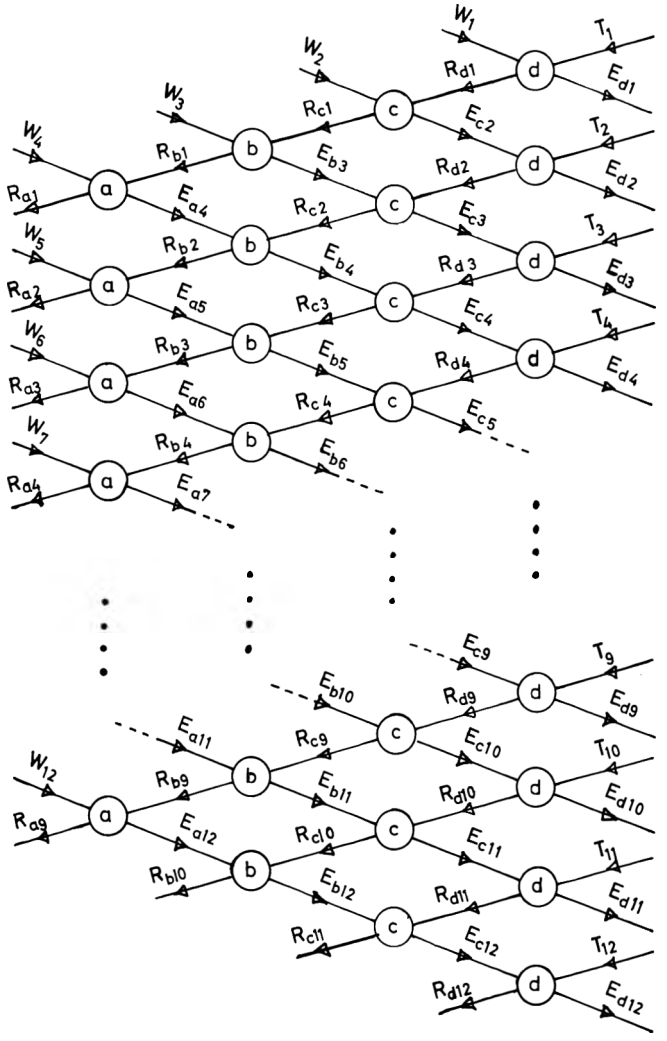

Figure 2. Operational plan for extraction runs. Encircled letters represent mixing tanks; T, W, E, R, represent batches of tea, water, extract and solid residue respectively differentiated by subscripts which denote the originating vessel and/or batch of input.

The extraction apparatus and its layout are shown diagrammatically in Fig. 1. Four identical water-jacketed stainless-steel mixing vessels (a, b, c, d), each having an internal height-to-diameter ratio of approximately 2:1 and a nominal capacity of 45 litres, were fitted with six-bladed paddle stirrers driven from below at about 100 rpm. The stirrers were mounted parallel and close to the vessel bottoms which sloped towards the outlet ports which in turn led through 3-way taps in a glass pipeline to the inlet of a Monopump (e) having a pumping

rate of 40 litres/min. From the pump outlet a flexible hose ran to the inlet of an elevated decanter-type centrifugal separator (f) having a bowl diameter 150 mm giving a liquid hold-up of less than 1 litre and a centrifugal field of 3070 g when rotating at 6000 rpm. From the outlets of the decanter separate routes were provided for gravity flow of solid and liquid; for solids transfer, a swivelling chute (g) gave access to any of the mixing tanks or a collecting bucket (j), while for liquids, a flexible hose (h) was long enough to reach at will any of the mixing vessels or the open-topped glass holding vessel (i), capacity 50 litres. The mixing vessels were electrically heated and provided with lids to reduce evaporative loss. All connecting pipework was 38 mm internal diameter.

All extractions were carried out at 80 ± 2°C and with one exception (considered later) followed the plan detailed in Fig. 2 which essentially involves forty-two mixing and separating operations arising from stagewise extraction of twelve equal batches of as-received leaf and the solid residues arising therefrom with twelve equal batches of water and their derived extracts. The vessels were used in sequence diagonally from right to left proceeding from top to bottom, i.e. in the order d, c, b, a, for the first thirty-six operations and d, c, b, d, c, d, thereafter. Batches of unextracted leaf (denoted T_1.....T_{12}) were always added to vessel d which generally contained previously prepared and reheated extract from vessel c (E_{c2}.....E_{c12}) but at start-up contained the first batch of water (W_1). After mixing for a pre-determined 'primary-contact' time the mixture was pumped to the decanter and the solid residue (R_{d1}.....R_{d12}) separated from extract (E_{d1}.....E_{d12}) which was continuously recycled through the mixing tank to flush out solids which would otherwise have deposited in the tank and pipeline. After solids discharge ceased the recycling extract was diverted from the system to storage and the residue added to vessel c for the next extraction with extract previously obtained from vessel b (E_{b3}.....E_{b12}) or exceptionally, at first, water (W_2). After mixing, separation and extract recycle as above, the solid residues from c (R_{c1}.....R_{c11}) were added to vessel b for similar extraction with extracts from vessel a (E_{a4}.....E_{a12}) or water (W_3) after which the residue was passed to vessel a for the final extraction with water (W_4.....W_{12}). Thus as the solid passed sequentially through vessels c, b, a, extracts were routed to vessels d, c, b, in preparation for the next round of extractions; water only being used in vessel a. The amount of water used was 23 kg per batch in all runs at which level it took about 40 sec to pump out a mixing tank. Solids collection was complete in about 2 min from the onset of pumping, half being collected in the first 50 sec. Extract was recycled for 3.5 min and on drainage left 1.5 litres hold-up in the system divided equally between the decanter and pipework.

Four runs were made according to the above scheme, three with primary contact times of 2 min at overall water-to-tea ratios (as-received basis) of 10:1, 50:7, 5:1 and one with a primary contact time of 1 min at water-to-tea ratio 5:1. A further run was made with a primary contact time of 2 min at 5:1 ratio using a modified starting programme. This involved replacing the first three rounds of extraction (diagonals beginning $T_1T_2T_3$) by a sequence of six operations starting at the top left hand element of Fig. 2 using vessels in the order a, b, a, c, b, a,

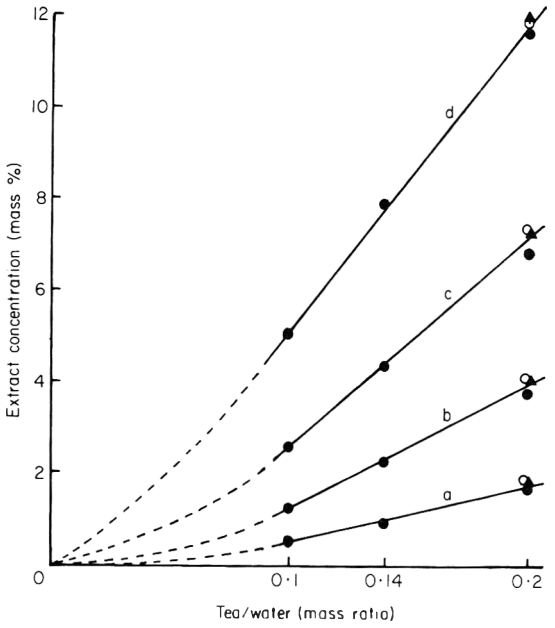

Figure 3. Stage extract concentrations. ●, runs 1, 2 and 3; ▲. run 4 (1 min primary contact time); ○, run 5 (modified start).

replacing R_{b1}, R_{c2} and R_{d3} by T_1, T_2 and T_3 and diluting extracts E_{a4}, E_{b4}, E_{c4} with about half their volume of water before passing to the next stage. In this way the starting concentrations of soluble tea solids in extracts and residues from vessels a, b, and c exceeded the final values and there was a descent towards the steady state in contrast to the former scheme in which concentrations rose towards the steady state. (In retrospect it is seen that it would have been better not to have diluted E_{c4}, when all initial concentrations would have exceeded the steady state.) From the fourth right-to-left diagonal of Fig. 2 onwards the procedure was identical in all runs. For the last four operations in each stage all extracts and residues were weighed and samples taken for gravimetric determination of total solids content of extracts and water content of residues.

Results

In the following treatment runs are numbered 1 to 5 in the order of reference in the experimental section and the letters a, b, c, d relate to outputs from vessels similarly designated. To emphasize trends, results for each stage in each run have been averaged and where possible are presented graphically.

Dealing first with extracts, Fig. 3 shows the average concentration of extract leaving each vessel as a function of the overall tea-to-water mass ratio. The

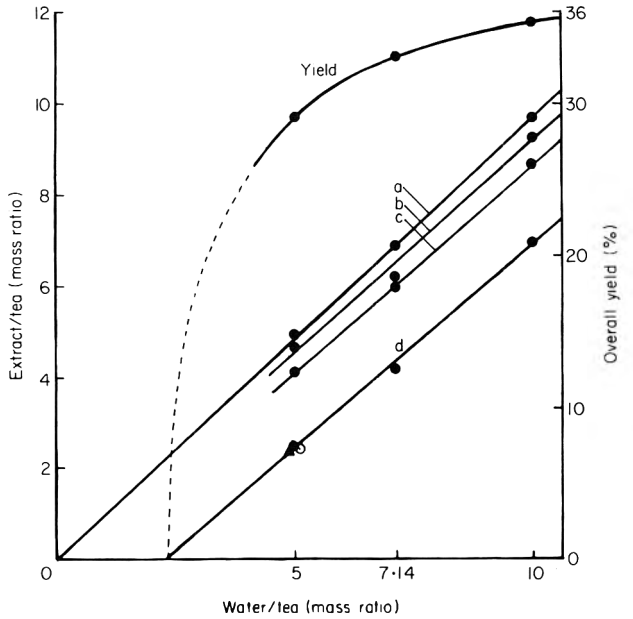

Figure 4. Masses of extract and overall yield. Key as Fig. 3.

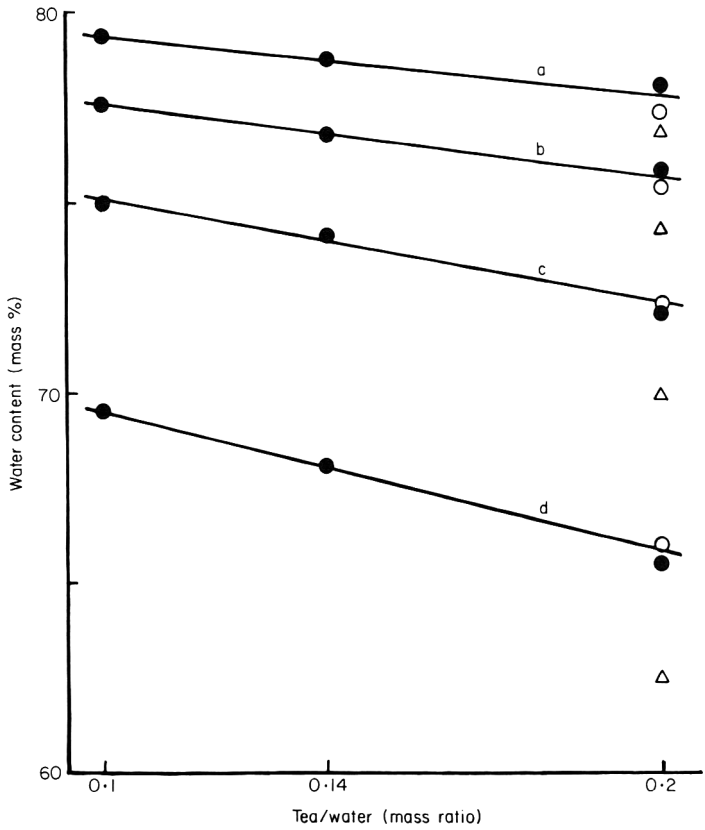

Figure 5. Water content of decanted solid residues. Key as Fig. 3.

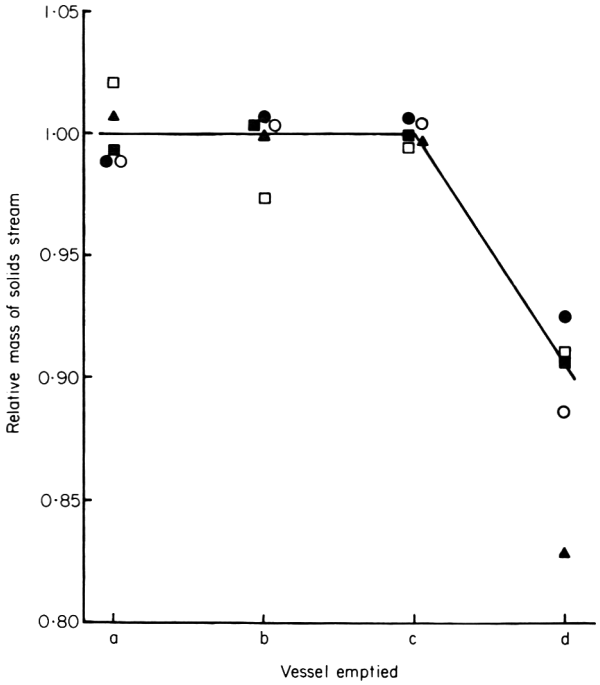

Figure 6. Relative masses of solids decanted from each stage. □, run 1; ■, run 2; ●, run 3; ▲, run 4; ○, run 5.

broken lines are a plausible extrapolation to the origin. Fig. 4 gives the amount of extract leaving each stage per unit mass of as-received tea extracted as a function of overall water-to-tea mass ratio. Inversion of the independent variable relative to Fig. 3 allows physically meaningful extrapolation of essentially linear graphs; the line for vessel a passing the origin and that for vessel d making an intercept (by least squares) of 2.3 on the water-to-tea axis, giving an indication of the net liquid retention by leaf after the first extraction and separation. The output from vessel d taken in conjunction with extract concentrations from Fig. 3 gives the overall yield which is also shown as a function of water-to-tea ratio in Fig. 4. The broken line indicates the probable extrapolation towards zero yield at low water-to-tea ratios. Where results for runs 4 and 5 are omitted from Fig. 4 they are indistinguishably different on the scale of the graph from those of run 3. In particular, yield was identical for these runs within 0.1% because of compensating differences in amount and concentration of extract.

Percentage yield (Y) also correlates at better than the 99.9% probability level with tea-to-water ratio (T/W) according to the relation:

$$Y = 41.8 - 63.7\ T/W \qquad (1)$$

This equation permits extrapolation of yield in the direction of decreasing values of T/W and gives an intercept on the yield axis insignificantly different

from the average value 41.4% found for maximum yield by direct extraction in sealed bottles at high dilution.

Considering next the solid residues leaving the decanter, Fig. 5 gives the average water content as a function of overall tea-to-water mass ratio for solids extracted in each vessel. The lines drawn are least squares plots for results from runs 1, 2, 3 and 5; results from run 4 are noticeably lower. The amount of solids leaving the decanter per unit mass of as-received tea extracted was essentially constant for vessels a, b and c in any run but varied slightly from run to run. Average values for the above vessels in runs 1, 2, 3, 4 and 5 were 2.80, 2.81, 2.72, 2.93 and 2.80. Relative amounts of residue decanted from the various vessels expressed as mass of solids discharged divided by the average for vessels a, b and c in the same run are given in Fig. 6 to illustrate the constancy of residue flow in stages other than the first. On average the amount of residue from vessel d was only about 90% of that from the other vessels in the same run.

Discussion

A conspicuous feature of the results is the occurrence of well-defined linear trends in stage behaviour which suggest consistency in operation and simplicity of interpretation. Further evidence of the former comes from the essential similarity of results from runs 3 and 5 in which the steady state was approached from different directions for vessels a, b, and c. In the following discussion the more obvious implications of the trends will be considered first followed by treatment of operational error and generalization of stage performance.

Linearity of extract concentration with tea-to-water ratio in Fig. 3 suggests that within stages tea leaf behaves incrementally as though it were a solid containing a fixed proportion of solubles. Decreasing slope in going from d to a correctly indicates a decreasing solubles content of residue with increasing numbers of extractions. Linearity does not extend to the origin however and extrapolation of the straight lines in that direction leads to the physical absurdity of negative extract concentrations at zero tea-to-water ratios. Since lines for all stages must pass the origin it follows that they are curved at low concentrations and extrapolation of the observed linear relations underestimates extract concentration in that region. This indicates an apparently increased solubles content of residues as previously undissolved matter goes into solution at high dilution as it did in the equilibrium extractions reported in part I. The intercepts on the tea-to-water axis found by linear extrapolation decrease in going from a to d which is consistent in direction with increasing evaporative loss of water as extract passes through the system from a to d and increasing dissolution of undissolved solubles as leaf passes from d to a. Both effects are thought to have occurred.

A surprising feature of Fig. 3 at first sight is the close similarity of extract concentration for primary contact times of 1 and 2 min, particularly for the first

extraction in vessel d where rates of solution are fastest. For corresponding average contact times (i.e. including the average time of discharge) of 2 and 3 min, earlier small-scale single-stage extraction reported in part III had shown about 15% difference in the amount of solid passing into free solution. In the present work such a difference could have been offset by increased absorption of extract by leaf at the longer residence time. This is just a peculiarity of the extraction ratio and number of stages used. In the 5:1 extraction (but no others) the average amount of solid in free solution was a maximum in vessel c and fell slightly by absorption into residue after extraction in vessel d, so the additional fall for extractions with 2 min primary contact could have compensated for increased dissolution when compared with 1 min primary contact.

Considering next Fig. 4, as might be expected the absorption of extract by residue is most pronounced in the first extraction of leaf (i.e. in vessel d), extrapolation suggesting that a water-to-tea ratio of 2.3 must be exceeded to get any free extract. This amount of water will be the lower limit of moisture content of the separated residue (which occurs at zero yield) plus any evaporative losses of water from the system. After satisfying this minimum water requirement for extract production the amount of the latter increases proportionally to the excess of water but not quite in a 1:1 ratio because of increase in extract retention by the leaf as solubles are displaced. As the number of extractions of a particular batch of leaf increases so does the amount of extract obtained until after four extractions (i.e. in vessel a) it nearly approaches the amount of water used. In a sense the leaf is becoming saturated with water and is approaching the upper limiting moisture content for a residue completely extracted of solubles.

The water content of stage residues for runs 1, 2, 3, and 5 in Fig. 5 vary in the opposite way to extract concentrations in Fig. 3 in that the highest concentrations are associated with the lowest moisture contents and the slopes are opposite in sign. Taken with the evidence of Fig. 6 for the constant relative mass of solids in stages other than the first, it appears that as solubles are extracted from leaf they are replaced by an equivalent mass of water. Results from run 4 are anomalous in that water contents are consistently lower for all stages. This might reflect the reduced time for absorbing liquor by leaf in that run but normally this would not make much difference after the first extraction. Alternatively it could have been due to accidental alteration of the pond setting or differential speed of the decanter or arise from differences in the properties of the leaf extracted. The latter seems most likely since, despite the reduced moisture content of residues in run 4, the mass of residues was the highest of the series. Returning now to Fig. 6, differences between masses of residues from first and later extractions are about 17 and 9% for extraction times of 2 and 3 min respectively in reasonable agreement with the net liquid uptake found in the single-stage extractions mentioned earlier in which there was approximately 8% difference in uptake between extraction times of 2 min and 3 min and also between 3 min and 12 min. Clearly for the first extraction swelling of leaf is incomplete but in later stages the mass flow may be considered constant

which is a convenient simplification for calculating extractor performance. It may be recalled that approximate constancy of residual mass after extraction was also found for gravity drainage of a fixed bed in part II.

Before making further analysis of the present work, because of the inherent inaccuracy of the scale of operation and the experimental technique employed, the self-consistency of results for each run was tested by drawing up overall and stage materials balances for tea solids and water. The overall balances showed both gains and losses of solids, the average imbalance between measured input and output being a loss of 1.5% of the solids input as leaf with a standard deviation of 3.5% indicating a general lack of precision but no statistically significant departure from zero. On the other hand the overall water balances always showed considerable systematic losses amounting on average to 14.3% of the total water input with a standard deviation of 1.7%. Such losses of water without loss of solids will have arisen by evaporation of water from hot recycling liquor, discharging solid and stored extract awaiting the next round of extractions. Tests carried out with water alone following the extraction time-schedule gave total losses of 9% with a standard deviation 0.9%, most of the loss occurring during recycle. In extraction runs the balance of loss (about 5% of input) will have occurred from solids streams.

The individual stage balances showed no significant difference in water loss between runs in the same vessel or between vessels a, c, and d in the same run but vessel b was anomalous in always giving about twice the loss of the others. The stage balances for tea solids always showed systematic imbalance which, although distributed about a mean of zero with a standard deviation of 4% of the tea solids input, correlated at better than the 99.9% probability level with the difference between final concentrations in the operation considered and its antecedent. The regression coefficient indicated the average imbalance could be explained by hold-up of 3 kg of extract, a view confirmed by dismantling the apparatus which consistently showed the hold-up of free liquor and solids to be about 1.5 kg and 2 kg respectively. The stage solids imbalance was thus essentially soluble solids in held-up extract, half retained by the pipework and half absorbed by solids held-up in the decanter discharge line mainly around the scroll conveyor outlet.

In view of the systematic losses indicated above, it is clear that the bare experimental results cannot be taken at face value unless they are to be applied to processes where the losses are known to be similar. To extend their application and to interpret stage behaviour it is desirable to reduce the results to a loss-free basis and to investigate the distribution of tea solids between the two phases present during extraction. The simplest approach is to consider the system to comprise three components: soluble tea solids, insoluble tea solids and water. Tea solids are then present as three classes: (i) soluble solids in free extract, (ii) soluble solids in extract absorbed by leaf and (iii) insoluble and undissolved soluble solids in leaf. The amount of solids in class (i) and the combined amounts of classes (ii) and (iii) were readily derived from the experimental results and were available as items in the stage mass balances. To

partition solids between the last two classes it was arbitrarily assumed that all water in the residue was present as extract identical with the free extract from which the residue was separated. This assumption in no way affects the distribution of soluble solids between leaf and extract which was based on direct measurement but it will underestimate the amount in class (ii) and overestimate the amount of solubles in class (iii) because it does not allow for the inevitable concentration gradients in a non-equilibrium situation. However from observed rates of solution (part III) the effect is likely to be small at the residence times employed, but its existence can be recognized by calling the calculated values for classes (ii) and (iii) 'effective' values. In passing it will be noted that an approximation of this kind namely the application of an equilibrium concept to a non-equilibrium situation is always implied in the widely-practiced design of multi-stage separation process from equilibrium data whenever stage efficiencies are introduced to allow for disequilibrium.

To generalize stage performance most interest centres round the form of any relation which may exist between the composition of extract-free residue and the concentration of extract from which it was separated. Both these valuables are subject to uncertainty because of errors and omissions in measurement reflected in the imbalance of stage materials flows. For example estimates of extract concentration are affected by evaporative loss of water so that assuming accurate analysis and sampling the 'true' concentration at separation from solid lies between two extremes: the measured value which would apply if all water loss from extract was complete before separation, and a lower value for which all stage loss of water occurred from extract after separation. The latter was quite improbable but had the advantage of being easily calculated from the ratio of stage water loss to mass of extract collected. It was never less than 90% of the measured value so a mean of the two values, having a maximum proportional error 5% and averaging 98% of the measured value, was taken as a satisfactory estimate of extract concentration at separation.

