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Harmonization of legislation on foodstuffs, food additives and contaminants

II. Achievements and programme

R. HAIGH

Summary

In an earlier article the consultative procedures used by the Commission for the development of its proposals were described in some detail (Haigh, 1978) and the role of the European Parliament and Economic and Social Committee placed in its legal context. The present article summarizes the achievements of the Community in the sector and gives pointers for the future.

Achievements and programme

If harmonization is to enrich the choice of the consumer and is to remove those technical obstacles to the development of a 'common market' the legislation has to treat subjects relevant to these aims and in a way which does not jeopardize the just rights of food producers, and consumers in the Member States. The programme is consequently fluid and has been modified several times over the years to take into account changed conditions. It is and will continue to be devised on the basis of a pragmatic choice of those products within a given sector that can possibly and sensibly be studied.

Free movement of all goods is neither a practical reality nor is it necessary for products which are of but local importance and whose producers have no aspirations to wider sales.

Information of potential problems may be transmitted to the Commission using the procedures described in Part I (Haigh, 1978) by the industries concerned, by consumer groups – who see different treatments of the same subject in different Member States, or by Government departments. Each of these groups could also make concrete proposals. The Commission itself has within its departments many experts who themselves may be able to anticipate a problem in a particular sector. All the advice the Commission receives is

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weighed carefully, examined by the various consultative groups and where appropriate a proposal for legislation is made to the Council.

In some cases it has been possible to include practically all forms of a product within the legislation so that all varieties can be traded freely in the Member States (e.g. preserved milk). In other cases a partial method has been adopted which controls only certain of the products within a sector (e.g. while sucrose is comprehensively controlled by the Directive on certain sugars, fructose is not).

A more flexible approach may be found in the optional method of harmonization under which a product complying with the provisions of the legislation may move freely in the Community, while at the same time allowing similar products with minor differences in composition or quality to move freely in one or other Member States – subject, of course, to that State's national legislation. The legislation thus developed ensures the free movement of goods without interference with national specialities or preferences. This approach has had considerable success in other sectors and is the basis of the Commission's proposal on jams and jellies.

Harmonization of food legislation is usually classified into 'vertical' (i.e. treatment of a specific group of similar products, for example emulsified sauces or coffee) or 'horizontal' (i.e. subjects of general application to all foods: additives, contaminants, labelling, etc.). The solution to such 'horizontal' problems has been shown to facilitate the subsequent discussion on 'vertical' subjects, and future activities will include a substantial effort on 'horizontal' topics.

HORIZONTAL

1. Additives

So far four groups of food additives are controlled comprehensively by Community legislation – colouring matters (Anon., 1962), preservatives (Anon., 1963), anti-oxidants (Anon., 1970) and emulsifiers (Anon., 1974a). Similar provision can be found in each of these Directives requiring that only substances enumerated may be used within the Community. The substances which Member States have agreed are given an 'E' number (see Table 1). With the notable exception of the Directive on Emulsifiers (etc.) an important and sometimes controversial provision requires that the use of these substances must not be subject to any general prohibition, although a Member State may suspend the authorization of any substance if new scientific information suggests that its use might endanger human health. In such circumstances the other Member States and the Commission must be informed so that a Community decision can be reached.

Member States are also instructed to take whatever measures are necessary to ensure that the substances in these classes are not placed on the market unless their packagings or containers are suitably labelled.

Table 1. Food additives and their EEC numbers (1 August 1978)

EEC No.	Common name
COLOURS	
E 100	Curcumin
E 101	Lactoflavin (Riboflavin)
E 102	Tartrazine
E 104	Quinoline yellow
E 110	Orange yellow S, sunset yellow FCF
E 120	Cochineal, carminic acid
E 122	Azorubine, Carmoisine
E 123	Amaranth
E 124	Cochineal Red A, Ponceau 4R
E 127	Erythrosine
E 131	Patent Blue V
E 132	Indigotin (Indigo carmine)
E 140	Chlorophylls
E 141	Copper complexes of chlorophylls and chlorophyllins
E 142	Acid brilliant green BS (lissamine green)
E 150	Caramel
E 151	Brilliant Black BN, Black PN
E 153	Carbo medicinalis vegetalis (charcoal)
E 160	Carotenoids:
	(a) alpha-, beta-, gamma-, carotene
	(b) Annatto (Bixin, Norbixin, Roucou)
	(c) Capsanthin, Capsorubin
	(d) Lycopene
	(e) Beta-apo-8′ carotenal (C 30)
	(f) Ethyl ester of beta-apo-8′ carotenoic acid (C 30)
E 161	Xanthophylls
	(a) Flavoxanthin
	(b) Lutein
	(c) Kryptoxanthin
	(d) Rubixanthin
	(e) Violoxanthin
	(f) Rhodoxanthin
	(g) Canthaxanthin
E 162	Beetroot red
	Betanin
E 163	Anthocyanins
E 170*	Calcium carbonate
E 171	Titanium dioxide
E 172	Iron oxides and hydroxides
E 173*	Aluminium
E 174*	Silver
E 175*	Gold
E 180†	Pigment rubine, lithol-rubin BK

* For surface colouring only.
† For colouring cheese rinds.

Table 1 – *continued*

The Member States shall, for diluting or dissolving the colouring matters listed in Annex 1, authorize the use of the following products only:

Sodium carbonate and sodium hydrogen carbonate	Sorbitol
Sodium chloride	Edible oils and fats
Sodium sulphate	Beeswax
Glucose	Water
Lactose	Citric acid
Sucrose	Tartaric acid
Dextrins	Lactic acid
Starches	Gelatin
Ethanol	Pectins
Glycerol	Ammonium, sodium and potassium alginates

L-ascorbic acid esters of the unbranched fatty acids C_{14}, C_{16} and C_{18} (authorized exclusively for the colouring matters listed under nos. E 160 and E 161).

This Directive shall not affect national rules concerning natural substances which are used in the manufacture of certain foodstuffs because of their aromatic, sapid or nutritive properties but which also have a subsidiary colouring property for example paprika, tumeric, saffron and sandal-wood in particular; colouring matters used for marking; colouring matters used for the shells of hard boiled eggs.

Member States may ... authorize the use in food of

the colours –

Brilliant Blue FCF
Brown FK
Chocolate Brown HT
Red 2G
Riboflavin-5′-phosphate
Yellow 2G

and for diluting and dissolving colouring matters –

ethyl acetate, diethyl ether, glycerol monoacetate, glycerol diacetate, glycerol triacetate, isopropyl alcohol, propylene glycol, acetic acid, sodium hydroxide, ammonium hydroxide.

