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On the conversion factors in thermal processes

I. J. KOPELMAN*, I. J. PFLUG AND D. NAVEH

Summary

Converting experimental or assumed heating parameters of one container size to another using the so-called conversion factor, CF, is an important practical step in the initial design of thermal processes. However, the current prevailing approach for conversion is limited to cans where the two containers are of metal and are processed essentially in the same heating medium. The present study develops the theoretical equations, verified experimentally, for the conversion factors in a broader spectrum of containers (e.g., glass jars to metal cans and vice versa) and different processing media (e.g., water to steam and vice versa). The developed relationships enable the thermal process engineer to use conversion factors in a simple manner for most practical processing conditions.

Introduction

Converting experimental or derived heating parameters of one container size to the heating parameters for another container size is an important practical step in the design and monitoring of thermal sterilization processes. Presently, the prevailing approach for such a procedure, using the so-called conversion factor, CF, is essentially based on the Schultz & Olson (1938) analysis for convection heating and the Ball & Olson (1957) analysis for conduction heating.

The approach to conversion factors divides the processed foods into two categories: those heated primarily by a convection mechanism and those heated primarily by conduction. Using this overall approach analysis, for convective heating products Schultz & Olson (1938) suggested that the heating rates (i.e., the temperature response parameters, f) for the same product packed in two different container sizes will be proportional to their respective volume/area

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ratio. For conduction heating, the Ball & Olson analysis suggests that the temperature response parameter, f, in any direction of the major axes of the body, can be derived from the expression $\frac{f\alpha}{R^2} = N_{Bi}$. The theory and analysis for conversion factors is fully delineated by Ball & Olson (1957). Using the above approach, conversion factors for a matrix of can sizes for conduction and convection heating were calculated and tabulated (Ball & Olson, 1957).

The limitation of the developed conversion factors is that they are valid only if the two containers are of metal and are processed essentially in the same heat transfer medium. Processing situations theoretically cannot be handled through the present conversion factor system where two different heating media are involved (e.g., water *vs* steam) or when the two containers are of a different type materal (e.g., glass jar *vs* metal container). Therefore, there is a need to develop conversion factor equations, especially for products heated primarily by convection, which will account for processing parameters such as different heating media or different container materials.

The purpose of this study is to: (a) discuss, in general terms, the conversion factor for conduction heating products and (b) develop from a theoretical basis the convection conversion factor equations or a wide spectrum of conditions as well as verify that these developed conversion factors give accurate results.

Analysis and discussion

Conversion factors for conducting heating

The theory of conversion factors for conduction-heated food products is well delineated by Ball & Olson (1957). The temperature response parameter, f, can be derived from $\frac{f\alpha}{R^2}$, which is a function of the N_{Bi}. In the range of N_{Bi} prevailing for conduction heating food products processed either in steam or agitated water, the $\frac{f\alpha}{R^2}$ for a cylindrical container (a finite cylinder) or a rectangular container (a finite slab) will assume a constant value. This constant value of $\frac{f\alpha}{R^2}$ is derived from similar $\frac{f\alpha}{R^2}$ constants for each specific one-dimensional configuration that defines the body. Since the $\frac{f\alpha}{R^2}$ for a given uniform body heated by conduction will assume a constant value whether processed in steam or agitated water, the respective conversion factor between these two media (for the same container size and type) will be approximately one. This assumes that the heated body as a whole (i.e., the product plus the container) has uniform

thermal properties. In the case of food products packed in metal cans (and to some extent also in flexible pouches), this assumption is reasonably accurate since the wall, due to its thickness, has a small temperature gradient across it as well as little effect on the overall heat capacitance of the system.

The situation is slightly different for glass jars. The thermal conductivity of glass is larger than that of food materials, and food materials have a smaller respective heat capacity. (The thermal conductivity of glass is about twice that of water and the respective heat capacity is approximately half, leading to a 4:1 ratio in thermal diffusivity.) An observation of a qualitative nature is that the converted f-value, based upon the jar's exterior dimensions and the thermal properties of the entire body taken uniformly to be that of the packed food, will probably result in a slightly larger (though close) f-value compared to the experimental value. The data given by Townsend *et al.* (1949) for conduction heating products packed in metal cans and glass jars substantiate this approach. Thus, it seems safe that one can convert heating data from metal cans to glass jars by assuming the glass wall to have the thermal properties of the packed food, i.e., to assume the jar with its external dimensions to be solid food.

If more accurate results are required, a quantitative approach should be used where in the food product packed in a glass jar is represented by a body with multilayer thermal properties. Analysis of such multilayer transient conduction systems requires special mathematical techniques, mainly numerical methods, such as a finite difference or finite elements. A complete analysis of the transient conduction heat transfer in such a case (leading to, among other things, the determination of appropriate conversion factors) is in progress by our group.

Conversion factors for convection heating

Convection heating is assumed for products with viscosities not greatly different from water heated in a non-agitated mode (e.g., juices, thin soups, and small particles in liquid such as peas in brine, etc.) and for products with a higher viscosity for agitated processes (e.g., thick soups, cream style corn, etc.). Under such conditions it is assumed that the container and its contents are heated in (or close to) a Newtonian manner (i.e., small, negligible temperature gradients within the product). Under such conditions the heat flow through the container to the product can be derived in the following lumped-sum form:

$$MC_p \frac{dT}{dt} = A\ U\ (T - T_i) \tag{1}$$

where

$$\frac{1}{U} = \frac{1}{h_o} + \frac{l}{k} + \frac{1}{h_i}. \tag{2}$$

Rearranging eqn (1) integrating it between the boundary conditions ($T = T_o$at

$t = 0$) and converting the logarithm from natural to base 10 yields:

$$2.303 \log \frac{T_1 - T}{T_1 - T_0} = \frac{A}{MC_p} U \, t. \tag{3}$$

Equation 3 yields a straight line on a semilogarithmic scale. Defining the temperature response parameter, f, to be the time required for the unaccomplished dimensionless temperature value $(T_1 - T)/(T_1 - T_0)$ to traverse one log cycle, one gets:

$$2.303 \log 10 = \frac{A}{MC_p} U f \tag{4}$$

or

$$f = \frac{2.303 \, MC_p}{A} \frac{1}{U}. \tag{5}$$

Substituting the value of U (eqn 2) into eqn 5 yields:

$$f = \frac{2.303 \, MC_p}{A} \left(\frac{1}{h_0} + \frac{l}{k} + \frac{1}{h_i} \right). \tag{6}$$

Equation (6) is the overall expression for the temperature response parameter, f, in convection heating, with the ratio between any two f-values being the respective conversion factor. Before proceeding, it is worthwhile to analyze the significance of the MC_p, l, and the A parameters in eqn (6).

The heat capacity, MC_p, represents the total heat capacity of the heated body, i.e., the container plus its contents. In the case of a metal container or a flexible pouch the heat capacity of the container is obviously negligible compared to that of the contents (for example, the heat capacity of a 303 × 406 can filled with an aqueous solution is approximately 0.01 and 1.1 Btu/lb°F for the container and contents respectively.) However, in cases where products are packed in glass jars, the container heat capacity is not negligible and should be taken into account. (For example, the heat capacity of a 303 × 508 glass jar filled with an aqueous solution is approximately 0.087 and 1.08 Btu/lb°F for the glass jar and contents respectively.)

The thickness of the glass, l, a major resistance to heat flow, is not uniform throughout the jar and may vary by as much as 30%. From the basic heat transfer equation, $Q = \frac{k}{l} A \Delta T$ (ΔT is the temperature gradient across the glass), it can easily be shown that the average thickness, l, is more acurately described as $A/l = \frac{1}{n} \sum_{i=1}^{n} (A/l)_i$, or if equally spaced across the glass surface, $1/l = \frac{1}{n} \sum_{i=1}^{n} 1/l_i$, rather than the usually taken arithmetic average of $l = \frac{1}{n} \sum_{i=1}^{n} l_i$.

The area A, represents the surface available for the transfer of heat. In computing the heat transfer surface one must resolve whether the compared heat transfer area of the containers should (or should not) include the top (i.e., $A = \pi DH + 2\pi D^2/4$ *vs* $A = \pi DH + \pi D^2/4$). There is significant evidence indicating that the heat transfer coefficients through the headspace (i.e., the top) are much smaller than those through the areas in direct contact with the internal fluid. This is especially true for convective heating systems where he heat transfer coefficient through the headspace is from 2 to 10 Btu/hr ft^2 °F and where the heat transfer coefficient through the internal liquid is approximately 100 Btu/hr ft^2 °F (Blaisdell, 1963: Evan & Board, 1954; Hidding, 1975). This suggests that when dealing with vertically-positioned nonagitated containers, the area of comparison should probably exclude the top (i.e., $A = \pi DH + \pi D^2/4$). On the other hand, for agitated processes or horizontally-positioned containers (e.g. Hydrostatic and Hydrolock retort systems) the considered area should include both the top and bottom surfaces. However, for most cases (excluding shallow containers) the method of area selection will have a relatively small effect on the conversion factors.

Solutions for conversion factors in convective heating are derived below for several practical cases. In general, the conversion factors deal with the same product packed in two different containers but exposed to a similar mode of turbulence-promoting forces (natural or forced). Under such circumstances one can assume that the internal heat transfer coefficient, h_i (primarily dominated by viscosity and sheer forces), has similar values in both containers.

The relationship between the *f*-values of a convective heating product packed in two different containers can therefore be derived by extracting the value, $\frac{1}{h_i}$ (eqn 6), from the equation for the first container (Index 1) and substituting into the quation for the second container (Index 2) yielding the general, overall relationship (eqn 7).

$$f_2 = \left(\frac{2.303\ MC_p}{A}\right)_2 \left[\left(\frac{fA}{2.303\ MC_p}\right)_1 + \left(\frac{l}{k}\right)_2 - \left(\frac{l}{k}\right)_1 + \left(\frac{1}{h_o}\right)_2 - \left(\frac{1}{h_o}\right)_1\right] \quad (7)$$

Equation 7 is the overall relationship between the *f*-values of two containers (the conversion factor being the respective ratio), taking into account the container wall properties (l/k) and the processing medium heat transfer coefficient, h_o.

Metal can to metal can

Processing two metal cans (or flexible pouches) in the same heating medium means the exterior heat transfer coefficients, h_o, are equivalent. The value of l/k

for metal is always negligible. Therefore, eqn (7) will become:

$$f_2 = \left(\frac{2.303\ MC_p}{A}\right)_2 \left(\frac{fA}{2.303\ MC_p}\right)_1 \tag{8}$$

or

$$CF = \frac{f_2}{f_1} = \frac{(MC_p/A)_2}{(MC_p/A)_1}. \tag{9}$$

Neglecting the heat capacitance of the metal, eqn 9 will yield:

$$CF = \frac{f_2}{f_1} = \frac{(V/A)_1}{(V/A)_2}. \tag{10}$$

Equation (10) is in the classical form expressed by Schultz & Olson (1938). The ratio (V/A) in eqn 10 can be expressed in terms of the linear dimensions for any given shape of a body.

Metal can to glass jar (and vice versa)

If the metal can and the glass jar are processed in the same heating medium, one can assume that the value of $1/h_0$ will be about the same. (The value of l/k for metal is negligible.) Eqn (7) is thus reduced to eqn (11), the conversion factor then being f_G/f_M. (G and M indicate glass and metal respectively).

$$f_G = \left(\frac{2.303\ MC_p}{A}\right)_G \left[\left(\frac{fA}{2.303\ MC_p}\right)_M + \left(\frac{l}{k}\right)_G\right] \tag{11}$$

The method of conversion from a glass jar to a metal can is performed in a similar manner, thus arriving with eqn 12:

$$f_M = \left(\frac{2.303\ MC_p}{A}\right)_M \left[\left(\frac{fA}{2.303\ MC_p}\right) - \left(\frac{l}{k}\right)_G\right]. \tag{12}$$

Glass jar to glass jar

If two glass jars have the same wall thickness and are processed in the same heating medium, the ratio of the f-values becomes trivial and is identical to the expression in conversion from metal to metal. That is,

$$\frac{f_1}{f_2} = \frac{(MC_p)_1/A_1}{(MC_p)_2/A_2} \tag{13}$$

where the MC_p represents the entire heat capacity (i.e., the heat capacity of the jar plus that of the contents) for the respective jars.

Conversion factor for two different processing media

When two containers are processed in two different media, the exterior heat transfer coefficient, h_o, should be taken into account. Clearly, the film heat transfer coefficient for condensing steam ($h_o > 3{,}000$ Btu/hr ft^2 °F) is at least one order of magnitude larger than that of agitated water ($h_o \sim 175$ to 400 Btu/hr ft^2 °F—Merril, 1948; Cowell *et al.*, 1959); therefore, the value, $1/h_o$, for steam can be neglected. The actual value of h_o for water should be selected based on experimental data, available correlations, or on an acceptable value from the literature.

The use of this conversion factor will be discussed with the following example of frequently-occurring situation. The heating parameter is required for a product packed in a glass jar to be processed in water. The heating data are available for the product packed in a metal can processed in steam. The following conversion equation is derived from eqn 7:

$$f_G = \left(\frac{2.303\ MC_p}{A}\right)_G \left[\left(\frac{fA}{2.303\ MC_p}\right)_M + \left(\frac{l}{k}\right)_G + \left(\frac{1}{h_o}\right)_G\right] \qquad (14)$$

Thus, the f-value or the conversion factor for other possible cases can easily be obtained by substituting the appropriate values into eqn 7.

Extensive experimental heat penetration data were reported by Townsend *et al.* (1949) for several products in many sizes of glass jars and metal containers processed in water and steam. We have used these data to verify our proposed derived conversion factor equations. Sets of data were tested using the appropriate conversion factor equations by inserting the appropriate thermal properties and correcting for the container dimensions (for headspace, can rims, double seams—see examples 1 and 2), as well as the glass thickness of jars (average of 2.0 mm for 202 × 309 or smaller and 2.3 mm for 208 × 401 or larger). The results (Table 1) indicate that good agreement exists between the converted f_h-values and the experimental values including converted f_h-values calculated over a wide range of container sizes, as well as simultaneously changing from one heating medium and container type to another.

Example 1. The f_h for a 401 × 411 metal can (A2-1/2), filled with 1% bentonite (convective heating) and processed in water, was reported by Townsend *et al.* (1949) to be 6.5 min. It is desired to predict the f_h for a smiliar-sized 401 × 411 jar filled with the same material and processed under similar conditions.

The thermal properties of the 1% bentonite solution were taken as those of water. Thermal conductivity of 0.6 and 9.25 Btu/hr ft^2 °F, specific heat of 0.2 and 0.12 Btu/lb °F, and density of 2.23 and 7.9 were used for glass and steel respectively. Corrections for headspace were taken to be 0.4 and 0.24 inches for the glass jar and the metal can respectively. Values of 0.25 and 0.13 inches were subtracted from the metal can's 401 × 411 nominal dimensions, reflecting rim correction for the height and thickness correction (of the double seam and of the wall) for the diameter to estimate the dimensions of the solution in the can.

Table 1. f_h Predicted based upon conversion factor equations

	Reference container				Desired Container						
								f_h, min.			
No.	Size	Heating medium	Type	f_h* Reported (min)	Size	Heating medium	Type	Predicted	Reported*	Percentage difference	Eqn used
1	202 × 214	Steam	Metal	4.5	202 × 309	Water	Glass	9.5	8.3	+ 10	14
2	211 × 210	"	"	4.1	208 × 401	"	"	10.5	10.2	+ 3	"
3	307 × 409	"	"	5.1	303 × 411	"	"	12.6	14.6	− 14	"
4	401 × 411	"	"	5.8	202 × 309	"	"	7.8	8.3	− 6	"
5	"	"	"	"	208 × 401	"	"	9.8	10.2	− 4	"
6	"	"	"	"	303 × 411	"	"	12.5	14.6	− 14	"
7	202 × 214	Water	"	3.2	202 × 309	"	"	7.0	6.9	+ 7	11
8	211 × 210	"	"	4.4	208 × 401	"	"	9.3	8.8	+ 6	"
9	202 × 214	"	"	3.2	202 × 309	"	"	6.9	6.9	0	"
10	"	"	"	1.8	"	"	"	5.4	6.0	− 10	"
11	"	"	"	2.8	"	"	"	6.5	7.1	− 8	"
12	211 × 210	"	"	3.4	208 × 401	"	"	8.4	8.9	− 6	"
13	401 × 411	"	"	6.5	401 × 411	"	"	13.3	13.8	− 4	"
14	"	"	"	5.5	"	"	"	12.3	13.4	− 8	"
15	208 × 401	"	Glass	8.9	211 × 210	"	Metal can	3.95	3.9	+ 1	12
16	"	"	"	"	202 × 214	"	"	3.3	3.8	− 13	"
17	401 × 411	"	"	13.4	401 × 411	"	"	6.11	6.0	+ 2	"
18	202 × 309	"	"	8.3	208 × 401	"	Glass jar	9.7	9.5	"	13
19	208 × 401	"	"	8.8	401 × 411	"	"	13.7	13.4	"	"
20	401 × 411	"	"	13.4	202 × 309	"	"	7.2	7.0	+ 3	"

*Data reported by Townsend *et al.*, (1949)

Knowing the glass wall thickness of the 401 × 411 glass jar ($l = 2.3$ mm) and using eqn (11), the predicted f_h was found to be 13.3 min, 4% less than the experimental value of 13.8 min reported by Townsend *et. al.*, 1949) (example 13, Table 1).

Example 2. The f_h for a 401 × 411 metal A2-1/2 can filled with 1% bentonite processed in steam was reported as 5.8 min (Townsend *et al.*, 1949). It is desired to predict the f_h for a 202 × 309 baby glass jar filled with the same material processed in water.

Substituting the thermal properties (outlined in example 1) and exterior water film coefficient, h_o, of 250 Btu/ft^2 °F into the appropriate equation (eqn 13) yielded an f_h of 7.8 min for the 202 × 309 glass jar as compared to the experimental value of 8.3 min reported by Townsend (Example 4 in Table 1). Though the above conversion was performed over a wide range of sizes (volumetric ratio of 401 × 411 to 202 × 309 is approximately 2:1) as well as when changing the heating medium, only a small difference (about 6% between the 7.8 min predicted and the 8.3 min measured) was determined.

The exterior heat transfer film coefficient in agitated water ranges approximately between 175 and 400 Btu/hr ft^2 °F. The exact value depends primarily upon the degree of turbulence and the body configuration. The selected value of the heat transfer coefficient will obviously affect the computed f_h. A significant decrease in the f-falue is expected when increasing the extermal heat transfer coefficient from 50 (minimum h_o for nonagitated water) to 200 Btu/hr ft^2 °F, after which the f-value rapidly approaches an asymptotic value.

In conclusion, the present study developed the theoretical equations for the conversion factors in a broader spectrum of containers (e.g., glass jar to metal can and vice versa), as well as processing media (e.g., water to steam and vice versa), and verified experimentally that the converted values provide accurate results. The developed equations enable the thermal process engineer to use conversion factors in a simple manner for most pratical processing conditions.

Finally, the application of any type of conversion factor should be used with care. One should bear in mind that for a given product (even processed under similar tubulence-promoting conditions) changes in type of container size, kind of filling, etc. can lead to unaccountable dissimilarities resulting in process deviation. As such, conversion factors should be used only as the preliminary step of process design and by no means should they be interpreted as a substitute for the final experimental validation step of the actual delivered lethality.

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Notations

A: Surface heat transfer area
CF: Conversion factor—the ratio between two heating rate parameters
D: Container diameter
f: The temperature response parameter—the time required for the straight portion of the semilogarithmic curve to traverse one log cycle
H: Container height
h_i: Internal heat transfer coefficient
h_o: External heat transfer coefficient
k: Thermal conductivity of wall material
l: Thickness of wall
MC_p: Heat capacity
N_{Bi}: Biot number
Q: Heat flow
R: Characteristic dimension
t: Time
T: Temperature, variable
T_r: Medium temperature
T_o: Initial temperature
U: Overall heat transfer coefficient
V: Volume of body
α: Thermal diffusivity

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Factors controlling the growth of *Clostridium botulinum* types A and B in pasteurized, cured meats

I. Growth in pork slurries prepared from 'low' pH meat (pH range 5.5–6.3)

T. A. ROBERTS, ANGELA M. GIBSON AND A. ROBINSON*

Summary

The effect of combinations of sodium chloride (2.5, 3.5, 4.5% w/v on water), sodium nitrite (100, 200, 300 μg/g), sodium nitrate (0, 500 μg/g), sodium isoascorbate (0,1000 μg/g, or equimolar with nitrite level) and polyphosphate (Curaphos 700; 0, 0.3% w/v), on the growth of *Clostridium botulinum* types A and B was studied in an experimental pork slurry system, without heating and after two heat treatments (80°C for 7 min and 80°C for 7 min plus 70°C for 1 hr) followed by storage at: 15, 17.5, 20 or 35°C for up to 6 months.

Statistical analyses showed that increasing salt or nitrite levels, adding isoascorbate or nitrate, using the highest heat treatment or decreasing the storage temperature all significantly reduced toxin production by *Cl. botulinum*. The addition of 0.3% polyphosphate (Curaphos 700) significantly increased toxin production. There were many significant two-factor interactions; the effect of increasing nitrite was relatively less if isoascorbate was present, at 4.5% salt, or at low storage temperature. The presence of isoascorbate also counteracted the increase in toxin production attributed to the presence of polyphosphate.

Introduction

Research into nitrosamine toxicity and carcinogenesis began after indications of human toxicity arising from the industrial use of dimethylnitrosamine (Barnes & Magee, 1954; Magee, 1973). Their possible occurrence other than in the industrial environment was not suspected until a serious liver disease occurred in Norway in 1960 (Sakshaug *et al.*, 1965) in sheep which had been fed fish meal preserved with nitrite, and which was later shown to contain dimethylnitrosamine (Ender *et al.*, 1964). That sufficient dimethylnitrosamine to kill animals

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could be formed raised the possibility that nitrosamines might also occur in foods for human consumption, and Ender & Ceh (1968) detected 0.5–4.0 μg/kg nitrosamines in smoked herring, kippers, smoked haddock, smoked sausages, bacon, smoked ham and fungi including mushrooms.

Subsequently nitrosamines have been reported in raw and smoked fish, wheat flour, milk, Tilsit cheese, alcoholic beverages, ham, frankfurters, bacon and other cured meats (Sebranek & Cassens, 1973; Gray, 1976; Gray & Randall, 1979), and in various industrial environments (Ember, 1980).

Formation of nitrosamines from secondary amines and nitrites under the conditions existing in the human stomach has been demonstrated (Sander, 1967; Sander, Schweinsberg & Menz, 1968) and simultaneous feeding to rats of secondary amines and sodium nitrite resulted in the production of tumours (Sander & Burkle, 1969). The possibility that nitrosamines may be formed from ingested nitrites and secondary or tertiary amines in the diet has led to the wish to minimize the amounts of nitrite and nitrate used in meat curing (Status Report, 1972). Nitrite *per se* appeared to be carcinogenic (Newberne, 1979) but a review of the data commissioned by the U.S. Food and Drug Administration and the U.S. Department of Agriculture revealed insufficient evidence to support this conclusion (Dickson, 1980).

The relative importance of cured meats as a source of nitrite and N-nitroso compounds should be kept in perspective. In addition to cured foods there are several sources of nitrite likely to contribute significantly to the total dietary intake. The relevance of such additional sources of nitrite, including fresh vegetables, dehydrated foods, cigarette smoke and polluted air, and endogenous sources such as saliva, intestinal contents and infections of the stomach or bladder has been summarized and reviewed by Tannenbaum (1979).

Nitrite gives cured meat its characteristic colour (Fiddler *et al.*, 1973a; Fujimaki, Emi & Okitani, 1975) and contributes to the flavour (Cho & Bratzler, 1970; Wasserman & Talley, 1972; Kueper & Trelease, 1974; Mottram & Rhodes, 1974). It is also important in preventing the growth of bacteria, particularly *Clostridium botulinum* (Steinke & Foster, 1951; Blanche-Koelensmid & van Rhee, 1968; Greenberg, 1972; Ingram, 1974, 1976) which occurs naturally in pork and cured products (Roberts & Smart, 1977). Changes in the permitted levels of nitrite will therefore affect the microbiological stability of the product and a fuller understanding of the factors which control the growth of microbes, particularly *Cl. botulinum*, is needed to ensure that the excellent safety record of cured meat products is maintained.

There is disagreement whether the nitrite added or the residual nitrite is the more important factor governing the control of *Cl. botulinum* in cured meats. There are numerous reports that the likelihood of toxin production by *Cl. botulinum* is better predicted from the initial nitrite level than the residual (Greenberg, 1972; Christiansen *et al.*, 1973; Hustad *et al.*, 1973; Bowen & Diebel, 1974; Pivnick & Chang, 1974). However, residual nitrite (at time of inoculation) was the critical factor in determining growth or inhibition of dormant spores over long periods of storage in laboratory media (Ashworth &

Spencer, 1972; Ashworth, Hargreaves & Jarvis, 1973). It has been suggested that prevention of growth of spores of *Cl.botulinum* is the consequence of germinated spores losing viability while nitrite remains, implying that the safety of canned meat is dependent upon sufficient residual nitrite remaining until the number of viable cells decreases below that needed to initiate growth (Christiansen, Tompkin & Shaparis, 1978).

In a laboratory medium, nitrite heated in the presence of a casein hydrolysate and a reducing agent was about ten times more inhibitory to *Cl.sporogenes* than filter-sterilized nitrite (Perigo, Whiting & Bashford, 1967). The heat treatment required to form the factor was greater than that to which pasteurized cured products are subjected, and the additional inhibitory properties were lost when pork was added suggesting that it might not be relevant in pasteurized meat products (Johnston, Pivnick & Samson, 1969). Spores which were inhibited by heated nitrite remained viable, and grew when transferred to fresh medium (Roberts & Smart, 1974). A similar, though less marked, effect which increased with the amount of heating, was found in meat (Ashworth & Spencer, 1972; Ashworth *et al.*, 1973). A nitrite derived inhibitor in meat was also demonstrated by Pivnick & Chang (1974), who stored canned meat until nitrite was no longer detectable and then introduced spores of *Cl.botulinum* previously heated in curing salts. The duration of spore inhibition was longer the greater the initial concentration of nitrite indicating that it had been converted to a substance able to prevent growth of *Cl.botulinum*.

The mechanism of nitrite inhibition is still unclear. It has been suggested that it reacts with –SH groups of enzymes such as dehydrogenases or coenzyme A thereby blocking energy metabolism (Castellani & Niven, 1955; Dainty & Meredith, 1973; O'Leary & Solberg, 1976). More recently Yarbrough, Rake & Eagon (1980) reported the specific inhibition of aldolase by nitrite. A complex formed between cysteine, ferrous ions and nitrite during autoclaving of laboratory medium inhibited vegetative growth of *Cl.perfringens* (Moran, Tannenbaum & Archer, 1975) and in aerobic systems nitrite may oxidize ferrous iron of an electron carrier(s) such as cytochrome oxidase to ferric iron (Rowe *et al.*, 1979).