Estimates of insoluble and undissolved solids in stage residues were subject to additional uncertainty from the stage solids imbalance. To circumvent this it was decided to derive the solids composition as far as possible from measurements on the extract streams which were thought to be more accurately characterized, for while there was evaporative loss of water from extract there was no reason to believe that there was any loss of solid except by hold-up which was relatively small and had been well measured. Since the leaf input to vessel d was accurately known, subtraction of the solids leaving the same vessel as extract gave the net flow of solids into the extraction system. Taking the system as approximating the steady state, the same net flow (with small corrections for stage hold-up of free extract) applied to all interstage transfers, so adding to this the input of solids as extract in any stage gave the solids flow in the stage residue. Subtracting from this the solids in absorbed extract finally gave the insoluble and undissolved solids. However at this point it was necessary to decide on an appropriate extract concentration and water content of the residue taking into account the stage water loss. As with estimates of extract concentration two

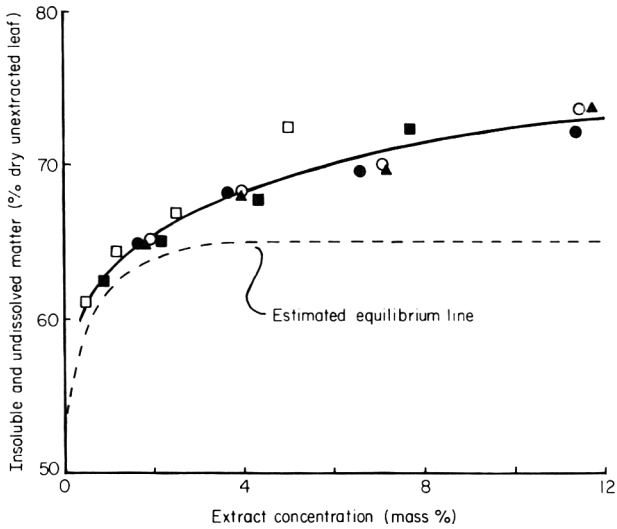

Figure 7. Effective insoluble and indissolved solids (dry tea basis) in stage residues as a function of extract concentration. Key as Fig. 6.

extreme limits were considered and a mean value of absorbed solids calculated from them. The upper limit of absorbed solids corresponded to all stage water losses occurring from solid after separation and the lower limit to all stage water losses occurring from extract after separation. Neither limit was particularly realistic, both being gross overestimates of any likely effect, however their influence on the value for insolubles and undissolved was relatively small, the range between extremes being on average 2.4% of their mean.

Values of effective insoluble and undissolved solids calculated by the above procedure and converted to a dry-tea basis are shown as a function of loss-corrected extract concentration in Fig. 7. The results correlate fairly well and with one exception lie close to the trend line drawn. For comparison the results of equilibrium extraction of a comparable but slightly more soluble tea taken from part I are shown as a broken line. The latter may be regarded as an approximation to the limit which would have been reached in the present work with a long stage residence time. It will be seen that while the two curves lack exact geometric similarity they are nevertheless broadly similar in form and converge as extraction proceeds and equilibrium is more closely approached. First extractions of leaf gave almost the same fraction of insoluble and undissolved matter in all runs, deviating from the mean value 72.8% by not more than 0.9%. The single run with 1 min primary contact is apparently indistinguishable from those carried out at the same extraction ratio with double the primary contact time. This is more likely to be due to low precision in the model and method of calculation than the absence of real differences in extent of solution. As mentioned earlier when discussing extract concentrations a proportional

difference of about 15% in the amount of solid dissolving, equivalent to a difference in insoluble and undissolved solids of about 4%, was expected in first extractions at these two times. This expectation was supported by values for insoluble and undissolved solids calculated from the measured residue flows from vessel d (neglecting imbalance) which gave an average of 71.0% for runs 1, 2 and 3 and 74.7% for run 4. (Unfortunately no reliable result could be obtained for run 5 by this means because of an exceptional solids imbalance for the vessel concerned.)

Comparison of the values for first extractions in Fig. 7 with the equilibrium solubles content of 35% at the concentrations involved indicates stage efficiencies approaching 80% for vessel d. Taking stage efficiency generally to be the decrease in undissolved solubles in any stage divided by the decrease achievable in that stage by going to equilibrium at the same concentration gives an average value for vessels other than d of about 55% making the overall average for the process about 60%. However considerable variation in stage efficiency occurred (standard deviation 18%) mainly due to low efficiencies of stages b and c (averaging 35%) following the attainment of high concentrations in stage d in runs 3–5. Hence it may be inappropriate to take a constant efficiency to characterize the entire process and it seems that stage output compositions are better represented by a variable efficiency which may be implied from the trend line for insolubles and indissolved content of residues. The use of the latter for design of extraction systems instead of equilibrium data and stage efficiency leads to many interesting results which it is hoped to outline in a concluding paper to the present series.

Finally it will be seen that the overall extent of solution of leaf after four stages was not too dissimilar from that occurring in a single equilibrium stage at the final concentration. Thus the overall (but not the detailed stage) behaviour would not be too badly represented by a model which assumes all solution occurs in the first stage to the equilibrium limit and subsequent stages merely wash out absorbed extract. However it is not to be supposed that this is always the case irrespective of the number of stages or is ever more than a gross over-simplication. A feature only partly resolved by the present work is the extent to which the yield can actually exceed the equilibrium dissolution in the first stage. This was discussed in part I when considering the interpretation of equilibrium measurements. The yield now reported of 35.3% and 33.0% (a.r. basis) for runs 1 and 2 and the positions of the terminal points for those runs in Fig. 7 suggest a modest contribution to yield from this cause.

Conclusions

(1) Batch simulations of continous countercurrent extraction of tea leaf with water in four physically separate stages gave good yields of soluble tea solids in extracts at high concentrations.

(2) The final extract concentration increased linearly with increasing overall

tea-to-water ratio but at the expense of yield which fell linearly with increase in the same ratio.

(3) Leaf residues from stages other than the first were approximately constant in amount showing that soluble tea solids are displaced by an equivalent mass of water as extraction proceeds, while in the first stage incomplete swelling of leaf resulted in about 10% less mass in the residue.

(4) The method of operation employed allowed considerable evaporative loss of water mainly from the extract streams and any application of the results has to take this into account.

(5) After correction for water losses, results for all runs may be unified by considering the system to compose three components: soluble tea solids, insoluble tea solids and water.

(6) A single curve, comparable to the equilibrium curve of part I, relates the insolubles and undissolved solubles content of stage residues to the concentration of extracts from which they were separated and may be taken as a basis for designing extraction systems with a stage residence times of 2–3 min at 80°C.

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References

Long, V.D. (1977) *J. Fd Technol.* **12,** 459.
Long, V.D. (1978) *J. Fd Technol.* **13,** 195.
Long, V.D. (1979) *J. Fd Technol.* **14,** 449.

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A study of the water activity lowering behaviour of some amino acids

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Summary

The water activities of aqueous solutions of L-lysine, L-proline and L-ornithine, have been experimentally determined. The data have been very well fitted to Norrish's (1966) equation for non-electrolyte solutions and correlating constants have been calculated and compared with values previously reported for other amino-acids. This comparison allows us to draw some conclusions regarding molecular structure of the amino acids and their a_w lowering ability.

Introduction

Intermediate moisture foods (IMF) have been the subject of numerous technical and scientific studies in the last decade (Heidelbaugh & Karel, 1975; Flink 1978; Davis *et al.*, 1976, Troller, 1979). These and various other studies covered several aspects such as microbial and chemical stability at reduced water activity (a_w), the technology of IMF and the selection of humectants for depressing water activity.

A knowledge of the a_w lowering effect of various potential humectants for IMF is an important area of research and has been the subject of various studies in this laboratory (Benmergui, Ferro Fontán & Chirife, 1979; Chirife, Ferro Fontán & Benmergui, 1980; Chirife & Ferro Fontán, 1980a, b). The prediction of the water activity in solutions of amino acids may be of potential interest in IMF formulation because the possibility of using amino acids for a_w control has been advanced (Davis, Birch & Parker, 1976). Whether or not this would be

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feasible, the prediction and understanding of the a_w lowering behaviour of amino acids may be useful for a better comprehension of the a_w lowering phenomena in aqueous solutions.

The knowledge of the a_w lowering behaviour of amino acids may be also of interest in connection with the mechanism by which certain microorganisms are able to survive and carry out their growth at reduced a_w values. It has been suggested (Measures & Gould 1976; Gould & Measures 1977) that an osmotically active compound accumulates intracellularly to counter the osmotic imbalance across the cell membrane when the cell is exposed to low a_w systems; these compounds include proline, γ-amino-butyric and glutamic acid in different osmotolerant bacteria.

This work reports the experimental determination of the water activity of aqueous solutions of L-lysine, L-proline and L-ornithine as well as its theoretical interpretation.

Experimental

Materials

L-proline was obtained from E. Merck, Darmstadt (West Germany); L-ornithine from BDH Lab. Chem. (Poole, England) and L-lysine from Lowell Lab. (Buenos Aires, Argentina). Solutions for a_w measurements were made using distilled water. The maximum amino acid concentration studied was, in each case, close to the limit of solubility of each compound.

Water activity measurements

The water activities of amino acid solutions were determined using the 'a_w-Wert Messer' manufactured by Firma LUFFT, Stuttgart, West Germany. In order to improve the reliability of the measurements the instrument was checked against five different standard saturated salt solutions in the a_w range of interest, and a calibration curve was obtained. The details of the procedure for a_w measuring as well as some statistical indicators of precision, have been published elsewhere (Chirife & Ferro Fontán, 1980a,b).

Results and discussion

In previous publications (Chirife *et al.*, 1979; Chirife & Ferro Fontán, 1980a,b) we have shown that Norrish's (1966) equation was a very good model for describing the a_w lowering curve of non-electrolytes. Norrish's (1966) equation may be written

$$a_w = X_1 \exp(-K X^2_2) \quad (1)$$

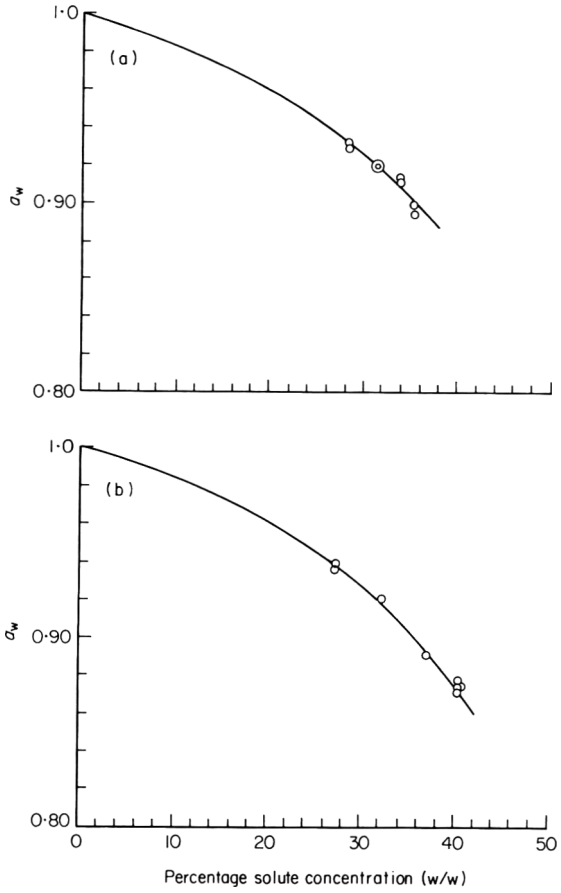

Figure 1. Experimental and calculated a_w data for solutions of (a) L-ornithine and (b) L-lysine at 25°C. O, Experimental; ——, calculated.

where X_1 and X_2 are molar fractions of water and solute respectively, and K is the correlating constant which indicates the departure from Raoult's law. Perhaps the most remarkable virtue of equation (1) is its extreme simplicity which needs only one parameter to characterize the a_w lowering curve of non-electrolytes. The wide range of applicability of this simple equation, both to type of non-electrolyte and water activity, (Chirife *et al.*, 1979; Chirife & Ferro Fontán, 1980a,b) not only facilitates the correlation of experimental data but also reduces the number of determinations needed to characterize the a_w lowering curve. In using Norrish's equation, however, it is convenient to utilize relatively concentrated solutions (e.g. $a_w < 0.93$) because the error in the evaluation of the K constant increases with decrease in the molar fraction of solute. For this reason all measurements reported in this work were made in solutions having a_w of values < 0.93. Figures 1 and 2 show experimental and calculated a_w data for solutions of L-lysine, L-ornithine and L-proline at 25°C. Calculated data were obtained by applying equation (1) to the experimental

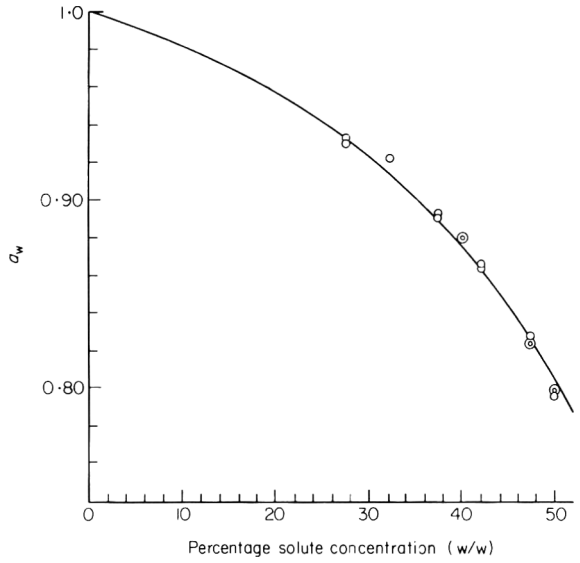

Figure 2. Experimental and calculated a_w data for solutions of L-proline at 25°C. O, Experimental; ——, calculated.

data. It can be observed that they are well fitted by Norrish's (1966) model. A computer program was used to calculate the best values of K which are shown in Table 1 as compared with values previously reported for other amino acids. It can be seen that all three amino acids here studied have negative deviations from Raoult's law as evidenced by the positive values of K.

Deviations from the ideal behaviour (Raoult's law) may be expected to be due to either of the following factors, (a) inequalities in the volumes of the molecules, and (b) disparities in the molecular forces (Denbigh, 1964). Norrish (1966), Chirife *et al.*, (1979) and Chirife & Ferro Fontán (1980b) showed that, other things being equal, the K constant may be correlated with the number of effective groups in the molecule, that is, those able to form hydrogen bonds with water. On these premises, the magnitude of K constant as shown in Table 1, may be used for further investigating the a_w lowering behaviour of amino acids. The data indicate that the diamino-monocarboxilic acids (lysine and ornithine)

Table 1. Values of K (equation 1) for various amino acids at 25°C

Amino acid	K	Reference
L-Lysine	9.3 ± 0.3	This work
L-Ornithine	6.4 ± 0.4	This work
L-Proline	3.9 ± 0.1	This work
α-amino-n-butyric acid	2.59 ± 0.14	Chirife *et al.* (1980)
β-Alanine	2.52 ± 0.37	Chirife *et al.* (1980)
Glycine	−2.02 ± 0.33	Chirife *et al.* (1980)

have K values substantially higher than the mono-amino-carboxilic ones (β-alanine, glycine and α-amino-n-butyric acid), indicating that the additional $-NH_2$ group plays a significant role for depressing a_w. Further, a comparison of K values between lysine (6 carbon atoms) and ornithine (5 carbon atoms) indicates that either the length of the aliphatic chain and/or the position of the second amino group relative to the carboxilic one, favours a negative deviation from Raoult's law. The same kind of effect appears when comparing the α-mono amino-mono carboxilic acids, namely glycine (2 carbon atoms) and α-amino-n-butyric (4 carbon atoms), as it can be observed in Table 1. The K value displayed by proline suggests that the α-imino group is relatively effective for depressing a_w.

Acknowledgments

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References

Benmergui, E.A., Ferro Fontán, C. & Chirife, J. (1979) *J. Fd Technol.* **14,** 625.
Chirife, J., Ferro Fontán, C. & Benmergui, E.A. (1980) *J. Fd Technol.* **15,** 59.
Chirife, J. & Ferro Fontán, C. (1980a) *J. Fd Science.* (in press)
Chirife, J & Ferro Fontán, C. (1980b) *J. Fd Science.* (in press)
Davis, R., Birch, G.G. & Parker, K.J. (eds) (1976) *Intermediate Moisture Foods*. Applied Science Pub.
Denbigh, K. (1964) *The Principles of Chemical Equilibrium*. Cambridge University Press, Cambridge, U.K.
Flink, J.M. (1978) *J. Fd Process. Preserv.* **2,** 324.
Gould, G.W. & Measures, J.C. (1977) *Phil. Trans. R. Soc. Lond.* **B 278,** 151.
Heidelbaugh, N.D. & Karel, M. (1975) In: *Freeze Drying and Advanced Food Technology*, (edited by S.A. Goldblith, L. Rey and W.W. Rothmayr), p. 618, Academic Press, London.
Measures, J.C. and Gould, G.W. (1976) In: *Intermediate Moisture Foods*, (edited by R. Davies, G.G. Birch & K.J. Parker). Applied Science Publishers, Barking.
Norrish, R.S. (1966) *J. Fd Technol.* **1,** 25.
Troller, J. (1979) *J. Fd Technol.* **Jan.** p. 72.

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A bioluminescence method for the determination of oxygen transmission rates through plastic films

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Summary

A new and fast bioluminescence method for the determination of oxygen transmission rates through plastic films and laminates is described. The method is based on the measurement of *in vivo* light intensity emitted by luminous bacteria confined in a pouch made of the tested plastic film. The measured light intensity was found to be linearly proportional to the oxygen transmissibility of different plastic films.

Introduction

Application of plastic films and laminates in flexible packaging in the food industry is expanding very rapidly. Plastic pouches and bags are widely used for dried, frozen and baked products as well as coffee and spices, and recently for liquids. The advantages of these films and laminates over the packaging materials previously used stem from the ease of their manufacture, excellent sealability, variety of permeabilities to different gases and vapours and their low cost.

Many foods undergo oxidation in the presence of atmospheric oxygen followed by off-flavour and off-taste formation which reduces their shelf life. (Karel, 1975). Fresh meats on the other hand, need a certain amount of oxygen in order to maintain their appealing red colour. Thus, the evaluation of oxygen permeability through a plastic package is of great importance.

In the conventional methods, oxygen permeabilities are obtained from measurements of the increase in the gas concentration (or pressure) in standard permeation cells (ASTM 1971). Such measurements are however time consuming and in the case of high gas barrier materials may become very lengthy.

In the present work, a new and very fast bioluminescence method for the determination of oxygen transmission rates through plastic films is described.

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Table 1. Description of tested films

Symbol	Material and thickness (μm)
A	LDPE* A, 114 μm
B	Nylon 6, 20 μm + LDPE 50 μm
C	Polyethylene Terephthalate (PET) 12 μm + LDPE 50 μm
D	PET 12 μm PVDC coated, + LDPE 50 μm
E	Biorented polypropylene (BOPP) 18 μm PVDC coated + LDPE 50 μm
F	LDPE A, 134 μm
G	LDPE 200 μm
H	Polybutylene (PB) 100 μm
I	Nylon 28 μm
J	K cellophane 25 μm two sides PVDC coated
K	40 phr DOP plasticized PVC 120 μm
L	Nylon 6, 30 μm + LDPE 50 μm
M	LDPE A, 75 μm
N	LDPE A, 90 μm
O	LDPE A, 200 μm

*Low density polyethylene fully described by Miltz & Ram (1971, 1973) and Ram & Miltz (1972).

The method is based on the fact that the *in vivo* luminescence of marine luminous bacteria is very sensitive to the presence of oxygen (Johnson, Shouwenburg & Van der Burg, 1939; Harvey, 1941; Hastings, 1952; Oshino *et al.*, 1972). Thus, when luminous bacteria are placed in a plastic pouch, the luminescence level depends on the oxygen transmission rate through the plastic film.

Experimental

Materials

Plastic films. All the plastic films and laminates studied in the present investigation were made by local food packaging manufacturers. Their composition and designation are summarized in Table 1.

Luminescent bacteria. Photobacterium leiognathi (strain 721) cells were grown in liquid complex medium (Ulitzur, Weiser & Yannai, 1979) with vigorous shaking at 25°C. At a cell density of 8×10^8 cells/ml. approximately (100 Klett Summerson Photometer units, filter 66), the culture was harvested by centrifugation at 10,000*g* for 10 min at 4°C. The cell pellet was suspended in the complex medium to give a final cell turbidity of 200 K.U. This bacterial suspension can be kept at 4°C for a few hours with only a little loss of activity.

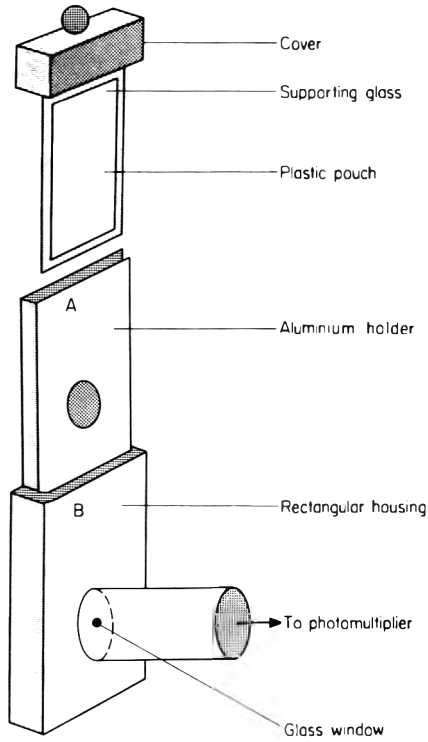

Figure 1. Holding device used for the bioluminescence method.

Methods

Permeability determination by a permeation cell. The increase in oxygen concentration with time in the originally oxygen-free compartment of a standard permeation cell (ASTM 1971) was measured for films A-F by means of a gas chromatograph. A Packard (Becker 406) GLC equipped with a dual thermal conductivity cell and a 12 ft × ⅛" copper column filled with molecular sieve 5Å 60/80 mesh were used. The helium carrier gas flow rate was 15 cc/min., the detector current 200 mA and the oven temperature 30°C.

Bioluminescence method. For the determination of oxygen transmissibility of plastic films by this method a simple device consisting of two parts was constructed (see Fig. 1). Part A is a U-type aluminium holder in which a round hole, 16 mm in diameter, was made. Part B is made of a rectangular housing with a glass window, also 16 mm in diameter, attached to a photomultiplier through a metal tube. All portions of the device were painted black to prevent light reflections.

From each of the plastic films to be tested a pouch, 80 × 30 mm in size, was prepared. A microscopic supporting glass was inserted into the pouch to keep the film streched. The pouch was placed in the holder (part A) which was

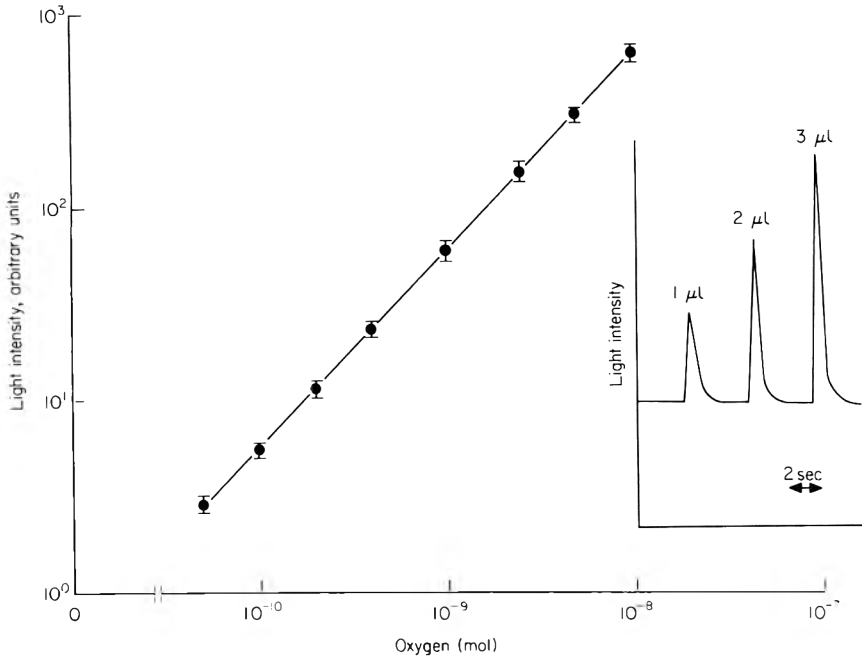

Figure 2. Effect of oxygen content on the bacterial bioluminescence.

inserted into the housing of part B. 5 ml of the bacterial culture (pre-warmed to 20°C) were injected into the pouch and the housing was then covered by an appropriate cover (see Fig. 1). The light intensity emitted by the bacteria was then followed until a steady reading was obtained (usually within 5 min). This reading was taken as the measured value.