EEC No.	Common name
PRESERVATIVES	
E 200	Sorbic acid
E 201	Sodium sorbate (sodium salt of sorbic acid)
E 202	Potassium sorbate (potassium salt of sorbic acid)
E 203	Calcium sorbate (calcium salt of sorbic acid)
E 210	Benzoic acid
E 211	Sodium benzoate (sodium salt of benzoic acid)
E 212	Potassium benzoate (potassium salt of benzoic acid)
E 213	Calcium benzoate (calcium salt of benzoic acid)
E 214	Ethyl *p*-hydroxybenzoate (ethyl ester of *p*-hydroxy-benzoic acid)
E 215	Sodium ethyl *p*-hydroxybenzoate
E 216	Propyl *p*-hydroxybenzoate (propyl ester of *p*-hydroxybenzoic acid)

Table 1 – *continued*

EEC No.	Common name
E 217	Sodium propyl *p*-hydroxybenzoate
E 218	Methyl *p*-hydroxybenzoate (methyl ester of *p*-hydroxybenzoic acid)
E 219	Sodium methyl *p*-hydroxybenzoate
E 220	Sulphur dioxide
E 221	Sodium sulphite
E 222	Sodium bisulphite (acid sodium sulphite)
E 223	Sodium metabisulphite (sodium pyrosulphite or sodium disulphite)
E 224	Potassium metabisulphite (potassium pyrosulphite or potassium disulphite)
E 226	Calcium sulphite
E 227	Calcium bisulphite (calcium hydrogen sulphite)
E 230*	Biphenyl (Diphenyl)
E 231*	Orthophenylphenol
E 232*	Sodium orthophenyl phenate
E 233†	2-(4′-thiazolyl)-benzimidazole (thiabendazole)
E 236	Formic acid
E 237	Sodium formate (sodium salts of formic acid)
E 238	Calcium formate (calcium salts for formic acid)
E 239‡	Hexamethylenetetramine
E 249	Potassium nitrite (solely in mixture with sodium chloride)
E 250	Sodium nitrite (solely in mixture with sodium chloride)
E 251	Sodium nitrate (alone or in mixture with sodium chloride)
E 252	Potassium nitrate (alone or in mixture with sodium chloride)
E 260	Acetic acid
E 261	Potassium acetate
E 262	Sodium diacetate
E 263	Calcium acetate
E 270	Lactic acid
E 280	Propionic acid
E 281	Sodium propionate (sodium salt of propionic acid)
E 282	Calcium propionate (calcium salt of propionic acid)
E 283	Potassium propionate (potassium salt of propionic acid)
E 290	Carbon dioxide

*Exclusively for the surface of citrus fruit (E 230 max. 70 ppm; E 231, E 232 max. 12 ppm as orthophenyl phenol).

† Exclusively for the surface treatment of bananas (max. 3 ppm) and citrus fruit (max. 6 ppm).

‡ Exclusively in provolone cheese (max. level 25 mg/kg expressed as formaldehyde).

Member States may ... maintain the provisions of their national laws relating to the use of:

formaldehyde in 'Grana padano' cheese provided that, when the product is marketed, it does not contain more than 0.5 mg/kg free or combined of formaldehyde.

Member States may ... authorize the use of:

hexamethylenetetramine in semi-preserved fish products (pH more than 4.5, max. level 50 mg/kg), caviar and other unsmoked fish eggs (max. level 1 g/kg).

This Directive shall not affect the provisions of national laws concerning:

(a) products used as foodstuffs but which may also have preservative properties, for example vinegar, sodium chloride, ethanol, edible oils, and sugars in particular;

Table 1 – *continued*

(b) nisin;
(c) products used for coating foodstuffs;
(d) products used to protect plants and plant products against harmful organisms;
(e) anti-microbial products used for the treatment of drinking water;
(f) antioxidants.

EEC No.	Common name
ANTIOXIDANTS	
E 300	L-Ascorbic acid
E 301	Sodium L-ascorbate (sodium of L-ascorbic acid)
E 302	Calcium L-ascorbate (calcium salt of L-ascorbic acid)
E 303	5, 6-diacetyl-L-ascorbic acid (ascorbyl diacetate)
E 304	6-palmityl-L-ascorbic acid (ascorbyl palmitate)
E 306	Tocopherol-rich extracts of natural origin
E 307	Synthetic Alpha-tocopherol
E 308	Synthetic Gamma-tocopherol
E 309	Synthetic Delta-tocopherol
E 310	Propyl gallate
E 311	Octyl gallate
E 312	Dodecyl gallate
E 320	Butylated hydroxyanisole (BHA)
E 321	Butylated hydroxytoluene (BHT)
SUBSTANCES WITH OTHER FUNCTIONS	
E 220	Sulphur dioxide
E 221	Sodium sulphite
E 222	Sodium bisulphite (acid sodium sulphite)
E 223	Sodium metabisulphite (sodium pyrosulphite or sodium disulphite)
E 224	Potassium metabisulphite (potassium pyrosulphite or potassium disulphite)
E 226	Calcium sulphite
E 322	Lecithins
ANTIOXIDANT SYNERGISTS	
E 270	Lactic acid
E 325	Sodium lactate (sodium salt of lactic acid)
E 326	Potassium lactate (potassium salt of lactic acid)
E 327	Calcium lactate (calcium salt of lactic acid)
E 330	Citric acid
E 331	Sodium citrates (sodium salts of citric acid)
E 332	Potassium citrates (potassium salts of citric acid)
E 333	Calcium citrates (calcium salts of citric acid)
E 334	Tartaric acid
E 335	Sodium tartrates (sodium salts of tartaric acid)
E 336	Potassium tartrates (potassium salts of tartaric acid)
E 337	Sodium potassium tartrate
E 338	Orthophosphoric acid
E 339	Sodium orthophosphates (sodium salts of orthophosphoric acid)
E 340	Potassium orthophosphates (potassium salts of orthophosphoric acid)
E 341	Calcium orthophosphates (calcium salts of orthophosphoric acid)
E 472 c	Citric acid ester of mono and di-glycerides of food fatty acids (citroglycerides)

Table 1 – *continued*

Substances in which antioxidants can be dissolved or diluted:

1. Drinking water, demineralized water, distilled water
2. Edible oils
3. Edible fats
4. Ethyl alcohol
5. Glycerol
6. Sorbitol
7. Propylene glycol (1,2-propanediol).

Member States may ... maintain the provisions of their national laws authorizing the use in foodstuffs of calcium disodium ethylene diamine-tetra-acetate, and ethoxyquin for the treatment of apples and pears (max level 3 mg/kg whole fruit).

EEC No.	Common name
EMULSIFIERS, STABILIZERS, THICKENERS AND GELLING AGENTS	
E 322	Lecithins
E 339	Sodium orthophosphates
E 340	Potassium orthophosphates
E 341	Calcium orthophosphates
E 400	Alginic acid
E 401	Sodium alginate
E 402	Potassium alginate
E 403	Ammonium alginate
E 404	Calcium alginate
E 405	Propane-1,2-diol alginate
E 406	Agar
E 407	Carrageenan
E 410	Locust bean gum
E 412	Guar gum
E 413	Tragacanth
E 414	Acacia
E 420	Sorbitol and sorbitol syrup
E 421	Mannitol
E 422	Glycerol
E 440(a)	Pectin
E 440 (b)	Amidated pectin
E 450	Sodium and potassium polyphosphates:
E 450(a)	Diphosphates: $Na_2H_2P_2O_7$; $Na_3HP_2O_7$; $Na_4P_2O_7$; $K_4P_2O_7$
E 450(b)	Triphosphates: $Na_5P_3O_{10}$; $K_5P_3O_{10}$
E 450(c)	Linear polyphosphates
E 460	Microcrystalline cellulose
E 461	Methylcellulose
E 463	Hydroxypropylcellulose
E 464	Hydroxypropylmethylcellulose
E 465	Ethylmethylcellulose
E 466	Carboxymethylcellulose
E 470‡	Sodium, potassium and calcium salts of fatty acids
E 471	Mono- and di-glycerides of fatty acids

