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Equations for fitting water sorption isotherms of foods: Part 1 – a review

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Summary

The purpose of the present work is to present a review of literature on equations for fitting water sorption isotherms of foods and food products. Twenty-three equations, which have been proposed in the literature for correlating equilibrium moisture content in food systems, have been compiled and analysed. Their origin, range of applicability (both to type of food and water activity) and use are discussed. It is hoped that this critical compilation may be a useful guide for those researchers interested in the mathematical description of the water sorption isotherms of foods.

Introduction

Equations for fitting water sorption isotherms in foods are of special interest in many aspects of food preservation by dehydration. Among them may be mentioned the prediction of drying times (King, 1968), the prediction of the shelf life of a dried product in a packaging material (Karel *et al.*, 1971; Labuza *et al.*, 1972), or the prediction of equilibrium conditions after mixing products with various water activities (Salwin & Slawson, 1959). Besides this practical interest, the isotherm equation is also needed for evaluating the thermodynamic functions of the water sorbed in foods (Iglesias *et al.*, 1976).

The physical chemistry of surfaces has provided the food scientists with a large number of theoretical isotherms proceeding from different molecular models that can fit various experimental water sorption results. Kuntz (1975), however, noted that, 'one should be suspicious of several of the assumptions that underlie this general approach'. He suggested that the most serious diffi-

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culty is that common adsorption theories ignore changes in the substrate surface. This is true because in most water food systems conformational changes are likely to occur during sorption (McLaren & Rowen, 1952; Bettelheim & Volman, 1957; Masuzawa & Sterling, 1968). Franks (1975) also noted that the derivation of detailed information from experimental sorption data must be always subjected to the limitations imposed by the particular adsorption theory utilized. He added, however, 'that this is not to say that empirical correlations using this isotherm cannot be obtained or should not be used'. In this way, numerous mathematical equations have been reported in the literature for describing water sorption isotherms of food materials. Each of the models proposed, empirical, semi-empirical or theoretical have had some success in reproducing equilibrium moisture content data of a given type of food and in a given range of water activity. It has to be mentioned, however, that the agreement of the experimental sorption data with the isotherm equation calculated for a particular sorption model does not provide a proof of the correctness of the theory, since, as it has been frequently observed, a curve may be described by many different equations.

The difficulty of having a unique mathematical model, either theoretical or empirical for describing accurately the sorption isotherm in the whole (or most of it) range of water activity and for different type of foods, is due to a number of reasons. The main ones are: (1) the depression of water activity in foods is due to a combination of factors each of which may be predominant in a given range of water activity (Karel, 1973), (2) moisture sorption isotherms of foods represent the integrated hygroscopic properties of numerous constituents whose sorption properties may change as a consequence of physical and/or chemical interactions induced by heating or other pre-treatments (Iglesias & Chirife 1976a, b), and (3) as a food sorbs water it usually undergoes changes of constitution, dimensions and other properties (McLaren & Rowen, 1952); water sorption also leads to phase transformations of the sugars contained in the food (Karel, 1973; Iglesias *et al.*, 1975a).

The purpose of the present work is to present a review of the literature on equations for fitting water sorption isotherms of foods. The origin, range of applicability (both to type of food and water activity) and uses of the compiled equations are discussed. It is hoped that this critical compilation may be of value for those researchers interested in the mathematical description of the water sorption isotherms of foods.

The following notation will be used throughout this work:

$A_w = p/p_0$ = water activity,
p = water vapour pressure exerted by the foods,
p_0 = vapour pressure of pure water at a given temperature,
M = equilibrium moisture content, usually dry basis,
T = temperature.

The isotherm equations are presented and discussed below in alphabetic order.

List of isotherm equations

(1) *The B.E.T. equation* (*Brunauer* et al., 1938)

The B.E.T. equation is:

$$\frac{A_w}{(1-A_w)M} = \frac{1}{M_m C} + \frac{A_w(C-1)}{M_m C} \tag{1}$$

where M_m = monolayer moisture content, C = constant related to the net heat of sorption. Equation (1) is probably the most popular food isotherm equation. The B.E.T. equation usually holds only between water activities from about 0.05 to 0.45, but this gives enough data so that the parameters M_m and C can be calculated (Labuza, 1968). Although the B.E.T. analysis is based on over-simplified assumptions (Labuza, 1968; Le Maguer, 1972) – certainly not expected to hold for water sorption in foods – the monolayer concept is useful because of its relationship with several aspects of the physical and chemical deterioration in dehydrated foods (Iglesias & Chirife, 1976a). In addition, the B.E.T. monolayer calculation is an effective method for estimating the amount of water bound to specific polar sites in dehydrated food systems (McLaren & Rowen, 1952; Duckworth & Smith, 1963). Recently, Iglesias & Chirife (1976a) reported 300 monolayer values corresponding to almost 100 different foods and food components. They showed that in all cases examined the monolayer values decreased significantly with increasing temperature. These authors also discussed the validity of the determination of B.E.T. monolayers for sorption isotherms having a low value of the C constant (the so-called type III isotherms), which usually correspond to high-sugar foods.

Although in the original B.E.T. theory (Labuza, 1968) the C term is related to the net heat of sorption for the first layer, Iglesias & Chirife (1976c) and Chirife *et al.* (1977) – after examining a wide number of food systems – disregarded the use of the B.E.T. equation to estimate the heat of water sorption in foods. Iglesias *et al.* (1977a) discussed the statistical procedure to be used for the evaluation of parameters M_m and C from the well known linear form of the B.E.T. equation (eqn (1)) and from a rearranged one proposed by Caurie *et al.* (1976), which is:

$$\frac{1}{(1-A_w)M} = \frac{1}{M_m} + \frac{1}{C M_m}\frac{(1-A_w)}{A_w}. \tag{2}$$

Iglesias *et al.* (1977a) showed that conventional (or unweighted) least squares should not be used in the last case (eqn (2)) in order to evaluate the parameters M_m and C.

(2) *The B.E.T. modified equation* (*Brunauer*, 1945)

This modification of the B.E.T. equation proposed that the radius of the capillary defines the upper limit for the number of layers of water that can be

built up within the capillary. The B.E.T. modified equation is,

$$M = \left[\frac{M_m C A_w}{1 - A_w}\right]\left[\frac{1 - (n+1) A_w^n + n A_w^{n+1}}{1 + (C-1) A_w - C A_w^{n+1}}\right] \quad (3)$$

where n = number of layers. Bushuk & Winkler (1957) found that a reasonably good representation of the wheat flour isotherm up to 0.80 water activity may be obtained in terms of eqn (3) if the number of sorbed layers (n) at saturation pressure was assumed to be five or six. Despite any theoretical significance, the improved fitting ability of eqn (3) over eqn (1) may be largely due to the suitable choice of a third parameter (n).

(3) *The Bradley equation*

In his theory, Bradley (1936) assumed that the sorptive surface is of polar nature. The first layer is sorbed because of strongly induced dipoles and these dipoles in turn polarize the second layer which in turn polarizes the third layer, etc.

In Bradley's equation,

$$\ln (1/A_w) = K_2 K_1^M \quad (4)$$

K_2 is a function of the sorptive polar groups and K_1 is a function of the dipole moment of the sorbed vapour. In addition, K_1 includes a term which is characteristic of the sorbed molecules on the sorptive sites. Hoover & Mellon (1950) found that their data of the sorption of water in proteins in the range of water activity 0.05 to 0.95 were fitted very well by the Bradley equation. Both constants, K_2 and K_1 were found to be temperature dependent. Ling (1965) applied the Bradley equation to fibrous proteins and Ling & Nagendank (1970) showed that the behaviour of water in frog muscle followed the Bradley multilayer adsorption equation. Equation (4) may be readily transformed into,

$$\ln \ln (1/A_w) = \ln K_2 + M \ln K_1 \quad (5)$$

which is a convenient form for testing, because a linear relationship should be obtained when plotting, $\ln \ln (1/A_w)$ versus M. Walker *et al.* (1973) reported that the Bradley equation fitted their adsorption data on myosin A and myosin B (two major muscle proteins) up to a water activity of 0.80. At higher water activities the plots showed deviations from the linearity suggested by eqn (5). Unfortunately, because of the ln ln transformation the apparent linearity of the plots reported by Walker *et al.* (1973) is not in itself sufficient to provide information on the goodness of fit of Bradley's equation as applied to their experimental data. A comparison between the experimental and calculated equilibrium moisture content isotherms would be more useful in this situation. Hansen (1976) reported Bradley isotherm plots (according to eqn (5)) of water sorption in ovalbumin and soy protein concentrates; the plots deviated from linearity above water activities of 0.70 to 0.85. Walker *et al.* (1973), Hansen

(1976) did not report quantitative information on the goodness of fit of Bradley's equation as applied to the experimental data.

(4) *The Caurie equation*

Caurie (1970), proposed the following equation based on purely mathematical manipulation,

$$\ln C = \ln A - r A_w \tag{6}$$

where r and A are constants and C = water concentration = 100 – % water/% water. According to Caurie (1970) eqn (6) is valid from zero water activity up to 0.85 water activity for most foods. In order to illustrate the application of his model, he plotted literature sorption data on gelatin and orange crystals according to eqn (6). Although negatively sloping straight lines were obtained, Caurie (1970) did not report any statistical parameter to illustrate the goodness of fit of his equation, nor did he show a comparison between the experimental and calculated moisture sorption isotherms. Instead, the simple examination of the straight lines reported suggests that the fit may be not as good as it was claimed.

(5) *The Chen equation*

The model developed by Chen (1971) is linked to the theory of drying. His analysis is based on the steady-state of the drying equation and is limited to situations where diffusion is the principal mode of mass transport. The equation developed is,

$$A_w = \exp [k + a \cdot \exp (bM)] \tag{7}$$

where k, a and b are constants. Chen (1971) tested his equation with experimental water sorption data on barley and sorghum and obtained good agreement between the observed and calculated results for most of the water activity range. The parameters, k, a and b were found to be temperature dependent. Chen & Clayton (1971) applied Chen's equation to various materials including, corn flaxseed, oats, rice, rye and wheat and reported quantitative information on the goodness of fit for the equation. A reasonably good fit was observed in a number of cases. The merits of this equation for describing equilibrium moisture contents should be judged, however, considering that it has three parameters which need to be determined for each material.

(6) *The Chen's modified equation*

In applying the three-parameters Chen equation (Chen, 1971),

$$A_w = \exp [k + a \cdot \exp (bM)]$$

to a number of materials, Chen & Clayton (1971) found that the values of constant k were very close to unity, so Chen's equation may be simplified and reduced to a two-parameter equation as follows,

$$A_w = \exp[-a \cdot \exp(-bM)]. \quad (8)$$

Chen & Clayton (1971) applied this equation to cereal grains and other field crops and evaluated the goodness of fit of the equation. They found that for the seventeen materials (including varieties) studied, the three-parameters Chen equation (Chen, 1971) had a better fit than the simplified two-parameter equation. The latter, however, was found to have an improved fit over Henderson's equation (Henderson, 1952).

A fact which apparently has not been noted in the literature is that Chen's simplified equation is mathematically equivalent to that by Bradley (Bradley, 1936). Bradley's equation may be written,

$$\ln(-\ln A_w) = \ln K_2 + M \ln K_1.$$

The simplified Chen's equation may be written as,

$$\ln(-\ln A_w) = \ln a - bM. \quad (9)$$

It is obvious that both equations are equivalent.

It is worth mentioning that Chen's equation and Chen's modified one were mostly tested with experimental sorption data in cereal grains and other field crops.

(7) *The Chen and Clayton equation*

In an attempt to predict the temperature dependence of the isotherm, Chen & Clayton (1971) empirically modified Chen's equation (Chen, 1971) and proposed a four-parameter equation for relating temperature, water activity and moisture content. The proposed equation is:

$$A_w = \exp[-k_1 T^{m_1} \cdot \exp(-k_2 T^{m_2} M)] \quad (10)$$

where k_1, k_2, m_1 and m_2 are constants. Chen & Clayton (1971) found that the proposed equation described adequately the temperature dependency of corn isotherms between 4.4 and 60° C. The equation was also shown to fit the experimental data better than the Day & Nelson's (1965) four-parameter equation; this last one is also used for predicting the temperature dependence of the isotherm and is discussed elsewhere in this paper.

(8) *The Chung and Pfost equation*

Chung & Pfost (1967) proposed a model of the form,

$$\ln A_w = -\frac{a}{RT} \exp(-bM) \quad (11)$$

where a and b are constants. This model is based directly upon an assumption about the way in which the free energy change for sorption is related to moisture content. The equation cannot be used to predict the effect of temperature since the use of the T term does not eliminate the temperature dependence of parameters a and b (Agrawal *et al.*, 1969; Nellist & Hughes, 1973). By removing the term RT eqn (11) becomes:

$$\ln A_w = -a' \exp(-bM) \tag{12}$$

which is identical to the Chen's modified equation, and hence mathematically equivalent to the older Bradley's equation, as was shown before. Recently, Young (1976) evaluated different mathematical models, including the Chung–Pfost one, to describe sorption and desorption isotherms of Virginia-type peanuts. He found that the Smith (1947) and Young & Nelson (1976a) equations (discussed elsewhere in this paper) were able to fit the experimental data better than the Chung and Pfost equation.

(9) *The Day and Nelson equation*

Henderson's equation (Henderson, 1952, discussed elsewhere in this paper) is:

$$1 - A_w = \exp(-gTM^n)$$

where g and n are constants. Day & Nelson (1965) observed that the T term in Henderson's equation does not eliminate the temperature dependence of constants g and n. They omitted the term T and related constants g and n to an empirical power function of temperature. The proposed four-parameters equation is:

$$1 - A_w = \exp(-j_1 T^{h_1} \cdot M^{j_2 T^{h_2}}) \tag{13}$$

where j_1, j_2, h_1 and h_2 are constants.

As discussed previously, Chen & Clayton (1971) tested the applicability of this equation to describe the temperature dependence of corn desorption isotherms in a wide temperature range.

(10) *The Hailwood and Horrobin equation*

In an attempt to interpret the water adsorption isotherms of proteins, Hailwood & Horrobin (1946) assumed a simplified model of two kinds of adsorption sites in the polymer. The first one is a hydrate formation on certain polar groups and the second one is a solid solution of water in the polymer. Consequently, they proposed a three parameter equation which in order to

facilitate computation may be put in the form:

$$A + B A_w - C A_w^2 = A_w/M \tag{14}$$

where A, B and C are constants.

Hailwood & Horrobin (1946) obtained good agreement between calculated and observed values for water adsorption in wool, nylon and silk through an extended range of water activity (about 0.10 to 1.0 water activity). McLaren & Rowen (1952) criticized the theoretical assumptions on which the Hailwood and Horrobin equation was developed and suggested that the agreement between experimental and calculated values must be largely due to a suitable choice of the three parameters A, B and C. Bettelheim & Volman (1957) applied the Hailwood–Horrobin equation to water sorption isotherms of pectic substances up to about 0.30 water activity.

(11) *The Halsey equation*

Halsey (1948) developed the following equation to provide an expression for condensation of multilayers at a relatively large distance from the surface,

$$A_w = \exp(-a/RT\theta^r) \tag{15}$$

where a and r are parameters and $\theta = M/M_m$. Equation (15) was developed by Halsey (1948) on theoretical ground as a criticism of the B.E.T. theory. Halsey assumed that the potential energy of a molecule varies as the inverse rth power of its distance from the surface. He also stated that the magnitude of the parameter r characterizes the type of interaction between the vapour and the solid. If r is large, the attraction of the solid for the vapour is very specific and does not extend far from the surface; when r is smaller the forces are more typical van der Waals and are able to act at a greater distance. As this equation was shown by Halsey to be a good representation of adsorption data that conform to the B.E.T. type I, II, or III shapes (Gregg & Sing, 1967), Iglesias *et al.* (1975b) and Iglesias & Chirife (1976d) applied it to a wide variety of food isotherms. It was observed earlier by Iglesias *et al.* (1975b) that the use of the RT term does not eliminate the temperature dependence of constants a and r. Consequently, they simplified the original Halsey's equation to the form:

$$A_w = \exp(-a'/\theta^r) \tag{16}$$

where a′ is a constant.

Iglesias & Chirife (1976b) further simplified eqn (16) to the form,

$$A_w = \exp(-a''/M^r) \tag{17}$$

where a″ is a constant. Iglesias *et al.* (1975b) and Iglesias & Chirife (1976d) found that Halsey's equation could be used to describe reasonably well the sorption behaviour of sixty-nine different food materials in the approximate range

of water activity 0.10 to 0.80. The total number of experimental isotherms to which they applied satisfactorily the Halsey's model amounted to 220.

(12) *The Harkins-Jura equation*

Harkins & Jura (1944) proposed an equation for the adsorption isotherm the validity of which is restricted to regions in which the adsorbed molecules form a condensed film. The relationship may be expressed in the form:

$$\ln A_w = B - A/M^2 \tag{18}$$

where A and B are constants. Plotting $\ln A_w$ versus M^{-2} should give a straight line and from the value of A (slope of the straight line) the monolayer moisture can be calculated (Labuza, 1968; Labuza, 1975). Labuza (1968) suggested that the approximation of this equation is not very good for most food materials, and usually it does not hold above a water activity of 0.40 to 0.50.

(13) *The Haynes equation*

Haynes (1961) gave an empirical equation to describe the sorption isotherm of biological materials. Haynes' equation is:

$$\ln p = (a + bM) \ln p_0 + (c + dM + gM^2) \tag{19}$$

where a, b, c, d and g are constants. This equation may have some merit for fitting purposes, but it requires five constants to be evaluated for each material.

(14) *The Henderson equation*

One of the most widely used models relating water activity and amount of water sorbed is Henderson's equation, (Henderson, 1952). This empirical equation may be written as,

$$1 - A_w = \exp - (kM^n) \tag{20}$$

where k and n are constants.

Equation (20) may be written,

$$\ln [-\ln (1 - A_w)] = n \ln M + \ln k \tag{21}$$

so a plot of $\ln(-\ln(1 - A_w))$ versus amount sorbed should give a straight line. However, Rockland (1969) observed that two or three 'localized isotherms' may be distinguished when the experimental sorption data are plotted according to eqn (21). This observation led Rockland (1969) to suggest that moisture sorption isotherms are composed of generally three 'localized' isotherms and that each of them may represent a special type of water binding. After analysing a very wide number of food isotherms, Iglesias & Chirife (1976e) confirmed

Rockland's (1969) observation in the sense that application of eqn (21) can be used to characterize (in most cases) three 'local' isotherms. However, in disagreement with Rockland (1969), Iglesias & Chirife (1976e) conclude that local isotherms cannot be used to give a precise and unequivocal definition of the physical state of water in foods.

It has to be admitted that, for fitting purposes, the utility of Henderson's equation would be severely restricted if two or more pairs of constants were needed to define the sorption isotherm. Nevertheless, Iglesias & Chirife (1976d) found that in many cases the experimental sorption data may be fitted by eqn (21), and although this may result in a loss of accuracy, the error introduced in this way may be in several cases small enough for fitting purposes.

These authors made a comparison between Henderson's and Halsey's equations. Literature data for 220 food isotherms comprising sixty-nine different materials were utilized to compare both equations. They found that in 70.4% of the isotherms examined Halsey's equation gave a better fit than Henderson's one, while in 21.9% of the cases the reverse was true. For 7.7% of the cases both equations gave a similar fit. Other authors who have applied Henderson's equation to various food materials, include Lafuente & Piñaga (1966), Agrawal *et al.* (1969), Chen & Clayton (1971) and Young (1976).

Singh & Ojha (1974) applied the Henderson equation to desorption equilibrium moisture curves of groundnut and chillies at various temperatures. They measured equilibrium moisture contents at only three relative humidities (in a narrow range) and used these values for evaluating the constants in Henderson's equation. These constants were then used for extrapolating the limited data up to the saturation pressure level, or to the low pressure one. This procedure is not valid because as it was previously discussed, the value of the Henderson's parameters depends on the region of the isotherm used for the evaluation. Singh & Ojha (1974) used Henderson's original formulation which included a temperature term,

$$1 - A_w = \exp(-c\,TM^n) \tag{22}$$

and evaluated the constants c and n for the experimental data at different temperatures. As expected, their results showed that the use of the T term does not eliminate the temperature dependence of parameters c and n. Surprisingly, Singh & Ojha (1974) conclude that Henderson's equation 'correctly describes the temperature dependency of the experimental isotherms', when the reverse was observed.

(15) *The Iglesias and Chirife equation*

Iglesias & Chirife (1976f) noted that the analysis of sorption phenomena in high-sugar foods, like most fruits, is complicated by the dissolving of sugars and by this reason the theoretical prediction of the isotherm is difficult. Accordingly, they proposed an empirical equation for describing the water sorption

behaviour of various fruits and related high-sugar items. The proposed equation may be written,

$$\ln (M + \sqrt{M^2 + M_{0.5}}) = b A_w + p \tag{23}$$

where $M_{0.5}$ is the moisture content at $A_w = 0.5$ and b and p are constants. Iglesias & Chirife (1976f) found that the proposed equation described adequately equilibrium moisture contents for seventeen isotherms comprising nine different high-sugar foods. Among the foods tested were, banana, grapefruit, peach, pear, pineapple and strawberry.

(16) *The Halsey's modified equation*

In order to develop a simple equation for describing the temperature dependence of the isotherm, Iglesias & Chirife (1976g) empirically modified Halsey's equation (discussed previously in this work). They proposed a three-parameter equation,

$$A_w = \exp [-\exp (bT + c) M^{-r}] \tag{24}$$

where b, c and r are constants, and found that it may be used to predict reasonably well the effect of temperature on water sorption isotherms of some food materials. Among the foods tested were, chicken, corn, fish protein concentrate, laurel, nutmeg, thyme and wheat flour. Iglesias & Chirife (1976g) stated that the merits of eqn (24) should be judged considering that it is simpler than the usual four-parameter equations reported in the literature to characterize the effect of temperature on the isotherms (see Day & Nelson (1965), and Chen & Clayton (1971)).

(17) *The Kuhn equation*

Quast & Karel (1972) used the following equation for correlating equilibrium moisture contents in potato chips up to about 0.32 water activity,

$$M = \frac{a}{\ln A_w} + b \tag{25}$$

where a and b are constants. Equation (25) is based on Kuhn's (1967) theoretical analysis of adsorption phenomena, who developed a theory which contains several determining features of all adsorption processes.

Labuza *et al.* (1972) also used eqn (25) for describing equilibrium moisture contents in dry milk (up to about 0.5 water activity) but reported some inconsistency of the Kuhn equation at low water activity.

(18) *The linear equation*

In view of the particular shape of most food isotherms it is obvious that the simple expression,

$$M = a + b A_w \tag{26}$$

where a and b are constants, may be of value only for describing a given portion of the isotherm. However, this may be enough for some practical situations like storage life predictions of packaged dried foods, where often only a relatively small portion of the isotherm needs to be considered. In their packaging predictions studies, Labuza *et al.* (1972) indicated that the straight line adequately described the lower portion of tea isotherm (up to about 0.55 water activity), and Mizrahi *et al.* (1970) showed that a straight line may be used for describing the sorption isotherm of cabbage between about 0.07 to 0.25 water activity. Becker & Sallans (1956) showed that a linear equation may be used to adequately describe the desorption isotherm of wheat between 0.12 to 0.65 water activity. Iglesias *et al.* (1977b) tried to describe the lower portion of air dried beef isotherm (between about 0.10 to 0.45 water activity) but the results were not entirely satisfactory.

(19) *The Mizrahi equation*

This equation,

$$A_w = \frac{a + M}{b + M} \tag{27}$$

where, a and b are constants, was found by statistical analysis of the sorption isotherm data to fit a two parameter equation. The equation was applied by Mizrahi *et al.* (1970) to describe the sorption isotherm of dehydrated cabbage in the approximate range of water activity 0.07 to 0.55.

