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Water binding by potato starch

C. VAN DEN BERG, F. S. KAPER, J. A. G. WELDRING AND I. WOLTERS

Summary

A tentative rough model for water binding in potato starch based on vapour sorption isotherms and calorimetric data is proposed. Heats of immersion and derived thermodynamic quantities are given. A constant differential net heat of sorption for the first 10% of water on dry starch was found. Sorption isotherms for water vapour of native and gelatinized potato starch showed good agreement with the Guggenheim sorption equation up to water activity 0·93 and with the Brunauer Emmet and Teller sorption equation up to water activity 0·3. The water binding mechanism was absorption, without evidence of capillary condensation, up to water activity of 0·95.

Introduction

The hydration of potato starch has been studied more than that of any other starches, not surprisingly because of its wide industrial applications. Potato starch has the highest water-binding capacity of any starch, because it has lowest degree of association between the starch molecules. The hydration of starches has been reviewed by Urquhart (1959), Schierbaum (1960a, b), Schierbaum & Taufel (1962a,b, c, d; 1963a, b, c) and briefly by Badenhuizen (1971).

Because natural starches cannot be brought entirely into crystalline form, it is not yet possible to develop sound molecular models of water bound by the polymer, as has been possible with considerable success for some crystalline proteins, e.g. collagen. Isotherms for water vapour sorption and desorption and calorimetric data still provide the most significant information, on a macroscopic scale, on mechanisms of water binding, as well as being of direct practical significance (Berendsen, 1975).

Several sorption isotherms have been published for potato starch in native and gelatinized form (Schierbaum, 1960a, b; Fish, 1957; Nemitz, 1962; Saravacos & Stinchfield, 1965; Morsi, Sterling & Volman, 1967; Chilton & Collison, 1974; Rakoski, 1911). Unfortunately, not all of these can be directly compared, mainly because of the different preliminary treatments. Drying at high temperatures, for instance, is known

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to reduce the water-binding capacity of starches (Sair & Fetzer, 1944; Schierbaum, 1960b).

Available data concerning the heats of sorption of starches have been thoroughly reviewed by Schierbaum & Taufel (1962a, b). Since then values for the heats of dehydration (desorption) of starches have been reported (Collison & Dickson, 1971; Oleneva *et al.*, 1973).

Schierbaum & Taufel (1963c) conclude at the end of a series of ten papers about starch hydration that potato starch binds the first 25% of water (mass/mass on a dry basis) by 'adsorption' and further water up to about 45–50% by 'capillary condensation'. At higher moisture contents, according to these authors, water is virtually 'free', that is it has no net heat of sorption.

Hellman & Melvin (1950) concluded that starch grains are not porous to nitrogen, although the grains are admittedly much more accessible to water. Nitrogen-specific surface areas of less than 1% of the specific surface areas for water vapour were reported. The same behaviour is widely known for other food materials (Labuza, 1968). Therefore it would be better to talk here of *ab*sorption (a volume phenomenon) rather than *ad*sorption (a surface phenomenon).

The word 'sorption', which has become popular in the food literature during the last two decades, does not distinguish between the two phenomena as such. Although much work has already been done, we hope that the results presented here will help to elucidate this problem area.

We here report part of our research into water binding by starch including results on:

water vapour sorption and desorption isotherms;

heat of immersion of native potato starch;

the influence of gelatinization followed by freeze drying on the isotherms.

The results are examined for fit with some of the more important current adsorption theories—the BET (Brunauer, Emmett & Teller, 1938) and the Guggenheim (1966) models of adsorption and pore structure analysis by the *t* method (Hagymassy, Brunauer & Mikhail, 1969). Finally, a tentative model is proposed for water binding by potato starch which, we think, conflicts with current ideas. The procedure of gelatinization followed by freeze drying (GF) was chosen because it ruptures the native starch structure completely. Also the effect may be compared with that of typical processing steps for starch-containing foodstuffs.

Materials and methods

Starch

Potato starch (analytical grade; ash 0·3%, moisture 19% on d.m.), obtained from BDH (London), has been used directly and after gelatinization followed by freeze drying. The starch was gelatinized by a standard procedure—heating of *c.* 100 g of 5% starch suspension in a water bath at 100°C with continuous stirring.

After setting, the gel was left for 10 min in the water bath, after which it was poured

into a Petri dish (layer thickness 6 mm), cooled, frozen at $-15°C$ and freeze dried for *c.* 18 hr at an absolute pressure of $1{\cdot}3\ N/m^2$ and a heating plate temperature of 50°C. After this treatment the GF sample still had roughly the original volume of the gel.

Sorption apparatus

Sorption isotherms for water vapour were measured with calibrated McBain Bakr balances, equipped with quartz springs (Quartz & Silice, Paris; sensitivity 1 mg per millimetre of extension). The sorption chambers (Fig. 1), kept at a constant temperature of $20 \pm 0{\cdot}05°C$, were equipped with a radiation shield of aluminium foil.

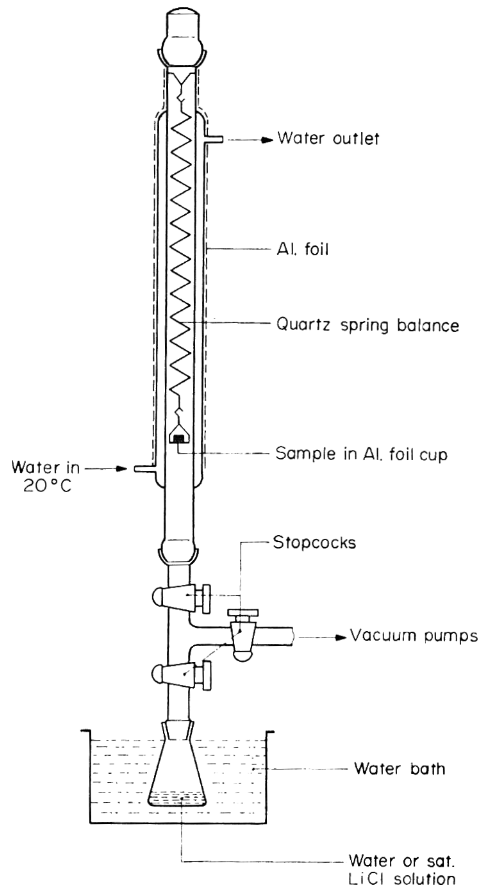

Fig. 1. Schematic diagram of glass apparatus showing the McBain Bakr sorption balance suspended in vacuum tube with device for establishing water vapour pressure.

Relative humidities were controlled, using saturated lithium chloride solution for measurement up to a relative humidity (r.h.) of 30% and higher with cooled distilled

water. The temperature of these solutions, kept constant within 0·05°C, was measured with copper-constantan thermocouples. The sorption chambers were connected to a manifold and standard equipment used in high-vacuum techniques. Samples were placed in a clean aluminium foil cup; for the gelatinized samples, the cup had to be covered with Teflon foil to avoid sticking of the starch. Sample weights were measured by means of a cathetometer, with accuracy better than $\pm 0{\cdot}045$ mg. The effect of buoyancy is included in this. Control experiments showed that up to 99% r.h. no measurable amount of water was sorbed by the quartz spring and sample cup. The accuracy of r.h. control is almost a linear function of r.h., being better than $\pm 0{\cdot}05\%$ r.h. near 5% r.h. and $\pm 0{\cdot}15\%$ near 100% r.h.

Water vapour tables of Perry (1950) and lithium chloride data of Young (1967) were used. On checking the upper range of r.h. (14–30% r.h.) of Young's data, these were found to be very accurate.

Isotherm measurement

Before sorption, the starch samples (*c.* 100 mg) were dried at 20°C by evacuating for four to five days in the sorption chamber at an absolute pressure of *c.* 2×10^{-4} N/m^2 in the manifold. After three to four days, the mass of the samples was usually constant. The criterion for equilibrium was a measured change of less than 0·02 mg in 24 hr. The dry mass obtained was 0·15% higher than that obtained by a standard drying procedure (International Organisation for Standardisation, 1968). In the isotherms, no corrections were made for this. After the initial conditioning, water vapour was admitted with care into the sorption chamber and the equilibrium water content of the sample was estimated. Generally an equilibrium time of 24 hr was found to meet the criterion. The GF samples usually required a somewhat longer time for desorption. The samples went through a complete sorption-desorption cycle using small increments or decrements in r.h. of *c.* 2–12%, depending on the curvature of the isotherm. Afterwards, the same dry sample mass was found. For the GF samples, a double sorption–desorption cycle (GF1 and GF2) was completed.

Calorimeter

To estimate heats of immersion, a Tronac Adiabatic Calorimeter, Model 450–2, was used. This equipment consists essentially of a 50-ml Dewar vessel with distilled water, containing a thermistor and a stirrer equipped with an ampoule holder and breaker. The vessel was completely immersed in a water bath kept at $20 \pm 0{\cdot}001$°C.

The content of the ampoule, being 0·5–1 g of native starch, was completely mixed with an excess of water after breaking the ampoule. The heat was released within 5–30 sec. The reading was calibrated electrically. The standard deviation of ten measurements was $+0{\cdot}13$ J/g dry starch. Not incorporated in this deviation is a possible error for conditioning.

Heats of immersion

Native potato starch of different water contents obtained by sorption and desorption was examined. The samples in open ampoules were dried before conditioning for six to seven days under *c.* 2×10^{-4} N/m^2 closed and weighed to obtain the dry mass. Then the sorption samples were brought directly to the desired r.h. The desorption samples were placed over distilled water for seven days and then exposed to the desired r.h. All conditioning was performed under vacuum in desiccators with saturated salt or sulphuric acid solutions (data from Young, 1967; International Critical Tables, 1928; Gal, 1967). The equilibration time allowed was at least seven days. This conditioning resulted in samples comparable to those used for the preparation of sorption isotherms.

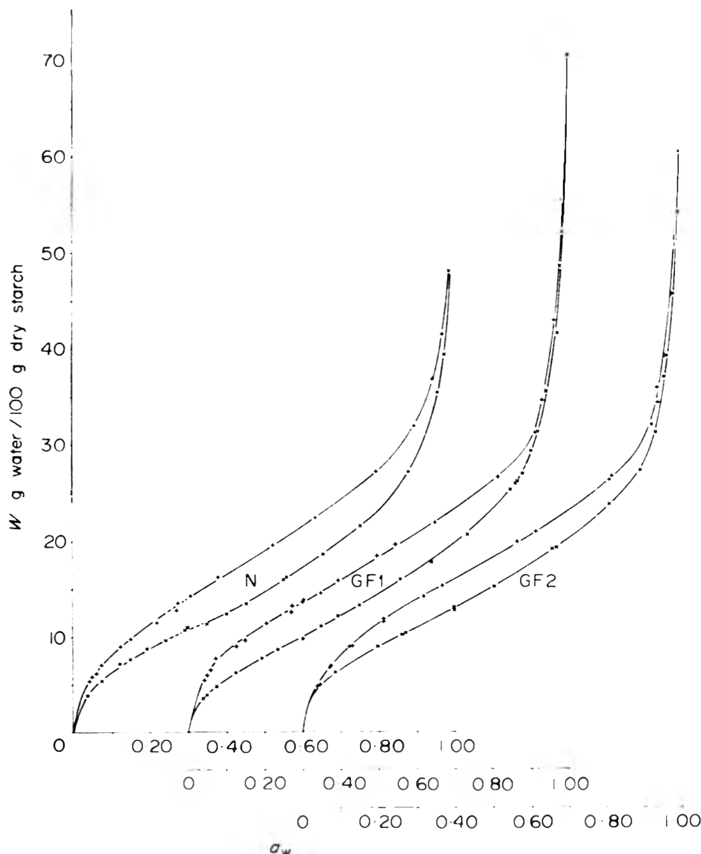

FIG. 2. Sorption (×) and desorption (+) isotherms of potato starch at 20°C. N, native; GF1, gelatinized and freeze dried, first cycle; GF2, gelatinized and freeze dried, second cycle; *W*, mass quotient of water to dry starch; a_w, water activity.

Results

Isotherms

The sorption and desorption isotherms for water vapour with potato starch, native (N) and gelatinized, freeze dried (GF1 and GF2) are given in Fig. 2. The actual experimental values are available on request from the authors.

GF samples of potato starch showed reproducible isotherms only after the macrostructure had collapsed. During the first sorption, the GF sample showed a rather sudden strong shrinkage at a water activity (a_w) of 0·91, the volume falling to about 40%. Above this a_w, the sample needed more time to attain sorption equilibrium. Other starches (wheat, tapioca and rice) behaved similarly. Figure 2 shows that the sorption curve for the native starch and the desorption curves (native and GF1) are not smooth near $a_w = 0{\cdot}3$ (i.e. the first derivative of the $W - a_w$ plot is discontinuous. These deviations were found to be reproducible and are definitely not due to experimental error.

Sorption models

If the sorption process occurs at active sites distributed throughout the material (three-dimensional surface) instead of at surface sites only, the well known adsorption equation of Brunauer *et al.* (1938) (BET) may still be applied, provided that volume statistics are assumed instead of surface statistics. The derivation of the equation via statistical mechanics remains identical (Cassie, 1945; Hill, 1960).

The same holds for the Guggenheim adsorption equation (Berendsen, 1975), which was formulated originally by Anderson (1946) as a modified BET equation with a third parameter k, theoretically justified by Guggenheim (1966):

$$V/V_m = Cka_w/(1 - ka_w)\{1 + (C - 1)\, ka_w\}$$

The meaning of the symbols is similar to that in the BET model. As adapted here, they are as follows:

V = amount of absorbed vapour per gram sorbent, at equilibrium (moisture W);
$V_m = V$, when all active sites bear one molecule of water;
$a_w = P_w/p_{0,w}$ = water activity;
C = absorption coefficient, equal to exp. $([H_1 - H_m]/RT)$ multiplied by a constant, which is usually set at 1;
$H_1 - H_m$ = difference in heats of sorption between the first and following molecules, respectively. These following molecules are assumed to be the bulk liquid in the BET theory (i.e. $H_m = H_{\text{liquid water}}$);
k = a factor correcting for the different sorbate structure as compared to bulk liquid.

In applying these equations, it should be remembered that the assumptions of these theories are not entirely valid for all swelling biopolymers. The BET theory in particular assumes a highly idealized model of sorption.

Table 1 gives the results of comparison of the isotherms with the BET equation, according to the least-squares method. The relative root mean squares (RMS) give an objective indication of agreement in percentage deviation. The smallest RMS determined the valid a_w region.

TABLE 1. Results of isotherm analysis by the BET equation (for meaning see text)

	Valid a_w region	Number of measuring points	C	V_m	V_m expressed as part of monohydrate	RMS (%)
Sorption						
Native	0–0·30	7	19·69	8·51	0·77	0·94
GF1	0–0·45	10	18·22	7·77	0·70	1·90
GF2	0–0·39	4	22·09	8·40	0·76	0·89
Desorption						
Native	0–0·27	6	20·06	10·63	0·96	0·42
GF1	0–0·30	9	21·01	10·55	0·95	1·34
GF2	0–0·32	5	15·85	11·31	1·02	2·25

Table 2 gives the same information for the Guggenheim equation. The delimitation of the applicable a_w range was determined by a RMS better than 4%. Above a_w 0·95, the RMS increases sharply. The GF1 sorption isotherm shows a somewhat different behaviour with RMS slightly above 4 in the intermediate region.

TABLE 2. Results of isotherm analysis by the Guggenheim equation (for meaning see text)

	Valid a_w region	Number of measuring points	C	k	V_m	V_m expressed as part of monohydrate	RMS (%)
Sorption							
Native	0–0·96	17	20·37	0·761	9·64	0·87	2·23
GF1	0–0·94	21	18·13	0·782	8·99	0·81	3·81
GF2	0–0·92	10	21·03	0·755	9·71	0·87	3·69
Desorption							
Native	0–0·94	14	17·31	0·672	13·67	1·23	3·16
GF1	0–0·93	17	19·03	0·662	13·27	1·19	2·71
GF2	0–0·81	7	17·37	0·610	14·19	1·28	1·65

The amounts of water bound by the starch, if V is V_m, are 0·7–1 molecule of water per molecule of glucose residue. These values show that this amount cannot be bound by pure 'adsorption', but are still in good agreement with the BET and Guggenheim models of adsorption.

Stability of the starch

In assessing the results it must be borne in mind that estimation of water vapour sorption equilibria of natural biopolymers is difficult, not only because of the tedious nature of the measurements and the observed hysteresis phenomena, but also because of instability of the polymer during water vapour sorption. In addition to changes in the preliminary treatment of the sample, the procedure of isotherm measurement may also have an appreciable influence on sorption equilibrium. For instance, when we measured sorption equilibria over small intervals of a_w, as we did in our isotherm measurements, we obtained values systematically at variance with those found in 'integral' sorption. Here equilibrium was determined in one step starting at a_{w0}. (See Table 3.) A much longer time (*c.* 20 hr) was necessary to attain sorption equilibrium during interval sorption compared with integral sorption (*c.* 5 hr).

TABLE 3. Difference in water-binding capacity through integral and interval sorption in gram water per 100 g dry starch for native potato starch at 20°C

a_w	Interval sorption (read from the isotherm)	Integral sorption
0·2010	8·89	9·24
0·2013	8·90	9·19
0·6510	18·57	19·18
0·6521	18·60	19·27

Pore structure analysis

To examine whether capillary effects are important in the upper part of the isotherm, isotherms were analysed against the t curves for water vapour (Hagymassy *et al.*, 1969). This t method, originally developed by de Boer *et al.* (see de Boer, 1972) for inorganic catalysts, has proved to be a valuable tool in pore structure analysis in general. The method is essentially the comparison of the t plot of the material under investigation with the t plot of a non-porous reference material (t curve). Plots, indicating the average thickness of the sorbed layer as a function of sorbate activity, are calculated from the isotherm. A positive deviation of the obtained t plot from the reference t curve is explained by capillary condensation; a negative deviation is explained by inactivation

of sorption sites, e.g. filling of micropores. The application of the t method here is based on the following assumptions for simplification:

C (BET) may be taken as a standard of equal sorptive behaviour;
non-rigidity of the polymer does not influence the method appreciably;
the method may also be applied for water vapour, being a more complex adsorbate than nitrogen, for which the method originally had been developed;
the method is equally applicable, with the same parameters, for internal and external surfaces.

The results of this isotherm analysis are given in Table 4. For practical reasons, we compared the isotherms and did not make a standard t plot. A difference of less than 5% from the t curves was considered to be insignificant, taking into account errors due to experiment and comparison. For $a_w < 0{\cdot}15$, the method is unreliable (Broekhoff, 1969).

Up to high a_w values, the sorption isotherms of native and GF2 starch fitted well with the t curves. The GF1 isotherm, which cannot be considered reproducible, is influenced by the low V_m (BET) value. After collapse, the isotherm is more similar. The desorption isotherms were consequently influenced by the higher V_m (BET) (Table 1).

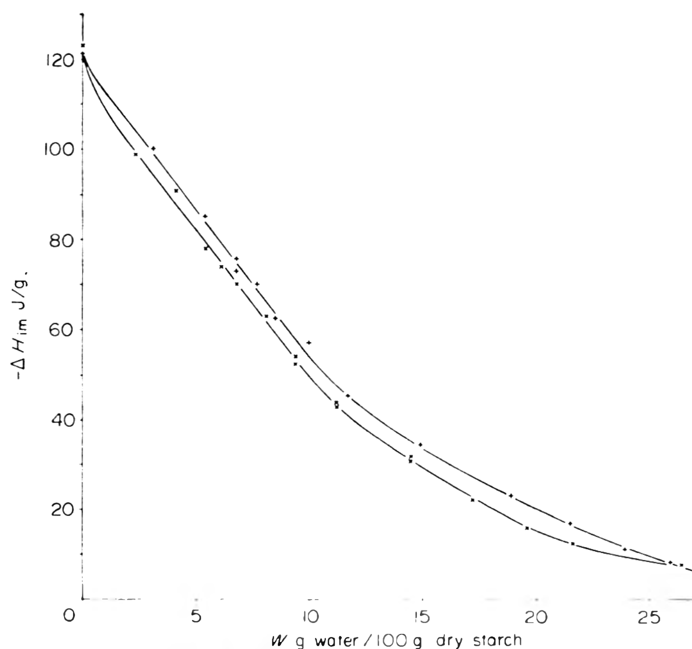

Fig. 3. Heats of immersion (ΔH_{im}) for dry native potato starch as a function of mass quotient of water to dry starch. ×, sorption; +, desorption.

Thermodynamic properties

Figure 3 gives the measured heats of immersion—ΔH_{im} (integral net heat of sorption) —for dry native potato starch. Assuming thermodynamic equilibrium, differential thermodynamic quantities, being the differential net heat (ΔH,) the differential net Gibbs-free energy (ΔG) and the differential net entropy (ΔS) of sorbed water can be derived from the heats of immersion and the vapour sorption isotherm. ΔH was derived from Fig. 3 by graphical differentiation. ΔG is given by the isotherm from:

$$\Delta G = (RT/M)\ln a_w$$

and ΔS is given by the equation:

$$\Delta S = (\Delta H - \Delta G)/T.$$

Figure 4 gives these properties for sorption. The differential net entropy for desorption was slightly lower than for sorption. Values below 2% for water content were considered unreliable.

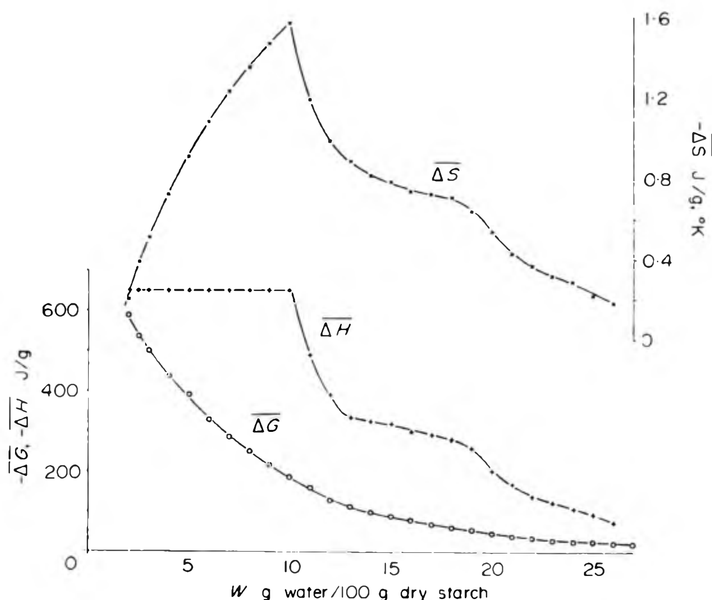

FIG. 4. Differential thermodynamic functions for sorption of water vapour by native potato starch at 20°C as a function of mass quotient of water to dry starch. $\overline{\Delta H}$ (left ordinate), the differential net heat of sorption; $\overline{\Delta G}$ (left ordinate), the differential net Gibbs-free energy of sorption; $\overline{\Delta S}$ (right ordinate), the differential entropy of sorption.

Discussion

Sorption equilibrium and hysteresis

There was a reproducible wide hysteresis loop between the sorption and desorption isotherms. It narrowed progressively at a_w below 0·35. The widest loop was found for

native starch, being 4·5% water on a dry starch basis at a_w 0·5. This width is about twice that reported by Schierbaum (1960a) and similar to that reported by Das, Sethi & Chopra (1972), who found a reproducible loop even during seven successive sorption-desorption cycles on a soluble starch.

A comparable difference between interval and integral sorption has been reported for cellulose derivatives by Beever & Valentine (1958) and for wool by Downes & Mackay (1958). The latter authors could not find this discrepancy during desorption. A possible explanation for this phenomenon could therefore be the 'activation' of sorption sites by the heat developed during sorption when coming from very low moisture contents.

The irregularity in the isotherms observed near a_w 0·3 could indicate a transition in the sorption process. It appears just above the point corresponding to the monohydrate, with a small difference between sorption and desorption.

Gelatinization followed by freeze drying

The influence of gelatinization and freeze-drying on the isotherm is small. Only at a_w above 0·94 is the water-holding capacity of the treated GF starch substantially larger; possibly steric hindrance of the starch grain limits its swelling. On desorption, the native starch holds some extra water between a_w 0·94 and 0·30. Before collapse, the GF1 starch held a little less water than GF2 and native starch. Thus, in general, the water-binding properties of the starch at a_w below 0·94 are hardly influenced by the disorganization of the native starch structure produced by gelatinization.

Capillary condensation

Pore-structure analysis by the t method (Table 4), as well as data from mercury penetration and nitrogen adsorption (unpublished results), show that potato starch is non-porous and therefore capillary condensation cannot play a significant role in water binding below a_w 0·95.

Schierbaum's conclusion about capillary condensation (1960b) is not justified, because it is mainly based on a comparison with the empirical Freundlich equation of adsorption.

Heat of immersion

Our results for the heats of immersion (Fig. 3) agree well with those reported in the literature (Schierbaum & Taufel, 1962b). A small but significant difference is visible between sorption and desorption. The linear part of the graph at low moisture contents is noteworthy, the more so because it implies that the first derivative (the differential net heat of sorption) is a constant. The same appears with the results from Schierbaum & Taufel (1962b) except when plotted with the mass quotient of moisture to dry starch on the abscissa. Collison & Dickson (1971) have also reported constant heats of dehydration for potato starch at lower moisture contents. This observation indicates that about

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TABLE 4. Results of isotherm analysis by the t method; $V/V_m > V/V_m$ Ref. might be an indication for capillary condensation; indicated figures are a_w ranges

	Difference > 5% $V/V_m < V/V_m$ Ref.	Difference < 5% $V/V_m = V/V_m$ Ref.	Difference > 5% $V/V_m > V/V_m$ Ref.
Sorption			
Native	—	0·15–0·99	—
GF1	—	0·15–0·55	0·55–0·85
		0·85–0·90	0·90–0·99
GF2	—	0·15–0·95	0·95–0·99
Desorption			
Native	0·80–0·975	0·15–0·80	0·975–0·99
GF1	0·70–0·975	0·15–0·70	0·975–0·99
GF2	0·60–0·975	0·15–0·60	0·975–0·99

Difference $= (V/V_m - V/V_m \text{ Ref.})/(V/V_m) \times 100\%$.

V/V_m Ref. = reference t curve for water vapour as presented by Hagymassy *et al.* (1969).

the first 10% of water (0·9 monohydrate) is bound by the potato starch in the same manner. In terms of sorption it might indicate also that only one type of active site governs the first part of the sorption process. This is in fact a major assumption of the BET and Guggenheim models of adsorption.

Thermodynamic quantities

Our results for the thermodynamic quantities are of the same order of magnitude as values reported by Morsi *et al.* (1967), which were derived by the Clausius-Clapeyron equation, but they show a different curvature.

Figure 4 indicates that water is bound by native potato starch in two ways up to 19% of water in starch on a dry basis. The water contents at the transitions are slightly lower than those corresponding to one and two molecules of water per glucose residue respectively (11·1 and 22·2 g water per 100 g dry starch).

The strong decrease in entropy (ΔS) suggests that the first molecule especially is highly immobile. This molecule has a constant molar net heat of vapour sorption of 55·8 kJ/mol water. The heat of sublimation of ice is very similar (51·2 kJ/mol), consisting only of the energy of two hydrogen bonds and van der Waals forces. Following the argument of Collison & Dickson (1971), the first water molecules could well be bound to the starch by at least two hydrogen bonds.

Water-binding model for potato starch

A tentative model of water binding might be roughly as follows. The sorption mechanism is almost entirely governed by active sites, the glucose residues of the starch

polymer. These residues bind the first water molecule strongly, presumably by two hydrogen bonds. On progressive sorption, a second water molecule is sorbed near the first one. The binding energy of the second molecule is less than two hydrogen bonds and the decrease in entropy suggests a larger mobility than for the first molecule. By steric hindrance, the starch matrix swells strongly during the uptake of this second molecule (Hellman, Boesch & Melvyn, 1952). Hysteresis is large in this region. Here possibly Urquhart's assumption (1959) is valid that hydrogen bonds between starch chains are broken and starch-water bonds are formed. Hysteresis is explained qualitatively when this happens at different water activities. Additional water molecules are sorbed between the starch molecules, resulting in large conglomerates of water molecules—the swelling or imbibition water.

Conclusions

(1) Water vapour sorption and desorption isotherms of potato starch are well described by the theoretical sorption equations of Brunauer, Emmet & Teller below a_w 0·30 and of Guggenheim below a_w 0·93.
(2) The first 10% of water is bound by a constant differential net heat of sorption, indicating that the water-binding mechanism is probably governed by the glucose residues as active absorption sites.
(3) Capillary condensation does not play any significant role in water binding by potato starch up to a_w 0·95.
(4) Gelatinization followed by freeze-drying results in only minor differences in water-binding behaviour up to a_w 0·94. Above a_w 0·95, the gelatinized samples bind considerably more water.
(5) Preliminary treatment may have an important influence on sorption or desorption equilibria of water vapour.
(6) Sorption isotherms of water vapour by potato starch (native and GF2) are identical with isotherms of adsorption for water vapour by inorganic non-porous materials with similar C (BET) values.
(7) A small difference is observed in water vapour sorption equilibrium between interval and integral sorption.
(8) Small irregularities are observed at a_w 0·3 in both sorption and desorption isotherms of potato starch.