Table 1 – *continued*

EEC No.	Common name
E 472(a)	Acetic acid esters of mono- and di-glycerides of fatty acids
E 472(b)	Lactic acid esters of mono- and di-glycerides of fatty acids
E 472(c)	Citric acid esters of mono- and di-glycerides of fatty acids
E 472(d)	Tartaric acid esters of mono- and di-glycerides of fatty acids
E 472(e)	Mono- and diacetyltartaric acid esters of mono- and di-glycerides of fatty acids
E 472(f)	Mixed acetic and tartaric acid esters of mono- and di-glycerides of fatty acids
E 473§	Sucrose esters of fatty acids
E 474§	Sucroglycerides
E 475	Polyglycerol esters of fatty acids
E 477	Propane-1,2-diol esters of fatty acids
E 481§	Sodium stearoyl-2-lactylate
E 482§	Calcium stearoyl-2-lactylate
E 483§	Stearyl tartrate

‡ Exclusively in the manufacture of 'Dutch' type rusks (max. level 1.5% of the flour used). May be present at not more than 6% (expressed as sodium oleate) in E 471, E 472(b), E 473, E 474, E 475 and E 477.

§ Not to be used in bread unless permitted by national law.

Member States may . . . authorize the use in food of the following:

Karaya gum (synonym: sterculia gum)
Partial polyglycerol esters of polycondensed fatty acids of castor oil
Sorbitan monopalmitate
Sorbitan monostearate
Sorbitan tristearate
Polyoxyethylene (20) sorbitan monolaurate (synonym: polysorbate 20)
Polyoxyethylene (20) sorbitan monopalmitate (synonym: polysorbate 40)
Polyoxyethylene (20) sorbitan monostearate (synonym: polysorbate 60)
Polyoxyethylene (20) sorbitan tristearate (synonym: polysorbate 65)
Polyoxyethylene (20) sorbitan mono-oleate (synonym: polysorbate 80)
Polyoxyethylene (8) stearate
Polyoxyethylene (40) stearate
Glyceric esters of fatty acids obtained from soya oil oxidized under heat
Ghatti gum
Xanthan gum
Extract of quillaia
Lactylated fatty acid esters of glycerol and propylene glycol
Sorbitan monolaurate
Sorbitan mono-oleate
Dioctyl sodium sulphosuccinate
Ammonium phosphatides (synonym: emulsifier YN)

This Directive does not affect national legislation on:

(a) foodstuffs which have emulsifying, stabilizing, thickening or gelling properties, such as, for example, eggs, flour and starches;

(b) emulsifiers used in release agents;

(c) acids, bases and salts which, when added to a foodstuff during manufacture, change or stabilize the pH;

Table 1 – *continued*

(d) blood plasma, modified starches, edible gelatine and hydrolysed food proteins and their salts.
(e) certain products containing pectin derived from ... (defined) ... fruits.

The Directives have been adopted over a period of 12 years and these labelling provisions are not the same in each case. Special problems exist for one class which are not relevant to another and ideas have developed since the adoption of the first of the series (on colouring matters). Broadly speaking, however, the number and/or name of the substance and the name and address of a manufacturer or seller within the Community must be indicated on the container with a declaration to the effect that the product is for 'food use'.

Recently the Community finalized its work on the establishment of specific purity criteria for 'E' numbered additives. General purity criteria limiting the amounts of heavy metals and other potentially dangerous elements are stipulated in each Directive.

The methods of analysis needed to verify that the general and specific criteria of purity are satisfied, the procedure for taking samples and the methods for the qualitative and quantitative analysis for these substances on food, have yet to be adopted (using the procedure involving the Standing Committee for Foodstuffs). They are the subject of active consideration at the present time in Commission working parties. Since 1962 there has been a considerable development in methods of toxicological assessment and the criteria used to guarantee that, on the basis of the available evidence, the use of a particular additive does not endanger the health of the consumer. The texts have been modified several times to take into account changed circumstances and to recognize the availability of new toxicological data on several of the listed substances. The opinion of the Scientific Committee for Food is now a recognized part of the procedure. Indeed, its advice is requested not only in relation to food additives, but also to foodstuffs (see Table 2).

The accession to the Community of Denmark, Ireland and the United Kingdom with differences of techniques of usage of additives (and in particular different lists) complicated the adoption of 'community lists', and the Commission concluded that this would require, in the case of colouring matters, a complete review of substances in the Directive. The Directives on Antioxidants and Preservatives were reviewed on an *ad hoc* basis. The review of the Directive on Emulsifiers (etc.) is not yet completed (see below).

Colouring matters

An essential criterion for the review of the Directive was the advice of the Scientific Committee for Food. The Committee was asked to examine the available toxicological data on all colouring matters in the Directive or requested for inclusion within the Directive. The Committee broadly classified the colouring matters into three groups – those which could be accepted, those

Table 2. Published opinions of the Scientific Committee for Food

Reports of the Scientific Committee for Food (1st series)

Sodium methyl parahydroxybenzoate, potassium nitrite and potassium propionate
(opinion expressed 15 November 1974)

Mercury in food
(opinion expressed 16 November 1974)

Rapeseed oils
(opinion expressed 16 November 1974)

Revision of the Directive on colouring matters authorized for use in foodstuffs intended for human consumption
(opinion expressed 27 June 1975)

Vinyl chloride monomer
(opinion expressed 27 June 1975)

Ethoxyquin
(opinion expressed 13 November 1975)

Reports of the Scientific Committee for Food (2nd series)

Amaranth
(opinion expressed 27 February 1976)

Some chemically modified starches
(opinion expressed 27 February 1976)

Research necessary on long chain fatty acids and oils and fats used in food
(opinion expressed 2 April 1976)

Thiabendazole
(opinion expressed 2 April 1976)

Propyl gallate
(opinion expressed 2 July 1976)

Reports of the Scientific Committee for Food (3rd series)

Guidelines for toxicological evaluation of a substance for materials and articles intended to come into contact with foodstuffs

Reports of the Scientific Committee for Food (4th series)

Saccharin
(opinion expressed 24 June 1977)

Calcium disodium ethylenediamine tetra-acetate
(opinion expressed 24 June 1977)

Colouring matters authorized for use in foodstuffs intended for human consumption
(opinion expressed 16 September 1977)

Formaldehyde in 'Grana Padano' cheese
(opinion expressed 20 October 1977)

Reports of the Scientific Committee for Food (5th series)

Elements of information given to the Commission on the use of additives for which no Acceptable Daily Intake has been allocated
(16 March 1978)

Fine Bakers' Wares, Rusks, Pastries and Biscuits
(opinion expressed 1 May 1978)

which could not be accepted and those for which they had certain reservations and which should be subjected to further toxicological studies before the end of 1978.

The Council accepted the Commission's proposal to prohibit the unacceptable colouring matters. Until the revision of the toxicological data on the 'temporarily acceptable' colouring matters takes place in 1978/79 one objective of the Directive (that is to authorize within the Member States only those substances listed in the Directive) cannot be attained and the Directive permits individual Member States to use, for the time being, certain colouring matters not yet accepted at Community level.

Purity criteria are specified for each colouring matter and diluent listed in the Directive. The colouring matter may only be marketed if its number is indicated on the packaging or container with the phrase 'colouring matter for foodstuffs'.