To test the response of the system to varying oxygen concentrations, a special stainless steel cell was designed to replace the plastic pouch. This cell was of the same size and shape as the U-type holder but was closed from all its sides except the top which was closed by a rubber septum. The window in the cell was formed by glueing a piece of glass on top of the hole. The cell was filled with the bacteria suspension and 1–50μl of 3% NaCl solution, in distilled water, saturated with air at 20°C, were injected by a Hamilton syringe exactly at the centre of the glass window. The resulting luminescence was then measured and recorded (see Fig. 2).

Results and discussion

The response of the luminous bacteria to different amounts of oxygen is shown in Fig. 2. It can be seen that a linear relationship between the light intensity and injected oxygen, in the studied range of 5×10^{-11}–5×10^{-8} mol, exists. Figure 2 also shows (insert) that all the oxygen introduced into the system is consumed

Table 2. Experimental oxygen transmissibilities and light intensities

Film	OTR*	LI†	OTR/LI	OTR ratio‡
A	95.5	960	0.1005	1
B	1.8	18.3	0.0956	55.1±6%
C	2.9	28.2	0.1042	32.8±5%
D	1.5	15.3	0.0961	65.6±5%
E	2.3	23.1	0.1000	41.8±2%
F	89.4	910	0.0982	1.07±2%

*Oxygen transmission rate cc/100 in^2 × 24 h × atm.

†Light intensity, arbitrary units.

‡Ratio between the OTR of film A and that of the corresponding film. The percentage stands for the maximum deviation between the LI (film A)/LI (corresponding film) and the ratio OTR (film A)/OTR (corresponding film) given in this column.

within one second. It is therefore clear that when a culture of such luminous bacteria is confined in a plastic pouch, all the oxygen diffusing into the bacterial suspension through the plastic film is consumed in a very short period. The oxygen concentration inside the pouch can therefore be assumed to be negligible at any time and thus the light emitted by the bacteria reflects the rate of oxygen transfer through the plastic film.

In Table 2 the oxygen transmission rates (OTR) measured by the standard method as well as the light intensities (LI) emitted by the bacterial cultures confined in the pouches made of films A-F are summarized. Each reported value in the table is an average of at least two measurments. The permeability of film A was found to be 433 (cc × mil)/(100 in^2 × 24 hr × atm), well comparable to published data (*Modern Plastics Encyclopedia*, 1978). As the thickness of this film was 0.114 mm (4.488 mil), its oxygen transmissibility was therefore 96.48 cc/(100 in^2 × 24 hr × atm). Oxygen transmission rates (transmissibilities) rather than permeabilities are given in Table 2 as most of these films were laminates consisting of two or three different layers and therefore no permeabilities could be defined. The calculated OTR/LI ratios (also given in Table 2) are shown to be constant (within ±5% of the average) for all the films. This means that the measured light intensity is proportional to the rate of oxygen transmission through the film. Therefore, if a film with a known OTR is used as a reference, the OTR of any unknown film can be determined based on this reference, as the ratio between the measured light intensities is also the ratio between the oxygen transmissibilities of the films.

Film A in Table 2 was used as the standard reference throughout the present work. This film was made of polymer A, a well characterized low-density polyethylene, previously described by Miltz & Ram (1971, 1972, 1973). The ratios between the OTR of this film and the corresponding values of the other films are also given in Table 2 (last column). To test the validity of the method the LI in the case of the above mentioned films (A-F) was measured several

Table 3. Light intensities and calculated OTR values for different films

Film	LI*	LIR†	COTR‡
A	950	1	—
G	475	2.0	48.2
H	755	1.3	76.6
I	15.9	59.8	1.6
J	16.0	59.4	1.6
K	257	3.7	26.1
L	12	79.2	1.2
M	1500	0.6	153.1
N	1200	0.8	122.1
O	750	1.3	76.0

*Light intensity, arbitrary units.

†Light intensity ratio, LI (film A)/LI (corresponding film).

‡Calculated Oxygen Transmission Rate, based on the transmissibility of film A, cc/100 in^2 × 24 hr × atm.

additional times on different days and using different bacterial cultures. Although somewhat different values were obtained each day, the ratios between the LI for film A and the corresponding value for each of the other films remained constant. A well characterized film of known OTR has to be used, therefore, as a reference each time a set of experiments is being carried out. In Table 3, the OTR values calculated from the light intensities measured for several additional films used for food packaging are given.

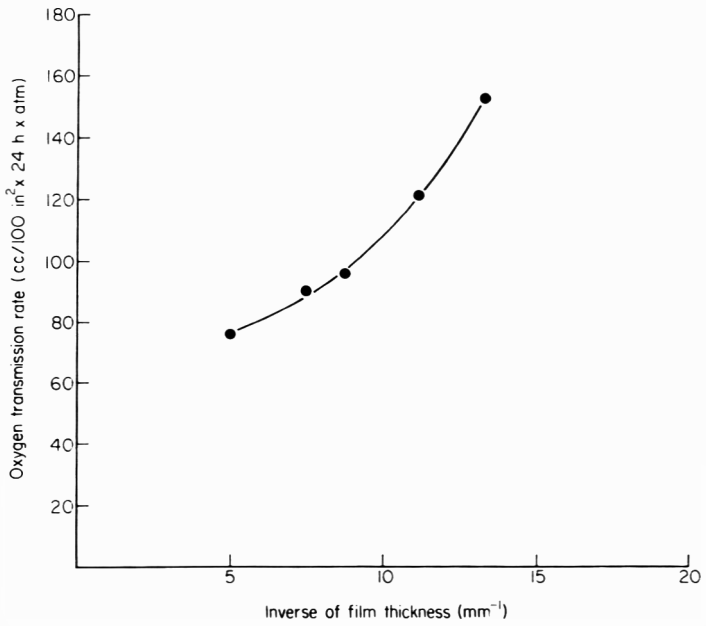

Figure 3. Effect of film thickness on the oxygen transmission rate.

The effect of film thickness on the OTR through films made of polymer A (films A,F,M,N,O) as measured by the new method, is shown in Fig. 3. It can be seen that a non-linear relationship between the OTR and the inverse of film thickness exists. Theory, on the other hand predicts a linear relationship between these two parameters (Stancell 1971). It should be mentioned however, that even though the same polymer was used for preparation of the five films, they were obtained from different sources and therefore their time-temperature history could never be the same. Differences in extrusion temperatures, in cooling rates and in blow and draw ratios will change the morphology of the polymer resulting in differences in its permeability. Such differences have a much greater effect for semicrystalline polymers like polyethylene than for amorphous ones. The permeability or the transmissibility, of each film should therefore be experimentally determined if accurate information is required.

It should be mentioned that the contact between one side of the polymeric film with the aqueous bacteria suspension may influence the transmissibility of the film. However, due to the very low diffusion rates of liquids in polymers and due to the short duration of the test, this effect is probably very small. It is worthwhile to note that although *P. leiognathi* bacteria were used in the present investigation, any other known species of luminous bacteria could be applied as well. The concentration of the bacterial suspension is also not critical as long as the light emitted is linear with oxygen content and a standard reference film is used. Luminous bacteria can be easily isolated from the sea or obtained from bacterial cultures collection [e.g. ATCC 7744 for *Photobacterium fischeri*]. The isolation, maintenance, handling and cultivation of these bacteria is described in detail by Hastings & Nealson (1977).

To summarize, the method described in the present communication enables one to determine the oxygen transmissibility of a plastic film or laminate in a very simple and fast manner. Once a steady state situation has been established only a few minutes are required for such a determination as compared to hours, days and even weeks that may be needed by the conventional methods. The reproducibility of the assay is very good and only one reference film is required in order to calculate the oxygen transmissibility of any unknown film.

References

ASTM 1971. Gas Transmission Rate of Plastic Film and Sheeting, D-1434, **35,** 460.
Harvey, E.N., (1941) *Ann. Rev. Biochem.* **10,** 531.
Hastings, J.W. (1951) *J. Cell. Comp. Physiol.* **39,** 1.
Hastings, J.W. & Nealson, K.H. (1977) *Ann. Rev. Microbiol.*, **331,** 549.
Johnson, F.H., Shouwenburg, K.L. & Van der Burg, A. (1939) *Enzymologia,* **7,** 194.
Karel, M. (1975) In: *Principles of Food Science,* Vol. 2, p. 399. Marcel Dekker, New York.
Miltz, J. & Ram, A. (1971) *Polymer,* **12,** 685.
Miltz, J. & Ram, A. (1973) *Polym. Eng. & Sci.,* **13** (4):273.
Modern Plastics Encyclopedia (1978) Engineering Data Bank.

Oshino, R., Oshino, N., Tamura, M., Kobilinsky, L. & Chancel B. (1972) *Biochemica et Biophysica Acta*, **273,** 5.
Ram, A. & Miltz, J. (1972) *Intern. J. Polymetic Matter*, **2,** 39.
Stancell, A.F. (1971) In: *Polymer Science and Materials*, Willey Interscience, New York.
Ulitzur, S., Weiser, I & Yannai, S. (1980) *Mutat. Res*, **74,** 113.

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The microbiology and storage stability of vacuum packed lamb

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Summary

Lamb joints (shoulders, loins and legs) stored in vacuum packs at 0–1°C remained unspoiled for 6 weeks, but since the aerobic storage life at 5°C (retail shelf life) of lamb after holding in vacuum packs at 0–1°C for 6 weeks was only 2 days, vacuum storage should be limited to a maximum of 4 weeks in practice. *Brochothrix thermosphacta*, Moraxella spp, and Moraxella-like organisms were predominant on aerobically spoiled lamb and the preservative effect of vacuum packing resulted from the inhibitory effects of the high carbon dioxide (>20%) and low oxygen concentrations (<1%) which developed in the packs. *Brochothrix thermosphacta* was not completely inhibited, however, and with lactic acid bacteria was the possible cause of cheesy/sour odours which terminated vacuum packed storage life.

Introduction

Large quantities of beef are distributed from abattoirs to retail outlets as boneless primal joints, vacuum packed in plastic materials of low gas permeability. This system of marketing reduces the space required for transportation and storage, makes more efficient use of skilled butchers and can provide quantities of different cuts to suit the specific requirements of each retail outlet. Vacuum packing gives the added advantage of extending shelf life to at least 8 weeks at 0°C. Lamb is rarely jointed and vacuum packed at the abattoir because the smaller carcass is easily distributed uncut and the fewer types of joint are readily prepared at the retail outlet. Packaging costs for lamb are higher because the joints are small and bone must be covered by a protective material to prevent puncturing of the pack. Nevertheless, vacuum packing could be economic where a long shelf life is required, such as in international trade.

Information on the shelf life and microbiology of vacuum packed lamb is

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scarce compared to the numerous publications on beef but Rhodes & Shepherd (1966), Barlow & Kitchell (1966) and Reagan *et al.* (1971) have indicated that the shelf life extension is less than for beef. In the present study we have examined the microbiology and storage stability of cuts of lamb in vacuum packs using different packaging materials, and after subsequent storage in air to simulate retail display.

Materials and methods

Meat

Nineteen Suffolk Cross lambs (8–9 months of age) were slaughtered at the Meat Research Institute abattoir and the carcasses hung for 5 hr at ambient temperature (15°C) followed by 19 hr at 1°C. After confirming that the pH of the *M. longissimus dorsi* was normal (5.5–5.9), each carcass was butchered into leg (femur removed), shoulder and loin joints.

Packing and storage

The loin joints from one carcass were loosely wrapped in 2.5 mil polythene (O_2 permeability 3,200 cm^3/m^2-day-atm. O_2) for aerobic storage at 5°C. to simulate retail display.

Joints from eighteen carcasses were vacuum packed in one of three materials: A, Cryovac BB1 (O_2 permeability at 20°C and 90% r.h. 23 cm^3/m^2-day-atm. O_2, CO_2 permeability 52 cm^3/m^2-day-atm CO_2; W. R. Grace Ltd, London); B, Nylon/Polythene/Surlyn (O_2 permeability 13 cm^3/m^2-day-atm. O_2, CO_2 permeability 38 cm^3/m^2-day-atm. CO_2; Smith & Nephew Plastics Ltd, Hull); C, Synthene 38C/3627 (O_2 permeability 73 cm^3/m^2-day-atm. O_2, CO_2 permeability 201 cm^3/m^2-day-atm. CO_2; Smith & Nephew Plastics Ltd). Two of the materials of similar oxygen permeability (A and B) were used to compare different systems of evacuation and sealing: material A was evacuated using a nozzle system (Cryovac 300 vacuum packing machine: W. R. Grace Ltd) sealed with a metal clip and heat shrunk, material B was evacuated and sealed in a vacuum chamber (Swissvac Major; Soplaril Ltd). Material C was also evacuated and sealed in a vacuum chamber but was used to investigate the effect of higher permeability to oxygen. Comparison between the materials was made by packing joints from the opposite sides of six carcasses in A and B, with six carcasses similarly packed in A and C and six carcasses in B and C.

Vacuum packs were kept at 0° – 1°C and joints from three carcasses (one from each pair of packaging materials) were sampled after each of 1, 2, 4, 6, 8 and 10 weeks' storage. Following each period of vacuum packed storage loins were wrapped in 2.5 ml polythene, stored at 5°C (retail display), and examined periodically until spoilage (off-odour or slime) was observed.

Analysis of gas in packs

The volume of gas in the vacuum packs and the concentrations of oxygen, carbon dioxide and nitrogen were estimated as described by Dainty *et al.* (1979) on a gas chromatograph (Model 69–552 GOW–MAC Instrument Co. Shannon, Ireland) after dilution with a known volume of helium carrier gas.

Drip

The volume of liquid (drip) in each pack was measured after storage.

Assessment of appearance and odour

The packs were examined in daylight and their appearance recorded. Odour was assessed by a panel of four who assessed the onset of spoilage.

Microbiological examination

Joints from the left hand side of each carcass were examined before packing and all joints except those that were punctured were examined after storage.

Fat and lean areas (10 cm^2) were sampled separately using the template and swab method (Kitchell, Ingram & Hudson, 1973) and the swabs shaken with 10 ml of diluent (0.1% (w/v) peptone, 0.85% (w/v) NaCl; pH 7.0). Standard drops (0.017 ml) of suitable decimal dilutions of the swab suspension were spread on the surface of plate count agar (PCA, Oxoid) + 1% NaCl incubated at 25°C for 5 days to give the total viable count; MacConkey Agar No. 3 (Oxoid CM115) + 1% glucose incubated at 25°C for 48 hr to enumerate Gram-negative bacteria; Cavett's (1963) modification of acetate agar (AA; Rogosa, Mitchell & Wiseman, 1951), incubated at 25°C for 5 days under 95% H_2 + 5% CO_2 to enumerate aciduric lactic acid bacteria; and streptomycin thallous acetate actidione agar (STAA; Gardner, 1966), incubated at 20°C for 48 hr to enumerate *Brochothrix thermosphacta.*

Thirty isolates taken at random from the lowest countable dilution on the PCA + 1% NaCl plates from selected joints were identified as described by Shaw & Harding (1978) to determine the predominant bacteria.

Results

Internal gas atmospheres

The total gas volumes in packs of materials B and C were considerably lower than in A showing that the vacuum chamber method removed more air from the bags; concentrations of carbon dioxide (produced by respiration of meat and

Table 1. Gas volumes and oxygen and carbon dioxide concentrations in vacuum packs of lamb during storage at 0–1°C

	Packaging material*								
	A			B			C		
Weeks storage	O_2	CO_2	Total gas volume	O_2	CO_2	Total gas volume	O_2	CO_2	Total gas volume
1	0.4†	20.3	64†	1.7	28.5	18	1.3	36.8	10
2	0.8	20.7	46	1.8	23.9	17	1.6	35.6	24
4	0.9	17.0	73	0.2	30.3	14	0.2	43.0	21
6	0.2	22.7	74	0.2	36.6	28	0.4	34.2	15
8	N D	28.8	45	N D	47.3	44	0.1	41.3	23
10	0.7	27.0	77	N D	42.0	38	0.4	41.0	33

*A, Cryovac BBl; B, nylon/polythene/surlyn; C, Synthene 38C/3627.

†Each gas concentration (% by vol) and total gas volume (cc) is the mean result of two loins, two shoulders and two legs.

N D, not detected.

bacteria) were therefore lower in packs of material A (Table 1). Oxygen, most of which was consumed by respiration was detected at similar low levels in all materials.

Appearance and odour

Fifteen packs were punctured during storage. This did not affect the appearance of the lamb after 1 week, but after 2 weeks or longer the meat in half of the punctured packs had an unacceptable brown appearance. Fruity or sickly sweet off-odours were detected in punctured packs after 6 weeks. In intact packs, joints maintained an acceptable purple lean colour and fat remained bright for 8 weeks. Brown discolouration of the lean and a dull fat colour were seen after 10 weeks, in 5 of the 17 packs. The appearance of the joints was aided by the low volumes of drip in the packs; many packs contained no measurable drip and the greatest volume detected was 15 ml.

No off-odours were detected in intact packs until 8 weeks when 10 of 17 joints were spoiled; after 10 weeks 12 of 17 joints examined were spoiled. Off-odours were cheesy, sour, and sometimes sulphidic, and were strongest in the region of the leg joints from which the femur had been removed. No differences in storage stability or appearance arose from the three different packaging materials.

Table 2. Bacterial numbers on joints of lamb during vacuum packed storage at 0–1°C in three materials

Weeks storage	Total viable count			Presumptive *Brochothrix thermosphacta*			Presumptive aciduric lactic acid bacteria			Presumptive Gram-negative bacteria		
	A*	B	C	A	B	C	A	B	C	A	B	C
0	3.1†	3.1	3.2	<0.7–2.0	<0.7–1.3	<0.7–2.0	<0.7–2.3	<0.7–2.3	<0.7–1.9	<0.7–3.5	<0.7–3.5	<0.7–3.0
1	3.0	3.1	3.2	<0.7–2.1	<0.7–3.1	<0.7–1.7	<0.7	<0.7–0.7	<0.7	<0.7–2.5	<0.7–2.8	1.6
2	3.4	3.1	3.7	<0.7–3.0	<0.7–2.1	<0.7–2.2	<0.7–2.2	<0.7–1.9	<0.7	1.6	1.5	1.9
4	4.2	4.2	4.1	3.6	3.5	3.4	<1.0–2.6	<1.0–3.5	<1.0–1.8	2.5	2.5	2.6
6	4.9	4.9	4.9	4.4	4.5	4.5	<1.0–3.4	<1.0–3.6	<1.0–3.5	2.8	2.7	3.1
8	4.6	5.4	5.1	3.7	4.4	4.1	2.6	2.9	2.9	3.2	3.3	2.9
10	5.9	5.6	5.9	5.2	5.2	5.7	3.9	3.9	3.7	3.3	2.8	4.0

*Packaging material – see Table 1.

†Values are either the mean or the range of $\log_{10}$ counts/cm² on the fat and lean surfaces of two leg, two loin and two shoulder joints.

ble 3. The incidence of micro-organisms isolated on PCA + 1% NaCl from lamb loins vacuum packed in nylon/polythene/surlyn during rage at 0–1°C

eks rage	Meat surface	Total viable count (log_{10} no/cm²)	Micrococcus spp.	*Brochothrix thermosphacta*	Non-aciduric lactobacilli	Pseudomonas spp.	Yeasts	Coryneforms	Enterobacteriaceae	Acinetobacter spp.	Moraxella-like organisms	Staphylococcus spp.
		3.8	80	–	–	–	14	3	3	–	–	–
		3.7	51	23	4	–	–	4	4	4	–	10
	Fat	4.7	7	93	–	–	–	–	–	–	–	–
		5.9	–	7	93	–	–	–	–	–	–	–
		3.2	71	–	–	–	13	13	–	–	–	3
		3.8	15	–	–	36	4	–	–	30	15	–
	Lean	5.8	–	100	–	–	–	–	–	–	–	–
		6.4	–	57	17	23	–	–	–	–	3	–

Table 4. The incidence of microorganisms isolated on PCA + 1% NaCl from lamb loins stored in air at 5°C with and without prior storage in vacuum packs at 0–1°C

Weeks storage in vacuum packs	Days' storage in air	Meat surface	Total viable count	Percentage of isolates: *Brochothrix thermosphacta*	Moraxella spp	Moraxella-like organisms	Pseudomonas spp	Non-aciduric lactobacilli	Entero-bacteriaceae	Acinetobacter spp
0	8		7.4	3	54	40	3	–	–	–
1	7		6.9	77	–	–	17	–	6	–
2	6	Fat	6.4	93	–	–	3	3	–	–
6	3		7.5	94	3	–	–	–	3	–
10	3		7.7	97	–	–	3	–	–	–
0	8		6.8	68	8	24	–	–	–	–
1	7		6.5	40	33	17	7	–	–	3
2	6	Lean	6.9	54	7	30	–	7	–	–
6	3		7.5	90	–	–	10	–	–	–
10	3		8.0	13	–	–	84	3	–	–

Aerobically stored loins kept for 7 days at 5°C when wrapped in polythene without prior vacuum packed storage. Vacuum packed storage for 1, 2, 4 and 6 weeks decreased the subsequent aerobic shelf life to 6, 4, 3 and 2 days respectively, after which a sickly sweet off-odour became evident.

Microbiology

The mean total viable count on the fat surface of joints after cutting was $2.5 \times 10^3/cm^2$ which is similar to that found by Patterson (1968) on chilled lamb carcasses in a commercial abattoir. Total viable counts did not change significantly during the first 2 weeks of vacuum packed storage and then increased up to 10 weeks (Table 2).

Presumptive numbers of *B. thermosphacta,* aciduric lactobacilli and Gram-negative bacteria detected on selective media during storage are shown in Table 2. The total viable count of the microflora developing on the meat surface was not accounted for entirely with these media because in many samples the sum of their counts was considerably less. In the fresh samples this was due to the high proportion of micrococci which were detected by identification of isolates from the total viable count plates (Table 3). *Brochothrix thermsophacta* was also more common on stored samples than was shown by the selective medium; at 6 weeks 93% and 100% of isolates on PCA + 1% NaCl from fat and lean respectively were *B. thermosphacta* (Table 3), whereas the selective count on the same samples was only 20% and 60% of the total viable count. In addition, on the sample examined after 10 weeks, there were many non-aciduric lactobacilli which could not be recovered on the selective medium used for lactic acid bacteria. Combining information from the identification of isolates on PCA + 1% NaCl with the selective media counts shows that there was an initial period in which limited growth of Gram-negative bacteria occurred after which *B. thermosphacta* became dominant until after prolonged storage when non-aciduric lactobacilli also became very numerous. There was no difference in bacterial growth on joints in the three packaging materials (Table 2).