(20) *The Oswin equation*

This is a mathematical series expansion for sigmoid shaped curves, and may be written as follows:

$$M = a\left[\frac{A_w}{1 - A_w}\right]^n \tag{28}$$

where a and n are constants. Labuza *et al.* (1972) used this equation to correlate equilibrium moisture content data in non-fat dry milk and freeze dried soluble tea up to about 0.5 water activity.

(21) *The Smith equation*

It has been shown by Smith (1947) that the final curved portions of the water sorption isotherms of various bio-polymers were closely described by the equation,

$$M = M_b - M_a \ln (1 - A_w) \tag{29}$$

where M_b and M_a are constants. Becker & Sallans (1956) found that Smith's equation could be used to describe adequately the desorption isotherm of wheat between 0.5 and 0.95 water activity. Young (1976), applied the Smith equation to adsorption and desorption isotherms of Virginia-type peanuts, and concluded that the equation may be used for describing the isotherms as long as water activities are above 0.30.

(22) *The Strohman and Yoerger equation*

Strohman & Yoerger (1967) derived the following equation:

$$\ln A_w = a \ln p_0 \exp b (M) + c \exp (d M) \tag{30}$$

where a, b, c and d are constants. It is to be noted that Chung & Pfost's equation (eqn (11)) has a similar but simpler form that eqn (30); their origin, however, is different.

(23) *The Young and Nelson equation*

Young & Nelson (1967a) developed a theory relating equilibrium moisture content to water activity and temperature, which in addition does take account of hysteresis. They considered that the water is held in the food by three mechanisms. First, there can be a unimolecular layer of water molecules bound to the surface of the cells. Second there can be multimolecular layers, and third there can be moisture within the cells. This last, moisture, because of resistance to movement through the cell wall, produces the hysteresis effect. The relationship consists of the following set of equations:

$$M_s = A(\theta + \alpha) + \beta\phi \tag{31}$$

and

$$M_d = A(\theta + \alpha) + \beta\theta A_{w_{max}} \tag{32}$$

where s and d refer to adsorption and desorption respectively, and $A_{w_{max}}$ is the water activity from which the desorption commenced originally. The

quantities θ, α and ϕ are given by the following relationships,

$$\theta = \frac{A_w}{A_w + (1 - A_w)E}$$

$$\phi = A_w \theta$$

and

$$\alpha = -\frac{E A_w}{E - (E-1)A_w} + \frac{E^2}{(E-1)} \ln \left[\frac{E - (E-1)A_w}{E}\right] - (E+1) \ln (1 - A_w).$$

The equations involve the use of three parameters, A, β and E which have to be evaluated from the experimental data. According to Young & Nelson (1967a, b) the temperature dependence of constants A and β can be calculated from the change of water density with temperature, and the E constant should vary according to,

$$\ln E_2 = \frac{T_1}{T_2} \ln E_1$$

where the subscript '1' refers to a reference temperature. Young & Nelson (1967a, b) fitted experimental sorption data in wheat to the derived equations using a combination of an iteration and a multiple regression technique. They found that eqns (31) and (32) adequately described the experimental data for both sorption and desorption processes in wheat. Although the temperature shift in the isotherm predicted by the theory seemed in general to be similar to those found experimentally, Young & Nelson (1967b) believed that the experimental data were insufficient to draw any definite conclusions on this aspect. Certainly, additional studies appear to be needed to establish the real value of Young and Nelson's equation for predicting the effect of temperature on the isotherm.

Young (1976) also used Young and Nelson's equation to fit experimental data of water sorption and desorption in Virginia-type peanuts.

Ngoddy & Bakker-Arkema (1970) proposed an isotherm equation for biological materials based primarily on the B.E.T. and capillary condensation theories and indirectly on the Polanyi's potential theory. The isotherm equation so developed is somewhat complex for fitting purposes and is not discussed here.

Conclusions

Twenty-three isotherm equations were compiled which have been reported in the literature for fitting water sorption isotherms of foods. Some of the equations are theoretical, others semi-empirical and others were obtained by curve fitting of the experimental data. Several of the equations reported are equi-

valent or similar in aspect although their origins are different. Some of these equations have been widely used, while others have had little or no success.

This review suggests that an 'overall-all' evaluation of this large number of equations is needed in order to have a more precise (and quantitative) definition of their fitting abilities as applied to different types of foods. This is done in part 2 of this series, in which a statistical analysis is carried out of the goodness of fit of various of the compiled equations as applied to a wide variety of foods.

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Principles of aerobic treatment of food processing wastes: nitrogen and phosphorus relationships

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Summary

Efficiency of treatment of food processing wastes by an activated sludge process was explored in a model treatment unit. Effects of varying the ratio of chemical oxygen demand (COD) to phosphorus and nitrogen were examined. It was found that the mean cell residence time is an important factor in the operation of the process. The results suggest that municipal sewage may be a useful source for the additional nitrogen and phosphorus required for the efficient treatment of food wastes.

Introduction

Treatment alternatives for food processing wastes offer more possibilities for the sanitary engineer than almost any other type of wastewater. With food processing wastes it is clearly possible to consider waste residues as potential valuable by-products which may be suitable for some type of reuse. Because many of these wastewaters are high in organic content and contain small amounts of inorganic nutrients, unique treatment problems are encountered. Generally, the nutrients nitrogen and phosphorus may be considered to be limiting to microbial growth and hence, removal of organic matter as measured by the biochemical oxygen demand (BOD) or chemical oxygen demand (COD). To increase the removal of these two parameters nitrogen and/or phosphorus are sometimes added to food processing wastewaters. As a result, it is possible to add sufficient nitrogen and phosphorus to make carbon (represented by BOD and COD) the growth limiting nutrient. If an excess of nitrogen and phosphorus are added, however, these elements can escape with the treated

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wastewater and cause another form of pollution of streams and lakes termed eutrophication.

The purpose of this presentation is to illustrate how the operation of aerobic, biological wastewater treatment processes, specifically the completely mixed activated sludge process, may be altered to optimize treatment efficiency for nutrient deficient wastewaters. It will also be shown how direct discharge of food wastes to municipal sewers, with only minimal pretreatment, may be advantageous to both industry and municipality, and may reduce total treatment costs for both. To accomplish these objectives a laboratory scale aerobic treatment process was operated under highly controlled conditions. Wastewater composition was varied to study the effect of the ratio of COD to nitrogen and phosphorus on treatment efficiency. In the following sections a discussion of the experimental procedures followed and results obtained are considered with respect to their implications for treating food processing wastes.

Materials and methods

To illustrate the manner in which varying the ratio of organic to inorganic matter will affect wastewater treatment, a laboratory model activated sludge unit was operated under a variety of loading conditions. For clarity of presentation this section is subdivided to consider separately the laboratory apparatus, feed solution, initial start-up, experimental methods and data analysis.

Laboratory apparatus

Two different sized, but similarly constructed, model treatment units were used in this study. A schematic diagram of the equipment used is shown in Fig. 1. Each treatment unit contained an aeration compartment that was separated from a settling compartment by an adjustable baffle. Microorganisms were mixed thoroughly in the aeration tank through the action of air supplied through two porous diffuser stones. The settling tank was maintained under quiescent conditions so that microorganisms were not discharged in the overflow. Raw wastewater was continually pumped into the process at a constant rate from a 5-gallon carboy and also collected in a similar vessel. Both of these containers and all rubber tubing were disinfected daily to prevent biodegradation of the wastewater before entering the aeration basin, and during storage of the effluent. Due to growth of microorganisms in the treatment unit as a result of biodegradation of the wastewater, it was necessary to waste a prescribed amount of organisms each day. Sludge was wasted from the aeration tank by removing a prescribed mass of organisms each day, and it was possible to operate the unit until steady state conditions were attained. Steady state conditions were considered to be attained when the aeration basin microorganism

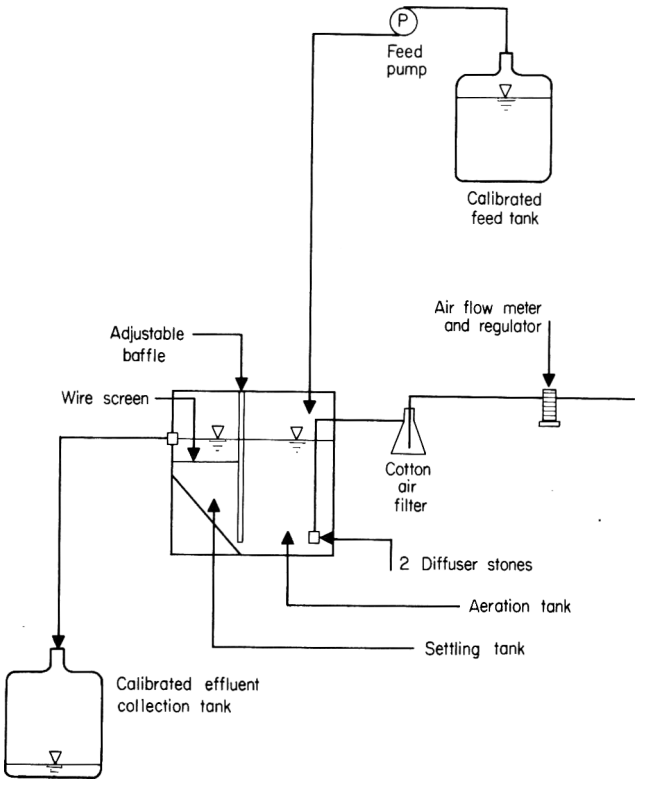

Figure 1. Experimental activated sludge unit with internal cell recycle.

concentration, effluent nitrogen, and effluent phosphorus values were constant over a period of time between 7 to 10 days. Measurements of parameters used to monitor the process were collected over this time span and then averaged to represent a single datum point.

Feed solution

Two very similar synthetic wastewaters were used in this study. The wastewater used to study the relationship of the COD:P on treatment performance had an influent COD of 325 mg/l. By varying the amount of phosphorus added it was possible to maintain COD:P of 32.6:1, 63.6:1 and 126.5:1. For the second wastewater it was desired to evaluate the effect of the ratio of COD to total Kjeldahl nitrogen (TKN) on treatment performance. In this case influent COD was maintained constant at 300 mg/l. The amount of nitrogen added to the wastewater was varied to establish a COD:TKN of 7.89:1 and 5.07:1. The organic components of the wastewater were supplied by bacto-peptone nutrient broth. Inorganic nutrients were supplied with the addition of various salt solutions. A detailed listing of all components of the wastewater may be found elsewhere (Stall & Sherrard, 1976; Benninger & Sherrard, 1978).

Initial start-up

Microorganisms utilized to start-up the experimental system were originally obtained from full-scale treatment plant facilities. These organisms were acclimated to the wastewater used in this study by operating on a fill-and-draw basis for a period of several weeks. Once acclimated organisms were obtained, and their concentration had risen to approximately 1500 mg/l, continuous flow operation was initiated. From this time on each of the parameters shown in Table 1 were measured on a daily basis.

Table 1. Parameters monitored daily

I.	Influent feed
	Chemical oxygen demand
	Alkalinity
	NH_3-N concentration
	Total Kjeldahl nitrogen
	Total phosphorus
	Soluble ortho-phosphate
	pH
II.	Filtered effluent
	Chemical oxygen demand
	Alkalinity
	NH_3-N concentration
	Total Kjeldahl nitrogen
	NO_3^--N concentration
	Soluble ortho-phosphate
	pH
III.	Unfiltered effluent
	Suspended solids concentration
	Total phosphorus
IV.	Biological reactors
	Total system microorganism concentration
	Ditto (intermittent)

Analytical procedures

All of the parameters listed in Table 1 were measured according to procedures described in 'Standard Methods' (1971). For total phosphorus determinations the persulphate digestion method followed by the stannous chloride method was used. Soluble orthophosphate determinations were made with the stannous chloride method. Colour measurement was made by using a spectrophotometer set at a wavelength of 650 nm. Nitrate-nitrogen determinations of filtered effluent were made using the cadmium reduction method. Colour development was measured using a Klett-Summerson Colorimeter and a #54 Klett filter. Unfiltered influent and filtered effluent total Kjeldahl and ammonia-nitrogen concentrations were also determined. Subsequent to the

preliminary distillation step, the acidimetric method of ammonia determination was used. For alkalinity determinations a pH meter was used to accurately determine the end point hydrogen ion concentration. Microorganism or suspended solids concentrations were obtained using the membrane filter technique.

Data analysis

Mathematical relationships developed for the completely mixed activated sludge process by Sherrard, Schroeder & Lawrence (1974) were used to analyse experimental data.

The mean cell residence time, which is related to the net microorganism specific growth rate, was used as the basis for comparison of experimental results. Mean cell residence time was determined by the expression

$$\theta_c = \frac{VX}{Q_w X + Q_{eff} X_{eff}} \tag{1}$$

where θ_c = mean cell residence time (days), V = volume of total reactor (litres), Q_w = wasted microorganism flow rate (litres/day), Q_{eff} = effluent wastewater flow rate (litres/day), X = total reactor microorganism concentration (mg/l), and X_{eff} = effluent liquid microorganism concentration (mg/l).

Each treatment unit was operated at steady state conditions over a range of θ_c values for a specified wastewater composition.

The observed microorganism yield coefficient, which is very useful to use in calculating waste sludge production, was found with the relationship

$$Y_{obs} = \frac{Q_w X + Q_{eff} X_{eff}}{Q(C_0 - C)} \tag{2}$$

where Y_{obs} = observed yield coefficient, Q = influent wastewater flow rate (litres/day), C_0 = influent wastewater COD concentration (mg/l), and C = effluent wastewater COD concentration (mg/l).

Efficiency of removal of organic material, total Kjeldahl and ammonia nitrogen, and phosphorus were found by evaluating

$$E = \frac{100\,(C_0 - C)}{C_0} \tag{3}$$

where E = designated removal efficiency (%), C_0 = influent wastewater COD, TKN, NH_3-N or phosphorus concentration (mg/l), and C = effluent wastewater COD, TKN, NH_3-N or phosphorus concentration (mg/l).

To test the validity of the data obtained it was linearized according to the expression

$$\frac{1}{\theta_c} = Y_{max}\, U - b \tag{4}$$

where Y_{max} = true or maximum yield coefficient, U = specific organic utilization rate ($days^{-1}$), and b = microorganism maintenance energy or decay coefficient ($days^{-1}$).

Results and discussion

Because the impact of both the ratio of organic matter to phosphorus and organic matter to nitrogen on treatment plant performance were evaluated in this study, each of these factors will be considered separately for clarity.

Phosphorus

By operating the model activated sludge unit at steady state conditions over a wide spectrum of operating conditions, i.e. mean cell residence times, at each of the three COD:P ratios selected, it was possible to assess the relationship of phosphorus to treatment plant performance. Regulations for discharge of effluent require high COD or BOD removal in wastewater treatment. Because many food processing wastes are deficient in phosphorus, phosphorus may limit COD removal unless it is added in the correct amount. Alternatively, it may be possible to adjust the θ_c of process operation. Even when the ratio of COD:P remains constant, varying the θ_c may cause phosphorus to become limiting at certain values of θ_c. This point is illustrated in Fig. 2 where COD

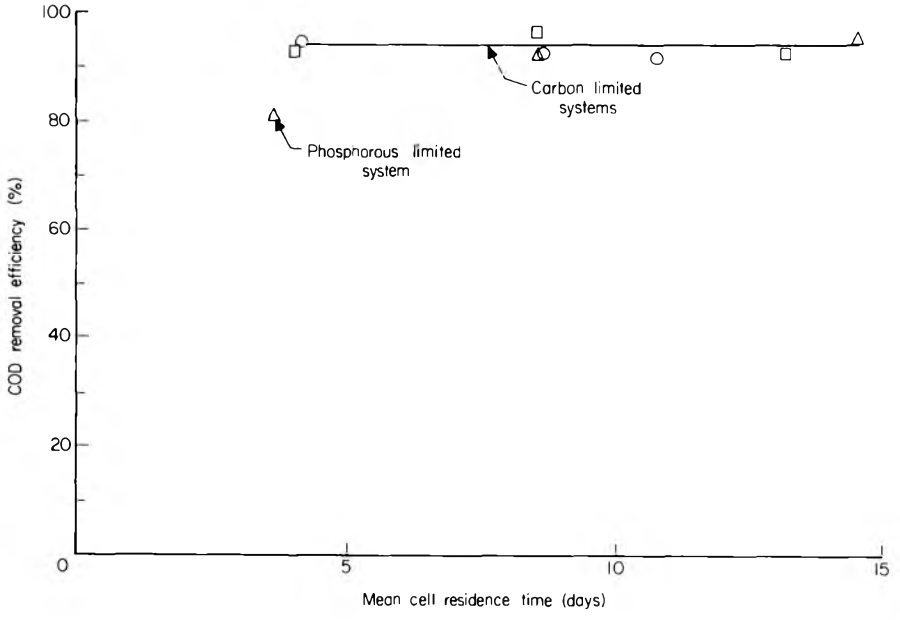

Figure 2. Effect of mean cell residence time on chemical oxygen demand removal efficiency. C_0 = 325 mg/l, θ = 8 hr; COD:P – ○ 32.6:1, □ 63.6:1, △ 126.5:1.

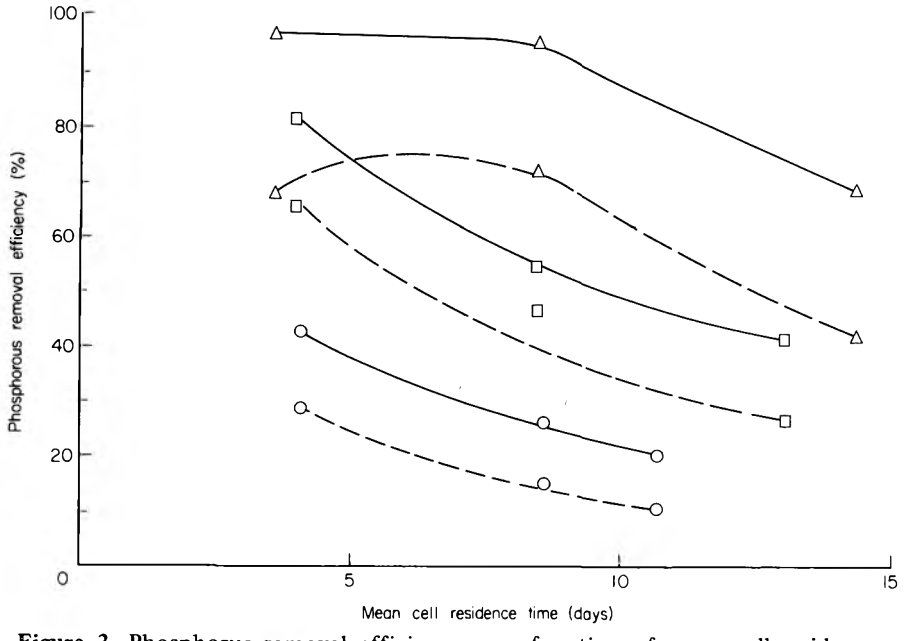

Figure 3. Phosphorus removal efficiency as a function of mean cell residence time. —— soluble PO_4^{3-} removal, – – – total P removal. COD:P – ○ 32.6:1, □ 63.6:1, △ 126.5:1.

removal efficiency is plotted as a function of θ_c. At a low θ_c and high COD:P ratio it is shown that the phosphorus may limit the microbial growth expected. As a result organic components of the wastewater are not completely metabolized and larger amounts of COD appear in the effluent. By changing the treatment process to a high θ_c greater amounts of COD can be removed, but less phosphorus will be removed. This may be seen more clearly in Fig. 3 where phosphorus removal efficiency is plotted as a function of θ_c. From this graph it can also be seen for a given wastewater that phosphorus removal efficiency can be varied by simply varying the θ_c of process operation.

Considering both of these figures together will lead to the conclusion that discharges of nutrient deficient wastes, i.e. some food processing wastes, to municipal sewers would be beneficial for both the food industry and the municipality. This would occur because the combined municipal-food waste sewage would have a higher COD:P ratio which would result in larger quantities of phosphorus being removed during treatment. Because removal of phosphorus would be increased, the municipality would spend less money by comparison with other more costly tertiary treatment methods.

Disadvantages may arise from the addition of food processing wastewaters to municipal sewage, however. Several of these disadvantages include higher oxygen requirements, greater sludge production, and bulking sludge if the COD:N or COD:P ratio are raised too high.

Shown in Fig. 4 is the relationship between the observed yield coefficient and θ_c. From this figure it can be further ascertained that the amount of waste

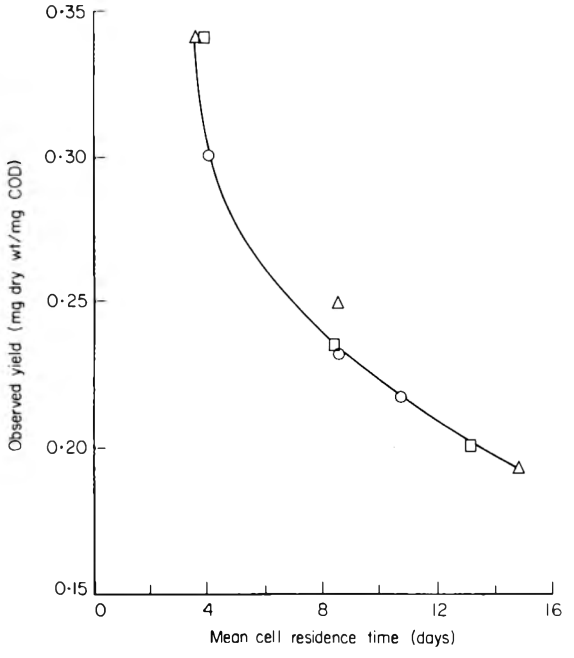

Figure 4. Effect of mean cell residence time on the observed yield coefficient; COD : P ratios as in Fig. 3.

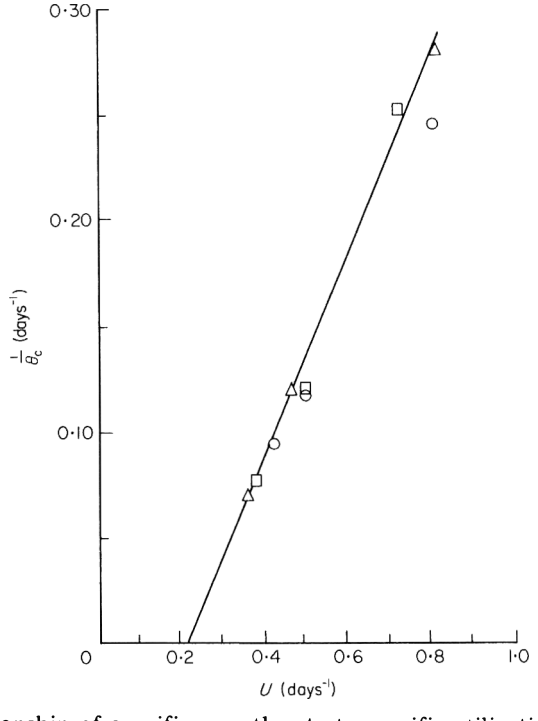

Figure 5. Relationship of specific growth rate to specific utilization rate; $1/\theta_c = 0.446\,U - 0.096$; COD : P ratios as in Fig. 3.

sludge produced, and hence phosphorus incorporated into the sludge, will vary with the θ_c of process operation. This occurs because the value of the observed yield coefficient decreases as θ_c increases.

Linearization of the data obtained is shown in Fig. 5. Based on the results obtained a maximum yield value of 0.446 mg bacteria/mg COD utilized and a bacterial maintenance energy coefficient of 0.096 $days^{-1}$ were obtained.

Nitrogen

For the experiments in which the influence of the COD:TKN ratio on wastewater treatment performance was evaluated, it was found that COD removal efficiency was high over the range of conditions studied. Carbon was always the limiting nutrient, irrespective of the θ_c utilized, for COD:TKN ratios of 7.89:1 and 5.07:1. The removal efficiency of TKN was found to vary with both the COD:TKN ratio and with θ_c. This may be seen more clearly in Fig. 6. As shown, as the COD:TKN ratio increases, a larger percentage of nitrogen is removed even though the same total amount of nitrogen is removed in waste sludge. This occurred because the influent COD was constant and the amount of influent TKN was varied. As can also be seen with the data for phosphorus, the expression of results as removal efficiency can often lead to erroneous conclusions.