Preservatives

The Directive lists permitted preservatives (and substances intended mainly for other purposes but which may have a subsidiary preservative effect). As in the case of colouring matters for a few preservatives a Community solution has not yet been found.

As far as the labelling of preservatives sold as such is concerned, the provisions differ from those on colouring matters by the requirement to state both name *and* EEC number, and by the obligation to list the percentage of any particular preservative in a mixture. A statement has to be included that the preservatives are 'for foodstuffs (restricted use)' as a reminder to the potential user that the presence of a preservative on the list does not imply complete freedom of use in any foodstuff. Indeed in some cases the Directive itself controls on a Community basis the uses to which preservatives can be put. For example certain preservatives are specifically mentioned as being for use on citrus fruit and the control measures to be used for the qualitative and quantitative analysis of their residues are regulated (Anon., 1967).

Other methods of analysis and sampling have not yet been adopted. Purity criteria for preservatives have already been established (Anon., 1965).

There is some ambiguity as to whether additives to be used on citrus fruit (and other fruits and vegatables) should be classed as preservatives or pesticides, or even as a separate category of 'fruit treatment agents'. The Commission intends to propose measures to clarify the situation.

Antioxidants

The same format was applied in the development of the Community rules on antioxidants. A comprehensive annex lists the antioxidants, substances having an antioxidant effect and also other functions, substances capable of increasing the antioxidant effect of other substances, and substances in which each of

these groups may be dissolved or diluted. Labelling requirements for sales to subsequent users are comparable to those specified for preservatives. Purity criteria for antioxidants have recently been adopted by the Council (Anon., 1978d). Methods of analysis and sampling have not yet been proposed.

Emulsifiers and similar substances

The Council Directive on emulsifiers, stabilizers, thickeners and gelling agents is unique in its not inconsiderable list of substances, the use of which could not be agreed at Community level in 1974 but which may be authorized by individual Member States should they so wish. This problem has to be reconsidered during 1978 and the Commission is consulting with interested organizations in order to arrive at a Community decision by June 1979. In other respects the Directive is substantially similar to other food additive Directives. Purity criteria for emulsifiers (etc.) have recently been adopted by the Council (Anon., 1978c). Methods of analysis and sampling have not yet been proposed.

As yet only these four classes of additives are controlled by Community legislation, but this is not to say that other classes are not under consideration.

Flavourings

Flavourings have been used for a long time in the food industry. For the manufacture of their products, food processors have used substances to give characteristic flavour and aroma to render them more appetizing. These substances may be naturally derived or artificially synthesized and the Commission is at present developing a proposal for their classification. Discussions are being held with a group of government experts and with professional organizations having particular interest in the subject (e.g. flavouring producers, food manufacturers, consumers, etc.) on the best means of implementing this proposal. The Scientific Committee for Food has been consulted but has not yet given its advice.

Solvents

The extraction of natural flavours, colours (and other additives), and the extraction of foodstuffs (e.g. for the decaffeination of coffee, treatment of oils and fats) requires the extensive use of solvents. These processes leave unavoidable residues in the final food which if not controlled properly could be a potential risk to health. The question is under consideration by the Commission. Solvents are also used as diluents for additives and as carriers for certain foodstuffs. This application may require a different approach to that which might be envisaged for 'extraction solvents' which could more appropriately be consi-

dered as contaminants, at least as far as the labelling of the final food is concerned.

Acids, bases and salts

The Commission has recently issued a preliminary working document on the listing of acids, bases and salts which follows the principles which have been accepted in other additive Directives.

Special problems

From time to time particular problems may arise on food additives (perhaps not generally subject to Community legislation). These problems may be drawn to the attention of the Commission by the ways mentioned earlier. The European Parliament is particularly attentive in these matters and it can be expected that such 'special problems' as amaranth, asbestos (as a filter aid) and saccharin will continue to be presented to the Commission. These topics receive the same careful scrutiny as would be given during a comprehensive 'class' review. The result of these examinations is not always Community legislation. In the case of saccharin, for example, the Commission recommends (Anon., 1978b) that Member States follow the advice of the Scientific Committee for Food.

Conditions of use of food additives

The adoption of Directives on classes outlined above will substantially include the main categories of additives used within the Community. The Commission is exploring the possibility and practicability of implementing the second stage referred to in all existing food additive Directives, before considering other classes (e.g. anticaking agents and artificial sweeteners) that is to say the development of what are known as 'conditions of use'.

So far, with few exceptions, no attempt has been made to list the foods in which the permitted additives may be used, and inevitably free movement of goods is hampered by national provision on the matter. If the consumer is to be protected against widespread or excessive use of a particular additive (which might be completely inoffensive at normal use levels, while being potentially dangerous if used in excess), the conditions for its use have to be specified on a Community basis – at least in products which are able to circulate freely in all Member States. For this exercise to be effective, accurate knowledge of the levels of use which are technologically necessary and the dietary intake of food by the population of the Community has to be obtained in order to compare this information with the toxicological assessment of the situation.

The differences in dietary habits and the difficulties in obtaining accurate information of levels of use for all foods in each Member State create massive obstacles to the pursuance of the objective, which so far have not been removed.

2. Contaminants

Packaging materials

The Directive on the approximation of the laws of the Member States relating to materials and articles intended to come into contact with foodstuffs (Anon., 1976a), lays down the general principles on the basis of which legal differences may be eliminated by the subsequent development of Directives on specific subjects such as the substances to be authorized in the production of packaging materials, purity standards for such substances, limits of migration of components of packaging materials into foodstuffs and general rules to ensure the implementation of these specific requirements. Special provisions for regenerated cellulose, ceramics, etc., are at various stages of advancement in Council or Commission working parties. The Directive is unique in the food sector in requiring the advice of the Scientific Committee for Food as a prerequisite to proposing modifications, and in the introduction of the idea of a provisional approval by the individual Member States prior to the adoption of a measure by the Community.

This basic Directive requires that materials and articles must not transfer any constituents to foodstuffs in quantities which could endanger human health. Administration of large doses of vinyl chloride monomer to experimental animals has been shown to produce harmful effects and the Scientific Committee for Food advised that VCM in polyvinyl chloride and related polymers should be reduced as far as possible. In applying the Committee's advice the Council has recently adopted a Commission proposal limiting VCM to a maximum of 1 mg/kg in the final material or article (Anon., 1978a).

Erucic acid

Although *erucic acid* can hardly be considered a contaminant of rapeseed oil, its level in mixtures of oils or in food products could be interpreted in this way.

When as a result of concern over the possible harmful effects of this acid, the Scientific Committee for Food advised that it would be prudent to reduce its intake in humans, the Council fixed the maximum level of erucic acid in oils and fats intended as such for human consumption and in foodstuffs containing added oils or fats (Anon., 1976b).

Heavy metals and similar materials

The Commission initiated discussions on the contaminants in food some time ago and its programmes repeatedly acknowledge the necessity of controlling contamination by such materials as mercury, lead, arsenic, cadmium, asbestos and mycotoxins. Although discussions with interested organizations continue to take place (for example the Scientific Committee for Food has advised on mercury and is studying asbestos) no Directive has yet

been proposed to the Council. The work is hampered by a lack of knowledge of the intake of foodstuffs containing these contaminants. As noted above, there is control of heavy metal contamination of food additives by the provisions in the Directives on colours, preservatives, antioxidants and emulsifiers.