The predominant bacteria on spoiled aerobically stored joints are shown in Table 4. Without prior vacuum packed storage the dominant types were Moraxella spp and Moraxella-like organisms on the fat, and *B. thermosphacta* and Moraxella-like organisms on the lean. When joints had been previously stored in vacuum packs, *B. thermosphacta* was always the dominant organism on the fat and was detected with Moraxella-like organisms or pseudomonads on the lean.

Discussion

This study has demonstrated that primal cuts of lamb can be kept in vacuum packs at 0° – 1°C for up to 6 weeks without spoilage, compared with 1–2 weeks

at 4°C and 3–4 weeks at −1.5°C – 0°C for hung carcasses (International Institute of Refrigeration, 1979). The maximum period for which meat can be stored in vacuum packs is limited by storage stability in the pack itself and the retail shelf-life in air that is subsequently required. Reagan *et al.* (1971) concluded that 8 days was a reasonable maximum period in the vacuum pack because longer storage resulted in a high proportion of samples with bacterial counts over their designated spoilage level (1×10^6 /cm^2) within 2 days' retail display at 0°C. However, this criterion of spoilage was based on data from meat which had been stored entirely under aerobic conditions, and may not be applicable to that which has had prior storage in the vacuum pack. Defining shelf-life as the time for which meat can be held without development of off-odours, we found this to be 4, 3 and 2 days in retail display at 5°C with 2, 4 and 6 weeks' vacuum packed storage respectively. Hence 4 weeks seems to be the maximum period for which it is advisable to store lamb in vacuum packs at 0–1°C. Where necessary, packs could be kept for 6 weeks by using −1°C combined with a high standard of hygiene during slaughtering and cutting.

The shelf-life extension obtained by vacuum packaging was the consequence of the inhibition of the aerobic spoilage microflora by the gas atmosphere which developed in the packs. *Brochothrix thermosphacta*, a main constituent of the aerobic spoilage flora of lamb in the present study and in those of Barlow & Kitchell (1966) and Newton, Harrison & Smith (1977), grows less rapidly in low oxygen atmospheres (Newton *et al.*, 1977) and would have been thus affected in many packs after 2 weeks. The 20% carbon dioxide attained after 1 week would have an additional slight inhibitory effect (Roth & Clark, 1975). Inhibition of *B. thermosphacta* by lactobacilli seems unlikely. This has been demonstrated in vacuum packed beef by Roth & Clark (1975), but 10^7 lactobacilli/cm^2 are necessary (Newton & Gill, 1978), and this level was never attained on the lamb. Inhibition of pseudomonads, Moraxella spp and Moraxella-like organisms was almost certainly due to the carbon dioxide which is generally effective against Gram-negative bacteria (Sutherland *et al.*, 1977).

The gas atmosphere in the packs did not, however, totally inhibit *B. thermosphacta* and pseudomonads, whose slow growth has also been reported on vacuum packed beef (Sutherland, Patterson & Murray, 1975; Dainty *et al.*, 1979). Patterson & Gibbs (1978) detected *B. thermosphacta* as the dominant organism on vacuum packed lamb after 6 weeks' storage at 1–2°C, and this was also true of the packs in the present study. It is possible that *B. thermosphacta* and non aciduric lactobacilli produced the cheesy/sour odour evident in many spoiled packs, as both produce short chain fatty acids (Dainty *et al.*, 1979) to which this type of odour may be attributed. The continued growth of *B. thermosphacta* could also be the main cause of the gradual reduction in aerobic shelf-life which occurred with increase in prior storage time in the vacuum pack.

Although lower concentrations of carbon dioxide developed in the packs evacuated by the nozzle system, they were still adequate to inhibit gram negative bacteria (Sutherland *et al.*, 1977), and there was no evidence to distinguish between this and the vacuum chamber evacuation. No detrimental

effects were detected from employing a packaging material with a gas permeability at the high end of the range used for vacuum packing.

The scope for using vacuum packing to extend the shelf-life of lamb, and the microbiological principles behind it are thus established. We encountered only one practical problem in this work – that of puncturing which resulted in discolouration and spoilage. This was due to the sharp edges of cut bones and could be solved by covering these before packing.

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References

Barlow, J. & Kitchell, A.G. (1966) *J. appl. Bact.* **29,** 185.
Cavett, J.J. (1963) *J. appl. Bact.* **26,** 453.
Dainty, R.H., Shaw B.G., Harding, C.D. & Michanie, S. (1979) In: *Cold Tolerant Microbes in Spoilage and the Environment,* p.97. Academic Press, London.
Gardner, G.A. (1966) *J. appl. Bact.* **29,** 455.
International Institute of Refrigeration (1979) In: *Recommended Conditions for cold storage of perishable produce,* 3rd edn. International Institute of Refrigeration, Paris.
Kitchell, A.G., Ingram, G.C. & Hudson, W.R. (1973) In: *Sampling-Microbiological Monitoring of Environments,* p.48 Academic Press, London and New York.
Newton, K.G., Harrison, J.C.L. & Smith, K.M. (1977) *J. appl. Bact.* **43,** 53.
Newton, K.G. & Gill, C.O. (1978) *J. appl. Bact.* **44,** 91.
Patterson, J.T. (1968) *Rec. agric. Res. Minist. Agric. Nth. Ir.* **17,** 1.
Patterson, J.T. & Gibbs, P.A. (1978) *J. Fd Technol.* **13,** 1.
Reagan, J.O., Jeremiah, L.E. Smith, G.C. & Carpenter, Z.L. (1971) *J. Fd Sci.* **36,** 764.
Rhodes, D.N. & Shepherd, H.J. (1966) *J. Sci. Fd Agric.,* **17,** 287.
Rogosa, M., Mitchell, J.A. & Wiseman, R.F. (1951) *J. Bact.* **62,** 132.
Roth, L.A. & Clark, D.S. (1975) *Can. J. Microbiol.* **21,** 629.
Shaw, B.G. & Harding, C.D. (1978) *J. appl. Bact.* **45,** 39.
Sutherland, J.P., Patterson, J.T. & Murray, J.G. (1975) *J. appl. Bact.* **39,** 227.
Sutherland, J.P., Patterson, J.T., Gibbs, P.A. & Murray, J.G. (1977) *J. Fd Technol.* **12,** 249.

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Discrimination between synthetic and natural ethyl alcohol in spirits and fortified wines

D. J. McWEENY AND M. L. BATES

Summary

Synthetic alcohol from petrochemical sources has a low ^{14}C content as compared to fermentation alcohol produced from contemporary carbohydrates. The ^{14}C contents of seventy-eight samples of potable spirits and fortified wines are reported and discussed in relation to their usefulness in detecting the use of synthetic alcohol. The importance of correcting for the effects of congeners during the ^{14}C-assay is discussed.

The ^{14}C level at which the use of synthetic alcohol might be presumed will vary according to the methods employed in producing the beverage.

Introduction

Fortified wines (e.g. sherries, ports, vermouths) are alcoholic beverages in which some of the alcohol is derived from yeast fermentation of grapes and some from the separate addition of distilled spirit. In some products, such as port from the Douro valley, the distilled spirit added is such that it imparts special characteristics to the product, but in others, such as madeira, a high quality neutral alcohol is used. Frequently, legislation restricts the type of alcohol which can be added. For example, in the EEC the alcohol used in fortified wines (called liqueur wines in EEC law) must be obtained from the distillation of wine. The manufacturer is often further limited in his choice of alcohol by local traditional manufacturing practices which are sometimes also enshrined in legislation so that the characteristics of a particular product may be maintained. Similar restrictions apply to the manufacturer of gin, vodka and other spirit drinks made from high quality neutral alcohol obtained from cereal grain, potatoes, molasses, fruit, wine, or other agricultural raw materials. It is sometimes necessary to distinguish between the alcohol of agricultural origin as

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is routinely used in these drinks and alcohol produced via ethylene from oil or natural gas (synthetic alcohol). Often, this presents little difficulty as even the highest quality commercially available agricultural alcohol contains sufficient quantities of congeners (higher alcohols, esters, aldehydes, etc, produced in the fermentation) to distinguish it from synthetic alcohol, which contains no such impurities. However, if the alcohol has been already incorporated in an alcoholic drink, this method cannot be used.

The present work describes the use of an alternative method based on the ^{14}C content of the ethyl alcohol in a number of alcoholic beverages on sale in U.K. during 1975. The procedure relies on the principle, first employed by Faltings (1958), that the ^{14}C content of ethyl alcohol derived from a fossil carbon source will be almost zero but in alcohol obtained by fermentation of a carbohydrate it will be determined largely by the ^{14}C content of the CO_2 in the atmosphere from which the carbohydrate was photosynthesized. Prior to 1956 this was approximately 13 dpm/g carbon (L'Orange & Zimer, 1968). As a result of nuclear weapons testing the level rose to almost twice that value in 1963–4 (Baxter & Walton, 1971) and has decreased steadily since then. In 1972 the ^{14}C content of biogenic acetic acid was reported as 20 dpm/g carbon (Kaneko, Ohmari & Massai, 1973). The method employed in the present work is based on a double distillation procedure which produces a spirit containing at least 88% ethanol; this is then assayed for ^{14}C by a liquid scintillation counting technique. The importance of including internal standards in the assay procedure is emphasized by the observed 'quenching' effects due to congeners.

Experimental

Preparation of alcohol samples

Sample sizes were chosen so as to contain approximately 60–90 ml of alcohol, e.g. 500 ml of wine or 250 ml of liqueurs and spirits. Where liqueurs had a high sugar content it was found advisable to dilute these with water (1:1) to avoid charring during the distillation stage. Samples were distilled slowly through a glass-helix packed fractionating column and the first 100 ml of distillate collected. In order to ensure a sufficiently high alcohol content for the ^{14}C assay stage the distillate was re-distilled and the fraction boiling at 78.5 ± 0.2°C was collected. The alcoholic strength of the distillate was determined by a specific gravity method. The absolute volume of alcohol delivered from a 5 ml bulb pipette was determined by calibration with 'Analar' ethyl alcohol; the alcohol delivered was weighed to 0.1 mg and the volume obtained by reference to standard tables. Using this calibrated pipette replicate 5 ml aliquots of the second distillate from each sample were weighed and their alcoholic strength calculated from tables. The concentrations were all in the range 88–95% w/v ethyl alcohol.

Table 1. ^{14}C content (dpm/gC) of ethyl alcohol in various alcoholic drinks

Commodity	No. of samples	Range	Mean	Standard deviation	Samples below 16.0
Spirits and liquers	37	12.3–23.0	19.13	±2.16	3 (12.3, 13.9, 15.6)
Fortified wines (sherries, vermouths, etc)	13	6.6–20.4	17.47	±3.41	1 (6.6)
Tonic wines	3	14.1–19.4	17.20	±2.76	1 (14.1)
Wines	15	16.6–21.7	19.23	±1.56	0
Miscellaneous products (perry, mead, whisky/wine blends, etc.)	10	13.9–20.8	18.62	±2.44	2 (13.9, 15.0)

Overall mean – all samples = 18.75 dpm/gC; standard deviation = ± 2.42; n = 78.
Mean of samples >16.0 dpm/gC = 19.31; standard deviation = ± 1.44; n = 71.
Mean of samples >15.0 dpm/gC = 19.24; standard deviation = ± 1.50; n = 72.
*Counting error; s.d. = 0.4 dpm/gC.

^{14}C assay procedure

Each sample was assayed in duplicate by liquid scintillation counting in a Phillips LS A1 scintillation counter. The vials contained 10 ml distillate and 10 ml scintillator (0.5% PPO, 0.05 dimethyl POPOP in toluene) and were counted for 100 min. Blanks contained 10 ml of synthetic alcohol (95%) in place of the distillate. Standards contained U-^{14}C-hexadecane (Radiochemical Centre, Amersham, U.K.) and after the initial 100 min count this material was added to each vial as an internal standard in order to measure the 'quench' factor for each sample. After application of corrections for background, 'quenching' and alcohol content of the sample the results were expressed as disintegrations per min per gram of carbon (dpm/gC). Typically the standard deviation due to the random nature of radioactive decay was ± 0.4 dpm/gC.

Results

The assay procedure

Preliminary experiments established that with the toluene-based scintillator system employed in this work phase separation in the counting vials was likely to occur if the alcohol content of the distillate was less than 83% w/v; the double distillation procedure ensured this figure was always comfortably exceeded for all the beverages examined.

'Quenching' was observed in distillates from all the beverages examined. This

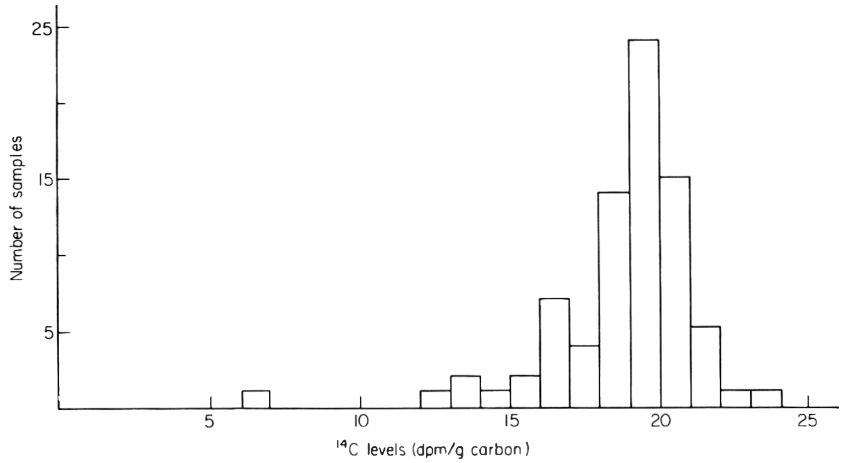

Figure 1. Incidence of ^{14}C levels from 0 to 25 dpm/gC in ethyl alcohol from alcoholic beverages.

absorption of photons by the sample material varied in severity according to the beverage it was derived from. Presumably this reduction in count rate was due to 'congeners' which appeared in the distillate in quantities sufficient to absorb a significant proportion of the photons. Typically 'quenching' is due to substances which absorb at visible or near-u.v. wavelength and the effect was exemplified by a test in which an 0.1% solution of crotonaldehyde (Σ_{max} = 285 nm) caused a 13.8% reduction in count rate due to ^{14}C standard. 'Quench' corrections on the alcoholic distillates varied from 5 to 15%.

^{14}C *content of beverages*

The abundance of ^{14}C in the ethyl alcohol derived from seventy-eight samples of alcoholic drinks is shown in Table 1. The ^{14}C contents vary from 23.0 dpm/gC to 6.6 dpm/gC. The frequency with which ^{14}C contents were encountered is shown, at 1 dpm/g carbon intervals, as a histogram in Fig. 1. The results indicate that of the seventy-eight samples examined 74% (fifty-eight samples) had ^{14}C contents in the range 18.0 to 20.9 dpm/gC. 91% (seventy-one samples) had ^{14}C contents greater than 16.0 dpm/gC. The seventy-one samples with ^{14}C content greater than 16.0 dpm/gC had a mean of 19.31 dpm/gC (s.d. = 1.44); the seventy-two samples with $^{14}C > 15.0$ dpm/gC had a mean of 19.24 (s.d. = 1.50).

Discussion

Alcohol with a ^{14}C content of 6.6 dpm/gC observed in one of the products examined in the present study is 50% of the pre-1956 levels and only 30% of levels existing in the early 1970's; it must be regarded as clear evidence of the use of synthetic alcohol in the fortification of the product. Levels of ^{14}C

significantly below the pre-1956 level of around 13 dpm/gC seem to be *prima facie* evidence of the use of synthetic alcohol since the effects of environmental factors which may reduce ^{14}C levels are thought to be relatively small. Levels of ^{14}C below contemporary levels in 1974 – about 17 dpm/g carbon, according to Resmino & Volunterio (1974) – could conceivably arise from the use of alcohol produced from pre-1956 carbohydrates; this would have a low ^{14}C content and if used for fortification of products it could give rise to ^{14}C contents in the range 13 to 17 dpm/gC. The extent to which pre-1956 alcohol was used commercially for fortifying the products on retail sale in 1974 is not readily ascertained.

After 1956 the ^{14}C content of natural ethyl alcohol rose steadily until about 1961 when it reached about 25% above pre-1956 figures. It then rose sharply and in 1963–64 reached twice the pre 1956 level (Baxter & Walton, 1971). These changes occurred as a result of ^{14}C release during the various nuclear weapons tests conducted during this period. The changes were quite rapid and inevitably there is uncertainty about the precise levels of ^{14}C likely to be encountered in spirits and fortified wines produced during this period. However with the passage of time it must become increasingly unlikely that alcohol from the pre-1963 period will be available in commercially significant quantities for use in production of spirits and fortified wines. Once that point has been reached and all 'natural' alcohol can be expected to contain post-1963 levels of ^{14}C then the ^{14}C contents encountered should be somewhere between the current level and the 26 dpm/g 'peak' figure; certainly they should not be significantly below current levels. However there are factors which may preclude the setting of a stringent lower limit on ^{14}C content which can be applied universally to all alcoholic beverages as an indicator of the use of synthetic alcohol. From their method of production certain drinks (e.g. sherries and blended whiskies) necessarily contain alcohol derived from atmospheric CO_2 several years before the time at which they are sold. The ^{14}C content of atmospheric CO_2 has decreased steadily from levels which gave a ^{14}C content of 26 dpm/gC in alcohol produced in 1965 to levels of around 17 dpm/gC in 1974 (Resmino & Volunterio, 1974); consequently those drinks which are matured for an extended time before sale should have a higher ^{14}C content than those which are not matured. This is illustrated by the ^{14}C figures for whisky samples in the present study which are 19.3, 22.5, 21.8, 20.2 and 23.0 dpm/gC as compared to a mean of 18.75 for all the drinks examined. So long as the ^{14}C content of atmospheric CO_2 continues to fall the level of ^{14}C in the alcohol of 'matured' drinks should exceed that of products which are consumed soon after primary production. Accordingly the level of ^{14}C which must be exceeded if the use of synthetic alcohol is not to be suspected should be higher for 'matured' drinks than for 'contemporary' drinks.

Inevitably the actual ^{14}C levels which may be chosen as indicating evidence of the use of synthetic alcohol in 'matured' drinks and in 'contemporary' drinks will depend upon careful definition of the measurement procedures to be used and on the degree of certainty required. The latter is necessarily a judgement which will not be determined solely on scientific grounds but it seems possible

that in practice any debate on the alternative approaches to this judgement may be rendered unnecessary by the current and expected future prices of feedstocks for the production of synthetic alcohol. These seem likely to make the cost of synthetic alcohol economically unattractive for use in spirits and fortified wines in the forseeable future.

References

Baxter, M.S. & Walton, A. (1971) *J. Fd Sci.* **36,** 540.
Faltings, V. (1952) Angew Chemie. **64,** 605.
Kaneko, T., Ohmari, S. & Massai, H. (1973) *J. Fd Sci.* **38,** 350.
L'Orange, R. & Zimer, K.E. (1968) *Naturwissenschaften,* **55,** 35.
Resmino, P. & Volunterio, G. (1974) *Riv. Vitic. Enol. Agr.* **10,** 1.

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Determination of 3, 4-benzopyrene and benzanthracene (PAH) in phenolic smoke concentrates

D. J. SILVESTER

Summary

The use of reversed phase high pressure liquid chromatography (HPLC) coupled to fluorimetry has been used for the determination of 3, 4-benzopyrene and benzanthracene in smoke concentrates used for food flavouring.

Introduction

Smoke concentrates are now increasingly being used in the food industry to impart a 'smoke flavour' to foodstuffs notably meat, fish, and cheese. The 'smoke flavour' is derived from phenolic materials which are produced when wood is burned in conditions of oxygen deficiency and the smoke flavour can be imparted to the food either by conventional direct smoking or by constituents/concentrates derived from wood smoke condensates sprayed onto the food. At the same time that the phenolic flavour materials are formed, small quantities of polynuclear aromatic hydrocarbons (PAHs) are also produced and since some of these, especially 3, 4-benzopyrene and benzanthracene are carcinogenic, determination of their levels in artificial smoke flavours becomes important. It has already been shown by Lijinsky & Shubek (1965) that 3, 4-benzopyrene, benzanthracene, and other PAHs exist in traditionally smoked fish and it is on this basis that 'smoke concentrates' could be used to overcome the problem of PAHs in smoke flavoured food.

Studies on phenolic concentrates used as the basis for 'smoke flavours' were initiated with respect to the development of a method for analysis of 3, 4 benzopyrene and benzanthracene with a sensitivity of better than 2 parts/10^9 and without using thin layer chromatography due to its inherent problems of quantification.

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Table 1. Reproducibility

Run No.	Area 3, 4-benzopyrene	Area B(α)A
1	15.00	21.54
2	15.17	21.08
3	15.30	21.31
4	15.09	19.80
5	15.20	20.54
Average	15.15	20.86
s.d.	0.10	0.62

Table 2. Linearity

	Analysis results (μg/ml)	
Sample solution (μg/ml)	3, 4-benzopyrene	B(α)A
1.0	1.01	1.00
0.8	0.79	0.83
0.6	0.57	0.56
0.4	0.41	0.38
0.2	0.21	0.22
0.1	0.09	–

Table 3. Recovery

Run	Percentage recovery of 10 parts/ 10^9 added 3,4-benzopyrene
1	92.3
2	91.5
3	91.6
4	92.5
5	85.4

Table 4. Materials tested

Name	Retention time (min)	Relative response factor	Comments
Benzene (Solvent)	9.08	14800 approx	
Phenanthrene	11.87	7.048	
Anthracene	13.25	1.150	
Fluoranthene	17.30	0.734	
9-Methyl anthracene	18.20	0.545	
Pyrene	19.56	1.780	
2-Methyl anthracene	20.98	1.124	
P-Terphenyl	27.39	10.580	
Chrycene	30.21	–	Merged peak
4, 5-Benzopyrene	30.21	–	Merged peak
Perylene	30.21	–	Merged peak
Benzanthracene	47.52	0.320	
3, 4-Benzopyrene	56.36	0.161	
Naphthalene	–	–	Not detectable
Fluorene	–	–	Not detectable
O Terphenyl	–	–	Not detectable
M Terphenyl	–	–	Not detectable
2, 6-Dimethyl napthalene	–	–	Not detectable
2, 3-Dimethyl napthalene	–	–	Not detectible
2-Methyl napthalene	–	–	Not detectable
2-Ethyl napthalene	–	–	Not detectable
1-Methly napthalene	–	–	Not detectable

Materials and methods

Apparatus

A Perkin Elmer 1000 M Fluorimeter with LC flow through cell and excitation filter of 280 nm and emission filter to pass wavelengths above 390 nm is coupled to a potentiometric recorder of 10 mV FSD and interfaced to a Perkin Elmer Sigma 10 Data Station for quantification. The HPLC colur n outlet is connected to the LC flow through cell with narrow bore 1/16 inch stainless steel tube to prevent peak broadening.

Column

A pre-packed column of 25 cm by 4.6 mm i.d. containing Partisil 10 ODS II (Whatman Company) was used. The packing material consists of 10 μm silica support with octadecyl silane bonded through Si-O-Si groups to the silica support. The carbon loading for this column is 15%, the high level of Si-OH group covering being needed to prevent peak tailing from 3, 4-benzopyrene.