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Microbial growth response to water sorption preparation

K. M. ACOTT AND T. P. LABUZA

Summary

Intermediate moisture food systems were prepared by both an adsorption and desorption method to similar a_ws. Hysteresis occurred above an a_w of 0·86. Four organisms, *Candida cypolytica*, *Pseudomonas fragi*. *Staphylococcus aureus* and *Aspergillus niger*, when inoculated in a pork system, showed more rapid growth in systems prepared by a desorption process than by an adsorption process at the same a_w. In a solid chicken cube system, the same effect was found but the difference was less between the adsorption and the desorption systems. These results indicate that the method of addition of water to a food system as well as the a_w is important in determining the response to growth.

Introduction

The growth requirement of water for a micro-organism has been related to the water activity (a_w) of the system. The work in this area has been recently reviewed by Troller (1973). A micro-organism may be identified by a minimum a_w for growth (Scott, 1957), but this may vary with respect to the solute used to adjust the a_w, the pH and the moisture content of the growth medium (Marshall *et al.*, 1971; Kushner, 1971). This a_w may also vary depending on the state of the water present, which can be affected by the method of preparation of the food substrate.

Food systems prepared to a given a_w by desorption and adsorption processes of water addition have different moisture contents. Desorption–prepared systems have more water at the same a_w as compared to systems prepared by adsorption of water from the vapour state. The hysteresis effect is due to the structural and solubility characteristics of the food system. This hysteresis has been discussed in depth by Labuza (1968) and was shown by Wolf, Walker & Kapsalis (1973) to exist to a large extent. Labuza, Cassil & Sinksey (1972b) and Plitman *et al.* (1971) studied the range of the minimum growth a_ws of micro-organisms in model food systems in which a large hysteresis effect was evident. From those studies, the a_w growth minima for organisms inoculated in adsorption systems were projected to be much higher than for the desorption systems, although

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absolute values were not determined. These works exemplified the fact that the method of preparation can affect microbial growth with respect to water content.

This phenomenon has practical interest related to food processing and also with respect to basic questions about water and its properties and interactions with food. The present study was done to further examine the effect of the preparation method with respect to water addition on the growth response of four organisms, *Pseudomonas fragi*, *Candida cypolytica*, *Staphylococcus aureus* (F265) and *Aspergillus niger* in two intermediate moisture food systems. The systems used were (1) an infusion-soaked, solid chicken cube prepared by a method modified from that described by Hollis *et al.* (1968) and (2) a pork slurry system designed by Labuza *et al.* (1972b). The systems were prepared, inoculated with a known population of test organism, held in storage at 21°C at the a_w of preparation and sampled to measure changes in the viable microbial population.

Materials and methods

Basic food systems

The pork slurry was based on Heinz pork baby food (3·88 g H_2O/g solids) in which a constant amount of glycerol to solids ratio was maintained. Water was added to reach various a_ws for the direct mix (DM) system. Table 1 shows the model system composition. After mixing the direct mix (DM) systems, 5–6 g portions were transferred to sterile 2 oz screw-cap jars. Half of these were frozen at −20°C and then freeze-dried at 75°F, 200 μ Hg for 18 hr. No significant glycerol loss occurred under these conditions as determined by GLC. The dry samples were then rehumidified by adding an amount of water which was 5% less than that needed to reach the desired a_ws. The jars were

TABLE 1. Pork system composition

A_w system*	Amount (g)			Moisture content g H_2O/g solids	
	Pork	Glycerol	H_2O added	DM†	FDR‡
0·67	5	7	0·0	0·50	0·42
0·71	5	7	0·4	0·58	0·46
0·81	5	7	1·4	1·01	0·87
0·86	5	7	3·0	1·39	1·11
0·92	5	7	7·0	2·28	1·27
0·97	5	7	12·0	5·40	4·90

* As measured after four weeks' storage.
† DM (direct mix) desorption system.
‡ FDR (freeze-dried rehumidified) adsorption system.

then held in desiccators of appropriate a_w for equilibration before being capped, sealed with tape and put into storage at 21°C with the DM samples. Table 1 shows a significant hysteresis effect with respect to water content. These values were obtained after two weeks' equilibration.

The solid food system was based on infusion-soaked chicken cubes. Sterile, cooked white chicken meat was cut into 1·3-cm cubes using aseptic technique. The chicken was soaked at 21°C for 18 hr in infusion solutions (see Table 2) on a shaker at 100 rpm. The ratio of chicken to soak infusion was 1 : 10 (w/w). The infusion was drained from the chicken. After equilibration for one day in open sterile 2-oz jars at the appropriate a_w, the samples were inoculated with the test organism. The other half was freeze dried under the same conditions as for the pork model system. When dry, the cubes were

TABLE 2. Composition of infusions (%—w/w)

a_w of resulting chicken cubes*	0·75	0·79	0·83	0·86	0·90	0·93	0·97
H_2O	41·8	48·3	54·8	61·8	74·3	78·8	88·6
NaCl	2·3	2·1	1·8	1·6	1·0	0·8	0·4
Chicken bouillon	4·1	3·7	3·2	2·6	1·9	1·8	0·8
Glycerol	51·4	45·6	39·8	33·8	22·6	18·3	10·9

* Measured by VPM.

rehumidified in desiccators over saturated salt solution. These were designated as the adsorption (FDR) systems. Inoculation was done after about two to four weeks' equilibration of the samples in the desiccator. The samples were stored open in the desiccators. The inoculation was kept small enough to prevent any significant change in moisture content. As shown in Fig. 1, after four weeks hysteresis was only present above a_w 0·86.

Inoculation and sampling procedure

The microbial suspensions were prepared in the same way for inoculation into both IMF systems. The bacteria were grown in 100 ml of trypticase soy yeast extract (TSY) broth at 21°C for 18 hr on a shaker. The mould was streaked onto a TSY agar prescription bottle slant with 30 ml of sterile water. The population of each suspension was estimated by measuring the turbidity at 450 nm. Necessary dilutions were made to give the desired initial viable population in the food system.

The microbes were added to the direct mix systems during the mixing stage of the pork model food system, and at the end of the infusion soak in the chicken cube system. All adsorption (FDR) systems were inoculated via part of the water in the partial rehumidification of the dried samples to prevent any death that would have occurred in

freeze drying. Specifically, they were inoculated by dropping 0·03 g of a suspension of the organism on to 5·0 g of the sample. To prevent possible loss of moisture during storage, the samples were then stored in the desiccators. The systems were inoculated to an initial viable population of 10^4–10^5 CFU/g IMF system.

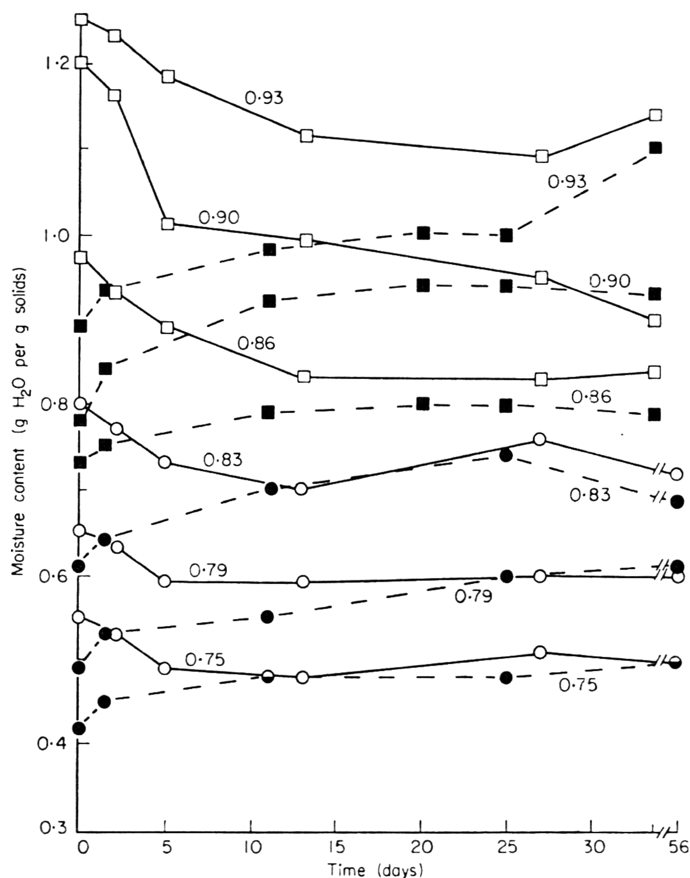

FIG. 1. Effect of storage time on moisture equilibration of chicken cubes at different a_ws at 21°C. – – –, adsorption systems; ———, desorption systems. □, DM; ■, FDR.

Sampling was done by either diluting 1 g of pork model system with 9 ml of phosphate buffer (0·125%) or by blending 5 g of chicken with 45 ml sterile phosphate buffer. Further dilutions were made in phosphate buffer and duplicate 0·1 ml aliquots were spread on TSY agar plates. The plates were incubated at a temperature near optimum for each micro-organism. The same procedure was used for the mould since it is impossible to separate the mycelial mass from the solid food. If growth occurs, an increase in colony forming units will also occur.

a_w—*moisture content measurement*

The water activity of the samples were measured after equilibration and during storage by the vapour pressure manometer technique of Taylor (1961). In this method the actual vapour pressure above a sample is measured in a vacuum system and is accurate to $\pm 0{\cdot}01$ a_w. The moisture content was measured by extracting a 5-g sample with 80 ml of anhydrous methanol and injecting a 50 μl aliquot into a gas-liquid chromatograph equipped with a $\frac{1}{4}$-in diameter × 1 ft Poropak Q column (Waters Associates, Framington, Mass.) and a thermal conductivity detector. It was found that a_w did not significantly change during storage ($\pm 0{\cdot}01$) and that moisture equilibration took over two weeks as shown in Fig. 1. It should be noted that the difference in water content for the chicken cubes is very small after thirty days so that hysteresis is at a minimum, except above a_w 0·86.

Results and discussion

The study of the effect of method of addition of water on growth response in the IMF pork model system was done basically to expand the work reported by Labuza *et al.*

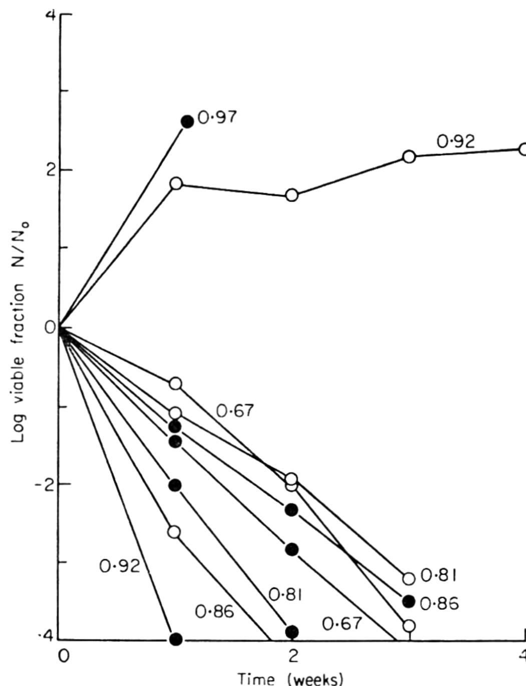

FIG. 2. Log of the viable fraction of *Staphylococcus aureus* at 21°C in a pork slurry model system as a function of time at different a_ws. DM (○) refers to a desorption prepared system; FDR (●) refers to an adsorption system.

(1972b). The results show the same general effects on the four organisms tested. As an example, Fig. 2 shows the growth response of *S. aureus* in the pork model food system vividly illustrating the effect of method of preparation. The direct mix (DM) system at a_w 0·92 shows growth, while the FDR system at the same a_w shows a rapid loss of

TABLE 3. Growth response of microbes in pork and chicken model systems*

System preparation		*Pseudomonas* sp.			*Candida* sp.			*Staphylococcus* sp.			*Aspergillus* sp.		
		1972**	Present		1972	Present		1972	Present		1972	Present	
			Pork	Chicken		Pork	Chicken		Pork	Chicken		Pork	Chicken
0·97	DM***	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
	FDR****	NT	NT	+	NT	NT	+	NT	+	+	NT	NT	+
0·92–93	DM	NT	–	+	NT	+	+	NT	+	+	NT	+	+
	FDR	NT	–	–	NT	0	+	NT	–	+	NT	+	+
0·90	DM	+	NT	NT	+	NT	NT	+	NT	0	+	NT	NT
	FDR	–	NT	NT	–	NT	NT	–	NT	0	0	NT	NT
0·88	DM	NT	NT	NT	NT	NT	0	NT	NT	0	NT	NT	NT
	FDR	NT	NT	NT	NT	NT	–	NT	NT	–	NT	NT	NT
0·85–86	DM	NT	–	–	NT	0	–	NT	–	–	NT	+	NT
	FDR	NT	–	–	NT	–	–	NT	–	–	NT	–	NT
0·83–84	DM	+	NT	NT	+	NT	NT	+	NT	NT	+	NT	+
	FDR	–	NT	NT	–	NT	NT	–	NT	NT	0	NT	+
0·81–82	DM	NT	–	–	NT	–	–	NT	–	NT	NT	–	+
	FDR	NT	–	–	NT	–	NT	NT	–	NT	NT	–	+
0·79	DM	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	+
	FDR	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	0
0·75	DM	–	NT	–	–	–	–	0	NT	–	+	NT	0
	FDR	–	NT	NT	–	–	NT	–	NT	–	0	NT	–
0·67–68	DM	–	–	NT	–	NT	NT	–	–	NT	0	–	NT
	FDR	–	–	NT	–	NT	NT	–	–	NT	0	–	NT
Duration of study (weeks)		8	2	6	8	5	4	8	4	6	8	8	14

* + indicates at least 1·5 log cycle increase in viable number.
– indicates at least 1·5 log cycle decrease in viable number.
0 indicates no change in viable number within 1·5 log cycle.
NT indicates not tested.
** Labuza *et al.* (1972b).
*** Direct mix.
**** Freeze-dried rehumidified.

viability, confirming the previous work of Labuza *et al.* (1972a, b). It also should be noted that the inactivation rate decreased as the a_w decreased. Below the minimum for growth (0·92) the inactivation rate at a_w 0·86 was faster than at a_w 0·67 for the direct mix system, but not the FDR system. The reason for this reversal is unknown because of a lack of understanding of the inactivation mechanisms involved.

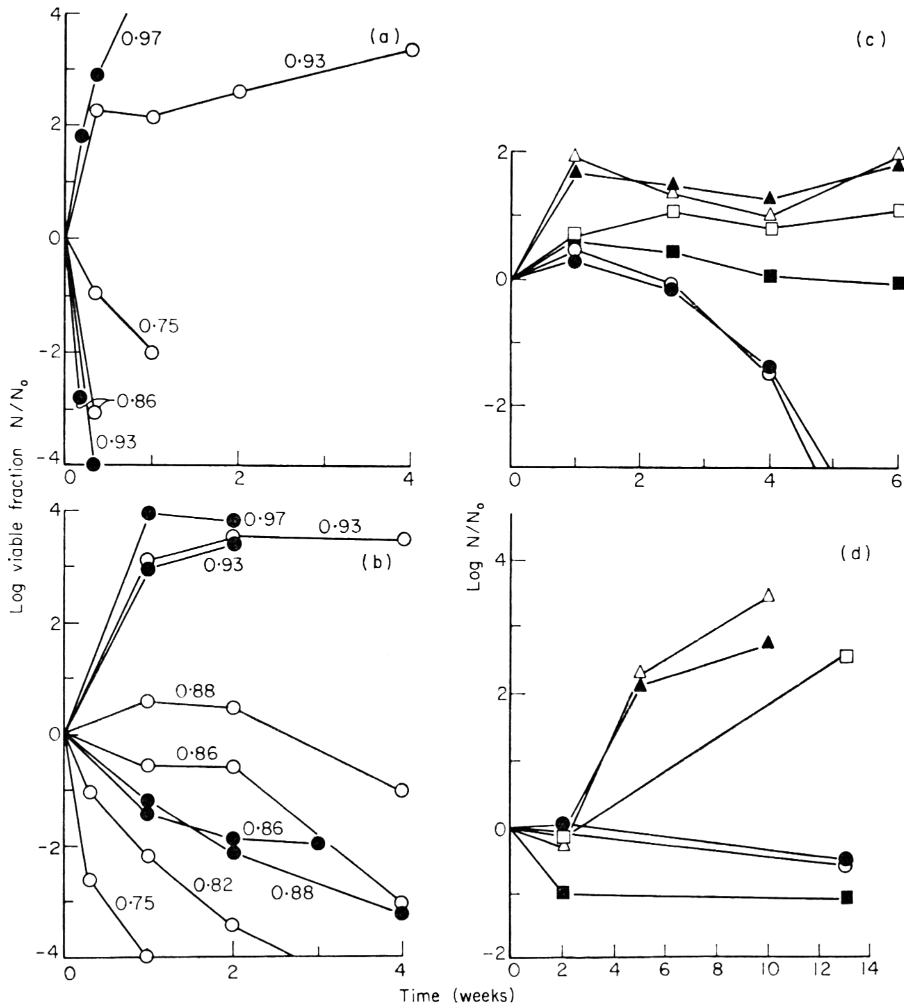

FIG. 3. Log of the viable fraction of the micro-organism at 21°C in a chicken cube IMF system as a function of time at different a_ws. DM refers to a desorption prepared system; FDR refers to an adsorption system. (a) *Pseudomonas fragi*; (b) *Candida cypolytica*; (c) *Staphylococcus aureus*; (d) *Aspergillus niger*. (a) and (b) ○, DM; ●, FDR. (c) A_w: 0·86: DM, ○, FDR, ●; 0·90: DM, □, FDR, ■; 0·93: DM, △, FDR, ▲. (d) A_w: 0·75: DM, ○, FDR, ●; 0·79, DM, □, FDR, ■; 0·83, DM, △, FDR, ▲. (c) and (d), chicken cubes at 23°C.

The overall results for the pork slurry systems are presented in Table 3 and compared to the previous study. In the present study, more glycerol was needed to achieve a comparable a_w because of a difference in the pork composition (i.e. less salts). As shown by Plitman *et al.* (1973) and Acott & Labuza (1975), glycerol itself may have an inhibitory effect on microbes beyond that of lowering the a_w. The high glycerol to solids ratio in this case has resulted in a change in the growth response. As seen in Table 3, for each organism there exists an a_w at which there is a difference in growth response in the pork slurry. The a_w at which this occurs is higher in the present study probably due to the antimicrobial effect of glycerol. Since the systems were equilibrated prior to inoculation for two to four weeks and a hysteresis exists, the growth response difference could be due to the amount of water present.

The chicken cube food system represents a typical IM solid food in which the effect of the addition of water can be studied as it affects microbial behaviour. The results are shown in Fig. 3 and Table 3. *P. fragi*, at a_w 0·93, grew in the DM chicken system but was rapidly inactivated in the FDR system, demonstrating the effect of the water addition method. This species is very sensitive to stress. Leistner (1970) lists the limiting growth a_w as being 0·96, which is higher than found here. This difference may be due to the method of a_w determination employed by the various investigators in the review of Leistner (1970) or to a specific glycerol effect. In any case, the hysteresis effect on microbial growth is evident at a_w 0·93.

The yeast shows a growth minimum close to that in the literature (0·88). The moisture addition effect was not as dramatic as occurred with *Pseudomonas fragi*. The effect on *Staphylococcus aureus* growth response in the solid food was not as distinct as in the liquid food system either. However, the difference is greatest at the a_w which seems to be at the border-line of growth (0·88–0·90). The results for *Aspergillus niger* show a very obvious effect at a_w 0·79. The low frequency of plating for mould was determined by the observation of the visual appearance of the mould on the chicken, which was used as a criteria. These results show that the effect of preparation method on growth response also occurs in a solid food even where hysteresis is much less or non-existent as with the mould at a_w 0·79.

Conclusion

This study was done to determine whether the method of preparation of an IM food with respect to water addition can affect the growth response of micro-organisms. Usually, a food prepared by a desorption technique has a higher moisture content at a given a_w than does one prepared by adsorption methods. In a liquid slurry system studies with four organisms showed that the organisms had a higher a_w requirement for growth in the adsorption system confirming previous work. The same phenomenon, to a lesser degree, occurred in a solid food system which had very little moisture hysteresis. This shows that a_w preparation method is important. In addition, the results also indicate

that the total amount of water as well as the availability as defined by water activity is important for control of biological reactions as has been shown for chemical deteriorative reactions (Labuza *et al.*, 1972a). Thus, one must know something of the history of the system. These results also suggest why literature values of growth limiting a_w are variable. Different nutrient compositions, humectants and preparation techniques are usually used and they are not directly comparable. Future work should suggest whether the mechanism of growth control is involved with membrane transport or with a direct effect on metabolic pathways.

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Selected physical properties of glucose syrup fractions produced by reverse osmosis

I. Specific rotation, average molecular weight, solubility rate

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Summary

A series of glucose syrup fractions in the range 15–67 D.E. were produced by reverse osmosis. Specific rotation, average molecular weight and solubility rate were determined for each fraction. Similarly, these parameters were determined for a series of commercially produced glucose syrups and the data for both series subjected to a statistical analysis to establish any differences between the series for a particular property.

No significant differences were established either for specific rotation or solubility rate but a significant difference was established between the average molecular weight of a fraction and a commercial syrup of the same D.E.

The glucose syrup fractions were later found to contain relatively large amounts of inorganic salts and, after demineralization, specific rotation and average molecular weight were redetermined. A significant difference was established between fractions before and after demineralization for both these properties, thus showing the importance of demineralization when measuring these parameters.

Introduction

For many years glucose syrups have been produced by acid or acid/enzyme hydrolysis of starch. The degree of hydrolysis is measured by the dextrose equivalent (D.E., i.e. reducing power calculated as dextrose and expressed on a dry weight basis) of the final product—the higher the D.E. the greater the hydrolysis.

Acid hydrolysis of starch follows a well defined path and D.E. is related to the quantity and type of sugars present (Palmer, 1970). If an enzyme hydrolysis is used then no such strict relationship is found and by specific enzyme selection a glucose syrup rich in one particular sugar may be produced (e.g. high maltose syrups).

We have recently described a procedure using the principles of reverse osmosis to produce a series of glucose syrup fractions, from 15–67 D.E., in a two-part separation process using a commercially produced 43 D.E. glucose syrup as starting material

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(Birch & Kearsley, 1974). No detailed description of the physical properties of the resulting fractions has yet been reported although the approximate carbohydrate compositions of each are known.

The physical properties of a glucose syrup dictate to a large extent how it will be used commercially and the carbohydrate composition of a glucose syrup is the overriding criterion which governs these properties. Since the carbohydrate composition of a fraction produced by reverse osmosis is different from that of a commercial syrup of the same D.E., it is to be anticipated that its physical properties might also differ. Examples of the carbohydrate composition of fractions and commercial syrups which correspond with these in D.E. are given in Table 1.

TABLE 1. Carbohydrate composition of selected fractions and commercial glucose syrups

	Carbohydrate composition				
Glucose syrup	Mono-saccharides	Di-saccharides	Tri-saccharides	Tetra-saccharides	Higher saccharides
15 D.E. fraction	0·3	1·3	3·5	6·3	89·5
15 D.E. commercial	3·7	4·4	4·4	4·5	83·0
25 D.E. fraction	4·8	7·6	9·5	9·7	68·4
25 D.E. commercial	7·7	7·5	7·2	7·2	70·4
48 D.E. fraction	23·8	19·7	14·1	7·8	34·6
48 D.E. commercial	25·8	16·6	12·9	10·0	34·7
65 D.E. fraction	47·8	22·9	11·9	5·8	11·7
65 D.E. commercial	42·5	20·9	12·7	7·5	16·4

Reverse osmosis therefore appears to offer a means whereby the concentrations of certain oligosaccharides in glucose syrups might be controlled, and hence any corresponding change in physical or chemical properties might be enhanced or prevented.

This report relates to the measurement of selected physical properties of a series of glucose syrup fractions and a series of commercial glucose syrups with a view to establishing any differences between the two by statistical analysis of the resulting data (Acton, 1950).

Materials and methods

Materials

Commercial glucose syrups were obtained from Corn Products Ltd, Manchester in the form of heavy syrups—75–80% solids. These were dried at 65°C under vacuum to constant weight (0% moisture) to produce solid samples.

Glucose syrup fractions produced in a pilot scale reverse osmosis unit supplied by Paterson Candy International using cellulose acetate membranes as previously de-

scribed (Birch & Kearsley, 1974) were obtained in solid form by a similar process, after concentration to 80% solids by evaporation under vacuum.

Methods

Specific rotation was calculated from the optical rotation produced by a 1% solution of the syrup at 22°C using the sodium *D*-line. Optical rotation was measured on a Bendix NPL automatic polarimeter, type 1430, using a 10-mm cell and sucrose as standard.

Average molecular weight was calculated from the depression of freezing point produced by a known weight of solute in a known weight of solvent (Glasstone & Lewis, 1965).

Ash values were determined as residue after ignition of the respective solid (*Standard Analytical Methods of the Member Companies of the Corn Industries Research Foundation*, 1963).

Solubility rate. Brennan & Priestley (1972) used the change in refractive index of a sugar solution, as the sugar dissolved, as a measure of the rate of solubility. This had inherent sampling difficulties as such for our application, but by taking the principle further, a suitable method was devised based on the fact that optical rotation increases as concentration increases for the same sugar sample. The basic apparatus is shown diagrammatically in Fig. 1.

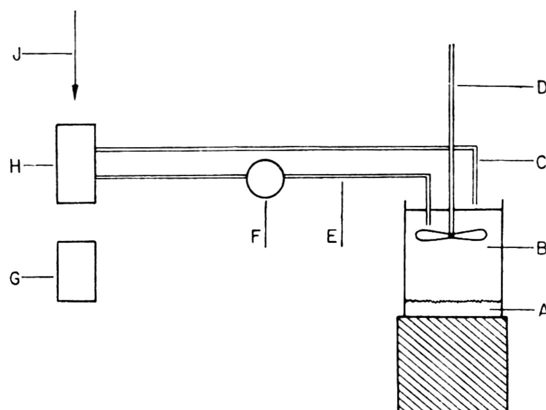

Fig. 1. Apparatus for solubility determination. A, Glucose syrup solids; B, distilled water; C, return pipe from flow-cell; D, constant speed stirrer; E, feed pipe to flow-cell; F, peristaltic pump; G, detector to chart recorder; H, automatic polarimeter flow-cell; J, plane polarized light.

A sample of each glucose syrup solid was comminuted in a zero humidity chamber and the part of this sample which passed through a 60-mesh sieve (British Standard 410) but was retained by a 120-mesh sieve was used in the determination. This was to ensure a reasonably constant particle size between 124 and 251 μm.

Twelve grams ($\pm 0{\cdot}1$ g) of each sample was spread evenly over the bottom of a 300-ml cylindrical container and 250 ml distilled water added carefully down the side of the container to ensure minimum disturbance of the solid and thus minimum initial dissolution. In practice, however, as D.E. increased, up to 20% of the solid was dissolved by this addition but, since the rate of dissolution was calculated from the constant rate period of the dissolution curve, this was not considered of significance.

To ensure constant sampling a constant speed stirrer (150 rpm) with feed and return pipes to and from the flow cell of an automatic polarimeter were fixed by clamps to enable the container with water and solids to be raised to the same relative position for each determination. The stirrer speed was set at 150 rpm from preliminary trials since at this speed the dissolved solute and solvent were mixed but no great disturbance of the undissolved solute occurred. A peristaltic pump was fitted in the feed circuit to drive the liquid through the flow cell of the polarimeter and the optical rotation at zero time (stirrer off) determined. After switching the stirrer on, further readings were taken until a constant value was obtained, i.e. solute totally dissolved. The readings were directly proportional to optical rotation and hence concentration, and these readings were thus used directly in the calculation of percentage dissolved at a particular time. For example, the reading after t min was x and the final reading (total dissolution) was y. Thus after t min $x/y \times 100\%$ of the sample had dissolved.

Demineralization of the glucose syrup fractions was accomplished by passing a 15% solution of the glucose syrup through a column of a mixed ion-exchange resin (Zerolit DM-F: BDH Chemicals Ltd). Ash values were determined on these demineralized fractions and the process was considered satisfactory if the ash value was equal to, or less than, 0·05%. Dextrose equivalent was also redetermined to ensure no sugars had been absorbed on to the column. In no case did a significant change in D.E. occur.

Results and discussion

Specific rotation

Duplicate measurements of specific rotation were made for each glucose syrup fraction and each commercial glucose syrup. The results are shown in Fig. 2.

The specific rotation of a sugar is a fundamental characteristic of that sugar and when plane-polarized light is passed through a glucose syrup solution the plane of polarization is rotated as a result of the optical activity of the carbohydrate present. From this figure for optical rotation, the specific rotation ($[\alpha]_D$) of the syrup can be calculated (*Standard Analytical Methods of the Member Companies of the Corn Industries Research Foundation*, 1963). The specific rotation is related to the carbohydrate composition of the glucose syrup and is a guide to the degree of hydrolysis and thus D.E. of that syrup. Whilst specific rotation is related to D.E. it is not constant for a specified D.E. due to different methods of manufacture producing different carbohydrate spectra at the same D.E. It is, however, a quantity which is approximately linearly related to D.E.