Sodium ascorbate and sodium isoascorbate (erythorbate) have been used in cure mixtures to accelerate cure colour formation. Both are reducing agents and prevent the formation of undesirable oxidation products thereby stabilizing colour and flavour (Fiddler *et al.*, 1973b). Ascorbic acid reacts with nitrite forming dehydroascorbic acid and nitric oxide (Mottram *et al.*, 1975) making it less available for the nitrosation of secondary amines (Mirvish *et al.*, 1972; Fiddler *et al.*, 1973a, b; Fan & Tannenbaum, 1973). Both ascorbate and isoascorbate could therefore be regarded as antinitrosating reagents (Tompkin, Christiansen & Shaparis, 1978a). The proportion of the ascorbic anion to nitrite is important (Mirvish *et al.*, 1972) and a ratio ascorbic acid : nitrite greater than 2 : 1 inhibited nitrosamine formation at pH 4.0 (Fan & Tannenbaum, 1973), while at ratios below 2 : 1 it was only partially inhibited. Mottram *et al.* (1975) demonstrated that nitrosation of dimethylamine during curing and canning was

suppressed approximately 80% when 1 mol ascorbate per mol nitrite was present in the curing brine. Ascorbate had no effect on the ability of nitrite to inhibit the formation of toxin by *Cl.botulinum* in wieners (Bowen & Deibel, 1974; Bowen, Gerveny & Deibel, 1974). In bacon ascorbate decreased the efficacy of nitrite to inhibit *Cl.botulinum*, especially at levels of ascorbate greater than 500 μg/g (Bowen & Deibel, 1974). Other workers have claimed that ascorbate and nitrite act synergistically. The addition of 1% ascorbic acid reduced the sodium nitrite tolerance of *Cl.botulinum* in laboratory medium (Baird-Parker & Baillie, 1974) and nitrite and ascorbic acid interacted to enhance the effectiveness of nitrite in mildly salted or medium salted bacons (Crowther *et al.*, 1977). Similarly isoascorbate enhanced the antibotulinal effect of nitrite in perishable canned cured meats (Tompkin *et al.*, 1978a). Storage at low temperatures (4.4, 10°C) prior to temperature abuse (27°C) increased nitrite depletion in the presence of isoascorbate, and the authors suggested that this could increase the risk of growth of *Cl.botulinum* if a product is temperature abused after a period of refrigerated storage (Tompkin *et al.*, 1978b).

In the U.K. the use of ascorbic acid (or its sodium or calcium salt) is permitted only as an antioxidant (Antioxidants in Food Regulations, 1978). Isoascorbic acid (erythorbic acid) as an antioxidant was reviewed in 1964 (Food Standards Committee, 1964) but its use was not recommended since ascorbic acid was already permitted. Isoascorbate is therefore not permitted in the U.K. although it is commonly used in cure mixtures in the U.S.A. (Tompkin *et al.*, 1978a). There are few comparisons of the effectiveness of ascorbate and isoascorbate in foods: superior antioxidant properties are claimed for ascorbic acid, particularly in processes involving heat, and it stabilizes the colour of cured meats more effectively than isoascorbic acid (Borenstein, 1965).

The mechanism by which ascorbate/isoascorbate enhances the antibotulinal effect of nitrite is not understood. Reducing agents, such as ascorbate or cysteine, enhanced the antibotulinal effect of nitrite in underprocessed meat slurry (Johnston & Loynes, 1971) perhaps by transferring the nitroso group directly to components of the bacterial cell, or by the subsequent release of nitric oxide. Tompkin, Christiansen & Shaparis (1978c) suggested that isoascorbate, ascorbate, cysteine and EDTA bind cations essential for the repair of nitrite injured cells.

The value of nitrate in cured meats in addition to nitrite has also been questioned. In unheated cured products the shelf-life of vacuum packed bacon (pH 5.45–6.05) stored at 5 or 15°C was similar in the presence or absence of nitrate (*ca* 500 μg/g) (Taylor & Shaw, 1975), but nitrate (*ca* 500 μg/g) sometimes reduced the rate of microbial growth in vacuum packed collar bacon (pH 5.90–6.45) stored at 5 or 15°C (Shaw, 1974; Taylor, Shaw & Jolley, 1976). Nitrate had little effect on growth of clostridia in those bacons (Roberts, 1975; Roberts & Smart, 1976). In heated cured products 156 μg/g nitrate had little effect on microbial growth, including putrefactive anaerobes (Silliker, Greenberg & Schack, 1958). Levels of nitrate up to 450 μg/g did not significantly affect toxin production by *Cl.botulinum* in experimentally produced wieners

(Hustad *et al.*, 1973) and Roberts & Ingram (1977) reported the effect of nitrate (500 μg/g) added to pasteurized pork slurries inconsistent. Duncan & Foster (1968a, b) reported that nitrate (up to 1000 μg/g) was probably not significant in the control of *Cl. botulinum* in canned luncheon meats, and Greenberg (1972) found nitrate (up to 200 μg/g) showed no antibotulinal activity in canned hams. At a given nitrite level, nitrate (500–2000 μg/g) had a slight but statistically significant effect in delaying development of *Cl. botulinum* toxin in canned comminuted ham (Christiansen *et al.*, 1973) but some nitrate was reduced to nitrite.

Polyphosphates are used commercially to aid water retention, which is claimed to improve tenderness, juiciness and flavour (Spencer & Smith, 1962) and decrease cooking shrinkage (Monk, Mountney & Prudent, 1964; Mountney & Arganosa, 1963). Some are claimed to be antimicrobial (Stauffer Chemical Co., 1979). There are relatively few reports on the effect of polyphosphates on growth and toxin production of *Cl. botulinum*. Polyphosphate (0.5% Curaphos 700) significantly reduced toxin production in a meat slurry system containing 3.5%, but not 2% salt, while 0.3% was less inhibitory (Jarvis, Patel & Rhodes, 1977). There was a significant interaction between polyphosphate and nitrite and the effect of polyphosphate was also related to pH change in the meat.

The number of cells used as the inoculum also influences the extent of growth of *Cl. botulinum*. Christiansen *et al.*, (1974) detected toxin at all nitrite levels tested (up to 340 μg/g) in bacon inoculated with 19 000 spores *Cl. botulinum* per g (4300 after processing) whereas 210 spores *Cl. botulinum* before processing (52 after processing) failed to produce toxin at 170 or 340 μg/g nitrite when stored at 27°C. An inoculum of 100 spores/g meat produced toxin in the presence of 150 μg/g nitrite (added), but not 200 μg/g, whereas 5000 spores/g produced toxin in meat product containing 400 μg/g but not 500 μg/g nitrite (Christiansen *et al.*, 1973). There was no effect of spore inoculum (10^1, 10^3 or 10^5) on the number of samples containing toxin after incubation at 17.5, 20, 22.5 or 25°C, but at 15° an inoculum of 10^3 caused a higher proportion of samples to contain toxin than did 10^1 (Roberts, Jarvis & Rhodes, 1976).

Storage temperature is also important. Storage of canned comminuted meat at 7°C prevented spoilage and development of botulinal toxin for up to 6 months, whereas similar product stored at 27°C became toxic within 2–3 weeks (Christiansen *et al.*, 1973). *Clostridium botulinum* type A (inoculum 5000 spores) failed to produce toxin in liver sausage at 18°C for up to 3 months, whereas at 30°C it became toxic within 20 days (Steinke & Foster, 1951). *Clostridium botulinum* is inhibited more readily by curing salts at reduced storage temperatures (Roberts, Jarvis & Rhodes, 1976; Ingram, 1974).

The importance of pH at any given nitrite level was demonstrated by Castellani & Niven, (1955) who showed that the nitrite sensitivity of *Staphylococcus aureus* increased with reducing pH values from 6.9 to 5.05. Reducing the pH value from 7.0 to 6.0 or 5.5 lowered the salt tolerance of vegetative cells of *Cl. botulinum* types A, B & E (Baird-Parker & Freame, 1967). In studying varying levels of salt and nitrite in combination, less growth and

toxin production of *Cl.botulinum* occurred at lower pH values in laboratory media (Roberts & Ingram, 1973; Roberts, 1974) and in vacuum packed Dutch smoked ring (pork) sausage (Blanche-Koelensmid & van Rhee, 1968).

The pasteurization process given to large hams (over *ca* 1 kg) and some other cured meat products is insufficient to destroy bacterial spores, but their growth is prevented by a combination of factors including the severity of heat treatment, the storage temperature, pH, salt and nitrate levels. Heated spores are more sensitive to salt and nitrite than unheated spores (Roberts & Ingram, 1966; Jarvis *et al.*, 1976).

The factors discussed above should not be considered individually. Although factors were known to act in combination or to interact in controlling the growth of clostridia (Bulman & Ayres, 1952; Riemann, 1963; Baird-Parker & Freame, 1967; Riemann, Lee & Genigeorgis, 1972), Roberts & Ingram (1973) first expressed semi-quantitatively the triple interaction of pH × NaCl × $NaNO_2$. In principle, therefore, the same degree of stability and safety with respect to *Cl.botulinum* can be achieved by many different combinations of pH, NaCl, $NaNO_2$ and storage temperature.

The purpose of this study was to acquire sufficient data to determine the minimum sodium nitrite concentration required to control *Cl.botulinum* taking into account realistic commercial conditions of pH, NaCl, storage temperature, heat process and other additives such as sodium isoascorbate, polyphosphate and sodium nitrate.

Pasteurized cured meats have an excellent record with respect to food-poisoning, and consideration of the growth of *Cl.botulinum* in the presence of commercially realistic combinations of the above factors could lead to a model able to express the relative effects of changes in formulation, and against which proposed chemical additives or substitutes for nitrite could be tested.

Materials and methods

Meat slurry system

The pasteurized pork slurry system described by Rhodes & Jarvis (1976) was used. Fresh pork leg was defatted by hand, cut into small pieces and minced through a 4-mm plate of a 'Crypto Mincer' (Model No. AB12) and stored in 1 kg amounts at − 10°C. Prior to use it was thawed at 1°C for 15 hr, then held for 4 hr at room temperature (approximately 18–20°C). The thawed, minced pork was homogenized with water containing the chemical additives (pork:water 1.0:1.5) using a 'Hobart' bowl chopper (Model No. VCM25, Hobart Manufacturing Co., 115 Brunswick Park Road, New Southgate, London N11) for 30 sec at low speed and 45 sec at high speed. The resultant slurry was filled into 28 ml screw-capped bottles ('universal' bottles) using an 'Albro' single head vacuum operated filler (Albro Filling and Engineering Co. Ltd., Wharfe Road, Ponders End, Middlesex). After dispensing the capped bottles were left overnight

(18–20hr) at 1°C, then held at room temperature for 1 hr to allow the contents to reach about 15°C prior to inoculation and heating. This procedure of preparation and storage at 1°C, and inoculation and heating the following day, ensured that all slurries were treated similarly and that the slurry temperature was about 15°C at the time of inoculation.

Organisms

The inoculum consisted of a mixed spore suspension of equal numbers of *Cl. botulinum* type A: NCTC 3806, NCTC 9837, NCTC 7272, 62A (National Canners Association, Berkeley, California, U.S.A.) 33A (D. Berkowitz, U.S. Army Natick Laboratories, Natick, Mass., U.S.A.) and type B: NCTC 3807, NCTC 751, 213B (National Canners Association) CN1356 and CN5009 (P. D. Walker, Wellcome Research Laboratories, Beckenham, Kent).

Spore production

Strains NCTC 9837, 62A, 33A, NCTC 3807, NCTC 751, 213B and CN 5009 sporulated on Blood Agar Base (Oxoid Ltd.) containing 5% (w/v) defibrinated horse blood (Wellcome Reagents Ltd., Beckenham, Kent), incubated at 30°C for 5 days, harvested centrifuged and washed three times with sterile distilled water. Strains NCTC 7272, NCTC 3806 and CN 1356 were cultured in 250-ml conical flasks using a 2-phase system similar to that described by Bruch, Bohrer & Denny (1968) comprising 100 ml of sterile solidified TPAYC agar (Trypticase 5%, Bacto-peptone 0.5%, ammonium sulphate 1.0%, Yeast Extract 0.2%, cysteine hydrochloride 0.05%, Agar 3%) overpoured with 100 ml of sterile 0.05% cysteine hydrochloride, and inoculated with 2 ml from an actively growing cooked meat medium culture. Strain CN 1356 was incubated at 35°C for 4 days, and strains NCTC 7272 and NCTC 3806 at 35°C for 3 days. Spores were harvested by centrifugation, washed three times in sterile distilled water and stored at 4°C as concentrated suspensions.

Preparation of spore inocula

Equal numbers of spores of each strain were mixed and diluted with sterile water to give final concentrations of 2×10^3 spores/ml and 2×10^1 spores/ml: 0.5 ml of one of these suspensions was inoculated into the centre of each bottle using a 1-ml Cornwall continuous pipetting syringe (Beckton Dickenson Ltd.), withdrawing the needle during inoculation to distribute the spores evenly through the slurry in the bottle.

Heat treatment and replication

Unheated (UH). Five replicates per inoculum level were placed unheated at each storage temperature.

LOW heat treatment (80°C/7 min). There were ten replicates per inoculum level and storage temperature.

HIGH heat treatment (80°C/7 min + 70°C/1 hr). There were ten replicates per inoculum level and storage temperature.

The number of replicates for heated samples was reduced to five for slurry 37 *et seq.*

The LOW heat treatment raised the centre temperature of the slurry in the bottles from 15°C to 70°C in 7 min. In the HIGH heat treatment (80°C/7 min + 70°C/1 hr) bottles of slurry were heated at 80°C for 7 min to raise the centre temperature to 70°C, then transferred to a water bath at 70°C where the temperature was maintained for 1 hr. After heating the bottles were cooled at room temperature for 1 hr and then at 1°C for 4 hr to reduce the temperature of the slurry to below the lowest storage temperature (i.e. below 15°C).

Experimental plan

The factors investigated were:	
NaCl (% w/v on water)	2.5, 3.5, 4.5
$NaNO_2$ (μg/g slurry)	0, 100, 200, 300
$NaNO_3$ (μg/g slurry)	0, 500
Sodium isoascorbate (μg/g slurry)	0, 1000, equimolar with $NaNO_2$
Polyphosphate* (% w/v slurry)	0, 0.3
Inoculum (spores per bottle)	0, 10^1, 10^3
Heat treatment	0, 80°C/7 min, 80°C/7 min + 70°/1 hr
Storage temperature (°C)	15, 17.5, 20, 35

Sodium isoascorbate was used throughout to enable these data to be compared with U.S. publications. There is no reason to suppose that it differs from sodium ascorbate with respect to its anti-botulinal effects.

Equimolar isoascorbate was used additionally in early experiments since Mottram *et al.*, (1975) found ascorbate equimolar with nitrite in the curing brine the most effective ratio to suppress the nitrosation of dimethylamine.

A complete list of slurries prepared is shown in Table 1.

Examination of stored samples

Spoilage. During storage the bottles were examined for spoilage typical of the growth of *Cl. botulinum* at intervals of: 7 days at 35°C, 14 days at 20°C, 21 days at 17.5°C and 28 days at 15°C.

*Curaphos 700, Fibrisol Service Ltd., Colville Road, London W3 8TE

Table 1. Complete list of slurries prepared including chemical analysis of slurries immediately after preparation

Slurry	pH value	NaCl (% w/v water) Added	NaCl (% w/v water) Detected	Nitrite (μg/g) Added	Nitrite (μg/g) Detected	Nitrate μg/g) (Added)	Iso-asco-bate (μg/g) (Added)	Poly-phosphate (% w/v) (Added)	Fat (%)	H_2O (%)
3	5.66	2.5	2.18	100	91	—	—	—	1.11	87.86
4	6.06	2.5	2.10	100	88	—	1000	0.3	1.42	86.84
5	5.69	2.5	1.87	100	73	—	1000	—	1.15	88.20
6	6.08	2.5	2.08	100	90	—	—	0.3	1.21	88.65
7	5.81	2.5	2.17	—	—	—	—	—	1.60	88.09
8	6.22	2.5	2.22	—	—	—	1000	0.3	1.70	87.48
9	5.81	2.5	2.17	100	79	—	Equ -*	—	1.70	87.57
10	6.19	2.5	2.21	100	83	—	Equi-	0.3	1.67	87.22
11	5.69	2.5	2.23	200	186	—	—	—	1.58	87.57
12	5.72	2.5	2.18	200	174	—	Equi-	—	1.54	87.59
13	6.04	2.5	2.28	200	178	—	—	0.3	1.93	87.59
14	6.03	2.5	2.23	200	203	—	Equi-	0.3	2.05	87.23
15	5.68	2.5	2.28	200	175	—	1000	—	2.29	87.42
16	6.10	2.5	2.47	200	175	—	1000	0.3	2.44	87.12
17	5.62	2.5	2.39	300	273	—	—	—	1.79	87.80
18	6.03	2.5	2.37	300	282	—	—	0.3	1.88	87.39
19	5.89	2.5	2.42	300	270	—	Equi-	—	1.75	87.19
20	6.29	2.5	2.26	300	253	—	Equi-	0.3	1.73	88.09
21	5.83	2.5	2.28	300	262	—	1000	—	2.37	87.54
22	6.24	2.5	2.08	300	239	—	1000	0.3	1.77	87.72
23	5.86	3.5	3.03	—	—	—	—	—	2.69	86.50
24	6.25	3.5	2.96	—	—	—	1000	0.3	2.60	86.72
25	5.92	3.5	3.17	100	99	—	—	—	2.84	86.18
26	6.36	3.5	3.25	100	112	—	—	0.3	2.44	86.52
27	5.73	3.5	3.13	100	82	—	1000	—	2.04	86.70
28	6.11	3.5	3.03	100	79	—	1000	0.3	1.87	86.70
29	5.72	3.5	2.95	200	175	—	—	—	1.69	87.22
30	5.74	3.5	3.02	200	155	—	1000	—	1.70	86.52
31	6.20	3.5	3.08	200	271	—	—	0.3	1.87	86.89
32	6.22	3.5	3.04	200	246	—	1000	0.3	1.48	87.20
33	5.66	3.5	3.16	300	254	—	—	—	1.61	87.19
34	5.74	3.5	3.02	300	277	—	1000	—	1.73	86.80
35	6.03	3.5	3.32	300	273	—	—	0.3	1.74	86.33
36	5.93	3.5	3.12	300	241	—	1000	0.3	1.53	86.88
37	6.25	2.5	2.21	200	157	500	—	—	1.29	87.91
38	6.12	2.5	2.24	200	177	500	1000	0.3	1.28	88.16
39	5.69	3.5	3.13	200	183	500	—	—	0.81	87.59
40	6.07	3.5	2.98	200	157	500	1000	0.3	0.86	87.34
41	5.92	2.5	2.37	100	104	500	—	—	0.78	88.23
42	6.10	2.5	2.22	100	84	500	1000	0.3	0.81	87.75

Table 1 *continued*

Slurry	pH value	NaCl (% w/v water) Added	NaCl (% w/v water) Detected	Nitrite (μg/g) Added	Nitrite (μg/g) Detected	Nitrate (μg/g) (Added)	Iso-ascorbate (μg/g) (Added)	Poly-phosphate (% w/v) (Added)	Fat (%)	H_2O (%)
43	5.83	3.5	3.14	100	99	500	—	—	0.88	87.56
44	5.97	3.5	3.03	100	80	500	1000	0.3	0.73	87.26
45	5.71	2.5	2.36	100	78	—	—	—	1.34	87.65
46	6.16	2.5	2.23	100	56	—	1000	0.3	0.98	87.48
47	5.74	2.5	2.19	300	254	—	—	—	1.19	88.02
48	6.14	2.5	2.14	300	238	—	1000	0.3	1.08	87.72
49	5.69	2.5	2.12	100	84	500	—	—	1.02	87.74
50	6.13	2.5	2.06	100	73	500	1000	0.3	0.98	87.50
51	5.54	4.5	4.24	—	—	—	—	—	1.50	85.70
52	5.61	4.5	4.06	100	89	—	—	—	1.01	86.34
53	5.60	4.5	3.84	200	177	—	—	—	1.76	86.00
54	5.60	4.5	3.78	300	248	—	—	—	2.18	85.40
55	5.84	4.5	3.82	200	150	500	—	—	1.90	85.70
56	6.13	4.5	3.23	200	134	—	1000	0.3	0.84	86.43
57	5.83	4.5	3.82	200	150	—	1000	—	1.24	86.12
58	6.00	4.5	3.79	200	165	—	—	—	0.96	86.16
59	6.03	4.5	3.63	200	154	500	1000	—	1.12	86.08
60	5.72	4.5	3.93	200	162	500	—	0.3	1.34	85.66
61	5.98	4.5	3.34	200	150	500	1000	0.3	1.10	85.94
62	5.40	3.5	3.15	100	92	500	1000	—	1.17	86.77
63	5.94	3.5	3.21	100	111	500	—	0.3	1.18	86.42
64	5.82	2.5	2.25	100	75	500	1000	—	1.72	87.46
65	6.16	2.5	2.11	100	81	500	—	0.3	1.55	87.36
66	5.71	3.5	3.13	200	160	500	1000	—	1.49	86.69
67	6.10	3.5	3.10	200	184	500	—	0.3	1.67	86.45
68	5.79	2.5	2.27	200	172	500	1000	—	1.07	87.85
69	6.12	2.5	2.15	200	177	500	—	0.3	1.18	87.49
70	5.77	3.5	3.02	300	239	500	1000	—	1.53	86.47
71	6.13	3.5	2.92	300	263	500	—	0.3	1.48	86.46
72	5.96	4.5	3.66	100	62	—	1000	0.3	1.51	85.75
73	5.69	4.5	3.94	100	71	—	1000	—	1.51	86.03
74	5.84	4.5	4.00	100	88	—	—	0.3	1.79	85.75
75	5.92	4.5	3.87	100	69	500	1000	0.3	1.53	85.82
76	5.70	4.5	4.06	100	70	500	1000	—	1.29	86.32
77	6.05	4.5	3.76	100	87	500	—	0.3	1.17	86.01
78	5.80	4.5	3.81	300	227	500	1000	—	1.46	85.96
79	6.12	4.5	3.64	300	223	500	1000	0.3	1.42	86.30
80	5.85	4.5	3.89	300	225	—	1000	—	1.20	86.23
81	6.11	4.5	3.92	300	237	—	1000	0.3	1.10	86.03
82	6.06	4.5	3.99	300	268	—	—	0.3	1.39	85.89

Table 1 *continued*

Slurry	pH value	NaCl (% w/v water) Added	NaCl (% w/v water) Detected	Nitrite (μg/g) Added	Nitrite (μg/g) Detected	Nitrate (μg/g) (Added)	Iso-ascorbate (μg/g) (Added)	Poly-phosphate (% w/v) (Added)	Fat (%)	H_2O (%)
83	6.01	4.5	3.95	300	269	500	—	0.3	1.24	85.77
84	6.05	2.5	2.29	300	283	—	—	0.3	1.22	86.79
85	5.64	2.5	2.29	100	92	—	—	—	1.07	87.00
86	6.13	2.5	2.11	100	81	—	1000	0.3	1.10	89.21
87	5.71	2.5	2.33	300	285	—	—	—	1.43	87.18
88	6.12	2.5	2.20	300	228	—	1000	0.3	1.76	86.63
89	5.80	4.5	4.19	100	91	500	—	—	1.11	86.11
90	6.15	3.5	2.85	100	81	—	—	0.3	1.04	86.77
91	6.19	3.5	3.19	200	167	—	—	0.3	1.09	86.77
92	6.21	3.5	3.29	300	263	—	—	0.3	0.79	87.19

All analytical results are the mean of three samples.
*Equi = equimolar with $NaCl_2$.

Spoilage was assessed on the following arbitrary scale:
0 = no visible change
1 = slight gas production
2 = gas production
3 = marked gas production, some proteolysis
4 = marked gas production, proteolysis, pink colouration of the meat
5 = marked gas production, marked proteolysis, a plug of bright pink meat, and straw coloured liquid

Bottles scoring '5' on this scale were stored for a further week and then tested for toxin.

Detection of Cl.botulinum toxin. At the end of the 6-month storage period, all bottles which had been inoculated with *Cl.botulinum* were tested for the presence of toxin, irrespective of the degree of 'spoilage' evident.

Bottles were centrifuged at 3000 rev/min for 10 min, and the supernatant stored −18 to −20°C prior to toxin testing. The thawed supernatant (0.5 ml) was injected intraperitoneally into 20–24 g mice (white, strain CELP, Carworth, Europe). Death, accompanied by pinching of the waist, characteristic gasping respiration, and posterior paralysis was presumptive indication of the presence of botulinal toxin. Selected samples were neutralized with monovalent type A and B antitoxin. If symptoms were not observed within 4 days of injection, botulinal toxin was presumed to be absent and the mice remaining were sacrificed.

The non-specific death of mice, which sometimes occured in heavily spoiled samples, was largely eliminated by diluting with gelatin-phosphate buffer (equal

amounts of 0.4% gelatin and 0.2 M Na_2HPO_4 (Crisley, 1964)) containing 100 μg/ml chlortetracycline (Lederle) (Roberts, Thomas & Gilbert, 1973). Samples containing no liquid phase were mixed with an equal volume of gelatin-phosphate buffer, stored at 1°C for 2 days to extract the toxin, centrifuged, and 0.5 ml of supernatant tested for toxin.

Chemical analyses. Nitrite was determined by the official method of the Society of Analytical Chemistry (Hansen, 1973). Moisture and fat content were determined by the British Standard 4401 (1970) method for analysis of meat products. Chloride was determined using a Radiometer chloride meter (Model CMT 10 Radiometer, Copenhagen, Denmark) after calibration against a standard curve (British Standard 4401, 1970). The pH values of slurries were determined electrometrically using a Radiometer pH meter (Model 26; Radiometer, Copenhagen, Denmark). A list of the analysed concentration of additives is included in Table 1. Salt, nitrite, pH, moisture and fat were determined on samples immediately after dispensing into bottles. Nitrite levels were assayed on uninoculated samples immediately after heat treatments and monitored throughout the storage period. The results of nitrite monitoring throughout storage comprise thirty-three tables recording up to five nitrite estimations per treatment combination, approximately 1500 analyses*.

Statistical analysis

Reduction of the spoilage data

The bulk of the spoilage data made necessary a more compact representation of the observed results for each treatment combination. Although at any given incubation time the rate and degree of spoilage were of interest, it was more practical to produce a single spoilage score for each treatment combination as follows: at any given time the mean spoilage score of the set of replicates was

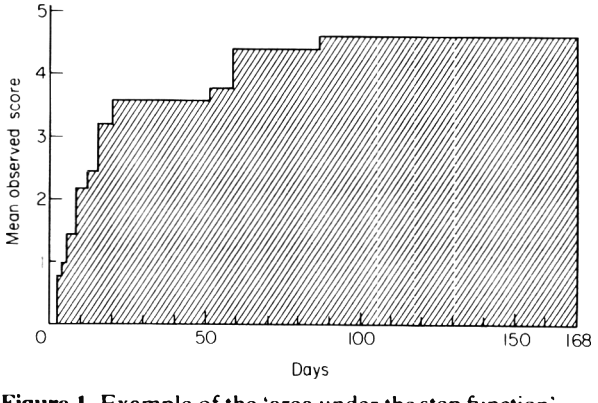

Figure 1. Example of the 'area under the step function'.

*These data are stored on magnetic tape (prepared using an ICL System 4 computer) copies of which are available from the authors, on request, at cost of tape plus handling.

generated, assuming that a bottle remained at the last recorded score until a change in score was observed. An example of the mean step function so produced is shown in Fig. 1, and the area under that step function was taken as the measure of spoilage for that treatment combination.

Since preliminary analyses showed that whatever sensible statistics were calculated the results were very similar, it was decided to use the above score since it contained information on all the aspects of the data that were of interest, such as the time spoilage increased and the rate of that increase. Other statistics such as means of the raw spoilage scores at various stages (e.g. 1 month, 3 months, 6 months) exhibited some, but not all, of the characteristics of the adopted statistic.

Processing the toxin data

The proportion (p) of samples containing toxin was calculated to provide a single value representative of each treatment combination. Since the size of the errors associated with each observeration p is not constant for different values of p we have employed the angular transformation

$$y = \frac{180}{\pi} \arcsin (p^{1/2})$$

to induce a homogeneous Normal error structure required for the subsequent analysis of variance (Bartlett, 1947).