3. Foodstuffs for special nutritional uses

The Council Directive implementing the first of the Community rules on foodstuffs for particular nutritional uses (Anon., 1977a) gives guidelines for the preparation of subsequent specific Directives on particular groups of 'dietetic' (or dietary) foodstuffs. At the moment no such 'specific' Directives (e.g. low sodium foods) have been adopted.

4. Labelling

In order to resolve many of the obstacles to the development of Directives in particular food sectors (i.e. 'vertical' Directives) the Commission has proposed to the Council measures on the labelling, presentation, and advertising of foodstuffs. The Council is devoting a considerable amount of attention to this subject in order to find agreement as soon as possible.

VERTICAL

The achievements of the Community and the principal items on the programme dealing with vertical questions can be summarized as follows.

Cocoa and Chocolate products

Community rules have been established by Directive (Anon. 1973a), on definitions and rules on composition, manufacture, specifications, packaging and labelling. The adoption of this Directive which was the first of the 'verticals' was complicated by the differences in production techniques and consumer preferences in the several Member States. Its adoption took many years, partly at least because its format was critically examined as a precedent for other foodstuffs Directives.

Certain sugars

The Directive (Anon., 1973b) on certain sugars specifies standards for: semi-white sugar, white sugar, sugar solution, invert sugar solution, invert sugar syrup, glucose syrup, dried glucose syrup and dextrose (anhydrous and monohydrate).

Permitted weights of packages are specified and limits for residual sulphur dioxide are also prescribed.

Honey

The Directive (Anon., 1974b) defines the terms 'honey' and makes provision for the different varieties which may be marketed under appropriate names. The main information which should appear on labels is specified.

Fruit juices and certain similar products

The Directive (Anon., 1975) controls the composition manufacturing specifications and reserves descriptions for: fruit juices (including reconstituted juice), concentrated fruit juice, fruit nectars and dried fruit juice.

Preserved milk

The Community rules control the composition manufacturing specifications and reserved description for certain partly dehydrated and certain wholly dehydrated milks defined within the Council Directive of 18 December 1975 relating to the subject in question (Anon., 1976c).

Coffee extracts

The Directive on coffee and chicory extracts (Anon., 1977b) includes provisions on extraction rate, weight, and labelling of these products and their mixtures. Decisions on the type of solvent which might be used in their preparation were postponed until the question on the use of solvents has been resolved 'horizontally'.

FUTURE ACTIVITIES

The management of Directives already adopted by the Council requires the attention of the Commission if resolution of the outstanding problems, solutions to which could not be found when the Directives were adopted, is to be a practical reality. Indeed, every adopted Directive contains provisions of this sort which are presently being re-examined. This involvement has meant that the resources in material and manpower available for new work do not permit rapid development of Community legislation on all the topics detailed in the Council Resolution of 17 December 1973 on Industrial Policy (Anon., 1973c), nor to complete the examination of such topics that have been under consideration. Indeed, it is clear that some subjects (e.g. beer and bread) are not likely to be 'harmonized' in the near future — and there are many who would agree that it would not be a bad conclusion to abandon these topics for all

time! On the other hand the Council seems likely to agree 'EEC' standards on mineral waters and jams and jellies. Commission working parties are actively examining the possibility of standards for frozen foods and modified starches; standards for caseinates may shortly be transmitted to the Council. There is also a strong possibility that preliminary work in Commission working parties on oils and fats, and soft drinks may give some positive results during 1978 or 1979.

As for other foodstuffs, if pragmatism is to be the key and if the Commission is to be able to treat problems as they arise, it is impossible to adhere to an exact programme and timescale, and it would be misleading to be more precise. For this reason alone it is obvious that this list of future activities is necessarily incomplete and it is incumbent on those with a particular interest to keep abreast of affairs using the possibilities offered by the consultations referred to in the first part of this article.

References

Anon. (1962) Council Directive of 23 October 1962, on the approximation of the rules of the Member States concerning the colouring matters authorized for use in foodstuffs intended for human consumption. *Official Journal of the European Communities*, No. 115 of 11.11.1962, p. 2645/62.

Modifications:

Directive 65/469/EEC of 25 October 1965. *O.J.* 178 of 26.10.1965, p. 2793/65.
Directive 67/653/EEC of 24 October 1967. *O.J.* 263 of 30.10.1967, p. 4.
Directive 68/419/EEC of 20 December 1968. *O.J.* L309 of 24.12.1968, p. 24.
Directive 70/358/EEC of 13 July 1970. *O.J.* L157 of 18.7.1970, p. 36.
Treaty of Accession. *O.J.* L73 of 27.3.1972, p. 14.
Directive 76/399/EEC of 4 April 1976. *O.J.* L108 of 26.4.1976, p. 19.
Directive 78/144/EEC of 30 January 1978. *O.J.* L44 of 15.2.1978, p. 20.

Anon. (1963) Council Directive 64/54/EEC of 5 November 1963, on the approximation of the rules of the Member States concerning the preservatives authorized for use in foodstuffs intended for human consumption. *Official Journal of the European Communities*, No. 12 of 27.1.1964, p. 161/64.

Modifications:

Directive 65/569/EEC of 23 December 1965. *O.J.* 222 of 28.12.1965, p. 3263/65.
Directive 66/722/EEC of 14 December 1966. *O.J.* 233 of 20.12.1966, p. 3947/66.
Directive 67/427/EEC of 27 June 1967. *O.J.* 148 of 11.7.1967, p. 1.
Directive 68/420/EEC of 20 December 1968. *O.J.* L309 of 24.12.1968, p. 25.
Directive 70/359/EEC of 13 July 1970. *O.J.* L157 of 18.7.1970, p. 38.
Directive 71/160/EEC of 30 March 1971. *O.J.* L87 of 17.4.1971, p. 12.
Directive 72/2/EEC of 20 December 1971. *O.J.* L2 of 4.1.1972, p. 22.
Treaty of Accession. *O.J.* L73 of 27.3.1972, p. 14.
Directive 72/444/EEC of 26 December 1972. *O.J.* L298 of 31.12.1972, p. 48.
Directive 74/62/EEC of 17 December 1973. *O.J.* L38 of 11.2.1974, p. 29.
Directive 74/394/EEC of 22 July 1974. *O.J.* L208 of 30.7.1974, p. 25.
Directive 76/462/EEC of 4 May 1976. *O.J.* L126 of 14.5.1976, p. 30.
Directive 76/629/EEC of 20 July 1976. *O.J.* L223 of 16.8.1976, p. 3.
Directive 78/145/EEC of 30 January 1978. *O.J.* L44 of 15.2.1978, p. 23.

Anon. (1965) Council Directive 65/66/EEC of 26 January 1965, laying down specific criteria of purity for preservatives authorized for use in foodstuffs intended for human consumption. *Official Journal of the European Communities,* No. 22 of 9.2.1965, p. 373/65.

Rectification:

O.J. 126 of 12.7.1965.

Modifications:

Directive 67/428/EEC of 27 June 1967. *O.J.* 148 of 11.7.1967, p. 10.
Directive 76/463/EEC of 4 May 1976. *O.J.* L126 of 14.5.1976, p. 33.