In Fig. 7 a plot of the forms of nitrogen present as a function of the θ_c of

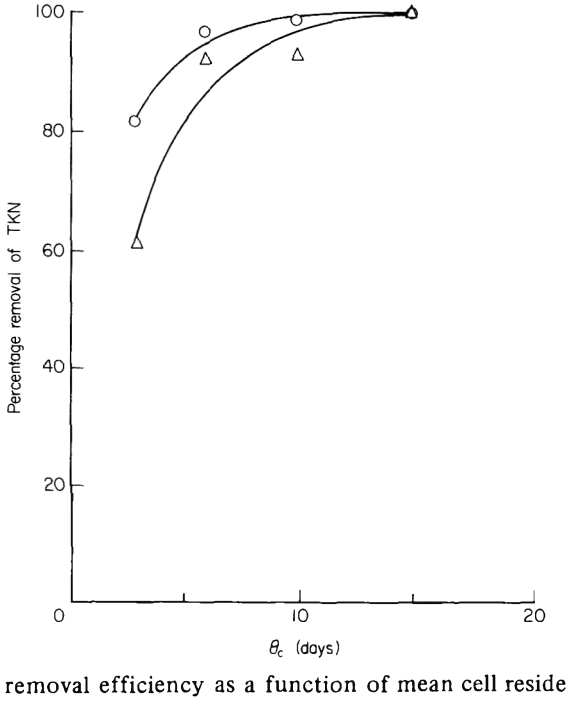

Figure 6. TKN removal efficiency as a function of mean cell residence time and COD:TKN; ○ COD:TKN = 7.89:1, △ COD:TKN = 5.07:1.

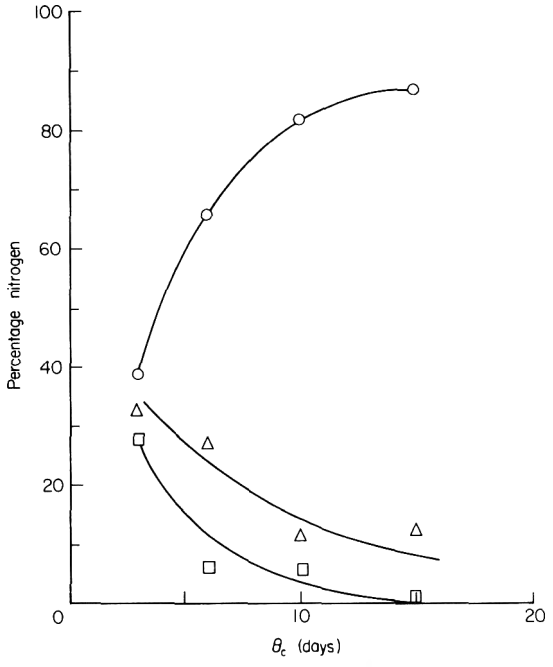

Figure 7. Percentage distribution diagram of nitrogen as a function of mean cell residence time for a COD:TKN = 5.07:1. ○ NO_3^- – N in effluent, △ N in waste sludge, □ TKN in effluent.

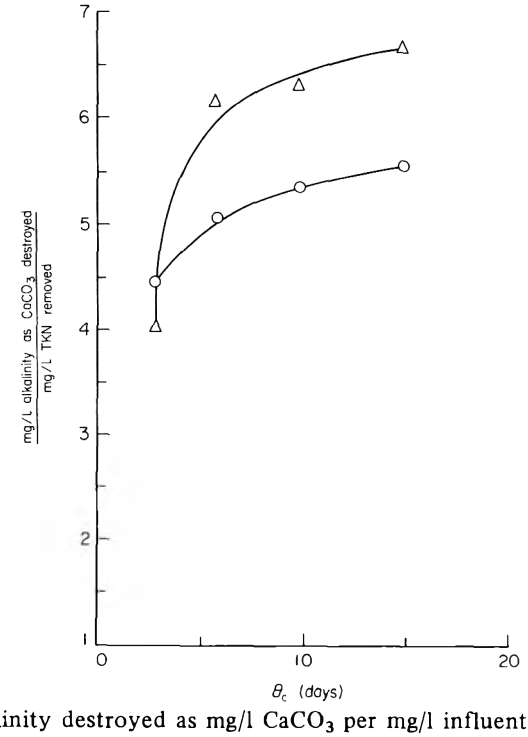

Figure 8. Alkalinity destroyed as mg/l $CaCO_3$ per mg/l influent TKN as a function of mean cell residence time and COD:TKN; ○ COD:TKN = 7.89:1, △ COD:TKN = 5.07:1.

process operation is shown. At low values of θ_c significant amounts of nitrogen are present in waste sludge and as effluent TKN. As the value of θ_c increases a large amount of the influent TKN is converted to nitrate-nitrogen due to nitrification and much less nitrogen is incorporated into the sludge.

For food processing wastewaters that do not have much buffering capacity (alkalinity) the results shown in Fig. 8 are of significance. Due to oxidation of organic carbon to CO_2 and ammonia-nitrogen to nitrate-nitrogen the pH can be expected to drop due to alkalinity destruction. If sufficient buffering capacity is not present it is possible for the pH value to fall to a value not suitable for microbial growth. From Fig. 8 it is possible to calculate the amount of alkalinity that should be added to a process to counteract the amount destroyed.

Data from this portion of the investigation were also linearized to determine whether or not the results were significant. As shown in Fig. 9, the data did linearize and the values of Y_{max} and b were 0.438 mg bacteria/mg COD utilized and 0.036 $days^{-1}$, respectively.

Summary and conclusions

In many instances food processing wastewaters are deficient in the elements nitrogen and phosphorus, and as a result, these elements are often added to attain high treatment efficiency. Because of the unfavourable stoichiometric composition of these wastewaters (high carbon concentration) their addition to municipal sewers may in certain instances improve the composition of municipal sewage to the degree that nitrogen and phosphorus removal costs may decrease significantly. Alternatively, for treatment facilities designed to purify food processing wastewaters, the use of municipal sewage as a source of the elements nitrogen and phosphorus should always be considered.

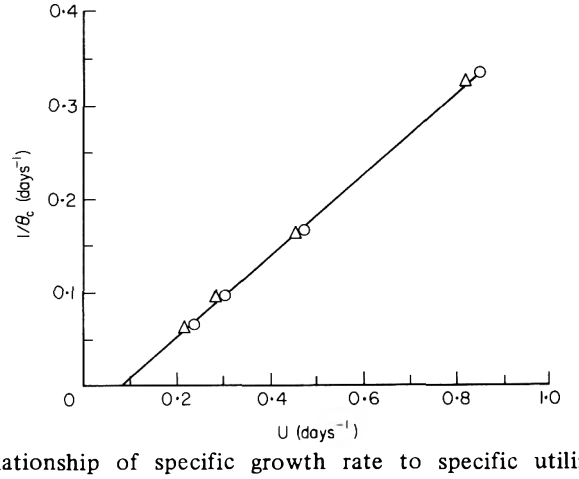

Figure 9. Relationship of specific growth rate to specific utilization rate. ○ COD:TKN = 7.89:1, △ COD:TKN = 5.07:1; $1/\theta_c = 0.438U - 0.035$, Y_{max} = 0.438, b = 0.036.

Specific conclusions resulting from this study include:

(a) the amount of nitrogen and phosphorus required for efficient biological wastewater treatment is variable and dependent on the mean cell residence time of process operation because of variable sludge production.

(b) for a given wastewater, larger quantities of nitrogen and phosphorus will be incorporated into sludge at lower mean cell residence times than at higher mean cell residence times.

(c) the amount of alkalinity addition needed to account for its destruction due to nitrification is a variable and dependent on θ_c.

(d) the amount of nitrogen incorporated into microorganisms or converted to ammonia or nitrate is a variable quantity that depends on both the COD : N ratio and θ_c.

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Differences in baking quality between wheat flours

F. MACRITCHIE

Summary

A study was made of six pairs of wheat flours, members of each pair being similar in protein content and other characteristics but differing in baking performance. Interchange of flour components showed that the origin of quality differences in all cases resided in the gluten protein. Measurements of loaf volume as a function of lipid content were made using defatted flours reconstituted with variable amounts of flour lipids. Loaf volumes for the poorer quality flours were below those for the paired samples over ranges of lipid content which varied from a small region near the natural lipid content to the complete range. Since lipid fractions behaved identically when exchanged between pairs of flours, this effect apparently reflected a gradation in protein quality. Preliminary results indicated that differences in protein quality were associated with the glutenin fraction although further work is needed to test the generality of this conclusion. No relation was found between baking quality and the amount of lipid bound by dough mixing.

Introduction

Wheat flours show a wide range in their capacity for bread-making and much work has been directed towards explaining this phenomenon. The work of Finney (1943) established that loaf volume, generally considered to be the most objective indicator of baking quality, varied linearly with protein content while the slope of the regression line depended on wheat variety. The term 'protein quality' has often been adopted to rationalize differences in performance between flours of similar protein content, although the cause of these differences has not been clearly identified. The lipid component has frequently been implicated in flour quality. Curves of loaf volume as a function of lipid content resemble Morse curves, showing minima at lipid contents intermediate

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between those of the defatted and whole flours (MacRitchie & Gras, 1973). The effects of lipid fractions have been more closely catalogued than specific protein fractions (Daftary *et al.*, 1968; MacRitchie & Gras, 1973). However, studies (Fisher *et al.*, 1966) have tended to show that, despite the important role that lipids play in baking, quality differences between flours cannot usually be ascribed to differences in the lipid component. Among the other flour components shown to affect flour quality, starch (Kulp, 1972; D'Appolonia & Gilles, 1971) and pentosans (Casier *et al.*, 1969; Cawley, 1964; D'Appolonia, Gilles & Medcalf, 1970) have been the most prominent.

The present work was undertaken with the aim of arriving at some generalizations about reasons for variation in baking quality. For this purpose, a selection was made of flours which performed unusually poorly considering their protein content. Each of these flours was then paired with a flour similar in analytical characteristics, but giving a good baking performance. Particular attention was given to matching protein content and starch damage as closely as possible. Each pair of flours was then subjected to a study aimed at determining the origin of the differences.

Methods and materials

Details of the six pairs of flours are given in Table 1. Protein and lipid percentages are quoted on a 14% moisture basis. For each pair, A is assigned to the flour of good performance and B to the poorer flour. Flours 1, 2 and 3 were from commercial mills and thus contained several wheat varieties. Flours 4, 5 and 6 were pure varieties which were milled on a laboratory Buhler mill. All

Table 1. Data for flours

Flour	Variety or main varieties	Protein content (%)	Lipid extractable from flour (%)	Lipid extractable from freeze dried dough (%)	Bound lipid (%)
1A	Falcon, Heron	10.5	1.17	0.33	72
1B	Falcon, Heron	10.0	1.23	0.74	39
2A	Eagle, Timgalen	11.1	1.25	0.74	41
2B	Eagle, Timgalen	11.1	1.28	0.35	73
3A	Timgalen, Gatcher	11.4	1.32	0.76	42
3B	Timgalen, Gatcher	11.4	1.34	0.79	41
4A	Gamut	11.3	1.09	0.65	40
4B	Robin	10.7	1.31	0.67	49
5A	Raven	11.5			
5B	Eagle	10.9			
6A	Festiguay	11.6			
6B	Gatcher	11.3			

flours were stored in plastic containers at 3°C. Control tests showed that the baking properties of the flours did not change measurably over the periods during which they were studied. Fermentograms of doughs, measured as described previously (MacRitchie, 1976) showed that gas production was not a limiting factor for any of the flours studied.

The methods for extraction of lipid, reconstitution of flours and test baking were the same as those described previously (MacRitchie & Gras, 1973; MacRitchie, 1976) except that test loaves were baked in triplicate, improvements in techniques making it possible to maintain the standard deviation per mean loaf volume at about 4 cm^3. For measurement of loaf volume at constant lipid and varying protein contents, flours were prepared by mixing appropriate proportions of whole flour, whole flour containing twice its natural lipid content and gluten from defatted flour to give the required compositions. Glutens were washed from defatted flours by hand kneading doughs in water maintained at 15°C. The starch and solubles fractions were separated by centrifugation and all fractions were freeze dried. Gluten protein was fractionated by stirring vigorously with thirty times its weight of 0.1 M acetic acid in a Janke & Kunkel Ultraturrax mixer for 2 min followed by centrifugation. The supernatant, which contained the fraction which will be referred to as the gliadin fraction, and the residue (glutenin fraction) were dialysed exhaustively against distilled water at 3°C and freeze dried.

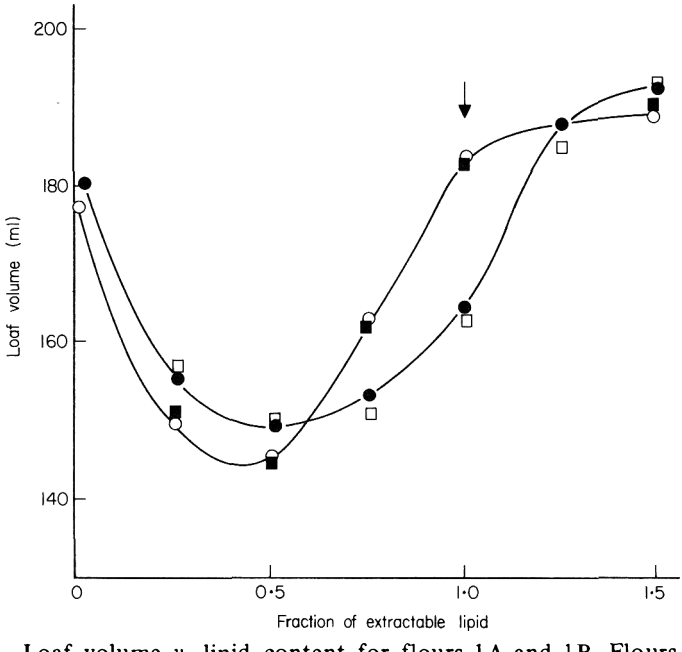

Figure 1. Loaf volume *v.* lipid content for flours 1A and 1B. Flours reconstituted with own lipids: ○ defatted flour 1A + 1A lipid, ● defatted flour 1B + 1B lipid; flours reconstituted with interchanged lipids: ■ defatted flour 1A + 1B lipid; □ defatted flour 1B + 1A lipid. Arrow indicates natural lipid contents of flours.

The percentage of lipid bound as a result of dough mixing was determined as follows. Thirty-five grams of flour and 20 g of water were mixed to peak consistency in a mixograph. The dough was freeze dried and powdered. The amount of lipid extractable by chloroform was then determined on the original flour and of the freeze dried dough. Three extractions of 10 g of flour (dry weight) were carried out, the extract was filtered twice and the lipid determined gravimetrically after complete evaporation of the solvent in a rotary evaporator. Results are quoted as means of triplicate determinations, the average standard deviation of the mean being approximately 4% of the mean value.

Results

The loaf volume-lipid content relations, obtained by reconstituting defatted flours with varying amounts of flour lipids for flours 1A and B, are shown in Fig. 1 together with the points obtained when lipid fractions were interchanged between the two flours. A systematic interchange of the gluten, starch and solubles fractions between flours 1A and 1B gave the results shown in Table 2. Loaf volumes were measured over a range of protein contents with the lipid contents fixed at their natural values (Fig. 2). Regression analysis gave figures of 6.5 and 5.8 cm^3 volume per percent of protein for the regressions of loaf volume on protein content for flours 1A and 1B respectively. Similar behaviour to that of flours 1A and 1B were shown by flours 2A and 2B. Figures 3 and 4 show loaf volume–lipid content curves for flours 3A, 3B and 5A, 5B respectively. Similar procedures to those followed for flours 1A and 1B revealed that differences in performance between these two pairs could also be completely ascribed to the gluten protein. Flours 6A and 6B gave results closely similar to 5A and 5B. Flour 4B showed behaviour intermediate between 1B and 3B; i.e. volume was depressed at the natural lipid content of the flour but was only

Table 2. Effects of loaf volume of interchanging components between flours 1A and 1B at natural lipid contents of flours

Gluten	Starch	Solubles	Loaf volume (cm^3)
1A	1A	1A	182
1A	1A	1B	182
1A	1B	1A	183
1A	1B	1B	180
1B	1B	1B	161
1B	1B	1A	160
1B	1A	1B	163
1B	1A	1A	163

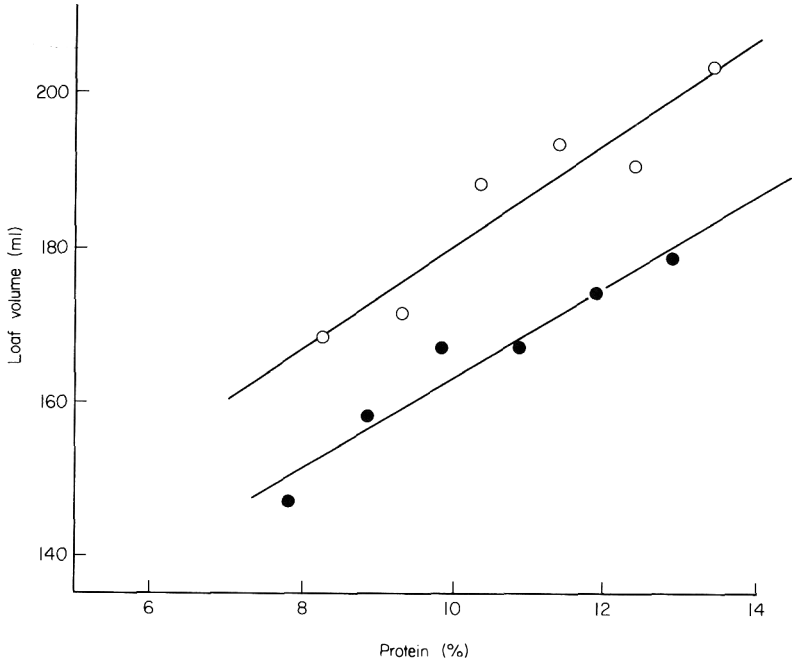

Figure 2. Loaf volume *v.* protein content for flours 1A and 1B. Lipid contents of flours are fixed at their natural values. ○, flour 1A; ●, flour 1B.

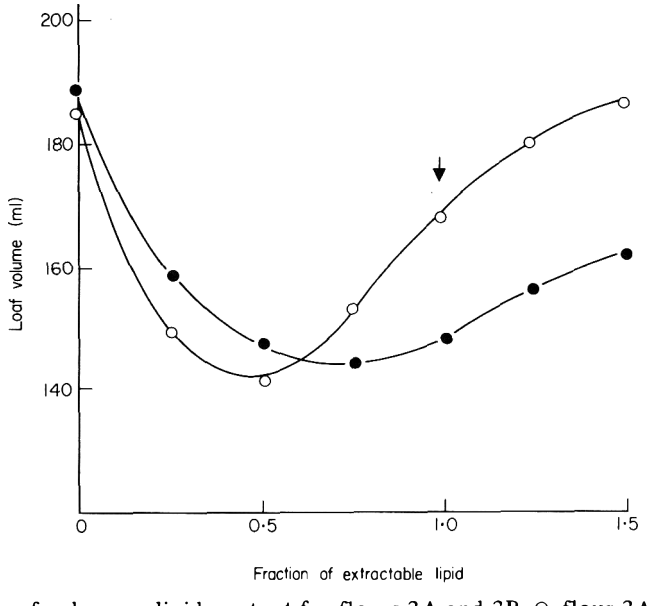

Figure 3. Loaf volume *v.* lipid content for flours 3A and 3B. ○, flour 3A; ●, flour 3B. Arrow indicates natural lipid contents of flours.

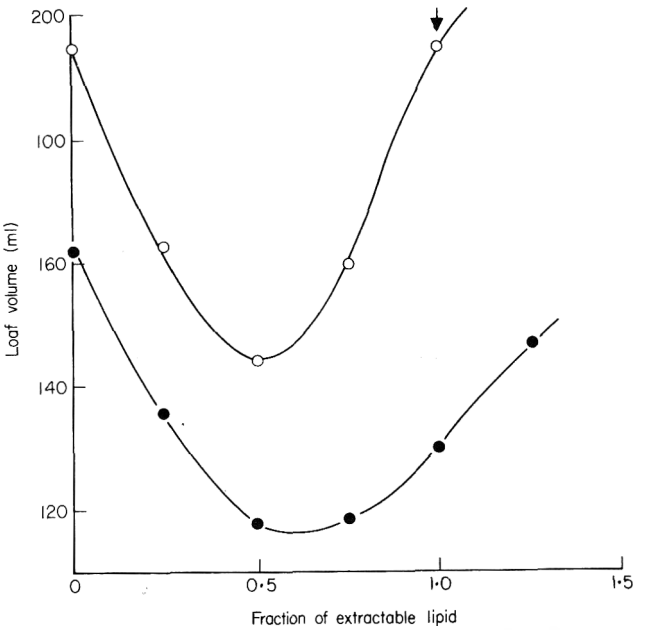

Figure 4. Loaf volume *v.* lipid content for flours 5A and 5B. ○, flour 5A; ●, flour 5B. Arrow indicates natural lipid contents of flours.

slightly below that of flour 4A at higher lipid contents (allowance being made for protein content).

For flours 1–4 (i.e. all pairs which differed in loaf volume over only part of the range of lipid content) the percentage lipid bound during dough mixing was determined and the results are included in Table 1.

Glutens from defatted flours 5A and 5B were separated into crude gliadin and glutenin fractions. The protein contents of corresponding fractions were similar for the two flours, the gliadin and glutenin containing approximately 60 and 40% respectively of the total gluten protein. The two fractions were

Table 3. Effects of loaf volume of interchanging protein fractions between defatted flours 5A and 5B

Gluten	Gliadin	Glutenin	Starch + solubles	Loaf volume (cm^3)
5A			5A	201
	5A	5A	5A	173
	5A	5A	5B	170
	5A	5B	5A	149
5B			5B	162
	5B	5B	5B	146
	5B	5B	5A	142
	5B	5A	5B	168

interchanged between the two flours and a baking test was carried out. Results are shown in Table 3. The dough properties of these reconstituted flours showed reduced times to reach peak consistency together with stickiness and excessive extensibility, characteristic of overmixed doughs.

Discussion

The differences in performance between the flours in each of the six pairs examined can be entirely ascribed to the gluten protein. This result is consistent with conclusions reached by previous workers; e.g. Finney (1943). The behaviour shown in Fig. 1 where the loaf volume–lipid content curve is displaced to higher lipid contents for the poorer flour suggested the possibility that a protein–lipid interaction might be involved. No support for this was obtained from the lipid binding measurements. All values fall close to 40% for bound lipid except for two high values near 70% for flours 1A and 2B, i.e. one which performs well, the other poorly. Therefore, no correlation between baking performance and lipid binding (as measured here) can be inferred from these results. In the case of flours 5 and 6, it seems unlikely that lipid–protein interactions are important in view of the large differences between the defatted flours in each pair.

There appears to be a trend in behaviour which may reflect a gradation in protein quality. A flour such as 1B (Fig. 1) gives depressed loaf volumes compared to a control flour such as 1A over a small range of lipid content near the natural value for the flour. For flour 3B (Fig. 3), loaf volume is depressed over a greater range of lipid content while in the case of flour 5B (Fig. 4), loaf volume is deficient at all lipid contents. It would appear that for a flour with its natural amount of lipid, conditions are the most critical for obtaining a good loaf volume. Thus, when comparisons are made at the same lipid contents, flour 1B gives loaf volumes equal to flour 1A when its lipid has been extracted or when extra lipid has been added, suggesting that there is a comparatively minor deficiency in the quality of its gluten. At the other extreme, flour 5B gives a loaf volume inferior to flour 5A even when the lipids have been extracted; i.e. under what appears to be the most favourable conditions for good loaf volume. It may be concluded that the gluten protein of flour 5B is of lower quality than that of flour 3B which in turn is inferior to the protein of flour 1B.