Models used in the analyses of variance

The same type of factorial linear model was used in all the analyses. Although spoilage and toxicity may be considered to depend on the various factors in some complex way described by a suitable formula, no formal regression model was used since several factors appeared at only two levels. Hence the simple factorial linear model described below sufficed to elicit the significant effects.

The models allowed for interactions of up to three factors and took the following form

$$\begin{aligned} y_{ijk\ldots rst} = {} & \text{MEAN} + (\text{NITRITE})_i + (\text{NITRATE})_j + (\text{POLYPHOSPHATE})_k \\ \ldots\ & + (\text{SALT})_r + (\text{ISOASCORBATE})_s + (\text{HEAT TREATMENT})_t + \\ & (\text{NITRITE} \times \text{NITRATE})_{ij} + \ldots + (\text{ISOASCORBATE} \times \text{HEAT} \\ & \text{TREATMENT})_{st} \\ & + (\text{NITRITE} \times \text{NITRATE} \times \text{POLYPHOSPHATE})_{ijk} + \ldots \\ & + (\text{SALT} \times \text{ISOASCORBATE} \times \text{HEAT TREATMENT})_{rst} + \epsilon_{ijk\ldots rst} \end{aligned}$$

In the above model $y_{ijk\ldots rst}$ represents the response to the combination of the factor levels on the right of the equation where the subscripts indicate the appropriate levels. For example, i may take the values 0, 100, 200, 300, and j may

take the values 0 or 500. In the toxin analyses y is $\frac{180}{\pi}$ arcsin $(p^{1/2})$ where p is the proportion of units toxin positive at the appropriate combination. In the spoilage analyses, y is the score obtained from the area beneath the step function in Fig. 1. Separate models of the above type were produced for each of the analyses.

Results

An example of toxin data accumulated after 6 months' storage is presented in Table 2. The total raw data (equivalent to thirty tables) are too extensive to include in this paper, and are stored on magnetic tape*.

Preliminary statistical analyses on spoilage data from slurries 1 to 36 (see Table 1) led to the following conclusions:

(1) Replication: spoilage scores (within a treatment combination) were highly consistent. Consequently in slurries 37 *et seq* the number of replicates per treatment combination was reduced from ten to five.

(2) Isoascorbate levels: in the early experiments isoascorbate was included at concentrations equimolar with nitrite (approximately 313, 626, 940 μg/g for 100, 200, 300 μg/g nitrite respectively) in addition to 1000 μg/g. Statistical analyses showed no significant differences between the effect on spoilage of equimolar isoascorbate and 1000 μg/g: hence the latter concentration was used in subsequent slurries.

(3) Heat treatment: there was no significant difference between spoilage in the absence of heating and after the LOW heat treatment; consequently the zero heat treatment was omitted from the full analyses of variance of spoilage data.

In a second series of experiments, to be reported later, only the 10^1 inoculum level was used to relate more closely to naturally occurring levels of *Cl.botulinum*, (Roberts & Smart, 1977). The statistical analyses below relate solely to an inoculum of 10^1 to facilitate direct comparison with later experiments.

Statistical analyses

The data were separated into two groups prior to analyses of variance:

Group 1: spoilage data;

Group 2: toxin data.

Allowance was made for fitting interactions of up to three factors. As might be expected with such a large design numerous effects were significant, although many, especially some of the two- and three-factor interactions, were very small compared with the effects of the individual factors. Factors and interactions significant at the 1% level are summarized in Table 3 where '+' indicates that the factor or interacting factors significantly affected spoilage or toxin production. They are discussed below.

*Prepared using an ICL System 4 Computer. Copies will be made available, on request, from the authors at the cost of the tape plus handling.

Table 2. An example of toxin data accumulated after 6 months' storage. Five replicates per treatment containing toxin at a particular spoilage entries are the number of replicates containing toxin at a particular spoilage score

Slurry No.	NaCl (% on water)	$NaNO_2$ (μh/g)	Sodium isoascorbate (μg/g)	Polyphosphate (% w/v)	$NaNO_3$ (μg/g)	*15°C								17.5°C								20°C								35°C							
						†10^1					10^3				10^1					10^3					10^1					10^3					10^1					10^3				
						‡1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
45	2.5	100	—	—				0		3				2		1			0		0					5			0		0		1	1				5					1								5
46	2.5	100	1000	0.3	—				0	2						5				0	3					5					1	3				2	3				0	0	3					4
47	2.5	300	—	—	—		1	1	0				1			3				0	4					5			0		3				1	4				0	1	2					5
48	2.5	300	1000	0.3	—			1	2							4				0	3					5			0		1					5					0				1			4
49	2.5	100	—	—	500			1						5					0	1						4				4					4					1							3
50	2.5	100	1000	0.3	500			0						4						2						5			1	1					0	4				0	2					0	3
51	4.5	—	—	—	—				0					0						0				0					0				3					0						0	1
52	4.5	100	—	—	—			0	0				0	0					0	0				0				0	0				0	0					0							4
53	4.5	200	—	—	—	0	0				0	1					0					0	0				1	2				1	1					0						1	2
54	4.5	300			500	0					0					0	0					0					0				0	0					0		1					5
55	4.5	200	1000	0.3	—	0	0				0					0						0	0				0	0				0	0			1		0							
56	4.5	200	1000	0.3	—	0	0				0					0						0	0				0	1	2			0										0				3	0
57	4.5	200	1000	—	—				0				0					0				0	0				0				0					0	0				0	1					
58	4.5	200	—	0.3	—			0					0					0	0				0	0	0			0	1				5					0							2
59	4.5	200	—	0.3	500	0	0				0					0	0	0				0	0				0				0	1					0	0						1
60	4.5	200	1000	—	500	0	0	0			0					0					0	0				0					0					0	0							

* = Storage temperature.
† = Inoculum level.
‡ = Spoilage score.

Table 3. Factors and interactions significantly affecting[1] spoilage and toxin production in pork slurry of 'low' pH (5.54–6.36)

3.1	Salt		Nitrite		Nitrate		Poly-phosphate		Iso-ascorbate		Heat treatment		Storage temperature	
	S	T	S	T	S	T	S	T	S	T	S	T	S	T
Salt	+	+												
Nitrite	+	+	+	+										
Nitrate	+	+	+	+	−	+								
Polyphosphate	+	+	+	−	−	−	−	+						
Isoascorbate	+	+	+	−	−	−	−	−	+	+				
Heat treatment	+	+	+	−	−	+	−	+	+	−	+	+		
Storage temperature	+	−	+	−	+	−	−	−	+	+	+	−	+	+

Significant 3-factor interactions affecting spoilage and toxin production

3.2	Spoilage	Toxin
Salt × nitrate × nitrate	+	+
Salt × nitrate × polyphosphate	+	+
Salt × isoascorbate × polyphosphate	−	+
Heat × isoascorbate × polyphosphate	+	−
Nitrate × isoascorbate × polypohosphate	−	+

S = = Spoilage.
T = Toxin production.
+ = Factor or interaction was statistically significant ($P = < 0.01$).
− = Factor or interaction was not statistically significant ($P = < 0.01$).
[1] = Polyphosphate significantly increased toxin production: all other factors and interactions significantly reduced toxin production and spoilage.

Most factors considered singly were highly significant although there were some notable exceptions e.g. nitrate and polyphosphate did not significantly affect spoilage. All individual factors significantly affected toxin production (see below).

In Table 3 interactions are listed as significant when the effect of combined factors was not equal to the sum of the effects of the single factors. In the case of interactions which were not significant, the effect of factors combined approximately equalled the sum of the effects of those factors considered singly. The majority of significant interactions were antagonistic, i.e. the combined effect of factors interacting was greater than that of either factor alone, but smaller than the sum of the effects of those factors. There were no synergistic interactions.

Some gaps in the design had occurred. In slurries containing both nitrite and nitrate the residual nitrite concentration increased during storage at 15, 17.5 and 20°C especially in the absence of isoascorbate and at the HIGH heat treatment. In several of these slurries the level of nitrite permitted in foods (200 μg/g) was exceeded. Combinations of nitrite (300 μg/g) + nitrate (500 μg/g) which would have led to nitrite concentrations exceeding that permitted level were not tested. To investigate the effect of nitrate it was necessary to omit from the statistical analysis the 300 μg/g level of nitrite. This was not considered important from the practical viewpoint since statistical analyses of data from slurries 3–36 clearly demonstrated that spoilage in slurries containing 300 μg/g was not significantly less than in those containing 200 μg/g.

Group 1. 'Low' pH slurries—spoilage analysis (Table 4)

Spoilage was reduced significantly by increasing nitrite from 100 to 200 μg/g but not reduced further by increasing nitrite to 300 μg/g (see above). Successively decreasing storage temperature or increasing salt level significantly decreased spoilage. The addition of isoascorbate significantly reduced spoilage.

Table 4. Means of spoilage data from slurries prepared from pork of 'low' initial pH (5.5–6.3). 1. Single factors: figures given are the mean of the 'area under the step function' (see statistical analyses, 'reduction of spoilage data') i.e. the smaller the figure the less spoilage

Treatment	Levels tested				Least significant difference (LSD)	
					$P = <0.05$	$P = <0.01$
Sodium nitrite (μg/g)	100	200	300			
	267.7	233.3	NS[1]		13.94	17.98
*Sodium nitrate (μg/g)	0	500				
	25[illegible]0	250.1			13.94	17.98
Sodium isoascorbate (μg/g)	0	1000				
	287.5	213.6			13.94	17.98
Heat treatment	UH	LOW	HIGH			
	NS[2]	282.9	218.2		13.94	17.98
Sodium chloride (% w/v on water)	2.5	3.5	4.5			
	321.2	240.1	190.3		17.08	22.03
*Polyphosphate (% w/v)	0	0.3				
	253.0	248.0			13.94	17.98
Storage temperature	15	17.5	20	35		
	171.2	208.9	259.7	362.2	19.72	25.44

NS[1] 300 μg/g not significantly different from 200 μg/g.
NS[2] not significantly different from low heat.
UH = Unheated.
LOW = 80°/7 min.
HIGH = 80°/7 min + 70°/1 hr.
Treatment levels are significantly different when their means differ by more than the LSD.
*Treatment not significant.

There was no significant difference between spoilage of the unheated slurries or after LOW heat treatment, but increasing heat treatment to HIGH significantly reduced spoilage. The addition of nitrate or polyphosphate did not significantly affect spoilage.

Mean spoilage scores of the two-factor interactions are shown in Table 5.

Table 5. Means of spoilage data from slurries prepared from pork of 'low' initial pH (5.5–6.3). Effect of two-factor interactions: figures given are the mean of the 'area under the step function' (see statistical analyses 'Reduction of spoilage data') i.e. the smaller the figure the less spoilage

Treatment interaction	Level tested				Number of replicates	Least significant difference (LSD) $P=<0.05$	Least significant difference (LSD) $P=<0.01$
Nitrate × nitrite		0	500				
	100	279.8	255.6		96	19.52	25.83
	200	221.1	244.5				
Isoascorbate		0	1000				
× nitrite	100	315.2	220.2		96	19.52	25.83
	200	259.7	206.9				
*Isoascorbate		0	1000				
× nitrate	0	280.3	221.6		96	19.52	25.83
	500	294.6	205.6				
Heat treatment		LOW	HIGH				
× nitrite	100	311.0	224.4		96	19.52	25.83
	200	254.7	212.0				
*Heat treatment		LOW	HIGH				
× nitrate	0	277.1	224.8		96	19.52	25.83
	500	288.6	211.6				
Heat treatment		LOW	HIGH				
× isoascorbate	0	337.3	237.7		96	19.52	25.83
	1000	228.4	198.7				
Salt × nitrite		2.5	3.5	4.5			
	100	372.1	253.0	178.0	64	24.14	32.11
	200	270.3	227.2	202.5			
Salt × nitrate		2.5	3.5	4.5			
	0	342.3	226.8	183.8	64	24.14	32.11
	500	300.1	253.4	196.7			
Salt ×		2.5	3.5	4.5			
isoascorbate	0	373.5	271.4	217.4	64	24.14	32.11
	1000	268.9	208.8	163.1			
Salt × heat		2.5	3.5	4.5			
treatment	LOW	371.3	271.1	206.2	64	24.14	32.11
	HIGH	271.2	209.1	174.3			
Polyphosphate		0	0.3				
× nitrite	100	279.3	256.2		96	19.52	25.83
	200	226.8	239.9				
*Polyphosphate		0	0.3				
× nitrate	0	248.7	253.2		96	19.52	25.83
	500	257.3	242.9				

Table 5 *continued*

Treatment interaction	Level tested					Number of replicates	Least significant difference (LSD) $P = <0.05$	Least significant difference (LSD) $P = <0.01$
*Polyphosphate		0	0.3					
× isoascorbate	0	289.7	285.3			96	19.52	25.83
	1000	216.4	210.8					
*Polyphosphate		0	0.3					
× heat treatment	LOW	290.5	275.2			96	19.52	25.83
	HIGH	215.5	220.9					
Polyphosphate ×		0	0.3					
salt	2.5	294.1	348.3			64	24.14	32.11
	3.5	256.9	223.3					
	4.5	208.1	172.4					
Storage temperature		15	17.5	20	25			
× nitrite	100	173.6	214.9	286.2	396.1	48	28.16	37.64
	200	168.8	202.9	233.2	328.4			
Storage temperature		15	17.5	20	35			
× nitrate	0	158.1	212.2	278.4	355.2	48	28.16	37.64
	500	184.4	205.7	241.0	369.3			
Storage temperature		15	17.5	20	35			
× isoascorbate	0	183.9	239.9	295.6	430.5	48	28.16	37.64
	1000	158.5	178.0	223.8	294.0			
Storage temperature		15	17.5	20	35			
× heat treatment	LOW	187.0	233.0	292.6	418.9	48	28.16	37.64
	HIGH	155.5	184.9	226.8	305.6			
Storage temperature		15	17.5	20	35			
× salt	2.5	187.1	270.5	342.3	485.0	32	34.82	46.94
	3.5	162.0	189.8	263.6	345.1			
	4.5	164.6	166.5	173.3	256.7			
*Storage temperature		15	17.5	20	35			
× polyphosphate	0	173.5	215.4	265.9	357.3	48	28.16	37.64
	0.3	169.0	202.5	253.5	367.2			

*No significant interaction.

Within a given interaction the difference of any two means is significant if it is greater than the LSD.

Interactions involving nitrite. Although the effect of increasing nitrite level was significant, the effect of increasing nitrite was less in the presence of other significant factors such as: isoascorbate, low storage temperature, high salt concentration, or HIGH heat treatment. In the presence of isoascorbate, or at 3.5 or 4.5% salt, at 15 and 17.5°C, or after HIGH heat treatment, increasing the nitrite from 100 to 200 μg/g achieved no further reduction in spoilage. Polyphosphate and nitrate did not significantly affect spoilage overall, but they both interacted significantly with nitrite, and in their presence, raising the nitrite level from 100 to 200 μg/g did not significantly reduce spoilage.

Interactions involving isoascorbate. Isoascorbate was significant in many interactions and its effect often masked the effects of increasing levels of other

significant factors (e.g. nitrite). Adding isoascorbate at any storage temperature reduced spoilage by the same order as reducing the storage temperature to the next lowest tested, storage at 15°C resulting in least spoilage. The addition of isoascorbate at the LOW heat treatment reduced spoilage to the same extent as raising the heat treatment to HIGH in the absence of isoascorbate, but the combination of isoascorbate and HIGH heat treatment resulted in the least spoilage. Similarly the addition of isoascorbate at 2.5 or 3.5% salt reduced spoilage to the same extent as increasing salt by 1%, but 4.5% salt plus isoascorbate resulted in the least spoilage.

Interactions involving salt. At 15 and 17.5°C the effect of increasing salt level was reduced. At 15°C there was no significant difference between the spoilage at 2.5 and 4.5% salt levels. Alternatively if high salt (4.5%) levels were maintained there was no significant difference between spoilage at 15, 17.5 or 20°C. The addition of nitrate significantly decreased spoilage only at 2.5% salt. Polyphosphate significantly increased spoilage at 2.5% salt whereas at 4.5% salt it significantly decreased spoilage. Increasing salt levels by 1% reduced spoilage by the same order as raising the heat treatment from LOW to HIGH. A combination of HIGH heat plus high salt (4.5%) resulted in least spoilage.

Interactions involving storage temperature. The increase in spoilage attributable to raising storage temperature was less at 200 μg/g nitrite than at 100 μg/g, or if HIGH heat treatment was used.

Three-factor interactions were also investigated, but very few were significant and their effects were small compared with the single main effects. These three-factor interactions were probably only significant because of the large main effects of the individual factors, and are described only for completeness. At 2.5% salt, nitrate reduced the significant effect of increasing nitrite from 100 to 200 μg/g. The addition of nitrate counteracted the interaction between salt (2.5 and 4.5%) and polyphosphate. At HIGH heat treatment level the addition of polyphosphate counteracted the reduction in spoilage attributable to isoascorbate.

Group 2 toxin analyses (Table 6)

Increasing nitrite from 100 to 200 μg/g, salt by 1%, the heat treatment from LOW to HIGH, or adding isoascorbate or nitrate all significantly reduced toxin production. Lowering the storage temperature to 15°C significantly reduced toxin production but 20°C storage was not significantly different from 35°C. The addition of polyphosphate significantly increased toxin production in these 'low' pH slurries.

Mean toxin scores for the two-factor interactions are shown in Table 7.

Interactions involving isoascorbate. The toxin reduction achieved by increasing salt was less when isoascorbate was present. At 15 and 17.5°C there was no further significant reduction in toxin production when isoascorbate was

Table 6. Means of transformed toxin data from slurries prepared from pork of 'low' initial pH (5.5–6.3). 1. Single factors: figures given are calculated from the percentage toxin-positive samples (see Statistical analyses 'Processing the toxin data'), i.e. the smaller the figure the less toxin

Treatment	Levels tested				Least significant difference (LSD)	
					$P = <0.05$	$P = <0.01$
Sodium nitrite (μg/g)	100	200	300			
	30.09	17.93	NS[1]		2.744	3.539
Sodium nitrate (μg/g)	0	500				
	27.21	20.81			2.744	3.539
Sodium isoascorbate	0	1000				
(μg/g)	29.17	18.84			2.744	3.539
Heat treatment	UH	LOW	HIGH			
	27.55	30.18	14.30		3.360	4.334
Sodium chloride	2.5	3.5	4.5			
(% w/v on water)	36.35	24.58	10.99		3.360	4.334
Polyphosphate (% w/v)	0	0.3				
	19.53	28.48			2.744	3.539
Storage temperature	15	17.5	20	35		
	15.33	23.35	29.03	28.32	3.880	5.005

NS[1] 300 μg/g not significantly different from 200 μg/g.
Treatment levels are significantly different when their means differ by more than the LSD.

added, but at the higher temperatures (20 and 35°C) addition of isoascorbate significantly reduced toxin production.

Interactions involving salt. Increasing nitrite from 100 to 200 μg/g had no significant effect on toxin production at 4.5% salt. At 2.5% salt addition of nitrate significantly decreased toxin production, but there was no significant decrease at 3.5 and 4.5% salt. At 2.5 and 3.5% salt more toxin production occurred after the LOW heat treatment than in unheated slurries or after the HIGH heat treatment. At 4.5% salt there was no significant difference between heat treatments. At 4.5% salt the addition of polyphosphate had no effect on toxin production.

Interactions involving nitrate. In the presence of nitrate the LOW heat treatment resulted in significantly more toxin production than the unheated, but the HIGH heat treatment resulted in the least toxin production. The effect of increasing nitrite from 100 to 200 μg/g was less in the presence of nitrate.

The three-factor interactions, although significant, were small compared with the effects of the significant single factors and are discussed only for completeness.

At 2.5 and 3.5% salt in the presence of nitrate there was no reduction in toxin production when nitrite was raised from 100 to 200 μg/g. In the presence of isoascorbate the addition of polyphosphate negated the beneficial effect of

Table 7. Means of transformed toxin data from slurries prepared from pork of 'low' initial pH (5.5–6.3). Effects of two-factor interactions: figures given are calculated from the percentage toxin-positive samples (see 'Materials and methods', 'Processing the toxin data') i.e. the smaller the figure the less toxin

Treatment interaction		Level tested			Number of replicates	Least significant difference (LSD) $P = <0.05$	Least significant difference (LSD) $P = <0.01$
Nitrate × nitrite		0	500				
	100	37.04	23.14		288	3.80	5.01
	200	17.38	18.48				
*Isoascorbate × nitrite		0	1000				
	100	36.76	23.42		288	3.80	5.01
	200	21.59	14.27				
*Isoascorbate × nitrate		0	1000				
	0	32.48	21.93		288	3.80	5.01
	500	25.87	15.75				
*Heat treatment × nitrite		UH	LOW	HIGH			
	100	34.00	36.51	19.75	192	4.65	6.13
	200	21.10	23.84	8.84			
Heat treatment × nitrate		UH	LOW	HIGH			
	0	31.39	28.77	21.46	192	4.65	6.13
	500	23.71	31.58	7.13			
*Heat treatment × isoascorbate		UH	LOW	HIGH			
	0	31.96	28.24	17.33	192	4.65	6.13
	1000	23.15	22.11	11.27			
Salt × nitrite		2.5	3.5	4.5			
	100	46.06	30.08	13.36	192	4.65	6.13
	200	26.84	18.32	8.63			
Salt × nitrate		2.5	3.5	4.5			
	0	44.68	23.82	13.12	192	4.65	6.13
	500	28.22	25.34	8.87			
Salt × isoascorbate		2.5	3.5	4.5			
	0	44.41	29.34	13.77	192	4.65	6.13
	1000	28.49	19.82	8.22			
Salt × heat treatment		2.5	3.5	4.5			
	UH	38.24	34.91	9.50	128	5.75	7.62
	LOW	47.83	27.69	15.01			
	HIGH	23.28	11.14	8.47			
*Polyphosphate × nitrite		0	0.3				
	100	24.96	35.21		288	3.80	5.01
	200	14.10	21.76				
*Polyphosphate × nitrite		0	0.3				
	0	22.19	32.22		288	3.80	5.01
	500	16.87	24.75				
*Polyphosphate × isoascorbate		0	0.3				
	0	25.43	32.92		288	3.80	5.01
	1000	13.63	24.05				
Polyphosphate × heat treatment		0	0.3				
	UH	19.35	35.75		192	4.65	6.13
	LOW	23.90	36.45				
	HIGH	15.34	13.25				

Table 7 *continued*

Treatment interaction	Level tested					Number of replicates	Least significant difference (LSD) $P = <0.05$	Least significant difference (LSD) $P = <0.01$
Polyphosphate		0	0.3					
× salt	2.5	28.56	44.33			192	4.65	6.13
	3.5	20.59	28.57					
	4.5	9.43	12.56					
*Storage temperature		15	17.5	20	35			
× nitrite	100	21.17	29.98	36.57	32.63	144	5.43	7.19
	200	9.49	16.72	21.50	24.01			
*Storage temperature		15	17.5	20	35			
× nitrate	0	15.69	26.67	33.89	32.57	144	5.43	7.19
	500	14.97	20.03	24.17	24.06			
Storage temperature		15	17.5	20	35			
× isoascorbate	0	18.50	25.31	34.24	38.64	144	5.43	7.19
	1000	12.16	21.39	23.82	17.99			
*Storage temperature		15	17.5	20	35			
× heat treatment	UH	17.95	23.78	32.32	36.16	96	6.65	8.80
	LOW	20.54	32.79	37.29	30.08			
	HIGH	7.50	13.48	17.48	18.72			
*Storage temperature		15	17.5	20	35			
× salt	2.5	25.96	36.68	41.62	41.53	96	6.65	8.80
	3.5	16.12	25.83	31.04	25.34			
	4.5	3.91	7.54	14.44	18.09			
*Storage temperature		15	17.5	20	35			
× polyphosphate	0	11.59	18.23	23.09	25.21	144	5.43	7.19
	0.3	19.07	28.47	34.98	31.43			

Within a given interaction the difference of any two means is significant if it is greater than the LSD.
*Interaction not significant.

nitrate. The salt, nitrate, polyphosphate interaction was significant because at 3.5% salt the addition of nitrate significantly increased toxin production. At 3.5% salt and in the absence of isoascorbate there was no increase in toxin production due to the addition of polyphosphate.

Discussion

U.K. regulations governing nitrite levels in cured meats (Preservatives in Food Regulations, 1979) have remained unaltered since the Preservatives in Food Regulations, 1975, through the Preservatives in Food Regulations (Amendments) 1977 and state only the maximum level of nitrite (200 μg/g) and

nitrate (500 μg/g) without specifying when the examination should be made. These regulations do not take into account other chemical additives such as salt, polyphosphate or ascorbate/isoascorbate. Levels of nitrite and nitrate were reviewed and a recommendation has been made to divide cured meats into four classes, each with different nitrite and nitrate levels (Food Additives and Contaminants Committee, 1978).

Our results have identified the most significant single factors and many significant two-factor interactions, supporting earlier reports that factors combine or interact to control the growth of clostridia (Bulman & Ayres, 1952; Riemann, 1963; Baird-Parker & Freame, 1967; Roberts & Ingram, 1973).

Many of the three-factor interactions were difficult to interpret and it is suspected that this may be due to differences between batches of pork. Similar lack of consistency in the results of such experiments have previously been reported (Tompkin, Christiansen & Shaparis, 1977: Rhodes & Jarvis, 1976). The following general conclusions may be drawn:

(1) Increasing nitrite significantly reduced spoilage and toxin production. The relative effect of nitrite was smaller in combination with other significant factors such as 4.5% salt, isoascorbate, or storage at 15°C but a combination of 200 μg/g nitrite with either 4.5% salt, or isoascorbate (1000 μg/g) or 15°C storage always resulted in least spoilage and least toxin production.

(2) Nitrate alone did not significantly affect spoilage but decreased toxin production. Chemical analysis indicated this to be a result of its reduction to nitrite. Several significant interactions involved nitrate.

(3) Increasing salt concentration significantly decreased spoilage and toxin production. At high salt levels other significant factors, e.g. unit increase in nitrite concentration, had less effect (see (1) above).

(4) Polyphosphate (Curaphos 700) did not affect spoilage overall but significantly increased toxin production in these 'low' pH slurries. This increase in toxin production was counteracted by the addition of isoascorbate, or nitrate, or raising nitrite or salt concentration, or the heat treatment to the next highest level tested.

(5) Isoascorbate significantly decreased spoilage and toxin production. In its presence the effect of many other significant factors (e.g. increasing salt, nitrite, heat treatment, storage temperature) were reduced. Spoilage and toxin production were least when isoascorbate was present in combination with 200 μg/g nitrite or 4.5% salt, or after HIGH heat treatment or after storage at 15°C.

(6) HIGH heat treatment significantly reduced spoilage and toxin production but little difference was observed between the effects of LOW and unheated levels.

(7) The effect of storage temperature was significant and the lowest storage temperature (15°C) resulted in least spoilage and toxin production. At low storage temperatures other significant factors (e.g. increasing salt or nitrite) were relatively less effective, although least spoilage and toxin production

occurred when storage was at 15°C in combination with 4.5% salt or 200 μg/g nitrite.

From these conclusions it would appear possible to reduce the input level of nitrite providing certain other conditions were met, e.g. if salt levels are kept high (4.5%), if isoascorbate were added (1000 μg/g) or if storage below 15°C could be guaranteed. Isoascorbate (1000 μg/g) was a highly significant factor; its presence furthered the reduction in toxin production attributable to other factors such as salt, nitrite or low storage temperatures.

Significant interactions were always antagonistic, i.e. the combined effect of the two interacting factors was less than would be expected by summing their individual effects. These data illustrate that it is possible to manipulate one significant factor (e.g. salt, or isoascorbate, or storage temperature) to minimize the concentration of another (e.g. nitrite) yet maintain an equivalent degree of control i.e. shelf stability ('spoilage') or likelihood of toxin production. These data were obtained in an experimental system which may not simulate all cured meat products. However, further limited experimentation should reveal by how much products differ from this system in their response to changes in concentration of additives, thereby making these relative effects more readily applicable.