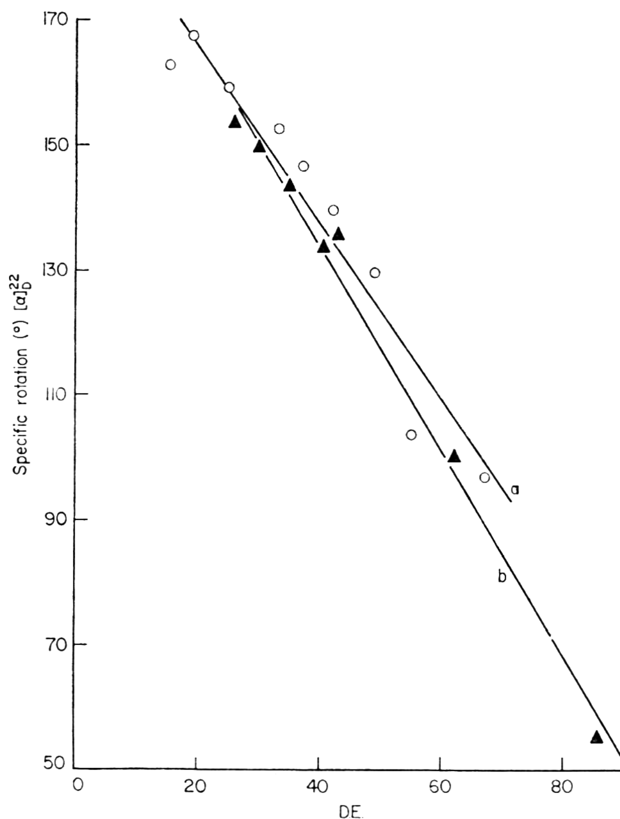

Fig. 2. Specific rotation *v.* D.E. for glucose syrup fractions and commercial glucose syrups. ○ (a), Glucose syrup fractions; ▲ (b), commercial glucose syrups.

and hence an indicator of degree of hydrolysis (Birch, 1968; Birch & Kheiri, 1971).

Since each series of samples was produced by a totally different method, it might be anticipated that the specific rotations of the fractions would be different from the commercial syrups at the same D.E. However, a statistical analysis of the data revealed no difference between the series. This was attributed to the wide spread of points for each series about the best fit regression line of Y on X, since as explained above the relationship between specific rotation and D.E. is only approximate.

Average molecular weight

A linear relationship was found between freezing point depression and D.E. (Fig. 3) for each series of glucose syrups and a log/log relationship between average molecular weight and D.E. (Fig. 4) for each series.

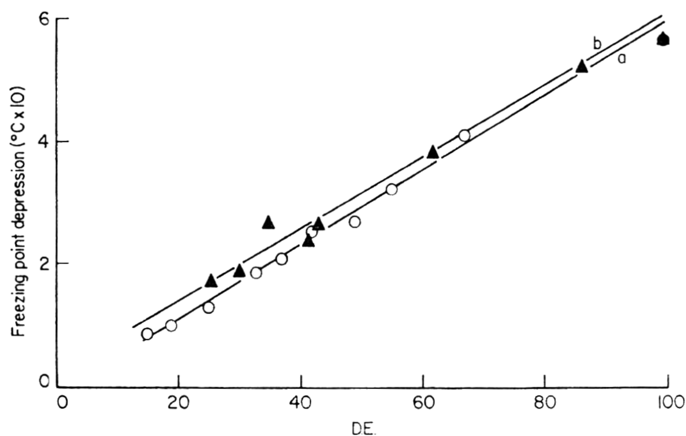

FIG. 3. Freezing point depression *v.* D.E. for glucose syrup fractions and commercial glucose syrups. ○ (a), Glucose syrup fractions; ▲ (b), commercial glucose syrups; ⬤, dextrose.

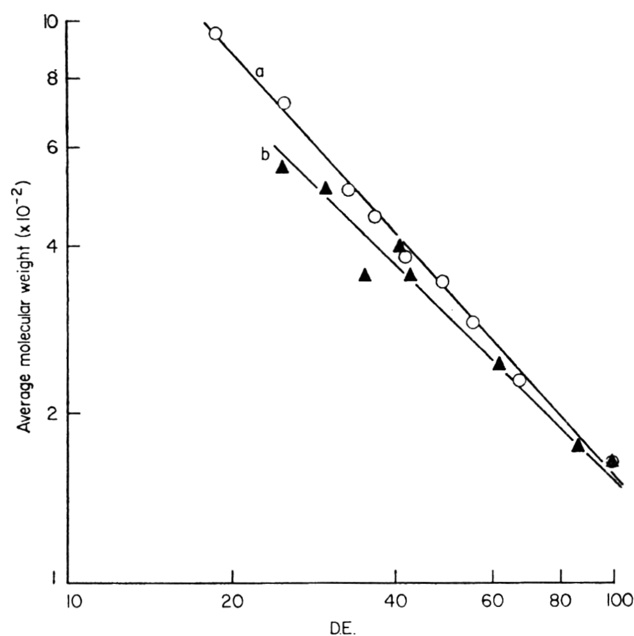

FIG. 4. Average molecular weight *v.* D.E. for glucose syrup fractions and commercial glucose syrups. ○ (a), Glucose syrup fractions; ▲ (b), commercial glucose syrups; ⬤, dextrose.

A statistical analysis of the data shows a significant difference at the 5% level and thus one may conclude that the average molecular weight of a glucose syrup fraction would be greater than a commercial glucose syrup at the same D.E.

The average molecular weight of a glucose syrup is a useful guide to its ingredient value in many food formulations and an indication of its carbohydrate spectrum. It was earlier established that a glucose syrup fraction had a different carbohydrate spectrum from a commercial syrup of the same D.E. (see Table 1). Thus a different average molecular weight would be expected.

Since average molecular weights are different between the series, properties related to molecular weight, e.g. hygroscopicity, may also be expected to differ.

It was noted in the determinations of average molecular weight that the experimental values obtained were substantially lower than theoretical values calculated on the basis of the carbohydrate composition of the sample. This was attributed to the mineral content of the fractions causing larger depressions of freezing point and thus lower molecular weights than would be found for the pure syrup. It was thus considered necessary to determine the mineral content of each syrup by the determination of the ash value (see below).

Solubility rate

A graph of percentage dissolved *v.* time was drawn for each sample, a typical example being shown in Fig. 5.

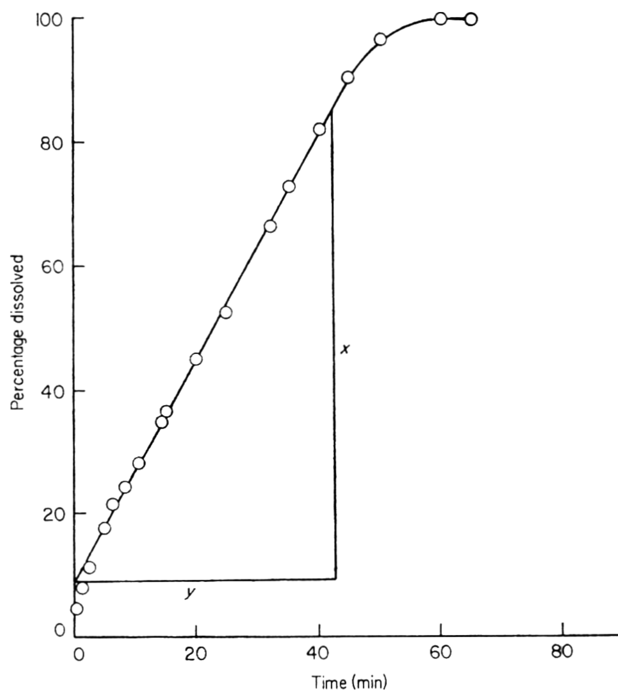

FIG. 5. Percentage dissolved *v.* Time—typical curve (15 D.E.). Rate of dissolution $= x/y = 76 \cdot 5/42 \cdot 5 = 1 \cdot 81\%$/min.

As can be seen there was a finite solubility rate at time zero, followed by a constant rate period and finally a slower rate of dissolution prior to total dissolution. The slope of the line during the constant rate period was calculated and this value related to D.E. for each sample. The results are shown in Table 2, these being the average of duplicate determinations for each sample. It will be appreciated that the higher this value, the faster the rate of dissolution. Dextrose was used as a 100 D.E. sample for reference. The results show a linear relationship between solubility rate and D.E. for each series. However, statistical analysis of the data did not reveal any difference between the solubility rate of a fraction and that of a commercial syrup at the same D.E.

TABLE 2. Solubility rate *v.* D.E.

Glucose syrup fractions		Commercial glucose syrup	
D.E.	Solubility rate	D.E.	Solubility rate
15·1	1·83	25·5	2·28
18·8	2·09	30·0	2·92
25·1	2·66	35·2	3·48
32·8	3·01	41·3	3·60
36·7	2·91	43·0	2·90
41·8	2·77	61·7	4·28
48·8	3·46		
55·2	3·58		
67·2	4·51		
Dextrose	6·18		

Ash values

The data are shown in Fig. 6 for the series of glucose syrup fractions. The ash values for some commercial syrups are also given for reference; the value for the starting material corresponds to the 43 D.E. position.

Commercially produced glucose syrups contain amounts of inorganic salts, principally composed of sodium chloride from the neutralization process after the hydrolysis, and calcium and potassium compounds. Since no neutralizing process or other chemical changes occur in the reverse osmosis fractionation of glucose syrups, the only sources of inorganic salts are from the starting material (commercial 43 D.E. glucose syrup) and from the mains water supply. The mains water supply in the area concerned is very hard and thus contains appreciable amounts of inorganic salts (280–300 ppm $CaCO_3$).

High D.E. fractions of glucose syrup would be expected to contain significantly more inorganic salts than low D.E. fractions since the salts, being generally of a low molecular

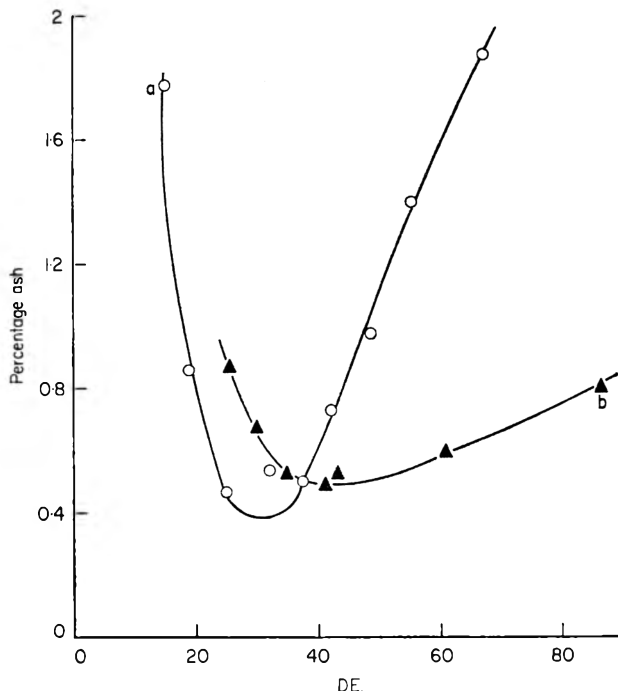

Fig. 6. Percentage ash *v.* D.E. for glucose syrup fractions and commercial glucose syrups. ○ (a), Glucose syrup fractions; ▲ (b), commercial glucose syrups.

weight, would pass through the membrane with the low molecular weight (hence high D.E.) components of the syrup.

The procedure used in the manufacture of these fractions of glucose syrups was such that at about 30 D.E. (the trough of the ash *v.* D.E. curve) water was added to facilitate the removal of the lower molecular weight components (Birch & Kearsley, 1974)—a washing out process—and thus the inorganic salt content was increased due to this addition of hard water. Up to this point the mineral content was decreasing with D.E. as minerals passed through the membrane. A corresponding increase in the ash value of the fractions down to 15 D.E. was shown as more tap water was added. The increase in ash value from 43–67 D.E. was attributed to the salts which were present at the start of the operation passing through the membrane with the low molecular weight components. The rise in ash value between 32 and 15 D.E. could be prevented or reduced by passing the feed water to the reverse osmosis unit through an ion-exchange filter.

Having established the high inorganic salt content in the fractions, they were demineralized to find the effect of the salts on the molecular weight determination since this was considered to be the physical parameter most likely to be affected. Specific rotation was also redetermined to establish any significant changes owing to demineralization.

Average molecular weight: demineralized fractions

The data are given in Table 3, the values for molecular weight being listed before and after demineralization.

Statistical analysis of the data revealed a difference at the 5% level of significance between the glucose syrups plus minerals and the demineralized glucose syrups. This is in fact as expected since in the first instance, i.e. glucose syrup and minerals, the minerals being of low molecular weight would cause a large depression of freezing point. With pure glucose syrup, the average molecular weight would be higher due to no 'small molecules' being present.

TABLE 3. Average molecular weight *v.* D.E. for fractions before and after demineralization

D.E.	Average molecular weight	
	Before demineralization	After demineralization
15·1	1082	1212
18·8	960	1019
25·1	721	760
32·8	506	581
36·7	452	558
41·8	385	479
48·8	346	392
55·2	291	313
67·2	230	244

Therefore for accurate determinations of molecular weight it is essential to demineralize the glucose syrup; otherwise misleading results are obtained.

Specific rotation: demineralized fractions

The data are shown in Table 4, the values for specific rotation being given before and after demineralization.

Statistical analysis of the data revealed a difference at the 5% level of significance between the two series.

This would appear contrary to the fact that inorganic salts are optically inactive and their presence or absence should not affect the optical rotation of a sugar solution containing them. However, their presence would affect the weight of solute used in the determination, i.e. 100 g of 67 D.E. glucose syrup fraction before demineralization would in fact contain only 98·2 g carbohydrate, the rest being minerals. If the results for specific rotation are calculated from the weight of pure carbohydrate before de-

TABLE 4. Specific rotation *v.* D.E. for fractions before and after demineralization

D.E.	Specific rotation ($[\alpha]_D^{22}$)	
	Before demineralization	After demineralization
15·1	162·8	175·0
18·8	168·3	178·2
25·2	159·8	173·5
32·8	152·6	162·5
36·7	146·8	156·7
41·8	139·9	147·3
48·8	130·0	133·8
55·2	105·8	107·2
67·2	97·0	95·5

mineralization, the results do not change significantly from those calculated using the weight of carbohydrate and minerals. Thus it would appear that the minerals in some way change the specific rotation either by inducing a conformational change in individual glucose residues (Angyal & Pickles, 1972) or possibly by forming helical complexes with oligomers. The latter hypothesis appears most likely since the difference is greatest in the low D.E. samples (i.e. higher molecular weight) before and after demineralization.

Acknowledgments

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Selected physical properties of glucose syrup fractions obtained by reverse osmosis

II. Hygroscopicity

M. W. KEARSLEY AND G. G. BIRCH*

Summary

A series of glucose syrups were prepared by the reverse osmosis fractionation of a commercial 43 D.E. glucose syrup and the carbohydrate composition of each determined by a paper chromatography technique. These fractions were dried to zero moisture content and the hygroscopicity of each determined.

The method of drying of the fractions was found to influence the rate of moisture uptake but not the final moisture content in each sample and the presence of inorganic salts in a sample caused an elevation of rate of absorption of moisture and also final moisture content.

An increase in time to reach an equilibrium moisture content was noted as dextrose equivalent increased.

Five commercial glucose syrups were compared with glucose syrup fractions of similar dextrose equivalent, and found to be more hygroscopic in all but one sample.

Samples of dextrose and maltose were also studied and were found to have abnormal moisture absorption curves if not dried correctly.

Introduction

During storage food materials may absorb or desorb moisture and this may be beneficial or harmful according to the use of the material. For example, invert sugar absorbs moisture during storage and can be used in cake icings to extend their freshness. However, the absorption of moisture by hard candies results in a sticky, opaque product and is thus undesirable (Duck & Cross, 1957).

The property of moisture absorption which occurs under normal atmospheric conditions is referred to as hygroscopicity and any dry crystalline solid which is soluble in water and does not form a crystalline hydrate will, when exposed to the atmosphere, tend to absorb moisture with the formation of a saturated solution (Cleland & Fetzer, 1944).

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Moisture absorption has previously been studied for different chemical structures within the carbohydrate field. Thus, Browne (1922) measured the absorption of moisture by starch, cellulose, agar and other carbohydrates whilst Whittier & Gould (1930) obtained data on lactose, sucrose, glucose and galactose. Sokolovsky (1937) determined the effect of humidity on the hygroscopic properties of selected sugars and caramel, and Dittmar (1935) the effect of humidity on sucrose, invert and other sugars. Grover (1949) studied the water vapour pressure of selected carbohydrates in relation to atmospheric water vapour pressure to determine whether foodstuffs containing these carbohydrates would gain or lose moisture under normal atmospheric conditions.

Cleland & Fetzer (1944) obtained absorption and desorption moisture equilibrium data for invert syrup and glycerol and compared the data obtained with that obtained for starch hydrolysates. Mahdi & Hoover (1965) conducted a study of the rate of moisture gain or loss and the equilibrium moisture contents of ten humectants at eleven different temperature and relative humidity storage conditions.

Whilst some of the above papers related to the hygroscopic properties of glucose syrups there is a dearth of information regarding them in general and more information would obviously be technologically desirable concerning these versatile food constituents.

More recently, Donnelly *et al.* (1973) determined the hygroscopicities of a series of D-Glucose polymers and also of several sugar mixtures to correlate the humectant properties of glucose syrups with their carbohydrate composition, whilst Johnson & Srisuthep (1975) determined the hygroscopicities of glucose polymers G_3–G_{10} and correlated this with molecular weight.

Two basic procedures have been used to determine the hygroscopic tendencies of carbohydrates (Donnelly *et al.*, 1973). The first, used by Browne (1922), Dittmar (1935) and Sokolovsky (1937), involved exposing dry samples in weighing bottles to different constant relative humidity environments. By measuring the gain in weight until a constant value was obtained the hygroscopicity of each sample was determined.

The second, used by Whittier & Gould (1930), involved measuring the vapour pressures of saturated equilibrated solutions of sugars at constant temperature and calculating the relative humidity levels corresponding to each pressure. From this the hygroscopic tendency of each sugar was determined.

Cleland & Fetzer (1944) and Mahdi & Hoover (1965) drew attention to the absorption of moisture by glucose syrups in shallow dishes. 'Surface sealing' or 'skin' formation stopped the flow of water vapour to the bulk of the sample and final equilibrium depended on diffusion of the moisture through the sample. In this case, amount of sample and physical condition of the sample became important factors. To avoid misleading results Cleland & Fetzer (1944) dispersed the samples on diatomaceous earth and Mahdi & Hoover (1965) cast thin coatings of the samples on aluminium plates.

Materials and methods

Materials

A series of glucose syrup fractions were prepared by reverse osmosis/ultrafiltration for use in the study (Birch & Kearsley, 1974).

Five commercial glucose syrups from Corn Products Ltd, Manchester were used for comparison purposes.

Analar dextrose and maltose, from British Drug Houses Ltd were used in the work.

Methods

Sample preparation. The commercial syrups were dried under vacuum at 65°C and comminuted to approximately the same sample size in a zero humidity cabinet.

The glucose syrup fractions were concentrated under vacuum and treated similarly. A series of spray dried fractions (15–42 D.E.) was also produced for comparison with the oven-dried samples of the same D.E. A sample of each fraction was also demineralized (Kearsley & Birch, 1975) and included in the study.

Hygroscopicity determinations. All determinations were carried out at 75% relative humidity (R.H.) by placing a saturated solution of sodium chloride plus excess solute in a desiccator and leaving for one week at 27°C to reach equilibrium. Five grams of each sample were weighed into a tared aluminium dish (diameter 3 in, depth 1 in and spread evenly over the bottom. The dish and contents were dried to constant weight at 65°C under vacuum and the weight of sample noted accurately. The dish was then placed in the 75% R.H. atmosphere and weighed every 24 hr for fourteen days by which time constant weight had usually been attained. All measurements were performed in duplicate and each sample was placed in a different desiccator to prevent any sample interaction.

Moisture content or moisture gain of the sample was expressed as:

$$\frac{\text{gain in weight of sample}}{\text{dry weight of sample}} \times 100\%$$

Results and discussion

It was initially intended to spray dry all glucose syrup fractions to produce solid samples but with the higher D.E. fractions the phenomenon of hygroscopicity made this method impractical. It was found that fractions below 43 D.E. could easily be spray-dried but above this, concentration under reduced pressure followed by drying at 65°C in a vacuum oven was necessary.

The carbohydrate composition of a fraction determines its moisture absorption and in general the greater the proportion of low molecular weight components in a fraction the more moisture is absorbed. The carbohydrate compositions of some glucose syrup

TABLE 1. Carbohydrate composition of fractions and final moisture content (after demineralization) after fourteen days

D.E.	Carbohydrate (%)					Final moisture content (%)
	Glucose	Maltose	Maltotriose	Malto-pentaose	Higher saccharides	
15·1	0·3	1·3	3·5	6·3	89·5	14·43
18·8	0·7	3·4	6·0	9·2	80·7	15·06
25·2	4·8	7·6	9·5	9·7	68·4	16·67
32·7	12·8	10·7	10·0	8·0	58·4	19·22
36·7	12·4	14·1	11·9	8·6	53·0	19·57
41·8	15·3	14·6	16·2	11·0	42·9	21·03
43·0*	18·8	15·4	11·1	8·1	47·1	24·18
48·8	23·8	19·7	14·1	7·8	34·6	23·53
55·2	32·8	20·4	13·3	8·7	25·4	26·32
67·2	47·8	22·9	11·9	5·8	11·7	27·68
78·0	61·6	25·4	10·0	1·6	1·4	34·24

* Starting material.

fractions are given in Table 1, with the final moisture content after 14 days' exposure to the 75% R.H. atmosphere.

It was noted above that two methods of drying were used for the fractions and to ensure all samples were alike, all fractions below 43 D.E. were also prepared by oven drying.

To establish any difference in moisture uptake between methods of drying each fraction so prepared was placed in the 75% R.H. atmosphere. A typical result is shown in Fig. 1, which shows the data obtained with a 42 D.E. fraction.

From this it can be seen that the method of drying affects the initial rate of moisture uptake but not the final moisture content of the sample. Spray dried samples have, by comparison with oven-dried samples, very small particle sizes which facilitate moisture uptake whilst the oven-dried samples, with larger particle sizes, are prone to 'surface sealing' and thus slower moisture uptake.

The shape of the moisture absorption curve in general shows an initial rapid uptake of water followed by a 'plateau' or slower rate of absorption, cf. solubility curves in Kearsley & Birch (1975).

It was found that although the higher D.E. fractions absorbed more moisture than the lower D.E. fractions over a fourteen-day period, the low D.E. fractions attained the 'plateau' more quickly. This is shown in Fig. 2 for four fractions.

We have previously noted (Kearsley & Birch, 1975) that the presence of inorganic salts in the glucose syrup fractions affected their physical properties and thus the

hygroscopicity of the demineralized fractions was determined. The results for a 33 D.E. fraction are given in Fig. 3.

The final moisture contents of a series of glucose syrup fractions before and after demineralization are given in Fig. 4.

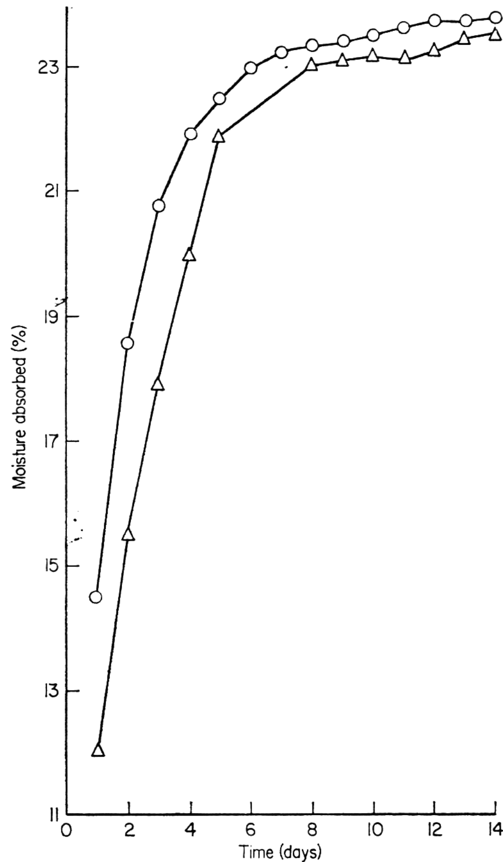

Fig. 1. Moisture absorption curves for spray-dried (○) and oven-dried (△) 42 D.E. glucose syrup fraction.

The presence of inorganic salts causes an increased rate of moisture uptake and also an elevated final moisture content after fourteen days. Inorganic salts have low molecular weights compared with the glucose syrup fractions and since low molecular weight is associated with extreme hygroscopicity in carbohydrates the presence of these salts might be expected to cause increased hygroscopicity. However, it seems more likely that the observation results from some form of interaction between the minerals and the sugars.

Charley *et al.* (1963) described the formation of complexes of sugars with iron and Rendleman (1966) the formation of complexes of sugars with alkali and alkaline earth metal salts. Angyal & Pickles (1972a, b) described the conformational changes in individual glucose residues in polymers induced by minerals, and helical complexes of oligomers and minerals. These helices would be more prevalent in the lower D.E. fractions and the differences in hygroscopicities between fractions with minerals and demineralized fractions is found to be greatest in the lower D.E. fractions. These

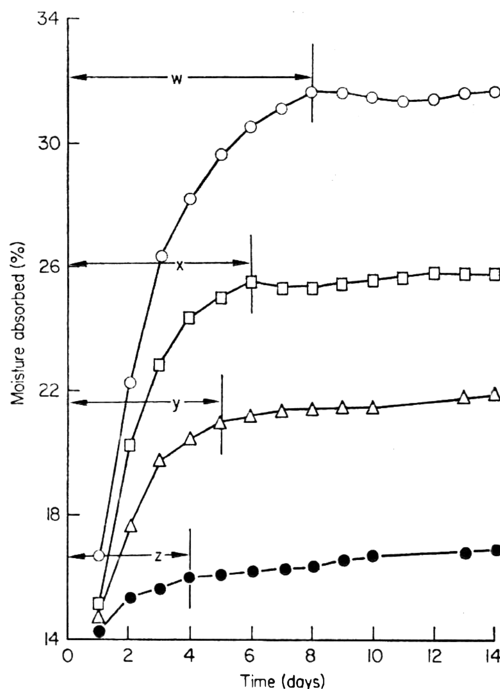

FIG. 2. Moisture absorption curves for 15, 33, 49 and 67 D.E. glucose syrup fractions. ○, 67 D.E.; □, 49 D.E.; △, 33 D.E.; ●, 15 D.E. w, 8 days; ×, 6 days; y, 5 days; z, 4 days.

complexes are already known to affect other physical properties of the sugars, for example, optical rotation (Kearsley & Birch, 1975). The formation of such complexes cannot therefore be ruled out and may well explain the observations in this report.

Five commercial glucose syrups were also used in the experiments for comparison with the fractions. The final moisture contents of these are compared with fractions of a similar D.E. in Table 2.

It is seen that in the first four examples the commercial syrups are more hygroscopic than the corresponding fractions. However, the 86 D.E. commercial syrup had a lower final moisture content than a 15 D.E. fraction which is a contradiction in terms of

earlier findings. The carbohydrate composition of this and the 78 D.E. fraction are given in Table 3.

The obvious differences between the two are in the proportions of mono-, di- and trisaccharides. The 86 D.E. syrup is virtually all monosaccharide and this high proportion could be responsible for its low hygroscopicity.

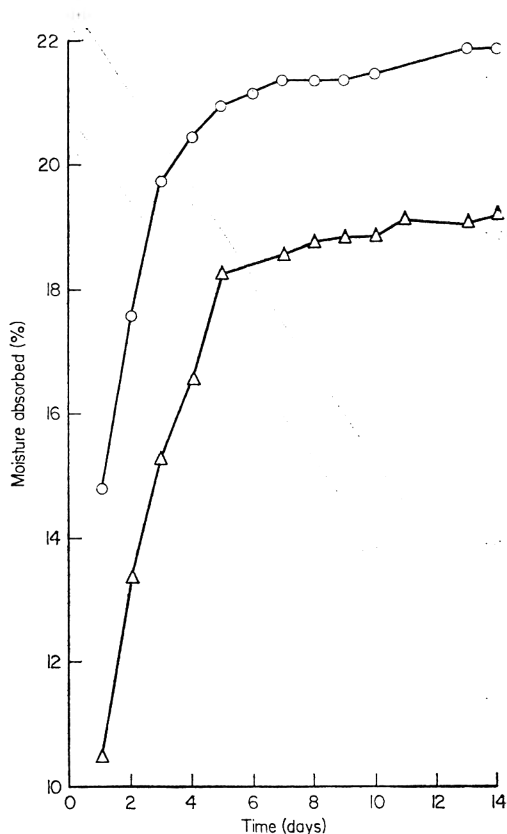

FIG. 3. Moisture absorption curves for 33 D.E. glucose syrup fraction before (○) and after (△) demineralization.

The hygroscopicity of dextrose and maltose were determined under the same conditions as the syrups. The dextrose and maltose were dissolved in water, evaporated to a heavy syrup and oven-dried. The importance of thorough drying of the samples is shown in Fig. 5 where samples of hydrated dextrose and maltose are compared with samples having zero moisture content.