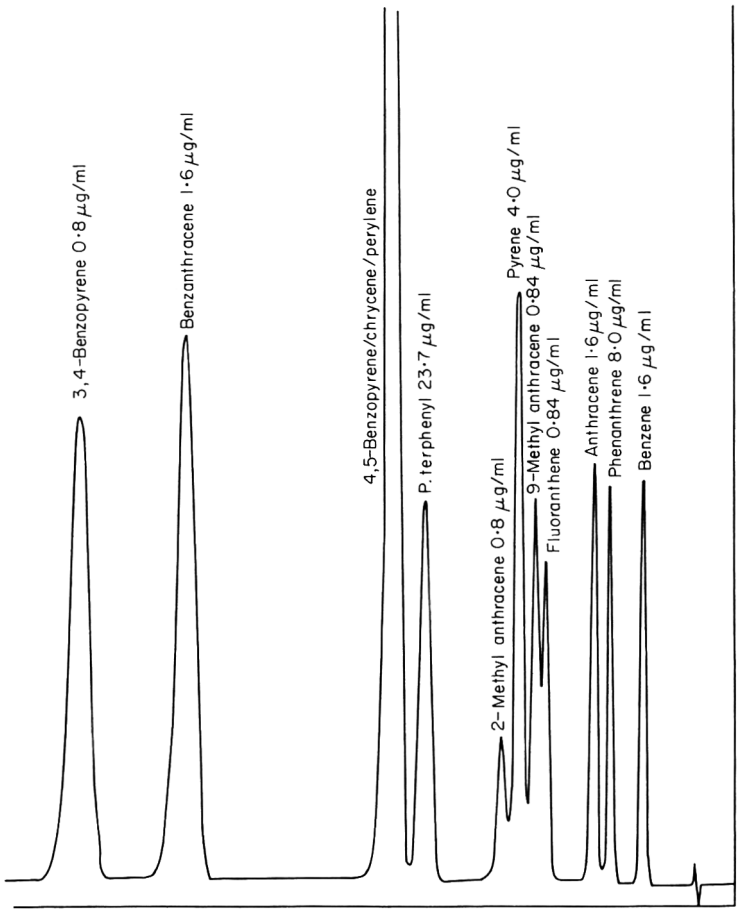

Figure 1. Injection 100 μl of solution containing given concentrations of standards.

HPLC operating conditions

The PAH containing solutions are injected into the column by a 100 μl loop injector (Rheodyne 7010) and eluted from the column by an isocratic mixture of 35% methanol, 35% acetonitrile, balance water by volume and delivered by an Altex 110A pump (Anachem Ltd) at 2 ml/min. A pre-column of Co–Pell ODS is used between the injector and analytical column, both columns being heated to 35°C by a water jacket and thermocirculator (Churchill Inst.).

Reagents

The model compounds (see Table 4 for list) were purchased from Phase Separations (Pfaltz & Bauer), Aldrich Chemical Company, Koch Light, and

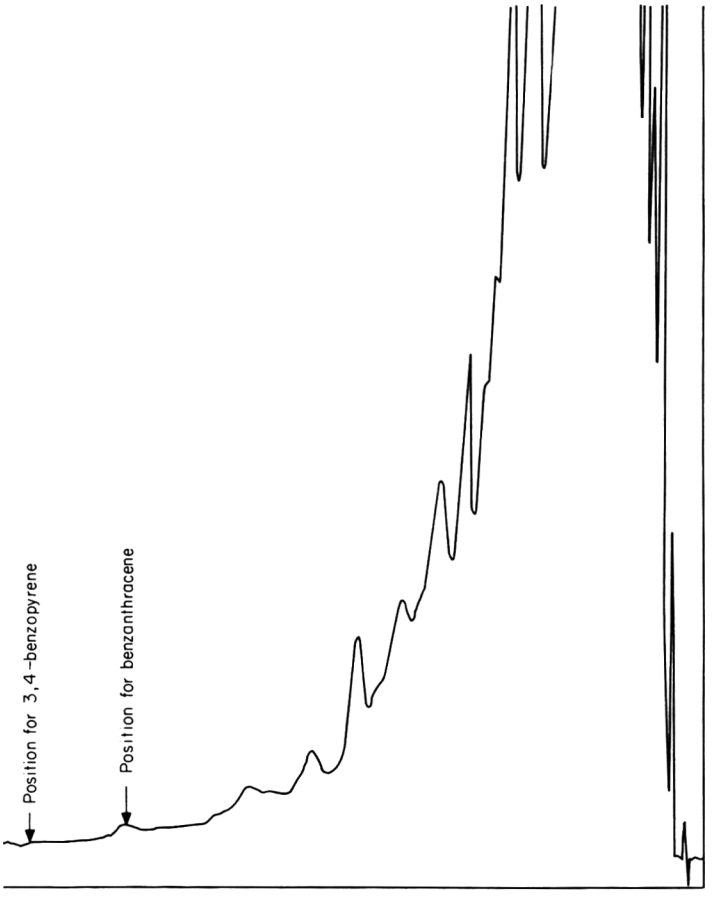

Figure 2. Typical analysis of smoke concentrate.

Cambrian Chemicals. All compounds were found to be chromatographically pure and were used as supplied.

Methanol, acetonitrile and hexane were all redistilled in glass before use; water was standard laboratory distilled.

Alumina used in the purification stage is BDH chromatographic grade Brockmann Activity 1 Neutral.

Quantification

Chromatographic peaks were quantified using a Perkin Elmer Sigma 10 Data Station which provides a written report of peak retention time and respective area. Since the machine is also a mini-computer, it can be used to provide a print-out of PAH levels in smoke extracts directly or as concentration in solution when used in the program mode.

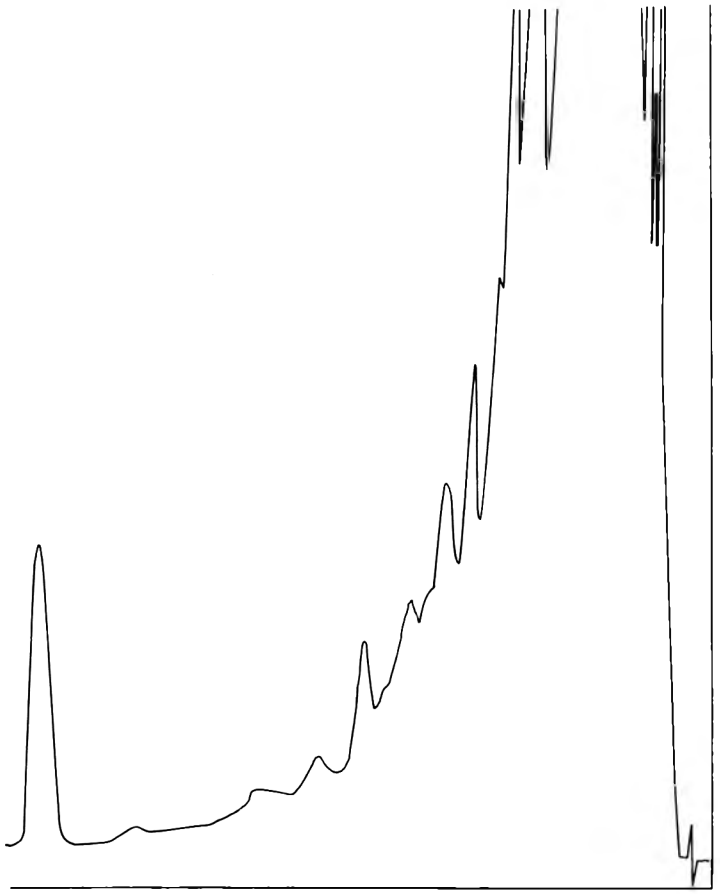

Figure 3. Analysis of smoke concentrate with added 10 parts/10^9 3,4-benzopyrene.

Sample preparation

A well mixed sample (50 g) of smoke concentrate was transferred to a 1 litre conical separator using 500 ml of 1 M (4%) sodium hydroxide (AR) solution to dissolve the sample. The separator was shaken to completely dissolve the sample in the solution as it is prone to form a tarry plug in the separator. A 200 ml portion of hexane was added, the separator shaken for 2 min, and the two layers allowed to separate (approx. 15 min) then the lower layer was run into a second separator. To the second separator was added another 200 ml of hexane and the separator shaken for 2 min, followed by 15 min to allow the layers to separate. The aqueous (lower) layer was discarded and the hexane extracts from the two separators bulked together. The now empty separator was washed with 2 × 250 ml of 1M NaOH solution and this was added to the bulked hexane. The separator was shaken for 2 min then the layers were allowed to separate, after which the lower aqueous layer was discarded. The hexane was then washed with 1 × 200 ml of water followed by 2 × 100 ml of 80% orthophosphoric acid.

In each case the separator was shaken for 2 min and the layers allowed to separate before the lower acid layer was discarded. The hexane layer was finally washed with 2 × 300 ml of water and the aqueous layer discarded.

An alumina purification column was prepared by slurrying 50±1 g of alumina with 100 ml of fresh hexane. The slurry was transferred to a 2 cm diam. sinta-glass column (Gallenkamp Company) ensuring that all of the alumina is transferred to the column. The hexane layer is then allowed to fall to within 0.5 cm above the alumina.

The hexane solution in the separator was then passed down the alumina column, collecting the eluate in a 500 ml conical flask. The separator was washed with a small quantity of hexane and this was also passed down the column. Finally the column was washed with 25 ml of hexane, this also being collected.

The eluate should be a colourless solution and the yellow band which forms in the alumina must not reach within 2 cm of the bottom of the column.

The eluate was then evaporated in a 25 ml pear-shaped flask on a Rotavapor (Orme Scientific) rotary evaporator used at 40°C and 50 mmHg pressure only allowing the eluate to come to dryness at the final stage. To the flask 1.0 ml of acetonitrile was added and the flask swirled to dissolve any material in it. The acetonitrile is used for injection into the HPLC system.

It is important to ensure that the extraction and analysis are carried out on one day due to deterioration of stored PAH samples.

Results

With temperature controlled conditions, the retention times and peak areas of the peaks were found to be very reproducible and since an isocratic system is used, the retention times rather than retention volumes were used for peak identification.

Figure 1 shows the relative peak positions of the PAHs which flouresce under the given conditions whilst Fig. 2 shows the chromatogram obtained by analysis of a smoke concentrate. Due to the large number of peaks in the early part of the chromatogram and since 3, 4-benzopyrene and benzanthracene are known carcinogens, whilst the carinogenic properties of the other compounds detected are as yet unknown, it was decided to concentrate on these two components only at present.

Figure 3 shows analysis of the same smoke concentrates as used in Fig. 2 but with added 10 parts/10^9 3, 4-benzopyrene.

Discussion and conclusions

Early trials of the extraction method of White, Howard & Barnes (1971) on which the extraction used is based, caused some difficulties due to the use of fluorisil reagent recommended in their method for the column purification

stage. Our own work showed that 3, 4-benzopyrene was absorbed onto the fluorisil and could not be removed by using hexane as eluent. A similar situation also occurs with silica gel whilst alumina was found to absorb colouring materials whilst allowing the PAHs to be eluted using hexane as eluent.

The use of more polar eluents for the removal of the PAHs from silica and flourisil results not only in the removal of the PAH but also of a large amount of the colouring material.

The present system offers a relatively simple method for the analysis of 3, 4-benzopyrene and benzanthracene in smoke concentrates down to a level of 1 part/10^9. Further work is required to try to improve the sensitivity of the method and the separation in relation to the other PAHs. Ideas under review are the use of 254 nm excitation with >350 nm emission for sensitivity increase and the use of gradient elution and/or other columns to improve the selectivity of the system.

References

Lijinsky, W. & Schubek, P. (1965) *Toxicol. App. Pharmacol.* **7,** 337.
White, R.H., Howard, J.W. & Barnes, C.J., (1971) *J. Agric. Food Chem.* **19,** 1.

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The production of volatile acids from glucose by soy yeast (*Saccharomyces rouxii*) NRRLY—1096

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Summary

The glucose metabolism of *Saccharomyces rouxii* NRRLY–1096, the soy yeast, under aerobic and anaerobic conditions, was investigated. The study was carried out at pH 4.5 and 7.0 with cells previously grown at either pH. The pH at which the cells had been grown as well as the pH at which glucose was fermented were found to affect the pattern of fermentation. A typical yeast type fermentation was obtained with cells grown at pH 4.5 and fermenting glucose at the same acid pH. An ability to fix CO_2 for formic acid synthesis was demonstrated by cells grown at neutral pH. Acetic acid was produced by cells fermenting glucose at neutral pH irrespective of the pH at which they had been grown. Various schemes of glucose fermentation were therefore proposed. From results of the present study, optimal conditions for the formation of the desired fermentation products, resulting in the flavour and bouquet of good quality soy sauce, are suggested.

Introduction

Saccharomyces rouxii is the yeast commonly used in the soy sauce industry. Its ability to assimilate glucose and other sugars in the presence of 18% NaCl has been studied by Onishi and found to vary from strain to strain. Apart from suggesting that sodium chloride affected yeast metabolism to alter the fermentation pathway, no attempt was made by Onishi (1961) at obtaining a balance sheet for the amount of glucose fermented. In the present study, the other factors, such as pH and aeration were investigated. The knowledge of how these factors could affect the metabolic pathways could be used to promote the formation of the desired fermentation products and thus influence the flavour and bouquet of the soy sauce produced.

The moromi stage of soy sauce fermentation by yeast and bacteria occurs under semi-anaerobic conditions, with the brewing mixture sometimes agitated

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by stirring. The degree of aeration during this stage of fermentation would influence the proportion of various metabolic products, and would very likely contribute towards the distinctive flavour of soy sauces produced by various fermentation processes. An understanding of the glucose metabolism in the absence and presence of air could be basic to the formulation of optimal conditions for the brewing of soy sauce.

From growth studies, pH 4.5 appears to be the optimum pH (Yong *et al.*, 1978), but the pH of the soy mash into which the yeast is first inoculated after the koji fermentation is around pH 6.8. Glucose metabolism of the yeast under these conditions was therefore investigated.

Materials and methods

Purtiy and source of chemicals

All chemicals used were of 'Analar' grade or the purest grade available. They were obtained mainly from Merck Chemical Company, Germany and Sigma Chemical Company, U.S.A. Chemicals for yeast culture were from Oxoid, England or Difco, U.S.A.

Yeast culture, media and culture methods

These as described previously (Yong *et al.*, 1978). Cells were grown in media containing 5% glucose and 10% NaCl at pH 4.5 or pH 7.0 at room temperature (28°C) for 3 days with shaking on a rotatory shaker. They were starved overnight in glucose-free media before use.

Determination of cell weight

Cells were harvested, washed free of growth medium and resuspended in 0.002 M $MgSO_4$. A quick estimate of the approximate weight of cells was then obtained by measuring the optical density of an aliquot of the cell suspension and comparing it with a standard curve. Appropriate dilutions with buffer were then made to obtain the desired cell density of *ca* 15 mg/0.2 ml. Corresponding dry cell weight was determined by drying 0.2 ml cells overnight at 60°C to constant weight.

Preparation of buffers

Phosphate buffer was prepared with 0.1 M monopotassium phosphate and 0.1 M disodium phosphate in carbon dioxide-free distilled water. With a mixture of these two solutions, pH 7.0 buffer solution was obtained.

Phthalate buffer pH 4.5 solution was made with a mixture of 0.1 M potassium biphthalate and 0.1 N NaOH solution in carbon dioxide-free distilled water.

Determination and identification of acids

The volatile acids were separated by steam distillation according to the method of Friedmann (1938). They were then identified by thin layer chromatography.

For steam distillation, the samples were acidified with 10 N H_2SO_4 and protein precipitated with 10% sodium tungstate. Solid $MgSO_4$ was added to increase volatility. The distillation, with the condenser cooled with circulating ice water, was carried out such that about 100 ml of distillate was collected within 30 min. This distillate was made to volume and aliquots titrated with 0.01 N NaOH to determine the acid equivalents.

For thin layer chromatography, the distillate was evaporated to dryness in a flash evaporator on a water bath at 48°C. The concentrated samples were then dissolved in a small volume (0.5 to 1.0 ml) of 95% ethanol and aliquots applied to microcrystalline cellulose thin layer plates of 0.25 mm thickness. These plates were prepared by washing them thoroughly with detergent, drying then cleaning with alcohol to remove all traces of grease. To obtain thickness of 0.25 mm, 25 g microcrystalline cellulose (from Applied Science Laboratory, State University, Pa., U.S.A.) was mixed with 110 ml water in a homogenizer and stirred for 1 min. Air bubbles in the slurry were then removed with a vacuum pump by placing the container in a flask connected to the pump. The slurry was then poured into the trough of a thin layer chromatographic applicator and layered evenly onto the prepared plates. The plates were then air-dried overnight.

After application of the samples, the thin layer chromatographic plates were developed overnight. Several solvent systems were tried and isopropanol-ammonia-water in the ratio of 8:1:1 was found to be the most suitable for the separation of the acids identified as acetic and formic acids. By using 0.1% bromocresol green (0.1 g in 100 ml 95% ethanol) as the spray agent, the acid spots appeared as yellow spots on a blue background especially when the plates were exposed momentarily to ammonia fumes.

Experimental and results

The aerobic and anaerobic metabolism of glucose by *Sacch. rouxii* NRRL Y-1096 was studied by following the gaseous exchange in the Warburg apparatus and analyzing the metabolites (if any) in the medium at the end of the experiment. The medium was also analyzed for glucose by the glucose oxidase method of Hugget & Nixon (1957) to confirm that the glucose added had been completely metabolized when each experiment was terminated. Cells grown at either pH 4.5 or pH 7.0 were used and a comparison was made between glucose metabolism at pH 4.5 and pH 7.0 by these cells. M/10 phthalate buffer was used for experiments at pH 4.5 and M/10 phosphate buffer at pH 7.0. Anaerobic conditions were obtained by flushing the Warburg flask with oxygen-free

nitrogen. Warburg flasks with double side arms were used so that glucose could be placed in one and 2 N sulphuric acid in the other. The acid was tipped into the reaction medium at the end of each experiment to stop the reaction and also to release any CO_2 which might be retained in the phosphate buffer. The experiments were carried out at 30°C. The production of CO_2 was measured by the direct method of Warburg (cited in Umbreit *et al.*, 1964).

Anaerobic glucose metabolism

The fermentation of 10 μ mol glucose was followed and the results of various experiments are summarized in Table 1. In all the experiments, CO_2 accounted for all the gaseous output which were totally absorbed by KOH. There was an absence of hydrogen production. Ethanol, a common product of glucose fermentation by yeasts, was also found to be present in the incubation medium at the end of all the experiments. The amounts of ethanol produced were determined by the micro-diffusion method of Conway (1962).

The reaction medium from each experiment was also assayed for glucose and volatile and non-volatile acids. In all the experiments there was no glucose left in the medium, and volatile acids were found to account for all the acids present. Formic and acetic acids were identified by thin layer chromatography. Gas liquid chromatography did not give a good separation. Attempts at determining the acids quantitatively by chemical analysis proved unsuccessful and they were only determined as acid equivalents by titrating with standard N/100 NaOH after being separated from the reaction mixture by steam distillation. Details of the procedures used have been recorded under 'Materials and methods'.

From the results in Table 1 it may be seen that cells of *Sacch. rouxii* NRRL Y–1096 which had been cultured at pH 4.5 and allowed to ferment glucose at pH 4.5 produced 2 mol each of ethanol and CO_2 per mol glucose. This type of glucose fermentation is typical of yeast cells. The same batch of cells, however, produced acetic acid and lesser quantities of the other two products per mol of glucose fermented if the experiment was carried out at pH 7.0. The acid was produced to reduce the neutral pH of the medium towards the more favourable acid pH. There was only one identifiable acid spot on the thin layer chromatography plates and it had the same R_F value as the standard acetic acid spot. The total amount of acid equivalents present in the steam-distilled samples of the reaction medium showed that the acid produced could be entirely acetic acid (see Table 1 and proposed schemes of fermentation).

When cells grown at pH 7.0, fermented glucose at pH 4.5, 2 mol of ethanol with a little less than 2 mol CO_2 and traces of volatile acids were produced per mol of glucose metabolized. Although the amount of acid produced was to small to be identified, it was probably formic acid. There was more acid produced when the same batch of cells fermented glucose at pH 7.0 (Table 1). About the same amount of ethanol was produced per mol glucose as that produced by cells which had been grown at pH 4.5 fermenting glucose at this

Table 1. Glucose fermentation by *Sacch. rouxii* NRRL Y–1096: 0.2 ml cells (15 mg dry wt) into 2.0 ml buffer were placed in 25 ml Warburg flasks with side arms containing 10 μ mol glucose (0.2 ml) and 0.2 ml 2N H_2SO_4 respectively.

pH level of medium for		Glucose fermented (μmol)	Acid (H^+), μequiv	CO_2 (μmol)	C_2H_5OH (μmol)
Growth	Reaction				
4.5	4.5	10	0.0	20.2 ±0.5	20.1 ±0.8
4.5	7.0	10	4.25 ±0.05	16.5 ±0.5	17.6 ±0.6
7.0	4.5	10	0.85 ±0.06	19.7 ±0.6	20.2 ±0.7
7.0	7.0	10	14.50 ±0.22	7.2 ±0.3	16.6 ±0.5

Results are from three experiments done in duplicate and expressed as mean ± s.e.mean.

pH. The amount of CO_2 produced, however, was halved. By thin layer chromatography both formic and acetic acids were identified as having been produced. Unfortunately it was not possible to determine the actual amounts of either acid present. The colour intensity of the formic acid spot on the thin layer chromatography plates was, however, noticeably more intense than the acetic acid spot separated from the samples from these experiments. Based on the stoichiometry of the reaction and the amounts of ethanol and CO_2 produced, the acid equivalents obtained from metabolizing 1 mol glucose are most likely to be due to 1 mol formic acid and *ca* 0.5 mol acetic acid.

From the results of these experiments, various schemes are proposed for the fermentation of glucose by *Sacch. rouxii* NRRL Y–1096 under different conditions. The number of carbon equivalents are shown within brackets.

Aerobic glucose metabolism

The aerobic metabolism of glucose by *Sacch. rouxii* NRRL Y–1096 was studied by following the gaseous exchange in the Warburg apparatus and analyzing the metabolites (if any) in the medium. No glucose was detected in the medium at the end of the experiments. The experiments were carried out with cells grown at pH 4.5 and pH 7.0 as in the experiments on the anaerobic metabolism of glucose to determine whether pH had an effect on the aerobic metabolism of glucose. Results of these experiments, carried out on 5 μ mol glucose, are summarized in Table 2. Calculated from O_2 uptake and CO_2 output,

Table 2. Aerobic glucose metabolism my *Sacch. rouxii* NRRL Y-1096. 0.2 ml cells (15 mg dry wt) into 2.0 ml buffer were placed in 25 ml Warburg flask with side arms containing 5 μmol glucose (0.2 ml) and 0.2 ml 2N H_2SO_4. Flasks for measuring oxygen uptake had 0.2 ml 10% KOH in centre well with fluted Whatman filter paper.

pH level medium for		Glucose uptake (μmol)	Carbon dioxide (μmol)	Oxygen (μmol)	RQ CO_2/O_2	Glucose assimilator (%)
Growth	Reaction					
4.5	4.5	5	8.17 ±0.20	7.82 ±0.19	1.04 ±0.02	72.77 ±0.68
4.5	7.0	5	7.82 ±0.15	7.42 ±0.17	1.05 ±0.04	73.93 ±0.59
7.0	4.5	5	6.29 ±0.13	5.96 ±0.11	1.05 ±0.03	76.93 ±0.61
7.0	7.0	5	6.80 ±0.16	6.63 ±0.15	1.02 ±0.03	77.33 ±0.70

Results are from three experiments performed in duplicate and expressed as mean ± s.e.mean.

the respiratory quotient was found to be unity in all cases. There was also an absence of metabolic products (such as acids, alcohol, esters) in detectable quantities in the reaction medium at the end of the experiments. In subsequent bulk experiments, glycerol was found to be produced in small quantities.