The above conclusions are based entirely on controlling *Cl. botulinum* types A and B. In pasteurized meat products there may be other considerations which make the suggested alternatives undesirable or inappropriate.

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Factors controlling the growth of *Clostridium botulinum* types A and B in pasteurized, cured meats

II. Growth in pork slurries prepared from 'high' pH meat (range 6.3–6.8)

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Summary

A pork slurry system was used to study the combined effect of the following factors on growth of *Clostridium botulinum*: sodium chloride (2.5, 3.5, 4.5% w/v on water), sodium nitrite (100, 200, 300 μg/g), sodium nitrate (0, 500 μg/g), sodium isoascorbate (0, 1000 μg/g) a polyphosphate (Curaphos 700; 0, 0.3% w/v) and heat treatment (80°C/7 min, 80°C/7 min + 70°C/1 hr) with storage at 15, 17.5, 20 and 35°C for up to 6 months.

Increasing nitrite, salt or heat treatment, adding isoascorbate, polyphosphate or nitrate, or reducing storage temperature significantly reduced toxin production. There were many two-factor interactions and the relative effect of increasing nitrite became less in the presence of isoascorbate or high salt levels. Increasing salt or heat treatment, adding nitrate or decreasing storage temperature had less effect if isoascorbate was present. The addition of polyphosphate enhanced the effect of adding isoascorbate.

Introduction

In a laboratory medium pH was an important factor in determining whether growth of *Clostridium botulinum* occurred (Roberts & Ingram, 1973). The difficulties in controlling the pH of a meat slurry prevented its study as a variable but two pH levels have been compared. In an initial study (Roberts, Gibson & Robinson, 1981) no attempt was made to select meat within a narrow range of pH values and slurries prepared from that pork had a mean pH of 5.93 (range 5.54–6.36). For the experiments reported below, only pork of 'high' initial pH (6.3 and above) was used, resulting in slurries with a mean pH 6.51 (range 6.27–6.72).

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Pork of high pH is less stable bacteriologically than that of normal pH. (Bem & Leistner, 1976; Taylor & Shaw, 1975; Gardner, 1971). Taylor, Shaw & Jolley (1976) reported that inclusion of nitrate in the curing brine improved the shelf-life of vacuum packed bacon produced from high pH pork, but had little effect on that from pork of normal pH.

Bacterial taints in cured meat have long been associated with pork of high initial pH (Callow, 1937; Ingram, 1952; Gardner & Patterson, 1975). Collar, which contains small muscles of relatively high pH, is commonly used in pasteurized cured meats. The knowledge that nitrite is a relatively less effective antimicrobial agent with increasing pH (Castellani & Niven, 1955; Blanche-Koelensmid & van Rhee, 1968; Roberts & Ingram, 1973) makes a fuller understanding of the implication of using high pH pork in ham production highly desirable, particularly with respect to the control of *Cl.botulinum* and the current wish to use the minimum amount of nitrite commensurate with bacteriological stability and safety.

Materials and methods

Meat slurry system

Meat of initial pH 6.3 and above was stockpiled until sufficient was obtained for the whole experiment. It was defatted by hand, cut into small pieces and minced, ensuring thorough mixing. The resultant homogenous batch of pork mince (pH *ca* 6.4) was weighed into 1 kg amounts and stored at $-10°C$. Preparation of the slurry is described by Roberts, *et al.*, (1981), excepting that the ratio of pork:water was 1:1.8. The higher pH pork bound water more strongly and the proportion of water was increased to facilitate dispensing the slurry. The mean pH of slurries prepared was 6.51 (range 6.27–6.72).

Inoculum

An inoculum of 2×10^1 spores/ml (comprising a mixture of five strains each of types A and B *Cl.botulinum*) was used throughout these experiments, 0.5 ml inoculated into each bottle. Details of the strains of *Cl.botulinum* and preparation of spores suspensions are given in Roberts *et al.*, (1981).

Heat treatment

The bottles were heated immediately after inoculation, using two heat treatments: 80°C/7 min (LOW) and 80°C/7 min + 70°C/1 hr (HIGH) with five replicate bottles at each treatment combination.

Experimental plan

The factors investigated were:

NaCl (% w/v on water)	2.5, 3.5, 4.5
$NaNO_2$ (μg/g slurry)	100, 200, 300
$NaNO_3$ (μg/g slurry)	0, 500
sodium isoascorbate (μg/g slurry)	0, 1000
polyphosphate (Curaphos 700, % w/v)	0, 0.3
inoculum (spores/bottle)	10
heat treatment	LOW, HIGH
storage temperature (°C)	15, 17.5, 20, 35

A complete list of slurries prepared is shown in Table 1.

Table 1. Complete list of slurries prepared including chemical analysis of slurries immediately after preparation

Slurry	pH value	NaCl (% w/v water) Added	NaCl (% w/v water) Detected	Nitrate (μg/g) Added	Nitrate (μg/g) Detected	Nitrate (μg/g) (Added)	Isoascorbate (μg/g) (Added)	Polyphosphate (% w/v) (Added)	Fat %	H_2O %
93	6.42	2.5	2.03	100	79	—	—	—	1.18	88.21
94	6.48	2.5	1.89	200	144	—	—	—	1.07	88.31
95	6.44	2.5	1.98	300	230	—	—	—	0.95	89.11
96	6.63	2.5	2.15	100	76	—	—	0.3	0.97	88.66
97	6.35	2.5	2.24	100	74	—	1000	—	1.11	88.77
98	6.58	2.5	2.51	100	79	—	1000	0.3	1.16	88.73
99	6.61	2.5	2.62	200	166	—	—	0.3	1.08	88.65
100	6.44	2.5	2.16	200	169	—	1000	—	1.05	88.77
101	6.60	2.5	2.51	200	323	—	1000	0.3	0.80	88.67
102	6.56	2.5	2.36	300	358	—	—	0.3	0.93	89.11
103	6.41	2.5	2.46	300	351	—	1000	—	0.86	88.95
104	6.59	2.5	1.96	300	289	—	1000	0.3	0.71	88.97
105	6.34	3.5	3.44	100	85	—	—	—	0.66	88.40
106	6.39	3.5	3.30	200	141	—	—	—	0.78	88.23
107	6.41	3.5	3.17	300	254	—	—	—	0.65	88.35
108	6.55	3.5	3.73	100	86	—	—	0.3	0.72	88.34
109	6.35	3.5	3.14	100	63	—	1000	—	0.79	88.32
110	6.55	3.5	3.34	100	70	—	1000	0.3	0.79	87.59
111	6.58	3.5	3.52	200	160	—	—	0.3	0.56	88.17
112	6.42	3.5	3.36	200	154	—	1000	—	0.78	88.37
113	6.54	3.5	3.32	200	144	—	1000	0.3	0.64	88.30
114	6.54	3.5	3.29	300	220	—	—	0.3	0.80	87.99
115	6.43	3.5	3.32	300	212	—	1000	—	0.95	87.92
116	6.56	3.5	3.19	300	224	—	1000	0.3	0.79	87.94
117	6.34	4.5	3.61	100	68	—	—	—	0.89	87.72
118	6.38	4.5	3.78	200	145	—	—	—	0.89	87.41
119	6.41	4.5	4.27	300	238	—	—	—	1.00	87.41
120	6.55	4.5	4.43	100	82	—	—	0.3	0.94	87.30
121	6.50	4.5	3.89	100	61	—	1000	—	0.91	87.27

Table 1. *continued*

Slurry	pH value	NaCl (% w/v water) Added	NaCl (% w/v water) Detected	Nitrate (μg/g) Added	Nitrate (μg/g) Detected	Nitrate (μg/g) (Added)	Isoascorbate (μg/g) (Added)	Polyphosphate (% w/v) (Added)	Fat %	H_2O %
122	6.66	4.5	4.19	100	69	—	1000	0.3	0.83	87.34
123	6.64	4.5	4.06	200	142	—	—	0.3	0.94	87.01
124	6.56	4.5	4.11	200	166	—	1000	—	0.99	87.35
125	6.53	4.5	4.43	200	167	—	1000	0.3	0.88	87.28
126	6.56	4.5	3.96	300	245	—	—	0.3	0.95	87.21
127	6.40	4.5	4.05	300	234	—	1000	—	0.94	87.53
128	6.58	4.5	3.46	300	193	—	1000	0.3	0.95	86.98
129	6.46	2.5	2.47	100	95	500	—	—	0.95	88.93
130	6.47	2.5	2.23	200	173	500	—	—	0.92	89.19
131	6.50	2.5	2.50	300	271	500	—	—	0.95	89.10
132	6.67	2.5	2.52	100	95	500	—	0.3	1.01	89.33
133	6.43	2.5	2.80	100	70	500	1000	—	0.98	88.82
134	6.70	2.5	2.24	100	70	500	1000	0.3	0.93	88.68
135	6.72	2.5	2.17	200	159	500	—	0.3	1.11	88.33
136	6.52	2.5	2.18	200	148	500	1000	—	0.97	88.76
137	6.59	2.5	2.61	200	205	500	1000	0.3	0.77	89.02
138	6.66	2.5	2.78	300	245	500	—	0.3	0.83	88.85
139	6.52	2.5	2.41	300	214	500	1000	—	1.00	88.85
140	6.70	2.5	2.72	300	223	500	1000	0.3	0.88	88.40
141	6.46	3.5	3.36	100	82	500	—	—	0.96	88.15
142	6.46	3.5	3.35	200	159	500	—	—	0.91	88.05
143	6.50	3.5	3.03	300	249	500	—	—	0.83	88.27
144	6.65	3.5	3.27	100	81	500	—	0.3	0.92	88.01
145	6.51	3.5	3.27	100	67	500	1000	—	0.91	88.17
146	6.72	3.5	3.74	100	68	500	1000	0.3	0.89	87.90
147	6.72	3.5	3.26	200	159	500	—	0.3	1.03	87.73
148	6.58	3.5	3.21	200	137	500	1000	—	0.56	88.52
149	6.48	3.5	3.05	200	150	500	1000	0.3	0.94	87.79
150	6.52	3.5	2.98	300	260	500	—	0.3	0.86	88.08
151	6.35	3.5	2.72	300	199	500	1000	—	0.81	88.25
152	6.48	3.5	2.81	300	226	500	1000	0.3	0.90	87.95
153	6.31	4.5	3.68	100	78	500	—	—	0.95	87.33
154	6.28	4.5	3.69	200	160	500	—	—	0.73	88.06
155	6.27	4.5	3.47	300	221	500	—	—	0.93	87.44
156	6.43	4.5	3.12	100	65	500	—	0.3	0.91	87.28
157	6.35	4.5	3.48	100	66	500	1000	—	0.89	87.36
158	6.59	4.5	3.61	100	73	500	1000	0.3	0.97	87.03
159	6.55	4.5	3.71	200	170	500	—	0.3	0.98	86.95
160	6.40	4.5	3.41	200	140	500	1000	—	0.90	87.45
161	6.53	4.5	3.58	200	143	500	1000	0.3	0.98	87.33
162	6.54	4.5	3.90	300	264	500	—	0.3	0.95	87.20
163	6.41	4.5	3.39	300	213	500	1000	—	0.93	87.40
164	6.55	4.5	3.75	300	234	500	1000	0.3	0.93	87.12

All analytical results are the mean of two samples

NB. Previous experience (Roberts *et al.*, 1981) suggested that a combination of 300 μg/g nitrite and 500 μg/g nitrate would result in residual nitrite levels above the maximum permitted in foods (Preservatives in Foods Regulations, 1979) but it was included to provide a fully crossed design for subsequent analyses of variance.

Results

Spoilage and toxin

Data were stored and tabulated as described by Roberts *et al.*, (1981). Only one table of the twenty needed to represent all spoilage and toxin data is presented (Table 2)*.

Table 2. An example of toxin data accumulated after 6 months' storage. Five replicates per treatment combination, slurry, heated 80°C/7 min. The entries are the number of replicates containing toxin at a particular spoilage score

Slurry No.	NaCl (% on water)	$NaNO_2$ (μg/g)	Sodium isoasco. (μg/g)	Polyphosphate (% w/v)	$NaNO_3$ (μg/g)	*15° †10¹ ‡1 2 3 4 5	17.5° 10¹ 1 2 3 4 5	20° 10¹ 1 2 3 4 5	35° 10¹ 1 2 3 4 5
93	2.5	100	—	—	—	3	1 1	5	5
94	2.5	200	—	—	—	1 2	2	1 3	4
95	2.5	300	—	—	—	0 0	0 0 1	0 2	0
96	2.5	100	—	0.3	—	2 1	0 1	4	5
97	2.5	100	1000	—	—	0	0 0	0 0 0	0 0 1
98	2.5	100	1000	0.3	—	1 0 0	0	0 0	0 1
99	2.5	200	—	0.3	—	4 0	3 0	5	5
100	2.5	200	1000	—	—	0	0 0 0	0 0	0 0
101	2.5	200	1000	0.3	—	0 0	0 0 0	0	0 0 0
102	2.5	300	—	0.3	—	0 0	0	0 0	0 0 3
103	2.5	300	1000	—	—	0 0 0	0 1	0 0 1	0 0 1
104	2.5	300	1000	0.3	—	0 0	0 0	0	0 0
105	3.5	100	—	—	—	5	1 3 1	1 4	4
106	3.5	200	—	—	—	0 0	5	3 1	5
107	3.5	300	—	—	—	0	0 0	1 0 2	0 0
108	3.5	100	—	0.3	—	1 1	1 2	4	5

*Storage temperature.
†Inoculum (spores/bottle).
‡Spoilage score.

*The complete data are stored on magnetic tape and are available from the authors, on request, at cost of the tape plus handling.

Statistical analyses

The data were processed and analysed as described by Roberts *et al.*, (1981), and a summary is presented in Table 3.

Table 3. Factors and interactions significantly affecting* spoilage and toxin production in pork slurry of 'high' pH (6.27–6.72)

3.1

	Salt		Nitrite		Nitrate		Polyphosphate		Isoascorbate		Heat treatment		Storage temperature	
	S	T	S	T	S	T	S	T	S	T	S	T	S	T
Salt	+	+												
Nitrite	+	+	+	+										
Nitrate	−	−	−	+	−	+								
Polyphosphate	+	−	+	+	+	−	+	+						
Isoascorbate	+	+	+	+	−	+	+	+	+	+				
Heat treatment	−	−	−	+	−	+	−	−	−	−	−	+		
Storage temperature	+	−	+	+	−	−	+	−	+	+	+	−	+	+

Significant three-factor interactions affecting spoilage and toxin production

3.2	Spoilage	Toxin
Salt × nitrate × polyphosphate	+	+
Nitrate × isoascorbate × polyphosphate	+	+
Salt × storage temperature × polyphosphate	+	−
Salt × nitrite × polyphosphate	+	−
Salt × nitrate × isoascorbate	+	−
Nitrite × polyphosphate × isoascorbate	+	−
Nitrite × isoascorbate × storage temperature	+	−
Nitrate × isoascorbate × storage temperature	−	+
Nitrate × isoascorbate × heat treatment	−	+
Nitrite × isoascorbate × salt	−	+
Nitrite × isoascorbate × nitrate	−	+

S = Spoilage.
T = Toxin production.
+ = Factor or interaction was statistically significant ($P = < 0.01$).
− = Factor or interaction was not statistically significant ($P = < 0.01$).
*All factors and interactions significantly reduced spoilage and toxin production.

Analyses of variance of spoilage data

Increasing nitrite or salt levels, decreasing storage temperature, or adding isoascorbate or polyphosphate significantly decreased spoilage. The addition of nitrate or increasing heat treatment from LOW to HIGH had no significant effect on spoilage (Table 4).

Mean spoilage scores of the two-factor interactions are shown in Table 5.

Table 4. Means of spoilage data from slurries prepared from pork of 'high' initial pH (6.4). Single factors: figures given are the means of the 'area under the step function' (See Roberts *et al.*, 1981). 'Reduction of spoilage data', i.e. the smaller the number the less spoilage

Treatment	Levels tested				Least significant difference (LSD)	
					$P = <0.05$	$P = <0.01$
Sodium nitrite (μg/g)	100	200	300			
	357.7	314.1	293.0		12.16	15.69
*Sodium nitrate (μg/g)	0	500				
	326.5	316.7			9.92	12.80
Sodium isoascorbate	0	1000				
(μg/g)	377	266.2			9.92	12.80
*Heat treatment	LOW	HIGH				
	326.1	317.0			9.92	12.80
Sodium chloride	2.5	3.5	4.5			
(% w/v on water)	363.5	323.8	277.5		12.16	15.69
Polyphosphate	0	0.3				
(% w/v)	374.5	268.7			5.92	12.80
Storage temperature	15	17.5	20	35		
	230.6	272.3	333.3	450.1	14.04	18.11

Treatment levels are significantly different when their means differ by more than the LSD.
*Treatment not significant.

Interactions involving isoascorbate. When isoascorbate was present little further reduction in spoilage was achieved by increasing nitrite. Similarly, increasing salt had little effect in the presence of isoascorbate. When both polyphosphate and isoascorbate were present the reduction in spoilage was significantly less than the sum of the effects of the individual factors, but was still least when both were present. The rise in spoilage when storage temperature was increased was less in the presence of isoascorbate.

Interactions involving polyphosphate. In the presence of polyphosphate no further reduction in spoilage was achieved by increasing nitrite from 200 to 300 μg/g. The reduction in spoilage attributable to polyphosphate (Curaphos 700) was greatest at 3.5% salt (cf. means of polyphosphate and salt interaction in Table 5. At 3.5% salt the addition of polyphosphate reduced the spoilage mean

Table 5. Means of spoilage data from slurries prepared from pork of 'high' initial pH (6.4) Effects of two factor interactions: figures given are the means of the 'area under the step function' (see Roberts *et al.*, 1981). 'Reduction of spoilage data' i.e. the smaller the number the less spoilage.

Treatment interaction		Levels tested			Number of replicates	Least significant difference (LSD) $P=<0.05$	Least significant difference (LSD) $P=<0.01$
*Nitrate × nitrite		0	500				
	100	368.2	347.2		96	17.01	22.51
	200	320.9	307.3				
	300	290.4	295.5				
Isoascorbate ×		0	1000				
nitrite	100	437.8	277.7		96	17.01	22.51
	200	370.2	258.0				
	300	323.1	262.8				
*Isoascorbate ×		0	1000				
nitrate	0	380.5	272.5		144	13.76	18.1
	500	373.6	259.8				
*heat treatment		LOW	HIGH				
× nitrite	100	367.5	347.9		96	17.01	22.51
	200	321.2	307.0				
	300	289.7	296.2				
*Heat treatment		LOW	HIGH				
× nitrate	0	336.2	316.8		96	13.76	18.11
	500	316.1	317.3				
*Heat treatment		LOW	HIGH				
× isoascorbate	0	385.4	368.6		144	13.76	18.11
	1000	266.9	265.4				
Salt × nitrite		2.5	3.5	4.5			
	100	401.6	364.0	307.5	64	21.04	27.98
	200	349.9	332.3	260.1			
	300	338.9	275.0	265.0			
*Salt × nitrate		2.5	3.5	4.5			
	0	364.1	332.5	282.9	96	17.01	22.15
	500	362.9	315.0	272.2			
Salt ×		2.5	3.5	4.5			
isoascorbate	0	461.9	378.3	290.9	96	17.01	22.15
	1000	265.1	269.2	264.2			
*Salt ×		2.5	3.5	4.5			
heat treatment	LOW	374.0	329.5	275.0	96	17.01	22.51
	HIGH	353.0	318.0	280.1			
Polyphosphate		0	0.3				
× nitrite	100	412.7	302.7		96	17.01	22.51
	200	376.3	251.9				
	300	334.6	251.4				
Polyphosphate		0	0.3				
× nitrate	0	369.5	283.5		144	13.76	18.11
	500	379.5	253.9				
Polyphosphate ×		0	0.3				
isoascorbate	0	441.1	312.9		144	13.76	18.11
	1000	307.9	224.4				

Table 5. *continued*

Treatment	Levels tested					Number of replicates	Least significant difference (LSD) $P = <0.05$	$P = <0.01$
*Polyphosphate ×		0	0.3					
heat treatment	LOW	379.6	272.7			144	13.76	18.11
	HIGH	369.4	264.7					
Polyphosphate		0	0.3					
× salt	2.5	413.8	313.1			96	17.01	22.51
	3.5	387.4	260.1					
	4.5	322.4	232.7					
Storage temperature		15	17.5	20	35			
× nitrite	100	236.4	292.5	369.1	532.8	48	24.54	32.81
	200	235.7	270.0	325.6	425.1			
	300	219.8	254.3	305.3	392.5			
*Storage temperature		15	17.5	20	35			
× nitrate	0	231.8	273.3	347.4	453.5	72	19.84	26.39
	500	229.4	271.2	319.2	446.8			
Storage temperature		15	17.5	20	35			
× isoascorbate	0	246.8	301.9	390.4	568.9	72	19.84	26.39
	1000	214.4	242.6	276.2	331.4			
Storage temperature		15	17.5	20	35			
× heat treatment	LOW	226.5	226.0	342.2	469.9	72	19.84	26.39
	HIGH	234.8	278.6	324.4	430.3			
Storage temperature		15	17.5	20	35			
× salt	2.5	243.3	302.0	388.0	520.5	48	24.54	32.81
	3.5	226.7	275.3	344.3	448.8			
	4.5	221.9	239.5	267.6	381.1			
Storage temperature		15	17.5	20	35			
× polyphosphate	0	270.6	318.4	386.5	522.6	72	19.84	26.39
	0.3	190.7	226.2	280.2	377.7			

Within a given interaction the difference of any two means is significant if it is greater than the LSD.
*No significant interaction.

by *ca* 127 *but* at 2.5 and 4.5% salt the reduction was only *ca* 90-100). The combination 4.5% salt + polyphosphate resulted in the least spoilage. Spoilage increased less with rising storage temperature in the presence of polyphosphate. Although the overall effect of nitrate was not significant there was a significant interaction between polyphosphate and nitrate resulting in less spoilage when both were present.

Interactions involving storage temperature. The effect of increasing nitrite was relatively less at lower storage temperatures, but a combination of 15°C and 300 μg/g nitrite resulted in least spoilage. Similarly the consequence of increasing salt was relatively less at 15 and 17.5°C, but least spoilage occurred with 4.5% salt and 15°C storage. There was no significant effect of heat treatment but

spoilage at 35°C was significantly reduced when the HIGH heat treatment was used.

Interactions involving salt. Salt and nitrite both significantly decreased spoilage but in combination the effect of increasing one became less at higher levels of the other. Increasing salt by 1% achieved a similar reduction in spoilage to that achieved by increasing the nitrite level by 100 μg/g.

Although the three-factor interactions were statistically significant, they were small compared with the main effects. Many interactions were significant simply because of the large individual effects of the main factors such as isoascorbate.

Analysis of variance of toxin data

The addition of isoascorbate, polyphosphate or nitrate, increasing nitrite, salt levels, or heat treatment, and decreasing storage temperature all significantly reduced toxin production (Table 6).

Table 6. Means of transformed toxin data from slurries prepared from pork of 'high' initial pH (6.4). Single factors: figures given are calculated using the percentage toxin-positive samples (see Roberts *et al.*, 1981). 'Processing the toxin data' i.e. the smaller the figure the less the toxin

					Least significant difference (LSD)	
Treatment	Level tested				$P = <0.05$	$P = <0.01$
Sodium nitrite	100	200	300			
(μg/g)	23.99	12.43	5.99		2.86	3.69
Sodium nitrate	0	500				
(μg/g)	17.04	11.24			2.34	3.01
Sodium isoascorbate	0	1000				
(μg/g)	25.07	3.20			2.34	3.01
Heat treatment	LOW	HIGH				
	16.38	11.90			2.34	3.01
Sodium chloride	2.5	3.5	4.5			
(% w/v on water)	20.09	15.15	7.18		2.86	3.69
Polyphosphate	0	0.3				
(% w/v)	19.45	8.82			2.34	3.01
Storage temperature	15	17.5	20	35		
	6.19	11.36	16.37	22.62	3.30	4.26

Treatment levels are significantly different when their means differ by more than the LSD.

Mean toxin scores of the two factor interactions are presented in Table 7.

Table 7. Means of transformed toxin data from slurries prepared from pork of 'high' initial pH (6.4). Effect of two-factor interactions; figures given are calculated using the percentage toxin positive samples (see Roberts *et al.*, 1981). 'Processing the toxin data', i.e. the smaller the figure the less toxin.

Treatment interaction	Level tested				Number of replicates	Least significant difference (LSD)	
						$P = <0.05$	$P = <0.01$
Nitrate × nitrite		0	500				
	100	30.09	17.90		96	4.00	5.30
	200	15.72	9.14				
	300	5.30	6.67				
Isoascorbate ×		0	1000				
nitrite	100	41.28	6.71		96	4.00	5.30
	200	23.89	0.96				
	300	10.04	1.94				
Isoascorbate ×		0	1000				
nitrate	0	29.87	4.20		144	3.24	4.26
	500	20.27	2.21				
Heat treatment		LOW	HIGH				
× nitrite	100	28.12	19.87		96	4.00	5.30
	200	15.43	9.42				
	300	5.58	6.40				
Heat treatment		LOW	HIGH				
× nitrate	0	21.32	12.76		144	3.24	4.26
	500	11.44	11.04				
*Heat treatment		LOW	HIGH				
× isoascorbate	0	28.08	22.06		144	3.24	4.26
	1000	4.67	1.73				
Salt × nitrite		2.5	3.5	4.5			
	100	29.41	25.80	16.77	64	4.95	6.58
	200	20.91	14.93	1.44			
	300	9.94	4.71	3.32			
*Salt × nitrate		2.5	3.5	4.5			
	0	23.15	19.62	8.34	96	4.00	5.30
	500	17.02	10.68	6.01			
Salt ×		2.5	3.5	4.5			
isoascorbate	0	36.16	26.58	12.47	96	4.00	5.30
	1000	4.01	3.72	1.89			
*Salt ×		2.5	3.5	4.5			
heat treatment	LOW	21.32	19.28	8.52	96	4.00	5.30
	HIGH	18.85	11.01	5.38			
Polyphosphate		0	0.3				
× nitrite	100	32.79	15.19		96	4.00	5.30
	200	17.54	7.32				
	300	8.02	3.96				
*Polyphosphate		0	0.3				
× nitrate	0	21.05	13.02		144	3.24	4.26
	500	17.85	4.63				

Table 7. *continued*

Treatment	Levels tested					Number of replicates	Least significant difference (LSD)	
							$P = <0.05$	$P = <0.01$
Polyphosphate		0	0.3					
× isoascorbate	0	33.41	16.73			144	3.24	4.26
	1000	5.48	0.92					
*Polyphosphate ×		0	0.3					
heat treatment	LOW	22.43	10.32			144	3.24	4.26
	HIGH	16.47	7.33					
*Polyphosphate		0	0.3					
× salt	2.5	26.19	13.98			96	4.00	5.30
	3.5	20.95	9.34					
	4.5	11.20	3.15					
Storage temperature		15	17.5	20	35			
× nitrite	100	11.08	20.27	27.19	37.43	48	5.78	7.72
	200	6.95	9.42	14.28	19.07			
	300	0.55	4.38	7.65	11.37			
*Storage temperature		15	17.5	20	35			
× nitrate	0	7.92	13.48	21.35	25.40	72	4.67	6.21
	500	4.47	9.23	11.40	19.84			
Storage temperature		15	17.5	20	35			
× isoascorbate	0	10.91	20.32	29.44	39.60	72	4.67	6.21
	1000	1.48	2.39	3.30	5.64			
*Storage temperature		15	17.5	20	35			
× heat treatment	LOW	8.57	13.18	18.66	25.10	72	4.67	6.21
	HIGH	3.82	9.54	14.09	20.15			
*Storage temperature		15	17.5	20	35			
× salt	2.5	12.31	15.36	20.55	32.12	48	5.78	7.72
	3.5	5.17	12.72	20.94	21.76			
	4.5	1.11	5.99	7.72	13.99			
*Storage temperature		15	17.5	20	35			
× polyphosphate	0	9.15	15.66	24.06	28.93	72	4.67	6.21
	0.3	3.24	7.05	8.69	16.32			

Within a given interaction the difference of any two means is significant if it is greater than the LSD.
*No significant interaction.