The moisture absorption curves for anhydrous dextrose and maltose follow established patterns with the dextrose reaching its plateau after about twelve days, the time

expected for a high D.E. glucose syrup. Maltose, on the other hand, remained at a very low moisture content (less than 1%). Donnelly *et al.* (1973) reported maltose as having a much lower moisture absorbance than other oligomers agreeing with the above findings. The hydrated samples show a very rapid increase in moisture content and both

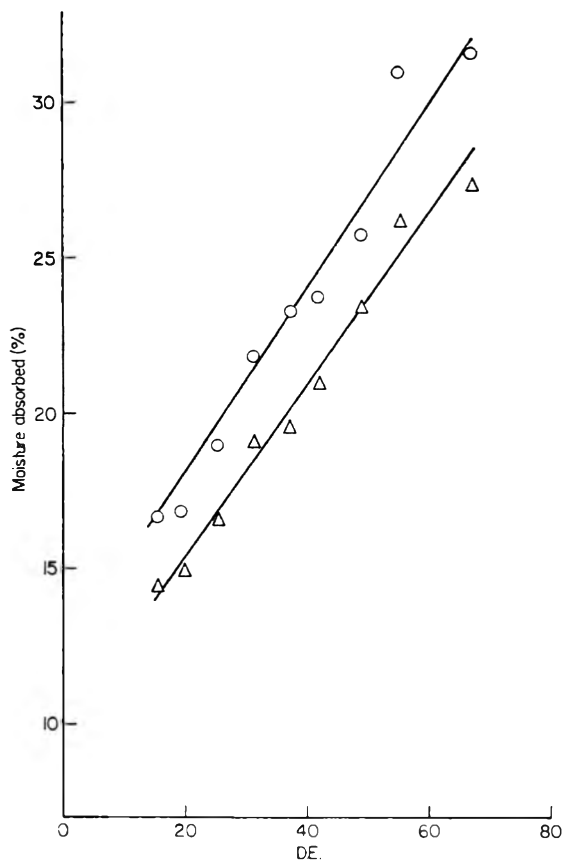

FIG. 4. Moisture absorbed *v.* D.E. for all fractions after fourteen days before (○) and after (△) demineralization.

were liquid after a one-day exposure. After this a crystallizing effect occurred and the moisture content fell until both were again completely solid.

Johnson & Srisuthep (1975) described a similar occurrence with dextrose and maltose at $60 \mp 5\%$ R.H. when both had maximum absorption of moisture after 15 min followed by decreased moisture content after 90 and 270 min. Furthermore, allotropic modifications of sugars in general appear to be well documented (e.g. Birch, 1965).

TABLE 2. Final moisture contents of commercial glucose syrups and glucose syrup fractions

Glucose syrup*	Final moisture content (%)†
25·5 D.E. Commercial	19·58
25·1 D.E. Fraction	16·67
35·2 D.E. Commercial	21·36
36·7 D.E. Fraction	19·58
43·0 D.E. Commercial	24·18
48·8 D.E. Fraction	23·53
61·7 D.E. Commercial	30·63
67·2 D.E. Fraction	27·68
86·4 D.E. Commercial	13·14
78·0 D.E. Fraction	34·24

* All samples demineralized.
† After fourteen days.

Since dextrose represents 100 D.E., the ultimate step in the hydrolysis of starch one would expect that, if the hygroscopicity increases as D.E. increases then dextrose itself (100 D.E.) would have a moisture content in excess of 30%. In reality it is less than 10% and like the 86 D.E. commercial syrup a contradiction to the rule. The 86 D.E. syrup contained 83% dextrose and it seems likely that dextrose is responsible for this behaviour. Dextrose is chemically the one anomalous member of the carbohydrate spectrum of glucose syrups, being devoid of a glycosidic linkage. However, whether or not this constitutes a valid explanation of the anomalous results obtained with dextrose must await further studies with chemically defined glycosides.

Thus it would appear that up to a point hygroscopicity increases as D.E. increases (about 80 D.E.) but above this the final moisture content of the sample falls rapidly.

As well as the final moisture content of the sample, the physical condition of the sample is important.

TABLE 3. Carbohydrate composition of 86·4 D.E. commercial glucose syrup and 78·0 D.E. glucose syrup fraction

Carbohydrate %	Glucose syrup	
	78 D.E. fraction	86 D.E. commercial
Glucose	61·6	82·6
Maltose	25·4	1·7
Maltotriose	10·0	1·1
Maltopentaose	1·6	1·8
Higher saccharides	1·4	12·8

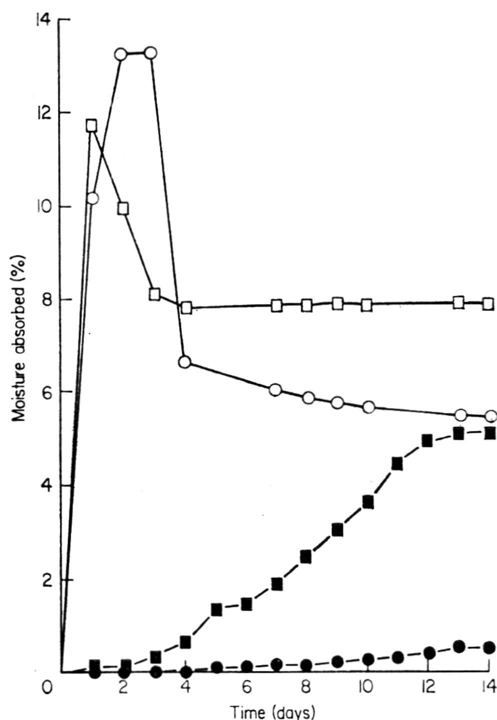

FIG. 5. Moisture absorption curves for dextrose and maltose; hydrated and anhydrous. □, Hydrated dextrose; ■, anhydrous dextrose; ○, hydrated maltose; ●, anhydrous maltose.

Up to 30 D.E. after fourteen days' exposure at 75% R.H. the samples were like hard, sticky, opaque candies. Above 30 D.E. all were liquid up to 78 D.E., the higher the D.E. the less viscous the end product. The 86 D.E. syrup, dextrose and maltose were hard, non-crystalline and opaque.

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An investigation of the bacteriological quality of bakery cream

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Summary

Samples of the cream that was supplied to four Ayrshire bakeries, the whipped cream from the mixing bowl and from the savoy bag were examined for bacteriological content. Poor bacteriological quality of the cream supplied to the bakery was the main cause of high mesophilic and psychrotrophic colony counts in the final product. Contamination was shown to occur, in some cases, in the mixing bowl and the savoy bag. Of the tests examined in this work, the proteolytic count of the water agar test was found to be the most useful indicator of bacteriological quality at different stages of production. A bacteriological standard is proposed for cream and whipped cream.

Introduction

In Great Britain, there is no statutory bacteriological standard for any designated creams, whether heat-treated or raw. Public Health Laboratory Service working parties have recommended the methylene blue reduction test as an advisory test for cream although they recognized that this test gave anomalous results (Report, 1958, 1970). Seiler (1971) has emphasized the necessity for using strict hygiene and refrigeration in the preparation of products that contain cream, but over the last twenty years, the bacteriological quality of whipped cream has been ignored except in Italy and Sweden. D'Arca Simonetti *et al.* (1968) found an improvement in the hygiene quality of the cream supplied to bakeries over a five-year period. However, these and other workers agreed that a high percentage of the whipped cream samples that they examined were unfit for human consumption (Fabio & Quaglio, 1963; Quesada & Soldati, 1969; Eng-Törnquist, 1969; Eng-Törnquist, Nordenskiöld & Åkerstrand, 1971). The following work is the result of a survey of the bacteriological quality of cream and whipped cream obtained at four bakeries over a twelve-month period. The object was to establish whether a bacteriological standard could be proposed for the cream in freshly produced products.

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Methods

Samples

Samples were obtained from bakeries A and B on nine occasions and from bakeries C and D on ten occasions over three consecutive four-monthly periods: 1, May–August; 2, September–December; 3, January–April. The bakeries obtained cream from three creameries P, Q and R. Creamery Q supplied ultra-high heat treated cream (UHT). Creamery P supplied cream with 48% fat while creamery R supplied cream at both 40% and 48% fat.

The samples were obtained using sterile spatulas, and were taken (i) from the bulk cream that was in current use, (ii) from the mixing bowl and (iii) from the nozzle of the savoy bag. Bacteriological examinations were made within an hour of the sample being taken.

In the Tables 2 and 3, the samples are identified by the bakery letter (A, B, C or D), the period when the sample was taken (1, 2 or 3), and the stage at which the sample was taken (i, ii or iii). If, during any one period, two samples from a bakery are considered, they are designated a and b.

Bacteriological methods

British Standard methods (British Standard 4285 : 1968; Supplement No. 1 (1970) to B.S. 4285 : 1968) were used for the colony count, presumptive coliform test, lipolytic and staphylococcal counts, all tests being incubated at 30°C for 72 hr. A confirmatory coliform count was done in violet red-bile agar (VRB) and incubated at 30°C for 24 hr. The water agar test (Taylor, 1971, 1975) was carried out at 30° and also at 5°C in conjunction with a colony count, both of which were counted at seven and fourteen days. Other tests used for cream were the methylene blue and phosphatase tests (Statutory Instrument 1571 : 1963). Since it was not convenient to use the statutory methylene blue test for whipped cream, this test was also done using the 1 : 10 suspension of cream or whipped cream in 2% sodium citrate recommended by the British Standard method for the colony count determination and the 1 : 10 suspension in 10% sterile Oxoid skim milk that was required for the water agar test. All the bacteriological tests were duplicated using sodium citrate solution or skim milk to prepare the first 1 : 10 dilution, with the exception of the water agar test, $\frac{1}{4}$ strength Ringer solution being used to make all subsequent 1 : 10 dilutions.

Results

The results of the samples obtained from the four bakeries showed interesting contrasts, not only in the bacterial quality of the cream and the whipped product but also in relation to the visible condition of the plant (Table 1). At bakery A, the bulk cream was bacteriologically poor, the colony count varying with the time of year. The appearance

TABLE 1. The condition of the plant, source and bacteriological quality of cream supplied and maintenance of hygiene at four bakeries

	Bakery			
	A	B	C	D
Superficial condition of plant	Average	Poor	Average	Very good
Cream source (dairy)	P	Q	R	R
percentage fat	48	23	40	48
colony count				
Period (1) May–August	10^7–10^8	$< 10^3$	10^3–10^5	10^3
(2) September–December	10^5–10^6	$< 10^3$	$< 10^3$†	10^3
(3) January–April	10^3*	$< 10^3$	$< 10^3$†	10^3
Method of whipping	Conventional beater	Conventional wire beater	Conventional beater	Automatic gas
Bacterial contamination during processing	+	−	+	+ or −

* Changed to cream supply R.
† Changed to cream supply Q.

of bakery B was poor, but by using ultra-high heat treated cream (UHT) and strict attention to hygiene, a product was obtained that was nearly bacteriologically sterile. The cream supplied to bakeries C and D came from the same source. Dairy R separated cream at 48% fat content for bakery D and then diluted this cream to 40% fat content with pasteurized milk for bakery C.

Typical mesophilic bacteriological results for samples of bulk and whipped cream are shown in Table 2. Samples A2 i, ii and iii were obtained from bakery A during the latter part of the year when the cream (A2 i) was of rather better bacteriological quality than it had been during the summer. Although the colony count of the cream was about 10^6 colonies/g, all tests, with the exception of the methylene blue test, showed that contamination occurred during the whipping process (A2 ii, iii). The methylene blue reduction is unable to give a worse result than the 0 hr that was obtained with the cream. Even when cream from another source was used, it was still possible to show that the hygienic standard could be improved and that contamination with faecal coliforms occurred during the whipping process (A3 i, ii, iii). The samples collected at bakery B were of a consistently high standard (B2 i, ii, iii). On one occasion there was a suggestion that contamination occurred during whipping, when a colony count of 450 colonies/g was obtained, but no change was found in any other test. During period 1, the cream at bakery C was of poor bacteriological quality (C1 i). This was surprising since the cream was prepared by diluting, with pasteurized milk, cream of 48% fat content that was normally of good bacteriological quality (e.g. D1 i, D3 i). Contamination occurred at

TABLE 2. Specific results after incubation at 30°C for the colony count, presumptive coliform test, coliform count in violet red-bile agar (VRB), lipolytic count, proteolytic count of the water agar test (WA), and methylene blue reduction test (MB) using cream (c), sodium citrate (cit) and skim milk (sm) dilutions for samples of cream (i), whipped cream from the mixing bowl (ii) and savoy bag (iii) from four bakeries A–D

Sample	Colony count	Presumptive coliforms	VRB count	Lipolytic count	WA	MB c	MB cit	MB sm
A2 i	$0{\cdot}96\times10^6$	+1/1000	44 800	24 000	9000	0	>7½	0
ii	$4{\cdot}80\times10^6$	+1/1000	81 000	28 000	56 000		>7½	0
iii	$6{\cdot}30\times10^6$	+1/1000	49 000	114 000	43 000		>7½	0
A3 i	3900	−1/10	<50	200	25	7	>7½	>7½
ii	10 400	+1/10	300	600	204		>7½	7
iii	6600	+1/100	150	500	255		>7½	7
B2 i	<50	−1/10	<50	<50	<5	>7½	>7½	>7½
ii	<50	−1/10	<50	<50	<5		>7½	>7½
iii	50	−1/10	<50	<50	<5		>7½	>7½
C1 i	25 000	+1/1000	4200	8200	950	0	>7½	0
ii	118 000	+1/1000	2800	92 000	1300		>7½	0
iii	63 000	+1/1000	15 600	19 700	1500		>7½	0
C3 i	50	−1/10	<50	<50	<5	>7½	>7½	>7½
ii	1800	−1/10	<50	400	345		>7½	>7½
iii	1600	−1/10	<50	900	360		>7½	>7½
D1 i	7700	−1/10	<50	<50	<5	>7½	>7½	>7½
ii	7700	−1/10	<50	<50	<5		>7½	>7½
iii	8400	−1/10	<50	<50	<5		>7½	7½
D3 i	9000	−1/10	<50	<50	<5	>7½	>7½	7
ii	$1{\cdot}1\times10^6$	+1/100	700	300	140		>7½	4
iii	$1{\cdot}8\times10^6$	+1/1000	700	200	110		>7½	3

the whipping stage at bakery C (C1 i, ii, iii) even after the cream was changed to UHT cream (C3 i, ii, iii). After the change to UHT cream, there was no longer any contamination from presumptive or faecal coliforms (C3 ii, iii). At bakery D, the standard of hygiene was usually very good (D1 i, ii, iii), but occasionally, the standard lapsed and the presence of both presumptive and faecal coliforms could be demonstrated in the whipped cream (D3 ii, iii).

A comparison of colony and proteolytic counts incubated at 30° and 5°C showed that there was very little relation between the results obtained at these two temperatures (Table 3). A low colony count at 30°C was no indication either that the proteolytic count at 30°C or the colony and proteolytic counts at 5°C would be low (D3 ia, iia, iiia; D1 i, ii, iii; D2 i). Samples D2 i, ii and iii showed that the colony count at 5°C could be very much greater than the colony count at 30°C. However, on occasions, contamination

TABLE 3. The variations that occurred between the colony count, presumptive coliform test, coliform test, coliform count in violet red-bile agar (VRB), proteolytic count of the water agar test (WA) after incubation at 30°C, the colony count and water agar test after incubation at 5°C for seven and fourteen days and the methylene blue test (MB) using cream (c) or sodium citrate (cit) and skim milk (sm) dilutions for samples of cream (i), whipped cream from the mixing bowl (ii) and savoy bag (iii)

Sample	Incubation at 30°C		Incubation at 5°C				MB		
	Colony count	WA	Colony count		WA		c	cit	sm
			7 days	14 days	7 days	14 days			
A2 i	$1\cdot0\times10^6$	142 000	546 000	850 000	112 000	155 000	0	>7½	0
ii	$0\cdot9\times10^6$	140 000	687 000	950 000	139 000	145 000		>7½	0
iii	$0\cdot8\times10^6$	95 000	336 000	510 000	131 000	131 000		>7½	0
C2 i	<50	10	<50	<50	<5	<5	>7½	>7½	>7½
ii	3400	30	<50	200	<5	<5		>7½	>7½
iii	13 500	25	<50	100	<5	<5		>7½	>7½
D1 i	2300	295	275	550	210	235	>7½	>7½	>7½
ii	2900	170	375	800	160	210		>7½	6
iii	3100	260	250	550	340	390		>7½	6
D2 i	4400	1090	25 000	34 400	14 400	16 300	1½	>7½	4
ii	17 700	5650	24 000	30 000	12 000	12 000		>7½	4
iii	16 800	4240	17 000	23 000	12 700	12 700		>7½	4
D3 i a	3900	<5	800	1000	<5	170	>7½	>7½	>7½
ii a	3100	155	1100	1800	140	375		>7½	>7½
iii a	4800	60	600	1500	100	235		>7½	>7½
D3 i b	9000	<5	<50	100	<5	<5	>7½	>7½	7
ii b	$1\cdot1\times10^6$	140	$1\cdot6\times10^6$	$1\cdot9\times10^6$	<5	65 000		>7½	4
iii b	$1\cdot8\times10^6$	110	$1\cdot7\times10^6$	$1\cdot9\times10^6$	<5	45 000		>7½	3

was almost entirely mesophilic, only a few colonies growing slowly at 5°C (samples C2 i, ii, iii). The relation between the colony and proteolytic counts at 5°C were equally variable. Samples D3 iib and iiib had high colony counts that were readily countable after incubation for seven days, showing very little increase in count after incubation for a further seven days. The proteolytic count for these two samples constituted only a small proportion of the psychrotrophic population and required incubation for fourteen days to exhibit proteolysis. The 5°C colony counts for samples A2 i, ii and iii were of the same order as those for samples D3 iib and iiib but a considerable proportion of the population was actively proteolytic, showing proteolysis after incubation for seven days. In some cases, almost the entire psychrotrophic population was actively proteolytic (samples D1 i, ii, iii).

Discussion

The methylene blue test, as recommended by the Public Health Laboratory Service for the grading of cream, is not suitable as a test for whipped cream which cannot be poured. If the methylene blue test were to be used as a bacteriological standard for whipped cream, it would be necessary to make a dilution. The British Standards Institution recommend 2% sodium citrate as a suitable aqueous diluent for cream. A protein solution such as skim milk, is equally suitable for dispersing the fat in cream. In this survey, the methylene blue test gave very little relevant information about the samples that were examined (Tables 2 and 3). With two exceptions the cream samples were either of good bacteriological quality with a reduction time of not less than 7·5 hr, or so poor that the dye was reduced at 0 hr. The use of the sodium citrate dilution in the methylene blue test inhibited reduction of the dye, reduction only occurring in samples of the poorest bacteriological quality when complete reduction was obtained at 0 hrs. The use of the skim milk dilution gave results that were in better agreement with the bacteriological quality of the samples but the sensitivity of the test was poor. The use of either sodium citrate solution or sterile skim milk to prepare the first 1 : 10 dilution made no significant difference ($P > 0{\cdot}05$) to the results of any of the other tests.

Under modern conditions of cream production, the most common indicator organisms for poor hygienic conditions are psychrotrophic. When working with pasteurized milk, Taylor (1971) found that the growth of more than twenty proteolytic colonies in the water agar test after incubation at 30°C, indicated the presence of actively proteolytic psychrotrophs in the pasteurized milk. This standard seems to be equally applicable to the samples of cream considered in this work. However, the addition of other materials to the cream to assist whipping appeared to make the water agar test rather less specific for the whipped product. The results showed that a count of not more than 50 in the 30°C water agar test could be used to indicate the absence of proteolytic psychrotrophs while a count of 50–500 indicated the presence of proteolytic organisms although they might not be actively proteolytic. Since this test is simple to perform and gives a result within 20 hr, it is suggested that this test is suitable for use as a bacteriological standard for cream and whipped cream. A proposed standard is that under good hygienic conditions the proteolytic count at 30°C should not be more than 20 in cream and not more than 50 in whipped cream.

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Comparison of methods of freshness assessment of wet fish

Part I. Sensory assessments of boxed experimental fish

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Summary

Samples of cod were obtained from different fishing grounds at different seasons. They were stored in boxes with ice for periods of up to twenty days. Freshness assessments by sensory and non-sensory methods were carried out at regular intervals.

Results obtained on four freshness factors by a sensory panel are reported in this paper. The different factors give similar results, but considerable variations are found in fish from different catches.

Introduction

Freshness is important in determining the quality of wet fish and much effort has been expended in the search for suitable methods of assessment. Many methods, both sensory and non-sensory, have been tried—Cutting & Spencer (1968) give nearly 200 references—but few have proved successful. Sensory methods are the most acceptable and widely used but their disadvantages are the long training required and the fact that they are dependent on the persons applying them. When two parties are involved in a transaction, for example catcher and wholesaler, wholesaler and retailer, owner and official inspector, a method demonstrably independent of the tester is of great advantage. The present work was undertaken to find a suitable non-sensory method for use on fishmarkets and in similar situations.

Two main types of non-sensory method are available, chemical and instrumental. Although application of the former in the proposed situations presents some difficulties, particularly in relation to the time available for testing, it was felt advisable to include them in order to cover all potentially usable methods.

The chemical tests which previously had shown most promise were the measurement of hypoxanthine and trimethylamine concentrations. Of the instruments the Intelectron Fish Tester Mark V had been widely tested and a prototype of the Torry Fish Freshness Meter had just become available. These four methods were chosen for detailed investigations.

Fish from different fishing grounds at different seasons were tested in an attempt to

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establish the ground and seasonal effects on spoilage rates. As these effects were expected to be different for the different methods, sensory assessments were included in the experiment to provide a basis for comparison between the methods, because firstly sensory tests are widely used to evaluate freshness and secondly fish is ultimately eaten and its eating quality is judged by the senses. The concern here is, however, not directly with consumer preference, but with the chemical, physical and sensory changes occurring in fish after death. For standard catching and storage conditions these changes are closely related to the time since death and days-in-ice have therefore been chosen as the criterion of freshness. These can either be actual days-in-ice when these are known or equivalent days-in-ice calculated from results obtained by a particular method and a previously obtained relationship between that method and actual days-in-ice.

The date of catching and the subsequent history of treatment are generally not known for fish landed on a market. It was therefore necessary to obtain data on fish of known history before applying the methods to market fish.

Stowage in melting ice held in conventional aluminium boxes was considered to be the best and most reproducible method of obtaining fish handled in a standard manner; it was therefore chosen for the first stage of the work.

Other variables which were known or expected to influence results were recorded: position of fish in the box, length and pH.

Before this work, data were available showing how results obtained by the methods changed with increasing spoilage, but little was known on the relationships between the tests and their dependence on other variables. The present investigation was designed to expand considerably the information on these points.

The results from the sensory tests on fish of known history are reported in this paper; subsequent papers will report the results from non-sensory tests and tests on commercially caught fish.

Material and methods

Species

The main species investigated was cod (*Gadus morhua*). Some tests were also carried out on haddock (*Melanogrammus aeglefinus*).

Seasons and grounds

The fishing grounds chosen were those of greatest economic importance to the United Kingdom wet fish industry at the time of investigation. Samples were obtained at the seasons appropriate to each ground. Catching was by the Torry Research Station's own trawler, FRV Sir William Hardy.

In 1969, the first year of the tests, four trips were made; in 1970 five trips were made, two grounds being fished on each trip (or one ground being fished twice), the 1969 trips being repeated in the corresponding month.

Table 1 lists the seasons and grounds fished for each of the fourteen runs.

TABLE 1. Seasons and grounds of catches

Run no.	Date	Ground
1	February 1969	North Sea
2	June 1969	Iceland
3	September 1969	Bear Island
4	November 1969	Iceland
5	February 1970	Lofoten
6	February 1970	North Sea
7	April 1970	Lofoten
8	April 1970	Iceland
9	June 1970	Iceland
10	June 1970	Iceland
11	September 1970	Barents Sea
12	September 1970	Bear Island
13	November 1970	Iceland
14	November 1970	North Sea

Catching and storage

Fish were caught in hauls not exceeding 2 hr in duration. The fish were gutted as quickly as possible and stowed in conventional aluminium boxes. A layer of flake ice was placed at the bottom of each box, then a layer of fish, a second layer of ice, a second layer of fish and a top layer of ice. Layers of ice were at least 5 cm thick. The fish were separated before stowage into two size groups, the division being nominally at 62 cm length; this was allowed to vary with the actual size distribution of the catch. Two boxes of each size group were required for each age-in-ice to be tested.

Further hauls were made if necessary until the required amount of fish was caught. One to three hauls were sufficient, except for the two February North Sea runs (1 and 6) when nine hauls each were made. The boxes were stored in the fishroom at air temperatures of 2–4°C during the remainder of the voyage and after landing were transferred to a chillroom at 2°C where they remained until required for testing. The top layer of ice was replenished when necessary.

Sampling

The first tests were carried out on the day of landing or the following day, so that the age-in-ice of the freshest fish tested depended on the length of the journey from the fishing ground. Fish fresher than this would not normally be found on United Kingdom fish markets. Samples were taken at regular intervals until the fish had been 18 to 20 days-in-ice. The intervals between sampling were two days in 1969. In 1970 when

two runs were tested concurrently, the interval was increased to three days; the two runs were assessed on different days and every third day was allowed as a rest day for the sensory panel members.

The procedure and sampling scheme are described here for all the tests carried out; the methods themselves are described in the paper where their results are first reported.

On each sampling day, two boxes of large and two boxes of small fish were used. Three fish were chosen from each layer using tables of random numbers, making a total of twenty-four fish.

A box was taken from the chillroom, the top layer of ice carefully removed by hand, the three fish to be tested were determined. A reading by the Torry Fish Freshness Meter was taken on the first fish in its undisturbed position in the box, the fish was then lifted up, tested by the Intelectron Fish Testers (uncompensated and temperature-compensated models), and its length was measured. The fish was then identified by a numbered tag placed in its tail and put in another box containing a layer of flake ice. This procedure was repeated for the other fish in the layer, the other layer and the other boxes. Samples of muscle were taken for hypoxanthine determination and the fish were prepared for taste panel examination. Each fish was beheaded, a steak weighing 170–230 g was cut from behind the head and a fillet was taken from one side of the remainder. The pH of each steak was measured and after assessment by the sensory panel a portion of the fillet was taken for measurement of the trimethylamine content.

Sensory assessment

Each fish was assessed by a sensory panel of four to six members using the method developed by Shewan *et al.* (1953). The steak was steamed in a glass casserole in a boiling water bath; the head, fillet and remaining portion were assessed in the raw state. Each panel member assessed four freshness factors of the raw fish (general appearance, appearance of the flesh, texture, odour) and three of the cooked fish (odour, flavour, texture). The maximum scores for odour and flavour are 10 each, for appearance and texture 5 each, high scores indicating fresh fish.

On any sampling day, all raw fish were assessed at a single panel session, followed by a further session for the assessment of the cooked fish. When assessing raw fish, some panel members gave scores for all the factors on one fish before assessing the next fish, while others assessed one factor on all the fish before assessing the next factor. For cooked fish the former procedure was used by all panel members.

The assessments on cooked fish continued until the panel considered the fish to be unfit for tasting; the final assessments were done on fish of 12–16 days-in-ice. During 1969, raw assessments stopped at the same time but in 1970 were continued until the fish were 18–20 days-in-ice.

Panel means are used in all subsequent calculations. Individual scores of panel members were recorded and an analysis of panel performance is being prepared and will be published separately. For the purpose of the present investigation, the sensory

panel has been considered as an instrument providing a single result for each factor on each fish assessed.

The pH of each raw steak was measured using an Activion combined glass calomel electrode fitted to a Radiometer pH meter 29.

Length was measured with a graduated rule.

Results and discussion

Although all seven freshness factors were assessed, results are given only for general appearance (GA), raw odour (RO), cooked odour (CO) and cooked flavour (CF). Texture scores are known to vary in freshly-caught fish and are not therefore a reliable measure of freshness.

GA and RO, the only non-destructive sensory tests, were investigated in a subsequent experiment for their possible use on fish markets. To make the present results comparable, the scores for GA and appearance of the flesh were not added together in the manner of Ehrenberg & Shewan (1955).

A box of fish was treated as a sample unit; means and standard deviations for each test and the correlations between the tests were calculated for each box. An analysis of variance was carried out for each run, with days-in-ice, size and layer as fixed effects and box as a random effect. No significant between-box variances were found. There was a significant size effect for RO, CO and CF, the larger fish obtaining higher scores, equivalent to just under ½ day-in-ice. Layer, however, affects only GA, the top layer obtaining higher scores by the equivalent of about ¼ day-in-ice.

Within-box-layer standard deviations were pooled over all runs and are shown in Table 2 for separate age-in-ice ranges and pooled over all ages. All tests show an increase in standard deviations for longer stored fish.

TABLE 2. Within-box-layer standard deviations, s

Days-in-ice	GA	RO	CO	CF
2–6	0·15	0·18	0·16	0·22
7–9	0·17	0·24	0·21	0·25
10–12	0·17	0·27	0·24	0·25
13–16	0·19	0·28	0·24	0·26
Pooled	0·17	0·24	0·22	0·25

Within-box correlation coefficients were pooled over all boxes and all runs. The pooled correlation coefficient shows the extent of association between tests when used on fish of the same age-in-ice and same size group. Table 3 shows the association to be highest within the cooked assessments, lower within the raw assessments and lowest

between raw and cooked assessments. This may be due to the effect of the taste panel procedure described above, whereby all raw assessments are made together and all cooked assessments together.

TABLE 3. Pooled within-box correlation coefficients

GA			
0·3	RO		
0·1	0·1	CO	
0·1	0·1	0·7	CF

Table 4 shows the pooled within-box correlation coefficients between sensory scores, length and pH. Higher scores tend to be given to larger fish, as found by the analysis of variance, and to fish of lower pH, the association being greater for cooked assessment. Length and pH are themselves negatively correlated. Similar relationships between toughness of cooked fish, pH and length have been found in freshly caught cod by Kelly *et al.* (1966), Kelly (1969) and Love *et al.* (1974).

TABLE 4. Pooled within-box correlation coefficients

	Length	pH
GA	0·1	ns
RO	0·1	−0·1
CO	0·2	−0·2
CF	0·3	−0·3
pH	−0·2	

ns, not significant at the 5% level.