Table 2 shows that cells, grown at pH 7.0 and starved overnight, assimilated a greater amount of glucose than cells which had been grown at pH 4.5. The process of assimilation appears to be independent of the pH at which glucose is metabolized.

Discussion

The pH at which the yeast cells were grown, as well as the pH at which glucose was fermented, were found to affect the balance of the fermentation products. This is not surprising as enzyme activity is known to be affected by pH and, in this case, physiological adaptation was probably also involved. When the cells fermented glucose at the acid pH of 4.5, only ethanol and CO_2 were produced, with traces of acid, if the cells had been grown at neutral pH (proposed schemes Types 1 & 3). Acids were produced in larger quantities by the yeast cells fermenting glucose at neutral pH irrespective of whether they had been grown at the acid or neutral pH (proposed scheme Types 2 & 4). This is probably to reduce the pH of the medium to the optimum acid pH of 4.5 (Yong *et al.*, 1978). Also constant amounts of ethanol and acetic acid per mol glucose were produced by these cells.

Proposed schemes of glucose fermentation by *Sacch. rouxii* NRRL Y–1096

Type 1
Glucose fermentation at pH 4.5

$$\underset{(6\ C)}{1\ \text{Glucose}} \rightarrow \underset{(2\ C)}{2\ CO_2} + \underset{(4\ C)}{2\ CH_3CH_2OH}$$

Type 2
Glucose fermentation at pH 7.0 with cells grown at pH 4.5

$$\underset{(6\ C))}{1\ \text{Glucose}} \rightarrow \underset{(0.85\ C)}{0.425\ CH_3COOH} + \underset{(1.66\ C)}{1.66\ CO_2} + \underset{(3.52\ C)}{1.76\ CH_3CH_2OH}$$

Type 3
Glucose fermentation at pH 4.5 with cells grown at pH 7.0

$$\underset{(6\ C)}{1\ \text{Glucose}} \rightarrow \underset{(0.08\ C)}{0.085\ HCOOH} + \underset{(1.97\ C)}{1.97\ CO_2} + \underset{(4.04\ C)}{2.02\ CH_3CH_2OH}$$

Type 4
Glucose fermentation at pH 7.0 with cells grown at pH 7.0

$$\underset{(6\ C)}{1\ \text{Glucose}} \quad \underset{(1\ C)}{1\ HCOOH} + \underset{(0.9\ C)}{0.45\ CH_3COOH} + \underset{(0.72\ C)}{0.72\ CO_2} + \underset{(3.32\ C)}{1.66\ CH_3CH_2OH}$$

The acids, acetic and formic, appear to have been formed from glucose via pyruvate and CO_2 fixation in the same way as that of the bacteria *Clostridium thermoaceticum* (Li *et al.*, 1966, Thauer, 1972). In the proposed scheme Type 2, the stoichiometry of the ethanol and CO_2 produced is of the typical yeast type of glucose fermentation. The amount of acetic acid produced without any CO_2 and accounting for the rest of the glucose carbon is suggestive of acetic acid formation via pyruvate and CO_2 fixation. Similarly, the formation of formic acid with a corresponding reduction in the amount of CO_2 produced was indicative of formic acid formation by CO_2 fixation (proposed scheme Type 4). Although the acid formed in Type 3 was not identified, it is believed to be formic acid because of a decrease in the amount of CO_2 which should otherwise be equivalent to that of ethanol if acetic acid was produced. Also the stoichiometry of the reaction was more suggestive of formic acid rather than acetic acid. There was no evidence of a hydrogenlyase reaction in the fermentation of glucose by *Sacch. rouxii* NRRLY–1096 under the conditions studied.

Thus, from the results of the study of glucose fermentation by the yeast *Sacch.*

rouxii NRRL Y–1096, it would appear that if formic acid were an undesirable product in good quality soy sauce its formation could be prevented by inoculating the moromi with yeast cells previously grown at pH 4.5. By varying the pH of the moromi, it would be possible to control the acetic acid content of the soy sauce. If a sweet soy sauce is required, cells grown at pH 4.5 should be used to inoculate moromi acidified to pH 4.5 with lactic acid. If some acetic acid is desired, then the initial neutral pH of the moromi need not be acidified before inoculating the yeast which had been grown at pH 4.5. Perhaps the amount of acetic acid could even be controlled by acidifying the moromi to pH 4.5 as soon as the desired acetic acid content had been obtained. Of course it would always be possible to add artificially produced acetic acid into the final soy sauce to obtain the desired degree of tartness in the sauce. Optimum conditions for the semi-anaerobic moromi stage of soy sauce fermentation could therefore be easily achieved especially when the aerobic glucose metabolism of the yeast was not affected by the pH level at which the cells had been grown or by the pH level of the reaction medium.

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References

Conway, E.J. (1962) *Microdiffusion Analysis and Volumetric Error*, p. 248. Crosby Lockwood & Son Ltd, London.

Friedmann, T.E., (1938) *J. Biol. Chem.* **123,** 161.

Hugget, A. St. G. & Nixon, D.A. (1957) *Biochem. J.* **66,** 12P.

Li, L.F., Ljungdahl. L.G. & Wood, H.G. (1966) *J. Bacteriol.* **92,** 405.

Onishi, H. (1961) *Agr. Biol. Chem. Jap.* **25,** 341.

Thauer, R.K. (1972) *FEBS Lett.* **27,** 111.

Umbreit, W.W., Burris, R.H. & Stauffer, J.F. (1964) *Manometric Techniques*. 4th edn. Burgess Publishing Co. London.

Yong, F.M., Lee, K.H. & Wong, H.A. (1978) *J. Fd Technol.* **13,** 385.

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Quality attributes of frozen okra as influenced by processing and storage

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Summary

Sensory evaluation and chemical analyses were carried out on unblanched, steam-blanched (5 min, 100°C) and water-blanched (3 min, 98°C) okra held in frozen storage (−18°C) for 4, 8, 12 and 32 weeks. Hot water-blanched frozen okra compared favourably with fresh samples, even after 32 weeks, in colour, flavour and overall acceptability and was superior to steam-blanched and unblanched except in viscosity. Blanching, especially in steam, improved ascorbic acid retention during frozen storage. Little change in protein occurred.

Introduction

Okra (*Hibiscus esculentus* L.) is widely consumed as a vegetable in tropical and sub-tropical countries. Its stringy, gum-like consistency is particularly desirable in soups. In addition to supplying vitamins and minerals, okra is believed to be a significant source of protein in the tropics (Constantinides, 1976).

Okra is frequently preserved by canning, dehydration and freezing. Processed okra is generally believed to be inferior in quality to fresh okra. On the contrary, Olorunda & Tung (1977) observed only minor differences in the viscometric behaviour of fresh and frozen okra dispersions, and concluded that soup made from frozen okra would not be inferior in viscometric properties to that made from fresh okra.

This paper reports the effects of processing and storage on the organoleptic quality attributes of frozen okra as determined by sensory evaluation. Changes in ascorbic acid and protein levels during processing and storage were also investigated.

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Materials and methods

Freshly harvested, early maturing okra cultivar TAE 38 was obtained from the National Horticultural Research Institute, Ibadan, Nigeria. After trimming, washing and draining, duplicate samples (500 g) of randomly selected okra were blanched either 3 min in water at 98°C or 5 min in steam (100°C). The blanching treatment had previously been determined to be adequate based on the peroxidase test (Ruck, 1969). For comparison unblanched okra was also used. The samples were packed in 0.1 mm gauge polyethylene bags, heat sealed and stored at −18°C for 4, 8, 12 and 32 weeks. At the end of each storage period, the samples were removed for sensory evaluation and chemical analyses.

Sensory evaluation

Sensory tests were carried out on cooked samples by an eleven-member panel using a multiple comparison difference analysis (Larmond, 1977). A standardized cooking procedure with no condiment other than table salt was used for all samples. Samples (100 g) were chopped and placed in 200 ml boiling water. One level tablespoonful of salt was added, the mixture stirred, and allowed to cook for 5 min. The panellists rated the cooked samples for colour, flavour, viscosity and overall acceptability on a nine-point scale where 9 and 1 represented 'like extremely' and 'dislike extremely' respectively. Each panellist rated each sample twice with the field fresh sample serving as reference.

Ascorbic acid analysis

Total ascorbic acid was determined by the indophenol method (Ruck, 1969). Ascorbic acid in fresh or frozen okra samples (100 g) was extracted with 0.4% oxalic acid and the extract titrated with standardized sodium 2:6 dichlorophenolindophenol.

Protein determination

Nitrogen in 2 g duplicate samples of oven-dried okra was determined by the Kjeldahl method using copper and selenium catalysts. Protein was calculated as 6.25 × Kjeldahl N.

Results

The mean values for the multiple comparison analysis for selected quality attributes of frozen okra are shown in Table 1. For each storage duration (4, 8, 12 or 32 weeks at −18°C) okra blanched 3 min in water at 98°C was rated significantly better in colour than unblanched okra or that blanched 5 min in

Table 1. Sensory scores for selected quality attributes of frozen okra stored at −18°C

Sensory attribute	Blanching treatment	Storage duration (weeks)* 4	8	12	32
Colour	Unblanched	2.2b	3.1b	1.5c	1.9c
	Water-blanched	6.4a	6.6a	6.2a	5.6a
	Steam-blanched	2.7b	2.4b	3.5b	3.1b
Flavour	Unblanched	3.9ab	3.4b	3.0b	2.9b
	Water-blanched	4.5a	4.9a	5.2a	5.1a
	Steam-blanched	2.8b	2.1b	1.9b	3.6b
Viscosity	Unblanched	3.5a	3.8a	3.8a	5.6a
	Water-blanched	2.6b	1.2b	2.4b	3.0b
	Steam-blanched	4.2a	3.3a	4.0a	4.0b
Overall acceptability	Unblanched	2.5b	2.6b	2.8b	2.8b
	Water-blanched	4.2a	3.8a	4.7a	4.8a
	Steam-blanched	2.8b	3.6ab	3.2b	3.2b

*Means within a column for each attribute followed by the same letter are not significantly different at 5% level of probability by Tukey's test. Higher values indicate greater preference.

steam. Panellists, preferred the 'bright green' colour of water-blanched samples to the 'dull green' and sometimes 'brownish green' colour of unblanched and steam-blanched samples. For each treatment (water-blanched, steam-blanched and unblanched), the duration of storage had no appreciable effect on the colour of frozen okra.

For all storage durations, water-blanched okra consistently received better ratings for flavour than steam-blanched and unblanched samples. Except for unblanched okra held for 32 weeks at −18°C, which several panellists described as 'slightly bitter' or 'sour', steam-blanched okra was generally rated slightly inferior in flavour to unblanched okra (Table 1).

Okra blanched 3 min in water at 98°C received consistently poorer scores for viscosity than unblanched okra or that blanched 5 min in steam irrespective of the duration of storage. However, virtually all the panellists still considered the viscosity of water blanched okra acceptable. In general, steam blanched okra compared favourably in viscosity with unblanched.

The storage duration at −18°C had no appreciable effect on the overall acceptability of unblanched, steam-blanched or water-blanched okra. For each storage period, water-blanched okra was more acceptable than unblanched or steam-blanched okra (Table 1). Steam-blanched okra was rated slightly better in overall acceptability than unblanched okra.

The effect of storage at −18°C on the ascorbic acid content of unblanched, steam-blanched and water-blanched frozen okra is shown in Fig. 1. Total ascorbic acid declined in unblanched okra from approx 35 mg/100 g at harvest, to less than 20 mg/100 g after 8 weeks, and less than 5 mg/100 g after 12 weeks

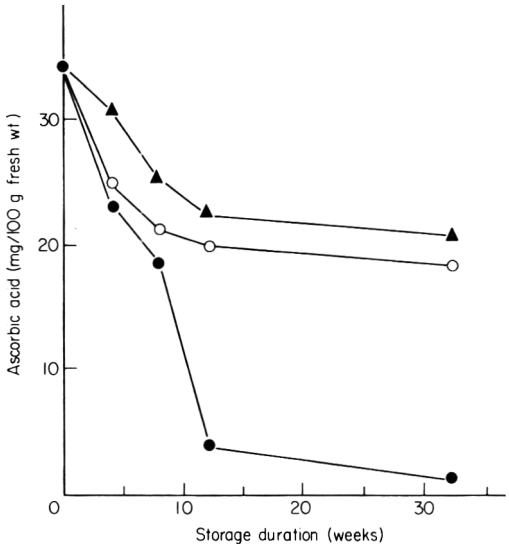

Figure 1. Effect of blanching and storage on ascorbic acid content of frozen okra held at −18°C. ▲, Steam-blanched; ○, water-blanched; ●, unblanched.

at −18°C. By 32 weeks, virtually all the ascorbic acid had been lost. In contrast, okra blanched 3 min in water at 98°C and that blanched 5 min in steam retained 54% and 63% respectively of the initial ascorbic acid content after 32 weeks at −18°C. The greatest loss in ascorbic acid occurred during the first 12 weeks of storage. For each storage period, steam-blanched okra retained more ascorbic acid than that blanched in hot water (Fig. 1).

There was a slight decline in the protein content of unblanched okra held at −18°C for 12 and 32 weeks (Table 2). Protein remained relatively stable for up to 32 weeks in blanched samples.

Table 2. Effect of blanching and storage on crude protein (g/100 g dry wt) in frozen okra held at −18°C

Blanching treatment	At harvest	Storage duration (weeks)*			
		4	8	12	32
	15.52±0.17				
Unblanched		14.42±0.05	14.32±0.05	12.64±1.09	11.58±1.51
Water-blanched		14.70±0.12	14.68±0.13	14.53±0.98	14.37±0.42
Steam-blanched		14.96±0.49	14.97±0.09	14.25±0.24	13.50±1.32

*Values shown represent the mean and standard deviations of duplicate samples.

Discussion

Okra in the fresh state deteriorates rapidly at warm temperatures because of its high respiration rate and cannot be stored at low temperatures, below 7°C, for any appreciable length of time, because of its susceptibility to chilling injury (Lutz & Hardenburg, 1968). The results obtained from this study indicate that soup made from hot water-blanched (98°C for 3 min) frozen okra, even after 32 weeks in frozen storage (−18°C), would not be inferior in colour, flavour and overall acceptability to that made from fresh okra.

Hot water blanching caused a reduction in viscosity of frozen okra presumably as a result of the leaching of mucilaginous substances. However, this reduction in viscosity was not sufficiently severe to markedly reduce the overall acceptability of the product relative to fresh okra. This is in agreement with previous work by Olorunda & Tung (1977) who observed only minor differences in the viscometric behaviour of fresh and frozen okra dispersions, and concluded that soup made from frozen okra would not be inferior in viscometric properties to that made from fresh okra. Steam blanching improved viscosity but resulted in poorer flavour, presumably because of loss of volatile flavour compounds, and colour.

The large loss of ascorbic acid (up to 97% after 32 weeks at −18°C) in unblanched okra is presumably largely due to ascorbic acid oxidase activity during frozen storage (Hatzler & Guerrant, 1952). Blanching significantly improved ascorbic acid retention in frozen okra, especially steam blanching, in which case leaching losses are minimized (Noble & Gordon, 1964; Cain, 1967). Proteolytic enzyme activity in frozen storage may also explain the small loss in protein observed in unblanched okra after 12 and 32 weeks at −18°C.

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References

Cain, R.F. (1967) *Fd Technol.* **21,** 998.

Constantinides, S. (1976) *Time Magazine* (Can. edn), May 31 issue.

Hartzler, E.P. & Guerrant, N.B. (1952) *Fd Res.* **17,** 15.

Larmond, E. (1977) *Laboratory Methods for Sensory Evaluation of Food* Pub. 1637, Canada Dept. of Agric., Ottawa.

Lutz, J.M. & Hardenburg, R.E. (1968) *The Commercial Storage of Fruits, Vegetables and Florist and Nursery Stocks*, U.S.D.A. Handbook No. 66, Washington.

Noble, I. & Gordon, J. (1964) *J. Am. Dietet. Assoc.* **44,** 120.

Olorunda, A.O. & Tung, M.A. (1977) *J. Fd Technol.* **12,** 593.

Ruck, J.A. (1969) *Chemical Methods for Analysis of Fruit and Vegetable Products*. SP 50, Summerland Research Station, Canada Dept. of Agric.

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Effects of processing on the thiamin, riboflavin and protein contents of cowpeas (*Vigna unguiculata* (L) Walp).

I. Soaking, cooking and wet milling processes

J. K. EDIJALA*

Summary

The effects of soaking, cooking and decortication and conversion to a paste product (moin-moin) on the thiamin, riboflavin and protein contents of six cowpea varieties were investigated. The effect of soaking was not significant but cooking resulted in considerable losses of the two B-vitamins; some of the lost vitamins, especially riboflavin, were detected in the cooking water. Decortication resulted in high losses of the vitamins for the brown varieties of cowpea. The retention of the vitamins in moin-moin was good. Changes in the protein content of the cowpea products as a result of processing were not significant.

Introduction

Cowpeas, a grain legume or pulse and commonly referred to in Nigeria as 'beans', are an important source of dietary protein. Cowpeas can contribute up to 80% of the total dietary protein intake in some parts of Nigeria, particularly the Western areas (Luse & Okwuraiwe, 1975).

The cowpea grains are prepared in a variety of ways and are consumed either singly or, more often, in combination with the chief staples such as rice, cassava products (e.g., garri), yam or plantain. The effects of the various processing treatments on the nutritional and eating quality of the final products have to-date received little attention.

This paper reports the results of an investigation into the changes in thiamin, riboflavin and protein in some cowpea products obtained after soaking, cooking and wet milling, including decortication and grinding for the preparation of the steamed paste product (moin-moin).

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Table 1. Thiamin, riboflavin and crude protein contents of the raw dry cowpea grains

Cowpea variety	Moisture* (%)	Thiamin per 100 g dwb† (mg)	Riboflavin per 100 g dwb† (mg)	Protein (N × 6.25) (%)
ACC 73001‡	14.1	0.96	0.27	26.0
ACC 70001§	14.0	0.96	0.18	27.8
ACC 68002‡	13.9	1.14	0.20	27.9
ACC 64546§	13.7	0.99	0.17	27.9
ACC 64456§	13.5	0.85	0.17	27.6
CA.X§	14.8	1.24	0.20	26.3
Mean ± s.e. mean	14.0	1.03 ± 0.06	0.20 ± 0.02	27.3 ± 0.4

†dwb = Dry weight basis.
*High moisture content due to humid storage conditions.
‡Brown cowpea variety.
§White cowpea variety.

Materials and methods

Six cowpea varieties were used for the investigation; five of the varieties, *viz*:- ACC 73001, ACC 70001, ACC 68002, ACC 64546 and ACC 64456 were obtained from the National Cereals Research Institute, P.M.B. 5042, Ibadan, Nigeria. The sixth variety, 'California blackeye' which is referred to as CA.X in this text, was purchased locally in Glasgow.

Thiamin and riboflavin contents were determined by a modified fluorimetric procedure (Edijala, 1979). Protein (N × 6.25) was determined using Tecator equipment (Tecator Ltd., Bristol) for digestion (approximately 0.2 g cowpea flour was digested with 5 ml conc. H_2SO_4 + 3.5 g K_2SO_4 and 0.1 g $CuSeO_3$ as catalyst for 30 min at 450°C) and for distillation. Moisture content was determined by drying 2–5 g cowpea flour or the mashed cowpea product for 5 h at 100°C. All the results are the average of duplicate or triplicate analyses.

Preliminary trials were carried out to establish soaking and cooking times for the individual varieties of cowpeas, i.e. the time taken to achieve no further increase in weight of the strained cowpeas after holding in cold or boiling water, respectively. The soaking times for maximum water uptake ranged from 5½ to 6½ h (mean = 6 h) and by then the dry grains had doubled their original weight. On the other hand, the cooking times ranged from 40–65 min (mean = 52 min) and by then the weight of the grains had increased by a mean factor of 2.2, i.e. more than doubled their original weight.

Soaked grains

The dry cowpea grains, about 20 g, were soaked for their required soaking time in 100 ml tap water at room temperature. The soaked grains were drained

Table 2. Effect of soaking at room temperature on thiamin and riboflavin contents of cowpeas

Cowpea variety	Soaking time (min)	Moisture (%)	Thiamin per 100g dwb* soaked grains (mg)	Thiamin change (%)	Original thiamin recovered in soaking water (%)	Riboflavin per 100g dwb* soaked grains (mg)	Riboflavin change (%)	Original riboflavin recovered in soaking water (%)
ACC 73001	330	56.2	1.10	+14.6	2.1	0.26	−3.6	14.8
ACC 70001	390	58.6	0.83	−13.5	2.1	0.15	−16.7	5.6
ACC 68002	330	55.9	1.07	−7.0	2.6	0.16	−20.0	25.0
ACC 64546	360	56.0	1.00	+1.0	1.0	0.18	+5.9	11.8
ACC 64456	360	54.0	0.82	−3.5	5.9	0.15	+17.6	11.8
CA.X	390	60.0	1.32	+6.5	1.6	0.21	−5.0	15.0
Mean ± s.e. mean	360	56.8	1.02 ± 0.08		2.6	0.18 ± 0.02		14.0

*dwb = Dry weight basis

Table 3. Effect of cooking at atmospheric pressure on the thiamin and riboflavin contents of cowpeas

Cowpea variety	Cooking time (min)	Moisture (%)	Thiamin per 100g dwb* cooked grains (mg)	Thiamin loss (%)	Original thiamin recovered in cooking water (%)	Riboflavin per 100g dwb* cooked grains (mg)	Riboflavin loss (%)	Original riboflavin recovered in cooking water (%)
ACC 73001	40	65.8	0.56	41.7	28.1	0.14	48.1	37.0
ACC 70001	55	62.7	0.42	56.3	17.7	0.11	38.9	38.9
ACC 68002	50	60.0	0.39	66.1	29.6	0.11	45.0	65.0
ACC 64546	55	63.3	0.42	57.6	14.1	0.10	41.2	41.2
ACC 64456	65	64.9	0.27	68.2	27.1	0.08	52.9	52.9
CA.X	45	63.9	0.61	50.8	27.4	0.11	45.0	55.0
Mean ± s.e. mean	52	63.4	0.45 ± 0.05	56.8	24.0	0.11 ± 0.01	45.2	48.3

*dwb = Dry weight basis

and mashed in a mortar with pestle. The cowpea mash, 2–3 g, was used for the vitamin determination.

The soaking water, 70–75 ml and at pH 6.3–6.5, was adjusted to pH 4.5 with 1N HCl and made up to 100 ml with pH 4.5 2N acetate buffer solution. To a 50 ml aliquot, 5 ml 2% aqueous enzyme (Clarase – MKC Enzymes, Miles Laboratories, Slough) solution was added, incubated for 20 h and used for the vitamin determinations.

Cooked unsoaked grains

The dry grains, about 20 g, were added to boiling water, 400–500 ml depending on variety, in a cooking pot of about 700 ml capacity and heated for their required cooking time. The drained cooked 'beans' were mashed and 5–6 g of the mash was used for the vitamin determinations.

The cooking water, 10–40 ml and at pH 6.0–6.2, was adjusted to pH 4.5 with 1N HCl and made up to 100 ml. To a 25 ml aliquot, 25 ml pH 4.5 2N acetate buffer solution was added and treated in the same way as the soaking water above.