Interactions involving isoascorbate. The effect of isoascorbate was highly significant and if it was present no further significant reduction in toxin production was achieved by: increasing salt from 2.5 to 4.5%, nitrite from 100 to 300 μg/g, adding nitrate, or decreasing storage temperature from 35 to 15°C.

Interactions involving nitrite. Increasing nitrite or salt levels decreased toxin production but when salt and nitrite were both present the effect of increasing one in the presence of high levels of the other became less. In the presence of polyphosphate, or after HIGH heat treatment, or at 4.5% salt, increasing nitrite from 200 to 300 μg/g nitrite did not further reduce toxin production. Adding polyphosphate at 100 μg/g nitrite was equivalent to increasing nitrite to

200 μg/g. Increasing heat treatment from LOW to HIGH achieved a similar reduction in toxin production to that achieved by increasing nitrite from 100 to 200 μg/g after LOW heat treatment. At 15°C storage there was no significant reduction in toxin production when nitrite was increased from 100 to 200 μg/g but a further increase to 300 μg/g significantly reduced toxin production. At 17.5°C there was a significant reduction when nitrite was increased from 100 to 200 μg/g, but no further reduction when nitrite was increased to 300 μg/g.

Interactions involving nitrate. The reduction in toxin production achieved by increasing nitrite was relatively less in the presence of nitrate. Although increasing heat treatment from LOW to HIGH, or adding nitrate, significantly reduced toxin production, there was no significant difference between the effect of LOW heat plus nitrate and HIGH heat plus nitrate.

There were very few significant three-factor interactions, and their effects were small in comparison with the effects of the single factors. Almost all the significant three-factor interactions involved isoascorbate and were probably only significant because of the large main effect of isoascorbate.

Discussion

All single factors studied significantly reduced toxin production, but increasing heat treatment from LOW to HIGH, or adding nitrate, did not reduce spoilage significantly.

Conclusions from this study using 'high' pH pork are essentially similar to those from the study using meat of an initial 'low' pH (Roberts *et al.*, 1981). The most important difference was the effect of polyphosphate (Curaphos 700). In 'low' pH slurries polyphosphate did not significantly affect spoilage and significantly increased toxin production, although this increase could be counteracted by the addition of isoascorbate or nitrate, or raising nitrite or salt concentration, or the heat treatment to the next highest level tested. In 'high' pH slurries polyphosphate significantly decreased both spoilage and toxin production. We have found no explanation in the literature for this effect.

Difference in experimental plan for 'high' and 'low' pH slurries make a statistical comparison between the two difficult, but the Grand Means for spoilage or toxin production at the two pH levels may be compared: There was considerably more spoilage overall in 'high' pH slurries (Grand Mean 321.6) than in 'low' (Grand Mean 250.5). There was, however, little difference between the Grand Means for toxin production at the two pH levels (Grand Mean: 'high' pH 14.14, 'low' pH, 10^1 inoculum level, 13.47). The similarity between the Grand Means for toxin production may be a consequence of the reversal of the effect of polyphosphate, when at 'low' pH levels it increased toxin production but at 'high' pH it decreased toxin production. A further contributory effect may have been isoascorbate, which was significant at both pH levels but at 'high' pH was the most significant single factor controlling spoilage and toxin production.

There were no true synergistic interactions and all significant interactions were antagonistic (i.e. the combined effect of the two interacting factors was less than would be expected by summing their individual effects, but the combined effect of the two factors interacting was always greater than the effect of either factor acting alone).

The overall trends may be summarized:

(1) Increasing nitrite decreased spoilage and toxin production. In the presence of other significant fctors, e.g. salt or isoascorbate, no further decrease in spoilage or toxin production occurred when nitrite levels were increased.

(2) Nitrate significantly reduced toxin production but did not significantly affect spoilage. Chemical analysis of residual nitrite levels during storage indicated that nitrate was reduced to nitrite, hence significant effects attributed to nitrate alone are most certainly due to increased nitrite levels.

(3) Increasing salt level reduced toxin production. At high salt levels increasing nitrite (100–200 μg/g) or heat (LOW-HIGH) did not significantly affect spoilage or toxin production.

(4) The addition of isoascorbate significantly reduced spoilage and toxin production. In its presence other significant factors (increasing nitrite or salt concentration or heat treatment, adding nitrate, or decreasing storage temperature) became relatively less effective, but least spoilage or toxin production occurred when isoascorbate was present.

(5) The inclusion of polyphosphate significantly decreased spoilage and toxin production and it interacted with isoascorbate, nitrite and salt.

(6) Increased heat treatment significantly decreased toxin production, although in the presence of other significant factors (high salt or high nitrite or isoascorbate or polyphosphate) the effect of increasing heat treatment was not significant.

(7) Lowering the storage temperatures significantly reduced spoilage and toxin production although low storage temperature reduced the apparent effectiveness of other factors, but storage at 15°C always resulted in least spoilage or toxin production.

Care must be exercised comparing these data on growth of *Cl. botulinum* in 'high' pH pork slurry with those from 'low' pH pork slurry (Roberts *et al.*, 1981). The tabulated mean spoilage and toxin scores in one paper must not be compared directly with those in the other. Within each paper the change in magnitude of score with treatment or treatment combination can be used to measure the overall effect of that treatment or treatment combination: e.g. increasing salt 2.5–4.5% reduces the mean from *ca* 20 to 7 (Table 6). Therefore increasing nitrate (100–300 μg/g) has a similar effect as increasing salt (2.5–4.5%) and the interacton between salt and nitrate (Table 7) illustrated that raising salt from 3.5 to 4.5% with nitrite at 100 μg/g can be regarded as equivalent to raising nitrite from 100 to 200 μg/g whilst maintaining salt at 3.5%.

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Separation of anthocyanins by adsorption on acid alumina

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Summary

The conditions have been determined for achieving an effective separation on acid alumina of polyhydroxylated anthocyanins from others.

The adsorption isotherms for some anthocyanins are also given and the behaviour of methanol, ethanol and propanol solutions has been examined.

Introduction

In recent years there has been growing approval for the use of natural red colorants in place of synthetic ones (Anon., 1959; Baker, Johnston & Barber, 1974; Esselen & Sammy, 1973; Shewfelt & Ahmed, 1977), mainly because of the health risk associated with the latter (Anon., 1970).

The stability of anthocyanins, red colorants of plant origin which also possess pharmaceutical properties (Cantarelli & Panelli, 1968; Proserpio, 1974; Bonacina & Pacchiano, 1974), depends not only on environmental factors, such as pH, temperature, partial oxygen pressure, the nature of the other components present (Adams, 1973; Timberlake & Bridle, 1976; Hrazdina, 1974) and the type of light radiation (Nordstrom, 1956; Sastry & Tischer, 1952; Riva *et al.*, 1974), but also on the glycosidation and nature of the B ring of the pigments (Robinson *et al.*, 1966; Pifferi *et al.*, 1979). In addition, anthocyanins with at least two ortho hydroxyls in the B ring have different colour shades (Harborne, 1967). From the above one can see the potential interest in the separation, purification, or both, of polyhydroxylated pigments on both the laboratory and industrial scale.

Anthocyanins have been separated on ion-exchange resin (Amberlite CG-50) (Chiriboga & Francis, 1970), polyvinylpyrrolidone (Hrazdina, 1975; Peri &

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Bonini, 1976) and also on alumina (Nebelsky *et al.*, 1949; Birkofer, Kaiser & Donike, 1966), however for the latter the conditions for optimal separation have not been determined.

Our study of some parameters (pH, kind of solvent, adsorption time, etc.) was aimed at achieving the most efficient separation on acid alumina of polyhydroxylated anthocyanins from others, independent of the type of glycosidation.

Materials and methods

Materials

Acid alumina (E. Merck, W. Germany) with a granule size larger than 200 mesh was used. The surface area, determined by the one-point method with a Sorpty apparatus (C. Erba, Italy), which employs the low-temperature gas adsorption (BET) technique, was found to be 93 m^2/g with an estimated error of $\pm 5\%$. Nitrogen was the adsorbent. The alumina used for the separation was reactivated by calcination at 600°C for 6 hr.

The anthocyanin sources were grape *(Vitis vinifera)* in the form of the commercial solid oenocyanin with 6% colouring matter (Reggiana Enocianina, Italy) and methanol extracts of eggplant *(Solanum melongena)* as the source of the delphinidin derivatives and red carnation *(Dianthus caryophyllus)* as the source of the pelargonidin derivatives. As a source of malvidin and peonidin derivatives we used the oenocyanin fraction that was not adsorbed by the acid alumina, in accordance with the pigment separation method mentioned below. This fraction was concentrated *in vacuo* and purified of traces of Al^{3+} by ion exchange (Pifferi & Vaccari, 1980). All reagents used were at least reagent grade.

Adsorption method

We prepared 1.25 ‰ solutions of solid commercial oenocyanin in methanol, in ethanol, and in propanol. These were agitated for 60 min at 25°C and filtered. The resulting clear solutions were used to prepare the samples of different water contents (A solutions) (see Tables 1 and 2). The known volumes of added water already contained the known quantities of 2N HCl or 2N NaOH that were needed to bring the mixtures to the desired pH values. The corresponding alcohol–water mixtures were prepared in a similar manner (B solutions).

We placed 1.0 g of acid alumina and 25.0 ml of a different B solution into each of 100 ml amber-glass centrifuge tubes with screw caps. These were agitated every 15 min up to the predetermined equilibration time for that particular trial, correcting the pH where necessary with the 2N HCl or 2N NaOH solution.

After the alumina had been thus equilibrated, 25.0 ml of the corresponding A

solution (i.e. that containing the same quantity of water) was added. The tubes were placed in a water bath thermostatically maintained at 25°C and were agitated every 30 min. The reference samples were prepared and treated analogously, except that the alumina was omitted. For the tests without equilibration, each A solution was immediately added to the corresponding alumina—B solution mixture, the pH level being corrected where necessary. The same method was used to investigate the adsorption of the eggplant and red carnation pigments.

Analysis of the supernatant

After centrifugation at 4500 rev/min (2700 *g*) for 10 min, a portion of the supernatant was analysed by two-dimensional chromatography on Whatman 3MM paper using the solvents *n*-butanol—acetic acid—water (BAW) (120:30:50 v/v) and acetic acid—37% HCl—water (AAW) (15:3:82 v/v) and the usual methods of aglycone analysis (Harborne, 1967). Quantitative analysis of the supernatant was performed by adding 2 ml of 2N HCl to 2 ml of solution and determining the absorbance at 520 nm using a Perkin Elmer model 402 spectrophotometer and then an $\bar{\epsilon}$ (mean molar extinction coefficient) of 28 000 Niketic-Ateksic & Hrazdina, 1972) in the calculation.

The pH of the supernatant was determined after centrifugation with a Metrohm Herisau model E 500 pH meter.

Separation of the pigments

After the alumina and pigment solution was kept at 25°C at pH 4.90 for at least 16 hr (in accordance with the adsorption method cited), it was poured into a chromatographic column equipped with a porous septum which contained a 2-cm layer of alumina previously equilibrated to pH 4.90 in methanol—water (95.5 v/v). Elution with methanol—water (95.5 v/v) was repeated until the filtrate was free of anthocyanins. The polyhydroxylated pigments adsorbed on the alumina were recovered with methanol—HCl (0.5–2.0 O N) (95:5 v/v).

Results and discussion

The data in Table 1 show that the alumina exerted a clear-cut buffer action, which was less intense for the oenocyanin solutions. This buffer action could be linked to the presence of both base and acid sites on the surface of the alumina (Hindin & Weller, 1956; Sacconi, 1963). In support of this hypothesis, we noted that samples 1 and 2 took on a violet colour, which can be attributed to the solubilization of Al^{3+} ions and the consequent formation with the anthocyanins of chelates having a different absorption spectrum. One can attribute the

Table 1. Percentage adsorption after 24 hr of oenocyanin from $CH_3OH—H_2O$ (95:5 v/v) at different pH levels, without alumina equilibration. Buffer effect of acid alumina on $CH_3OH—H_2O$ (95:5 v/v) mixtures at various initial pH levels

	With oenocyanin			Without oenocyanin	
Sample	Adsorption (%)	pH level at 0 hr	pH level after 24 hr	pH level at 0 hr	pH level after 24 hr
1	39.9	0.50	0.60	1.10	1.60
2	41.9	2.30	2.60	2.00	2.60
3	64.0	3.50	4.00	3.00	3.60
4	83.0	4.50	4.40	4.00	4.25
5	94.0	5.00	4.50	5.00	3.95
6	97.0	5.50	4.80	—	—
7	94.1	6.00	4.60	6.00	3.85
8	90.4	7.00	6.00	8.00	4.70

decrease in buffer action to the effect of the other components of the crude oenocyanin on the alumina, since they, too, are susceptible to adsorption, especially acids (citric, tartaric, and malic) and polyphenols.

From Table 1 it can also be seen that with a 5% (v/v) water content in the solvent mixture and with no equilibration of the alumina and anthocyanins demonstrated maximum adsorption at an initial pH of *ca* 5.50. Similar results were obtained with 90 min of equilibration. Table 2 confirms in fact that with a longer equilibration time there was a tendency for the maximum adsorption to occur at *ca* pH 5.50 and a water content of *ca* 3% (v/v), although at lower adsorption values than those in Table 1.

When the equilibration was prolonged to 24 hr, there were poorer results at all pH values, with lower selectivity and greater irregularity in the adsorption trend. Vice versa, the chromatographic analyses of the supernatants showed that maximum selectivity in polyhydroxylated anthocyanin adsorption occurred at pH 4.90, after 90 min of equilibration and a 5% (v/v) water content.

Table 2. Adsorption of oenocyanin on acid alumina equilibrated for 90 min as a function of the pH level, percentage water (v/v) in the CH_3OH solution and time

	pH 3.85					pH 4.90					pH 5.50				
	Percentage water (v/v)					Percentage water (v/v)					Percentage water (v/v)				
Time (hr)	0	3	6	10	15	0	3	6	10	15	0	3	6	10	15
2	41.9	46.4	42.4	47.0	42.9	43.9	49.2	41.9	40.4	45.3	14.3	22.7	24.0	17.9	22.5
4	50.2	59.4	54.7	54.1	51.2	65.6	63.9	64.2	60.4	62.9	61.3	61.4	62.4	56.7	52.6
8	—	—	—	—	—	—	—	—	—	—	66.6	67.3	67.0	61.5	58.5
18	71.3	76.3	71.1	67.5	65.9	74.3	73.4	66.3	69.2	72.7	76.4	77.3	75.5	70.9	65.3

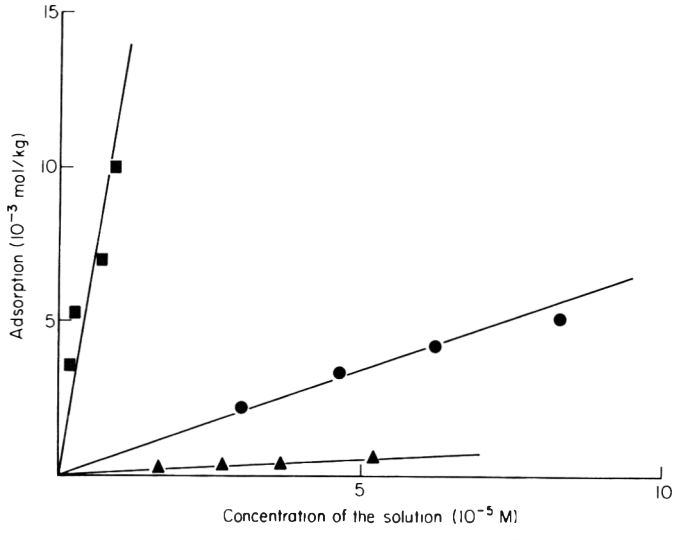

Figure 1. Adsorption isotherms at pH 4.90 in CH_3OH—H_2O (95:5 v/v) of the monoglucosides of delphinidin (■), malvidin and peonidin (●), and pelargonidin (▲).

An analysis of the adsorption isotherms reported in Fig. 1 demonstrated that the antocyanins containing the trihydroxylated aglycone delphinidin (Dp) were more strongly adsorbed than those containing malvidin (Mv) and peonidin (Pn) or pelargonidin (Pg), with solid phase/liquid phase separation constants at 25°C of 1100, 70, and 12 l/kg respectively.

The values for the analysis of the mixture of the malvidin and peonidin glycosides (i.e. the oenocyanin supernatant) and, although to a less degree, the pelargonidin glycosides (red carnation) were, however, slightly higher than the actual values, since the presence of anthocyanin fractions acylated with aromatic acids (Ribéreau-Gayon, 1958) increases adsorption. On the other hand, the differences in the separation constants are such to justify our regarding this difference in values having no effect on the general trend of the phenomenon, whereas there remains the advantage of the applicability of our method to natural systems, which are of greater interest from the technological viewpoint.

The possiblity of polyhydroxylated anthocyanins forming complexes at pH values greater than 3.00 with various metals (among which aluminium) with an anthocyanin/metal ratio of 2:1 (Bayer *et al.*, 1966; Monties, Marine-Font & Douillard, 1969) accounts for the behaviour of the delphinidin derivatives, whereas the adsorption of the malvidin and peonidin derivatives and the pelargonidin derivatives may be regarded as being purely physical in nature. This conclusion is supported by the reversibility of this adsorption by repeatedly washing the alumina with the solvent mixture.

Figure 2 shows the existence of two distinct retention phenomena, one chemical and one physical, for the adsorption of a oenocyanin solution on

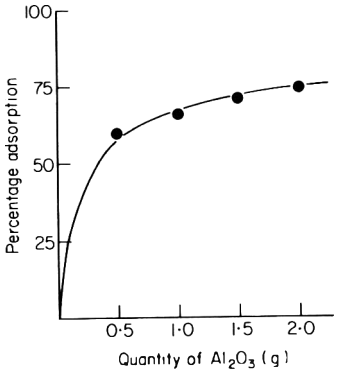

Figure 2. Percentage adsorption of oenocyanin as a function of the quantity of acid alumina.

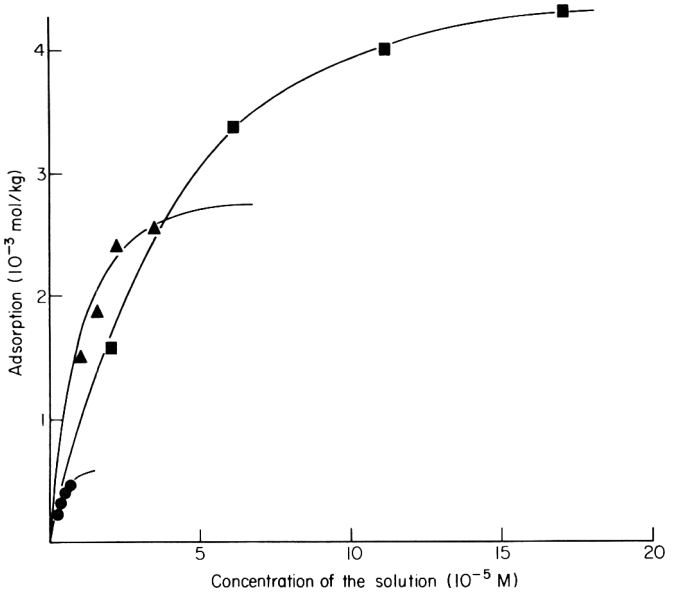

Figure 3. Adsorption isotherms of saturated solutions (at 25°C) of oenocyanin from alcohol—water (95:5 v/v). ■, Methanol; ▲, ethanol; ●, propanol.

increasing quantities of acid alumina. In the first curve section there is, in fact, a rapid increase in the percentage adsorbed, even for small quantities of adsorbent, which can be linked to chelate formation and which is limited by the quantity of polyhydroxylated anthocyanins present. The subsequent increase can be attributed, instead, to physical adsorption and is directly proportional to the quantity of alumina used.

Examining Fig. 3 one finds that the ethanol-water (95 : 5 v/v) mixture was the solvent that showed the greatest adsorption of anthocyanins at pH 4.90 at a given molarity of oenocyanin solution. Chromatographic analysis on paper also demonstrated complete retention of the polyhydroxylated pigments.

The greater efficiency of the ethanol-water system can be attributed to a combination of different effects, viz. a decrease in oenocyanin solubility as the

aliphatic chain of the alcohol is increased, the increase in the relative adsorption associated with the progressive reduction in solvent-solute competition for the adsorbent, and the increase in viscosity that occurs when passing from ethanol to propanol. With the latter the adsorption is strongly influenced by physical factors with a reduced accessibility of the microporous surface of the alumina (Lippens & Steggerda, 1970).

The results for ethanol-water mixture were especially interesting from the technological viewpoint, in that this alcohol is not toxic, as methanol is, and, compared to propanol, it is a better solvent for oenocyanin and has a lower boiling point, making it easier to distil.

We analysed the data of Fig. 3 with the aid of Langmuir's equation

$$1/Q = 1/\mathrm{K}\ M\ S_m + 1/S_m$$

Where M is the molarity of the oenocyanin solution at equilibrium, Q the quantity of adsorbed pigments, and S_m the quantity of pigment adsorbed in monolayer formation. When $1/Q$ was plotted against $1/M$, linear relationships were found for the range of concentrations used, and the following S_m values were calculated from the intercepts (Kapadia, Guess & Austin, 1964): $52 \cdot 10^{-4}$, $35 \cdot 10^{-4}$ and $1 \cdot 10^{-4}$ mol/kg for methanol, ethanol, and propanol solutions, respectively.

The physically adsorbed pigments (malvidin and peonidin, and pelargonidin derivatives) were eluted by repeatedly washing with an alcohol—water (methanol or ethanol) (95:9 v/v) mixture, whereas the polyhydroxylated pigments were eluted with acidified water or, more effectively, with an alcohol—HCl (0.5–2.0 N) (95:5 v/v) mixture. The polymerized pigments tended to remain irreversibly adsorbed; this method could also be used for partial purification.

The alumina can be readily regenerated by calcination at 600°C for 6 hr, with complete recovery of the initial adsorbency. Pigments eluted with acid solvents can be subsequently concentrated *in vacuo* and freed of traces of Al^{3+} by ion exchange (Pifferi & Vaccari, 1980). With our method it is therefore possible to separate polyhydroxylated pigments from others, purify them of decomposition and polymerization products, or both. The applicability of this method to natural extracts and its efficacy in ethanol solutions make it especially attractive for the food and pharmaceutical industries.

The considerable differences in the separation coefficients demonstrate the presence of two distinct retention phenomena, one chemical, with chelate formation, and the other physical. The ease of anthocyanin recovery and the complete, simple regeneration of the adsorbent, in addition to its low cost, make this method also applicable on an industrial scale.

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Potato flour as partial replacement of wheat flour in bread: baking studies and nutritional value of bread containing graded levels of potato flour*

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Summary

Potato flour (PF) is a material that does not differ significantly from wheat flour (WF) with regards to its physical appearance and chemical composition. For that reason it may be used in bread making. In this study mixtures of wheat flour and potato flour were prepared containing PF at levels of 0, 2, 4, 6, 8 and 10%. The farinograph properties of wheat flour affected by addition of potato flour were studied. Water absorption increased gradually from 62% for WF to 79% for blend with 8% PF. Other parameters such as development time, weakening of dough (Brabender units) and valorimeter value (W) were adversely modified by the addition of potato flour. The bread properties were studied using mixtures containing 0, 2, 4, 6, 8, 10 and 15% PF. The substitution of WF produced increases in water absorption, loaf weight and loaf volume as compared to all-wheat bread. In addition, bread containing PF retained moisture for longer periods than normal bread. Loaves made from wheat flour and 2, 4, 6, 8 and 10% potato flour were tested for their chemical composition and protein efficiency ratio (PER) in the rat. Moisture of bread increased with each increase in the level of potato flour substitution. The protein content of bread showed a progressive fall from 6.8% (at 4% level) to 6.3% (at 10% level). The protein efficiency ratio did not change significantly with the inclusion of potato flour up to 8% compared to all-wheat, but at the 10% level there was a significant reduction in this parameter ($P<0.05$).

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Introduction

Bread is an important component of the Chilean diet. Several authors have shown that this food contributes about 40% of the daily calorie intake and about 35% of the protein (SNS, 1976). However, the production of wheat in the country is insufficient. Therefore substantial quantities of this cereal must be imported every year (ODEPA, 1975). Making bread by partial substitution of wheat is not a new idea and it is worthwhile to mention some of the efforts made in the past to make bread from local materials, be they cereal flour or roots starches (Dendy, Clarke & James, 1970).

In Chile, potato flour appears to be one of the most promising substitutes in bread making in order to conserve wheat flour. This tuber is normally found in large surplus especially in the Southern provinces of the country. On the other hand some investigators have shown that potato protein is superior to most major plant proteins (Markakis, 1975; Meister & Thompson, 1976).

The present study was undertaken to assess the feasibility of using potato flour as a partial replacement of wheat flour and obtain basic information useful to the breadmaking industry. To this purpose the physical dough properties of blends of both materials and their baking properties were studied. In addition we investigated the chemical composition and biological quality of bread with graded levels of potato flour.

Materials and methods

Potato flour (PF) was obtained from a local supplier. Basically the PF production consists of: (a) selection of potatoes; (b) washing; (c) peeling; (d) dicing to facilitate the cooking; (e) cooking in a continuous cooker; (f) grinding and drying on a drumdryer. Commercial wheat flour of 78% extraction was purchased from a local mill. This flour was enriched with B-complex vitamins, iron and addition of 50 ppm of potassium bromate according to Chilean law. Potato flour was analysed for moisture, total ash and nitrogen by AOAC methods (AOAC, 1975). The wheat and potato nitrogen content in grams was multiplied by the factors 5.7 and 6.25 respectively to determine the protein content.

Physical dough properties of wheat flour and wheat flour/potato flour blends containing 2, 4, 6, 8 and 10% PF were determined with a Brabender farinograph (50 g flour, 50 g bowl), as outlined according to AACC methods (1968).

Breadmaking

Bread was prepared in the laboratory from a combinatin of wheat flour and potato flour. The levels of substitutions of wheat flour by potato flour were 2, 4, 6, 8, 10 and 15%. The breadmaking formula of 100 g wheat flour or the

blend, 4 g dry, skim milk, 3 g shortening, 10 ml of a salt/sucrose solution (1.5 and 4% respectively) and 10 ml of a 2% yeast suspension.

Bread (100 g pup loaves) was baked from the WF/PF blends using a National Complete Dough mixer by a straight dough baking procedure with a 3-hr fermentation and a temperature of 30°C. During the fermentation period, the dough was removed from the bowl after 1 hr and after a further 45 min and given the first and second punches, respectively.

The dough was rounded, molded, placed in baking pans and allowed to ferment for 55 min at 30°C. The loaves were baked for 25 min at 230°C in a Despatch oven with rotary plate and thermostat. Both weight and volume of the loaves were determined after cooling for 1 hr. The loaf volume was measured by a rapeseed displacement method. Loss of moisture was determined at 24, 48, 72 and 96 hr. The moisture content was measured in 10-g samples in an oven heated at 130°C for 90 min.