Regression analyses were carried out with age-in-ice as the independent variable and sensory score as the dependent variable. Tests for linearity showed no systematic deviation for CF while the RO curve showed a flattening for longer-stored fish. The effect of adding a quadratic term to the regression is generally small, the increase in the deviation accounted for by regression exceeds 2% in only three runs. When the age range for RO was shortened to that for CF, no systematic deviations from linearity were noted and the linear regressions calculated for this restricted time range were used for the subsequent comparison of methods. Table 5 shows the linear regression parameters, regression coefficient (b), intercept (a) and the standard deviation from regression (s_r), pooled over all runs. The regression coefficient is interpreted as the spoilage rate (fall in score per day-in-ice). Box means and fitted regression lines are shown in Figs 1–4.

TABLE 5. Linear regression parameters with days-in-ice

Test	a	b	s_r
GA	4·6	−0·20	0·21
RO	9·2	−0·34	0·31
CO	9·2	−0·33	0·33
CF	9·2	−0·33	0·33

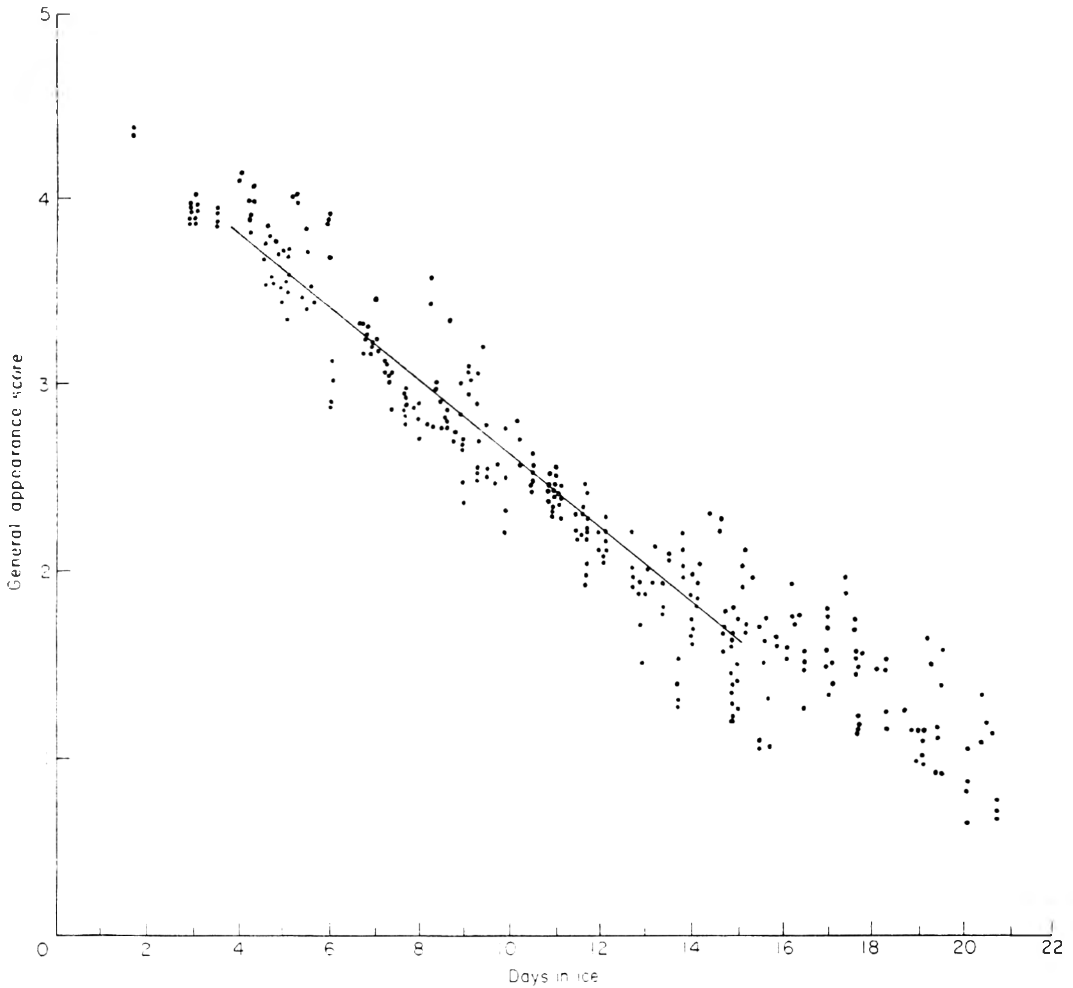

FIG. 1. Relationship between general appearance score and time of iced storage. Box means and fitted regression line.

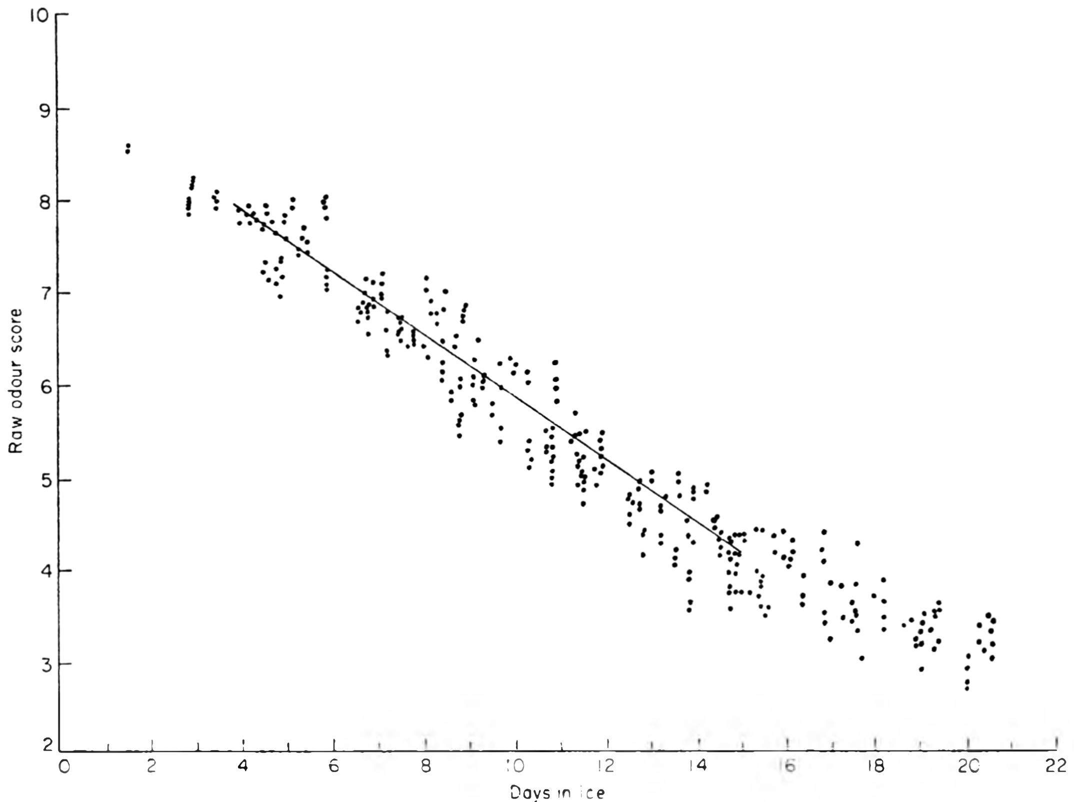

FIG. 2. Relationship between raw odour score and time of iced storage. Box means and fitted regression line.

To enable comparisons to be made between methods employing different scales, the ratio of the standard deviation within the sampling unit and the regression coefficient with time was calculated for each test. These ratios (s/b) are in fact standard deviations expressed in units of equivalent days-in-ice and they express the sensitivity of a test (Baines & Shewan, 1965) when the mean score within the sampling unit is to be estimated from the scores on a sample. Lower ratios correspond to more sensitive tests. In the calculation of the sensitivities for Table 6 the within-box-layer standard deviations pooled over all ages (Table 2) were used. As the between-layer effect was significant only for GA, where it was small, and as there are no significant between-box variances, the sample unit may encompass a number of boxes containing fish of the same age-in-ice and size group. It is generally sufficient to estimate the mean sensory score for the sampling unit, but there may be occasions where a prediction of the true days-in-ice is required from the sensory score. The prediction of true days-in-ice from sensory scores is the reverse of the usual regression prediction (Snedecor & Cochran, 1967). To obtain

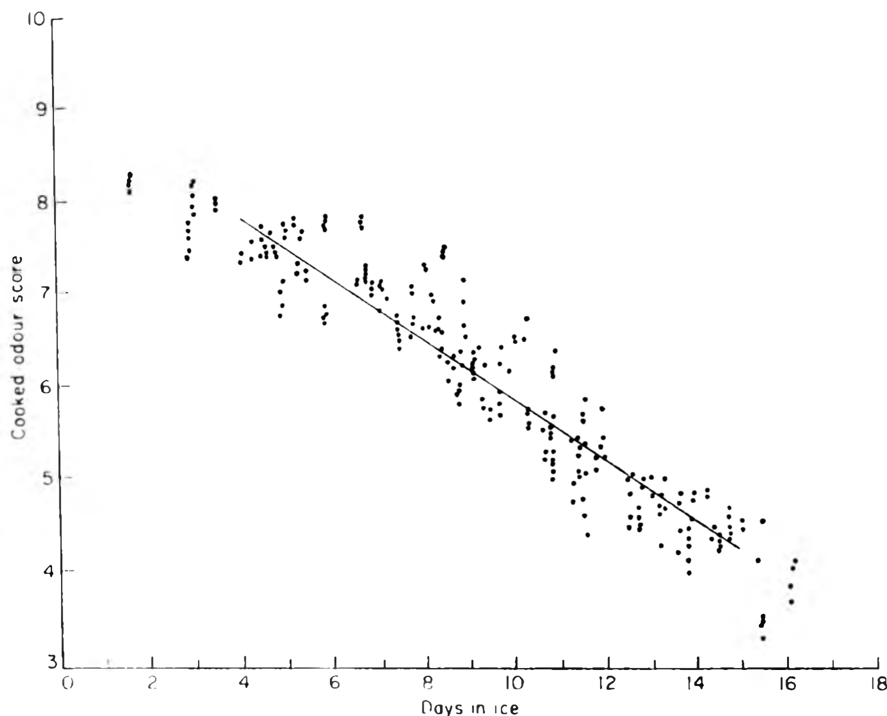

FIG. 3. Relationship between cooked odour score and time of iced storage. Box means and fitted regression line.

sensitivities for prediction the standard deviation from regression replaces the within-sampling-unit standard deviation.

Sensitivities for estimation and prediction are listed in Table 6. The higher value of sensitivity for GA is at least partly accounted for by the greater coarseness of its scale, half-units being differentiated by each panel member for a scale with maximum 5, compared with odour and flavour having the same possible discrimination with a maximum of 10.

The sample number required for estimation or prediction can be calculated for any required confidence limit and significance level. For an estimation to within $\pm L$

TABLE 6. Sensitivity of test, days-in-ice

Test	Estimate	Prediction
GA	0·9	1·1
RO	0·7	0·9
CO	0·7	1·0
CF	0·7	1·0

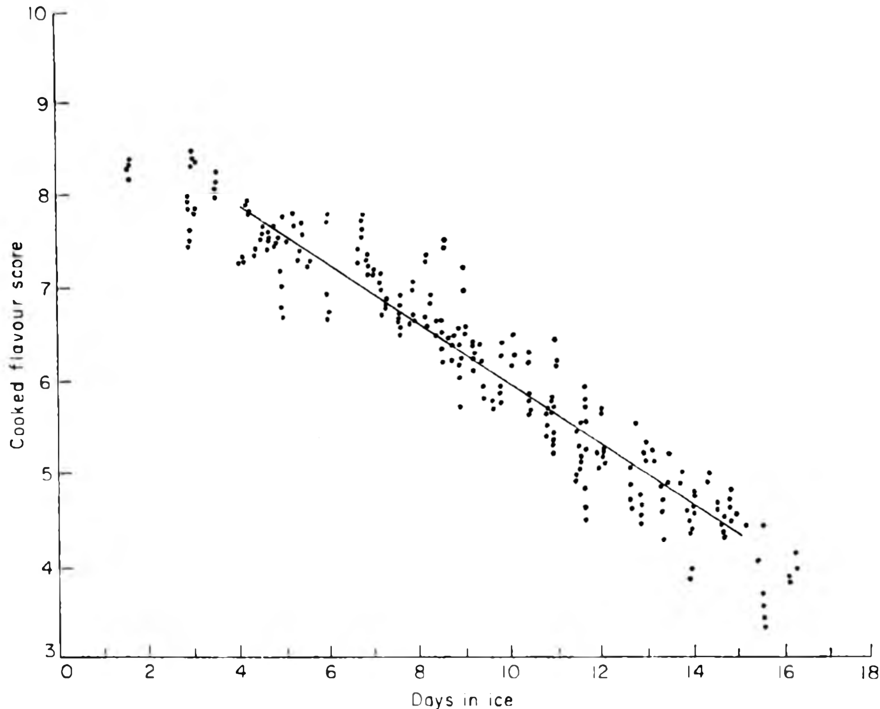

FIG. 4. Relationship between cooked flavour score and time of iced storage. Box means and fitted regression line.

days-in-ice it is $(t \times \text{sensitivity}/L)^2$, where t is Student's t corresponding to the desired significance level and the sample number used in calculating the sample standard deviation. The same expression is a good approximation for predictions whenever the regression coefficients are highly significant and the sample number in the original estimation is large compared with the sample number being determined from the expression. This expression is strictly valid only at the mean ages-in-ice. Larger samples are generally required at the extremes. In the present case, however, the differences are negligible. Table 7 lists the sample numbers required for 95% confidence limits of ± 1 day-in-ice.

In the calculation of sample numbers for estimation, the pooled standard deviations were used. In the range of 13–16 days-in-ice, where the standard deviations are larger than the pooled values, sample numbers for GA and RO are increased to 4 and 3. For constant sample numbers, the confidence limits would be increased or the significance levels decreased.

Separate regression lines were calculated for large fish and small fish in each run. The regression lines were in most cases significantly different, as expected from the analysis of variance results; the differences were generally in the elevation and not the slope, showing a consistent difference between sizes over the whole age range tested.

TABLE 7. Sample numbers, ±1 day-in-ice, 95% confidence

Test	Estimate	Prediction
GA	3	4
RO	2	3
CO	2	4
CF	2	4

Comparison of the regression analyses of the separate runs showed them to give significantly different results.

In Table 8 the days-in-ice corresponding to scores of 7 and 4½ for RO, CO and CF are shown for each run. To provide a comparison with GA, scores of 3·3 and 1·8 were assumed for that test. The range of age-in-ice for a given sensory score is 2½ to 3½ days. When a prediction of age-in-ice is required from a sensory score, the regression parameters for that particular batch of fish are generally not known and a previously determined estimate must be used. Using the pooled values of the parameters from Table 5, biases of up to 2 days can therefore be introduced. This bias is additional to the error of prediction discussed earlier. The differences in ages-in-ice predicted by the different factors in one run are about ½ day.

TABLE 8. Days-in-ice predicted in different runs

Test	GA	RO	CO	CF	GA	RO	CO	CF
Score	3·3	7·0	7·0	7·0	1·8	4·5	4·5	4·5
Run								
1	6·9	6·6	6·4	6·5	14·7	14·0	13·5	13·8
2	6·2	5·8	6·4	7·0	13·8	12·3	13·7	14·6
3	6·9	6·6	7·9	7·9	12·9	13·5	12·5	12·7
4	6·1	6·5	6·2	6·4	12·6	13·3	12·2	12·2
5	8·0	8·3	8·3	8·3	14·1	14·5	14·7	14·7
6	6·8	6·7	6·5	6·4	14·4	14·6	14·8	14·4
7	8·6	7·9	9·0	9·0	13·9	13·5	14·0	14·2
8	6·7	6·2	6·7	6·7	13·4	12·8	13·5	13·5
9	7·2	7·0	6·8	6·5	15·3	14·8	14·8	14·9
10	7·6	7·4	7·4	7·2	16·0	14·1	14·1	14·4
11	5·3	6·0	6·0	6·2	13·9	13·5	14·0	14·3
12	5·4	5·4	7·0	6·9	14·3	13·3	13·3	13·4
13	6·1	6·0	5·9	5·8	13·6	12·3	13·3	13·3
14	5·5	6·0	5·5	5·6	13·3	14·0	—	—
Pooled	6·6	6·5	6·6	6·7	14·3	13·8	14·1	14·0
Range	3·3	2·9	3·5	3·4	3·4	2·5	2·6	2·7

When the results for the four freshness factors are averaged, the ages-in-ice of fish corresponding to score 7 (or the equivalent for GA) are within ± 1 day of $6\frac{1}{2}$ days except for the two Lofoten runs which are between 8 and 9 days. The five runs where the score falls to 7 soonest were all carried out in September and November.

Score $4\frac{1}{2}$ is reached by all runs within ± 1 day of 14 days, except runs 3 and 4 (Bear Island, September and Iceland, November) which reach that score sooner. Baines & Shewan (1965) call the time to reach a flavour score of $4\frac{1}{2}$ the 'keeping time'. The keeping times calculated from the regression equations of Ehrenberg & Shewan (1955) are 15 days for Spitzbergen, 18 days for Lofoten and 20 days for North Sea fish. The keeping times in the present investigation are considerably lower and have a smaller range. They vary from 12–$15\frac{1}{2}$ days, close to the results of Shewan & Ehrenberg (1957) who found an average keeping time of $15\frac{1}{2}$ days for North Sea fish with no seasonal variation indicated.

Although some ground and seasonal effects are suggested by the present results, there are sometimes greater differences between runs from the same ground in the same month in successive years than between runs of different origin.

It is thus not possible to specify regression equations for particular grounds or seasons.

Acknowledgment

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The protein of intermediate moisture meat stored at tropical temperature

I. Changes in solubility and electrophoretic pattern

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Summary

Intermediate moisture beef was prepared by desorption (cook-soak-equilibration method) and stored at 38°C. The state of the proteins at processing and during storage was followed by solubility and electrophoretic studies. Two types of protein reactions—breakdown and crosslinking—were found to be going on simultaneously during storage. The crosslinking reactions over-rode the breakdown reactions as storage advanced. The state of the proteins depends on the balance between these reactions.

Introduction

The recent commercial success of intermediate moisture (semi-moist) pet foods has stimulated interest in intermediate moisture (i.m.) food technology. Most of the i.m. foods for humans have been developed in USA to meet the demand of the army for less bulky, shelf-stable, easy-to-prepare, nutritious foods, which are stable under tropical climates, (Hollis *et al.*, 1968; Brockmann, 1969, 1970; Pavey, 1972). The benefits of i.m. foods for military use have also interested the National Aeronautics and Space Administration (NASA) who are developing suitable foods for astronauts in the various space flights (Klicka, 1969; Lachance & Klicka, 1969; Labuza, 1973, 1974). Besides these logistic uses, some companies have formulated i.m. human foods which are now being test-marketed. Others are investigating i.m. foods for use in special diets such as those required in chronic renal failure.

Generally, i.m. foods contain moderate levels of moisture, of the order of 20–50% by weight (Potter, 1970). The concentration of solutes in the moisture are sufficient to decrease water activity below that required to support microbial growth. They are thus shelf stable without refrigeration and can be eaten without need for rehydration (Loncin *et al.*, 1968; Potter, 1970; Anon, 1972; Brockmann, 1973). This can be achieved either by controlled re-humidification of dried food (i.e. adsorption) or by osmotic dehydration of the wet food (i.e. desorption). With the adsorption technique, the drying

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procedure for the food to be re-humidified is very important and most workers use freeze-drying. This seriously limits the profitability and scope of application of this method especially in the developing countries. In the desorption technique the normal food is soaked in an infusing solution of higher osmotic pressure so that, after equilibration, the water activity is reduced to the desired level. The equilibration process can be quickened by raising the temperature as in the cook-soak-equilibration method of Hollis *et al.* (1968, 1969).

The simplicity of the desorption technique makes it a particularly beneficial food preservation technique in the developing countries.

With the present keen interest in, and prospects for, the development of i.m. foods for human nutrition, there is great need for more extensive studies on the quality and mechanisms of deterioration of such foods. The present studies have been undertaken from this viewpoint.

Materials and methods

Cook-soak equilibration

Post rigor bovine *Longissimus dorsi* trimmed of visible fat and connective tissue was cut into pieces (about 1 cm^3). These were placed in cans and immersed in 1·5 times their weight of infusing solutions containing NaCl (9·5%), antimycotic (0·5%) and predetermined amounts of glycerol and water to give, after equilibration, water activities of 0·82 to 0·86. Table 1 gives the composition of the infusing solutions.

TABLE 1. Composition of the infusing solutions

a_w	Water (g)	99% glycerol (g)	Salt (g)	K-Sorbate (g)	Total wt of solution (g)
0·82	496·7	412·4	95·0	5·0	1009·1
0·83	514·9	394·0	95·0	5·0	1008·9
0·84	534·1	374·7	95·0	5·0	1008·8
0·85	554·2	354·4	95·0	5·0	1008·6
0·86	575·3	333·0	95·0	5·0	1008·3

The cans were sealed and heated in a 77°C water bath to an internal temperature of 70°C, for 15 min (Pavey, 1972). To ensure complete equilibration the cans were tumbled for 16 hr on an end-over-end shaker at room temperature. The low water activities of the infusing solutions prevented microbial growth during this period. After equilibration, the solutions were retained for analysis and the meat pieces vacuum dried to remove surface moisture. This was achieved by the method described by Pavey (1972). The freeze-drier was used as a simple vacuum drier, i.e. without the heater and refrigeration units. The initial vacuum pressure was 5·0 mm Hg absolute but as drying

progressed, resulting in higher concentrations of solutes, the pressure declined to a final value of 0·5 mm Hg after 3 hr. The platen and product temperatures were about 20°C and −10°C respectively. The low product temperature arose from evaporation, under vacuum, of moisture but, due to the glycerol and salt contents, the meat did not freeze. All the cook–soak–equilibrated (i.m.) samples, apart from the quantities required for initial analysis, were stored in Cryovac impermeable PVDC bags (W.R. Grace Ltd, London) at 38°C in a thermostatically controlled hot-air oven.

Control procedure

Both uncooked and cooked beef was used for comparison. Part of the trimmed *L. dorsi* was blast-frozen at −20°C and stored at −10°C. At intervals samples were taken, heated in 1·5 times their weight of distilled water at 77°C for 15 min and tested along with the cook–soak equilibrated beef.

For the cooked sterile meat control, beef cubes from another *L. dorsi* muscle, were taken and divided into two sets of three lots. Each lot of the first set of three was put into a polythene bag and each of the second set in an impermeable Cryovac bag. These were immersed in a boiling water bath. After 40 min the liquor in each bag was drained off and the bag spun round to compress the meat and squeeze out more water. After draining, the bags were tied repeatedly to give a complex labyrinth to minimize air entry and contamination of the meat. The bags were re-immersed in the boiling water bath and cooked for a further 20 min to ensure complete sterilization. After cooking for a total duration of 1 hr, all the meat packs were put into a polythene bag and cooled in slush ice for about 15 min. One pack from each set was removed for initial analysis while the other packs were stored at 38°C alongside the i.m. meats.

Sampling

Samples were taken at three-week intervals; for the cooked sterile meat, this meant just taking one of the pre-packed lots in each set. In the case of the intermediate moisture beef the cubes were mixed within the pack and samples randomly taken.

At every sampling all the meat pieces, except those for microbial counts and moisture determination, were frozen in liquid N_2, milled by pulverization in solid CO_2 and kept in a refrigerator for the CO_2 to evaporate off. All subsequent tests were done in duplicate.

The pH of homogenates of the fresh, frozen, cooked and processed meats in water was measured with a glass electrode.

Moisture was determined by drying 5- or 10-g aliquots in a vacuum oven at 70°C and absolute pressure of about 50 mm Hg for 20 hr.

Nitrogen contents were determined by the macro-Kjeldahl method. Twenty per cent trichloroacetic acid (TCA) was used to separate non-protein nitrogen (NPN) from protein nitrogen in soluble systems (Sharp, 1963).

The microbial status of the meats was assessed by (a) total plate count on Nutrient Agar incubated at 37°C for two days, (b) halophilic count on Nutrient Agar plus 15% NaCl incubated at 37°C for two days, (c) *Staph. aureus* on Mannitol Salt Agar incubated at 37°C for two days and (d) yeasts and moulds on Malt Extract Agar incubated at 25°C for five days.

Solubility studies

After processing the concentration of nitrogen in the cooking solutions was determined.

Solubility in 0·1 M *KCl*. This solvent was used to determine the amount of any soluble (undenatured) protein, plus salt soluble NPN in the i.m. beef. One-gram samples were suspended in 10 ml of 0·1 M KCl and the nitrogen contents of both filtrate and residue determined.

Solubility in other weak solvents. The solubility of selected i.m. beef samples was also assessed in (a) one quarter strength Ringer's solution, usually used in determining soluble collagen as hydroxyproline (Herring, Cassens & Briskey, 1967) and (b) 1% β-mercaptoethanol often used to split disulphide bonds. With both solvents and with 0·1 M KCl solubilities were determined on mixing both at room temperature (20–25°C) and at 77°C for 70 min.

Solubility on heating in SDS-plus β-mercaptoethanol. A solution of sodium dodecyl sulphate (SDS) and β-mercaptoethanol is often used to solubilize denatured proteins for electrophoretic studies. 0·5 g of the meat sample was suspended in 50 ml of 3% SDS-plus-1%-β-mercaptoethanol, soaked for 30 min, heated in a boiling water bath for 30 min and centrifuged warm at 30 000 ***g***. The nitrogen contents of both the clear supernatant and the residue were determined.

Electrophoretic studies

Preparation of samples. Non-protein materials were extracted from the milled meat samples by the method of Parsons & Lawrie (1972). Fifty milligrams of the residual powder was dissolved in 10 ml of 3% SDS-plus-1%-β-mercaptoethanol as described by Penny & Hofmann (1971).

Gel electrophoresis. The protein extracts (i.e. the supernatants) were analysed in a slab of polyacrylamide gel as described by Young & Lawrie (1974). After the gels were carefully removed, by running water, from the glass support plates they were fixed for 2 hr in a 1 : 6 : 13 acetic acid-methanol-water solution, stained overnight in 0·5% Coomassie Blue, cleared in the above fixing solution to provide a translucent background and photographed. It was found that gels which were not fixed yielded comparable electrophoretograms and so this step was omitted for some samples.

Results

pH and water contents

The pH, after equilibration, was 5·6–5·7 and it remained constant throughout

storage. After processing, the glycerol solutions had the same pH as the i.m. beef.

The moisture contents of the i.m. meats after the light (surface) drying were 37–43%. During storage moisture retention was good, falling by only 2·5–6% in three months (Table 2).

TABLE 2. Moisture contents (% w/w) of i.m. beef during storage at 38°C

Weeks of storage	a_w 0·82	0·83	0·84	0·85	0·86	Frozen raw beef
0	37·40	37·55	40·75	43·31	42·26	68·90
3	38·06	38·60	40·98	42·90	43·59	68·90
6	35·21	34·57	39·47	41·74	39·89	68·92
9	35·87	37·05	40·36	42·01	39·83	69·45
12	32·29	31·58	37·17	39·91	38·09	69·50

Each value is the mean of two determinations which differ from the mean by less than 1%.

Microbiological status of the i.m. meats

During storage total plate counts fell sharply (about 100-fold) in the period 0–3 weeks at all a_ws (Table 3). From 3–6 weeks increases were observed due to increased yeast and mould contamination. But these too died down within three weeks (Table 3) and thereafter both total plate counts and yeasts and moulds remained low in number. Halophiles and *Staph. aureus* were virtually absent both before and during storage.

TABLE 3. Microbiological status of the i.m. meats

Weeks at 38°C	Total plate count/g of meat (a_w)					Yeast and mould count/g of meat (a_w)				
	0·82	0·83	0·84	0·85	0·86	0·82	0·83	0·84	0·85	0·86
0	2510	2810	1310	500	290	0	0	0	0	0
3	3	3	0	33	0	123	48	25	8	8
6	585	190	115	28	180	625	343	170	125	738
9	10	8	5	0	3	28	50	30	0	3
12	28	53	30	25	28	48	5	3	8	13

Loss of soluble meat-nitrogen during processing

Some soluble meat nitrogen diffused into the cooking solution during processing. The amounts of NPN that diffused out of the meat varied with a_w; however, the amounts were always less than observed on heating the frozen meat in water alone (Table 4).

TABLE 4. Grams of NPN recovered from 100 g of broth after processing

a_w of i.m. beef broth					Frozen beef control	
0·82	0·83	0·84	0·85	0·86	3 weeks' storage	9 weeks' storage
0·139	0·171	0·176	0·172	0·151	0·185	0·182

Total nitrogen content of the beef

On a wet weight basis the total nitrogen contents of the i.m. beef was similar to that of fresh, cooked beef (Table 5). The rather wide scatter of values for the i.m. samples is probably due to sampling variations arising from the need to use composite samples taken from different parts of two whole *L. dorsi* muscles. It has been shown that there are appreciable differences between parts of the *L. dorsi* muscles in ultimate pH and distribution of fat and protein fractions (Lawrie, 1961).