Cooked pre-soaked grains

The dry grains, about 20 g, were soaked for 6 h (the mean soaking time for the six cowpea varieties) and cooked by boiling for 40 min. This time was chosen for all the varieties as it has been a common belief that pre-soaking significantly reduced cooking time. The cooked grains and cooking water were treated as above for the vitamin determinations.

Wet milling

The dry grains, about 150 g, were soaked in five times their own weight of water for 6 h. The testa of the soaked grains were removed. A sample (20 g) of the washed decorticated grains was mashed and 1.5–2.0 g mash was used for the vitamin determinations.

For the preparation of moin-moin about 100 g decorticated grains with both intact and split cotyledons were milled in a blender with an equal volume of water to produce a smooth and moderately thick slurry. The homogenate was mixed with the 25–30 ml water used to rinse the blending cup and distributed into 100 ml beakers which were then covered tightly with aluminium foil. The beakers were immersed (but not submerged) in boiling water in a cooking pot for 40 min. The beakers were then removed and allowed to cool; the contents formed a semi-solid paste and 5–6 g samples were taken for vitamin determinations. The cowpea to water ratio, W/V, which produced moin-moin of good consistency for the six cowpea varieties was approximately 1:1.3.

Results and Discussion

Thiamin, riboflavin and protein contents of the raw dry grains were established and the evaluation of the effects of the processing methods was based on the original contents of these nutrients (Table 1).

The mean thiamin and riboflavin contents of the dry grains, 1.03 mg and 0.20 mg/100 g dry weight, respectively, agreed with values reported by Ogunmodede & Oyenuga (1969) and Watt & Merril (1975). These vitamins appear to be present in cowpea grains in an approximate ratio of 5:1.

The crude protein content of the individual varieties was not significantly different from the mean value of 27.3% (Table 1) but was slightly higher than previously reported values of 25% (Oyenuga, 1968; Edijala, 1976).

Effect of soaking

Soaking for 5½ to 6½ h, depending on variety, did not significantly affect the thiamin and riboflavin contents of the cowpea grains. For some varieties, there were losses in thiamin of up to 13.5% while others showed increases of up to 14.6% (Table 2). These results did not confirm the 30% thiamin loss after a 5 h soaking period reported by Ogunmodede (1972). The soaking water contained a negligible amount of thiamin (1–6%).

The pattern of riboflavin changes in the soaked grains was similar to that of thiamin. However, the soaking water contained more riboflavin than the corresponding values found for thiamin (Table 2).

Effect of cooking

Cooking by boiling the grains for their established cooking times (40–65 min) resulted in high losses of the two B-vitamins. Thiamin loss ranged from 41.7–68.2% (Table 3). Higher losses were recorded for the varieties requiring the longer cooking times, 50–65 min, indicating that the losses increased with cooking time. Thiamin recovered from the cooking water was 14–30% of the original value which implied that a large proportion of the vitamin was thermally destroyed.

Although riboflavin loss from the grains was high (38–52%), the amount recovered from the cooking water was, for most varieties, equal to the losses (Table 3). This observation therefore showed that whereas riboflavin may be lost from the grains by leaching into the cooking water, the vitamin, by virtue of its thermal stability, is not destroyed. Unlike thiamin, riboflavin loss showed no relationship with cooking time.

For both vitamins, however, the values leached into the cooking water were higher than the corresponding values found in the soaking water. It therefore follows that the losses of both vitamins, especially riboflavin, will be greatly reduced if the cooking water is consumed with the cooked grains. This is the usual practice in the consumption of 'boiled beans' (ewa) in Nigeria.

Table 4. Effect of pre-soaking on the thiamin and riboflavin contents of cooked cowpeas

Cowpea variety	Moisture (%)	Thiamin per 100g dwb* cooked pre-soaked grains (mg)	Thiamin loss (%)	Original thiamin recovered in cooking water (%)	Riboflavin per 100g dwb* cooked pre-soaked grains (mg)	Riboflavin loss (%)	Original riboflavin recovered in cooking water (%)
ACC 73001	67.7	0.43	55.2	41.7	0.13	51.9	55.9
ACC 70001	64.5	0.62	35.4	22.9	0.13	27.8	33.3
ACC 68002	62.3	0.49	57.4	37.4	0.08	60.0	65.0
ACC 64546	61.7	0.47	52.5	42.4	0.07	58.8	70.6
ACC 64456	62.0	0.43	49.4	42.3	0.09	47.1	47.1
CA.X	65.5	0.62	50.8	24.2	0.10	50.0	40.0
Mean ± s.e. mean	64.0	0.51 ± 0.04	50.0	35.6	0.10 ± 0.01	49.3	51.9

*dwb = Dry weight basis

Table 5. Changes in the thiamin content of decorticated cowpeas and their paste product (moin-moin)

Cowpea variety	Decorticated grains			Moin-moin		
	Moisture (%)	Thiamin per 100g dwb* (mg)	Thiamin change (%)	Moisture (%)	Thiamin per 100g dwb* (mg)	Thiamin loss (%)
ACC 73001	57.2	0.84	−12.5	79.4	0.65	32.3
ACC 70001	55.0	1.22	+27.1	79.8	0.95	1.0
ACC 68002	53.2	0.96	−19.8	79.4	0.76	33.9
ACC 64546	55.3	1.09	+10.1	76.9	0.81	18.2
ACC 64456	57.1	1.01	+18.8	80.8	0.84	1.2
CA.X	58.8	1.24	0.0	80.0	1.17	5.6
Mean ± s.e. mean	56.1	1.06 ± 0.06		79.4	0.86 ± 0.07	15.4

*dwb = Dry weight basis

Effect of cooking pre-soaked grains

The effects of this treatment on the two B-vitamins were similar to those of cooking the unsoaked grains. The mean thiamin content of the cooked pre-soaked grains was 0.51 mg/100 g dry weight and the mean thiamin loss was 50% (Table 4) compared with the corresponding values of 0.45 mg/100 g and 56.8% loss obtained for the cooked unsoaked grains (Table 3). These small differences are explained by the shorter cooking time of 40 min which was chosen for all the varieties. For instance, variety ACC 73001 was over-cooked and showed an increased loss while thiamin loss in CA.X which was just well-cooked remained unchanged even though the cooking time had been reduced by 5 min. The other four varieties were under-cooked and thiamin losses were consequently reduced. This observation once again showed the relationship with thiamin loss and cooking time. It was noted, however, that the amount of leached thiamin increased which was possibly due to the presoaking treatment which may have led to enhanced leaching during the subsequent cooking.

The effect of this two-stage treatment of the cowpeas on riboflavin was similar to that for thiamin. The mean riboflavin retained in the grains, 0.10 mg/100 g dry weight, the mean 49.3% loss, and the mean 51.9% recovered from the cooking water (Table 4) were similar to the corresponding values obtained for unsoaked cooked grains (Table 3).

Effects of decortication and preparation of paste product moin-moin

The mean thiamin contents of the raw decorticated grains and of moin-moin were 1.06 mg and 0.86 mg/100 g dry weight, respectively (Table 5). In relation to the original content of the dry grains, thiamin losses of 12–20% were observed only for the two brown varieties, ACC 73001 and ACC 68002, after decortication while the white varieties showed increases of 10–27%. Losses of thiamin in moin-moin for all the varieties ranged from 1–34% (mean = 15.4%) with the highest losses (32–34%) for the brown varieties (Table 5). The results therefore showed that there is a high concentration of the vitamin in the testa of the brown varieties of cowpeas. The mean loss of 15% for moin-moin was small compared to the mean 57% loss obtained for drained cooked whole grains (Table 3). There was, therefore, a high retention of thiamin in moin-moin as losses were due only to decortication and thermal destruction; loss by leaching could not occur.

The riboflavin content of raw decorticated grains and of moin-moin ranged from 0.12–0.18 mg and the mean values, 0.15 mg/100 g dry weight, were the same for both products (Table 6). The mean losses of 24% and 25%, respectively, for decorticated grains and moin-moin were similar. Like thiamin, riboflavin losses (30–40%) were highest in the brown varieties. Compared with the 45% loss in drained cooked whole grains (Table 3), moin-moin showed good retention of riboflavin. The relatively low loss of 25% was not due to

Table 6. Changes in the riboflavin content of decorticated cowpeas and their paste product (moin-moin)

Cowpea variety	Decorticated grains†		Moin-moin†	
	Riboflavin per 100g dwb* (mg)	Riboflavin loss (%)	Riboflavin per 100g dwb* (mg)	Riboflavin loss (%)
ACC 73001	0.18	33.3	0.16	40.7
ACC 70001	0.15	16.7	0.14	22.2
ACC 68001	0.12	40.0	0.14	30.0
ACC 64546	0.14	17.6	0.12	29.4
ACC 64456	0.14	17.6	0.14	17.6
CA.X	0.16	20.0	0.18	10.0
Mean ± s.e. mean	0.15 ± 0.01	24.2	0.15 ± 0.01	25.0

*dwb = Dry weight basis

†Moisture values for decorticated grains and moin-moin as shown in Table 5.

thermal effect or leaching but due to the effect of decortication and hence the losses were similar in both products (Table 6).

Protein content (N × 6.25)

All the processing methods showed small and uniformly increased protein contents for the six cowpea varieties. The mean increases on a dry weight basis compared with the protein content of the dry raw grains (Table 1) were:-

(a) Soaked grains, 1.2%; (b) cooked unsoaked grains, 3.7%; (c) cooked pre-soaked grains, 3.6%; (d) decorticated grains, 4.0%; (e) moin-moin, 2.6%.

Soaking and/or cooking in water appear to incur the preferential leaching of soluble carbohydrates so that the protein content of the whole or decorticated cowpeas becomes slightly more concentrated.

In conclusion, it would seem that the traditional methods for the preparation of cowpeas in Nigeria, as ewa or moin-moin, provide better ways of retaining the B-vitamin content than the European practice of discarding the cooking water after boiling legumes.

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References

Edijala, J.K. (1976) *Annual Report 1975/76*, National Cereals Research Institute, Ibadan.

Edijala, J.K. (1979) *The Analyst,* **104,** 637.

Luse, R.A. & Okwuraiwe, P.E. (1975) *Proc. Grain Legume Improvement Meeting,* June, 1975, p. 97, I.I.T.A., Ibadan.

Ogunmodede, B.K. (1972) *Nigerian J. Sci.* **6,** 23.

Ogunmodede, B.K. & Oyenuga, V.A. (1969) *J. Sci. Fd Agric.* **20,** 101.

Oyenuga. V.A. (1968) *Nigeria's Foods and Feeding-Stuffs,* 3rd Edn. Ibadan Univeristy Press, Ibadan.

Watt, B.K. & Merril, A.L. (1975) *Handbook of Nutritional Contents of Foods,* U.S. Dept. of Agric., p. 28, Dover Publications, New York.

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Effects of processing on the thiamin, riboflavin and protein contents of cowpeas (*Vigna unguiculata* (L) Walp)

II. Alkali ('potash') treatment

J. K. EDIJALA*

Summary

Alkali treatment of cowpeas using 'potash' (sodium sesquicarbonate) and sodium bicarbonate caused severe losses of thiamin and riboflavin but had no significant effect on the protein content in terms of total nitrogen. The vitamin losses were not dependent on the pH but on the concentration of the alkaline solutions. The reduction in cooking time by the use of alkali was significant only at high concentrations but the resultant products were not acceptable organoleptically. As the vitamin losses were high even with low concentrations of alkali the traditional Nigerian use of 'potash' is not advocated. In addition, there is a possible latent danger to health with 'potash' treatment of cowpeas because of the possible undesirable formation of lysinoalanine.

Introduction

The poor cooking characteristic of extended cooking time is one of the major factors which tends to limit the utilization of the grain legumes (pulses) for food. Dried pulses require cooking for 40–220 min, depending on species and varieties, with cowpeas being one of the pulses with the shortest cooking time (Rockland, Zaragosa & Oracca-Tetteh, 1979; Meiners *et al.*, 1976). Cooking times of 40–65 min, depending on variety, have been found for some cowpeas grown in Nigeria (Edijala, 1980).

The cause of hardness and the attendant extended cooking times of the pulses have been attributed to:

(i) development of 'hardshell' arising from unsuitable storage conditions with temperature > 21°C, moisture content > 11%, and storage time > 6–12 months (Burr, 1975; Quast & Da Silva, 1977);

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(ii) the presence of insoluble phytates of Ca and Fe (Araullo, 1974);
(iii) the interaction of phytic acid, Ca, Mg and free pectin (Muller, 1967);
(iv) the presence of high lignin content (Muller, 1967).

In the latter cases, Muller (1967) observed that during cooking soluble Na/K phytates present in the cotyledons interact with the insoluble Ca/Mg pectates present in the testa to form soluble Na/K pectates. This process increases the permeability of the testa to hot water and hence has a softening effect. Thus, the presence of an external source of Na/K ions, which acts as a fixing agent by replacement of the Ca/Mg ions of the insoluble pectates, reduced the softening time of the pulses except for those with high lignin content.

Based on this principle, patented processes have been described (Torrey, 1974) which made use of solutions of mixed alkaline salts and chelating agents to reduce the cooking time of the pulses. Further improvement of these processes, such as subjecting the pulses to intermittent vacuum infiltration in the alkaline solutions, has produced quick-cooking grain legumes which cook in 7–20 min. The nutritional and eating qualities of the products are claimed not to be significantly different from those of the untreated pulses cooked by the conventional method (Rockland & Metzler, 1967; Rockland *et al.*, 1974; Rockland, Miller & Hahn, 1977; Rockland, Zaragosa & Oracca-Tetteh, 1979).

'Potash', as it is erroneously called in Nigeria, is a naturally occurring sesquicarbonate of sodium ($Na_2CO_3 \cdot NaHCO_3 \cdot 2H_2O$) which is commonly used, in addition to soaking for 1–24 h, to effect reduction in cooking time of the pulses. Reports on the effect of 'potash' treatment on the nutritional quality of cowpeas are scanty. It was, however, reported that cooking cowpeas with 'potash' which is alkaline in solution, but without specifying the concentration and cooking time, lead to losses of thiamin and riboflavin (Ogunmodede, 1972). This study was undertaken to determine the effects of different concentrations of 'potash' (pH = 10) on the reduction of cooking time and on the contents of these vitamins and protein in California blackeye cowpeas. Concurrent studies were also made taking sodium bicarbonate as an example of a weaker alkali (pH 8.6).

Materials and methods

Cowpea variety – California blackeye (CA.X).

'Potash' was purchased in its crude form in a Nigerian market. It was ground into a powder and dried at 100°C for 5 h. Microanalytical results showed that it contained Na^+ (31.0%), CO_3^{2-} (25.2%) and HCO_3^- (5.54%) with a HCO_3^- : CO_3^{2-} ratio of 1.5.

Sodium bicarbonate (BDH).

Concentrations of 0.01, 0.05, 0.10 and 0.50% solutions of the alkaline salts, 'potash' and sodium bicarbonate, were used in cooking the cowpeas.

The cooking time of the cowpeas grains was determined at the different concentrations of the two alkaline solutions. The grains, 20 g, were cooked in

Table 1. Some characteristics of the alkali-treated cowpea grains

Solution	Alkaline conc. (%)	Cooking time (min)	Moisture uptake (g H_2O/100 g cowpea)	pH of cooking soln.		Organoleptic properties of the cooked product
				Pre-cooking	Post-cooking	
Tap water	0	45	131.6	7.0	6.0	Softy; fluffy paste; normal pleasant taste
'Potash'	0.01	40	134.6	9.8	9.1	Soft; fluffy paste; pleasant taste
	0.05	35	142.7	10.1	9.8	Very soft; moderately fluffy paste; fairly pleasant taste
	0.10	35	142.2	10.1	9.9	Too soft; viscous paste; unpleasant taste
	0.50	30	139.5	10.1	10.1	Too soft; very viscous and slimy paste; very unpleasant taste
Sodium bicarbonate	0.01	45	134.0	8.6	9.3	Texture and taste were the same as for 'Potash' except products obtained with 0.1 and 0.5% NaHCO were less severely affected
	0.05	40	143.5	8.6	9.3	
	0.10	35	144.7	8.7	10.1	
	0.50	35	142.7	8.6	10.2	

the boiling solutions and the cooking time was measured by the maximum water uptake method (Edijala, 1980). Grain to solution ratio, w/v, was 1:20.

Vitamin and protein determinations

About 20 g dry cowpea grains were cooked for 30–45 min, depending on the required cooking time, at the different concentrations of alkali.

The cooked grains were drained and mashed. The cowpea mash, 6–8 g, was analysed for the thiamin and riboflavin contents using the fluorimetric method of Edijala (1979). The cooking solutions, 10–40 ml and pH 9–10, were adjusted to pH 4.5 with 1N HCl and made to 100 ml. To a 25 ml aliquot, 25 ml pH 4.5 2N acetate buffer solution was added, followed by 5 ml 2% aqueous enzyme (Clarase) solution. The solution was then incubated for 20 h and used for the analysis of the vitamins.

Protein (N × 6.25) was determined by the Tecator digestion and distillation system. Moisture content was determined by drying 2–5 g of the cowpea mash at 100°C for 5 h.

All the results are the average of duplicate or triplicate analyses.

Results and discussion

Effect on cooking quality

Cooking the cowpea grains in the alkaline solutions increased the rate of moisture uptake which was dependent on the concentration of alkaline. For both alkalis the time for maximum moisture uptake (cooking time) was reduced from 45 min to 30 min with increasing concentration (Table 1). At the respective cooking times with the different concentrations, the amount of moisture absorbed had increased by 2–10% over that absorbed in boiling water (Table 1). The increased water retention is believed to have been induced by a higher degree of starch gelatinization due to the alkali treatment thereby permitting more inter- and intra-molecular H-bonding. The observed reduction in cooking time agrees with the findings of LaBelle & Hackler (1975) who showed that soaking grain legumes in alkaline salt solutions at high temperature (80°C) facilitates the removal of adsorbed gas and accelerates hydration; similarly, the observation of Muller (1967) that fixing agents containing Na/K ions could lead to the formation of soluble pectates resulting in increased permeability of the testa to hot water and hence reduced cooking time was demonstrated.

The low concentrations, 0.01–0.05% solutions, of both alkalis gave products which were acceptable in terms of texture and taste but the reduction in cooking time by 5–10 min was small. The high concentrations, 0.1–0.5% solutions, gave increased reduction in cooking time of 10–15 min but the products were slimy and viscous with an unpleasant taste. These detrimental effects were more severe with the 'potash' solution than with the bicarbonate solution at corresponding concentrations (Table 1).

Table 2. Effect of alkali treatment on the thiamin content of the cooked cowpea grains

Solution	Alkaline conc. (%)	Moisture (%)	Thiamin per 100 g dwb* (mg)	Thiamin loss (%)	Original thiamin recovered in cooking soln. (%)	Thiamin totally lost by thermal/alkaline effect (%)
	Raw, Dry	14.8	1.24	–	–	–
Tap water	0	63.9	0.61	50.8	27.4	23.4
'Potash'	0.01	66.4	0.54	56.5	16.1	40.4
	0.05	69.3	0.35	71.8	1.6	70.7
	0.10	67.8	0.24	80.7	1.6	79.0
	0.50	68.3	0.08	93.6	1.6	91.9
Sodium bicarbonate	0.01	67.3	0.46	62.9	16.1	46.8
	0.05	68.8	0.35	71.8	2.4	69.4
	0.10	69.0	0.25	79.8	2.4	77.4
	0.50	68.6	0.12	90.3	1.6	88.7

*dwb = Dry weight basis

Table 3. Effect of alkali treatment on the riboflavin content of the cooked cowpea grains

Solution	Alkaline conc. (%)	Riboflavin per 100 g dwb* (mg)	Riboflavin loss (%)	Original riboflavin recovered in cooking solution (%)	Riboflavin totally lost by thermal/alkaline effect (%)
–	Raw, Dry	0.20	–	–	
Tap water	0	0.11	45.0	55.0	10.0†
'Potash'	0.01	0.08	60.0	45.0	15.0
	0.05	0.07	65.0	45.0	20.0
	0.10	0.08	60.0	30.0	30.0
	0.50	0.06	70.0	15.0	55.0
Sodium bicarbonate	0.01	0.08	60.0	55.0	5.0
	0.05	0.09	55.0	40.0	15.0
	0.10	0.08	60.0	30.0	30.0
	0.50	0.07	65.0	20.0	45.0

*dwb = Dry weight basis.

†Overall effect of boiling the variety CA.X in water was a 10% gain in riboflavin (see pt. I, Table 2).

Effect on the vitamins

Thiamin. The alkali treatment resulted in increased loss of thiamin in the cowpea grains. The losses from the grains, depending on the concentration of the alkalis, ranged between 56.5 to 93.6% and 62.9 to 90.3% for 'potash' and bicarbonate, respectively, compared to the 51% loss obtained by boiling in water (Table 2). The losses were linearly related to concentration of both additives with relatively high correlation coefficients (r) of 0.84 and 0.82, respectively, for 'potash' and bicarbonate solutions.

The values of thiamin recovered from the cooking solutions were similar (1.6–16.1%) for both additives but were very much lower than the 27.4% recovered from cooking water (Table 2). The total thiamin loss, 40–92%, due to combined thermal and alkaline effects were also similar for both additives compared to the 23.4% loss given by thermal effect alone when boiled in water (Table 2). The total losses were also linearly related to concentration of alkali with similar r values of 0.74 and 0.72, respectively, for 'potash' and bicarbonate solutions.

Riboflavin. Riboflavin losses of 55–70% from the grains were similar for both additives and were also higher than the 45% loss obtained by boiling in water (Table 3). These losses were not highly correlated to the concentration of the alkalis as shown by the relatively low r value of 0.67 for both the 'potash' and bicarbonate solutions.

The riboflavin, 15–55%, recovered from the cooking solutions was similar for both additives; the 40–55% riboflavin recovered at low concentrations was not very different from the 55% riboflavin recovered from plain cooking water (Table 3).

The total losses of 15–55% and 5–45% riboflavin showed good correlation with concentration giving r values of 0.87 and 0.84 for 'potash' and bicarbonate solutions, respectively. In this case, unlike thiamin, the total loss was due not to the combined thermal and alkaline effects but to alkaline effect alone as the overall effect of boiling in water was a small gain in riboflavin (Table 3).

For both vitamins, the results have shown that the losses were independent of the pH of the alkaline solutions above pH 8 but were related to the concentration of the alkali. This is contrary to the report that the losses are linearly related to the pH of 'potash' solution between pH 7 and 9 (Ogunmodede, 1972). Moreover, the pre-cooking and post-cooking pH of the 'potash' solutions was constant at all concentrations due to its buffering property. However, the 'potash' solution which is a stronger alkali (pH 10), had a slightly more severe effect on the vitamins than the bicarbonate solution (pH 8.6) although the pH of both alkaline solutions at all concentrations was similar by the end of the cooking period (Table 1).

Nutritionally, as a result of the observed adverse effects of the alkaline treatment on the vitamins and the marginal reduction in cooking time at low concentrations at which organoleptically acceptable products were obtained, the use of 'potash' for cooking cowpeas or other pulses is not advantageous and should be discontinued.

Table 4. Protein content (N × 6.25) of the alkali-treated cowpea grains

Solution	Alkaline conc.	Protein per 100 g dwb* (g)	Protein change (%)
–	Raw, Dry	26.3	–
Tap water	0	26.7	+1.5
'Potash'	0.01	27.1	+3.0
	0.05	26.8	+1.9
	0.10	26.5	+0.8
	0.50	26.1	−0.8
Sodium bicarbonate	0.01	27.6	+4.9
	0.05	27.2	+3.4
	0.10	26.8	+1.9
	0.50	26.7	+1.5

*dwb = Dry weight basis.