Anon. (1967) Council Directive 67/427/EEC of 27 June 1967, on the use of certain preservatives for the surface treatment of citrus fruit and on the control measures to be used for the qualitative and quantitative analysis of preservatives in and on citrus fruit. *O.J.* 148 of 11.7.1967, p. 1.

Anon. (1970) Council Directive 70/357/EEC of 13 July 1970, on the approximation of the laws of the Member States concerning the antioxidants authorized for use in foodstuffs intended for human consumption. *Official Journal of the European Communities,* No. L157 of 18.7.1970, p. 31.

Rectification:

O.J. L18 of 22.1.1972, p. 12.

Modifications:

Treaty of Accession. *O.J.* L73 of 27.3.1972, p. 14.
Directive 74/412/EEC of 1 August 1974. *O.J.* L221 of 12.8.1974, p. 18.
Directive 78/143/EEC of 30 January 1978. *O.J.* L44 of 15.2.1978, p. 18.

Anon. (1973a) Council Directive 73/241/EEC of 24 July 1973, on the approximation of the laws of the Member States relating to cocoa and chocolate products intended for human consumption. *Official Journal of the European Communities,* No. L228 of 16.8.1973, p. 23.

Modifications:

Directive 74/411/EEC of 1 August 1974. *O.J.* L221 of 12.8.1974, p. 17.
Directive 74/644/EEC of 19 December 1974. *O.J.* L349 of 28.12.1974, p. 63.
Directive 75/155/EEC of 4 March 1975. *O.J.* L64 of 11.3.1975, p. 21.
Directive 76/628/EEC of 20 July 1976. *O.J.* L223 of 16.81976, p. 1.
Directive 78/609/EEC of 29 June 1978. *O.J.* L197 of 22.7.1978, p. 10.

Anon. (1973b) Council Directive 73/437/EEC of 11 December 1973, on the approximation of the laws of the Member States concerning certain sugars intended for human consumption. *Official Journal of the European Communities,* No. L356 of 27.12.1973, p. 71.

Anon. (1973c) Council Resolution of 17 December 1973, on industrial policy. *Official Journal of the European Communities,* No. C117 of 31.12.1973, p. 1.

Anon. (1974a) Council Directive 74/329/EEC of 18 June 1974, on the approximation of the laws of the Member States relating to emulsifiers, stabilizers, thickeners and gelling agents for use in foodstuffs. *Official Journal of the European Communities,* No. L189 of 12.7.1974, p. 1.

Modification:

Directive 76/612/EEC of 29 June 1978. *O.J.* L197 of 22.7.1978, p. 22.

Anon. (1974b) Council Directive 74/409/EEC of 22 July 1974, on the harmonization of the laws of the Member States relating to honey. *Official Journal of the European Communities,* No. L221 of 12.8.1974, p. 10.

Anon. (1975) Council Directive 75/726/EEC of 17 November 1975, on the approximation of the laws of the Member States concerning fruit juices and certain similar products. *Official Journal of the European Communities,* No. L311 of 1.12.1975, p. 40.

Anon. (1976a) Council Directive 76/893/EEC of 23 November 1976, on the approximation of the laws of the Member States relating to materials and articles intended to come into contact with foodstuffs. *Official Journal of the European Communities,* No. L340 of 9.12.1976, p. 19.

Anon. (1976b) Council Directive 76/621/EEC of 20 July 1976, relating to the fixing of the maximum level of erucic acid in oils and fats intended as such for human consumption and in foodstuffs containing added oils or fats. *Official Journal of the European Communities,* No. L202 of 28.7.1976, p. 35.

Anon. (1976c) Council Directive 76/118/EEC of 18 December 1975, on the approximation of the laws of the Member States relating to certain partly or wholly dehydrated preserved milk for human consumption. *Official Journal of the European Communities,* No. L24, of 30.1.1976, p. 49.

Anon. (1977a) Council Directive 77/94/EEC of 21 December 1976, on the approximation of the laws of the Member States relating to foodstuffs for particular nutritional uses. *Official Journal of the European Communities,* No. L26 of 31.1.1977, p. 55.

Anon. (1977b) Council Directive 77/436/EEC of 27 June 1977, on the approximation of the laws of the Member States relating to coffee extracts and chicory extracts. *Official Journal of the European Communities,* No. L172 of 12.7.1977, p. 20.

Anon. (1978a) Council Directive 78/142/EEC of 30 January 1978, on the approximation of the laws of the Member States relating to materials and articles which contain vinyl chloride monomer and are intended to come into contact with foodstuffs. *Official Journal of the European Communities,* No. L44 of 15.2.1978, p. 15.

Anon. (1978b) Commission Recommendation of 29 March 1978 to the Member States on the use of saccharin as a food ingredient and for sale as such in tablet form to the final consumer. *Official Journal of the European Communities,* No. L103 of 15.4.1978, p. 32.

Anon. (1978c) Council Directive 78/663/EEC of 25 July 1978 laying down specific criteria of purity for emulsifiers, stabilizers, thickeners and gelling agents for use in foodstuffs. *O.J.* L223 of 14.8.1978, p. 7.

Anon. (1978d) Council Directive 78/664/EEC of 25 July 1978 laying down specific criteria of purity for antioxidants which may be used in foodstuffs intended for human consumption. *O.J.* L223 of 14.8.1978, p. 30.

Haigh, R. (1978) *J. Fd Technol.* **13**, 255.

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J. Fd Technol. (1978) **13**, 511–519

Behaviour of some volatile compounds during storage of orange juice powder with low and intermediate moisture contents

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Summary

The changes in flavour of orange juice powder at low and intermediate moisture contents have been studied by sensory analysis, as a function of time and temperature (27–69°C). The development of some volatile components has also been studied by gas chromatography.

The volatiles, which show the most significant increase when a change in flavour becomes detectable by the taste-testing panel, are furfural and diacetyl, specially in the product with the lower moisture contents (2–15% water content). In the product with higher water contents (20–40%), the concentrations of these two volatiles exhibit a smaller increase, but the increase in the amounts of α-terpineol and 3-methyl-2-buten-1-ol contents become more important.

It is suggested that under these experimental conditions, the origin of diacetyl may be oxidation of acetoin.

Introduction

Several investigations have been devoted to the changes in orange juice during storage: Huskins, Swift & Veldhuis (1952) and Huskins & Swift (1953) have studied the changes affecting nitrogen, phosphorus and fatty constituents; Blair *et al.* (1952) have discussed the chemical reactions which can be responsible for changes in flavour; Rymal *et al.* (1968), Askar, Bieling & Treptow (1973) and Tatum, Nagy & Berry (1975) have investigated the modifications in volatiles composition. For orange juice in the dried state, Tatum, Shaw & Berry (1967) identified several volatiles formed in non-enzymatic browning processes. Knowledge is however limited concerning quantitative changes of volatiles,

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especially in the initial period of storage, when a taste-testing panel is still not able of detecting, or begins to detect, a change in flavour.

The behaviour of volatiles in orange juice powder with low and intermediate moisture contents, at room temperature or slightly higher ones, is, however, of great practical significance, since these conditions can be met during the storage of the dried product, or during the final steps of the drying processes. These final steps are known to be the most deleterious in drying operations of products such as fruit juices, due to the high temperatures which may then be encountered.