The chemical analysis included the following determinations: moisture was measured by direct reading in a Brabender oven. Ash and nitrogen were measured according to established methods (AOAC, 1975). To convert N to protein the factor 6.25 was used.

Biological methods

The control and experimental loaves with 2, 4, 6, 8 and 10% PF were tested for the protein efficiency ratio (PER) in rats according to the method of Chapman, Castillo & Campbell (1959). For this purpose the loaves were sliced and air-dried on racks for 72 hr at approximately 25°C and the ground through a Wiley mill. The ground bread was incorporated into experimental diets containing: ground bread 80%; corn oil, 10%; mineral mixture, 4%; vitamins, 1%; and cellulose 5%.

The experimental groups consisted of ten weanling rats. The animals were randomly assigned into groups, adjusted to give similar mean weights and then started on their assigned diets containing bread as the sole source of protein. The diet and water were offered *ad libitium* and food consumption and body weight were recorded weekly. Protein efficiency ratios (PER values) were calculated by dividing the overall body weight gain by protein intake.

Statistical analysis

Baking parameters and biological data were analyzed by the analysis of variance (Snedecor & Cochran, 1967) and Duncan's multiple range test (Duncan, 1955).

Results and discussion

The promimate chemical composition of wheat flour and potato flour are shown in Table 1. Protein content was 9.3% (N × 5.7) for wheat flour and 6.7 (N × 6.25) for potato flour. Table 2 lists the results of the farinograph studies on flour mixtures containing different levels of potato flour. The amount of water (absorption) required to centre the farinogram curve on the 500 BU (Brabender units) line increased steadily with every increment of potato flour from 62% for 0% PF to 79% for 10% PF, or a 27% increase. Changes in farinograph curve characteristics are illustrated in Fig. 1. The dough developing time decreased with the addition of potato flour. The control bread exhibited a value of 7.0 min which decreased gradually to 2.0 min for the highest level of replacement. These results may imply that the physical mechanical properties of the dough were severely affected by the incorporation of potato flour. The weakening of the dough increased gradually from 64.8 BU for the control to 202.5 BU for the 10% level of substitution, thus confirming the deterioration of the physical properties

Table 1. Gross chemical composition of wheat flour and potato flour (g/100 g)

	Wheat flour	Potato flour
Moisture	12.0	7.2
Crude protein	9.3 (N × 5.7)	6.7 (N × 6.25)
Ash	0.6	2.2
Fat	1.2	0.3

Table 2. Farinograph properties and baking characteristics of blends of wheat flour and potato flour (*)

	Potato flour level (%)						
	0	2	4	6	8	10	15
Water absorption (%)	62.0	64.5	65.5	69.8	73.2	79.0	—
Farinograph values							
Developing time (min)	7.0	5.3	3.4	1.6	1.7	2.0	—
Weakening (BU)	64.8	101.3	123.8	195.0	200.0	202.5	—
Valorimeter value (W)	63.8	54.3	43.8	25.3	23.8	24.0	—
Bread properties							
Loaf weight (g)	142.1	144.3	147.0	151.2	151.8	154.0	160.0
Loaf volume (ml)	695.5	749.4	760.0	761.9	770.6	771.2	779.4
Specific loaf volume (ml/g)	4.89	5.19	5.17	5.04	5.08	5.01	4.87
Loaf water absorbed (ml)	92.5	97.0	101.8	108.0	114.4	117.5	131.6

(*) No significant difference between underlined treatments at the 5% level of significance.

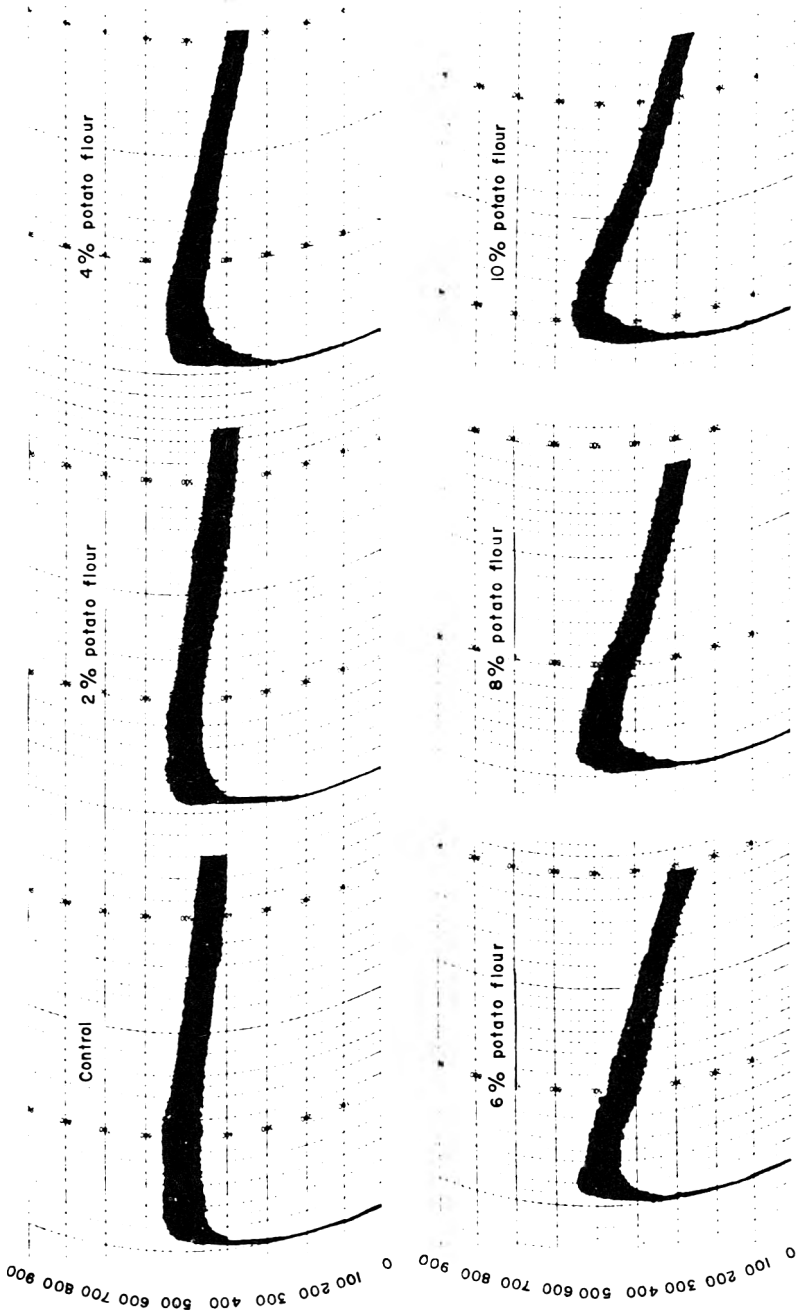

Figure 1. Farinogram of flour-water doughs. Absorptions for the commercial wheat flour with different levels of potato flour.

of the dough. The valorimeter value (W) which represents the physico-mechanical properties of the dough decreased from 63.8 to 24.0. According to Chinean regulations the minimum acceptable W value for wheat flour intended to make bread is 40 (INN, Chile, 1977). The values for the doughs containing 2 and 4% PF were well within the acceptable range. Higher levels of PF produced a significant decrease of valorimeter value ($P<0.01$). In summary all these parameters showed a gradual decrease in the physico-mechanical properties of the dough with increasing amounts of potato flour.

The experimental baking studies showed that the concentration of 2, 4 and 6% potato flour did not affect the handling of the dough. The blend containing 8% PF developed some degree of stickiness and the levels of 10% and 15% presented a great difficulty to dough hndling. The breadmaking studies showed that the volume of water absorbed increased significantly with the addition of PF with respect to the control ($P<0.01$). The all-wheat bread absorbed 92.5 ml of water and this value increased to 131.6 ml at the level of 15% substitution by potato flour. The weight of the loaves increases significantly ($P<0.01$) with the increasing levels of potato flour, except for the 2% level which did not change the loaf weight. The mean weight for the control loaves was 142.1 g and this value rose to 160 g at the highest level of substitution. This unexpected result could be explained by the presence in potato flour of some unknown factor which enhances loaf volume. This increase is obviously a result of the increased water absorption and retention by the dough. While this fact may be important from the economical standpoint, there remains the question of how it may affect other properties of bread. There was an increasing trend in the loaf volume with increasing levels of substitution by PF ($P<0.01$). The volume of the control bread was 695.5 ml. This figure increased to 779.4 ml for the 15% PF treatment, or an increase of 12%. This result may prove to be an advantage of potato flour incorporation in bread making over other materials that have been used to produce protein fortified breads which cause a decrease in loaf volume as the level of substituent increases (Tsen, Hoover & Phillips, 1971). The specific loaf volume (ml/g) of wheat flour bread was 4.89, this value increased slightly to 5.19 and 5.17 for the levels of 4 and 6% PF respectively and then decreased progressively to the value of 4.87 which is essentially equal to the basal value of 4.89.

Table 3 shows the loss of moisture from 24 through 96 hr. At 24 hr after baking the control bread contained 34.9% moisture, while bread containing potato flour had higher moisture values, reching 40.9% at the 10% level of potato flour. At 48 hr only control bread had fallen under the minimum acceptable value of 30% (INN, Chile, 1977). At 72 hr all loaves containing potato flour, except that with 10% PF, were above the minimum levels. At 96 hr only samples containing 2 and 4% potato flour had an acceptable level of moisture while those containing higher levels were slightly under that level. These results seem to indicate that bread containing potato flour retains more water and for a longer period than wheat bread. This may represent an important advantage over wheat bread which has a shorter shelf-life, due to moisture loss, since it may

Table 3. Moisture content of breads containing several levels of potato flour at 24, 48, 72 and 96 hr

	Potato flour levels (%)					
	0	2	4	6	8	10
24	34.9	37.0	37.1	36.0	38.2	40.9
48	29.3	33.3	34.9	33.2	37.5	37.9
72	28.8	31.5	31.1	31.2	35.7	29.5
96	28.5	30.6	30.4	27.1	29.9	29.0

Table 4. Chemical composition of bread containing potato flour

	Level of potato flour (%)					
	0	2	4	6	8	10
Moisture	33.3	36.8	37.3	37.3	37.5	38.6
Total ash	1.7	1.7	1.7	1.7	1.7	1.7
Protein (N × 6.25)	7.2	6.6	6.8	6.7	6.6	6.3
Ether extract	0.6	0.5	0.4	0.4	0.5	0.5

Table 5. Protein efficiency ratio (PER) of breads with different levels of potato flour

Potato flour level (%)	Initial weight (g)	Weight increase (g)	Protein intake (g)	PER*
0	37.9 ± 2.6*	21. ± 5.5*	22.2 ± 2.0*	0.97^{a} ± 0.2*
2	37.8 ± 2.5	20.6 ± 4.2	21.2 ± 3.4	0.98^{a} ± 0.1
4	37.7 ± 2.5	18.1 ± 3.8	20.3 ± 1.6	0.90^{a} ± 0.1
6	37.9 ± 2.3	20.5 ± 3.2	21.5 ± 2.7	0.99^{a} ± 0.2
8	37.8 ± 2.1	20.5 ± 3.8	20.5 ± 2.1	1.02^{a} ± 0.1
10	37.8 ± 2.1	17.8 ± 2.2	21.3 ± 2.0	0.85^{b} ± 0.1

*Means with the same letter re not significantly different ($P<0.01$).
*Mean ± s.d.

prevent losses of this valuable food especially in areas which rely heavily on wheat imports. The overall results reported here seem to suggest that the substitution of wheat flour by potato flour would not exceed 8%.

The chemical analysis of bread (Table 4) showed a trend to a higher water content with higher levels of potato flour, from 33.3% for the control to 38.6% for the 10% PF level. On the other hand, the protein content decreased with the incorporation of potato flour. Control bread contained 7.2% protein while bread containing potato flour showed values ranging between 6.8 and 6.3% for the levels assayed.

The protein evaluation test in rats (Table 5) showed that weight increase was low and similar in all groups. PER value for control bread was 0.97 which was

similar to values obtained previously in our laboratory (Yánez *et al.*, 1973). The substitution of wheat flour by potato flour did not modify this parameter up to the 8% level. However, bread containing 10% potato flour showed a significant decrease in PER ($P < 0.05$).

These results seem to support the feasibility of the incorporation of potato flour in bread making. The additon of PF at the levels of 2, 4, 6 and 8% did not impair the nutritive value of bread as shown by the PER method and proximal chemical composition. Beyond the 8% level the biological quality of the protein is significantly decreased.

These results suggest that PF may be used as a wheat flour extender in breadmaking inasmuch as it would enable the industry to save wheat without reducing the protein value of bread.

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Effects of processing of grated cassava roots by the 'screw press' and by traditional fermentation methods on the cyanide content of gari

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Summary

The extent of the loss of hydrocyanic acid (HCN) from grated cassava roots, selected from both the sweet and bitter varieties, was compared in the roasted grit (gari) derived from their fresh pulp which had been dewatered and fermented by two different processes; the quick (1-day) 'screw press' method (QSP) and the slow (3-day) traditional one (STD), respectively. The relative amounts of HCN which had disappeared after 1 day, in the case of QSP, and 3 days, in respect of STD, were comparable (92–100%) for either free (non-glycosidic) or bound (glycosidic) cyanide content of the two cultivars, indicating that the former method was more efficient than the latter in the detoxification of the grated pulp. The QSP method appeared to retain some of the bound cyanide while with the STD method, virtually no bound cyanide was detectable. About 83–91% of the total HCN content of the grated roots was present as free cyanide. It would seem that varietal differences in HCN contents of cassava may not be a critical factor in the preferential selection of the roots for 'garification'.

Introduction

Cassava (*Manihot esculenta* Crantz) roots, which usually contain the cyanogenic glycosides linamarin and lotaustralin are an important staple food for about 300 million inhabitants of the tropics (Nestel, 1973). Two main types of cassava are cultivated in Nigeria: namely; the 'sweet' variety which is associated with low content of cyanogenic glycosides and the 'bitter' variety which is known to have high cyanide content. The cyanogenic glycosides produce hydrocyanic acid (HCN) when the action of an endogenous enzyme

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linamarase is initiated by crushing or otherwise damaging the cellular structure of the plant (Conn, 1973).

The utilization of cassava roots for both human and animal nutrition appears to be limited by the presence of these cyanogenic glycosides. As a result, the roots have to be processed by a wide variety of traditional methods (Coursey, 1973) in order to reduce their toxicity and improve their palatability. Gari, the form in which cassava is most widely consumed in Nigeria, is a grit which is derived from the roots by grating, de-watering and fermentation of the grated pulp and then roasting of the resultant mash. It is probably the most important single traditional staple food in West Africa and it is estimated that about 70% of the total cassava crop grown in Nigeria is channeled into gari manufacturing (Olayide *et al.*, 1972). The annual output figure of this commodity in Nigeria has been estimated to be in the neighbourhood of 1.5–2 million metric tonnes, amounting to a monetary turnover of between ₦150 million and ₦200 million per year (Ngoddy & Kaplinsky, 1976).

The capacity to process cassava into gari in the villages, however, is a factor which tends to limit potential sales. A recent technological development which could remove the processing bottleneck has been the introduction of the 'screw press' which is used to expel water from grated roots by the application of high pressure by means of a metal press. This method of de-watering the grated pulp requires a few hours as opposed to the 3–4 days usually employed in the traditional system.

Although the use of the 'screw press' appears to be a quicker method than the traditional process, its application reduced considerably the time of fermentation; a process which is regarded as being largely responsible for reducing the hydrocyanic acid content of the grated pulp (Ketiku *et al.*, 1978). It therefore seems desirable to evaluate the effectiveness of both processing methods in the detoxification of grated cassava roots.

Materials and methods

Roots from the two different types of cassava, the so-called sweet and bitter varieties were harvested, peeled by hand and grated using locally manufactured impliments. The fresh pulp from each cultivar was pooled and equal weights of these were stuffed into jute bags which were similar in texture and dimensions. The bags were then subjected to varying degrees of downward pressure using either the 'screw press' or the popular traditional assembly.

In the case of the 'screw press' the jute bag, and its contents (4 replicates per cassava type), were placed on a stainless rectangular metal platform while another was screwed down vertically onto the jute bag until the plate could move down no further, and in such a way that the applied pressure did not burst the bag. In the traditional method each test jute bag, and contents, was placed on a metal platform and some pieces of heavy stones were then placed on top of the bag.

The experiments were terminated after 24 hr in the case of the 'screw press' and 72 hr in respect of the 'traditional press'. The fermented mash, in each case, was roasted, for the same period of time, into gari by village women hired for that purpose. Free and bound cyanide contents were determined in samples of the peeled root and in the pulp using the sensitive enzymic assay of Cooke (1978). In the case of gari, Cooke's method as described by Maduagwu (1979) was employed.

Results

Analysis of the grated roots showed that most of the total cyanide content of the pulp was present in the free (non-glycosidic) form; 83% and 91% respectively in the sweet and bitter varieties (Table 1). The remaining 17% and 9% were in the bound (glycosidic) form. The concentrations (mg/kg dry wt) of these two forms of cyanide in random samples of roots with no overt signs of damage were 20.46 ± 9.26 and 194.96 ± 70.12 in the sweet varieties and 127.13 ± 31.40 and 1301.27 ± 101.27 in the bitter cultivars. The traditional processing method appears to retain more of the 'free' cyanide and less of bound HCN in gari than the QSP method (Table 1). This situation is also reflected in the percentage losses of cyanide (Table 2). No bound cyanide was however detected in gari prepared by the STD method.

Table 1. Cyanide contents of grated cassava roots and their product (gari)

Test sample	Origin	Main experimental processing method	HCN concentration (mg/kg dry wt) ± s.d.		
			Total	Free	Bound
Root pulp	Bitter cassava	Grating	1375.40	1144.24	231.16
Root pulp	Sweet cassava	Grating	169.80	155.0	14.8
Gari	Bitter cassava	Screw press (24 hr)	4.45 ± 0.45	3.81 ± 0.16	0.64 ± 0.30
Gari	Sweet cassava	Screw press (24 hr)	3.36 ± 0.27	3.01 ± 0.06	0.35 ± 0.24
Gari	Bitter cassava	Traditional press (72 hr)	12.28 ± 0.85	12.56 ± 0.55	0
Gari	Sweet cassava	Traditional press (72 hr)	11.51 ± 0.38	12.02 ± 0.34	0

Table 2. Cyanide loss in gari prepared from grated cassava roots

Source of root pulp	Mode and duration of de-watering and fermentation of pulp	HCN loss (percentage dry wt)	
		Free	Bound
Bitter cassava	Screw press (24 hr)	99.67	99.72
	Traditional press (72 hr)	98.90	100
Sweet cassava	Screw press (24 hr)	98.06	97.64
	Traditional press (72 hr)	92.25	100

Discussion

From the data reported in this paper the 1-day QSP method appears to be more efficient than the STD process in terms of the rate of detoxification of grated cassava roots. The differences noted in the cyanide contents of gari prepared by both methods could be attributed either to the expulsion, with time, of larger volumes of cyanide by the screw press arising from the application of a higher and more uniformly distributed pressure to the grated pulp and/or to the fact that, owing to the differing fluid contents of the pulp after dewatering, different amounts of cyanide will be removed during the roasting procedure.

In the traditional method the amount of cyanide lost after 72 hr could be compared with that removed after 24 hr using the QSP method. The indication therefore is that the fermentation process, apart from imparting a desired flavour to gari may play only a minimal role in the detoxification of grated cassava roots. If however, cyanogenic glycoside *per se* can be assumed as being toxic then the traditional method from the point of view of non-retention of bound cyanide in gari (Table 1) could be regarded as more beneficial.

However, there is now some evidence that cyanide levels in gari may not be due to the presence of HCN in the free gaseous form but to an adjunct. For instance Maduagwu & Fafunso (1980) have shown good correlation between cyanide levels in gari and sugar or starch content or particle size.

The low values recorded for 'free' cyanide (Table 1) in gari have shown that varietal differences in HCN content may not be a critical factor in the selection of cassava roots for garification although from a strictly toxicological standpoint the use of low cyanide varieties is certainly desirable.

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Studies on the local techniques of yam flour production

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Summary

Traditional Nigerian techniques of yam flour production have been studied with a view to mechanizing the processes.

Results of the studies show that the processing time can be much reduced if mechanized. The results also show that parboiling is an essential and critical procedure in the processing. The effects of variations in processing parameters on the production are discussed.

Introduction

Yams belong to the genus Dioscorea of the family Dioscoreaceae, which has about 600 species of which *D. alata* L; *D. cayenenesis* Lam; and *D. rotundata* Pair, have the greatest economic importance.

The susceptibility of yams to injury, microbial attack and subsequent post-harvest loss has been reported by several authors, such as Coursey (1961), Adesuyi (1971), Booth (1974) and Passam, Read & Rickard (1976). Losses of up to 40–50% after 6 months of storage were recorded by Adesuyi (1971). Various studies have been directed towards limitation of losses of yam tubers. These include wound repair (Passam *et al.*, 1976) tuber curing (Adesuyi, 1971) and wound coating (Ige, 1978) but all these techniques for improving the storage of yam tubers still need many refinements before they can be put to any practical use. However, processing of the tuber into a stable product offers an alternative to storage in the fresh state.

Yam tubers are processed into several products for storage. The most popular products are 'Poundo yam' (Cadbury Nigeria Ltd), pounded yam flour (a product of the Food Science and Technology Department of the University of

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Ife) and yam flour (Elubo) produced locally by farmers using traditional techniques.

The first two products are attempts to prepare instant foods which reconstitute into products very similar to the traditional pounded yam which is one of the most highly favoured items of diet in West Africa. The third product called, 'Amala' in Yoruba when prepared is different from the first two products in colour, taste and texture.

The processing of yam tubers into yam flour is a long-established practice in Nigeria, especially the Western State, and the neighbouring countries, but the method has never been fully investigated scientifically to determine the parameters that affect the quality of the product. For instance, during the parboiling stage the local processors usually determine the parboiling temperature subjectively by dipping their hands into the pot, a procedure which is clearly unacceptable for large-scale processing.

The need to modernize this method by developing appropriate machinery and equipment for processing and handling of the products calls for some basic studies into the parameters that affect the product.

Previous work

The unique role of yams in the diet, economy, social and religious festivities of many countries in the tropics, especially in West Africa, has been well highlighted by Coursey (1967). Figures which he quotes also show that yam is a very good source of energy with high calorific and appreciable protein values. Most literature on post-harvest aspects of yams has been concerned with losses in tubers during storage. Coursey (1967) concluded that storage losses are more serious than is widely realized. Adesuyi (1971) recorded losses of between 40 and 50% after 6 months of storage.

As a result of the combination of a high degree of perishability, bulkiness, distance from production areas to consuming centres, and the seasonal nature of production, attention has been focused on the processing of the tubers into flour. Efforts have led to such relatively sophisticated products such as the Cadbury product, 'Poundo Yam' and the product of the Food Science and Technology Department of the University of Ife. The latter process involves washing, peeling, dicing, sulphiting, pregelling, cooling, drying, milling and bagging (personal observations).

Traditionally yams are prepared in several ways for immediate consumption, for example, boiled yam, yam chips (Dundu), yam balls, pounded yam and yam soup. The only traditional method for processing yam for storage has been processing into yam flour. This process involves washing, peeling, washing, slicing, parboiling, drying, milling and bagging, but the process has not been scientifically investigated to establish the processing parameters. The parboiling and drying stages are the two most critical in the processing.

Materials

White yam (*D. rotundata)* is mostly used for preparing yam flour and was used for this investigation. A kerosene stove was used for the parboiling and the parboiling temperatures recorded. Moisture content was determined by drying in an air oven at 100°C. A rig with sensing cantilever and measuring equipment (Ige, 1977) were used for measuring the crushing strength of the processed yam pieces.

Experimental procedure

Traditional processing method

One typical traditional processor of yam flour was observed while he carried out the operation from the stage of peeling to the stage where drying was about to begin. Other local processors were also interviewed on their processing procedures. Samples of dried pieces were obtained for moisture content determination.

Investigation on essential nature of the parboiling stage

Observations of the practices of traditional processors indicated that the parboiling stage is the critical one which determines whether the process is successful or not. Occasionally, the processing is terminated at this stage when it is realized that it has not been properly done. The possibility of eliminating the parboiling stage was investigated by drying peeled yam pieces in an oven at 80°C and in the sun, and by boiling the yam and drying under the above conditions. The products were then milled and prepared in the normal way in which the flour is prepared for the table.

Establishment of optimal parboiling temperature

Observations of the traditional processing method showed that the yam slices need to be of a distnctly flabby texture before the yam is considered to be adequately parboiled for the production of good flour. To achieve the same texture, several slices of yam were parboiled at temperatures of between 50 and 80°C, the yam slices being put into the parboiling vessel, covered with water, which was then raised to the set temperature. The vessel was then removed from the stove and the slices observed while the water was cooling. During the process at 60°C, samples of yam pieces were removed at intervals from the vessel for moisture content determination.

Establishment of the parboiling time for different thicknesses of yam pieces

Yam pieces ranging from 0.5 to 2.5 cm in thickness were put into vessels containing water and the temperature was raised to 60°C. The vessels were then removed from the stove and the texture of the yam pieces monitored until each reached the required degree of flabbiness. The average time for sets of yam pieces of the same thickness to reach the desired texture was determined.

Effects of treatments on rate of drying of yam pieces

Yam slices parboiled; unparboiled; and soaked in water at room temperature for the same time as the parboiling process were oven-dried under the same conditions at 100°C. Parboiled yam slices of varied thickness were dried under the same conditions, and the masses of the drying yam pieces were observed.

Establishment of optimal drying temperature

Preliminary investigation indicated that the rate of drying affects the hardness of the dried yam pieces and hence the milling properties. Parboiled and unparboiled yam slices of equal thickness were oven dried at temperatures ranging from 60 to 100°C. The products were then milled using the Peppink Deventer and Pascall Engineering mills wich are, respectively, hammer and burh mills. The milled products were then prepared in the normal way that yam flour is prepared for the table.

Effects of parboiling and parboiling time on the crushing strength of yam pieces

A rig which had been constructed previously for measuring the crushing strength of cowpea (Ige, 1977) was used for measuring the crushing strength of the yam pieces. The rig consisted of cantilever beam with mounted strain gauges. The strain gauges were wired to a Wheatstone bridge and connected to the balancing circuit of a Vis-recorder with an air damped galvanometer. The set-up was calibrated setting the gain on the recorder at 100.

From the yam pieces, representing fully parboiled samples, samples taken during the parboiling process and unparboiled samples, 1-cm cubes were prepared. Samples were also prepared from dried parboiled and unparboiled yam pieces. All samples were subjected to the crushing test.

Results

Traditional processing method

The parboiling stage usually lasts for about 8–13 hr. Processors usually commence processing after the day's work in the farm between 5 and 6 p.m. The yam tubers are sliced or at times left unsliced. They are peeled, washed and covered with water in vessels which are then heated over a wood fire and the temperature monitored manually. The fire under the vessels is removed when the water is uncomfortably hot and the pot is left till about 7 a.m. next day when the yam pieces are spread outside in the sun for drying. During the dry season, the operation can be started in the morning and drying commenced in the evening. Some of the yam pieces become overcooked during parboiling, and such pieces are usually discarded. The parboiled yam pieces are dried for about 2–5 days depending on climatic conditions. The moisture content of dried local samples was about 18% (w.b.).

The need for parboiling

All the samples that were not properly parboiled did not yield the characteristic 'amala' when prepared for the table, the colour, taste and texture being quite different. When the samples were in the unconstituted forms (flour) one could hardly detect the difference between them and the regular flour. The sample which was dried unparboiled resembled, rather than the expected 'Amala', another type of product called 'Lafun', usually prepared from cassava.