Effect on protein content

The protein content, in terms of total nitrogen, was not significantly affected by the alkali treatment. However, as with plain cooking water, slight increases in protein concentration which decreased with increasing concentration of alkali were observed (Table 4).

Although the protein, in terms of total nitrogen, was not adversely affected, it is possible that the traditional treatment of cowpeas with 'potash' could adversely affect the nutritional quality of the protein. Alkali treatment leads to reduction in protein digestibility and nutritional quality as a result of reduced availability of some essential amino acids such as cystine, histidine and, particularly, lysine. In addition, such treatment may result in the formation of lysinoalanine which is toxic to the kidneys (Chu, Pellet & Nawar, 1977; Nashef *et al.*, 1977; Struthers *et al.*, 1977; Woodard *et al.*, 1975). In view of these reports, the 'potash' treatment of cowpeas or other pulses poses a latent danger to health, especially when as much as 10% 'potash' relative to the weight of cowpea flour is used (Dolvo, Williams & Zoaka, 1976) for the preparation of Nigerian 'danwake'.

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References

Araullo, E.V. (1974) *Proc. Symp. on Interaction of Agric. with Fd Science*, pp. 131–142, Singapore.

Burr, H.K. (1975) *Nutritional Aspects of Common Beans* (Ed. by W.G. Jaffe) pp. 81–91, Archivos Latinoamericanos de Nutricion, Caracas.

Chu, N.T., Pellet, P.L. & Nawar, W.W. (1976) *J. Agric. Fd Chem.* **24,** 1084.

Dolvo, F.E., Williams, E.E. & Zoaka, L. (1976) *Cowpeas: Home Preparation and use in West Africa*, p. 41. IDRC, Ottawa.

Edijala, J.K. (1979) *The Analyst,* **104,** 637.

Edijala, J.K. (1980) *J. Fd Technol.* **15,** 435.

LaBelle, R.L. & Hackler, L.R. (1975) *Nutritional Aspects of Common Beans* (Ed. by W.G. Jaffe) pp. 217–232. Archivos Latinoamericanos de Nutricion, Caracas.

Meiners, C.R., Derise, N.L., Lan, H.C., Ritchy, S.J. & Murphy, E.W. (1976) *J. Agric. Fd Chem.* **24,** 1122.

Muller, F.M. (1967) *J. Sci. Fd Agric.* **18,** 292.

Nashef, A.S., Osuga, D.T., Lee, H.S., Ahmed, A.I., Whitaker, J.R. & Feeney, R.E. (1977) *J. Agric. Fd Chem.* **25,** 245.

Ogunmodede, B.K. (1972) *Nigerian J. Sci.* **6,** 23.

Quast, D.C. & Da Silva, S.D. (1977) *J. Fd Sci.* **42,** 370.

Rockland, L.B. & Metzler, E.A. (1967) *Fd Technol.* **21,** 26A.

Rockland, L.B., Miller, C.F. & Hahn, D.M. (1977) *J. Fd Sci.* **42,** 25.

Rockland, L.B., Zaragosa, E.M., Hahn, D.M., Booth, A.N. & Robbins, D.J. (1974) *IV Int. Congr. Fd Sci. & Technol.,* **7a,** 19. Madrid.

Rockland, L.B., Zaragosa, E.M. & Oracca-Tetteh, R. (1979) *J. Fd Sci.* **44,** 1004.

Struthers, B.J., Dahlgren, R.R. & Hopkins, D.T. (1977) *J. Nutr.* **107,** 1139.

Torrey, M. (1974) *Food Technology Review,* No. 13, (Ed. by M. Torrey) pp. 146–156. NDC, Park Ridge, New Jersey.

Woodard, J.C., Short, D.D., Alvarez, M.R. & Reyniers, J. (1975) *Protein Quality of Food and Feeds* (Ed. by M. Friedman), Vol. I, Pt. 2, pp. 595–616. Marcel Dekker, New York.

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Technical note: Energy utilization during microwave cooking

R. COLLISON AND NORMA J. BEER

Introduction

It was recently shown (Collison, 1979) that the heat absorbed by a number of different foods during cooking in an electric forced-convection oven is equal to the sum of the sensible and latent heats. The method of cooking, and the particular oven used, were selected because they were convenient for carrying out heat-balance measurements. This work has now been extended to microwave cooking using a wider variety of food commodities.

Materials and methods

Cooking methods

All the food was cooked in a Litton Menumaster (Systems 70/91) operating at a frequency of 2450 MHz. Food loads were between 200 and 300 g and cooking times between 95 and 270 seconds, depending on the amount and type of food. Details for individual foods were as follows:

Baked potatoes were cooked in their jackets. The average internal temperatures reached, varied from 90 to 97°C.

Cod fillets and pork sausages were cooked on paper plates, to average internal temperatures of 86°C, and 98°C respectively.

Egg custards were made from a mix of 1500 cm^3 milk, 150 g sugar, and 450 g of eggs, and heated in a plastic container to an average internal temperature of 98°C.

Sprouts, cabbage and cauliflower were all cooked in a plastic container with added water to an average internal temperature of 97°C, 97°C and 93°C respectively.

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0022–1163/80/0800–0455 $02.00

Table 1. Analysis of energy data (energy values in MJ per kg of initial food load.)

Commodity	Average Initial Load (g)	Electrical Input Energy	Microwave Output Energy	Sensible Heat	Latent Heat	Sensible and Latent Heat
Cod	270	2.14	0.92	0.30	0.64	0.94
Pork sausages	266	2.30	0.99	0.31	0.68	0.99
Egg custards	280	0.82	0.35	0.32	0.03	0.35
Baked potato	287	1.40	0.60	0.26	0.32	0.58
Sprouts	282 (+80g water)	1.80	0.77	0.42	0.33	0.75
Cabbage	211 (+100g water)	1.90	0.82	0.49	0.30	0.79
Cauliflower	267 (+80g water)	1.50	0.64	0.38	0.24	0.62
Potato mix (*)	244	2.70	1.16	0.32	0.87	1.19

*(All the commodities were prime cooked apart from the potato mix which was reheated.)

All the above foods were made up from fresh commodities and prime cooked. In addition, a potato mix prepared from 23 g dehydrated potato to 100 g boiling water was chilled to 4°C and subsequently reheated in the microwave oven.

Microwave output

The oven has a rated power consumption of 2.4 kW. The electrical input energy was taken as the product of rated power and cooking time divided by the food load.

The useful microwave output power was calibrated according to the method of Carter (1974). Water loads between 170 g and 300 g were heated in the oven and found to absorb between 1.00 kW and 1.02 kW; this represents an average conversion efficiency of 43%. The food loads used in the experiment all contained a water content within the range (170 g to 300 g) used in the calibration.

Sensible and latent heat

The sensible heat is the product of food load, average temperature rise, and specific heat. The latent heat is the product of the weight loss during cooking and the latent heat of water. Details of measurements and specific heat data were described previously (Collison, 1979).

Results and discussion

The energy data is shown in Table 1. The heat absorbed by paper plates or plastic containers is very small and has been ignored. Within experimental error, the microwave energy transmitted to and available for heating in the oven cavity is equal to the sum of the sensible and latent heats of the food for all commodities. This reinforces the earlier findings using an electric forced-convection oven (Collison, 1979) and it seems reasonable to suppose that this relationship will apply to all cooking methods. This makes it possible to measure the efficiency of energy utilization for any cooking process, provided reliable specific heat data are available. Conversely, it is possible to obtain specific heat data over the temperature range encountered in cooking from microwave experiments.

The energy absorbed per kg of food during microwave heating varies from 0.34 MJ for cooking egg custards to 1.14 MJ for reheating the potato mix. Variations within this range are largely attributed to differences in water evaporation during heating.

The efficiency of electrical energy utilization depends almost entirely on the efficiency of converting electricity to microwave energy. The electrical energy used for heating food in these experiments ranges from 0.39 to 2.7 MJ per kg. This compares with values of 0.39 to 2.2 MJ obtained by Taylor (1977) who used a variety of different electrical cooking methods with optimum food loads and with continuous batch cooking, conditions which favour economic energy utilization. It is unlikely that these conditions will be met in practical catering situations, whereas the efficiency in microwave cooking will apply in most practical situations.

References

Carter A.C. (1974) *Laboratory News,* **74,** 13.

Collison, R. (1979) *J. Fd Technol.* **14,** 173.

Taylor, D.G. (1977) *Catering Equipment and Systems Design* (ed by G. Glew) p.95. Applied Sciences Publishers, London.

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Technical note: Vitamin C content of some Papua New Guinean fruits

M. R. BAQAR

Introduction

Fresh fruits, unlike cocoa, copra, coffee and tea, do not play a significant role in Papua New Guinea's international and domestic economy. They are, however, often assumed to be of vital importance in improving the nutritional status of the population of Papua New Guinea by providing adequate content of vitamin C in the diet. There is as yet no report available on the vitamin C content of the fruits grown in Papua New Guinea. Use is normally made of interantional food composition tables when evaluating vitamin C intake. These tables, however, do not represent the vitamin C content of fruits grown under Papua New Guinean climatic conditions. This study examines the vitamin C content of fruits commonly grown and consumed in Papua New Guinea.

Materials and methods

Fresh avocado, banana, grapefruit, mandarine, orange and tomatoes were obtained from the Food Marketing Corporation in Lae. Five corner and paw-paw were collected from the campus gardens. Lime and pineapple were obtained from the Highlands Agricultural Experiment Station, Aiyura. Laulaus were purchased from the local fruit and vegetable market and guavas were collected from the trees growing in the Martin Luther Seminary in Lae.

All fruits were purchased or collected in the mornings and were immediately cleaned and analyzed. Vitamin C was estimated in the juice of grapefruit, lime, orange, tomato, five corner, mandarine, laulau and pineapple. Juice was extracted from the fruits by an electric juice extractor and a 10 ml aliquot was diluted to 50 ml with 3% metaphosphoric acid.

In other fruits, a representative sample (20–50 g) was blended with 150 ml 3% metaphosphoric acid and made upto 250 ml. Both juices and fruit extracts were filtered through fast filter paper. Duplicate aliquots were immediately titrated with 2, 6-dichlorophenol-indophenol until the end point persisted for 15 sec (Kefford, 1957).

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Table 1. Vitamin *C* content of some Papua New Guinean‡ Fruits.

Fruit	Botanical name	No. of samples analyzed	Vitamin C mg/100g with s.d.	Range mg/100g	Vitamin C for fruits used in tropical countries mg/100g**
Avocado	*Persea americana*	10	16±1	13.9 to 17.7	15 (5–30)
Banana	*Musa sapientum*	10	10±1	8.3 to 13.4	10 (6–30)†
Grapefruit	*Citrus paradisi*	10	48±3	42.5 to 54.0	40 (20–70)
Mandarine	*Citrus reticulata*	10	24±3	19.1 to 31.1	42 (35–80)
Sour orange	*Citrus aurantium*	10	20±11	18.0 to 29.1	42 (35–80)
Sweet orange	*Citrus sinensis*	20	61±6	52.7 to 72.2	45 (35–80)
Tomato	*Lycopersicon esculentum*	10	20±3	15.2 to 24.8	26 (8–31)
Five corner*	*Averrhoa carambola*	10	25±2	22.2 to 27.2	–
Pawpaw	*Carica papaya*	10	55±6	43.2 to 65.1	50 (18–180)
Lemon	*Citrus limon*	10	36±2	33.1 to 39.0	40 (25–60)
Pineapple	*Ananas comosus*	10	30±1	27.2 to 33.4	30 (8–165)
Laulau	*Eugenia megacarpa*	10	14±2	10.64 to 17.9	–
Guava (red)	*Psidium guajava*	20	52±9	41.0 to 66.4	200 (20–600)
Guava (white)	*Psidium guajava*	10	68±14	47.1 to 90.8	200 (20–600)

*Sour cultivar

**From Platt (1974)

†Figures in parentheses refer to range.

Results and discussion

The vitamin C content of the fruits analyzed is shown in Table 1. In some cases, a wide range of values was obtained for each fruit and the standard errors were relatively large. This is, however, not unexpected as the vitamin C content of fruits and vegetables has been found to be dependent upon type of cultivar, exposure to sunlight, stage of ripeness at harvest, and temperature of storage (Schuphan, 1965). The vitamin C values for all fruits, except guavas, compared favourably with the available food composition tables (Platt, 1974). The guavas grown in Papua New Guinea do not contain as large a quantity of vitamin C as do the Indian cultivars whose content is as high as 307 mg/100 g (Mangalam & Mudambi, 1972). Guava cultivars rich in Vitamin C should therefore be introduced in Papua New Guinea. Apart from guavas, sweet oranges and pawpaws are a good source of vitamin C. They are available at little cost almost throughout the year. Their greater use by the Papua New Guineans should be promoted.

All fruits used in this study are invariably consumed in their freshly picked state, and are grown on a limited scale. Commercial fruit production is expected to increase as a result of the wide spread introduction of other tropical fruits throughout Papua New Guinea (Charles, 1979). It is, however, unlikely that fresh fruit production will expand significantly and imports of temperate fruits, canned fruits and fruit juices will not be reduced in the foreseeable future unless an extensive research and extension programme is undertaken in Papua New Guinea.

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References

Charles, A.E. (1979) *Harvest*, **5**, 41.
Kefford, J.F. (1957) *CSIRO Fd Press. Quart.* **17**, 42.
Mangalam, N. & Mudambi, S.R. (1972) *Ind. Fd Packer*, **25**, 20.
Mudambi, S.R. & Rajagopal, M.V. (1977) *J. Fd Technol.* **12**, 189.
Platt, B.S. (1974) *Tables of Representative Values of Foods Commonly Used in Tropical Countries*. Medical Research Council Special Report Series No. 302, HMSO, London.
Schuphan, W. (1965) *Nutritional Values in Crops and Plants*. Faber and Faber, London.

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Book reviews

Food and Beverage Mycology. Ed. by Larry R. Beuchat. Westport, Connecticut: Avi, 1978 (Second printing 1979). Pp. xi + 527. ISBN 0–87055–247–3. Hardback $39.00, softback $21.50.

The majority of texts on the subject of food microbiology include sections on the fungi as something of an afterthought, and a book that brings together, under one cover, the various activities of the fungi in relation to food and drink has been long overdue. It is pleasant to record, therefore, that the hiatus has, at last, been filled by a multi-author text edited by Larry Beuchat, and it must surely be regarded as one of the outstanding contributions to the literature on food microbiology.

Thus, the editor has succeeded, not only in extracting contributions from a most able collection of authors, but has ensured also that the standard of the individual chapters is both uniform and informative. This latter point is especially relevant, because contributors to multi-author texts can easily succumb to the temptation of simply regurgitating a few platitudes from past literature, in the hope that the new format will cover the deficiencies. In the drafting of this present text, however, the trap has been studiously avoided, and each chapter offers the reader a view of the subject that is entirely fresh and authoritative.

The general tone of the book is set by the excellent introductory chapters, the first of which deals with the classification of those fungi relevant to the subject, while the second highlights the role of water activity as a major determinant of fungal growth and metabolism. The scene is thus set for a comprehensive series of treatises dealing with the behaviour of fungi as spoilage organisms of various commodities, as well as their involvement as vital agents in the production of alcoholic beverages, bread, speciality cheeses and traditional fermented foods. The book draws to a close on the rather sobering note of the biology and estimation of mycotoxins, and the resumé of fungal activity is complete.

Although each contribution is, of necessity, limited in scope, the attractive feature of this book is that essential detail has not been sacrificed. Relevant biochemical pathways are included as the narrative demands, while the employment of flow-sheets and/or diagrams enables the reader to acquire a considerable knowledge of individual topics. In addition, the references provided at the end of each chapter offer clear guidance for those seeking further information, and even the illustrations have been selected with more than usual care.

This book emerges, therefore, as a thoroughly readable and authoritative account of the importance of fungi in the food industry. The advent of the 'soft

cover' volume is also to be welcomed as a means of making the text more accessible to students, and it is certainly a book that seasoned microbiologists and newcomers alike will find a most useful addition to their bookshelves.

R. K. Robinson

Food Engineering: Principles and Selected Applications. By M. Loncin and R. L. Merson.
New York: Academic Press, 1979. Pp. xix + 494. ISBN 0–12–454550–5. £28.60.

This book is an updated and revised version of Loncin's earlier French and German edition, and makes a very welcome addition to the food engineering literature.

There are eleven chapters, varying both in length and in complexity, many appendices, an extensive bibliography (over 1300 references) and a selection of problems and their solutions. The approach is different from most other books in this subject area in that it deals with general principles as such, rather than with individual unit operations. Nevertheless reference to the unit operations can be quickly found using the index. The principles of heat, mass and momentum transfer are covered very comprehensively.

Chapter one is a brief chapter on operations of the food industry and points out that we should always be striving to optimize the quality of processed foods. Chapter two (2 pages only) deals with units and dimensions and could have well been incorporated into the symbols section. SI units are used throughout.

After a simple introduction chapters three and four plunge into 'Equations related to the transfer of mass heat and momentum, and the solution of these transfer equations'. An advanced mathematical treatment, similar to that presented in standard chemical engineering textbooks, is given. There are useful reviews on physical properties of food materials, such as diffusivity, thermal conductivity, thermal diffusivity and rheological properties. Chapter four deals with the solution of steady and unsteady state heat and mass transfer equations, momentum transfer equations and heat transfer between phases in turbulent flow.

Chapter five is a long chapter which can be broadly divided into three sections. The first deals with similarity, dimensionless groups, dimensional analysis and its application to scale-up. The second deals with momentum transfer and the theory and application of the following operations, namely, gravity settling, continuous sedimentation, centrifugation, separation, fluid flow, fixed and fluidized bed situations, filtration and agitation. The style is simple and even though little space is devoted to the description of equipment, except for centrifuges and separators, the many references will direct the reader to more detailed texts. The final section deals with heat and mass transfer

operations, both with and without phase changes. Major topics are the determination of heat film coefficients for different flow situations, the prediction of freezing and drying times, and a discussion on crystallisation. The chapter ends by mentioning transfer operations in non-Newtonian systems.

Chapter six deals with equilibrium between phases and gives a rigorous thermodynamic approach to the prediction of phase equilibrium conditions in non-ideal systems; sections are then devoted to relative volativity and distillation, and water activity.

Chapter seven, entitled the 'Evolution of driving forces', deals with the combination of equilibrium and operating conditions in sizing dryers, extractors etc. for batch, semi-batch and continuous operations. Concepts such as HTU (height of transfer unit), NTU (number of transfer units) and HETE (height equivalent to a theoretical extraction) are discussed: the chapter ends by considering the problems involved in partially miscible phases.

Chapter eight is on mechanical aspects and is more qualitative in nature; subject areas include description of particulate systems, mechanical separations, mixing, size reduction, homogenization and size enlargement.

Chapter nine, entitled "Applied Biochemical Kinetics", deals fairly briefly with enzyme kinetics and both the growth and thermal destruction of organisms. A recent method, proposed by Thijssen for calculating optimal processing conditions is outlined. The chapter ends with an analysis of residence times and dispersion of residence times in continuous processing operations.

Chapter ten deals with cleaning, disinfection and rinsing and is one of the few books offering a quantitative approach to this very important subject. The second half mentions different types of detergent and the practical achievement of good plant hygiene.

Chapter eleven is mathematical in nature and deals with optimization. It starts simply but quickly becomes fairly involved. There are sections on optimizing functions of several variables, with and without constraints, Lagrangian multipliers and the maximum principle of Pontryagin. The latter half of the chapter deals with programming methods, particularly linear programming. Worked examples are given, but I suspect that the majority of readers may by-pass this chapter because of its rather specialized nature. In my opinion this space could have been more effectively used either by explaining the mathematics found in other parts of the text or by extending the 'selected applications' part of the book. Nevertheless optimization and linear programming are now standard parts of chemical engineering courses, and their introduction in a food engineering text will serve to remind an older generation of food and chemical engineers of the changing patterns in education. This chapter may also be of interest to those involved with food economics.

There are twenty pages of appendices, tabulating physical properties of materials and mathematical functions. The bibliography is extremely comprehensive and the book ends with seventy nine selected problems and their respective solutions.

The book is not cheap; this must reduce its appeal to students. Nevertheless,

it would make a suitable textbook for chemical and biochemical engineering students; one in which the principles of transport and phase equilibrium are applied to particular problems of the food industry rather than to those of the petrochemical industry.

The book is very well presented and the approach is interesting. When dealing with mathematical aspects it resembles a conventional text book, but when dealing with food processing operations or techniques, it is written in the form of a review article. Therefore it would be very useful for the professional food scientist or technologist as it would quickly direct him to more detailed material. The text reads well but in some places I feel that relatively simple concepts have been over-complicated.

People used to conventional chemical engineering texts may find the symbols initially confusing. For example the following symbols are used: 'α' for heat film coefficient; 'α_o' for overall heat transfer coefficient; 'λ' for thermal conductivity; 'S' for the power law index; and 'b' for consistency index. It is similar in style and layout to *Food Process Engineering* (Leniger & Beveloo) and offers much more advanced treatment than most other food engineering books. Therefore it would make a very welcome addition to the food engineer's library, alongside those dealing in greater detail with equipment and applications.

M. J. Lewis

Applied Protein Chemistry. Ed. by R. A. Grant.
London: Applied Science Publishers Ltd, 1980. Pp. x + 332. ISBN 0–85334–865–0. £22.00.

This is a collection of eleven reviews on industrial protein technology, a field whose importance is steadily growing, particularly in the food sector. The topics covered are mainly commodity orientated. Four (on gelatin, blood protein, meat by-products and leather) deal with products of different parts of an animal carcase, to which are added one each on the proteins of egg, whey, leaves and soyabeans. The remaining three contributions are at a more general level, dealing with protein waste, industrial gel filtration and enzyme purification.

The publisher's claim that the topics chosen cover the main protein-based industries has some justification but there are obvious gaps, such as fish, wool and hair. Is cereal processing a protein-based industry or is the level of protein too low? Two topical areas with important implications for the future also spring to mind – single-cell protein and rapeseed protein. However, the book makes no claim to comprehensiveness.

No doubt a lack of homogeneity is inherent in the subject. The reviews range from well-established and commercially viable technology (e.g. J. H. Sharphouse on Leather), through soundly based but still emergent sectors (e.g. R.

Seal on Industrial Soya Protein Technology) to speculative operations still at the R & D level (e.g. N. W. Pirie on Leaf Protein).

In some respects the reviews are uneven. For factual data and direct value to production, development and research personnel some are outstanding but others less so. Particular attention should be drawn to those of M. T. A. Evans & J. F. Gordon (Whey Protein), P. Filstrup (Processes and Equipment for Protein By-products in the Meat Industry), R. A. M. Delaney (Industrial Gel Filtration of Proteins) and M. D. Scawen, A. Atkinson & J. Darbyshire (Large-scale Enzyme Purification). Of these, the second – an exceptionally clear practical exposition – would have been even better with a bibliography. Some of the other contributions, though useful, also suffer from incomplete referencing, brevity or occasional superficiality.

Most readers will have specialized interests which draw their attention initially to two or three of the eleven chapters. The special value of a book like this, however, is that, not only will one's knowledge of one's own subject be consolidated and brought up-to-date, but in browsing among unfamiliar topics, novel applications and ideas will stem from cross-fertilization.

The Editor is to be congratulated on having assembled a useful compilation – for technologists, students and research workers – surely something for everybody. The book is well produced and commendably free from misprints and errors. There is indeed room for a further volume exploring more of the many remaining areas of protein technology.

B. J. F. Hudson

Journal of Consumer Studies and Home Economics

CONTENTS

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

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

NON SI UNITS

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

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

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

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