In the present investigation, we have tried to correlate the changes in flavour, as observed by a tasting panel, with the behaviour of some volatiles. The product examined was freeze dried orange juice rehydrated to moisture contents ranging from 3 to 40%: the lower figures are similar to residual moistures of industrial dried fruit products; the higher ones correspond to moisture contents of the product during the final steps of drying operations.

Methods

Orange juice

A commercial orange concentrate (65°C Brix*) has been mixed with fresh juice (12° Brix), obtained in the laboratory by pressing fruits, in order to prepare a concentrate of good flavour (26° Brix – Product A). Product B was a fresh juice (12° Brix) obtained in the laboratory. Products A and B were frozen immediately after preparation and then freeze dried in operating conditions which were not deleterious to flavour.

Processing of samples

The dry material was conditioned in aluminium tubes (length 18 cm, diameter 3.5 cm, thickness 0.01 cm) which were closed by flattening the open end and covering it with adhesive tape. These tubes allowed the rather large amounts of material which were necessary for the panel (120 g per tested sample, in two tubes) to be submitted to abrupt changes in temperature.

The tubes were placed in thermostated and ventilated chambers for conditioning at moderate temperature (22–47°C) and in stirred water baths for conditioning at the higher temperatures (52–69°C). The temperature fluctuated less than ± 0.4°C. At the end of the desired heating time, rapid cooling of the tubes was obtained by immersing them in cold water. A part of the dry product, which was given as a reference sample to the panel, was stored at –35°C.

* ° Brix = sucrose content ($g\,l^{-1}$) of a solution which should give the same refractometer figure as the tested product.

The different moisture contents were obtained by adding water to the tubes before closing them. The residual moisture of the product after freeze drying, measured by the Karl Fischer method, was taken into account in the calculations of water contents (expressed as wt/wt%).

Sensory analysis

The rehydrated samples (10° Brix) were evaluated by a panel of twenty judges who were accustomed to the taste testing of juices. The judges were offered five samples (including the control) and were asked to rank them on a 1–9 scale. The results were submitted to the variance analysis method and to the Duncan test. The samples were exposed to each conditioning temperature for various time intervals. A critical time for flavour alteration (CTFA) was determined by interpolation between the time when the product was first judged to be different from the control (and rated lower) and the time of the preceding tasting when the sample was not found significantly different from the control.

Analysis of the volatiles

Extraction. The dry material (150 g) was rehydrated with 400 ml of distilled water; 2 ml of a 4-methyl-2-pentanone solution (100 ppm) were added to the juice as an internal standard. The volatiles were extracted by steam stripping under reduced pressure and at a temperature not exceeding 35°C. The mixture (300 ml) which was collected in traps at 0 and −196°C was saturated with $(NH_4)_2SO_4$, and volatiles extracted with ethyl ether. After separation from the aqueous phase, the ether phase was dried over Na_2SO_4, then concentrated to 10 ml by raising the temperature, and finally 0.5 ml under nitrogen flow. Chemical were of high purity grade (Merck).

Gas chromatography and mass spectrometry. The qualitative analysis of the volatiles was carried out with a gas chromatograph (Girdel 3.000) associated with a mass spectrometer (Varian Mat CH_5 type). The quantitative analysis was performed with a gas chromatograph (Packard 7.400) associated with an electronic integrator (Hewlett Packard 3380A) under the following conditions:

flame ionization detector (250 V),

glass column 6 m, diameter 2 mm, packed with Chromosorb W-AW80-100 mesh impregnated with 15% Carbowax 20M,

temperature of injector 220°C, detector 240°C,

temperature of column: isotherm at 68°C for 23 min, then increase of 2.3°C/min from 68 to 180°C,

flow rate of nitrogen 20 ml/min, hydrogen 25 ml/min, air 250 ml/min (N = 9.250 theoretical plates for diacetyl).

The extraction ratio was determined for furfural diacetyl and 4-methyl-2-pentanone, by comparing the amounts determined in the extracts obtained

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from a control juice, with a sample to which known quantities of these volatiles had been added.

The contents of other volatiles in the extract are expressed as peak areas, the unit area being the area of 4-methyl-2-pentanone for this sample.

Results

Influence of temperature and humidity on the CTFA

The CTFA values which have been observed at different temperatures for 2.6% residual moisture are given in Table 1.

Plotting the reciprocal logarithm of the CTFA versus the reciprocal of the absolute temperature (Fig. 1), the activation energy of the change of flavour can be calculated as 41.9 kcal mole^{-1}.

The CTFA decreased when water content was raised in the rather narrow range which has been examined (Table 2).

Table 1. Influence of temperature on CTFA (water content 2.6%)

Temperature (°C)	CTFA (hr)
69	0.3
61	2.2
53	12.5
45	120.0
37	240.0
32	840.0
27	1920.0

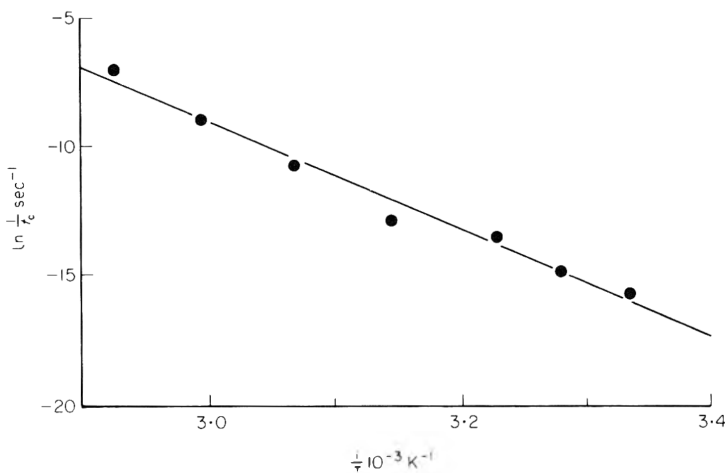

Figure 1. Influence of temperature on the critical time for flavour alteration (CFTA). T = absolute temperature (K), t_c = CTFA (s), residual moisture of the powder 2.6% (Product A).

Table 2. Influence of water content on CTFA (temperature 32°C)

Water content (%)	CTFA (hr)
2.6	840
4.2	480
6.8	360

Development of volatiles

Two chromatograms of samples of the control juice and of the same juices after heating at 61°C for 7 hr (residual moisture 3.5%), are presented in Fig. 2. Of the fifty-six peaks, sixteen have been identified.