Flours 5A and 5B were chosen for further fractionation studies of their protein because these flours showed a significant difference in loaf volume when defatted. By working with defatted flours it was possible to avoid complications introduced by differences in distribution of lipid between fractions. Although loaf volumes are depressed as a result of imperfect reconstitution, the results of Table 3 suggest that the origin of the differences in baking response lies in the more insoluble glutenin fraction. Similar effects of faulty reconstitution resulting from protein extraction have been reported by Shogren *et al.*

(1969). These problems need to be overcome or at least minimized before reliable conclusions can be reached concerning the contributions of specific protein fractions.

Acknowledgment

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Aqueous extraction of black leaf tea.

II. Factorial experiments with a fixed-bed extractor*

V. D. LONG

Summary

Tea extracts produced from black tea and water by one extraction in a single-stage, laboratory-scale, fixed-bed extractor at water-to-tea ratios 4:1 and 5:1 by mass had concentrations 6–12 mass% soluble solids corresponding to 11–23 mass% yield from leaf on an as-received basis. Concentration and yield are related functions of the water-to-tea ratio and the method of water addition. Observed trends are explained by quantitative theory which presents equations relating to perfectly stirred extraction and plug-flow percolation. The best performance, obtained by conditioning leaf before extraction with part of the extraction water, occurred under conditions of reduced convective mixing.

Introduction

As part of an investigation into the production of instant tea from black leaf, experiments were carried out to quantify aqueous extraction. Earlier work (Long, 1977) had established leaf solubilities applicable to the process design of equilibrium stage extractors, so it remained to characterize behaviour in systems with short contact times between leaf and water. The simple experiments described below were a first attempt at this using a conveniently available apparatus employed by Alexander & Rustidge (1966) in small-scale instant tea production for organoleptic tests. Although the method of extraction followed has little direct practical application, the results obtained well illustrate important properties of the extraction process and provide data for a tentative mathematical model with wider applications.

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Materials and methods

Experiments were carried out to a factorial design by measuring extract concentration and yield for duplicate runs at two levels of leaf content, two water-to-tea ratios and two methods of extraction.

The leaf extracted was the same blend of large-leaf orthodox teas used in earlier work (Long, 1977). The moisture content was 6% by mass and over 97% by mass of the leaf was in the size range 10–40 mesh B.S. sieve.

The extraction vessel was a steam-jacketed glass column of integral construction comprising an upper cylindrical working section 83 mm internal diameter and 610 mm high flanged at the top to take a glass lid and separated by a permanently-fixed, sintered-glass plate from a lower conical discharge section, overall height 100 mm, reducing to a vertical spout 20 mm diameter protruding from the bottom of the steam jacket and fitted with a short length of flexible plastic tubing closed by a screw clip. To expedite discharge of extract by filtration and residue by inversion, a disc of fine stainless steel gauze was placed on the upper surface of the sintered glass plate.

To make an extraction, a weighed amount of tea was introduced into the steam-heated column and left to heat partially for about 5 min. Next an amount of boiling distilled water, appropriate to the chosen water-to-tea ratio was poured onto the leaf, two different modes of addition being used: (i) adding all water in one batch, (referred to as extraction of unconditioned leaf), (ii) adding first an amount of water equal to twice the weight of tea leaf to swell the leaf, and after 3 min adding the remainder of the water, (extraction of conditioned leaf). Any extract seeping through the supporting plate immediately after addition of water was run off and reintroduced at the top of the column. Once the leaf began to swell, seepage was generally negligible. Ten minutes after the first addition of the water, tea extract was allowed to drain from the column for 5 min and the collected extract cooled rapidly. The volume of cooled extract was measured in a graduated cylinder, its specific gravity estimated by common hydrometer, and the total-solids content determined by evaporation of 10 ml aliquots in duplicate.

Using the above procedure, duplicate runs were made involving the following three factors at the two levels indicated: weight of tea, 200 g and 400 g; water-to-tea ratio, 4:1 and 5:1; state of leaf, conditioned and unconditioned.

Results

Average results for duplicate runs are shown graphically in Figs 1–3. Figure 1 correlates specific gravity (G) measured at 13°C by hydrometer with the extract concentration (c) expressed as a mass fraction. The solid line is the regression minimizing residual variance in specific gravity. It has the equation:

$$G = 0.406c + 1.0004. \quad (1)$$

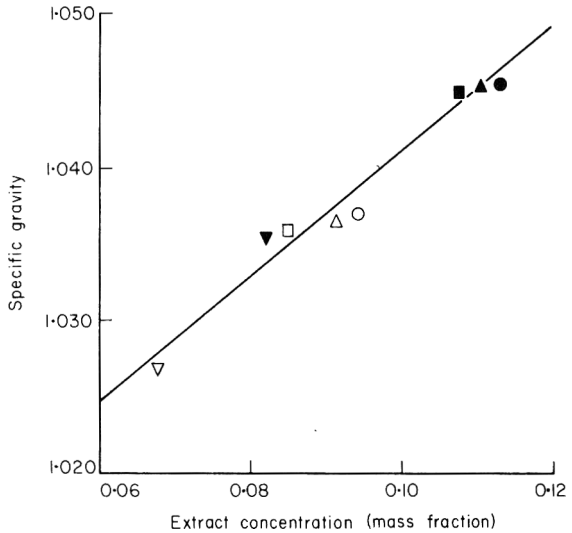

Figure 1. Specific gravity of extracts as a function of total solids content. Key: W/T = water-to-tea mass ratio; T = mass of tea extracted (g); U = unconditioned leaf; C = conditioned leaf.

▼ W/T = 4, T = 200, U; ■ W/T = 4, T = 200, C
▲ W/T = 4, T = 400, U; ● W/T = 4, T = 400, C
▽ W/T = 5, T = 200, U; □ W/T = 5, T = 200, C
△ W/T = 5, T = 400, U; ○ W/T = 5, T = 400, C

Figures 2 and 3 relate to derived results. Figure 2 shows the specific net liquid uptake by leaf (defined as the difference between the volumes of extracting water added and extract collected per g of leaf) as a function of extract concentration. Values for unconditioned leaf (shown as triangles) tend to be slightly lower than corresponding values for conditioned leaf. This was statistically significant at the 1% level by Student's *t*-test but is of little practical significance. Two results from 5:1 extractions appear to be anomalous but there is no statistically significant departure from the grand mean of 2.7 ml/g.

Figure 3 shows the gravimetric yield of tea solids in collected extract (expressed as a mass fraction of leaf extracted (6% moisture basis)) as a function of extract concentration. Lines have been drawn through the points merely to emphasize trends which will be discussed below in relation to elementary quantitative theory developed in the Appendix.

Discussion

The correlation between specific gravity and mass fraction tea solids is statistically very highly significant (correlation coefficient 0.974) but there is considerable scatter (residual standard deviation 0.0015) which largely reflects the

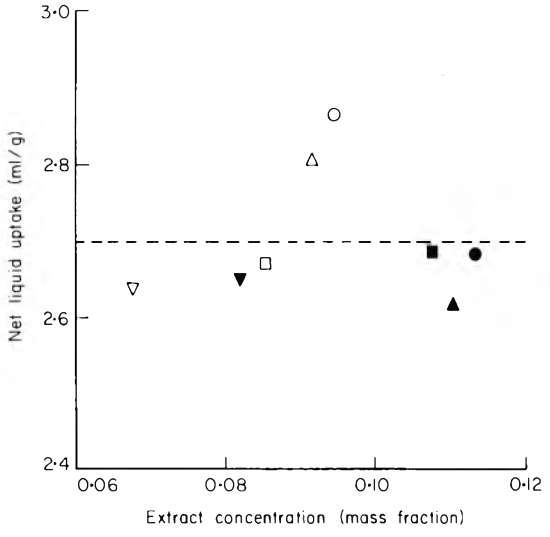

Figure 2. Net liquid uptake by leaf as a function of extract concentration. Key as for Fig. 1.

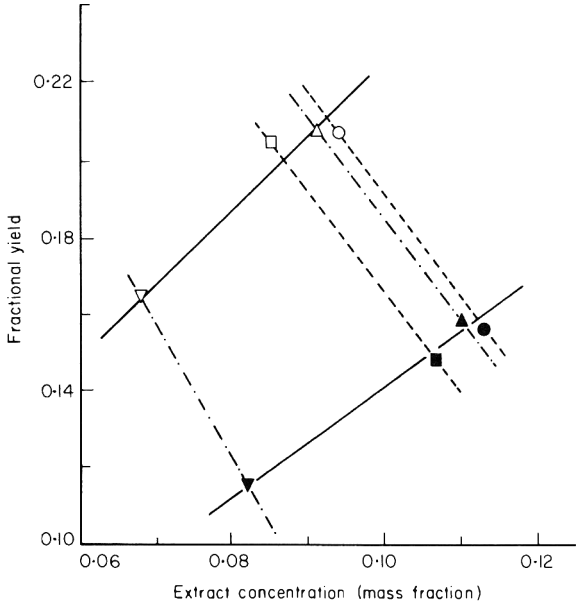

Figure 3. Yield of soluble solids in collected extract as a function of extract concentration. Key as for Fig. 1.

difficulty of observing the hydrometer stem in tea solutions. Thus determination of specific gravity by hydrometer will give only approximate estimates of total solids content. The value of the intercept in the above correlation matches the known specific gravity of water at 13°C relative to water at the hydrometer calibration temperature 15.5°C (Perry, 1963).

Probably a more fundamental correlation would have been between specific gravity and total solids content expressed volumetrically in g/ml (c_e), which gives the regression equation:

$$G = 0.380\, c_e + 1.0004. \tag{2}$$

This treatment gives a slightly higher correlation coefficient (0.978) with a corresponding small reduction in scatter (standard deviation 0.0014). Thus a minute part of the scatter in the first correlation is possibly due to non-linearity of the variables correlated. Obviously the present results are not sensitive enough to decide between the merits of the two forms of correlation by scatter alone; the matter is ultimately decided by extrapolation. If the intercept at zero concentration, which is identical for both correlations, is taken to be the apparent specific gravity of water, then the density of tea solution (ρ_s) may be expressed in terms of the density of water (ρ_w) by a relationship of the form:

$$\rho_s = \rho_w + \alpha c_e. \tag{3}$$

Substituting $\rho_s c$ for c_e gives the specific volume of solution (v_s) as:

$$v_s = 1/\rho_s = (1 - \alpha c)/\rho_w. \tag{4}$$

Hence by the well-known method of tangent intercepts (Moore, 1972) the partial specific volume of tea solids is $1 - \alpha/\rho_w$. Obviously if α is constant the partial specific volume is constant and tea forms an apparently ideal solution. The density of pure solute is then $\rho_w/(1 - \alpha)$. Taking ρ_w as approximately 1 g/ml and $\alpha = 0.38$, the density of dry soluble tea solids is estimated as around 1.6 g/ml in contrast with the value 1.4 g/ml predicted by extrapolation of eqn (1). Direct measurement of the density of freeze-dried tea solids by density bottle using petroleum ether as the suspending fluid and refluxing for a considerable period to ensure penetration of the porous solid by solvent confirmed the higher figure. Hence as far as density is concerned the extracts produced in the present work were indistinguishable from ideal solutions of a solid of density 1.6 g/ml. It may be of interest to mention here that later more precise work with aqueous solutions of commercial instant tea containing up to 40 mass % solute also showed constancy of partial specific volume of tea solids to within 2.5% of the specific volume of the undissolved tea measured in *n*-pentane. However, in this case the solid density was about 1.70 g/ml possibly reflecting differences in blend and extraction yield.

It is characteristic of extractions of tea leaf that the volume of extract obtained is less than the volume of water originally added. This is particularly noticeable when using low water-to-tea ratios as in this work. Taken to the extreme case it means that unless the amount of water added exceeds a certain limit, no extract will be collected at all, since the leaf will totally absorb its own extract. The specific net liquid uptake of Fig. 2 is a measure of extract absorption by leaf. Considering the crude and variable draining to be expected for gravity flow through an irregular bed and the nearly two-fold variation in

extract concentration, the value is remarkably constant around the mean value 2.7. The low value found reflects both the good draining character of the leaf and the long drainage time employed. The same leaf in continuous counter-current extraction having only a few seconds' gravity drainage before discharge would have up to twice this amount of liquor associated with it. The constancy of the result is particularly fortunate, it implies constancy of volume (and approximate constancy of mass) of residue which greatly facilitate extraction calculations and can be made the basis of a simple theoretical model (see Appendix) to explain the trends in the present results for concentration and yield, and provide a basis for further experiment.

The results for concentration and yield given in Fig. 3 show three clear trends. First, at constant water-to-tea ratio, yield increases approximately linearly with extract concentration. Secondly, for a given water-to-tea ratio and mass of tea extracted, conditioned leaf gives higher yield and concentration than unconditioned leaf; the advantage being particularly marked with the smaller amount of tea. Thirdly, for any given mass of tea and method of extraction, decreasing the water-to-tea ratio decreases yield while increasing concentration.

Considering first the relation between yield (Y) and extract concentration (c_e) at constant water-to-tea ratio (W), eqn (14) (Appendix) can be rearranged to give:

$$Y/c_e = W - V_n. \tag{5}$$

Now if the specific net liquid uptake (V_n) can be regarded as constant, a graph

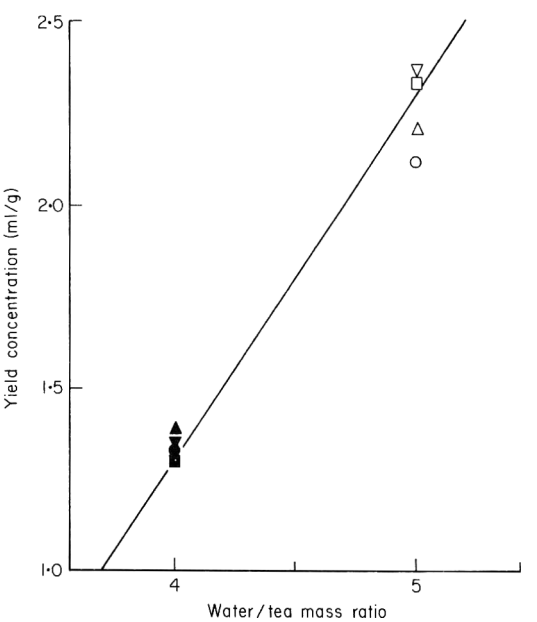

Figure 4. Fractional yield/volumetric concentration as a function of water-to-tea ratio. Key as for Fig. 1.

of Y/c_e plotted against W should be linear with a slope of unity and an intercept of V_n on the W axis when Y/c_e is zero. This is tested in Fig. 4 where a line of unit slope is drawn through the grand mean of all points (4.5, 1.800). Although not a perfect fit, it is clearly a good indication of the trend of the results. Extrapolation of the line to zero yield gives a value for V_n of 2.70 in complete agreement with the previous estimate. Thus the approximately linear relation between extract concentration and yield at a constant water-to-tea ratio is well accounted for by the relative constancy of the specific net liquid uptake.

It will be observed that the relation between extract concentration and yield given by eqn (5) does not predict by itself the particular concentration and yield to be expected in any extraction, but merely gives the locus of possible values. The precise value depends on the conditions of extraction. From Fig. 3 it will be seen that 400 g tea gave better results than 200 g, and conditioned leaf was superior to unconditioned leaf, although the latter difference was small when using 400 g tea. The explanation of these effects seems to be that the poorer the convective mixing in the column, the higher the yield and concentration. In fact, it was prior consideration of this possibility which led to adoption of conditioning as a mode of extraction in these experiments. Conditioning *in situ* causes the leaf to swell so that it is compressed against the column walls thereby reducing mixing when the bulk of water is added. Likewise the higher the bed of tea in relation to its diameter, the poorer the mixing. Thus it would appear that a lower limit of performance of an extractor should occur with perfect mixing and a higher limit with plug flow of liquid through the bed. To test this hypothesis a theoretical comparison has been made between extract concentrations in a well-stirred system and those resulting from plug flow of extract through a bed of swollen leaf. In both systems the tea was assumed to have the same soluble-solids content and physical properties, and to obey the same first order law of mass transfer.

For well-stirred systems having water-to-tea ratios of 4 and 5, concentration of extract was calculated as a function of time by eqn (21) (Appendix), taking the following typical values for the parameters involved:

effective soluble solids content of leaf	0.35 mass fraction
density of soluble solids	1.6 g/ml
extract concentration at zero time	0 g/ml
specific net liquid uptake	2.7 ml/g
rate constant for mass transfer	0.4 min^{-1}

Needless to say these values were taken for purposes of illustration only and may not be absolutely correct for the present conditions of extraction; this particularly applies to the rate constant. The results are shown as broken lines on Fig. 5. As may be predicted from the form of eqn (21), equilibrium is approached slightly faster at the lower water-to-tea ratio. The equilibrium extract concentrations were calculated to be 0.0830 and 0.0671 g/ml respectively, values slightly less than the average for runs with 200 g unconditioned

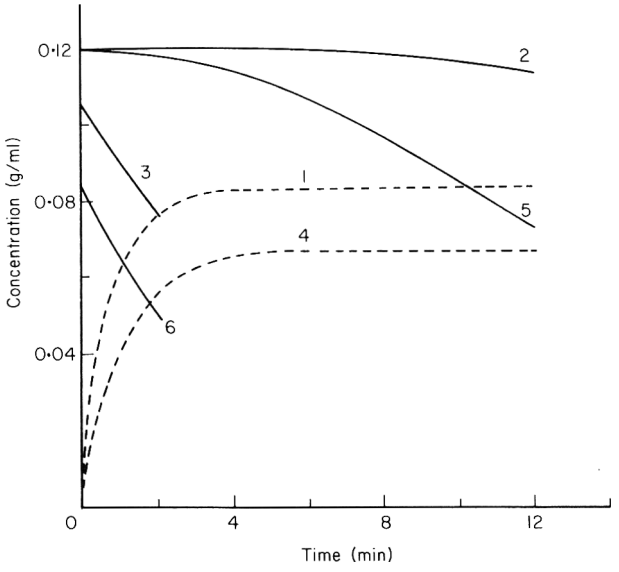

Figure 5. Theoretical comparison of extract concentrations obtainable by well-stirred extraction and percolations at two levels of water-to-tea ratio. Key: broken lines, well-stirred; solid lines, percolations; curves 1, 2 and 3: water-to-tea mass ratio = 4 : 1; curves 4, 5 and 6: water-to-tea mass ratio = 5 : 1.

leaf (i.e. 0.0850 and 0.0694 g/ml). By itself this level of agreement only signifies good selection of a figure for effective soluble solids content; if it had been thought worthwhile even closer agreement could have been obtained.

For the sake of simplicity in the calculation of plug-flow percolations it was assumed that leaf was initially conditioned with an amount of water equal to the net liquid uptake. Under such conditions, for a leaf having the properties previously detailed, the strength of tea extract initially absorbed by the tea would be about 0.12 g/ml. This then will be the maximum concentration extractable from the bed as defined. (However, if leaf is conditioned with tea extract percolating from higher zones, as may well happen in practice, higher local concentrations are possible near the bottom of the bed. The latter situation may be calculated also, but the computation is too complex for present use.) The parameter which determines the concentration of extract leaving a percolated bed is the volumetric flow rate of extract per unit mass of tea used to prepare the bed. This can be related to a specific overall water-to-tea ratio by considering a definite time of discharge of extract. For the present calculations, two times of percolation have been considered, viz: 2 and 12 min. These values represent the average observed time of drainage and total time of extraction and drainage respectively in the present experiments, and are therefore limits between which the actual percolation time lay.

The calculated volumetric concentrations of extract leaving the bed at various times are shown as solid lines in Fig. 5. As might be anticipated, concentration falls with increasing time and water-to-tea ratio. Clearly slow percolation is predicted to give higher concentrations than rapid percolation.

The reason for this is connected with the finite rate of mass transfer of tea solids, since if water passes the column rapidly there is less time to effect diffusion to unit volume of extract. Another prediction of interest is that percolation for a given period of time results in a higher average concentration of extract than with a well-stirred system operating for the same period of time and the same overall water-to-tea ratio. The average concentrations of extract found by integration were: 2 min percolation, 0.091 and 0.066 g/ml; and 12 min percolation, 0.119 and 0.103 g/ml at water-to-tea ratios of 4 : 1 and 5 : 1 respectively. The latter results are not too different from the average for 400 g of conditioned leaf: viz. 0.118 and 0.098 g/ml at the two water-to-tea ratios respectively. Thus it is concluded that conditions which restrict mixing will give highest extract concentrations, and that concentrations obtained from 400 g of conditioned leaf are probably not far removed from the maximum attainable in the present apparatus.

Finally, consideration will be given to the third feature of Fig. 3, i.e. decreasing yield and increasing concentration with decreasing water-to-tea ratio for constant mass of tea and mode of extraction. This aspect of extraction is difficult to treat generally because of the complex conditions of flow and mixing occurring in the bed being extracted. Even for the relatively simple case of plug flow, the general solution involves numerical methods which have to be specifically evaluated for each example. Thus the best that can be done theoretically is to show that the trend found is to be expected in specific cases, and to infer from this that it is probably generally true.

The simplest case to consider is the equilibrium condition in a well-stirred batch extraction. The equilibrium concentration is then given by eqn (17b) (Appendix). Substituting this expression in eqn (5) and rearranging gives the following expression for yield:

$$Y = \frac{S'(W - V_n)}{W + S'/\rho_{sol}}. \tag{6}$$

Now if $W \gg S'/\rho_{sol}$, this can be simplified to

$$Y \simeq S'(1 - V_n/W). \tag{7}$$

This solution clearly has the required property that as W decreases, Y decreases. Also on expansion, and reapplying equation (17b), we get:

$$Y \simeq S' - V_n c_e \tag{8}$$

which is simply a statement that in equilibrium, yield is equal to total effective solubles content less the solubles absorbed as extract which is approximated by $V_n c_e$. This result requires the relation between yield and concentration to be linear with a negative slope equal to V_n. In the present experiments, the nearest approach to the well-stirred condition occurred in the runs with 200 g of unconditioned leaf. For these runs, the correlation coefficient between yield and volumetric concentration was found to be -0.974 and the slope -3.03 ml/g tending well towards the predictions for the limiting case.

Table 1. Results of linear regressions of yield upon volumetric concentration for different modes of extraction

Mass of tea (g)	State of leaf	Yield at zero concentration (mass fraction)	Slope	Correlation coefficient
200	Unconditioned	0.374	−3.03	−0.974
200	Conditioned	0.379	−2.03	−0.858
400	Unconditioned	0.375	−1.84	−0.707
400	Conditioned	0.404	−2.07	−0.950

For other methods of extraction, it is also to be expected that yield will be some function of concentration and that maximum yield will be obtained at zero concentration and will be equal to the effective soluble solids content. Thus to a first approximation it is quite likely that yield can be represented in all cases by an equation of the form of eqn (8), but having a less negative slope. Linear regressions of yield upon volumetric concentration for all results before averaging, treating each condition of extraction separately, gave the coefficients shown in Table 1. Statistical significance at the 5% level was achieved in two cases only but considering the excessive distance of extrapolation and the scatter in the original data, the intercept is remarkably constant with an average value 0.383. Thus it would appear that Fig. 3 is really part of a larger diagram in which lines of constant water-to-tea ratio converge at the origin and lines of constant extraction conditions converge at a point corresponding to the effective soluble solids content of leaf and zero concentration. However, in view of the wide spectrum of solubility of tea solids it is not to be expected that such a diagram would be valid at low extract concentrations but extrapolation to a hypothetical solubles content does support previous evidence (Long, 1977) that at low water-to-tea ratios the tea-water system can be well approximated by a system of three components with a constant ratio of solubles to insolubles.