Establishing optimal parboiling temperature

Yam pieces subjected to parboiling temperatures of 50 and 55°C did not attain the required flabby texture, and darkened within a short time of removal from the parboiling vessel. Samples parboiled at temperatures of 65°C and above became nearly cooked and also did not attain the appropriate texture. A parboiling temperature of 60°C produced the required flabbiness. The moisture content of the yam pieces after reaching 60°C is plotted in Fig. 1 for three different experiments. Generally the moisture content was between 60 and 70% (w.b.). Initially the moisture content rose slightly and then decreased slightly towards the point when the yam pieces had been in the hot water for 30 min. Further experiments indicated that the maximum moisture content was likely to be at about the time when the appropriate degree of flabbiness was reached.

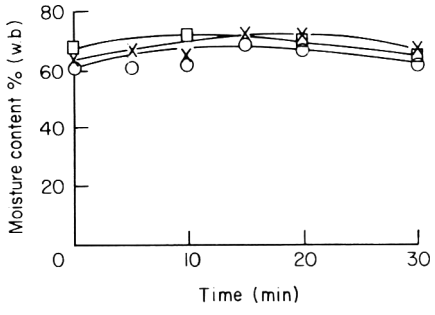

Figure 1. Moisture content of parboiled samples at 60°C. □, Experiment 1; ×, experiment 2; O, experiment 3.

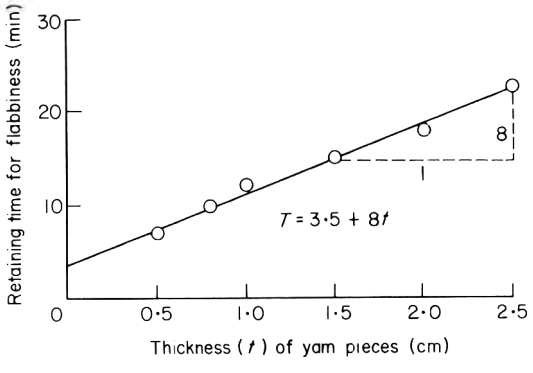

Figure 2. Retaining time for flabbiness after attaining temperature of 60°C.

Parboiling time for dfferent thickness of yam pieces

Figure 2 shows the average time taken to reach the required flabby texture when the pieces were retained in hot water the temperature of which had previously been raised to 60°C. The results suggest a linear relationship between retention time for flabbiness at 60°C and the thickness of the yam pieces. The fitted straight linear equation is given by: $T = 3.5 + 8t$ where t is th thickness of the slices and T is the time to obtain flabbiness after attaining a temperature of 60°C.

Effects of treatment on rate of drying

Figure 3a shows the rate of change in yam slices subjected to different treatments. The results show that the drying rate is almost unaffected by the treatment. However, the drying of the untreated yam pieces lagged behind that of the parboiled pieces because the latter's initial moisture content was higher.

The results of the effect of yam thickness on drying rate is plotted in Fig. 3b. This shows that as might be anticipated the rate of drying of the thinner slices is faster than that of the thicker slices.

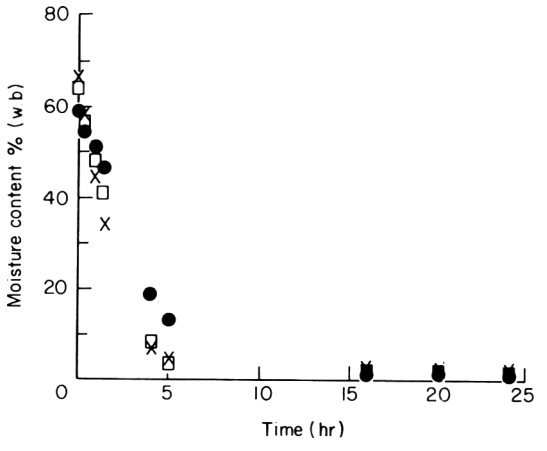

Figure 3a. Effect of different treatment on the rate of drying of yam pieces, ×, Unparboiled samples; ●, parboiled samples; †, samples soaked in cold water (30°C) water.

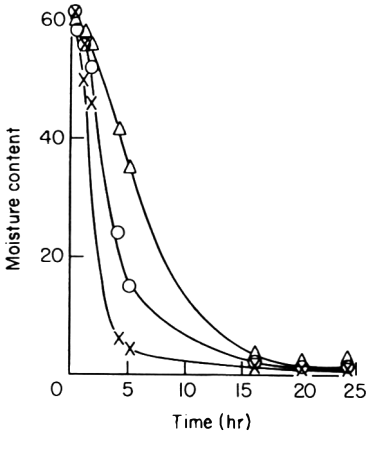

Figure 3b. Drying profile of different thicknesses of yam pieces. ×, 0.5 cm thickness; ○, 0.8 cm thickness; □, 1 cm thickness.

Establishment of optimum drying temperature

Observations show that drying of parboiled yam pieces at temperatures above 80°C tends to cook them and dry them, the colour becoming brownish and the pieces hard. Drying at temperatures between 60 and 80°C produced the best results, the pieces retaining their whitish colour. Drying under these conditions, for about 24 hr, resulted in fairly brittle products with a moisture content of about 13–16% (w.b.) which is considered a safe level for storage (the moisture content of yam pieces processed locally is about 18% w.b.). The flours made from samples dried at above 80°C were relatively coarse compared to those samples dried at temperatures below 80°C, and the coarse texture could be detected in products prepared for the table. Milled and prepared samples dried

between 60 and 80°C had a texture identical with the locally processed yam flour.

Effects of parboiling and parboiling time on the crushing strength of yam pieces

Figures 4a and 4b show the results of the effect of parboiling and parboiling time on the crushing strength of the yam pieces. The crushing strength appeared to increase slightly and then decrease fairly sharply with time. The time required to reach maximum strength appears to correspond to the time required for reaching flabbiness. The results also show that the crushing strength of dry parboiled pieces is less than the dry unparboiled pieces.

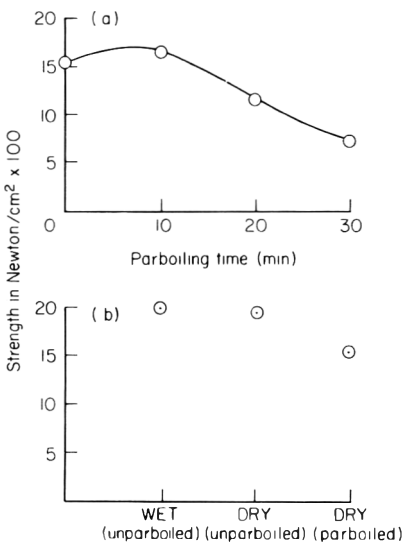

Figure 4a. Crushing strength of yam pieces after attaining a temperature of 60°C.
Figure 4b. Crushing strength of yam pieces.

Discussion

The significance of this study is that it shows that the processing of yam tubers into flour which is physically identical with the traditional flour known in Yoruba as 'Elubo' can now be mechanized.

A mechanical slicer can be designed to slice the peeled tubers into slices of predetermined sizes, and a parboiling tank can be designed to hold the temperature of its content at about 60°C and the retention time at 60°C can be determined for the given size from eqn $T = 3.5 + 8t$ as given previously. An artificial drier can be used which should be set at a temperature of between 60 and 80°C.

Conclusions

This study shows that the time needed for processing yam tubers into yam flour can be much reduced if the process is mechanized.

The results show:

Parboiling is an essential element in processing yam tubers into yam flour, locally called 'elubo'.

A flabby texture of parboiled yam pieces indicates that they are adequately parboiled.

The parboiling temperature should be held at about 60°C.

The parboiling time required depends on the thickness of the yam pieces, governed by the eqn $T = 3.5 + 8t$ as given previously.

The drying temperature should be between 60 and 80°C to obtain a fairly brittle product which will mill into powder form. Above this temperature the product becomes brownish and the grains are coarse.

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Effect of metal ions such as iron and copper on the formation of nitrogen heterocyclic compounds in sesame oil—α–amino acids model systems under frying conditions

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Summary

Deep-fat frying of foods in vessels made of metals such as iron and copper imparts varied aromas. Formation of pyrazine and pyridine compounds in sesame oil and α–amino acids model systems, with and without metallic ions such as iron and copper (10 ppm), heated under frying conditions, is reported. Metal ions have a catalytic effect on the formation of nitrogen heterocyclics and thus in changing the flavour profile.

Introduction

Peanut oil and sesame oil are commonly used as edible cooking media for deep-fat frying by a majority of the people in this country. During frying operations, the oil undergoes oxidation with the liberation of various carbonyls, which readily undergo reactions with food components particularly amino acids to form different flavouring compounds resulting in 'fried flavour'. Buttery *et al.* (1971) have demonstrated the formation of varous pyrazines and the pyridine compounds during potato chip frying. Vasundhara & Parihar (1980) also showed the formation of different pyrazines when peanut oil was heated with several α–amino acids under frying conditions. It is a common observation that frying or cooking foods in different metallic vessels imparts varied aroma and flavour. Commonly iron vessels are used and those of copper to a lesser degree. During continuous frying operations the oil is constantly heated and fresh oil is added to the same lot as and when required. As a result of this traces of the metal are dissolved in the oil and may influence the formation of flavour compounds.

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The effect of metal ions on fat oxidation has been studied by Patton, Barner & Evans (1959), Crossley, Heyes & Hudson (1962) and Waters (1971) and reviewed by Labuza (1971) and investigated by Sedlacek (1974). Shibamoto & Bernhard (1977) reported that the additon of metal ions such as copper and zinc accelerated browning reactions and reduced the yield of smaller less substituted pyrazines, but increased the yield of larger alkyl substituted poyrazines. This effect was attributed to acceleration of the dehydration reactions by the metal ions. The present paper reports the effect of metal ions such as iron and copper, when present in sesame oil in the range of 10 ppm, on the formation of nitrogen heterocyclic compounds in model systems consisting of sesame oil and α-amino acids heated under frying conditions.

Materials and methods

Reagents

All solvents used were of analytical grade. Glycine, alanine, phenyl alanine and isoleucine were of Analar BDH grade. Authentic pyrazines were obtained from Coca-Cola Company, U.S.A. as gifts and 2-acetyl pyridine from Koch Light, England. Silica gel G was of E. Merck Grade.

Apparatus

A Chromatolite ultraviolet lamp with short wave radiation 260 μm for locating the compounds on TLC plates, and a Perkin Elmer UV Spectrophotometer, model 124, for both u.v. and visible regions were used. Hilger Watt's Infragraph 4000 model was employed for recording i.r. spectra of various compounds. A gas chromatograph equipped with flame ionization detector, CIC make, Baroda was used for g.l.c. analysis.

Heating of sesame oil with α–amino acids and isolation of basic fractions

From standard aqueous solutions of ferrous sulphate and copper sulphate a known volume of each was taken in separate 500-ml round-bottomed flasks so that the metal concentration was 10 ppm in the final reaction mixture. Water was completely removed and 200 ml of sesame oil added. To different flasks 10 g each of glycine, alanine, phenyl alanine and isoleucine were added. The flasks were fitted with reflux water condensers and heated at 180°C for 5 hr. The basic fractions were isolated by dissolving the resultant mixture in 200 ml chloroform and extracting with 3×150 ml 5% HCl. The combined acid extracts were brought to pH 9.0 with 2% sodium hydroxide solution. The liberated basic

compounds were extracted with 3×200 ml methylene chloride. From the combined extracts, after drying over anhydrous magnesium sulphate, the solvent was carefully distilled off under reduced pressure. The residual mixture was made up of 5 ml with the same solvent and used both for t.l.c and g.l.c. analysis. The same procedure was carried out for blanks when no metal was incorporated.

Thin layer chromatography

The t.l.c. analysis was carried out according to the method of Vasundhara & Parihar (1980). The plates were developed with chloroform-ethyl acetate (6:4) and also with benzene-acetone (8:2) employing an ascending technique. The compounds were detected along with the authentics by spraying with Dragendorff's reagent as reported by Stahl (1969) and were identified by their R_F values as given in the table. From an unsprayed t.l.c. plate, the portions of the spots were marked under u.v. light, each spot separately scraped and extracted with methylene chloride. The concentrated solution of each individual compound superimposed with the authentic was subjected to two-dimensional t.l.c. using chloroform-ethyl acetate (6:4) and benzene-acetone (8:2) respectively, when they ran as single spots. The compounds were estimated using Dragendorff's reagent colorimetrically after the procedure of Vasundhara & Parihar (1980).

Three millilitres of the sample were applied on silica gel G plate as a streak and developed with chloroform-ethyl acetate (6:4), the separated bands were marked u.v. light, scraped separately, eluted with methylene chloride, the solvent evaporated off carefully and the residue used for both u.v. and i.r. analysis. Ultra violet spectra were noted in 90% ethanol and i.r. as thin film.

Gas liquid chromatographic anaylsis

One-microlitre samples were separated on 5% DEGS, S.S. column $6' \times 0.8''$ o.d. under isothermal conditions at 130°C, using nitrogen as a carrier gas, the flow rate being 55 ml/min. The amounts of various compounds were calculated from their peak areas when compared with those of known amounts of authentic compounds. The experiment was repeated three times and the mean values of the quantities of various compounds noted.

Results and discussion

A few commercial samples of heated sesame oil which had been used for frying were analysed for their maximum iron and copper contents to assess the level of metal ions. In none of the samples did the metal level exceed 10 ppm, and hence this level was chosen for addition of metal ions in model systems. Neutral amino

Table 1. The R_F values on t.l.c. retention times (min) on g.l.c. and the amounts (mg) of various heterocyclic compounds formed in model systems consisting of 200 ml sesame oil + 10 g amino acid + without or with 10 ppm Fe or Cu reaction mixtures. The combined average quantities by the t.l.c and g.l.c. analysis are given within the brackets

Compound No.	Name of the compound	R_F value on t.l.c. I	R_F value on t.l.c. II	Retention times by g.l.c. analysis and quantities: SO + glycine	SO + glycine + Fe	SO + glycine + Cu	SO + alanine	SO + alanine + Fe	SO + alanine + Cu	SO + phenyl alanine	SO + phenyl alanine + Fe	SO + phenyl alanine + Cu	SO + Isoleucine	SO + Isoleucine + Fe	SO + Isoleucine + Cu
1.	Pyrazine	0.44	0.81	—	—	—	—	—	—	1.4 (5.9)	1.4 (8.0)	1.4 (8.9)	—	1.4 (4.0)	1.4 (6.2)
2.	2,5-Dimethyl pyrazine	0.38	0.51	—	—	—	2.0 (4.6)	—	2.0 (8.0)	—	—	—	2.0 (5.0)	2.0 (4.1)	—
3.	2-Ethyl-6-methyl pyrazine	0.50	0.68	—	—	—	2.2 (4.0)	—	2.2 (4.2)	2.2 (3.2)	—	2.2 (4.0)	—	2.2 (4.1)	2.2 (8.0)
4.	2,3-Dimethyl pyrazine	0.28	0.48	—	—	—	2.6 (3.5)	2.6 (5.6)	2.6 (4.4)	2.6 (3.5)	2.6 (4.1)	2.6 (4.2)	—	2.6 (5.0)	2.6 (10.1)
5.	2-Methyl-3-isobutyl pyrazine	0.72	0.93	—	—	—	3.3 (3.10)	3.3 (6.0)	3.3 (4.6)	3.3 (3.0)	3.3 (3.1)	3.3 (3.0)	3.3 (4.3)	3.3 (4.1)	3.3 (7.0)
6.	2-Propyl-3,6,-dimethyl pyrazine	0.55	0.76	—	—	—	4.2 (3.6)	4.2 (5.1)	4.2 (5.9)	—	—	—	4.2 (5.0)	—	—
7.	2-Acetyl-pyrazine	0.60	0.96	5.4 (6.0)	5.4 (15.8)	5.4 (23.1)	5.4 (25.8)	5.4 (26.1)	5.4 (26.9)	5.4 (15.8)	5.4 (16.0)	5.4 (15.0)	—	—	5.4 (8.30)
8.	Tetramethyl pyrazine	0.33	0.54	—	—	7.6 (4.3)	—	—	—	—	—	—	7.6 (6.4)	7.6 (7.8)	7.6 (8.3)
9.	2-Acetyl pyridine	0.20	0.32	—	—	—	—	8.6 (6.0)	8.6 (5.3)	—	—	—	8.6 (13.0)	8.6 (12.8)	8.6 (14.0)
10.	2-Acetyl-3-methyl pyrazine	0.65	0.80	—	9.3 (4.2)	9.3 (4.3)	—	9.3 (4.9)	9.3 (5.6)	—	—	—	—	—	—

SO, sesame oil.

Thin layer chromatography solvent system: I, chloroform-ethyl acetate (6:4); II, benzene-acetone (8:2).

Table 1 *(cont'd)*

Ultra violet absorption maximum (μm)		Important i.r. peaks (μm)	
Isolated	Authentic	Isolated	Authentic
261	261	3.0, 3.4, 4.4, 5.1, 5.2, 5.3, 5.6, 5.8, 6.7, 7.6, 8.7, 8.9, 9.3, 9.8, 10.8, 11.2, 12.1, 13.9	3.1, 3.4, 4.4, 5.15, 5.25, 5.3, 5.6, 5.8, 6.7, 7.65, 8.8, 8.9, 9.3, 9.8, 10.8, 11.25, 12.1, 13.9
275.5	275	3.2, 3.3. 3.4, 3.5, 5.1, 5.5, 6.4, 6.65, 7.2, 7.5, 8.55, 9.6, 10.4, 11.3	3.2, 3.35, 3.5, 5.1, 5.5, 6.5, 6.65, 7.2, 7.48, 8.55, 9.6, 10.4, 11.35
276	275.5	3.4, 6.5, 6.8, 7.0, 7.5, 7.9, 8.6, 9.45, 9.8, 9.9, 10.3, 11.5, 11.7, 12.0	3.4, 6.5, .68, 7.1, 7.5, 7.9, 8.6, 9.4, 9.8, 9.9, 10.3, 11.5, 11.7, 12.0
274	275	3.0, 3.5. 4.75, 5.25, 7.0, 7.1, 8.0, 8.6, 9.3, 9.8, 10.2, 11.2, 11.8, 13.1	3.1, 3.5, 4.8, 5.25, 7.0, 7.1, 8.0, 8.63, 9.3, 9.8, 10.2, 11.2, 11.8, 13.1
290	290	3.39, 6.49. 6.85, 6.9, 7.2, 7.7, 7.9, 8.4, 8.6, 9.2, 9.4, 9.9, 10.1, 11.2, 11.8	3.4, 6.5, 6.85, 6.9, 7.2, 7.7, 7.95, 8.4, 8.6, 9.2, 9.4, 9.9, 10.1, 11.2, 11.8
279	279.5	3.4, 6.5, 6.85, 7.3, 7.4, 7.7, 8.6, 9.2, 9.4, 9.9, 10.5, 11.2, 11.8	3.4, 6.5, 6.9, 7.3, 7.4, 7.7, 8.6, 9.2, 9.4, 9.9, 10.5, 11.2, 11.8
271	269	5.9, 6.38, 7.75, 8.51, 9.05, 9.55, 9.70, 10.5, 11.70	5.9, 6.4, 7.75, 8.5, 9.05, 9.55, 9.70, 10.5, 11.70
279	279.5	3.0, 3.5. 5.2, 6.0, 6.5, 6.9, 7.0, 8.2, 8.4, 10.0, 11.1, 12.4, 13.8, 14.5	3.0, 3.5, 5.2, 6.0, 6.5, 6.9, 7.0, 8.2, 8.9, 10.00, 11.1, 12.4, 13.8, 14.5
228.5, 267	288, 267	2.85, 5.9, 6.4, 8.28, 9.96, 12.5, 13.3	2.9, 5.9, 6.4, 8.3, 9.45, 12.5, 13.3
290	290	3.37, 5.9, 6.47, 6.52, 7.1, 7.3, 7.85, 8.0, 9.2, 9.45, 9.70, 10.0, 10.3, 11.7	3.4, 5.95, 6.45, 6.50, 7.1, 7.3, 7.85, 8.0, 9.2, 9.4, 9.7, 10.0, 10.3, 11.7

acids were taken to eliminate the acidic or basic effects of amino acids which might influence the pyrazine formations. The extracts were run along with authentics on t.l.c. using two different solvent systems and the compounds thus resolved were characterized by their $R_F$value. They were further confirmed by two-dimensional t.l.c., when the isolated compounds along with the authentic compounds ran as single spot. All the compounds were colorimetrically estimated after the method of Vasundhara & Parihar (1980) and the mean values of three readings noted.

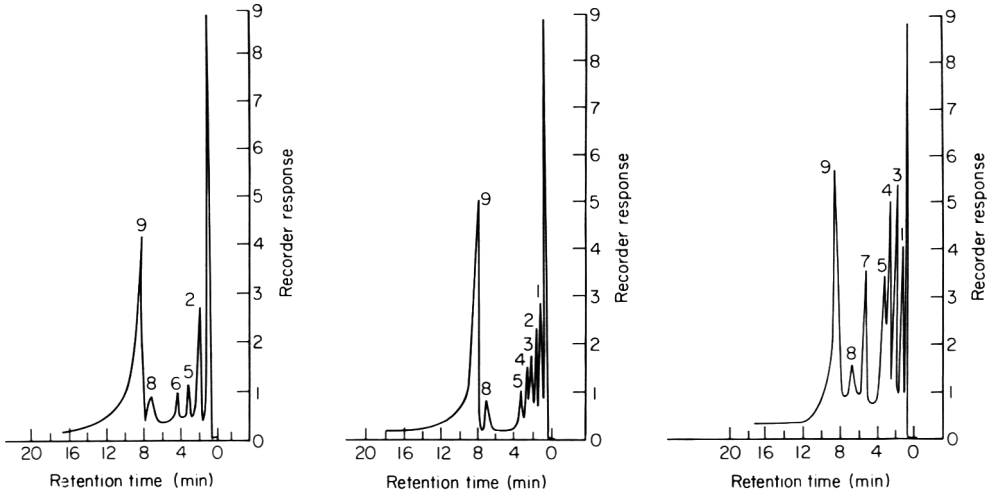
The basic extracts from the model system were subjected to g.l.c. and the retention times of different compounds present noted. The authentic compounds were also run under identical conditions. The retention times were distinct and the compounds were easily characterized. The mean vlues of the quantities of various compounds obtained from three sets of experiments from model systems were noted. Table 1 gives the combined average values of t.l.c. and g.l.c. of various compounds present. All the compounds isolated from model systems had distinct λ_{max}in the u.v. region and characteristic i.r. spectra, and thus could be easily differentiated and confirmed. There were least number of compounds in glycine system compared to other amino acid systems. In the case of glycine blank only 2-acetyl-pyrazine was detected, whereas addition of Fe and Cu considerably increased its yield. With Fe besides 2-acetyl-pyrazine, 2-acetyl-3-methyl-pyrazine was also confirmed. In the case of copper, in addition to those two pyrazines, tetramethyl pyrazine was also detected.

In the case of alanine blank six different pyrazines were identified and the majority of which was 2-acetyl-pyrazine. There was no significant change in the amount of 2-acetyl-pyrazine in the presence of metal ions. In the case of Fe-added system two of the lower molecular weight pyrazines viz. 2,5-dimethyl-pyrazine and 2-ethyl-6-methyl-pyrazine were missing. On the other hand 2-acetyl-pyridine and 2-acetyl-3-methyl pyrazine were detected. Thus five pyrazines and one acetyl-pyridine were found when Fe was used as a catalyst. Copper enhanced the yield of 2,5-dimethyl-pyrazine and resulted in a total of seven pyrazines and 2-acetyl-pyridine.

Sesame oil and phenylalanine on heating gave a total of five pyrazines, out of which 2-acetyl-pyrazine was in the larger proportion. Incorporation of metal ions affected the yield of pyrazine but did not alter appreciably the quantities of other compounds.

In the case of sesame oil–isoleucine system 2-acetyl-pyridine was detected as a major fraction. Metal catalyzed rections yielded a greater number of pyrazines, which were not detected in the blank. Typical gas chromatograms of sessame oil-isoleucine system, blank and with Fe and Cu are given in Figs 1, 2 and 3 respectively. The peak number corresponds to the compound number in Table 1.

Both the metal ions have a catalytic effect on the formation of nitrogen heterocyclic compounds. The catalytic effect of metal ions on the oxidation of fats and oils is well known. But the catalytic effect of metal ions on the formation of heterocyclic compounds and the mechanism involved is not clear. Probably



Figures 1–3. Gas liquid chromatographic separation of nitrogen heterocyclic compounds isolated from sesame oil-isoleucine system heated at 180°C for 5 hr. Fig. 1, blank; Fig. 2, with 10 ppm Fe; Fig. 3, with 10 ppm Cu. The peak numbers in the figures correspond to the compound numbers given in Table 1.

the metal ions when present in trace amounts may be exerting the catalytic effect by forming some intermediate complexes with carbonyl compounds as well as amino acids.

Acknowledgments

Thanks are due to Dr H. J. Takken, Naarden International Flavour Research Laboratory, Holland and to Dr M. Gianturco, Coca Cola Company, Atlanta, U.S.A. for sending the authentic pyrazine samples as gifts. The facilities for i.r. spectra provided by Central Food Technological Research Institute, Mysore are gratefully acknowledged.

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J. Fd Technol. (1981), **16**, 321–328

The hydraulic transportation of potato chips in horizontal pipelines

H. D. McLAIN*† AND G. McKAY‡

Summary

The pipeline transportation characteristics of uncooked potato chips in water have been studied and the results compared with correlations obtained for the transportation of plastic cuboids with the same cross-sectional dimensions. It was found that the general functional relationship relating pressure drop to flow velocity for plastic cuboids could be applied to potato chips. The constants in this relationship have been determined for the transportation of potato chips in pipelines of 95 and 152 mm diameters.

Introduction

Since the invention of the centrifugal pump, pipelines have been used for the hydraulic transport of solids. In the food industries a wide variety of shapes and materials are transported hydraulically: spheres—peas, cherries; cubes—diced carrots and potatoes; cuboids—chipped potatoes; conical and cylindrical shapes—whole carrots; flat discs—chopped carrots.

The problem investigated in this paper arose out of the need for hydraulic transportation of chipped potatoes in a plant producing these for catering establishments. A hydraulic transportation system was subsequently installed in this plant to convey the potato chips from the chipping machine to the blanching tank. There was no data readily available to the designer of a transportation system for food materials to enable him to design an optimum system. The design of such a system is usually based on recommendations given by the manufacturer of the equipment selected.

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Initially transportation studies (McKay & McLain, 1980) were carried out using plastic cuboids as the cuboid shape predominates in a sample of potato chips. The plastic cuboids proved to be good models for potato chips. This enabled the test apparatus, the experimental procedures and the flow relationships to be developed over long test periods. It would have been much more difficult using potato chips due to the breakdown and degradation which would have occurred. This present paper describes the behaviour of uncooked, chipped potatoes in pipelines of 95 and 152 mm diameters.

Materials and methods

The test apparatus consisted of a horizontal test section in which could be installed a pipe of 95 mm diameter or a pipe of 152 mm diameter. Part of each test pipe was transparent (glass or perspex) to enable the flow characteristics to be observed. A variable speed recessed impeller centrifugal pump was used to convey the mixture of water and chipped potatoes. The other main items of equipment were: feed and collection water tanks; a belt conveyer to feed in the potato chips; a micromanometer to measure the pressure drop along the pipe; a potentiometric chart recorder; a flow measuring device which enabled a sample of the mixture to be taken over a known time interval. The apparatus is shown in Fig. 1.