TABLE 5. Nitrogen content of the beef samples (g/100 g meat)

Weeks at 38°C	a_w of the i.m. beef					Frozen beef control (cooked for test)
	0·82	0·83	0·84	0·85	0·86	
0	4·438	4·564	4·074	4·410	4·578	4·998
3	4·116	4·816	4·438	4·634	4·500	4·928
6	4·320	4·634	4·144	4·200	4·606	4·956
9	4·354	4·746	4·046	4·396	4·284	4·956
12	4·480	4·606	4·606	4·578	4·396	4·970
Mean	4·342	4·673	4·262	4·444	4·483	4·962

Solubility studies

0·1 *M KCl.* None of the meat nitrogen solubilized by this weak solvent at room temperature (20–25°C) was precipitated by 20% TCA indicating absence of protein. As would be expected, thermal denaturation of meat sarcoplasmic proteins, as measured by insolubility, was complete in the cook-soak-equilibration as well as in conventional

cooking. The solubilized NPN was found to increase significantly during storage in both the i.m. beef and the cooked sterile beef. However, at all a_ws the NPN values were lower in the i.m. beef than in the conventionally cooked meat. The presence of air did not appear to have a significant effect on the course of NPN production as was also observed by Sharp (1963) in sterile raw beef stored at 37°C. Table 6 summarizes these findings.

TABLE 6. KCl soluble nitrogen (NPN) of the beef samples (g/100 g nitrogen)

Weeks of storage at 38°C	a_w of glycerol-preserved i.m. beef					Cooked sterile beef	
	0·82	0·83	0·84	0·85	0·86	In cryovac bag	In polythene bag
0	4·73	3·68	4·81	4·13	4·59	7·07	6·98
3	5·45	5·81	6·94	6·35	6·46	7·35	7·35
6	5·83	3·93	6·08	6·00	5·17	8·77	—
9	5·47	4·43	6·92	7·64	7·84	—	—
12	7·50	8·21	—	—	—	—	—

Other weak solvents. These gave essentially similar results as 0·1 M KCl (Table 7). Differences due to solvent and temperature of extraction were slight but differences in amounts solubilized immediately after processing and after eighteen weeks' storage were significant. All solvents extracted more NPN as storage progressed.

3% *SDS-plus-*1%*-β-Mercaptoethanol.* This solvent was found to be moderately effective for the i.m. beef. However, it is seen that with storage the percentage solubilized

TABLE 7. The effect of different solvents on the solubility of the nitrogen (g/100 g meat) in i.m. beef

Solvent	Temperature of extraction (°C)	0·83a_w beef		0·84a_w beef	
		Prestorage	18 weeks at 38°C	Prestorage	20 weeks at 38°C
0·1M KCl	25	0·280	0·406	0·224	0·376
	77	0·280	0·420	0·252	0·392
¼ Strength Ringer's	25	0·252	0·350	0·196	0·336
	77	0·280	0·372	0·196	0·364
1% β-mercaptoethanol	25	0·252	0·322	0·182	0·364
	77	0·238	0·434	0·196	0·392

In all cases the soluble nitrogen as judged by solubility in 20% TCA was NPN.

decreased quite markedly after three weeks. This is illustrated in Fig. 1 at $a_w = 0{\cdot}83$ and 0·80. Further studies with different meat samples have confirmed the decline in protein solubility during storage. These will be reported later. It is implicit from the high levels of solubility obtained that most of the nitrogen solubilized by SDS-plus-β-mercapto-ethanol is protein nitrogen. As can be seen from Fig. 1 there is no decrease in protein solubility upon storage at 38°C of beef cooked in water.

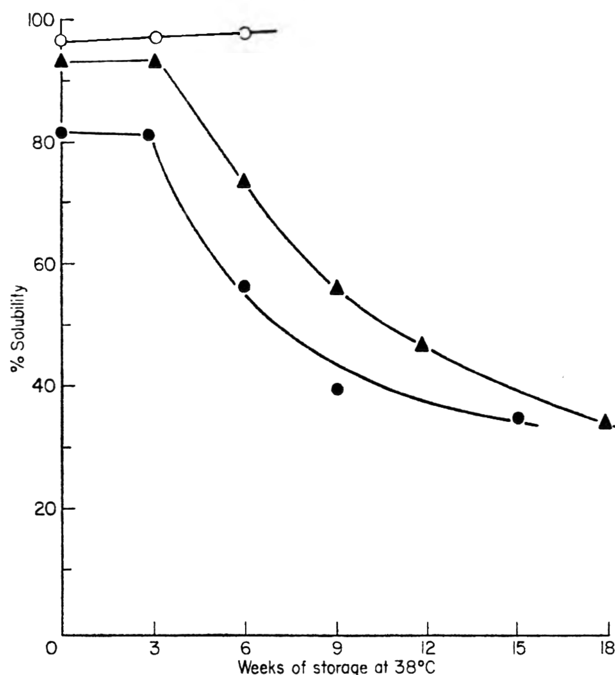

Fig. 1. Solubility of nitrogen in 3% SDS + 1% β-mercaptoethanol during storage at 38°C. Solubility is defined as (soluble N × 100)/total N. ▲, $a_w = 0{\cdot}82$; ●, $a_w = 0{\cdot}80$; ○, cooked beef. The results for a_w 0·80 were determined on a different bovine *L. dorsi*.

Electrophoresis

The polyacrylamide electrophoretograms of the protein extracts are shown in Plates 1 and 2. Within the a_w range studied, the electrophoretograms were independent of water activity.

Initially there were no differences between cook-soak equilibrated and ordinary cooked beef (Plate 1a). It is apparent that the proteins in the cooked and freshly processed beef are similar. Only at 0 and 3 weeks' storage is a direct comparison possible as nitrogen solubility was similar at these times (Fig. 1). Examination of Plate 1b indicates that during the 0 to 3 weeks of storage marked breakdown of the proteins occurred in the i.m. beef but not in the frozen beef cooked just prior to analysis. Also no

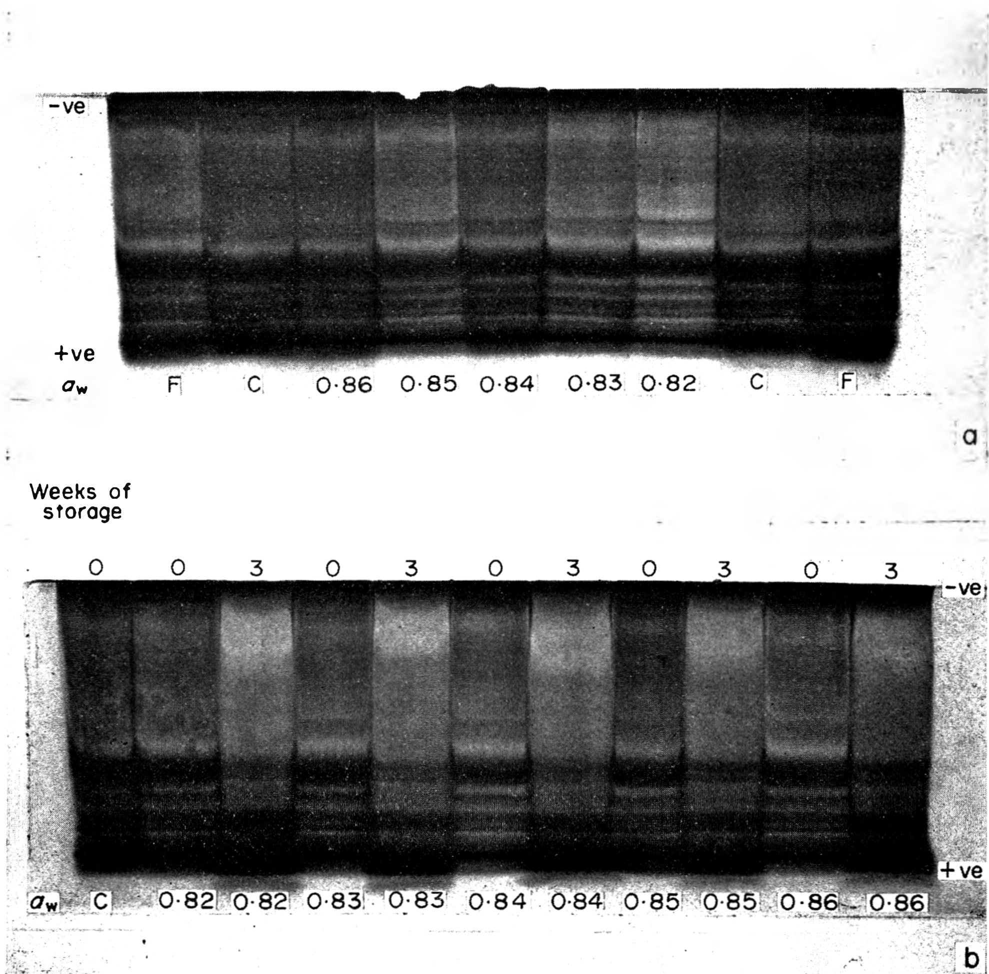

PLATE 1. (a) Polyacrylamide electrophoretograms of the SDS-β-mercaptoethanol extracted proteins from frozen (F), frozen and cooked prior to analysis (C) and freshly processed i.m. beef of different water activities (a_w). Sample origin (−ve) at the top. (b) Polyacrylamide electrophoretograms of the SDS-β-mercaptoethanol extracted proteins from i.m. beef after 0 and 3 weeks' storage at 38 °C and the a_ws shown. C is the frozen beef cooked prior to analysis. Sample origin (−ve) at the top.

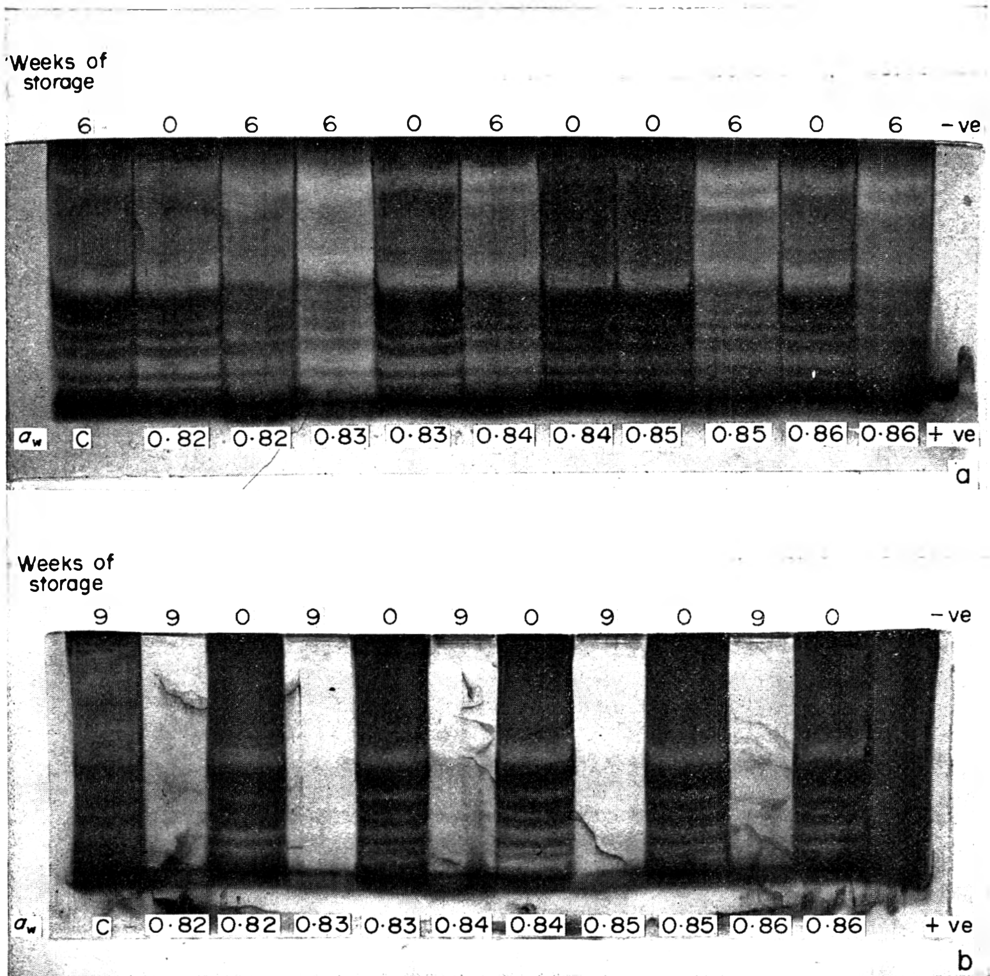

PLATE 2. (a) Polyacrylamide electrophoretograms of the SDS-β-mercaptoethanol extracted proteins from i.m. beef after 0 and 6 weeks' storage at 38°C and a_ws shown. C is the frozen beef cooked prior to analysis. Sample origin (−ve) at the top. (b) Polyacrylamide electrophoretograms of the SDS-β-mercaptoethanol extracted proteins from i.m. beef after 0 and 9 weeks' storage at 38°C and a_ws shown. C is the frozen beef cooked prior to analysis. Sample origin (−ve) at the top.

change was apparent in the protein of beef cooked in water and stored at 38°C for three weeks. After three weeks the decreased solubility (Fig. 1) plus continuing protein breakdown will explain the differences observed in the electrophoretograms of the i.m. beef (Plate 2a, b).

Discussion

It is apparent that the i.m. beef samples did not support microbial growth as shown by the decrease, upon storage, of the total plate count and the yeast and mould counts. This is also borne out by the constancy of the pH during storage. However, although the total nitrogen content of the samples was fairly constant during processing and storage changes in the nature of the proteins did occur.

The electrophoretograms indicate that from 0 to 3 weeks of storage considerable proteolysis occurred in glycerol-infused beef but not in beef cooked in water. The increases in nitrogen soluble in 0·1 M KCl agrees with this conclusion though the increase in nitrogen solubility (Table 6) is less than anticipated from the protein changes shown by electrophoresis (Plate 1b).

During prolonged storage it is also apparent that a fraction of the protein became less soluble in SDS + β-mercaptoethanol (Fig. 1) suggesting that stable crosslinks were being formed. This is supported by the decreased concentration of all bands in the electrophoretograms (Plate 2a, b). It is evident that as storage advances, the crosslinking reactions over-ride the breakdown reactions. The slight loss of water during storage of i.m. meats may well be a result of these changes in protein structure leading to a decreased water holding capacity. It is possible that some crosslinking occurs during the processing stage itself as the KCl-soluble nitrogen is less in the i.m. beef than in ordinary cooked beef (Table 6). Also less non-protein nitrogen diffuses from the meat into the cooking solution during cook-soak equilibration than in ordinary water cooking (Table 4); whether this is due to osmotic balances between the meat and the broth or due to binding of NPN in glycerol-infused beef is not certain. However, the similarity between the electrophoretograms of cooked and freshly processed meats (Plate 1a) suggest that such linkages, if formed at this stage, are (a) broken on heating in SDS + β-mercaptoethanol, (b) counteracted by other bonds rupturing or (c) of insignificant concentration.

What the nature of the protein breakdown and crosslinking reactions are one can only speculate but glycerol is known to crosslink proteins (Sheppard & Sweet, 1921; Hatschek, 1932; Gelatin Res. Soc. of America, 1950; Zubov, Zhurkina & Kargin, 1954). As the cooked sterile beef stored at 38°C did not show equivalent protein breakdown and crosslinking to that observed in the i.m. beef, it is concluded that both types of protein reactions are either inherent or accelerated in glycerol-infused meat at intermediate water activity. Further experiments to elucidate these reactions are described in the following paper.

Acknowledgment

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J. Fd Technol. (1975) **10**, 667–674.

The protein of intermediate moisture meat stored at tropical temperature

II. Effect of protein changes on some aspects of meat quality

Z. A. OBANU, D. A. LEDWARD AND R. A. LAWRIE

Summary

Further studies are reported on the quality of the i.m. beef prepared by cook-soak equilibration in glycerol.

During storage at 38°C, the amounts of all amino acids were fairly constant in the first six weeks but decreased thereafter. Conversely, soluble hydroxyproline increased, indicating that collagen was progressively degraded. The protein (and collagen) breakdown was associated with an increase in tenderness within the first three weeks of storage; thereafter the resistance to shear was variable and may well depend on the balance between the breakdown and crosslinking reactions. TBA values decreased in the 0–6 week period suggesting the probable involvement of carbonyls from lipid oxidation in some of the reactions.

It is concluded that texture and rancidity are not limiting factors on the storage life of lean beef for up to three months, but the crosslinking and breakdown reactions call for urgent consideration to be given to the nutritional value of the proteins in i.m. beef.

Introduction

In the previous paper (Obanu, Ledward & Lawrie, 1975) solubility of cook-soak-equilibrated intermediate (i.m.) beef in various solvents was used to investigate changes during processing and storage at 38°C. It was found that during storage of the cook-soak-equilibrated beef complexing reactions occurred leading to increased insolubility in sodium dodecyl sulphate/β-mercaptoethanol. From the results obtained it was inferred that both crosslinking and breakdown of the proteins occurred during storage; the breakdown reaction never yielded salt-soluble protein fragments but only non-protein nitrogen (NPN). Such accumulation of NPN due to autolysis has been shown in sterile raw beef and rabbit *L. dorsi* muscles during storage at 37°C (Sharp, 1963).

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Electrophoretograms of the i.m. beef proteins supported the view that both breakdown and crosslinking reactions occurred.

These gross changes in protein quality may well be of great importance regarding the eating and nutritional quality of the meat. For this reason further studies were made to elucidate any changes in (i) overall amino acid pattern since certain essential amino acids could become unavailable following the crosslinking reactions; (ii) the nature of the collagen present as this protein is a major factor in determining meat texture (Hill, 1966); (iii) 2-thiobarbituric acid (TBA) value, since this is often taken as an index of lipid oxidation (Tarladgis, Watts & Younathan, 1960; Tarladgis, Pearson & Dugan, 1962).

Materials and methods

Preparation and storage of the samples

The intermediate moisture beef samples were prepared and processed as described in the previous paper (Obanu *et al.*, 1975). Storage was in cryovac bags (W.R. Grace Ltd, London) at 38°C. Some of the uncooked beef *L. dorsi* was blast frozen at −20°C and stored at −10°C for use as a control. Cooked sterile control samples were also prepared from another *L. dorsi* muscle and stored at 38°C, in polythene and cryovac bags as described earlier (Obanu *et al.*, 1975). Samples were taken at three-week intervals, frozen in liquid nitrogen and pulverized with dry ice. These were left in a refrigerator for the CO_2 to escape. Some unpulverized cubes were retained for texture determination.

Automated amino acid analysis

0·5 g of the i.m. beef of $a_w = 0{\cdot}85$ stored for 0, 3, 6, 9 and 12 weeks and 0·5 g of a frozen control stored for nine weeks were digested with 6 N HCl at 110°C for 16 hr and the hydrolysate made up to 500 ml. To 50-ml aliquots 2 ml of standard Nor-leucine solution (1·0 μM/ml) was added and evaporated almost to dryness, under vacuum at 37°C. This was made up to 10 ml with sodium citrate or lithium citrate buffer (pH 2·2).

The acidic and neutral amino acids were eluted using 0·1-ml aliquots on a 30-cm column of locarte cationic resin (Locarte Co., London) at pH 2·58 and 34°C: the pH was changed to 3·65 after valine had eluted. For the basic amino acids, 0·1 ml was eluted on a 10-cm column of locarte cationic resin at 34°C and pH 4·19. For both runs the Locarte automatic amino acid analyser was used.

Determination of hydroxyproline

The pulverized meat samples were heated in one-quarter strength Ringer's solution at 77°C for 70 min (Herring, Cassens & Briskey, 1967). Both the supernatant and the residue were digested with 6 N HCl in a retort at 16–19 lb pressure (Goll, Bray & Hoeskstra, 1963). The hydroxyproline contents of both fractions were determined by the method of Woessner (1961).

Determination of texture

Texture was determined on 1 cm³ pieces by Volodkevich Shear Jaw Tenderometer (Volodkevich, 1938; Grünewald, 1957).

Determination of rancidity

Possible changes in rancidity of the samples were followed by the malonaldehyde distillation method of Tarladgis *et al.* (1960). This evaluation was combined with smelling and tasting of the samples.

Results

Amino acid contents

The amino acid contents of the i.m. beef at various stages of storage and of the frozen raw beef (cooked for test) are shown in Table 1. It is evident that the concentra-

TABLE 1. Amino acid contents (g/16 gN)

Amino acid	0·85 a_w beef stored at 38°C					9-week frozen beef (cooked in water for test)
	0 weeks	3 weeks	6 weeks	9 weeks	12 weeks	
Aspartic acid	6·88	7·47	6·15	4·46	4·32	8·76
Threonine	2·91	3·93	3·01	2·32	2·79	3·83
Serine	3·05	3·28	2·63	2·34	2·41	4·74
Glutamic acid	8·84	10·42	9·36	7·52	7·68	14·10
Proline	4·03	4·17	3·99	3·21	3·27	5·35
Glycine	3·86	4·07	4·07	3·35	3·59	5·54
Alanine	4·37	4·20	4·11	3·40	3·46	5·94
Cystine	0·73	1·08	1·19	0·55	0·60	1·37
Valine	3·53	3·74	3·80	2·46	2·57	5·00
Methionine	2·22	2·37	2·18	1·64	1·44	2·60
Iso-leucine	3·75	3·88	3·85	2·91	2·60	4·99
Leucine	6·97	7·37	8·26	5·59	5·53	8·83
Tyrosine	2·60	3·04	2·70	2·05	1·77	3·78
Phenylalanine	3·27	3·80	3·44	2·37	2·11	2·73
Lysine	7·03	8·11	7·46	6·28	6·27	8·62
Histidine	1·71	1·57	1·54	1·43	1·27	2·73
Unidentified peak* eluting between aspartic acid and threonine	0·60	0·41	0·54	0·18	0·19	0 17

* Unidentified peak: values quoted are in g/16 g N assuming a colour factor of 1·00 and molecular weight of 100.

tion of each amino acid eluted was lower after cook-soak equilibration in glycerol than after cooking in water. During the first six weeks of storage there appears to be little difference in the concentrations of the eluted amino acids. However, after six weeks storage there is a further decline in the amount of the amino acids determined following acid hydrolysis.

In the chromatograms of all the hydrolysates a small unidentified peak was observed between aspartic acid and threonine. It was over three times greater after the cook-soak equilibration than after cooking the frozen control in water (Table 1), but with six weeks' storage it decreased to a level similar to that seen in the control (Table 1). This unidentified peak was also seen as a minute immeasurable peak in fresh (non-frozen) beef *L. dorsi* analysed both before and after cooking in water; however, it was of a measurable size in the nine-week frozen control sample.

A small shoulder was also seen on the lysine peak in the frozen meat and this peak too

TABLE 2. (a) Changes in percent. soluble OHP* with storage

Weeks at 38°C	a_w of the i.m. beef					Frozen beef cooked in water
	0·82	0·83	0·84	0·85	0·86	
0	14·8	15·6	14·9	14·6	14·8	21·6
3	31·8	29·2	28·8	29·7	—	21·6
6	40·1	39·1	50·8	51·9	49·9	21·1
9	32·2	28·2	40·1	44·3	38·3	16·3
12	48·7	49·6	47·4	53·0	48·0	16·8

(b) Per cent soluble OHP* in cooked sterile beef† stored at 38°C

Weeks at 38°C	Meat in Cryovac bag	Meat in polythene bag
0	15·4	13·2
3	25·6	19·2
6	30·5	—

* Soluble OHP (%) = Soluble OHP × 100/ Total OHP where total OHP was determined on each sample and varied from 0·7 to 1·3 mg/g meat in the i.m. samples and 1·4 to 1·8 mg/g meat in the cooked beef.

† *L. dorsi* from a different animal.

increased in area wth desorption in glycerol. However, it disappeared within three weeks.

Collagen breakdown

Immediately after processing in glycerol the percentage of hydroxyproline (OHP) solubilized was independent of a_w and varied from 14·6 to 15·5. The percentage solubilized from beef frozen for various times and cooked prior to analysis was independent of storage time (Table 2) and varied from 16·3 to 21·6. This decrease in the i.m. samples was very highly significant ($P < 0{\cdot}001$).

The changes in levels of soluble OHP with storage are shown in Table 2(a). In all cases the soluble OHP increased to about 50% during storage for twelve weeks.

Table 2(b) shows that during storage of cooked, sterile beef at 38°C increased amounts of soluble OHP were formed. This agrees with the observation of Sharp (1963) that soluble OHP was formed during the aseptic storage of flamed raw rabbit *L. dorsi* at 37°C.

Texture measurements

From 0–3 weeks of storage the i.m. beef samples exhibited a decreased resistance to shear (i.e. became more tender) as shown in Table 3. However, after three weeks'

TABLE 3. (a) Effect of storage on the Volodkevich shear force area (cm²)* of i.m. and cooked beef

Weeks at 38°C	a_w of the i.m. beef					Frozen beef cooked for test
	0·82	0·83	0·84	0·85	0·86	
0	3·23	2·90	2·90	2·90	2·58	2·26
3	1·87	2·55	2·58	2·16	1·61	2·26
6	2·90	3·16	2·58	1·94	1·94	2·23
9	2·26	2·71	1·94	1·61	1·77	2·90
12	2·42	2·26	1·03	0·90	2·06	2·45

(b) Cooked sterile beef† stored 38°C

Weeks at 38°C	Meat in cryovac bag	Meat in polythene bag
0	1·36	1·36
3	1·94	1·81
6	1·48	—

* Each value is the mean of four samples.
† *L. dorsi* from a different animal.

storage the textural changes in i.m. beef were variable. Compared with the cooked controls, the i.m. meats showed increased brittleness with storage, i.e. the samples became less elastic (chewy). This could be an additional parameter to tenderness as measured by shearing.

Thiobarbituric acid (TBA) value

No rancidity was observed by smelling or tasting during twelve weeks of storage. However, the slight sweetness imparted to the flavour by the glycerol may make subjective assessment of rancidity difficult. Changes in TBA value during storage were observed (Fig. 1).

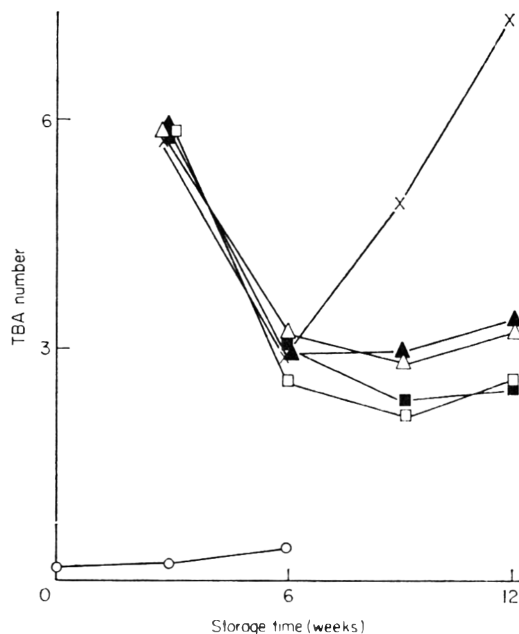

FIG. 1. Changes in TBA values of i.m. beef and cooked beef during storage at 38°C. TBA number is defined as milligrams malonaldehyde/100 g of meat. x, $a_w = 0{\cdot}86$; △, $a_w = 0{\cdot}85$; ▲, $a_w = 0{\cdot}84$; □, $a_w = 0{\cdot}83$; ■, $a_w = 0{\cdot}82$; ○, cooked and stored in air-impermeable cryovac bag. The TBA values of beef samples cooked and stored in polythene bags were similar to those stored in cryovac.

At a_w 0·80 to 0·85 the TBA value was highest immediately after manufacture (i.e. prestorage) but declined within the first six weeks of storage and then tended to level off. However, at $a_w = 0{\cdot}86$ the TBA values increased with prolonged storage and exceeded the prestorage level by the twelfth week (Fig. 1). From Fig. 1 it can be seen that in ordinary cooked beef there was no decline in TBA value; in fact over six weeks there is the expected increase.

Discussion

That the amounts of amino acids eluted decreased after treatment of the meat with glycerol suggests that they somehow combined strongly with the glycerol or some other compound to form products which were not converted back to free amino acids during acid hydrolysis. The unidentified peak (between aspartic acid and threonine) made more prominent in the processing may indicate the presence of such a complex, as also may the tiny shoulder observed on the lysine peak. However, the amounts of these unidentified amino acids are not sufficient to explain the large differences observed. The decrease in soluble OHP observed after cook-soak-equilibration may also indicate that some specific bonds are formed between the denatured proteins and possibly glycerol although the rapid release of OHP on storage suggests that this imino acid is not directly involved in these reactions.

Several glycerol-protein reactions are possible (Newman, 1968):

(i) formation of amino derivatives between the free amino groups and glycerol;
(ii) formation of ester linkages between exposed carboxyl groups and glycerol;
(iii) formation of thio derivatives from sulphur amino acids, particularly those with terminal sulphydryl groups, e.g. cystine;
(iv) alkyl substitution in which methyl groups, especially in dimethyl amino acids (e.g. valine and leucine), replace the non-functional hydrogen atoms of glycerol to form alkyl-(methyl-) glycerols.

Whatever the products are, it would appear that their formation increases during prolonged (> six weeks) storage. It is interesting to observe that the trend of amino acid contents with storage parallels the decline in solubility of i.m. beef in sodium dodecyl sulphate/β-mercaptoethanol (Obanu *et al.*, 1975). Possible reasons for the apparent decreases in amino acids eluted could be:

(i) increased insolubility and resistance to acid hydrolysis;
(ii) losses during acid hydrolysis due to humin formation common with high-carbohydrate samples (Light & Smith, 1963). As the bonds formed are apparently resistant to acid hydrolysis, the nutritional implications may be quite important.