It will be seen that the value of fractional effective soluble solids found by extrapolation is close to, but not completely consistent with, the value arbitrarily chosen for the comparative theoretical study. The latter was based on the undoubted oversimplifications of a simple first order solution law and idealized hydrodynamics and to get better agreement a more realistic model is required. From the level of effective solubles content found in the short contact times available it is clear that extraction is a rapid process as might be expected for a material which is probably only a dozen or so cells in thickness and is crushed in manufacture. The closeness of approach to equilibrium is seen by comparison with an extrapolated equilibrium yield of 39.2% on an as-received basis reported for a comparable tea by Prabhudesai (1969) after extractions at 97–100°C for 4 hr with water-to-tea ratios in the range 20:1 to 5:1. With regard to the hydrodynamics it is surprising that a medium which ultimately contained only 25–40% free liquid could sustain a level of mixing which even remotely approached perfect stirring in effect. However, mechanical

stirring produced little reduction in extract concentration beyond the lowest values reported here. Effects of stirring in a redesigned column and measurement of rate constants for the solution process will be considered in a subsequent publication.

Conclusions

(1) The relation between yield and extract concentration was found to be a function of water-to-tea ratio and the conditions of extraction.

(2) At a constant water-to-tea ratio, yield increased approximately linearly with increasing concentration, the highest values of both yield and concentration being associated with extraction conditions which restricted convective mixing to a minimum.

(3) With constant conditions of extraction, extract concentration decreased as water-to-tea ratio increased, while yield increased approximately linearly with falling concentration.

(4) The range of extraction conditions available lies between two theoretical extremes: a perfectly stirred system which gives the lowest concentrations and plug-flow percolation which gives the highest concentrations. Quantitative theory has been developed for both cases.

Acknowledgments

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Appendix

Simple quantitative theory of extraction

This treatment is the simplest quantitative approach with predictive value. It is not claimed that it is scientifically exact in all its aspects, but it will explain trends found in extraction. It should be viewed as a useful approximation, having simplicity as its principal merit.

(i) *General nomenclature.* Consider 1 g of original leaf extracted with W ml water to give extract at a fractional yield Y, with a specific net liquid uptake in the residue of V_n ml/g. Thus the volume of extract will be $(W - V_n)$ ml and the concentration $Y/(W - V_n)$ g/ml.

Let the solubles dissolving during extraction comprise a fraction S of the original leaf and let the densities of solubles and insolubles be ρ_{sol} and ρ_{insol} respectively.

(ii) *Volume of swollen leaf.* The volume of swollen residue (V_r) comprises the volume of absorbed water (V_a) plus the volume of leaf solids originally present reduced by the volume previously occupied by leaf solids now dissolved in free extract outside the leaf. Assuming tea solids form a perfect solution (i.e. the solubles occupy the same volume in solution as in solid state), then it follows that:

$$V_r = (S - Y)/\rho_{sol} + (1 - S)/\rho_{insol} + V_a. \tag{9}$$

Now the volume of free extract equals the volume of dissolved leaf solids plus a volume of water equal to the difference between the water-to-tea ratio and the volume of water absorbed by the leaf, i.e.

$$W - V_n = Y/\rho_{sol} + W - V_a \tag{10}$$

so on rearranging

$$V_a = V_n + Y/\rho_{sol}. \tag{10a}$$

Eliminating V_a between eqns (9) and (10a) gives:

$$V_r = S/\rho_{sol} + (1 - S)/\rho_{insol} + V_n. \tag{11}$$

Hence, if V_n is constant, V_r is also constant. It will also be seen from eqn (11) that the volume of the swollen residue is the sum of the net liquid uptake and the volume of original leaf, i.e.

$$V_r = V_n + 1/\rho_{leaf} \tag{11a}$$

where ρ_{leaf} is the density of original leaf.

(iii) *Mass of swollen leaf.* The mass of swollen residue (M_r) comprises the mass of absorbed water plus the mass of tea solids originally present less that mass now dissolved in free extract. Thus:

$$M_r = V_a \rho_w + 1 - Y. \tag{12}$$

Substituting for V_a from eqn (10a) and rearranging gives:

$$M_r = 1 + V_n \rho_w - Y(1 - \rho_w/\rho_{sol}). \tag{13}$$

Hence the mass of residue is a function of yield. However the final term on the right hand side of eqn (13) is small in comparison with the preceding term so mass is approximately constant if V_n is constant.

(iv) *Relation between yield and concentrations in extract and leaf.* Let c_e and c_r denote volumetric concentrations (g/ml) of soluble tea solids in free extract and leaf residue respectively.

It has previously been pointed out (in (i)) that :

$$c_e = Y/(W - V_n). \tag{14}$$

Thus if V_n is constant, c_e and Y are linearly related.

The concentration of soluble tea solids in the residue may be conveniently represented as resulting from the solution of all solubles in the absorbed water. This is undoubtedly an over-simplification since it neglects the presence of undissolved soluble solids, but can be made to correspond closely to reality over a limiting range of water-to-tea ratios and extract concentrations by a suitable choice of soluble solids content.

Thus the volume of extract held by the porous leaf matrix is $V_a + (S - Y)/\rho_{sol}$, which, from eqn (10a) is equivalent to $V_n + S/\rho_{sol}$.

It follows therefore that:

$$c_r = (S - Y)/(V_n + S/\rho_{sol}) \tag{15}$$

or, rearranging,

$$Y = S(c_{r,0} - c_r)/c_{r,0} \tag{15a}$$

where $c_{r,0} = S(V_n + S/\rho_{sol})$.

Obviously $c_{r,0}$ may be regarded as the starting concentration of extract in leaf. It is a mathematically-defined quantity which happens to correspond physically to the fictitious situation where all swelling is complete but extraction has not started.

(v) *Equilibrium concentrations and definition of effective solubles.* In general the equilibrium relation between leaf and extract can be expressed by a distribution coefficient λ defined as the ratio of equilibrium concentrations $(c_{r,eq}/c_{e,eq})$.

Thus from eqns (14) and (15),

$$\lambda = (S - Y_{eq})(W - V_n)/[Y_{eq}(V_n + S/\rho_{sol})] \tag{16}$$

where Y_{eq} is the equilibrium yield.

Now since S has been defined in terms of the soluble solids dissolving during extraction, and has in effect to be calculated from the terminal conditions of an extraction experiment, there will be no loss in rigour in adopting instead a value for 'effective' soluble solids S' based on the further restriction of $\lambda = 1$.

Thus on substituting $\lambda = 1$ and $S = S'$ in eqn (16), it follows that:

$$S' = WY_{eq}/(W - V_n - Y_{eq}/\rho_{sol}) \tag{17}$$

or

$$Y_{eq} = S'(W - V_n)/(W + S'/\rho_{sol}). \tag{17a}$$

Clearly as $W \to \infty$, $S' \to Y_{eq}$ so that S' could be thought of as the yield at infinite dilution. This is not a true physical picture, however, since S, to which S' is related, is a function of W. It is better to consider the equations at constant W, where it will be seen that $S' = Y_{eq}$ if $V_n + Y_{eq}/\rho_{sol} = 0$. By reference to eqn (10a) it will be seen that the latter condition corresponds to zero absorption of water. Thus the effective solubles content, at a particular water-to-tea ratio, is equal to the fictional yield calculated assuming all water is present as extract at the equilibrium concentration. The effective solubles content will then be a function of water-to-tea ratio, and at infinite dilution will correspond to the result found by soxhlet extraction. From eqns (14) and (17a), it follows that the equilibrium concentration is given by:

$$c_{e,eq} = S'/(W + S'/\rho_{sol}). \tag{17b}$$

(vi) *The approach to equilibrium in well-stirred conditions.* From eqn (11), the volume of tea solution contained by a porous leaf residue is $V_n + S'/\rho_{sol}$. Likewise, by definition, the volume of extract is $W - V_n$.

By the law of conservation of mass,

mass of soluble solids leaving residue = mass of soluble solids entering extract

Thus,

$$-(V_n + S'/\rho_{sol})\frac{dc_r}{dt} = (W - V_n)\frac{dc_e}{dt}. \tag{18}$$

Assuming the rate of mass transfer is proportional to concentration difference between solutions in the residue and extract, the rate of change in the concentration of solution in residue may be expressed as:

$$-\frac{dc_r}{dt} = K(c_r - c_e) \tag{19}$$

where K is the mass transferred per unit volume of solution in residue per unit concentration difference.

From eqns (18) and (19), the rate of increase in extract concentration may be simplified to:

$$\frac{dc_e}{dt} = AK(c_r - c_e) \tag{20}$$

where $A = (V_n + S'/\rho_{sol})/(W - V_n)$.

Rearranging eqns (19) and (20) and eliminating c_r gives a homogeneous linear equation with constant coefficients for which the roots of the auxiliary quadratic are 0 and $-(A + 1)K$.

Hence the solution has the form:

$$c_e = C_1 + C_2 \exp[-(A + 1)Kt].$$

The boundary conditions are:

$$c_e = c_{e,0} \text{ at } t = 0$$

and

$c_e = c_{e,eq}$ at $t = \infty$.

Therefore the required solutions are:

(a) extract concentration

$$c_e = c_{e,eq} - (c_{e,eq} - c_{e,0}) \exp[-(A+1)Kt) \tag{21}$$

(b) residue concentration

$$c_r = c_{e,eq} + (K/A)(c_{e,eq} - c_{e,0}) \exp[-(A+1)Kt]. \tag{22}$$

Thus the time required to reach a fraction f of equilibrium is given by:

$$t_f = \frac{\ln[1/(1-f)]}{(A+1)K}. \tag{23}$$

Clearly the larger the value of A (i.e. the smaller the value of W), the faster equilibrium will be reached, if K is constant.

(vii) *Percolation through a bed of swollen leaf (Plug flow).* Let the cross sectional area of the bed be A_b, and the porosity be E.

Using the same mass transfer law as in the well-stirred case, the rate of mass transfer ($\dot{m}_{\delta z}$) in an element of bed of height δz is given by:

$$\dot{m}_{\delta z} = A_b \delta z (1-E) K (c_r - c_e). \tag{24}$$

From a mass balance on the residue in the element, mass transferred equals depletion in concentration. Therefore,

$$\dot{m}_{\delta z} = -A_b \delta z (1-E) \left[\frac{V_n + S'/\rho_{sol}}{V_n + 1/\rho_{leaf}}\right] \times \frac{\partial c_r}{\partial t} \tag{25}$$

A mass balance on the extract present in the element shows the mass transferred to equal the net output from the element carried by flow, plus the accumulation of extract within the element. Therefore,

$$\dot{m}_{\delta z} = \frac{\dot{V} \partial c_e}{\partial z} \delta z + A_b E \, \delta z \, \frac{\partial c_e}{\partial t} \tag{26}$$

where $\dot{V}$ is the volumetric flow rate.

By equating eqns (24), (25) and (26), it is found that:

$$\frac{\partial c_r}{\partial t} = -\beta (c_r - c_e) \tag{27}$$

and

$$\frac{\partial c_e}{\partial z} + \frac{\gamma \partial c_e}{\partial t} = \alpha (c_r - c_e) \tag{28}$$

where $\alpha = A_b K (1-E)/\dot{V}$

$\beta = K(V_n + 1/\rho_{leaf})/(V_n + S/\rho_{sol})$

$\gamma = A_b E/\dot{V}$.

If the variables t, z are transformed to the new variables $\tau = \beta(t - \gamma z)$, and $\zeta = \alpha z$, eqns (27) and (28) simplify to:

$$\frac{\partial c_r}{\partial \tau} = c_e - c_r \tag{29}$$

and

$$\frac{\partial c_e}{\partial \zeta} = c_r - c_e. \tag{30}$$

The boundary conditions are:

When $\tau = 0$, $c_r = c_{r,0}$ for all $\zeta > 0$.

When $\zeta = 0$, $c_e = c_{e,0}$ for all $\tau > 0$.

The solution of eqns (29) and (30) has been obtained by Anzelius (1926). Fitting this solution to the present problem gives:

$$\frac{c_{r,0} - c_r}{c_{r,0} - c_{e,0}} = \exp(-\zeta) \int_0^\tau \exp(-s)\, I_0(2\sqrt{\zeta s})\, ds \tag{31}$$

$$\frac{c_e - c_{e,0}}{c_{r,0} - c_{e,0}} = \exp(-\tau) \int_0^\zeta \exp(-s)\, I_0(2\sqrt{\tau s})\, ds \tag{32}$$

where I_0 is a hyperbolic Bessel function of the first kind and zero order.

Charts of the functions appearing on the right hand side of eqns (31) and (32) have been published by Schumann (1929) and Furnas (1930) for certain values of τ and ζ. Alternatively, the functions may be computed from the series obtained by integrating by parts.

In the present work, interest centres mainly on the concentration leaving a bed of height h. The corresponding value of ζ is given by $\zeta' = \alpha h = A_b K(1 - E)h/\dot{V}$.

Now $A_b(1 - E)h$ is the true volume of residue resulting from a charge of tea of mass T (say).

Therefore, from eqn (11a) it follows that,

$$\zeta' = \alpha h = K(V_n + 1/\rho_{leaf})\, T/\dot{V}. \tag{33}$$

From the above equation, at constant $T/\dot{V}$, ζ', and hence leaving concentration, is independent of the mass of tea used.

Values of τ are given by: $\tau = \beta(t - A_b Eh/\dot{V}) = \beta(t - t_h)$ where t_h is the time taken for the percolating liquid to traverse the bed. Thus if t' is the elapsed time since the appearance of the first extract, then $\tau = \beta t'$.

Substituting for β, it follows that,

$$\tau = K\left(\frac{V_n + 1/\rho_{leaf}}{V_n + S'/\rho_{sol}}\right) t'. \tag{34}$$

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Organoleptic assessment of meat: a comparison of beef breeds

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Summary

Palatability of five beef breeds was assessed using a series of paired comparisons. The participants preferred Angus, Hereford, Friesian crossbred beef to Maine Anjou and Pie Rouge crosses. A method of analysis for non-homogeneous products such as meat is discussed.

Introduction

Consumer panels have been used with varying degrees of success to measure responses to food products. Often samples are distributed to participants to be evaluated one at a time in their homes (Kirton, 1968; Hendrix *et al.*, 1963). Useful data for meat are difficult to obtain with this method because, first, there is no control over the treatment of the product prior to cooking; secondly, cooking methods are very varied and may influence scores; and thirdly, there is often a blasé attitude by the taster towards scoring and returning the score sheets.

In this study, an approach similar to that of Simone & Pangborn (1957), which eliminates variable home factors, was used to test the participant's preference in meat from five beef breeds. This was considered desirable due to criticism that trained panels do not reflect the opinion of the consuming public.

Materials and methods

Meat to be evaluated came from 20-month old steers from a beef breeding programme. Eight animals from each of the following five breeds were used:

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purebred Angus, Hereford × Angus, Friesian × Angus, Maine Anjou × Angus, and Pie Rouge (French Simmental) × Angus. Purebred Angus is the most common beef breed in New Zealand, while Hereford × Angus is the beef cross most widely used, and Friesian × Angus is the most common dairy-beef cross in New Zealand. The Maine Anjou and Pie Rouge are recent introductions into New Zealand and were included because they had scored highest and lowest of several new breeds in a preliminary, trained panel evaluation (Moore, unpublished observations).

All animals were grazed on perennial ryegrass-clover pasture with winter hay supplements from weaning to slaughter.

The animals were slaughtered by conventional techniques. The right *M. longissimus* with fat cover was removed from the carcase within an hour of slaughter, vacuum-packed in polyethylene bags, and held for 24 hr at 10°C in single layers in an air-flow of 0.5–1.0 m/sec and 90–95% relative humidity (Schmidt & Gilbert, 1970). The muscles were then frozen to −18°C and stored for 2–6 weeks before being cut into 25 mm thick steaks. The labelled steaks were thawed at 0°C for 48 hr, then at 2°C for a further 24 hr.

The available carcases were blocked on the basis of weight, and a replicate comprised one such block with a carcase from each of the five breeds. Four steaks from each carcase were used for the breed comparison, and each of the ten possible pair-wise comparisons among five breeds made, with eight people tasting steak from the same breed pair of the same replicate. With eight such replicates, sixty-four people would assess a given breed pair and a total of 640 people were required in the programme, since each person was given only one comparison to make. Visitors to an annual Farmers' Field Day were enlisted for the tests. A simple statement of preference was sought from each. Of the 640 participants approximately 70% were male.

Three pairs of steaks were grilled simultaneously on racks 88 mm below the elements of domestic ovens set at 205°C. Steaks were cooked for 12 min, turned and cooked for a further 10 min, resulting in a medium-well done steak. Cooking was arranged so that there was a constant supply of hot steaks to be tasted. All steaks of a given replicate were cooked and tested before those of the next replicate, but the order of cooking of breeds was randomized. Each hot steak was cut into sixteen cubes approximately 20 mm square. Two cubes per sample per taster were used rather than one large cube, to enable the taster to assure himself of his judgment. The two cubes were placed on the appropriate side of a small plastic dish which was divided into regions labelled A and B. Breeds were assigned to A or B in a random manner so that in four out of the eight replicates each breed was A. The dish was presented to the taster, who was requested to indicate preference for sample A or B, or no preference, on a coded score sheet. There was no control over the order in which tasters arrived at the tasting site and no control over the sex or age of the tasters, although only those over 12 years were allowed to participate.

For analysis, where preference was expressed, the preferred sample scored +1 and the other sample of the comparison −1; where no preference was expressed,

both samples scored 0. These scores were added for individual carcases within replicates to obtain relative scores for breeds within replicates. The sum of scores for a replicate is zero. Two types of analysis were carried out; first, an analysis of variance using the summed score for each breed in each replicate enabled the comparison of mean scores of breeds against variation of breeds within replicates. Secondly, the five breeds for each replicate were ranked from highest to lowest score, with highest being assigned a value of 1 and lowest a value of 5. Rank sums were calculated across the eight carcasses and Friedman's Chi-squared test (Siegel, 1956) was applied.

Results and discussion

The data are summarized in Tables 1 and 2. Table 1 gives the number of positive responses for each breed. Both methods of analysis in Table 2 indicated that the differences between breeds approached significance ($P < 0.10$), and the Duncan's multiple range test (Duncan, 1955) on the analysis of scores indicated the breeds fall into groups: Angus × Angus, Hereford × Angus, and Friesian × Angus scoring similarly and preferred to the Maine Anjou × Angus and Pie Rouge × Angus.

Table 1. Summary of positive and negative responses for each of the ten comparisons.

Comparison	Response		
	Positive	Negative	Neither
Angus*–Hereford	29	27	8
Angus–Friesian	33	28	3
Angus–Maine Anjou	36	25	3
Angus–Pie Rouge	41	23	0
Hereford–Friesian	29	33	2
Hereford–Maine Anjou	39	18	7
Hereford–Pie Rouge	33	28	3
Friesian–Maine Anjou	44	19	1
Friesian–Pie Rouge	41	20	3
Maine Anjou–Pie Rouge	26	30	8

* Positive responses are for the first breed of each pair listed in the comparison.

The marked preference expressed for three of the breeds agrees with results of another study (Moore & Bass, 1978) in which a trained panel ranked these five cross-breeds as follows: Purebred Angus 1, Hereford 2, Friesian 4, Maine Anjou 5, and Pie Rouge 7 out of ten breeds compared. This ranking closely followed the order of preference obtained from the panel's scores for general acceptability on a 1–9 scale. In the case of the trained panel, the differences were highly significant ($P < 0.01$). The reasons for the preference in

Table 2. Breed preference rankings

Sire breed	Angus	Hereford	Friesian	Maine Anjou	Pie Rouge
Mean score	4.5[a]	4.4[a]	4.9[a]	−8.8[b]	−5.0[b]
Rank sum	19.5	18.0	21.5	33.0	28.0

[a,b]Scores with the same superscript are not significantly different from each other, $P < 0.10$ (Duncan, 1955).

the present study are not clear, as tasters were not asked to comment specifically on why they expressed their particular preference. The tenderness of the meat was similar and of an acceptable quality (MIRINZ tenderometer shear force values ranged from 30–40), therefore the tasters may have based their decision on some other characteristic, perhaps flavour.

Angus, Hereford and Friesian breeds are commonly used for beef production in New Zealand, so the tasters would have been familiar with the product from these breeds, and when faced with a slightly different meat may have expressed a preference for the meat with which they were familiar.

The use of paired comparisons in studies of this type is common when homogeneous products are being used (Bradley, 1976; Simone & Pangborn, 1957). However, in studies of consumer preferences for meat, the fact that there may exist animal to animal variation even within a breed must be recognized. The sensitivity of a tasting programme will depend on the number of carcases tasted, and the analysis of the present data was based on this premise. The scores for individual carcases within a replicate are in fact analogous to the 'preferences' calculated in Scheffé's (1952) paired comparison analysis. Therefore, in the present analyses of scores, breed differences are assessed relative to the variation in 'preference' across replicates.

This method of assessing participant reaction has several obvious advantages. First, the meat is treated similarly before serving; secondly, temperature and appearance at time of tasting can be controlled; and thirdly, treatments are compared pair-wise at the same time by the same person. Because an enlarged number of tasters can be accommodated, it is possible to form complex comparisons while demanding only simple decisions from each of them.

Acknowledgments

The authors are grateful to Dr A. H. Carter of Ruakura for supplying the animals for this study.

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Non-effect of dilute alkali solutions on the number average granule diameters of some legume starches

CHRISTOPHER G. ANDERSON*

Summary

Starches of field bean, garbanzo and lentil were isolated according to a detailed separational scheme (Anderson & Romo, 1976) which employed several different aqueous protein extraction media adjusted to pH values in the range of 5.5 to 9.5. Number average granule diameters were determined for the isolated starch granules using photomicroscopy in order to ascertain the effect of protein extraction medium alkali concentration, if any, on this parameter. It was established that the number average granule diameters of the three starches under investigation were not influenced by a change in alkali concentration of the protein extraction-medium within the pH range studied. Accordingly, garbanzo starch granules exhibited a pooled number average granule diameter of 24 μm with a standard deviation of 4 μm, while both lentil and field bean starch granules exhibited pooled number average diameters of 27 μm with a standard deviation of 7 μm. Granule size distributions based on more than 1100 separate measurements were also constructed for the three starches prepared at neutral pH in the form of histograms for the sake of comparison. The distributions for field bean and lentil are similar, both showing some skewness with that for field bean being most pronounced. The distribution for garbanzo, on the other hand, is more normal and much more narrow than the other two examples.

Introduction

The utilization of legumes other than soya as sources for the production of protein isolates may become important in the future. This is particularly true in countries like Chile, which produce little or no soya, but require protein

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concentrates with desirable functional properties for incorporation into manufactured foods.

Many of the legumes cultivated in Chile contain, in addition to usable amounts of protein, substantial quantities of starch which can be readily isolated and purified after extraction of protein. Legume starches then, represent potentially valuable products to be derived from some legumes. Hence, determination of the effect of protein extraction conditions on the physical properties of these starches is considered to be important in advancing their practical utilization.

One important protein extraction parameter which influences certain physical properties of some legume starches is the pH of the extraction medium used to solubilize protein. Protein isolates are normally prepared by extraction of legume flours with dilute aqueous alkali solutions. A recent study has shown (Anderson & Romo, 1976) that the concentration of alkali in the protein extraction medium can affect the protein content (as a contaminant) and colour of purified legume starches without affecting the yield.

Thus, in this communication another possible effect of protein extraction medium pH is considered, that is, the effect of this variable on the number average diameter of starch granules obtained from three Chilean varieties of legume: lentil bean (*Lens culinaris*), field bean (*Phaseolus vulgaris* var. Coscorron) and garbanzo bean (*Cicer arietinum*). The technique of photomicroscopy was employed to measure granule diameters and the pH range studied was 5.5 to 9.5. In addition, the population distributions of granule diameters were prepared for the three starches from measurements obtained at neutral pH, which are based upon more than 1100 separate measurements. These data are presented in the form of histograms.