The water and chip mixture was pumped from the feed tank through the horizontal test pipe, then up a short vertical section of pipe to the collecting tank. At this point a sample of the flow could be taken by a pneumatically operated scoop in order to determine the flow rate and the delivered solids concentration. In the collecting tank a screen separated the potato chips from

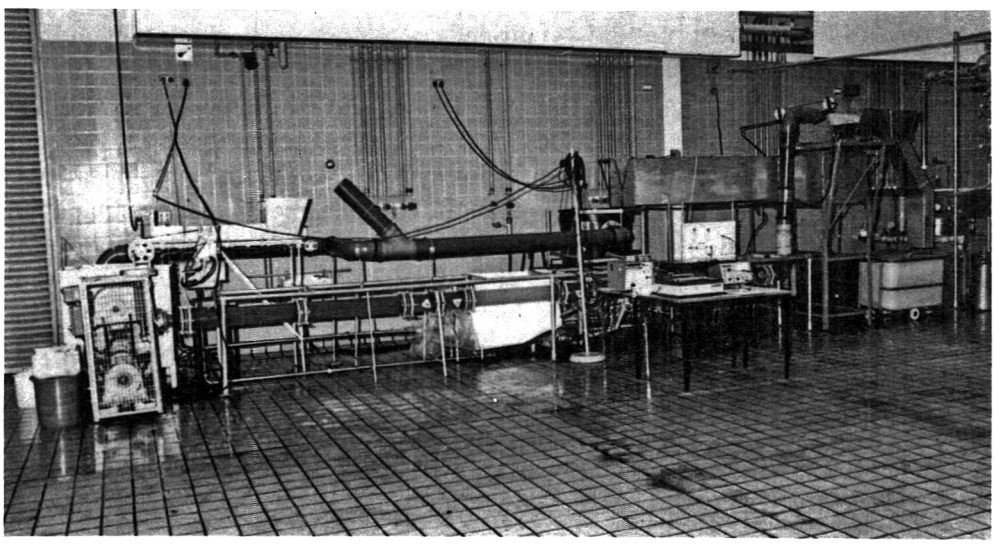

Figure 1. The test apparatus

the water. The water was then returned to the pump feed tank. A variable speed conveyor was used to feed the potato chips into the pump inlet. The pressure gradient along the test pipe was measured over a range of flow rates and chip concentrations.

The potato chips were tested as received from the processing factory. They had nominal cross-sectional dimensions of 12.5 × 12.5 mm. From each batch a sample was taken to determine the length and shape distribution and the specific gravity which ranged from 1.060 to 1.065.

Theory and results

It has been shown (McKay & McLain, 1980) that during he hydraulic transportation of cuboids of cross-sectional dimensions 12.5 × 12.5 mm, at particle concentrations from 0.05 to 0.25, the pressure drop is given by the functional relationship:

$$\frac{i-i_w}{i_w C} = \mathrm{x}\left[\frac{V_m}{(\mathrm{g}D_t(s\text{-}1))\,0.5}\right]^{-\mathrm{y}} \tag{1}$$

where the constants x and y depend on the particle length and the pipe diameter.

The results for potato chips showed that eqn 1 was followed and the constants x nd y for this are given in Table 1. The experimental results for the 95-mm pipe are shown in Fig. 2. The correlation lines for the results of the 152- and 95-mm pipelines fell between the correlation lines for the 25- and 51-mm long plastic cuboids, but closer to the 51-mm line than the 25-mm line. This result is consistent with the length distribution of the potato chips which ranged from 10 to 70 mm with the mean value around 45 mm.

Table 1. Constants from eqn 1 for the transportation of potato chips.

Pipe diameter (mm)	x	y	Correlation coefficient
152	116	2.79	0.95
95	95	2.56	0.96

Discussion

It is of interest to compare the results of Worster (1952), for the transportation of coal in 75- and 150-mm pipes with the results for potato chips. This is shown in Fig. 3. Coal and potato chips exhibit different characteristics. The relative pressure gradient over the clear water value, for the transportation of potato chips, in both pipe sizes tested, is greater than for the transportation of coal,

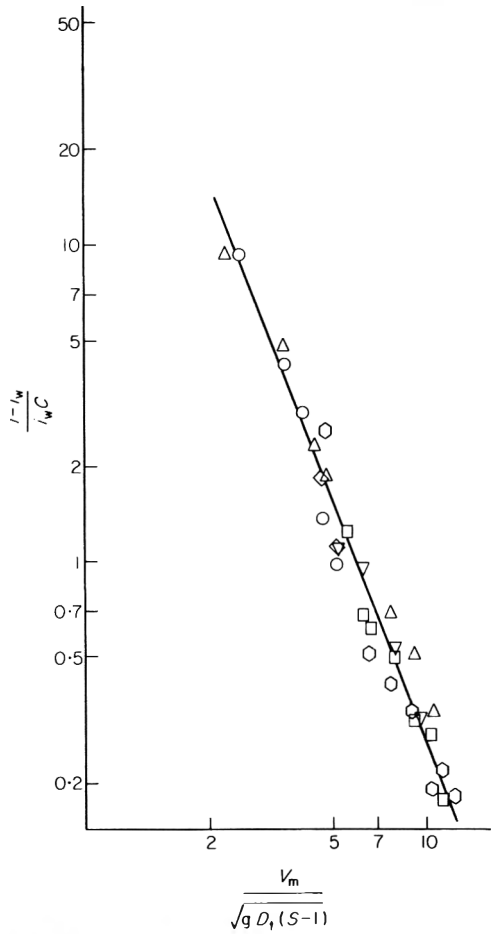

Figure 2. Plot of $\frac{i-i_w}{i_w C}$, against $\frac{V_m}{\sqrt{g D_t (S-1)}}$, for the transportation of potato chips in 95-mm pipe.

except at very low velocities in the 152 mm pipe. This difference in relative pressure gradient increases with flow velocity.

The comparison of the results of the 152- and 95-mm pipes, shows that the inclusion of the pipe diameter in the term $\frac{V_m}{\sqrt{g D_t(s\text{-}1)}}$ does not fully account for the effect of pipe diameter. Shook (1976) also indicated that the effect of pipe diameter is not properly accounted for in this way. However the advantage of representing the sesults in this manner is that they are immediately available for engineering design purposes (Babcock, 1971).

Velocity for minimum pressure loss

Using eqn 1, idealized curves of pressure gradient, i,, against mean flow velocity, V_m, may be drawn with volumetric particle concentration as the parameter. Figure 4 shows these plotted for the 152-mm pipe.

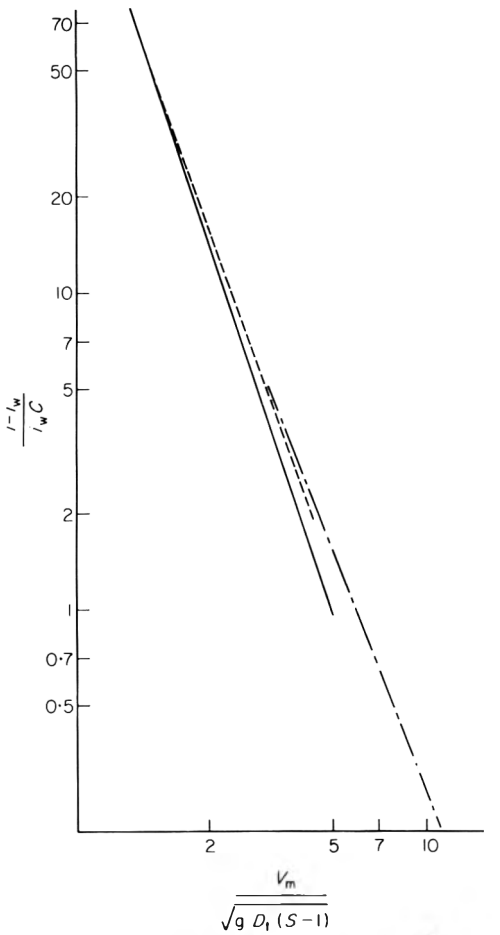

Figure 3. Comparison of the plots of $\frac{i-i_w}{i_w C}$ against $\frac{V_m}{\sqrt{g D_t (S-1)}}$ of potato chips in 152- and 95-mm pipes with the curve of Worcester for the transportation of coal. ——, Worster; ---, potato chips, 152-mm pipe; — · —, potato chips, 95 mm pipe.

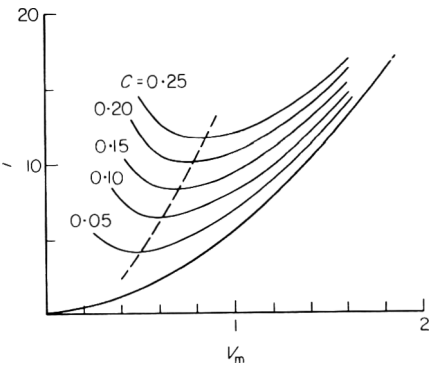

Figure 4. Idealized plot of pressure gradient, i, (mm H_2O/m), againstmean velocity, V_m, (m s^{-1}), for a range of particle concentrations of the transportation of potato chips, (sp. gr. = 1.065), in a 152-mm diameter pipe. ---, Minimum pressure drop conditions.

Table 2. Critical velocities, V_c, for potato chips (sp. gr. = 1.065)

	Critical velocity, V_c for potato chips, m s^{-1}	
C	152 mm pipe	95 mm pipe
0.05	0.51	0.33
0.10	0.61	0.43
0.15	0.70	0.50
0.20	0.78	0.56
0.25	0.83	0.62

At very low velocity the excess pressure gradient, above the clear water value due to the presence of solids, is high. As the flow velocity is increased the excess pressure gradient falls to a minimum velocity and then increases again. The velocity corresponding to the minimum pressure gradient has been called the critical velocity, V_c, and it is the optimum velocity for the operation of a pipeline, in respect of pumping power. Table 2 lists the critical velocities for the potato chips at five values of solids concentration. The critical velocities are greater in the large pipe. These values apply to potatoes of the specific gravity of the sample tested. In any particular industrial situation the specific gravity of the available material would have to be used to obtain the critical velocities.

Deposit velocity

It was not possible to determine the velocity corresponding to the onset of a permanent deposit on the bottom of the pipe. Attempts to determine this by slowly reducing the flow velocity resulted in pipe blockage which required partial dismantling of the pipeline in order to clear it. The onset of pipe blockage due to a stationary deposit was very rapid and is considered to be due to the size of chip particles but may also be partly due to the characteristics of the pump. In all cases pipe blockage occurred well below the critical velocity, V_c. In practice a plant would not be operated near the critical deposit velocity as the pressure gradient would be high and the solids delivery low. If the critical velocity, V_c, is considered as the minimum velocity for plant operation this will give an adequate margin of safety above the deposit velocity.

Application of the transportation results to pipeline design for potato chips

Equation 1 may be used to calculate the pressure gradient for the horizontal transport of potato chips using constants x and y from Table 1 for 95- and 152-mm diameter pipes. From this the overall pressure loss for the pipeline can be calculated.

It is recommended that the choice of pipe size should be made primarily on the quantity of potato chips which have to be transported. For pipes of diameters between 152 and 95 mm interpolation between the two sets of experimental results should be used. An estimate of the pressure loss in pipelines of greater diameter than 152 mm can be obtained from eqn (1) using x and y for the 152-mm pipe. A similar estimate can be obtained by using the equation for the 95-mm pipe for pipelines of lesser diameter. A greater uncertainty, however, in using a smaller pipe is the effect of reducing pipe diameter on the initiation of pipe blockage. Hence the use of smaller pipe sizes without experimental verification is not recommended.

The tests covered volumetric concentrations of 0.05 to 0.25 with the highest value achieved being 0.28. No pipeline problems occurred at the higher concentrations. However, the conveyor supply capacity and the pump intake capacity tended to restrict the upper value of concentration attained. It is suggested that a delivered concentration of 0.20 would be a good design point.

For minimum pressure loss the chips should be conveyed at the critical velocity, V_c. If the overall pressure loss is not great then the flow velocity may be increased above V_c to increase the transport capacity of the system. This will generally be the case in practice. In the calculation of V_c it is important to use the maximum density of the potatoes likely to be processed.

Vertical transportation was not specifically studied in this investigation. However the minimum transport velocity in a vertical pipe may be obtained from an investigation of the fluidization characteristics of potato chips (McLain & McKay, in press). Comparing the minimum transport velocity for vertical flow from these fluidization tests with the critical velocity, V_c, for horizontal transportation shows that V_c is greater than V_f. This means that if a system is designed to give horizontal transportation then conveyance is always assured in any vertical part of the pipe.

Nomenclature

C: solids concentration by volume.

D_f: diameter of transportation pipe (m).

g: gravitational constant (m s^{-2}).

i: pipeline pressure gradient for mixture of potato chips and water (mm H_2O/m).

i_w: pipeline pressure gradient for clear water (mm H_2O/m).

s: ratio of $\frac{\text{density of potato chips}}{\text{density of water}}$

V_m : mean velocity of mixture (m s^{-1}).

V_c : critical velocity for minimum pressure gradient (m s^{-1}).

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Book reviews

Advances in Fish Science and Technology Ed. by J. J. Connell *et al.* Farnham, Surrey, England: Fishing News Books Ltd, 1980. Pp. 528. ISBN 0 85238 108 5, £39.50 + £3.50 postage and packing.

The Jubilee Conference of the Torry Research Station held in July, 1979, was conceived, in part, as an appropriate method of commemorating 50 years of research and development activity into various aspects of fish science and technology. It was this special status of the conference that enabled the organizers to attract speakers from all over the world, and it is their papers, or at least some eighty of them, that have now been collated into a single conference volume.

The fact that the book, which has an A4 format, runs to over 500 pages gives some indication of the coverage that has been achieved, and the claim that the volume 'is an extensive compilation of valuable material which will stand, for many years, as the definitive account of the current state of advancement in fish science and technology' is one that few would dispute.

The actual text is divided into sixteen 'chapters', with each one being a collection of papers around a common theme. The 'state of the art' in relation to the handling, preservation and marketing of fish is dealt with in the first 'chapter', while the scientific background to, and/or consequences of, these processes forms the basis of the second of these two groups of review papers. This authoritative introduction paves the way for the remaining chapters, which offer a complete coverage of the chemistry, microbiology and processing of fish. The emphasis in every case is on recent research into the topic concerned, and one commendable aspect of many papers is the willingness of the authors to speculate constructively about possible future trends. However, the real value of these latter chapters lies in the depth of treatment which each subject receives, and it is this wealth of detail that makes this book such a useful addition to the literature on fish.

It is also, of course, a feature that poses problems for any reviewer, because expert appraisal of so many topics is wellnigh impossible. It is more than likely, therefore, that these few comments do less than justice to this excellent book, but if that is the case, then it was certainly not by intention. Thus, in short, this volume is a most fitting tribute to 50 years of scientific activity in relation to fish and fish products, not only academically, but also in respect of the quality of the finished text. Those responsible for the editing, publishing and printing of this work have achieved standards of clarity and accuracy of reproduction that, compared to many modern texts, make it a pleasure to read. This detailed

attention to presentation has, of necessity, been reflected in the price, but even so, anyone connected with the fishing industry or its subsidiary organizations will surely consider this book a worthwhile investment.

R. K. Robinson

Food Analysis Laboratory Experiments, 2nd edn. By C. E. Meloan and Y. Pomeranz.
Westport, Connecticut, U.S.A.L. The AVI Publishing Co. Inc. 1980. Pp. vii + 156 pp. ISBN 0 87055 351 8. $11.50 (paper).

The first impression of this book is that it provides an excellent, well-balanced series of experiments suitable for food science undergraduate courses. The book, or manual as the authors prefer to call it, is divided into three major parts: (1) methods demonstrating instrumental techniques, (2) selected standard methods and (3) appendices. However, from the contents these divisions are not obvious.

The experiments described cover a wide range of food analysis with some fifty experiments under thirty-five headings. The complexity of the experiments varies from simple extraction techniques to sophisticated instrumental methods including fluorimetry, flame photometry and high pressure liquid chromatography. This is the second edition of this book and the opportunity has been taken to include some of the recent advances in instrumental techniques.

The book has obviously been written with an American market in mind and as such will be found somewhat too colloquial for the United Kingdom. A chapter which starts 'To be sure, antihistamines are not foods . . .' or a section which states 'To a liberal arts student, corn is only to be used to feed cattle and hogs, but a truly educated person knows better', is unlikely to be taken seriously by students in this country.

The format of the book is good but the standard of diagrammatic material is very poor. Indeed a number of diagrams contain misprints or errors and some illustrations require extensive study to unravel the points that are being illustrated. The chapter on infrared spectrophotometry contains reproduced spectra, some with completely illegible captions, of a general quality that would not be acceptable in a thesis let alone a published book. In Appendix VI the authors suggest, very sensibly, the use of a standard system of abbreviations but appear unable to adopt their own advice in the text. A further consequence of the fact that this is an American book is that a number of the methods described are not those which are traditionally used in the United Kingdom. This is not to imply that they are inferior but rather that they are different from those methods currently in use in this country. A number of scientific terms are also used in a manner which appears inconsistent, for example spectroscopy and spectrophotometry are used interchangeably.

In summary the book undoubtedly contains a large amount of material highly

pertinent to food science undergraduate courses. However, the excessive colloquial style, coupled with the poor standard of typography and presentation detract from its merits. It is hoped that the 3rd edition of this manual will be written in a style more acceptable to a worldwide market.

R. Macrae

Food, People and Nutrition By Eleanor F. Eckstein.
Westport, Connecticut: The AVI Publishing Co. Inc., 1980. Pp. xii + 624.
ISBN 0 87055 355 0. $20.00 (paper).

The first points that strike the reader ploughing through this lengthy volume of 624 pages are the wide range of topics, the complexity of the issues and the underlying ecological theme which encompasses the interactions of food, people and nutrition. Although the book reflects 'nutrition experience' in the U.S.A., there is a wealth of relevant factual information which makes the volume useful to nutrition and food scientists, dieticians, nutrition counsellors, food marketers, students and professionals alike.

The author has successfully tackled the enormous job of combining the fundamental principles of nutritional science with the psychological, sociological, economic and political factors which determine food choice.

Nutritional science is particularly prone to misinterpretation, and facts are frequently reported out of context, sensationalized and used by the unscrupulous for gain. In this book, the author has taken on her shoulders the burden of analysing the plethora of fact and fiction, and presented to the reader her synthesis of sound scientific reasoning and common sense. She has taken a cautious approach to controversial points and, in most cases, avoided oversimplistic interpretations of complex issues, e.g., the relationship between diet and heart disease. Throughout the book the text is designed to assist the reader and focus attention on areas where interpretations of the available scientific data is particularly difficult.

The book is made up of six units comprising 33 chapters. Unit I—'Nutrient Supply and Demand Status' summarizes (1) the relationship between food consumption and life expectancy—a theme which is emphasized throughout the volume, (2) foods as sources of nutrients, and (3) the control of nutrient supply and demand from a physiological point of view. Unit II—'Nutritional Status: Effects on cells and specialized organs'—is an update of the more established and enduring tenets of known nutritional science and covers the role of energy and of macro- and micronutrients in health maintenance. Unit III— 'Food Ecology—Man's relations to the Food Environment'— provides the reader with a basic guide for answering the questions 'Why do people eat or not eat X?', 'What is food?' and 'What is poison?' This substantial section gives extensive coverage to man's emotional and psychological responses to food, a broader definition of the economic and political importance of food ('food is power') and

the aesthetic value of foods based on the psychological evaluation of the physical properties of foods. Unit IV—'Dietary Assessment'—includes excellent chapters on the uses and limitations of recommended allowances, nutrition data collection, diet recording, questionnaire formulation and food composition studies. Useful references abound. Unit V—'Nutritional Risk and Life Expectancy'—describes how poor nutrition at any stage of development can be a threat of life expectancy. Individual chapters cover nutrition during pregnancy, infancy, childhood, adolescence, young adulthood, middle age and old age. Unit VI—'Contemporary Issues: Food Ecology and Life Expectancy' —reviews the food and nutrition delivery systems—the complex series of events which bring food and nutrition delivery systems—the complex series of events which bring food to the consumer or, as the author puts it, the 'who, what, when, where and how food is served'. The final chapter reminds us that, for centuries, the popular notion was that a good diet was one that was filling. The North American attitude to food today, however, is a further demonstration that with increasing affluence there is a shift of emphasis from the need to satisfy hunger to one in which many human wants and motivations must be fulfilled. Anyone concerned in developing new and novel food products would do well to read this book. The single Appendix consists of the U.S.D.A. Home and Garden Bulletin 72, *Nutritive Value of Foods*, which is reproduced in its entirety as a supplementary reference to this text.

Perhaps the most salient paragraph in the book is on page 425, and it is worth quoting for its common sense: 'It is true that one should eat for health; it is also true that eating is an activity to be enjoyed. So limits must not be set. At the same time, it is inappropriate for normal people who are free from disease to allow negative admonitions to permeate their consciences in such a way as to become the basis for a severely restricted food array. What is needed is the wisdom to select the right amounts of a balanced diet.'

In summary, the volume is comprehensive, up-to-date, interesting and obviously based on the author's experience. Alas, the book could hardly be described as concise. It is long and, in places, verbose. It has much to recommend it, but as an opponent of 'diseased English', of which there are many examples, I am sure the author could have found an easier way of saying: 'Self-actualization is a predominant need in affirming generativity and opposing the tendency towards stagnation.' For those who are sufficiently motivated, this gem can be found on page 527!

D. P. Richardson

Food Control in Action. Ed. by P. O. Dennis, J. R. Blanchfield and A. G. Ward.
Barking, Essex: Applied Science, 1980. Pp. ix + 290. ISBN 0–85334–894–4. £18.00.

The publishers could well have titled this book 'Food for Thought', which its 281 pages certainly provide.

In the opening chapter dealing with the general philosophy of food control, the President of the Institute of Food Science and Technology, J. Ralph Blanchfield, is at pains to point out that food control is an attitude of mind, assisted by the arts and skills of science, tempered by experience. Food control is not a single, but a multiple mix of skill, knowledge and experience which, when adequately blended together, gives product control, and quality assurance to the consumer.

The book incorporates seventeen separate papers, including the discussions following, presented at the Institute of Food Science and Technology Summer Symposium held in July 1979. It provides a comprehensive summary of those factors which must necessarily be considered by anyone wishing to make a thorough study of the process of food control. Divided into four main areas of study, every effort has been made in the book to prevent unnecessary overlap, while preserving sufficient links to show the interaction between them. Separate papers illustrate the science, technology and methods used for food control under the general heading 'The Components', followed by four further papers dealing with constraints applied by legislation, cost, the manufacturing organization and the market itself. Whilst the constraints of cost and market forces are self-evident, the reader is introduced to other aspects which are not immediately apparent.

The overall influence of company manufacturing policy on the food control activity and the increasing need for scientific and technical input at all levels are well covered and serve to introduce the reader to the idea of one area of constraint having an effect on the others.

The paper dealing with the constraint of legislation is of particular value, summarizing the whole spectrum of interrelated legislation as it comes to a focus at plant level, from environmental considerations through the more obvious food standards and labelling legislation to the Health & Safety at Work Act.

The third section concentrates specifically on interactions between raw materials purchasing, the product and the process, quality assurance, production and productivity. Essentially, it deals with the very important issue of interface between the areas of responsibility and the need for a corporate approach to problems. As industries increase in size, areas of responsibility become more fragmented, making communication of critical importance if collective responsibility for the product and the organization as a whole is to be preserved.

This group of papers gives realistic and practical guidance based on experience of how best to achieve these essential objectives. The final paper in this section deals with the interface with the consumer via the distribution chain. The subject is of particular interest to a Public Analyst, highlighting the difficulties faced by the manufacturer in respect of minimum durability of products when distributed through chains of various lengths and suitability, and stresses the importance of adequate communication, and arrangements between manufacturers and distributors if satisfactory consumer links are to be maintained. In the discussion, the point is well made that the label is often the

only direct interface with the consumer. The final session of this symposium consists of four further papers dealing with the practice and application of the food controller's skills to the production of canned products, quick frozen meat and fish products, and chocolate, sugar and cereal confectionery. Some old ground is unavoidably covered in this session, but not tediously so, as the emphasis is directed toward working examples of systems integrating together the component parts of the food controller's skills. There are interesting contrasts of approach, from subjective assessments based on experience to highly organized and technically biased total product assurance systems, each with its role to play in particular industries. It is noteworthy that, regardless of sophistication, the question of adequate communication is still paramount.

In the seventeenth and final chapter, Professor A. G. Ward summarizes his views on the role played by food control now, and suggests future trends in the light of increasing recognition of its importance, and the need for adequate training and qualification of those engaged in it.

In conclusion, I believe the symposium, and through it the publication of this book, has achieved its objective in gathering together the views of many senior representatives of the food industry to produce an authoritative view of the current state of the art of food control. As such, this book must become essential reading, indeed a study guide, for those aiming for the Mastership in Food Control. From a different point of view, the book provides a valuable and interesting insight into the problems faced by the food industry and the wide variety of systems adopted for their control.

The symposium was restricted to the application of food control processes within the industry and it seems a pity that there was insufficient time to incorporate the view of the enforcement side which, in the term used, would represent 'external audit' on behalf of the consumer. Whether the idea of 'self regulation' will ever be achieved remains to be seen. However, as long as the constraints of cost and organizational policy apply, there must be some doubt as to its viability.

A. J. Harrison

Books received

A History of Refrigeration Throughout the World. By Roger Thévenot, translated by J. C. Fidler.
Paris: International Institute of Refrigeration, 1979 (from the original French edition of 1978). FFr.120.

Developments in Meat Science, Vol. 1. Ed. by R. Lawrie.
London: Applied Science, 1980. Pp. xii + 254. ISBN 0 85334 866 9. £17.00.

Economic Microbiology, Vol. 4, Microbial Biomass. Ed. by A. H. Rose.
London: Academic Press, 1979. Pp. xv + 459. ISBN 0 12 596554 0. £27.80.

Statistics and Experimental Design, 2nd edn. By G. M. Clarke.
London: Edward Arnold, 1980. Pp. xii + 188. ISBN 07131 2797 X. £6.50.
An elementary textbook in statistics for biologists, not requiring a knowledge of calculus. Worked examples and exercises are provided throughout the text.

Report of the Government Chemist, 1979.
London: H.M.S.O., 1980. Pp. 197. ISBN 0 11 5 2919 7. £6.50.

Elementary Food Science, 2nd edn. By J T. R. Nickerson and L.J. Ronsivalli.
Westport, Connecticut: AVI, 1980. Pp. x + 44[illegible]. ISBN 0 87055 318 6. $21.00 (paper).
A first textbook in food science, which covers some topics to a level slightly beyond the beginning of the first year of study of the subject at a British University, but deals with other areas (notably those of food chemistry) in a very elementary sub-A level manner.

Commercial Food patents, U.S., 1979. By H. B. North.
Westport, Connecticut: AVI, 1980. Pp. iii + 228 ISBN 0 87055 358 5. $33.00.
This book is designed to provide, under nineteen subject classifications, all of the significant U.S. patents relating to food. The abstracts given are those which appeared in the weekly issues of the official gazette.

UNIVERSITY OF GEZIRA – SUDAN

DEPARTMENT OF FOOD AND SUGAR TECHNOLOGY

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

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

SI UNITS

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

NON SI UNITS

inch	in	= 25·4 mm
foot	ft	= 0·3048 m
square inch	in^2	= 645·16 mm^2
square foot	ft^2	= 0·092903 m^2
cubic inch	in^3	= 1·63871 × 10^4 mm^2
cubic foot	ft^3	= 0·028317 m^3
gallon	gal	= 4·54611
pound	lb	= 0·453592 kg
pound/cubic inch	lb in^{-3}	= 2·76799 × 10^4 kg m^{-3}
dyne		= 10^{-5} N
calorie (15°C)	cal	= 4·1855 J
British Thermal Unit	BTU	= 1055·06 J
Horsepower	HP	= 745·700 W
Fahrenheit	°F	= 9/5 T°C + 32

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

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