The almost unimpeded release of soluble OHP from collagen during storage (Table 2) suggests that OHP may not be involved in the complexing reactions—at least not to the same extent as other amino acids. As collagen in meat is one of the major determinants of texture, the rapid release of OHP would suggest that the i.m. beef should become more tender in storage. This, in fact, is so up to three weeks' storage (Table 2). Also, previous results indicate that general protein degradation is occurring (Obanu *et al.*, 1975); this too will lead to increased tenderness. However, the crosslinking reactions thought to occur between some of the proteins (Obanu *et al.*, 1975) may lead to increased resistance to shear. This balance between breakdown and crosslinking may well explain the variable textural results obtained on prolonged storage. The results do, however, show that textural changes *per se* will not be a major factor in determining the accept-

ability of these products. In the ordinary cooked beef there was no apparent tenderization with storage even though collagen breakdown occurred (Table 2).

It is also obvious that rancidity development, in i.m. beef trimmed of visible fat, will not usually lead to a decrease in eating quality within three months, at least not at $a_w = 0{\cdot}80$ to $0{\cdot}85$. What is interesting about the TBA values is the apparent decline during storage. As the TBA value is a measure of the concentration of certain carbonyls in the food, it may well be that these carbonyls take part in the degradation or crosslinking reactions and so are not free to react with TBA. Whatever the nature of the protein degradation and crosslinking reactions, the observation that they occur calls for urgent consideration to be given to the nutritional value of the proteins in i.m. beef.

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On the nature and reactivity of the haematin complexes present in intermediate moisture beef

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Summary

The colour of intermediate moisture (i.m.) beef prepared by cook-soak equilibration in glycerol was studied. The freshly processed cook-soak-equilibrated beef samples were spectrally similar to ordinary cooked beef. With storage at 38°C and 28°C the spectra changed from that typical of cooked meat haemoprotein to one more typical of free haematin. This reaction produced a subjectively detectable change (lightening) in meat colour. The rate of change varied with water activity. However, the amount of haematin that could be extracted by 40% pyridine decreased during storage suggesting that if freed the haematin is either involved in further complexing reactions or broken down to pyrrole fragments and non-haematin iron. The nature and nutritional availability of the iron in the i.m. beef may depend on these reactions.

Introduction

It has recently been established that the haematin complexes present in cooked meat are formed by reaction between the haematin of myoglobin and haemoglobin and any of several of the denatured proteins present in cooked meat (Ledward, 1971, 1974). The pigments are thought to be diimadazole complexes of ferric haematin, these complexes being further stabilized by salt linkages, hydrogen bonds and hydrophobic interactions.

In the previous papers on the nature of protein in i.m. beef (Obanu, Ledward & Lawrie, 1975a, b) it has been established that, in intermediate moisture beef ($a_w = 0{\cdot}82$–$0{\cdot}86$), the proteins present are, in several respects, different from the proteins present in fresh and cooked beef and that with storage further changes occur. These differences are apparently due to both increased crosslinking and degradation of the proteins present in the glycerol-infused intermediate moisture samples.

From the nutritional point of view meat is an excellent source both of protein and iron. Marked changes in the nature of the proteins occur in intermediate moisture beef, and as the haematin is presumably bound to these proteins, a study of the haematin

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complexes present in these samples was considered desirable. As well as being important nutritionally the haematin complexes present will also be of importance in determining consumer acceptability of the product (i.e. the colour) and possibly the keeping quality of the samples as some haematin complexes may catalyse lipid oxidation (Kendrick & Watts, 1969).

Materials and methods

Preparation and storage of the samples

The intermediate moisture beef samples were prepared and processed as described previously (Obanu *et al.*, 1975a). Storage was in cryovac impermeable PVDC bags (W.R. Grace Ltd, London) at 38°C and 28°C. The control cooked beef was prepared as described (Obanu *et al.*, 1975a) and stored at 38°C in both polythene and air-impermeable cryovac bags.

Reflectance spectrophotometry

Reflectance spectra of the intact 1 cm^3 meat pieces were recorded against a MgO standard, using a Perkin-Elmer Model 124 double beam spectrophotometer. The range scanned was 700 nm to 340 nm at a rate of 60 nm per minute.

Solubility studies

Absorption spectra of the solutions obtained on homogenizing, for 60 sec, and filtering 1 g samples of the pulverized beef with 10 ml of 40% pyridine at neutral pH were recorded in a Unicam SP800 double beam spectrophotometer. The range scanned was 700–370 nm using a pathlength of 1 cm.

Subjective assessment of meat colour

After forty days' storage fifteen non-specialist judges ranked the meats of different water activities on a scale of 1 (brightest) to 7 (darkest). This assessment was done on a different *L. dorsi* muscle over a_w 0·80–0·86. To see the effect of storage at 28 and 38°C on colour portions were placed at −10°C, at three-week intervals, and subjectively assessed by three judges at the end of the experiment (twelve weeks). Due to the anti-freeze property of glycerol and depression of freezing point by the infused solutes, the i.m. samples did not freeze at this temperature.

Results

Reflectance spectra

Typical spectra for freshly cooked meat and processed intermediate moisture beef are shown in Fig. 1. It is seen that following processing the reflectance spectra are typical of cooked meat having distinct minima (absorption maxima) at about 415, 540 and 640 nm. However, upon storage the spectra of the processed meats became less

TABLE 1. Position of the Soret peak in i.m. beef (λ nm)*

Weeks at 38°C	a_w of the i.m. beef 0·82	0·83	0·84	0·85	0·86	Cooked sterile beef
0	420	416	415	415	412	410
3	410	405	400	380	395	410
6	395	398	385	380	380	410
9	385	385	385	385	375	—
12	385	390	382	380	380	—

* Whereas the Soret peaks in the initial i.m. samples and all conventionally cooked meat samples were sharp and distinct, the stored i.m. samples had broad flattened peaks with increased errors of measurement of about ± 5% (see Fig. 1).

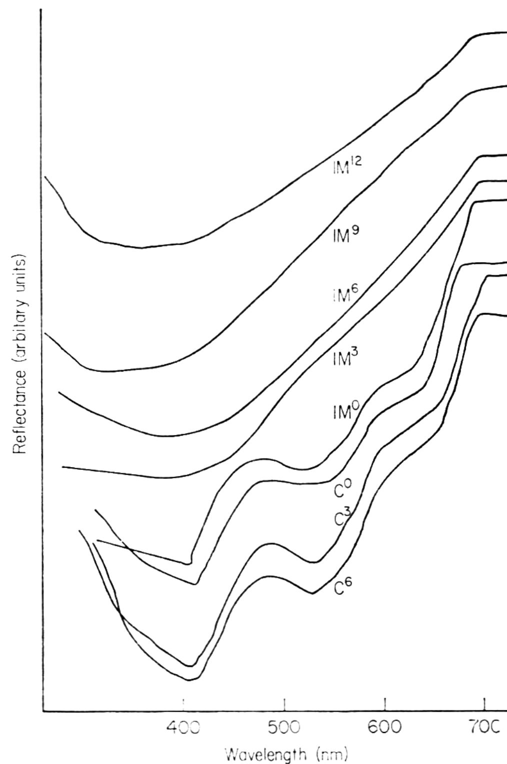

FIG. 1. Reflectance spectra of i.m. beef (IM) and cooked beef (C) stored at 38°C. The superscripts refer to the number of weeks of storage. The spectra of the i.m. meats during storage were similar at all a_ws studied. Also the spectra of the cooked beef were the same whether stored in polythene or cryovac bag. For clarity all spectra are arbitrarily displaced on the reflectance axis.

distinctive, the minima at about 540 and 640 nm disappearing while the Soret peak at about 415 nm became broader and shifted to lower wavelength. The shifts in the Soret peak are summarized in Table 1.

Although Aitken *et al.* (1962) have shown similar spectral changes in accelerated freeze-drying of pork at 80°C and 100°C no such changes occur in ordinary cooked beef over six weeks storage at 38°C (Fig. 1).

Solubility studies

Only limited solubility of the pigments was achieved in 40% pyridine, the spectra of the dilute solutions being typical of a pyridine haemichrome. However, the solubility of the freshly processed samples was only about 50% of that obtained with freshly cooked beef and during storage it decreased still further (Fig. 2). The cooked sterile meat showed a similar trend during storage at 38°C (Fig. 2).

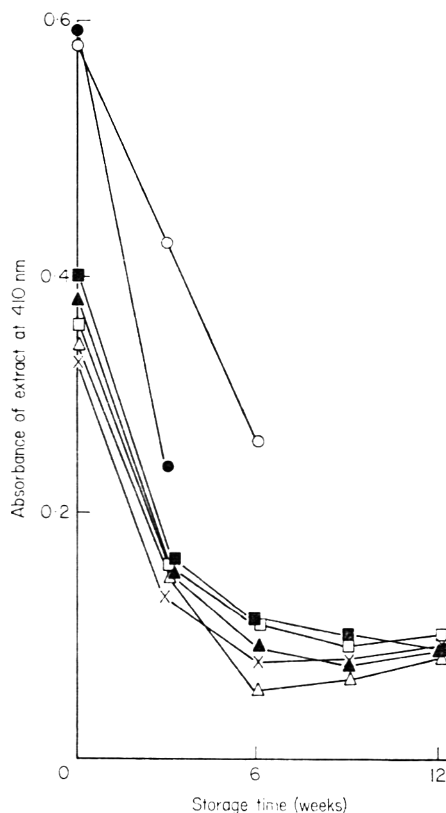

FIG. 2. The extractability by pyridine (40% v/v) of the haematin complexes in i.m. beef and cooked beef during storage at 38°C. X, $a_w=0{\cdot}86$; △, $a_w=0{\cdot}85$; ▲, $a_w=0{\cdot}84$; □, $a_w=0{\cdot}83$; ■, $a_w=0{\cdot}82$; ○, cooked and stored in air-impermeable cryovac; ●, cooked and stored in polythene.

Subjective evaluation

Initially there was no significant dependence of the colour on water activity (a_w). However, at all a_ws the samples, with storage, became progressively lighter in colour from the dark greyish brown colour of cooked beef to very pale yellow—a change which Lawrie (1974) attributes to the conversion of myoglobin to bile pigments. Colour changes were slower at 28°C than at 38°C. After forty days storage at 38°C the panel rated the samples of lower a_w as darker (more like fresh cooked or processed beef) than those at the higher a_ws. The rank averages are summarized in Table 2.

TABLE 2. Panel assessment of meat colour* after forty days' storage

Temp. of storage (°C)	a_w of the i.m. beef						
	0·80	0·81	0·82	0·83	0·84	0·85	0·86
28	4·90	5·23	5·60	2·27	3·80	2·07	4·20
38	6·47	6·27	4·73	1·87	3·60	1·60	3·60

Values are the means for fifteen judges ranking the samples 1 (brightest) to 7 (darkest).
* Different muscle to that used for the spectral analysis.

Discussion

In the previous two papers (Obanu *et al.*, 1975a, b) it was concluded that the proteins in glycerol preserved intermediate moisture meats may crosslink during processing, this crosslinking continuing and also protein degradation occurring during subsequent storage. The present results suggest that the i.m. cooked meat haemoproteins behave similarly. Thus the disappearance of the typical cooked meat haemoprotein spectrum during storage indicates that the haematin-protein interactions are weakened yielding, ultimately, a spectrum typical of free haematin (Schecter & Epstein, 1968). It is interesting to note that the disappearance of the characteristic protein-haematin Soret peak correlates with the subjective evaluation of the darkness of the meat, i.e. the disappearance of the peak corresponds to lighter meat. Both objective measurement of the Soret peak position (Table 1) and the subjective assessment after six weeks' storage at 38°C indicate that the haemoprotein breakdown is faster at the higher water activities.

If breakdown of the haemoprotein is occurring during storage one would expect the haematin to become more reactive and easily solubilized. This is not the case. Forty per cent pyridine, a solvent with a great affinity for haematin (Lemberg & Legge, 1949) is a less effective solvent of the haematin in processed meats than freshly cooked meat, its effectiveness diminishing as storage progressed from 0 to 6 weeks (Fig. 2). It would thus appear that the crosslinking reactions causing protein insolubility in these immediate moisture meats also causes insolubilization of the haematin so that although the haematin–protein interactions are diminished the haematin is still firmly held in the meat

matrix. An alternative explanation may be that the haematin is broken down to colourless or yellow pyrrole fragments and non-haematin iron during storage. However, if this is so it is unlikely that, in the intact samples, the reflectance minima would be seen at about 370–380 nm characteristic of free haematin. It is also apparent (Fig. 2) that insolubilization of the haematin occurs in cooked beef during storage and is faster, during storage at 38°C, in air-permeable (polythene) packs than in air-impermeable (cryovac) packs. Thus although there is no evidence of decreased haematin–protein interactions in cooked meat the changes which cause the haematin to be less reactive, or destroyed, during storage of i.m. meat also occur in cooked meat. Another factor which could affect the colour of these meats is non-enzymatic browning. If this type of reaction occurred one would expect a darkening (browning) in colour and significant flavour changes to be observed. However, during the first six to nine weeks of storage no darkening was observed, in fact the colour became lighter due to breakdown of the haematin pigments and no flavour changes were detected. It would thus appear that within the first two months or so of storage non-enzymatic browning is not a problem in i.m. lean beef from which visible fat (and connective tissue) has been removed. However, non-enzymatic browning was apparent after about twelve weeks and the samples were coffee-brown after eighteen weeks at 38°C. These Maillard type reactions may well contribute to the insolubilization reported in the previous papers (Obanu *et al.*, 1975a, b).

As the haematin complexes in meat are a nutritionally important source of iron it is obviously desirable that a study be made of the nutritional quality of *both* the protein and iron in these intermediate moisture meats.

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Home frozen strawberries

I. Influences of freezing medium, fanning, syrup temperature, soaking time, storage time and temperature, and rates of freezing and thawing on sensory assessments

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Summary

Cambridge Favourite strawberries were assessed for appearance, colour, texture and flavour after being frozen, mainly in room temperature 60% syrup, by various methods available in the home. Soaks of 4 and 16 hr in syrup prior to freezing significantly lowered scores for appearance and colour, but tended to improve texture and, for the 4-hr soak, flavour. A 4-hr soak in sugar also had an adverse effect on colour. Colour was worsened by a 3-hr fanning of the dry berries before immersion in syrup, but was improved by the use of cold syrup and by adding syrup to plain berries after the thaw. Appearance was worse if the syrup was replaced by dry sugar, to which water was added for tasting.

Generally no significant differences were found when berries were frozen at −18°C and at −32°C or when they were stored at these temperatures. Thawing rate appeared to have a greater influence, and a fast thaw in running water significantly improved appearance, though it tended to have an adverse effect on texture. As a rule there were no significant differences between storage periods except for a surprising improvement of appearance with time.

Flavour scores tended to decrease with storage.

Introduction

Cultivated strawberries are difficult fruits to freeze in the home. During freezing the cellular membranes are damaged and after thawing the cellular fluid drips from the tissues, making the berries unpleasantly mushy. In addition the freezing adversely affects the flavour and appearance of the berries. The most successful media so far found to improve quality are dry sugar and syrup. Reports in the literature conflict somewhat as to which is the best of these two media to use. Fieger, DuBois & Kalogereas

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(1946), Dawson, Harris & Alexander (1952) and Jacobson & Weller (1967) favoured dry sugar as they claimed that it improved flavour. Hohl (1947) found that it improved appearance and texture, and Jacobson & Weller (1967) reported an improvement in colour. MacArthur & Johnston (1947), MacArthur & Harper (1948), Stoll (1966) and Leach *et al.* (1970), however, obtained better results using syrup. There seems to be general agreement that a strong syrup gives better results than a weak one, and MacArthur & Harper (1948), Jacobson & Weller (1967) and Leach *et al.* (1970) favour the use of a 60% syrup (weight/weight). Armbruster (1967) compared sugar and syrup treatments and found no organoleptic differences for texture, but she reported greater cellular disorganization in two cultivars of strawberries when dry sucrose was used.

This paper deals with quality improvement in the home using, as controls, berries in 60% room temperature syrup which were frozen and stored at −18°C and thawed slowly. The effects of fanning dry berries before adding syrup, using cold syrup, pre-soaking the berries in syrup or sugar, adding after storage syrup or water to berries frozen plain or in sugar respectively, freezing and thawing at different rates, and storing at a lower temperature are experiments which are considered.

Materials and methods

Cheddar grown Cambridge Favourite strawberries were hulled, and sorted for size and maturity. Batches of twenty-six fruits, each containing the same range of variation, were packaged in flat layers in double polythene bags. Sufficient material was prepared for the storage time × treatment samples. Each experiment was duplicated with a second set of berries. The standard practice was to cover the berries with room temperature syrup (four parts fruit by weight : three parts 60% syrup by volume), sugar (ten parts fruit : 5·8 parts sugar) or to leave them plain. Berries covered with syrup were frozen immediately or after a 4-hr soak at room temperature. The berries were then frozen in a cold room at −18°C, stored at this temperature and, after storage, thawed slowly overnight in a refrigerator. Length of the storage periods and variations from the above regime are given with the results.

The material was assessed by approximately ten panelists. Bowls for appearance and colour were judged prior to the texture and flavour samples. Tasting was randomized, and marking was on a 7–1 hedonic (personal preference) scale, 7 being excellent, 6 good, 5 above average, 4 average, 3 below average, 2 poor, 1 objectionable. Results showing no significant trends have not been included in the tables.

Results

Effect of desiccation prior to freezing

Berries were frozen (1) in syrup (2) after soaking in syrup for 4 hr and (3) in syrup after they had been placed in a single layer and fanned for 3 hr. Results for the two

storage times (four and ten months) did not differ significantly from one another. Combined storage analyses showed that fanning had an adverse effect on colour (Table 1). Berries soaked 4 hr had significantly ($P=0{\cdot}05$) lower scores for colour and appearance than the non-soaked control material.

TABLE 1. Effects of fanning dry berries

Treatment	Appearance	Colour	Flavour
Frozen in syrup (0 hr soak)	4·23 b	4·35 b	4·05 ab
Frozen in syrup (4 hr soak)	3·78 a	3·95 a	4·30 b
Frozen in syrup after 3 hr fanning time	3·93 ab	3·83 a	3·85 a
s.e.	0·13	0·11	0·15

Mean scores followed by the same letter do not differ significantly from one another at the 5% level.
s.e., standard error.

Effect of soaking berries in chilled and room temperature syrups

Berries were soaked in syrup at room temperature or in chilled syrup at 2°C for 0, 4 or 16 hr. They were then frozen and stored seven months.

The use of chilled in place of room temperature syrup always increased colour scores. When material was soaked for 16 hr higher flavour and appearance scores were obtained for the chilled syrup (Table 2). Texture, however, was not improved by the cold syrup, presumably because there was less penetration of the sweetener into the cells, and scores for the 0 hr cold soak treatments were significantly lower than for the warm syrup.

TABLE 2. Mean scores for berries soaked in syrup at room temperature (R.T.) or at 2°C before freezing

Treatment	Appearance	Colour	Texture	Flavour
0 hr soak at R.T.	4·10 c	4·05 b	3·60 b	3·60 b
0 hr soak at 2°C	4·30 c	4·10 c	2·75 a	3·25 ab
4 hr soak at R.T.	3·40 ab	2·70 a	3·50 b	3·70 b
4 hr soak at 2°C	3·80 bc	3·65 b	3·20 b	3·75 b
16 hr soak at R.T.	3·10 a	2·20 a	3·65 b	2·55 a
16 hr soak at 2°C	3·90 bc	3·50 b	3·75 b	3·80 b
s.e.	0·21	0·22	0·22	0·26

Mean scores followed by the same letter do not differ significantly from one another at the 5% level.
s.e., standard error.

Four- and 16-hr soak treatments at room temperature worsened appearance and colour. The 16-hr treatment carried out at room temperature lowered flavour scores. Both the 4- and 16-hr cold soaks improved texture.

Comparisons of berries frozen plain, in syrup or in sugar and then tasted after being brought to the same sugar/water concentrations

Berries were soaked 0 or 4 hr in sugar or syrup before freezing, and others were frozen plain. After three, six and nine months storage the berries were thawed and water was added to the sugared berries, and syrup to the plain material, to equalize the sugar/water concentrations in each treatment. In another treatment syrup was added to plain berries just before thawing.

The panel rated the appearance of berries stored for nine months higher than for those stored three months ($P=0{\cdot}01$), and there was a signifiicent ($P=0{\cdot}05$) lowering of marks for flavour between the sixth and ninth month storage period. Combined results (Table 3) for storage times showed no significant differences between the treatments for texture or flavour, but the colour of the plain berries to which syrup was added after thawing was significantly higher than for the controls. There were indications that it was better to add syrup after, rather than before, thawing. Results also indicated that berries frozen in syrup had a significantly improved appearance and were marginally better than those which were frozen in sugar and had water added for tasting. Pre-soaking for 4 hr in sugar or syrup significantly lowered colour scores and, for berries in syrup, the appearance.

Effect of freezing rate

To compare freezing conditions in home freezers with and without super-speed switches for rapid freezing, bags of berries were frozen on shelves in cold rooms held at

TABLE 3. Mean scores for berries tasted in the same concentrations of syrup, but frozen plain, in syrup or in sugar

Treatment	Appearance	Colour
Frozen in syrup (0 hr soak)	4·47 b	4·07 b
Frozen in sugar (0 hr soak); water added after thawing	3·63 a	4·08 b
Frozen in syrup (4 hr soak)	3·75 a	3·63 a
Frozen in sugar (4 hr soak); water added after thawing	3·57 a	3·43 a
Frozen plain; syrup added before thawing	4·40 b	4·43 c
Frozen plain; syrup added after thawing	4·50 b	4·50 c
s.e.	0·12	0·12

Mean scores followed by the same letter do not differ significantly from one another at the 5% level.

s.e., standard error.

−18 and −32°C. Those frozen at −32°C were transferred to the −18°C room after 24 hr. Three experiments were undertaken:

(1) plain berries stored for four months were tested without added sugar;
(2) berries in dry sugar were tested after five months;
(3) berries in syrup were tested after three, six and nine months.

The berries in sugar and in syrup received the usual soaking and non-soaking treatments.

Storage analyses for the berries in syrup showed that appearance improved with storage and was rated highest ($P = 0{\cdot}01$) after the two longer periods. The rate of freezing had virtually no effect on quality. Significant differences between the two rates occurred only in the case of berries soaked in sugar for 4 hr; here the colour of the fast frozen material was rated highest ($P = 0{\cdot}05$). Quality was significantly affected by the soaking treatments and, with the exception of the appearance of berries frozen in syrup at −18°C, the 4-hr soak lowered scores for appearance and colour (Table 4).

TABLE 4. Mean scores for berries frozen at −18 and −32°C

Treatment	Appearance	Colour
Frozen in syrup (0 hr soak) at −18°C	3·88 b	3·97 c
Frozen in syrup (0 hr soak) at −32°C	4·07 b	3·80 bc
Frozen in syrup (4 hr soak) at −18°C	3·80 ab	3·67 ab
Frozen in syrup (4 hr soak) at −32°C	3·52 a	3·43 a
s.e.	0·10	0·11
Frozen in sugar (0 hr soak) at −18°C	4·50 b	4·10 c
Frozen in sugar (0 hr soak) at −32°C	4·45 b	4·20 c
Frozen in sugar (4 hr soak) at −18°C	3·40 a	2·70 a
Frozen in sugar (4 h soak) at −32°C	3·45 a	3·35 b
s.e.	0·18	0·18

Mean scores followed by the same letter do not differ significantly from one another at the 5% level.
s.e., standard error.

Storage temperature

Berries in syrup were frozen in a cold room at −18°C. The following day half the material was transferred to a room at −32°C. Duplicate sets of berries were tasted after four, seven and ten months. Prior to thawing, the material stored at the lower temperature was allowed to equilibriate at −18°C.

Material kept for seven and ten months had a significantly better appearance ($P = 0{\cdot}05$) than that stored for the shortest time. Combined results for the storage times showed no significant differences between the two temperature treatments.

Rate of thawing

Berries soaked in syrup for 0 and 4 hr were frozen for two, five and eight months. They were then thawed slowly overnight in the refrigerator, at a medium rate at room temperature, or rapidly by placing them under a cold running tap.

Berries stored for five and eight months had significantly higher scores for appearance ($P = 0{\cdot}001$) than those which had been stored only two months. Combined results for the three storage times (Table 5) showed significantly higher scores for appearance when berries were thawed at the fastest rate, but there were indications that texture was improved by slower speeds. With the exception of material thawed at a medium rate, soaking treatments tended to lower scores for appearance and colour, and there was a consistent trend for texture and flavour scores to be increased.

TABLE 5. Mean scores for berries thawed at different rates

Treatment	Appearance	Colour	Texture	Flavour
0 hr soak in syrup; slow thaw	3·97 ab	3·90 b	3·58 ab	3·70 ab
0 hr soak in syrup; medium thaw	3·88 ab	3·87 ab	3·53 ab	3·50 a
0 hr soak in syrup; fast thaw	4·32 c	3·93 b	3·35 a	3·72 ab
4 hr soak in syrup; slow thaw	3·73 a	3·58 a	3·90 b	3·93 b
4 hr soak in syrup; medium thaw	4·17 bc	3·92 b	3·58 ab	3·68 ab
4 hr soak in syrup; fast thaw	4·10 bc	3·73 ab	3·60 ab	3·80 ab
s.e.	0·11	0·11	0·13	0·14

Mean scores followed by the same letter do not differ significantly from one another at the 5% level.

s.e., standard error.

Discussion

The control method for freezing berries was to immerse them in 60% syrup, to freeze and store at −18°C, and to thaw slowly. In this work it was shown that a fast thaw improved appearance significantly, presumably because there was less time available for drip to occur before tasting.

The difficulty of producing all round quality improvement was realized. This was especially noticeable with the soaking treatments where a hold in syrup significantly lowered scores for colour and appearance but tended to improve flavour and texture. Unpublished Testing Machine (Instron) values also showed a trend for firmer texture following soaking. This increase in firmness may be the result of greater penetration of the syrup into the fruits. It is also possible that improved firmness is caused by increased drip loss and resultant compression of the tissues; this is supported by the finding of MacArthur & Harper (1948) that there is a greater drip loss following a pre-freeze hold

in syrup. Drip loss also seems to be responsible for a paling of the berries and for a deterioration of visual parameters. Thus soaking results in greater drip loss that leads to loss of appearance and colour, but to an improvement of texture. The adverse effect of syrup on colour was also evident from the marked colour improvement obtained by reducing the soaking time, i.e. by the use of cold syrup and by the addition of syrup to the fruit after, rather than before, freezing. Leach *et al.* (1970) also found that berries frozen plain or in water had a better colour than those frozen in syrup. Loss of colour following syrup treatment could be due to degradation of the anthocyanins by the sugars (Meschter, 1953; Mackinney, Lukton & Chichester, 1955). However, as colour deterioration seemed to be linked with increased drip loss further explanations are possible, i.e. that (a) prolonged plasmolysis, following immersion of the berries in strong syrup, weakens the protoplasm and reduces the water holding capacity of the cytoplasm and (b) the strong syrup draws cellular fluid out of the cells. Soaking for 4 or 16 hr in cold syrup had no perceptible effect on colour, presumably because of increased viscosity of the syrup and its failure to penetrate the cells.

MacArthur & Harper (1948) reported that berries developed a 'preserved' flavour following a pre-freeze hold of longer than 2 hr in syrup. Such a flavour deterioration was not found by our panel after a hold time of 4 hr, though a soak of 16 hr at room temperature did induce flavour loss. The trend towards flavour improvement which we observed following a 4-hr hold was probably due to sugar penetration and a more balanced flavour.

It is a well-known fact that overripe berries do not freeze well. Possibly the colour loss resulting from fanning berries for 3 hr before freezing was due to further ripening occurring during this time.

Freezing at a temperature below −18°C had no influence on quality. As the material was packaged in fairly bulky packs which reduce the speed of freezing, this was not surprising. Clearly much faster freezing is necessary to achieve the quality improvement such as that reported by Gutschmidt (1969) and Durif (1971).

Lack of significant improvement in quality after storing berries at −32°C, as opposed to −18°C, suggests that very little enzymic change occurs at −18°C which affects sensory evaluations. Crivelli, Rosati & Monzini (1969), however, reported that at −20°C there is still a loss of sugars and ascorbic acid which is considerably slowed down if the strawberries are stored at −30°C.

Apart from one experiment in which flavour was found to fall significantly at the end of the storage time, extension of the storage period to nine months had no apparent adverse effect on quality. The significant improvement in appearance found in two-thirds of the experiments was surprising. However, such marked changes were not detected in a further set of experiments (Hudson *et al.*, 1975) in which the berries were frozen in boxes and not frozen in a single layer as in these tests. Presumably when the berries are in a single layer they are not subjected to pressure from overlying material and will undergo some case hardening in regions where they are imperfectly covered

with syrup. Under these conditions a firmer, glossier appearance might be expected to develop with storage time.

Conclusions

We consider that the best sweetening medium for freezing strawberries is 60% syrup. The syrup should be cold and the berries are best if packaged as a flat pack and are frozen immediately. A fast thaw is recommended. Colour is improved by adding syrup after freezing. Further experiments should establish whether equally good quality is obtained by freezing berries plain and adding sugar for tasting.

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Home frozen strawberries

II. Influence of additives in syrup on sensory assessments and texture measurements

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Summary

Experiments were designed to improve the quality of home frozen strawberries (cultivar Cambridge Favourite) by the addition of easily obtainable substances to the 60% syrup in which the fruit was frozen. Additives used were 0·08% ascorbic acid, 1% calcium lactate, 0·08% ascorbic acid + 1% calcium lactate, 0·04% citric acid and 0·04% tartaric acid. Syrups were made up with hard and soft water. Fruit was stored at −18°C for approximately three and six months and various tests were used to assess quality.