Materials and methods

Materials

Samples of Chilean grown lentil, garbanzo and field beans were purchased from local markets and the starches, isolated according to the procedure described below, stored over silica gel in a desiccator until determinations were made. A Zeiss Standard Microscope equipped with a 6.3 × Planachromat objective, a 12× occular, a camera adapter and a 35 mm single lens reflex XE-1 Minolta Camera (without lens) were employed for measurement of granule sizes. All chemicals were reagent grade.

Extraction procedure

Starches were isolated and purified according to the previously described method of Anderson & Romo (1976).

Determination of granule sizes

Individual starches of lentil, garbanzo and field bean isolated utilizing several extraction media whose pH values were adjusted to 0.5 pH unit intervals between 5.5 and 9.5 ± 0.05 were separately introduced as a 5% suspension (H_2O) into a Hemacytometer (Spencer, bright line, improved Neubauer). This apparatus, which possesses a micrometer scale with the smallest division being equal to 50 μm, was placed on the specimen stage of the microscope (Schoch & Maywald, 1956) and the sample photographed at a magnification of approximately 75×. The pH of each sample was previously checked and found not to vary by more than 0.1 pH units from pH 7.0. After photographing the samples, the film was developed and mounted in slide frames. Each slide was introduced into a Kodak slide projector and the image projected onto a vertical surface covered with white paper. The projector was then positioned in such a way that the smallest division of the Hemacytometer (50 μm) corresponded to a projected measurement of 50 mm on the projection surface or such that 1 μm equalled 1 mm. The major axis or longest axis of 300 garbanzo and 400 lentil and field bean starch granules was then measured directly with a meter stick to the nearest 1 mm, each granule being marked on the paper as it was counted. Finally a number average and standard deviation were calculated in micrometers for each sample, the histograms prepared and the data subjected to 'Analysis of Variance' according to Snedecor & Cochran (1967).

Results and discussion

Leach, Schoch & Chessman (1961) determined that swelling of starch granules in aqueous alkali depends on the relative amounts of water, alkali and starch present in the suspension. In addition it was shown by these researchers that individual granules adsorb alkali in a very specific manner and gelatinize or swell when the quantity of adsorbed alkali exceeds a certain critical concentration. Later, Leach (1965) showed that the critical concentration of alkali is dependent on both the type of alkali and the species of starch employed.

In the case of alkaline protein extraction of starch containing legume seeds it can therefore be reasoned that as the pH of the extraction medium is increased, a point could be reached which satisfies the conditions for swelling defined by Leach *et al.* (1961). A three-dimensional irreversible structural change would occur in the granules and the gelatinization properties of the alkali treated starch would be modified (for example, see Schoch & Maywald, 1956). This may or may not be desirable depending on the starch's application.

Results of this study shown in Table 1 indicate that as the extraction medium pH is varied from 5.5 to 9.5 there is no change (within the limits of experimental error) in the number average granule diameters (major axis) of the three starches. This judgement is based on an analysis of variance according to Snedecor & Cochran (1967). Thus, calculated F values for garbanzo, lentil and

Table 1. Number average granule diameters and standard deviations in μm for lentil, garbanzo and field bean starches prepared at different pH values

Sample	Extraction pH	Mean (μm)	S.D. (μm)
Garbanzo starch	5.5	23.8	4.0
Garbanzo starch	6.0	24.2	4.0
Garbanzo starch	6.5	24.4	4.1
Garbanzo starch	7.0	24.0	3.9
Garbanzo starch	7.5	24.6	3.9
Garbanzo starch	8.0	24.3	3.9
Garbanzo starch	8.5	23.8	4.2
Garbanzo starch	9.0	24.1	4.0
Garbanzo starch	9.5	24.6	4.0
Mean		24.2	4.0
Lentil starch	5.5	27.0	6.9
Lentil starch	6.0	26.4	7.0
Lentil starch	6.5	27.5	6.9
Lentil starch	7.0	27.2	7.4
Lentil starch	7.5	27.1	7.0
Lentil starch	8.0	26.5	7.4
Lentil starch	8.5	26.7	7.1
Lentil starch	9.0	27.1	6.8
Lentil starch	9.5	27.7	6.9
Mean		27.0	7.0
Field bean starch	5.5	27.5	7.2
Field bean starch	6.0	27.1	7.6
Field bean starch	6.5	27.8	7.6
Field bean starch	7.0	27.4	7.0
Field bean starch	7.5	26.2	7.8
Field bean starch	8.0	26.9	7.1
Field bean starch	8.5	26.9	7.1
Field bean starch	9.0	27.0	7.0
Field bean starch	9.5	27.4	7.4
Mean		27.1	7.3

field bean starch granules were determined to be 1.8, 1.2 and 1.3, respectively, compared to a theoretical value for F of 2.51 (Snedecor & Cochran, 1967). Since the calculated F values in all three cases are less than the theoretical value for F ($f_1 = 8$ and $f_2 = \infty$) at the 1% level it is highly probable that no difference exists in the granule diameter means of starch samples prepared at different pH values for a given starch. Accordingly, the conditions for swelling of starch granules elucidated by Leach *et al.* (1961) are apparently never achieved under the experimental conditions employed in this study for protein extraction – even at the highest pH value (9.5).

One must be cautious in considering the possible implications of this result as it applies to the gelatinization properties of the starches. Because, although an irreversible three dimensional change in the granules would definitely

influence the gelatinization properties of a starch, the lack of any change does not necessarily mean that the gelatinization properties would not be affected by some other mechanism. For example, it is easy to imagine that gelatinization properties could be modified as a consequence of slight chemical degradation of the polymer chains produced by alkali attack. Hence in the future, it will also be important to determine the influence of protein extraction medium pH on the gelatinization temperature range of the three starches discussed in this and past publications.

Population distributions

Figures 1, 2 and 3 show the granule size distributions calculated on a population basis and presented in the form of histograms. The dstributions are based upon more than 1100 measurements of granule diameters of starches prepared by protein extraction at neutral pH. The figures show that lentil and field bean starches (Figs 1 and 2, respectively) have similar and more or less broad granule distributions, both demonstrating some skewness with that for field bean being most pronounced. On the other hand garbanzo starch granules exhibit a more normal distribution that is considerably more narrow than the other two examples. Additionally, field bean and lentil starch granules are much more irregularly shaped with the majority of the granules possessing an eliptical or kidney shaped configuration. Both of these starches contain a high proportion of fissured granules (greater than 50%), also. Conversely, garbanzo starch is made up of nearly spherical unfissured granules suggesting a possible new

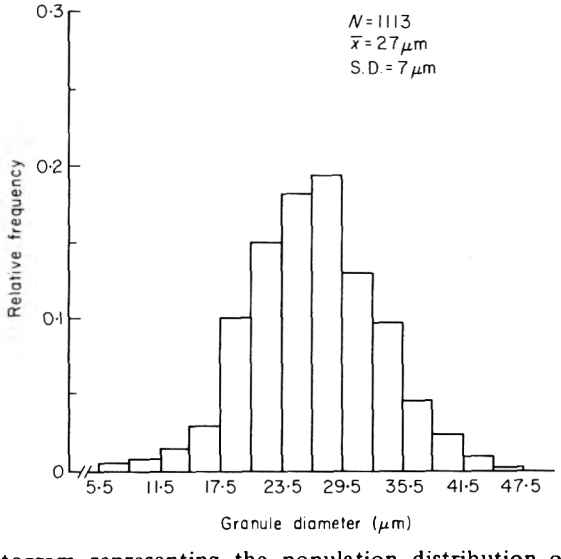

Figure 1. Histogram representing the population distribution of lentil starch granule diameters (major axis was measured).

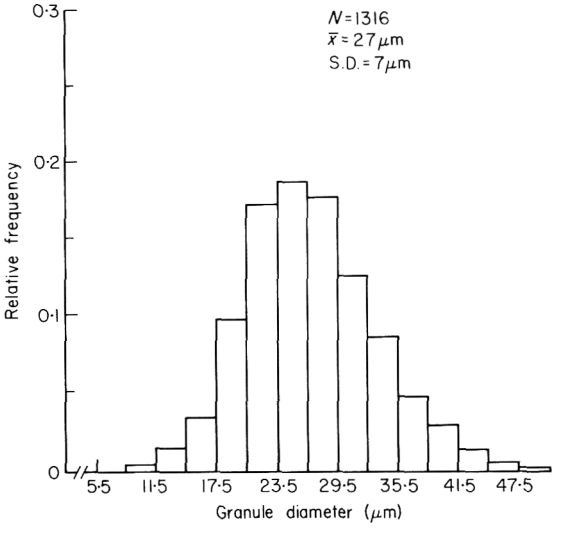

Figure 2. Histogram representing the population distribution of field bean starch granule diameters (major axis of granule was measured).

application for garbanzo starch which will be discussed in a subsequent publication. Incidentally, it should be noted that the values for the mean and standard deviation appearing in Figs 1, 2 and 3 have been rounded off to the nearest 1 μm.

Although population distributions are not shown for the pooled samples (i.e. a population distribution constructed by considering all granule diameter measurements for a given starch over the entire pH range of 5.5 to 9.5), when they are constructed, each is essentially statistically equivalent to its corresponding counterpart shown in Figs 1, 2 and 3. Comparison of data for the

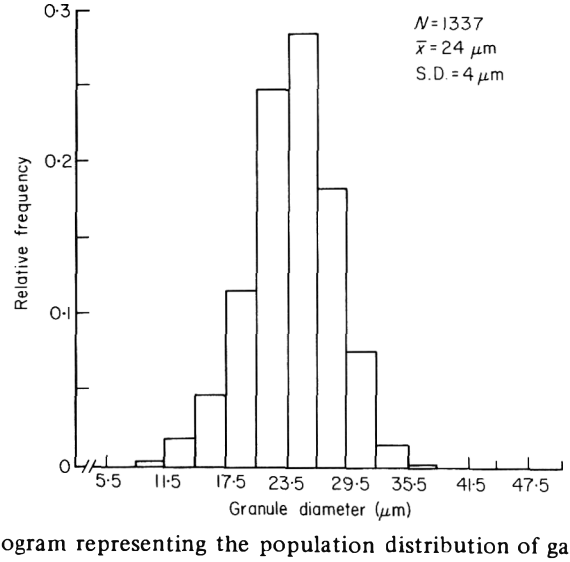

Figure 3. Histogram representing the population distribution of garbanzo starch granule diameters (major axis of granule was measured).

pooled samples to those obtained at neutral pH by means of the *t*-test provides no evidence for rejection of the null hypothesis (i.e. $\bar{\chi}_{pooled} = \bar{\chi}_{pH\ 7.0}$ considering a rejection level of $P < 0.01$). This result further strengthens the earlier conclusion that no difference exists between the means of granule diameters for starches prepared at different pH values for a given starch.

In conclusion, a brief word about sample size seems called for in view of the fact that Schoch & Maywald (1956) have reported that a minimum of 1000 granule measurements should be collected for corn and sorghum starches in order to obtain reproducible population distributions. The author agrees with this conclusion; however, it should be pointed out that it is not necessarily true that 1000 granule measurements be taken for starches from other sources. In this study, for example, only 300 measurements for garbanzo starch and 400 for lentil and field bean starches were necessary to obtain reproducible population distributions. Choice of these numbers is supported by the fact that it is possible to estimate the number of data needed to achieve a statistically significant mean value with a specified degree of confidence by setting a limit of accuracy in the estimate and having some idea of the sigma value for the population from which the sample comes (Snedecor & Cochran, 1967). The actual formula for computing sample size is:

$$n = A^2\sigma^2/L^2$$

where A = some number determined by what confidence is desired in the result and L = limit of accuracy in the result set by the investigator.

Normally, the value for σ is not known since it is a population statistic; however, if enough data are available σ can be estimated by the standard deviation of all sample means which in this study is based on more than 4700 individual measurements for lentil, 4900 for field bean and 4000 for garbanzo starch. At the 1% level, $A = 2.6$, $L = 1\ \mu m$ and σ of all samples for lentil, field bean and garbanzo = 7, 7 and $4\ \mu m$ respectively. These numbers translate to minimum sample sizes of 106, 328 and 356 for garbanzo, lentil and field bean, respectively. In all three cases then, the means of all samples surveyed are based on more than the minimum calculated sample size.

Acknowledgment

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Effect of concentrations and vacua on boiling points of fruit juices

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Summary

Boiling points of fruit juices affect the design and operation of juice evaporators. Hence boiling points of pineapple, mango and lemon juices were experimentally determined at solid concentrations of 5 to 45% and vacua of 0 to 70 cm Hg. As expected, boiling points increased as concentration increased. Duhring's plots are also presented. For the same concentration range, mango juice has the highest boiling point elevations followed by pineapple and lemon juices in that order.

Introduction

Concentration of fruit juices by evaporation is an important method of their preservation. For efficient and economic operation of an evaporator it is necessary that it be designed correctly. The basic equation used in the design of the evaporator is

$$q = UA\,\Delta t \tag{1}$$

where, q is the heat load on the evaporator, U the overall heat transfer coefficient, A the heat transfer surface area and Δt the temperature difference between steam or heating vapour and the boiling juice.

The heat load can, in general, be written as

$$q = m\,c_p(t - t_f) + m_v\,\lambda_v \tag{2}$$

where, m and m_v are, respectively, mass flow rates of feed and vapour evaporated, c_p is the specific heat of the feed, t the average boiling or evaporation temperature of the juice, t_f the feed temperature of the juice and λ_v the

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latent heat of evaporation. It may be observed that t will be higher than the evaporation temperature of water at the same pressure. Thus q is higher than when the boiling point elevation would be assumed negligible; the boiling point elevation of juice would decrease Δt in eqn (1). Thus if the boiling point elevation is not considered, the evaporator would be designed under capacity. In a multiple-effect evaporator, if the large number of effects are used or the boiling point elevations are high, as happens when the concentration increases, the sum of the boiling point elevations in a proposed evaporator could be greater than the total temperature drop available and operation under such conditions is impossible. Actual boiling points of juices at various concentrations and vacua must therefore be known.

Desrosier (1970) has given boiling points of typical fruit juice/sugar mixtures at various altitudes for soluble solid concentration range of 50 to 76%. The data are for unknown fruit/sugar mixtures and are of limited use in evaporator design. Information on boiling points of fruit juices as such are not readily available in the literature (Brennan *et al.* 1974). This paper presents boiling points of pineapple, mango and lemon juices at various concentrations and vacua, and Duhring's plots of boiling points of juices versus boiling points of water at the same pressure for these juices.

Materials

Juice was extracted from pineapple, mango and lemon fruits in a manually operated screw press. The juice was filtered in an ordinary cloth filter to remove suspended particles and concentrated to 10, 15, 25, 35, 45 and 60% solids (d.b.) in a rotary vacuum evaporator (manufactured by Veb Medizintechnik Leipzig, East Germany). The juice, before and after concentration, was stored at about 4°C when there was any substantial time gap in between two processes. The concentration was measured by either a vacuum oven method or by a sugar refractometer. Before use, the sugar refractometers, one ranging from 0 to 50% and the other from 40 to 85%, were calibrated for fruit juices against gravimetric results (Varshney & Barhate, 1977) following the method used by Towler (1976) for sodium caseinate solution.

Method

The Cottrell's apparatus as described by Prutton & Maron (1957) was used with additional arrangements to produce a vacuum. The apparatus consisted of a 10 cm diameter, 30 cm high aluminium boiling vessel fitted with a vapour condenser and a cork from which extended a shield 16 cm long, whose function was to prevent the condensed vapours from the vapour condenser from coming into contact with the Beckman thermometer. The thermometer itself was suspended in vapour above the juice and inside the shield. To assist equilibrium

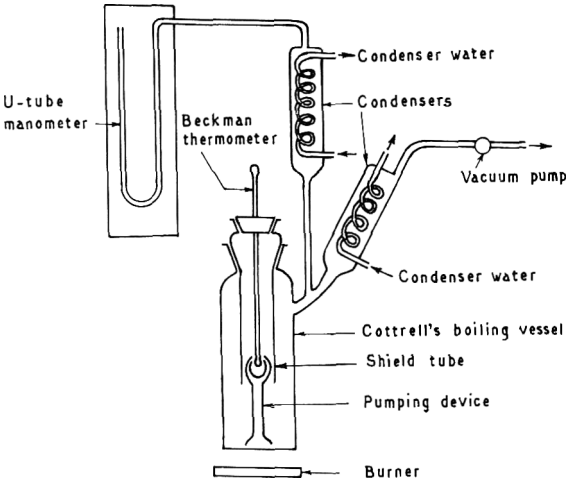

Figure 1. Schematic diagram of experimental boiling point apparatus.

between vapour and boiling juice at the thermometer bulb, a siphon pump was incorporated into the apparatus. The pump consisted of a tube having a funnel shaped base of diameter 6.25 cm and a bent U-shaped top 6.5 cm high with a gap of 2.5 cm between the two bifurcated outlets, which fitted around the thermometer stem as shown in Fig. 1. When the juice boiled the device continually pumped the liquid up in such a way as to keep the bulb always covered with a thin film of boiling liquid and the thermometer reached a steady equilibrium temperature in a very short time. The liquid would go up through the device due to convection along with the bubbles of gas formed and discharge on the bulb and flow down. The cork fitted in the mouth of the aluminium vessel had two holes, one for a thermometer and the other for connections to the vacuum pump and the U-tube manometer through a condenser. The vacuum line also had a condenser. The boiling vessel was heated by a gas burner.

The condensers used were of a spiral tube and shell type with the cooling water flowing through the tube coils and the vapor through the shells. As soon as the vapours entered the condenser from the boiling vessel containing juice, they were condensed and owing to gravity the condensate flowed down through the same tube since the vacuum in the system was constant. This maintained the concentration of the juice constant.

A positive displacement type vacuum pump was used to create a vacuum in the boiling vessel.

The performance of the apparatus was checked on water and sugar solutions whose boiling points at various concentrations and vacua are known (Spencer & George, 1945) and was found to be excellent. In the experiment a 500 ml sample of concentrated juice was filled in the boiling vessel, and all connections were made. The cooling water flow was started in both the condensers, the heating started slowly, and after 2–3 min the vacuum pump started to obtain a

vacuum of 70 cm Hg. When boiling started, maintaining the vacuum, the thermometer reading was taken when a constant temperature was attained for about 5 min. The vacuum was then decreased to the next lower value, i.e. 60 cm Hg and the procedure was repeated. The same was repeated at 50, 40, 30, 20, 10 and 0 cm Hg pressures. The heating was then stopped, and the vessel, allowed to cool down to the room temperature. The juice was removed and the concentration checked.

The juice of another concentration was then placed in the boiling vessel and the above procedure repeated.

Results and discussion

Boiling points of pineapple, mango and lemon juices at various concentrations and vacua are plotted in Figs 2–4. The plots are the change of boiling points with concentrations at different vacua. The boiling point in all the cases increased linearly with the concentration, and hence can be expressed mathematically as

$$T = mx + T_0$$

where T is boiling point, °C; x is mass concentration of solute in the juice, dry basis; and m and T_0 are constants. Regression analysis using the least square method resulted in the values of m and T_0 as tabulated in Table 1.

The boiling point is that the temperature at which the vapour pressure of the liquid is equal to the external pressure. The addition of a non-volatile solute in

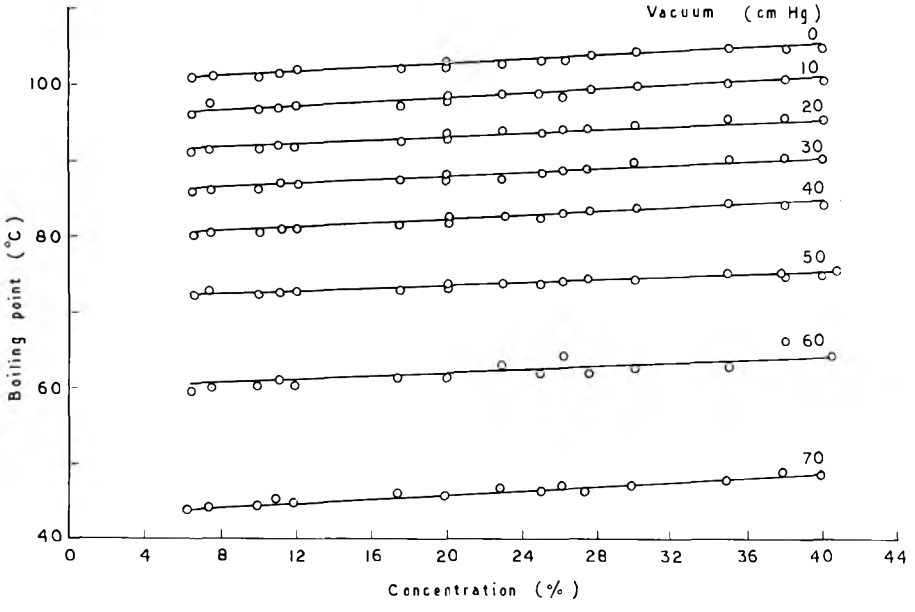

Figure 2. Variation of the boiling point of pineapple juice with concentration at different vacua.

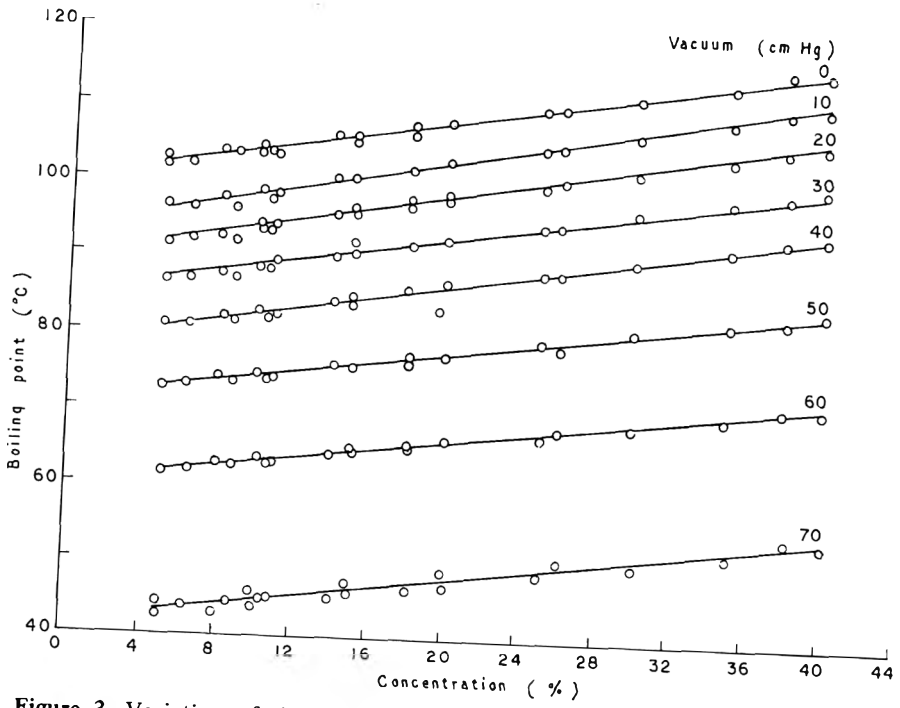

Figure 3. Variation of the boiling point of mango juice with concentration at different vacua.