A large consumer panel assessed overall preference and tended to favour citric acid and ascorbic acid + calcium lactate for the short and long storage periods respectively. Citric acid improved colour and appearance of drained berries. A texture panel found no treatment to give significantly firmer fruits than the controls. Universal Testing Machine results indicated the same findings, though there was a tendency for calcium lactate + ascorbic acid treatments to give increased firmness after longer storage. Between the two storage periods there occurred an increase in drip loss and, for treated berries in hard water syrup, an increase in firmness. When thawed berries were allowed to drip 4 hr a loss of visual appearance took place between the storage times.

Introduction

Many attempts have been made to improve the quality of frozen strawberries by the use of additives to the syrup or dry sugar in which the fruit was frozen. Pectic compounds, calcium salts and addition of acids have received attention, but the difficulty has been that of improving certain parameters without lowering others. Flavour is often altered undesirably by an additive. Many workers have tended to favour the use of pectic compounds (Baker, 1941; Grab, Wegener & Baer, 1948; Barton, 1951; Wegener, Baer & Rogers, 1951; Hoover & Dennison, 1955; Jacobson & Weller, 1967)

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and have often obtained improvements, especially in colour and texture. Colour improvement was thought (Grab *et al.*, 1948; Barton, 1951) to result from a gel formation on the surface of the fruit. Stoll (1966) and Leach *et al.* (1970) found that pectins had an unfavourable effect on flavour. The effects of calcium salts have also been widely tested (Baker, 1941; Grab *et al.*, 1948; MacArthur & Harper, 1948; Jacobson & Weller, 1967; Crivelli & Rosati, 1973). Generally there was little success in improving quality and Grab *et al.* (1948) reported that calcium chloride gave a metallic flavour. Polesello & Crivelli (1971) found calcium ascorbate to be one of the most useful additives for improving texture and believed that quality of frozen strawberries could be improved by immersing them in 2·5% calcium ascorbate under vacuum for 3 hr. Further work (Crivelli & Rosati, 1973) showed that immersion of the berries in 2% calcium ascorbate for 60 sec. before freezing did not noticeably improve quality. Ascorbic acid has also failed to produce improved results (Crivelli & Rosati, 1973; Hohl, 1947), but citric acid was of more value, and Wells, Martin & Tichenor (1972) obtained a better colour and greater retention of ascorbic acid in berries treated with up to 1% citric acid. Luther & Cragwall (1946) reported that citric acid markedly enhanced the effect of ascorbic acid in preventing browning. They believed that it prevented discolouration by lowering the pH sufficiently to inhibit enzyme activity and to retard oxidation of ascorbic acid. Wrolstadt, Putnam & Varsefeld (1970) are of the opinion that pH is the only objective measurement correlated with colour quality and that a pH of 3·51 or lower is necessary for an acceptable quality in strawberries after freezing. Meschter (1953) found strawberry anthocyanins to be most stable at pH 1·8; below this pH it was thought that browning induced by reaction between the sugars and pigment was more important than the stability contributed to the pigment by low pH.

In this paper the addition of common household substances, calcium lactate, and ascorbic, citric and tartaric acids, to strawberries frozen in 60% syrup has been examined. Concentrations were chosen so that when the substances were added to neat syrup their flavour was imperceptible. This has meant the use of concentrations much lower than those normally used. Both hard and soft waters have been used for making the syrups.

Methods

Materials, preparation, storage and thawing

Freshly picked Cambridge Favourite berries from Cheddar were hulled, and graded for size and ripeness. For each experiment sets of berries with the same range of variation were placed in plastic boxes and 60% (w/w) cold syrup was added in the same weight as the fruit. Waxed paper was placed on top of the berries and the boxes were closed and frozen and stored at −18°C. Syrup was plain (control) or had had one of the following additives dissolved in it:

A 0·08% ascorbic acid;
L 1% calcium lactate;
(A + L) 0·08% ascorbic acid + 1% calcium lactate;
C 0·04% citric acid;
T 0·04% tartaric acid.

Two types of water from the Bristol Waterworks Company were used for making the syrups:

H, moderately hard water of pH 7·8 and a total hardness as $CaCO_3$ of 200 ppm (Barrow treated);

S, relatively soft water of pH 6·9 and total hardness as $CaCO_3$ of 100 ppm (Bottlehead treated).

Throughout the paper code letters are used, e.g. S/C implies that citric acid was added to soft water syrup.

Hard water treatments were tested after three and six months (H3 and H6 respectively) and the soft water treatments after four and seven months (S4 and S7 respectively). The boxes were thawed overnight at 4°C and were brought to 10°C for sensory and Shear press assessments.

Organoleptic assessments

The thawed berries were placed in fresh strawberry-coloured syrup and were presented in paired dishes contained in pre-cooled (10°C) insulated boxes. Six pairs, composing the controls against the five treatments and against berries frozen in the other type of untreated syrup, were tested at one time. Two panels assessed quality:

(1) twelve experienced and sixty untrained tasters gave personal preferences once after each storage period; results from the two groups did not differ significantly and have been treated together;

(2) a panel of twelve experienced tasters assessed firmness at the end of each storage period; the experiment was duplicated.

Results were given values of 5, 4, 3, 2 and 1 corresponding to the treated berry being much preferred, slightly preferred, the same as, less liked or much less liked than the control material respectively.

Universal Testing Machine (Instron) tests

When berries thaw, they drip, lose their shape and the tissues become compacted. Penetration tests on such berries give high values for firmness. To overcome the difficulty each berry, equilibriated to 10°C, was placed in a small dish and covered with water at 10°C. If berries had compacted, they were gently pushed back into their original shapes. Following the work of Ourecky & Bourne (1968), a star shaped probe, diameter 0·9 cm, was chosen. Cross head and chart speeds of 5 and 40 cm/min respectively were used with a Full Scale Load of 100 *g*. The force (*g*) needed for the probe to penetrate 0·625 cm into the berries was calculated. Three replicates, each with twenty-four berries, were tested for each of the treatments at the four storage periods.

Drained weight, quality count, colour and appearance of thawed berries

Each treatment was replicated thrice for each storage period. After storage and thawing, the twenty-four berries in each box were placed on a raised wire basket in a humid atmosphere at room temperature. The underside of the basket was blotted and weights were taken after 0 and 2 hr 'drip' time. Percentage loss in weight of the fresh berries was calculated. After 4 hr drip time a 'quality count' was taken, and panelists were asked to score the numbers of good, medium and poor berries in each batch, respective marks of 3, 2 and 1 being given; for each basket the mean value for the twenty-four berries was estimated. At this stage the berries were also assessed for colour and appearance using a paired comparison technique in which untreated hard water samples were used as controls and were given a standard score of 3.

Results

Organoleptic tests for preference

The large consumer panel which assessed preference gave no clear indication that it preferred any particular treatment consistently throughout the storage periods (Table 1). Ascorbic acid + calcium lactate was marginally preferred after longer storage periods and H/(A + L) was marked significantly ($P = 0{\cdot}05$ in test t_1) higher than H in storage period H6. This treatment appeared to have less value for shorter times, especially for the soft water trials. There was evidently a synergistic effect between the two additives. They were not as effective on their own and in S4 the calcium lactate treatment had a significantly lower value than the control. It is possible that the flavour of the calcium lactate was having a perceptible adverse effect on flavour in the purer (soft) water. Comments from the tasting panel indicated that flavour was the basis for preference in 80% of the cases and texture in 15% of the preferences. For short storage times C tended to be the most favoured treatment.

Organoleptic tests for texture

Organoleptic assessments for firmness yielded little of significance and the method was considered disappointing for detecting textural differences induced by the treatments. In the separate storage analyses (Table 2) there were indications that H/L and H/(A + L) were slightly better than the control in H6, when H/(A + L) became significantly firmer than H/T. In the soft water treatments highest marks were given to the tartaric acid treatments; treatment S/(A + L) appeared to have no influence on the sensory texture, and scores tended to be lower than for the soft water controls. Combined analyses for the storage periods showed that treatments S/C and S/T were better than S/A ($P = 0{\cdot}05$).

Testing machine values for firmness

These tests proved to be of greater use for assessing firmness than the organoleptic

TABLE 1. Paired comparison preference assessments for berries in syrup

Treatment	H3			H6			S4			S7		
		t_1	t_2		t_1	t_2		t_1	t_2		t_1	t_2
H	3·00	—	—	3·00	—	—	2·86	NS	ab	2·96	NS	a
S	2·89	NS	a	3·15	NS	a	3·00	—	—	3·00	—	—
A	3·20	NS	a	3·04	NS	a	2·97	NS	ab	3·10	NS	a
L	3·06	NS	a	3·07	NS	a	2·70	**	a	2·99	NS	a
(A+L)	3·11	NS	a	3·26	*	a	2·89	NS	ab	3·18	NS	a
C	3·12	NS	a	2·97	NS	a	3·08	NS	b	3·13	NS	a
T	3·09	NS	a	2·96	NS	a	3·05	NS	b	3·06	NS	a
s.e.	0·113			0·106			0·103			0·106		

Test for significant difference between treatment mean and fixed control value 3·00:

$$t_1 = \frac{\bar{x}_i - 3}{\text{s.e. } (\bar{x}_i)}$$

Test for significant difference between two treatment means:

$$t_2 = \frac{\bar{x}_i - \bar{x}_j}{\text{s.e. } (\bar{x}_i - \bar{x}_j)}$$

Mean scores followed by the same letter do not differ significantly from each other at the 5% level.
s.e., standard error.
N.S., not significant.
Levels of significance: * 5%; ** 1%; *** 0·1%. One of these levels of significance preceded by ST(H) or ST(S) indicates the effect of storage time on the hard water and soft water treatments respectively.
For other symbols see Methods.

TABLE 2. Organoleptic firmness assessments for berries in syrup

Treatment	H3			H6			S4			S7		
		t_1	t_2		t_1	t_2		t_1	t_2		t_1	t_2
H	3·00	—	—	3·00	—	—	3·17	NS	a	3·09	NS	a
S	3·31	*	a	2·81	NS	ab	3·00	—	—	3·00	—	—
A	3·15	NS	a	3·13	NS	ab	2·77	NS	a	2·84	NS	a
L	3·08	NS	a	3·17	NS	ab	3·17	NS	a	3·02	NS	a
(A+L)	3·04	NS	a	3·19	NS	b	2·88	NS	a	2·96	NS	a
C	2·90	NS	a	2·83	NS	ab	3·17	NS	a	3·20	NS	a
T	3·04	NS	a	2·69	NS	a	3·21	NS	a	3·22	NS	a
s.e.	0·150			0·173			0·168			0·160		

For abbreviations and symbols see footnotes to Table 1.

method. Analyses of the storage times (Table 3) revealed no significant difference between the two soft water storage periods, but there was an interesting and highly significant ($P = 0{\cdot}01$) increase in firmness between the first and second hard water periods. This firming occurred only in the case of the treated samples and was not significant for H or S; H/T showed a marginal increase, H/A and H/L increased significantly at the 5% level, and both H/(A + L) and H/C increased at the 1% level. There was a slight tendency for H and S to have the higher values than the treated samples after storage period H3, and this no doubt accounted for the fact that at the end of H6 the hard water control was not noticeably different from the treated material. In H6 the only significant differences between the treatments were (1) that H/(A + L) was firmer than S and H/T and (2) H/L was firmer than S. In S4 S/(A + L) was also found to be greater than S/T, and a combined analysis for the soft water storage times revealed that S/(A + L) was firmer than S($P = 0{\cdot}05$). In fact the treatment (A + L) held high values throughout the tests, and for the last two storage periods had higher values than the other treatments. Both in these and the sensory firmness assessments we find that in H6 the treatment H/(A + L) is better than H/T. It is difficult to explain why the treatment S/(A + L) was not rated higher in the sensory tests.

TABLE 3. Force (g) needed for a probe to penetrate 0·625 cm into the berries

Treatment	H3	H6	S4	S7
H	24·11 a	25·74 abc	25·72 ab	23·68 a
S	24·16 a	23·26 a	24·41 ab	23·63 a
A	21·78 a	25·29 abc	25·65 ab	23·56 a
L	23·47 a	26·64 bc	25·69 ab	24·98 a
(A+L)	23·45 a	27·20 c	25·84 b	25·42 a
C	21·88 a	25·68 abc	25·03 ab	24·24 a
T	21·90 a	24·46 ab	23·79 a	24·69 a
s.e.	0·847	0·709	0·713	0·803
	ST(H)**		ST(S) NS	

For abbreviations and symbols see footnotes to Table 1.

Water retaining capacity

Berries which had been stored for three and four months and thawed, but not allowed to drip, showed no loss of liquid and there was a slight gain in weight, indicating absorption of syrup. At the end of the longer storage periods there were significant ($P = 0{\cdot}001$) losses of fluid which amounted to 7 and 9% in the hard and soft waters respectively. No significant differences occurred between the treatments, apart from a greater drip loss ($P = 0{\cdot}05$) in (A + L) than C when all four storage times were analysed together (Table 4).

Combined estimations of drip loss during storage, thawing and the 2-hr drip time showed no difference between the treatments but, for both hard and soft waters, there was a significant increase in loss of liquid ($P = 0{\cdot}001$) of approximately 3% between the two storage periods.

TABLE 4. Percent weight losses of berries and quality count

Treatment	Per cent weight loss of berries combined H3, H6, S4 and S7		Quality count after 4 hr drip time	
	During storage and thawing	During storage, thawing and 2 hr drip time	Combined H3 and H6	Combined S4 and S7
H	3·33 ab	22·99 a	2·04 ab	2·09 d
S	3·21 ab	23·31 a	1·95 a	2·03 bcd
A	2·94 ab	23·02 a	2·06 ab	2·00 abcd
L	2·73 ab	23·06 a	2·05 ab	1·96 abc
(A+L)	3·62 a	23·91 a	1·99 ab	1·88 a
C	2·57 b	22·93 a	2·15 b	2·08 cd
T	2·71 ab	23·69 a	2·00 ab	1·95 ab
s.e.	0·412 ST(H)*** ST(S)***	0·725 ST(H)*** ST(S)***	0·055 ST(H)***	0·044 ST(S) NS

For abbreviations and symbols see footnotes to Table 1.

Visual assessments for berries which had dripped 4 hr

In assessments of the numbers of good, medium and poor berries (quality count, Table 4) a significant loss ($P = 0{\cdot}001$) in quality occurred between the storage times for the hard water samples, but no such changes were observed for the soft water treatments. In the combined hard water analyses H/C was superior to S, whilst in the soft water analyses S/C and H were marked higher than many other treatments.

Paired comparison techniques were used for assessments of colour and appearance after the 4-hr drip time. A combined analysis of the water treatments (Table 5) shows a marked tendency for C to yield the best results and L and (A+L) the worst. Analyses for colour showed that H/C and S/C were superior to H/L and S/L respectively; S/C was greater than S/(A+L) and H; S and S/A were better than S/(A+L). Appearance scores showed similar results (H/C > H/T; S/C > S/L, S/(A+L), S/T and H); treatment T was poor.

TABLE 5. Paired comparison assessments for berries after 4 hr drip time

Treatment	Colour: Combined H3 and H6			Colour: Combined S4 and S7			Appearance: Combined H3 and H6			Appearance: Combined S4 and S7		
		t_1	t_2		t_1	t_2		t_1	t_2		t_1	t_2
H	3·00	—	—	3·00	—	—	3·00	—	—	3·00	—	—
S	3·26	NS	ab	3·33	NS	bc	2·80	NS	ab	3·34	NS	c
A	2·93	NS	ab	3·41	*	bc	3·17	NS	ab	3·09	NS	bc
L	2·72	NS	a	2·97	NS	ab	2·90	NS	ab	2·52	*	a
(A+L)	2·80	NS	ab	2·68	NS	a	2·89	NS	ab	2·77	NS	ab
C	3·36	NS	b	3·57	**	c	3·39	*	b	3·53	**	c
T	2·89	NS	ab	3·25	NS	abc	2·68	NS	a	2·79	NS	ab
s.e.	0·183 ST(H) NS			0·196 ST(S) NS			0·170 ST(H) NS			0·172 ST(S) NS		

For abbreviations and symbols see footnotes to Table 1.

Discussion

In paired comparison tasting techniques the control material retains a constant value and differences between storage times are masked. Quantitative tests are of more value in detecting these changes. Crivelli & Rosati (1971) found no differences in the drip lost from berries stored for three and eleven months at $-20°C$. Our results showed, however, that as storage time increased beyond three months the drip from the berries became greater after thawing. Increased drip loss implies loss of water holding capacity of the cells. This leaching will give rise to a loss of pigments from the cells (fading) and to a compression of the cells (increased firmness). Results showed that during storage firming took place and quality count was significantly lowered due to drip loss in hard water treated material. The (A+L) treatment was particularly effective in increasing firmness, but was of less use when used with soft water. Clearly the composition of the water had some influence on improved firmness and led to the conclusion that there is a synergistic effect on the berries between the ions (probably calcium) in the water and the additives. Further evidence for this was in the finding, obtained from both the texture panel and Shear press measurements, that the effect of calcium lactate in improving firmness after longer storage periods is enhanced in the presence of ascorbic acid. Similarly Polesello & Crivelli (1971) reported that ascorbic acid had a synergistic effect in the presence of calcium ions; they believe the acid helps to bind calcium ions to pectin molecules and so improves firmness. Doesburg (1965) attributed the firming effect of calcium salts to a precipitation of pectinic acids with a low degree of esterification, and to a reduction of the swelling power of the pectic substances. As our results

for the four combined storage times for the per cent weight lost immediately after thawing did show that the (A + L) treatment lost significantly more fluid than C ($P = 0{\cdot}05$), it would seem that increase in firmness is due to loss of cellular fluid. The tendency for the large consumer tasting panel to prefer the (A + L) treatment after six months' storage implies that texture was the parameter that changed most during storage.

One would expect colour deterioration to be correlated with the amount of drip, and the lowest colour scores were in fact found for treatments which produced most fluid loss, i.e. (A + L) and L. Since citric acid was the best additive for improving colour in berries which had been allowed to drip for 4 hr and was also a good treatment for preventing loss of cellular fluid, it is possible that the effect of the citrate in colour retention may be due to its power to increase water holding capacity of the cellular constituents. Further evidence for this arises from the findings of Doesburg (1965) which indicate that calcium-binding components, such as citric acid, might act upon the calcium in cell walls and cause a decrease in the available calcium and a swelling and solubilization of the pectic materials. Wrolstad *et al.* (1970) were, however, of the opinion that the only objective measurement which had a high correlation with colour quality was pH; our results, on the other hand, showed no increased colour retention with tartaric acid, which is a stronger acid than citric. Another possible explanation for the effectiveness of citric acid lies in the fact that citrate forms a stronger complex with metal ions than tartrate (Pollard & Timberlake, 1971), and since anthocyanins are known to complex with metal ions to give pink or purplish discolourations, the role of citrate in improving colour may be its ability to immobilize metal ions naturally present in the fruits and/or tap water.

Many authors (Sonderheimer & Kertesz, 1953; Pollard & Timberlake, 1971; Starr & Francis, 1973) have found that at high temperatures ascorbic acid causes degradation of anthocyanins. However, under low temperature conditions Wrolstad *et al.* (1970) found the acid to have no destructive effect on the pigments; our results support this finding.

Results showed that untreated hard and soft waters made up into syrups behaved relatively similarly. There was a slight trend for the harder water to give a firmer texture, which would be expected.

Throughout the experiments there was the indication that an improvement in colour and appearance is often associated with poorer texture and sometimes flavour. This is understandable if, as is thought, colour and appearance depend mainly upon fluid retention, whilst texture is improved if a certain amount of drip has taken place.

Results frequently showed marked changes in colour and appearance, whereas texture and flavour were not significantly altered. The reason for this may be that the first two parameters are judged with relative ease, whereas flavour is subject to personal preferences and texture is not easy to assess in materials such as thawed strawberries which contain large quantities of loosely held water.

Conclusions

For storage periods of up to three months the overall quality of home frozen strawberries can be marginally improved if 0·04% citric acid is added to the 60% syrup. For longer storage periods a mixture of 1% calcium lactate + 0·08% ascorbic acid was found to give the best results. Whereas citric acid is effective in improving colour, calcium lactate + ascorbic acid tends to increase firmness. Calcium lactate is not as effective when used alone. It thus seems likely that further overall quality improvement could be obtained by adding a mixture of calcium ascorbate + citric acid to the 60% syrup in which the berries are frozen.

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Book Reviews

Human Nutrition and Dietetics, 6th edn. By Sir Stanley Davidson, R. Passmore, J. F. Brock and A. S. Truswell.
Edinburgh: Churchill Livingstone, 1975. Pp. viii + 756. £9.00.

In the three years since the previous edition, several definitive changes can be seen in this highly esteemed textbook of nutrition: Professor Truswell has joined the team of authors, an extra 160 pages have been added and a linen-backed version has been introduced. The changes in the contents are best described by quoting from the Preface: 'Every section of the book has been carefully revised. There are three new chapters, on historical and geographical perspectives, on hyperlipidaemias, and on nutrition and cancer; and new sections on dietary fibre, novel proteins, and adult man.'

The new introductory chapter covering the history of man and his eating habits provides a gentle and interesting approach to the nitty-gritty of the science of nutrition. When one considers that a whole book can be devoted to a single vitamin or to trace elements, for example, it is appreciated that this one volume can only provide a detailed skeleton of nutritional science. *Human Nutrition and Dietetics* (1975) maintains its justifiable reputation as a reliable source of basic information and is in a class of its own; the nutritionist with a specialized interest can be assured of finding references (up to early 1974) for further reading.

Is this standard nutritional work of interest to the food technologist? As with earlier editions, ten chapters are devoted to 'Food' all of which are relevant to the profession and form a book within a book. Other chapters contain information of value to the food technologist, such as public health measures to improve the nutritional status of all sections of the community and how special dietary foods can be used in the treatment of disease and inborn errors of metabolism.

Is the new edition sufficiently different to warrant its purchase when one already owns the 5th edition? Of the completely new chapters and sections, probably the resumé on novel proteins is most relevant to food scientists. However, it is the overall up-dating which is the attraction and determining factor in making the 6th edition a 'must' for all readers with nutritional interests; a mention of the methyl ester of L-aspartyl-L-phenylalanine as a new artificial sweetener, the expansion of the section covering folic acid, the formula for determining the Biological Value of protein, are examples of these relatively small but useful additions. The 6th edition is characterized by the inclusion of a comprehensive summary of the respective Food Tables used by a large number of countries; this original reference source will be invaluable.

The only tentative reservation about the 6th edition of *Human Nutrition and Dietetics* is whether the linen-bound version will stand up to the constant use to which this essential reference book will inevitably be put.

K. Mary Clegg

Quality of Horticultural Products. By V. D. Arthey.
London: Butterworths, 1975. Pp. vii+228. £5.50.

Quality is of increasing importance to growers, pre-packers and the processing and preservation industries. The book is timely because interest in the subject has intensified following the United Kingdom's entry into the European Economic Community and the horticultural industry is now increasingly concerned with the standards and grades which apply to fresh produce in the Common Market. The close contractual relationship between growers and processors or growers and merchants, pre-packers or supermarkets is clearly described and the importance of quality in determining the price paid for crops such as early peas provides an excellent example of the importance of this factor. In addition to vegetables and fruits, other horticultural products such as cut flowers, bulbs and fruit trees are included and the scope of quality-determining factors comprises: nutritional value, colour, texture, condition, size, shape, form and style, yield, flavour, defects, plant health, processing qualities and seed quality where appropriate. The author is well qualified to deal with this complex subject being Head of the Agriculture Department of The Camden Food Preservation Research Association and responsible for the work of the Chipping Campden organization on quality appraisal of both raw and processed food products. The book is particularly directed to degree and diploma students of horticulture, food science and related subjects and to workers in the horticultural industry such as growers, advisers, processors and hopefully the consumer also. Its acceptance abroad may be prejudiced by the frequent use of imperial rather than metric or S.I. units.

Most chapters are well documented with references to original papers and authoritative texts, but unfortunately few to recent work, none to work published since 1972 and only four references each to papers published in 1971 and 1972.

Some readers may find that the chapters on Nutritional value and Flavour contain too much detail on certain aspects of these topics at the expense of broader principles. Future editions might, for example, provide more information, already available in specialized literature, on the extent to which fruits and vegetables, in the amounts that they are customarily consumed, supply our daily requirements of calories, mineral salts, vitamins, etc. and the extent to which nutrients and flavour are influenced by storage and processing, including cooking.

In spite of these minor criticisms, the book is very welcome as satisfying a definite need.

G. G. Freeman

Our Polluted Food, a Survey of the Risks. By J. Lucas.
London: Charles Knight, 1975. Pp. xi+237. £4.95.

This is a useful compilation. The author has collected figures from a variety of sources showing the concentration of such contaminants as pesticide residues, antibiotics and

hormones which have been found to occur in different foods. Figures are also given of the levels of naturally occurring radioactive elements as well as those derived from artificial sources. Analytical values for such elements as zinc, cadmium, mercury, lead, arsenic and selenium are helpful in making it possible to assess at what point the concentration in a food of plant or animal origin represents what can be expected to be present naturally, and when the level has reached a point at which the substance should be considered a contaminant. There are a number of helpful discussions of the relative importance which should be given to the presence of potentially harmful compounds at very low concentrations.

While the factual content of the book and the broadly based selection of references will be of value to members of the Institute, some of the more general statements of opinion, often of a pessimistic character, about the potential relationship of foods to cancer and cardiovascular disease are less helpful. This aspect of Dr Lucas' writing is perhaps exemplified in the title of the work which, in fact, tends to give a misleading impression of its main contents.

MAGNUS PYKE

Books Received

Statistical Methods of Quality Control. By A. K. S. JARDINE, J. D. MACFARLANE and C. S. GREENSTED.
London: Heinemann, 1975. Pp. ix + 195. £6.50 (hardback), £4.00 (limp).
A brief general introduction to statistical methods of quality control.

Fishery Products. Ed. by R. KREUZER.
West Byfleet: Fishing News (Books) for FAO, 1974. Pp. 462. £19.75.
The text of over eighty papers on many aspects of the utilization and marketing of fish and fish products read before an FAO conference in Tokyo in December 1973.

Methods of Sugar Analysis.
Peterborough: International Commission for Uniform Methods of Sugar Analysis, 1975. Pp. xi + 383. £6.00.
Proceedings of the 16th session of the commission covering all aspects of sugar analysis.

Food Processing Hygiene. By D. J. COOK and R. BINSTEAD.
London: Food Trade Press, 1975. Pp. 71. £2.50.
An elementary introduction to principles of food hygiene for students and technicians.

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Soybeans as a Food Source

W. J. Wolf and J. C. Cowan

The recent interest in soybeans as a source of food proteins and as the basis of unconventional foods stimulated the revision of this monograph. New developments in processing and technology are outlined and the growing potential of soybeans is discussed.

Selection from Contents Soybean production; Conversion to edible oil products; Food uses of soybean proteins; Physical and chemical properties; Nutritional properties; Food containing soy proteins; Problem areas.

Second Edition, 1975. 120 pages. £9.80.

CRC Press

Blackwell Scientific Publications

Oxford London Edinburgh Melbourne

WATER RELATIONS OF FOODS

Proceedings of an International Symposium held in Glasgow, September 1974

edited by R. B. Duckworth
Department of FoodScience and Nutrition
University of Strathclyde, Glasgow, Scotland

November/December 1975, xviii + 716 pp. £17.50/$45.25
0.12.223150.3

The aim of this volume is to make available to food scientists and technologists the information presented at the first symposium to deal comprehensively with all major aspects of the condition, properties and behaviour of the compositional water of foods.

These proceedings reflect the remarkable recent growth of knowledge in this field, which has resulted largely from the successful application of relatively new techniques such as nuclear magnetic resonance, dielectric methods and differential thermal analysis, but also in part from improvements in more traditional methods. Moreover, the book includes much novel information that has recently been obtained concerning the water relations of food micro-organisms and their responses to changes in the aqueous environment.

The topics range from the physico-chemical properties of water substance itself, and its interaction with other food constituents, through the water relations of food micro-organisms, to the influences that water and its properties can have on quality in both fresh and preserved foods—and the implications of this knowledge for current and possible future developments in food technology. Recent advances in methods of studying food water are also described, making the book a very useful practical as well as theoretical reference source for food scientists and technologists both academic and industrial.

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Water and its Molecular Interaction with other Constituents of Biological Systems

Methods and Criteria used in the Study of Water in Foods

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Non-enzymic Chemical Changes at Low and Intermediate Moisture Contents

Effects Associated with Freezing and Thawing

Aspects of the Condition and Properties of Water in Relatively Moist Food Materials

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JOURNAL OF FOOD TECHNOLOGY: NOTICE TO CONTRIBUTORS

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Typescripts (two complete copies) should be sent to the Editor, Dr H. Liebmann, c/o Research and Development Department, Metal Box Co. Ltd, Twyford Abbey Road, London NW10 7XQ. Papers should be typewritten on one side of the paper only, with a 1½ inch margin, and the lines should be double-spaced. In addition to the title of the paper there should be a 'running title' (for page headings) of not more than 45 letters (including spaces). The paper should bear the name of the author(s) and of the laboratory or research institute where the work has been carried out. The full postal address of the principal author should be given as a footnote. (The proofs will be sent to this author and address unless otherwise indicated.) The Editor reserves the right to make literary corrections.

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

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

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

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

SI UNITS

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

NON SI UNITS

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

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

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