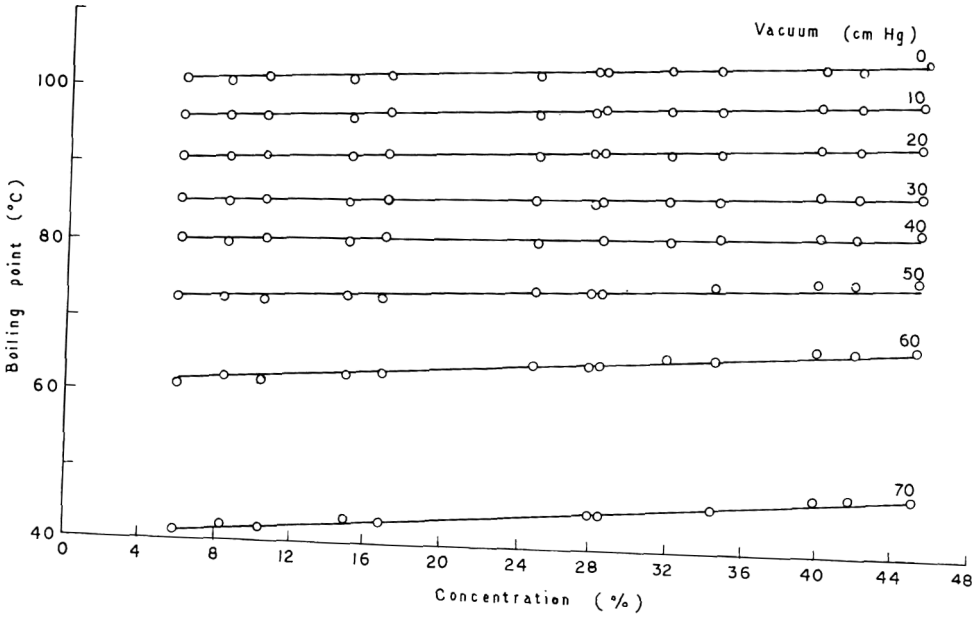

Figure 4. Variation of the boiling point of lemon juice with concentration at different vacua.

Table 1. Values of the constants m and T_0 in equation $T = m\,x + T_0$ for pineapple, mango and lemon juices' plots of Figs 2–4

Juice	Regression constants	Vacua (cm Hg)							
		0	10	20	30	40	50	60	70
Pineapple	m	0.128	0.132	0.189	0.130	0.125	0.093	0.105	0.138
	T_0	100.000	95.360	90.860	85.186	79.504	71.620	60.005	43.210
Mango	m	0.385	0.432	0.412	0.324	0.377	0.303	0.282	0.291
	T_0	100.040	93.370	89.900	85.280	78.880	71.270	60.580	42.500
Lemon	m	0.118	0.097	0.085	0.122	0.077	0.109	0.169	0.121
	T_0	100.100	95.490	90.320	83.280	79.240	71.570	60.185	41.370

a solvent lowers the vapour pressure of the solution in accordance with Raoult's law (Prutton & Maron, 1957). In consequence, the solution requires a higher temperature to be reached before the solution vapour pressure is equal to the external pressure, to cause it to boil. The added solute produces therefore a boiling point elevation, which obviously increases as concentration increases.

In evaporator calculations, apart from the boiling point, a knowledge of boiling point elevation is also required. A very useful plot to estimate boiling point elevation is Duhring's plot. Duhring's Rule states that the boiling point of a given solution is a linear fuction of the boiling point of water at the same pressure. Thus the resulting plots are straight lines and plots for different concentrations are roughly parallel (Foust *et al.*, 1960). Figures 5–7 show these plots for pineapple, mango and lemon juices at various concentrations. It may be noted that the resulting plots are straight lines and almost parallel to each other.

The boiling point of a liquid is affected by the pressure, solute concentration and hydrostatic pressure, increasing these increases the boiling point.

The boiling point elevation of a dilute ideal solution, due to solute concentration, is directly proportional to mole fraction of solute and is independent of the nature of the solute (Prutton & Maron, 1957) as shown below

$$\Delta T_b = \frac{R T_0^2}{\Delta H_v} . m$$

where R is the gas constant, T_0 the boiling point of the solvent, ΔH_v the latent heat of vaporization of the solvent per mole from the solution and m the mole fraction of solute.

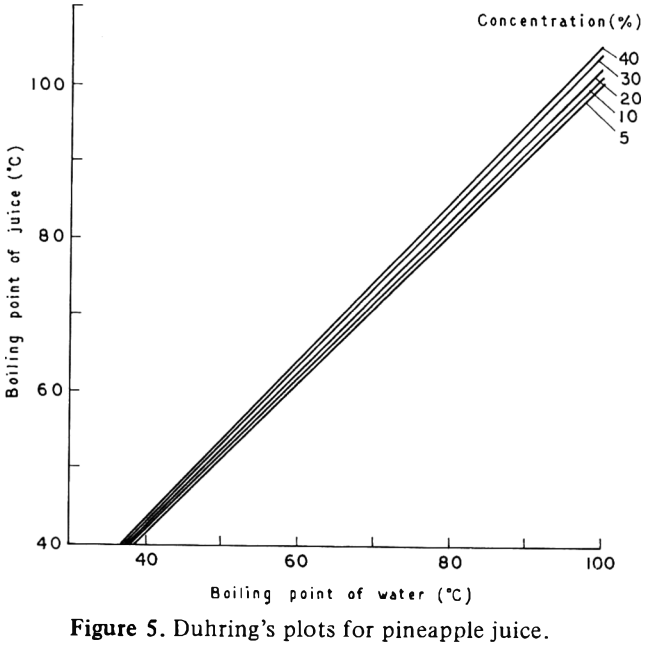

Figure 5. Duhring's plots for pineapple juice.

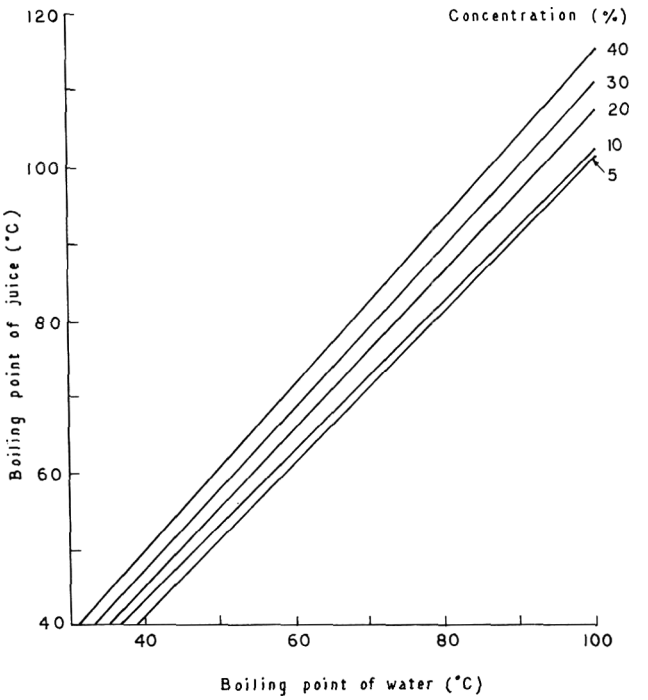

Figure 6. Duhring's plots for mango juice.

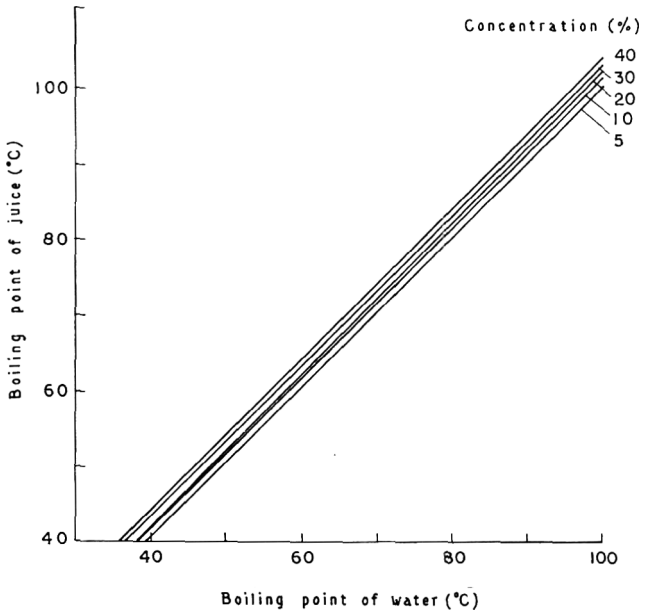

Figure 7. Duhring's plots for lemon juice.

The equation may be transformed for convenience as following

$$\Delta T_b = \left(\frac{R T_0^2}{\Delta H_v}\right)\left(\frac{1000 w_2}{w_1 M_2}\right)$$

where w_1 and w_2 are masses of solvent and solute respectively and M_2 is molecular weight of solute.

Thus the estimation of boiling point from the above equation will require accurate knowledge of the fraction of solutes in the solution, which in the case of juices is difficult because they contain many solutes. Further, the error in the estimation of the boiling point of juice from this equation is compounded as the concentration increases, since in the derivation of this equation many simplifications are made which at higher concentrations may cause substantial errors (Foust *et al.*, 1960).

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The performance of liners for retail wine casks

E. G. DAVIS

Summary

Properties of flexible film liners and valves used in wine casks were studied to assess their influence on the loss in quality of wine retailed locally in this type of package.

Oxygen permeability measurements were made at 25°C and 75% r.h. on two types of liner materials and three types of valves. A procedure involving treatment of the valve sealing surfaces with polydimethyl siloxane was developed to test valves under simulated conditions of use. Application of these results to an analytical model of a wine pack suggested that the rate of oxygen uptake by wine may be as high as 0.17 ppm/day and that the valves contributed 33–52% of this amount.

Theoretical considerations demonstrate that loss of SO_2 from the model by permeation was negligible.

Introduction

The wine cask is a recent innovation in the Australian retail liquor market. In this package, wine is sealed in a flexible film liner which is mechanically supported within a paperboard carton. A valve, fitted to the liner, protrudes through the carton so that wine may be 'poured' without breaking the liner seal and, since the liner is collapsible, entry of air is avoided. This design is advantageous since wine remaining in the pack is not exposed to oxygen which is known to be detrimental to wine quality (Rankine & Pocock, 1969; Prass & Virgo, 1976).

One problem associated with the marketing of wine in these casks is a decrease in shelf life compared with that obtained using traditional glass bottles. The industry seeks a minimum shelf life of 6 months but it has observed significant loss of free sulphur dioxide (SO_2) accompanied by the appearance of oxidized flavours after only 3 months storage in casks.

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Rankine (1966) discusses two reasons for adding SO_2 to wine; to inhibit growth of microorganisms, and to reduce oxidation. Most added SO_2 combines with constituents of the wine, but the residual free SO_2 functions as a preservative. It is apparent that the undesirable changes which determine the shelf life of wine become increasingly important as the level of free SO_2 declines.

Davis *et al.* (1973) showed that when dried apricots are packaged in containers that are permeable to gases and vapours, loss of SO_2 may occur during storage by permeation through the package material and by reaction with oxygen permeating into the package. Although the levels of SO_2 used in wine are much lower than those used in dried fruits, the possibility exists that similar mechanisms of loss may occur in wine.

This paper discusses some of the container properties which influence the loss of quality in wine packaged in retail casks.

Materials and methods

Materials

Schematic diagrams of the three types of valves tested are shown in Fig. 1. Each consisted of a flanged body to which the liner material was heat sealed and a press-on spigot which incorporated the pouring mechanism. In type C, the seal between the body and spigot also functioned as the pouring orifice whereas the other types had separate pouring orifices elsewhere in the spigots.

Type B valves were sealed to a liner made from a laminate of 15 μm polyamide/3 μm polyvinylidene chloride (PVDC)/64 μm polyethylene. Valves A and C were sealed to a triple-web liner consisting of two outer webs of 50 μm Saranex* 14 and an inner web of 50 μm ethylene-vinylacetate (EVA) copolymer.

Methods

Oxygen permeability measurements were made at 25°C and 75% r.h. using the concentration-increase apparatus described by Davis & Huntington (1977). Measurements on the valves were made by sealing in the cell a sample of film with valve attached so that the internal surface of the valve faced the inert-gas or measuring compartment of the cell. The permeability of the valve was then calculated from the value observed on this sample less the permeability of the area of film present.

Twelve valves of each type were tested. Half of these were tested without modification; the remaining half were tested after all body-to-spigot joints and pouring-orifice seals were treated by lightly coating with polydimethyl siloxane (Siliastic RTV†) which was allowed to cure for 72 hr.

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† Dow Corning Corp., Michigan, U.S.A.

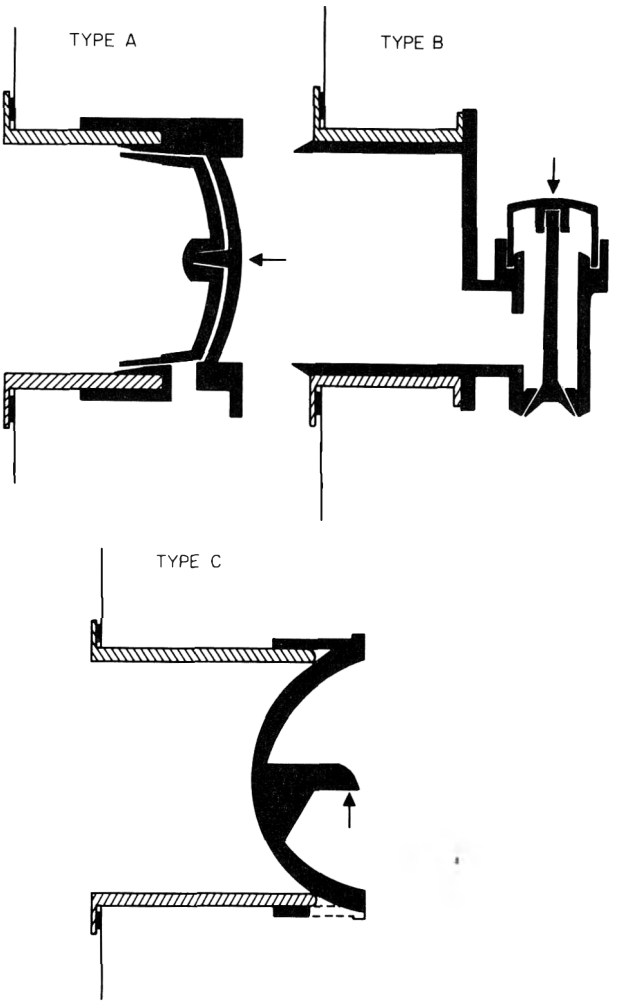

Figure 1. Diagram of wine-cask valves tested. Hatched and filled sections represent valve bodies and spigots respectively and arrows indicate where force is applied to open valves.

Analytical model

As a model, the calculation of SO_2 loss and oxygen pick-up are based upon a pack consisting of 4.5 kg of wine in a cask liner having a surface area of 0.25 m^2 and stored for 90 days at 25°C. The initial levels of free SO_2 and oxygen in the wine are assumed to remain constant at 50 ppm and 0.0 ppm respectively on a weight basis.

The quantity (Q) of SO_2 lost by permeation from the wine was calculated from the following relation:

$$Q = PA\,t\,\Delta p\,d\,\frac{10^6}{W}\ \text{ppm (w/w)} \qquad (1)$$

where P = the permeability of the liner material to SO_2, A = the liner area, t = the storage time, Δp = the partial-pressure difference of SO_2 across the liner surfaces, d = the density of SO_2, and W = the weight of wine. The oxygen pick-up by the wine was also calculated from eqn (1) by substituting the relevant values of P, Δp and d for oxygen.

The quantity (Qs) of SO_2 lost from the wine by solution in the liner material was calculated from the relation:

$$Qs = SA\ ld\ \frac{10^6}{W}\ \text{ppm (w/w)} \qquad (2)$$

where S = the solubility of SO_2 in the liner material and l is the liner thickness.

Results and discussion

Loss of SO_2

The permeation of gases and vapours through polymer films occurs by a 'solution-diffusion' mechanism under the influence of a difference in partial pressure of the penetrant across the film. So far as loss of SO_2 from inside a package is concerned, therefore, two processes have to be considered; the net transport over a specified time interval and the amount of SO_2 dissolved in the film material.

Solution of eqn (1) requires an estimate of P and Δp; A, t and W have been specified for the model and the known value of d for SO_2 is 2.93×10^{-3} g $\times$ cm^{-3}.

PVDC is the main barrier layer in laminated and coextruded films used for wine-cask liners. Davis, Rooney & Larkins (1975) have reported the permeability to SO_2 of two PVDC-coated films. The highest value observed was 3.8×10^{-2} ml (STP) $\times$ m^{-2} $\times$ hr^{-1} $\times$ kPa^{-1} at 25°C, thus providing an estimate of P.

Unpublished work by the author has shown that at 25°C there is a relation between the free SO_2 content of dried apricots and the concentration of volatile SO_2 which accumulates in the atmosphere surrounding the fruit. At a free SO_2 level of 50 ppm, the corresponding partial pressure of SO_2 is 2.3×10^{-3} kPa. No data were found in the literature on the relation between volatile and free SO_2 for wine, so the value of 2.3×10^{-3} kPa was used for Δp.

Substitution of the above data in eqn (1) gives a value of 3.1×10^{-2} ppm for the loss of SO_2 by permeation from the model wine pack over a period of 90 days.

The solution of eqn (2) for Qs requires an estimate of S for the liner material. Davis & Rooney (1971) measured the solubility of SO_2 in films of polyamide and polyethylene at partial pressures of 3.3 kPa and above. Extrapolation of these data to a partial pressure of 2.3×10^{-3} kPa yields S values of 2.1×10^{-3} ml (STP) $\times$ cm^{-3} and 3.3×10^{-5} ml (STP) $\times$ cm^{-3} for polyamide and polyethylene respectively. Substitution of these values and the known values for l in eqn (2) for each type of polymer in the laminated material gives a total loss of SO_2 of 5.4×10^{-3} ppm from the model pack.

These calculations show, therefore, that the expected loss of SO_2 from the model wine pack after a storage period of 90 days is only 3.6×10^{-2} ppm. Although several assumptions were made in deriving some of the data used in the calculations and the effect of the valve fitted to the cask liner was ignored, the calculated loss is too low to suggest that loss of SO_2 by permeation is responsible for the deterioration of wine packaged in cask liners which rely on PVDC as the barrier layer.

Uptake of oxygen

Another way that SO_2 may be lost is by reaction with oxygen entering the wine by permeation through both the liner material and the valve assembly. Oxygen permeability values of 2.05×10^{-3} ml (STP) $\times$ $m^{-2} \times hr^{-1} \times kPa^{-1}$ and 2.68×10^{-3} ml (STP) $\times$ $m^{-2} \times hr^{-1} \times kPa^{-1}$ were measured on the liner materials based on Saranex 14/Saranex 14/EVA and Nylon/PVDC/polyethylene respectively.

The oxygen permeability values observed on three types of valves before and after treatment with polydimethyl siloxane (DMS) are shown in Table 1. The differences between valves before treatment were highly significant, and treatment with DMS reduced significantly the oxygen permeability of each type of valve. After treatment with DMS, the oxygen permeabilities of the type A and type B valves were similar, and both types were significantly less permeable than type C.

The large variations found between untreated valves of the same type support the view that the transmission of oxygen occurs by two mechanisms; 'true' permeation through the valve material and diffusion through imperfections in the sealing surfaces. The only sample variables which could affect 'true' permeation are variations in the area and thickness of the valve material but, since the valve components are moulded, it is unlikely that these dimensions would differ sufficiently to account for the large valve-to-valve differences observed. In contrast, the dimensions of small passages or pores between the seal surfaces could vary widely and produce relatively large differences in the

Table 1. Oxygen permeability at 25°C and 75% r.h. of wine-cask valves before and after treatment with DMS

Valve type	Oxygen permeability $\times 10^4$ ml (STP) $\times$ $hr^{-1} \times kPa^{-1}$			
	Untreated		Treated with DMS	
	Mean	s.e.	Mean	s.e.
A	105	±26.7	3.28	±0.034
B	9.85	±2.98	3.24	±0.065
C	6.54	±0.450	5.52	±0.088

amounts of oxygen transmitted by diffusion. Thus, by the application of DMS, seal imperfections were filled and oxygen transmission could then occur only by permeation through both the valve material and the thin layer of DMS.

In use, the inside surfaces of valves are in contact with wine, and any imperfections in the sealing surfaces are filled with wine. The oxygen permeability of DMS is 1.19×10^4 ml (STP) $\times \mu m \times m^{-2} \times hr^{-1} \times kPa^{-1}$ (Sweeting 1971) which is similar to that of water as calculated from data on the solubility and diffusion of oxygen in water. The test procedure using DMS, therefore, should simulate end-use conditions, and avoid misleading results which may be observed on valves tested without modification.

To calculate the oxygen uptake by the model wine pack, eqn (1) requires modification to take into account the amounts of oxygen permeating through both liner and valve. The modified equation becomes

$$Q = (PA + P_1)\, t\, \Delta p d \frac{10^6}{W} \text{ ppm (w/w)} \tag{3}$$

where P_1 = the permeability of the valve. Using values of 21.2 kPa and 1.43×10^{-3} g $\times$ cm^{-3} for Δp and d respectively for oxygen, and permeability values reported for the type B valves treated with DMS and a liner made from a laminate of polyamide/PVDC/polyethylene, solution of eqn (3) yields a total oxygen uptake of 14.5 ppm over a storage period of 90 days.

With the above combination of liner and valve, the valve itself contributes 33% of the oxygen uptake of 14.5 ppm over 90 days. Similar calculations applied to a combination of valve C and triple-ply liner show that an oxygen uptake of 15.6 ppm could be expected of which the valve contributes 52%.

The above calculation is based on the assumption that all oxygen permeating into the pack is consumed by reactions with wine constituents so that the partial pressure of oxygen in the wine remains near zero. In support of this assumption, Rankine & Pocock (1969) reported that if wine is saturated with air at 20°C, all the dissolved oxygen is consumed within 14 days. Based on an oxygen content of 8.5 ppm, this represents an average consumption rate of 0.61 ppm/day which is higher than the rate of uptake of 0.17 ppm/day calculated for the model.

Rankine & Pocock (1969) state that oxygen is capable of combining with free SO_2 in wine in the ratio 1:4. On this basis, an oxygen uptake of 15 ppm would account for an SO_2 loss of 60 ppm. However, because of the equilibrium which exists between free and combined SO_2, this amount would represent the loss of total SO_2; the decrease in the level of free SO_2 in the wine would be less.

These observations suggest, therefore, that loss in quality experienced during retail marketing of wine in wine casks is due to the entry of oxygen by permeation through both the liner and the valve. With liner and valve materials in current use, the rate of entry of oxygen at 25°C may be as high as 0.17 ppm/day. Storage of packaged wine at temperatures below 25°C should reduce the rate of oxygen uptake but improved valve designs and the use of liner and valve

materials having oxygen permeabilities lower than those examined in the present studies would provide better solutions to the problem.

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Technical note: Cellulase and exo-amylase in experimental soy sauce fermentations

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Introduction

Previous reports have dealt with the production of a number of enzymes, including sucrase, endo-amylase (α-amylase), proteinase, lipase and tyrosinase during the growth of *Aspergillus oryzae* in the Koji (mould growth) stage of soy sauce production, and with their survival into the Moromi (brine fermentation) stage (Yong & Wood 1975, 1976, 1977*a, b*). The history, technology, microbiology and biochemistry of soy sauce have been reviewed by Yong & Wood (1974), and the relationships of the soy sauce fermentation to other food fermentations have been discussed by Wood & Yong (1974), Wood *et al.* (1975) and Wood (1977).

The present report deals with the participation of cellulase and exo-amylase in soy sauce fermentation. Production of both of these types of enzyme by members of the genus *Aspergillus* including *A. oryzae* has been long been known, but, so far as we have been able to ascertain, no evidence relating to the participation of cellulase in the soy sauce fermentation has been published. The disintegration of the initially intact beans during the Moromi fermentation suggested to us that considerable degradation of the cell walls must be taking place, and this would suggest cellulase involvement. In the case of exo-amylase, many writers on the soy sauce fermentation infer that this type of activity participates in the extracellular hydrolyses effected by the mould, and the levels of reducing sugar present in the latter stages of the Koji fermentation (Yong & Wood 1977a), can only be explained at all readily through the participation of exo-amylase. Morita *et al.* (1966) describe its presence in rice Koji.

In the course of the work which forms the basis of the present report, we were primarily concerned with technological aspects of the fermentation, and

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thus we were not concerned to differentiate the types of cellulose hydrolysis which were taking place, but preferred to employ the production of reducing power as a measure of overall cellulose degradation. Similarly we did not differentiate between amyloglucosidase and β-amylase activities, but again reported the total activity in terms of reducing power, a measurement which will also incorporate the production of reducing end-groups through α-amylase activity. To avoid ambiguity we have therefore called the two types of amylase activity exo- and endo-amylase respectively.

Methods

Microbiological methods, the production of Koji and Moromi, and sampling procedures employed in this study were all as detailed in previous reports (Yong & Wood, 1976, 1977a, b). The only mould used here was *A. oryzae* strain NRRL 1989. Moromi was prepared by correcting a mixture of Koji and brine to pH 4.5 with D-L-lactic acid, then inoculating with *Saccharomyces rouxii* strain NRRL Y-1096 (Yong & Wood, 1976).

Exo-amylase assay was based on the method of Morita *et al.* (1966), employing Sumner's method (Sumner, 1925) for measuring reducing sugars, as described by Yoshino & Takano (1954) for analysis of soy sauce. Enzyme extracts were made by suspending samples in a pH 5.0 buffer containing sodium acetate and acetic acid, which was 0.1 N with respect to total acetate. Solid material was removed by centrifugation at 10 000 r.p.m. for 30 min on an MSE 18 centrifuge operating at a bowl temperature of 5°C. The clear supernatant was decanted, and the residue reextracted as before; the combined extracts were made to known volume and further diluted as necessary with the buffer solution. The substrate for the assay was a 1% solution of soluble starch in 0.01 N acetate buffer, pH 5.0. Starch solution (0.5 ml) and enzyme extract (0.5 ml) which had been preheated separately for 10 min at 30°C, were mixed, and the mixture incubated at 30°C for 10 min. The 1% 3,5 dinitrosalicylic acid reagent (prepared according to Yoshino & Takano (1954) (1.0 ml) was then added, mixed and the tube immersed in boiling water for 5.0 min. Next the tube was cooled under running water for 1 min, then distilled water (10.0 ml) was added, the whole mixed, and the absorbance measured at 540 nm in a 10 mm path length cell. The absorbance was measured against a control prepared using distilled water (0.5 ml) in place of enzyme extract. Reducing sugars present in the extract were determined separately, and subtracted from the result obtained in the assay, thus giving the reducing sugar produced as a result of starch hydrolysis. All absorbance results were converted to reducing sugar content using a standard plot prepared with glucose. The enzyme unit was that amount of enzyme which produced 1.0 mg of reducing sugar calculated as glucose under the above conditions.

Cellulase assay was by a method based upon that of Gascoigne & Gascoigne (1960). The substrate was a 1% solution of sodium carboxymethylcellulose in

0.01 N acetate buffer, pH 5.0. Preparation of crude enzyme extract and the assay procedure were otherwise exactly as described for exo-amylase, except that an incubation temperature of 50°C was used. The unit of enzyme activity was also derived in a similar manner, by estimation of reducing sugar.

In reporting enzyme levels in Koji, results are expressed in terms of units per gram dry weight of Koji. Dry weight was determined according to the method described by Jacobs (1951). When assays were to be performed on Moromi procedures employed in this study were all as detailed in previous reports (Yong an aliquot of the supernatant liquid taken by pipette, and this sample was centrifuged to give a clear liquid; assay results are expressed in terms of enzyme units per ml of this supernatant liquid.

Results and discussion

Plots of the concentration of the enzymes against time for both Koji and Moromi are presented in Figs 1 and 2. Exo-amylase levels in Koji increased rather more rapidly than did endo-amylase levels (Yong & Wood, 1977a) after spore germination. There then followed a period of slowly rising exo-amylase concentration between the thirtieth and fiftieth hours of fermentation, and this in turn was succeeded by a steady decline in enzyme activity during the remainder of the fermentation. The decrease in enzyme in the latter half of the fermentation is in marked contrast to the behaviour reported for endo-amylase, which showed a fairly steady level during much of the fermentation, and a further increase towards the end of the fermentation.

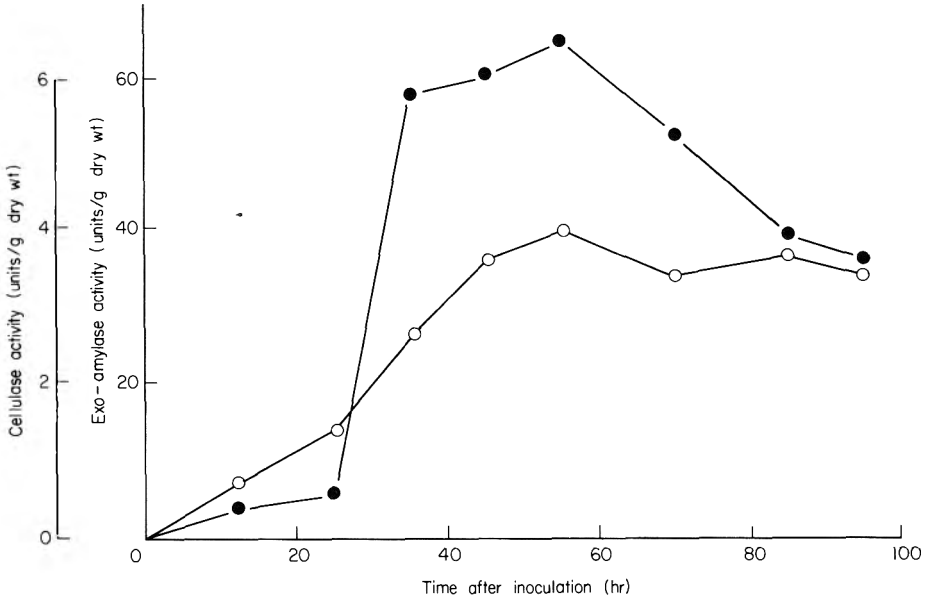

Figure 1. Cellulase (○) and exo-amylase (●) in soy sauce Koji.

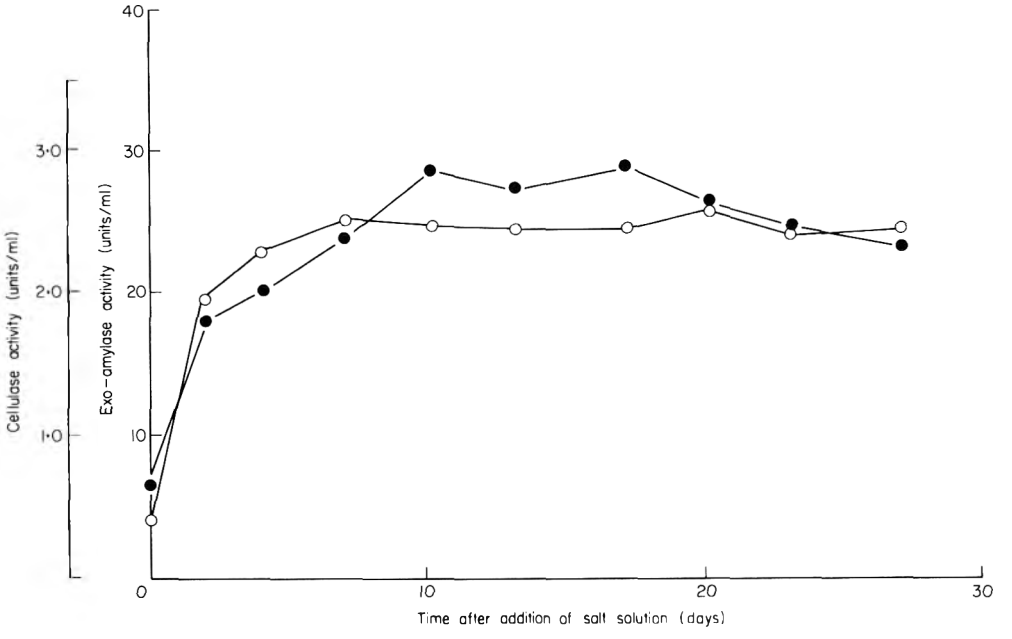

Figure 2. Cellulase (○) and exo-amylase (●) in soy sauce Moromi.

In the Moromi stage, both enzymes were rather stable. During the first 2 or 3 days there was the usual pattern of fairly rapid increase in enzyme level in the brine, due (we believe) to solubilization of enzyme present in the mould mycelium and substrate, then a slower increase to a plateau level sustained for the rest of the month-long incubation. In the case of exo-amylase, this stability was in marked contrast to the behaviour of the endo-amylase, which seemed to be less stable than any other enzyme examined in the Moromi (Yong & Wood, 1977b).

In summary therefore we conclude that exo-amylase and cellulase can be added to the list of carbohydrate-hydrolysing enzymes present in soy sauce fermentations conducted under the controlled conditions which are employed in our work. It can be readily inferred that they will also participate in commercial fermentations. They have an important contribution to make in releasing fermentable sugars for use by the mould and by the flavour-producing yeasts and bacteria which colonize the Moromi. Cellulose-degrading enzymes will also assist in increasing yields of solubilized carbohydrates and proteins from the soy beans and wheat flour, by aiding the disintegration of cell walls.

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Technical note: the determination of meat and soya proteins in meat products by peptide analysis

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Introduction

To date, many different methods of analysis of soya protein in meat products have been investigated. In general, they depend on differences in the physical and chemical properties of meat and soya protein molecules, such as electrophoretic mobility, molecular weight and immunochemical response. Denaturation of the proteins by heating, either during cooking or production processes, substantially alters their physical and chemical properties and consequently the sensitivity and accuracy of methods depending on these properties is greatly diminished. Thermal denaturation rarely changes the amino acid sequence but, more usually, causes aggregation of individual protein molecules into large groups which are then difficult to dissolve, and hence are not amenable to many analytical techniques. These problems may be overcome by hydrolysis of the proteins to peptides or amino acids, which can be dissolved more readily.

The hydrolysis of meat and estimation of liberated 3-methylhistidine and N^{ϵ}-methyllysine, which affords an index of the lean meat content of meat products, has been described (Hibbert & Lawrie, 1972; Rangeley & Lawrie, 1976, 1977). Hydrolysis of products containing meat and soya protein by digestion with trypsin has also been reported (Bailey, 1976). In this method products were autoclaved for 3 hr prior to digestion, thereby bringing them to a 'standard denatured condition' and rendering the results independent of manufacturers' processing treatments. The enzymatic cleavage of the protein chain yields a mixture of peptides, one of which is derived specifically from soya protein (SP-1). Separation of the peptide mixture on a cation exchange resin column, using an amino acid analyser, enables measurement of the SP-1 peptide and hence quantitative determination of the soya protein content of the meat product. When this method was applied in the Laboratory of the

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Government Chemist, in collaboration with the Unilever Research Laboratories, difficulty was observed in resolving the SP-1 peak from a preceding peak arising from meat. It was found also that column pressure tended to increase rapidly, possibly due to blocking by large peptides or undigested protein. To alleviate these problems Bailey's procedure was modified by further fractionation of the digests through an Amicon UM2 ultrafiltration membrane with a molecular weight cut off of 1000. A peak characteristic of soya protein was obtained which had a retention time 5 min less than that of authentic SP-1 (prepared in the Unilever laboratories). Chromatography on a longer column and reduction of the flow rate by half, resolved this peak into two components designated SP-2A and SP-2B (see Fig. 1(a)). The SP-1 peptide does not appear to pass through the membrane. A peptide characteristic of meat (MP-1) was also obtained (see Fig. 1(b)). This note reports a procedure in which measurement of these peptides enables simultaneous quantitation of the meat and soya protein contents of a mixed product.

Materials and methods

Meat and soya 'product' mixtures were prepared as follows: Lean beef (defatted by hand sorting) was minced and water, rusk, salt and polyphosphate added to form a meat mix (nitrogen level, 2.78%). A Unilever soya protein isolate (nitrogen level 13.4%) was then blended in various proportions with this mix to form 'products' containing 0, 2, 4, 8, 16, 24 and 32 g soya protein per 100 g total protein, and these were heat set in a steam oven at 100°C for 30 min. On cooling, the 'products' were cubed, frozen, freeze dried and finally powdered in a Hobart mill. The total protein contents of the dried powders were subsequently determined (N × 6.25). Nitrogen levels were determined by the Kjeldahl technique.

Product powder (1 g) was suspended in 5 ml trishydroxymethylmethylamine (0.05 M) and calcium chloride (0.0086 M) buffer (pH 8.1), placed in a universal bottle and autoclaved for 3 hr at 120°C. On cooling, tris/calcium chloride buffer (10 ml) and 1 ml aqueous trypsin solution (crystalline beef pancreas B.D.H.) (10 mg/ml) were added. Digestion was allowed to proceed at room temperature overnight, after which it was stopped by the addition of glacial acetic acid (2 ml). The suspension was centrifuged at 4000 rev/min (2700 g) for 15 min and the supernatant retained. The pellet was suspended in 1% acetic acid (10 ml) and recentrifuged for 15 min. The combined supernatants were diluted to 100 ml by addition of pH 5.50 sodium citrate buffer (0.067 M) and the resultant solution passed through an Amicon UM2 ultrafiltration membrane at 42000 kg per m^2 (60 p.s.i.). The ultrafiltrate was concentrated to dryness under reduced pressure at 50–55°C and dissolved in water (5 ml) before filtering through a 13 mm diameter millipore filter (0.45 μm pore size) in a Swinney holder prior to use. Aliquots (0.5 ml) were placed at the top of an Aminex A5 cation exchange resin column (6 mm i.d. × 455 mm) in a Technicon Auto Analyser and eluted at 59° with pH 5.50 (0.067 M) citrate buffer at a flow rate of

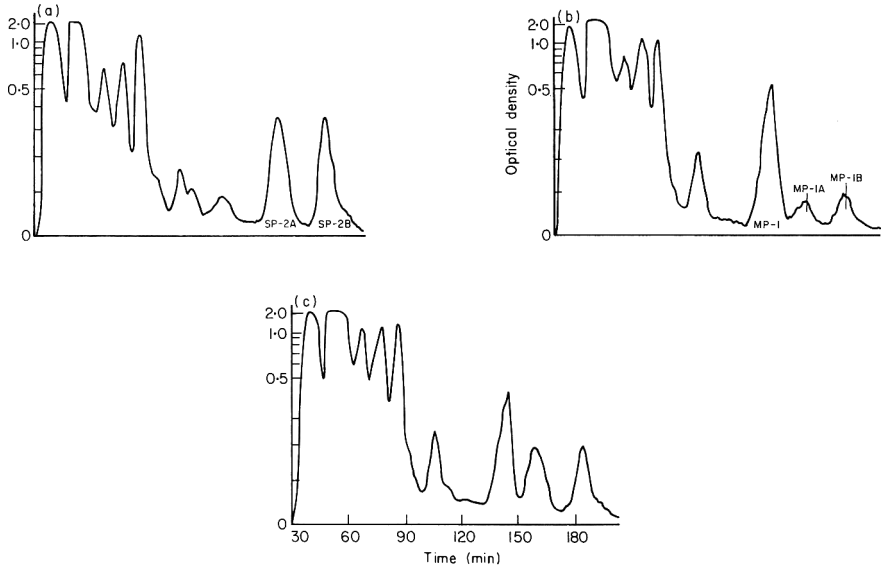

Figure 1. Amino acid analyser traces of trypsin digests of (a) Unilever soya protein isolate (b) meat mix (c) meat and soya 'product' containing 68% meat protein and 32% soya protein.

0.39 ml/min. Peptides were detected colorimetrically at 570 nm by reaction with ninhydrin. Samples of freeze dried and defatted lean beef, chicken, lamb, pork and a laboratory prepared canned meat and baked pie which contained 70 and 59 g soya protein per 100 g total protein respectively were digested and analysed.

Results and discussion

Figure 1 shows the aminoacid analyser traces of digests of (a) soya protein isolate, (b) meat mix standard and (c) a 'product' containing 68% meat protein and 32% soya protein. Trace (a) shows the two peaks designated SP-2A and SP-2B (retention time 160 and 185 min) characteristic of soya protein. Trace (b) shows the major meat peak MP-1 (retention time 146.5 min) and two minor peptides MP-1A and MP-1B (retention times 164 and 184 min respectively). Both the MP-1 and SP-2B peaks are assymetric indicating that they are not homogeneous. The trace of the mixed product (c) displays three peaks corresponding to MP-1, SP-2A superimposed on MP-1A and SP-2B superimposed on MP-1B. By comparison of the MP-1 peak from the soya/meat product with the meat mix standard it was possible to estimate the meat protein content of the sample. Similarly comparison of SP-2A and SP-2B from the mixed product with the soya isolate standard enabled an estimate of the soya protein content to be made. The true areas of the SP-2A and 2B peaks for the mixed product were obtained by subtracting the contributing areas of the underlying MP-1A and 1B peaks. The ratio of the areas of MP-1A and MP-1B to MP-1 do not vary greatly, hence the areas of MP-1A and MP-1B in the sample are related to the

areas of MP-1A and MP-1B from the standard by the ratio of the areas of the MP-1 peaks from sample and standard respectively. Thus the true area of the SP-2A peak from the mixed product sample is calculated:

True area SP-2A in sample = Measured area SP-2A in sample – (Area MP-1A in standard × Area MP-1 in sample/Area MP-1 in standard) and similarly for SP-2B and MP-1B.

A plot of the results of analysis of meat protein content of the series of meat/soya products against their actual meat protein contents was linear in the range 60–100% meat protein and could be represented by the equation $y = 0.87x + 5$ where y is the measured meat protein content (% total protein) and x the actual meat protein content (% total protein).

The MP-1 peak decreased in size slightly on storing the ultrafiltrates of the digests at −10° C for a period of 4 weeks or longer. Similar calculations for the estimation of soya protein contents, were correct to within ±15% of the theoretical % content.

The meat and soya protein contents of the laboratory prepared pie were found to be 60 and 40% respectively (theoretical values 59 and 41% respectively) and analysis of the laboratory prepared canned meat afforded values of 74% (meat) and 26% (Soya) (theoretical values 70 and 30% respectively). These were trial experiments, however, and the results may be somewhat fortuitous.

Samples of freeze dried chicken, lamb, pork and beef gave a peptide pattern almost identical to that of the meat mix standard (Fig. 1(b)); MP-1, 1A and 1B were all present in approximately the same ratio. If MP-1 is derived from the myofibrillar proteins actin or myosin, the method could be applied as an indicator of lean meat content.

These initial results indicate the potential of the technique as a method for the simultaneous estimation of the meat and soya protein of mixed products and provide a basis for further development. Other ingredients of meat products, such as heart, kidney, liver, milk and egg powder and various offals are also being examined by the technique to ascertain whether they give peptide peaks which might interfere with MP-1 or SP-2A and 2B. It is proposed to examine the tryptic digests of pure actin and myosin to establish whether or not the MP-1 peak arises from one of them.

Acknowledgment

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Book reviews

Laboratory Methods in Food and Dairy Microbiology. By W. F. Harrigan and M. E. McCance.
London: Academic Press, 1976. Pp. xii + 452. £9.20.

This is a revised and updated edition of a well established text. The book is written in three parts dealing with basic methods, with techniques of applied microbiology and describing schemes for the identification of microorganisms. There are appendices containing recipes for stains, reagents and media, tables of most probable numbers, a list of manufacturers and suppliers and, finally a selected bibliography.

The clear descriptions of methods have been written for operatives with no previous experience of microbiological techniques and nothing has been taken for granted. This approach to practical microbiology makes this book especially valuable for beginners, although it should also serve as a laboratory handbook for those who have been engaged in microbiology for some time. Of necessity the depth of approach has been limited by the size of the book, and those seeking further knowledge on a particular subject are referred to the bibliography. Safety precautions are listed at the start of the book.

The book contains a few minor errors. The chemicals listed as hazardous in the table of Appendix 1 do not include all those to be found in the recipes coded with (H). On the whole, however, as a handbook of practical microbiology for food microbiologists this edition of *Laboratory Methods in Microbiology* should prove to be a valuable source of information.

H. Dallyn

Meat Technology. By F. Gerrard.
London: Northwood Publications Ltd, 1977. Pp. xii + 402. £7.95.

This book was first published as a *Practical Textbook for Students and Butchers* in 1945; the present volume is the fifth edition. The author is perhaps the grandest of the 'grand old men' of the U.K. meat industry and the book is based upon a lifetime of experience centred on the small firm and the personal involvement of the manager with every aspect of meat from farm to customer. Thus it contains a wealth of information covering many areas but very few points are dealt with in any degree of detail. Better use should have been made of the new edition to eliminate many descriptions of out of date techniques, e.g. roping at stunning which was (as stated) made illegal in 1963, and by including newer developments in muscle seaming, hot boning, pre-packing and in methods of overcoming cold shortening. The structure of the book is confusing and chapter and section headings are often misleading; under 'Tempera-

ture Control' one finds an account of bacterial spoilage; under 'British Breeds' the Charollais, Blonde D'Aquitaine and Limousine rub shoulders with the Simental and Chianina, the relationship between gross sales and profit in a retail business appears in the chapter on 'Beef Cutting' and the index is unfortunately far from adequate to compensate.

This book is not intended for the food technologists and would prove of very limited value since it makes no attempt to present the scientific principles underlying production, preservation and marketing of meat as a food. This major sector of the food industry is now in turbulent development as the craft industry is confronted by the pressures of supplying urban population concentrations and today's life-styles which demand increasing efficiency in production and distribution to meet competition from an ever growing range of convenience foods. The technological response to this challenge must be based upon the scientific principles underlying the transformation of animal muscle into meat and students, while needing the introduction to the craft element amply provided in this book, must also seek out and study the theoretical basis underlying practice.

D. N. Rhodes

Books received

Clinical Nutrition. A Physiological Approach. By M. H. Overton and B. Lukert. Chicago: Year Book Medical Publishers Inc., 1977. Pp. xi + 171. £8.75, Paperback.

This book is aimed principally at medical students and clinicians. It attempts to bring together information on nutritional aspects of disease and treatments.

Everything You Should Know About Food. By C. Loewenfeld. London: Faber & Faber, 1978. Pp. 288. £3.75, Paperback.

The book is written for the non-professional public with an emphasis on home produced and organically grown foods.

World Review of Nutrition and Dietetics, Vol. 29. Toxicology and Nutrition. Ed. by C. H. Bourne. Basel: S. Karger, 1978. Pp. x + 190. US$53.

A collection of papers on toxicological aspects of nutrition. Amongst the subjects discussed are the following: natural toxic substances in food, metabolism for drugs and other foreign components by intestinal microorganisms, the effects of nutrition and enzyme induction in toxicology, drug, toxin and nutrient interaction, and dietary effects on the toxicity of food and environmental contaminants.

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Abbreviations. Abbreviations for some of the commoner units are given below. The abbreviation for the plural of a unit is the same as that for the singular unless confusion is likely to arise.

gram(s)	g	second(s)	sec
kilogram(s)	kg	cubic millimetre(s)	mm^3
milligram(s)		millimetre(s)	mm
(10^{-3} g)	mg	centimetre(s)	cm
microgram(s)		litre(s)	l
(10^{-6} g)	μg	millilitre(s)	ml
nanogram(s)		pound(s)	lb
(10^{-9} g)	ng	gallon(s)	gal
hour(s)	hr	milliequivalent	mEq
minute(s)	min	R_F values	R_F

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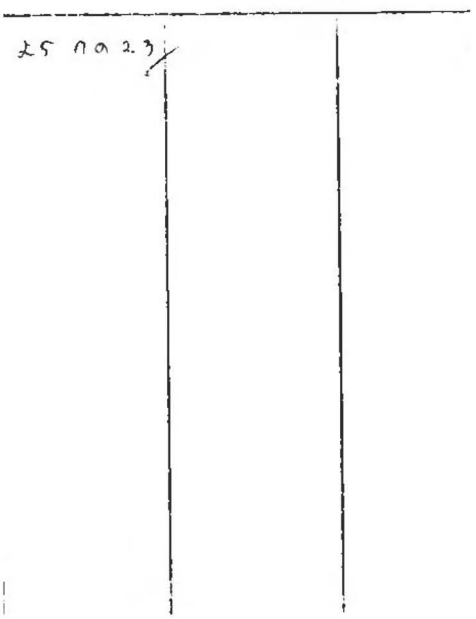

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