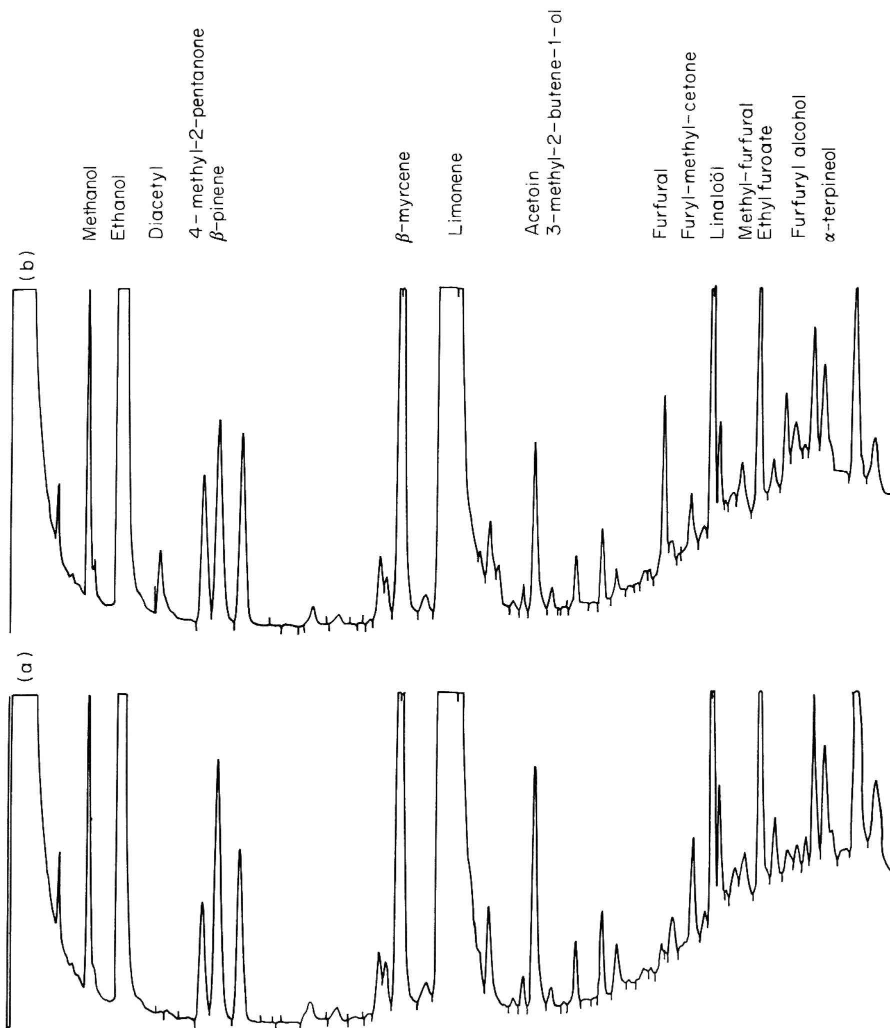

Figure 2. Chromatogram of the volatiles extracted after rehydration of orange juice powder (product A); (a) control, (b) after heating at 61°C for 7 hr (residual moisture 3.5%).

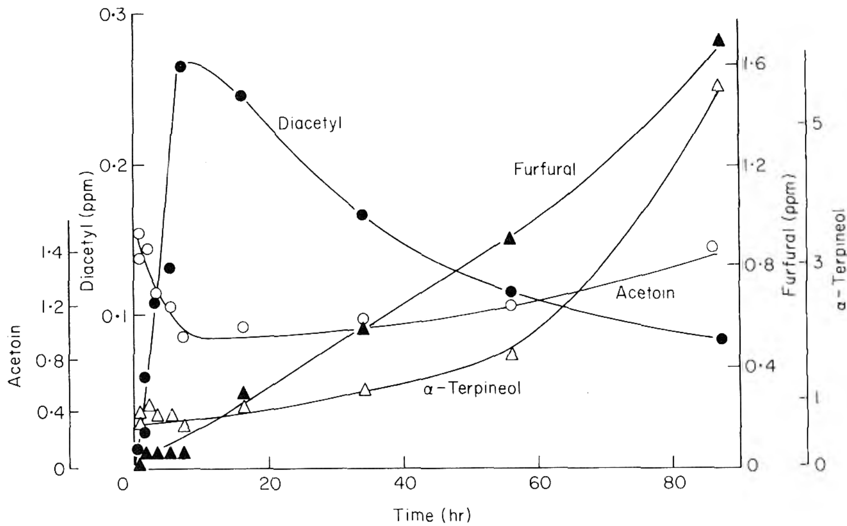

Figure 3. Evolution of volatiles content as a function of time of heating at 61°C (Product A) (residual moisture 3.5%) for acetoin and α-terpineol (arbitrary units).

Figure 3 shows the development of those volatiles which exhibit the largest changes during conditioning of the product at 61°C (water content 3.5%). Furfural shows an almost linear increase as a function of heating time; for diacetyl content a maximum is observed after about 7 hr; acetoin exhibits the opposite behaviour from diacetyl; α-terpineol content increases in the final stage of treatment.

The influence of water content is shown in Fig. 4.

After heating times equal to CTFA, or slightly shorter than that, furfural and diacetyl concentration increase, and the acetoin concentration decreases (Table 3). It can be seen in Table 3 that diacetyl and furfural are present in the control (A) as well as in control (B) which was freshly pressed orange juice.

Table 3. Volatile contents after heating times ⩽ CTFA (water content 3.5%)

Temperature (°C)	Heating time (hr)	Volatiles contents			
		Furfural (ppm)	Diacetyl (ppm)	Acetoine arbitrary	α-terpineol units
Control (juice A)	0	0.012	0.008	1.79	1.64
61°C (juice A)	0.5	0.024	0.027	1.61	1.75
Control (juice B)	0	0.013	0.010	2.86	0.162
37°C (juice B)	240	0.213	0.216	2.65	0.146
27°C (juice B)	1680	0.210	0.173	2.05	0.145

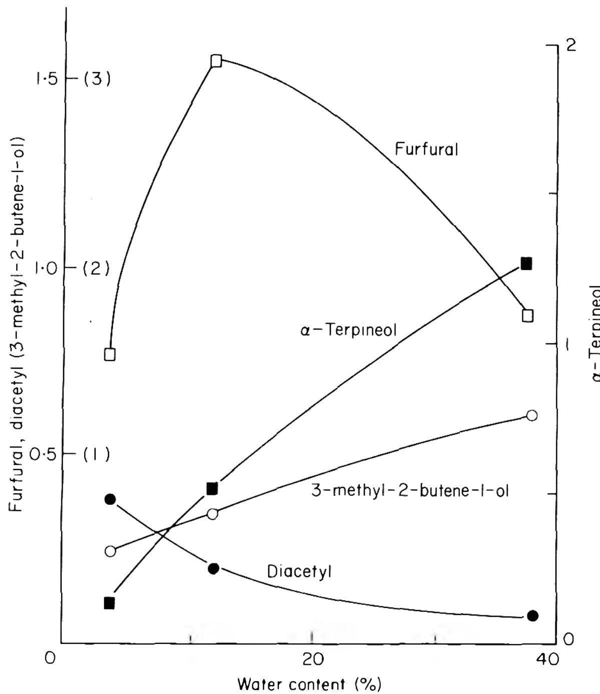

Figure 4. Influence of water content on the volatiles contents after heating at 61°C for 7 hr (product A) (arbitrary units).

Discussion

Furfural has been identified in orange juice powder after storage by Tatum *et al.* (1967) and Dinsmore & Nagy (1971). Shaw *et al.* (1966) have shown that this compound is formed during heating of a solution of fructose and ascorbic acid. Tatum, Shaw & Berry (1969) and Huelin *et al.* (1971) have demonstrated that its origin is the degradation of ascorbic acid.

It has been observed (Papanicolaou, 1975) that the destruction rate of ascorbic acid was highest for a certain water content (about 10% at 61°C). This similarity in behaviour with the furfural production suggests that the furfural originates mostly from degradation of ascorbic acid.

Diacetyl has been shown to be produced in orange juice by bacteria (*Lactobacillus* and *Leuconostoc*) or yeasts (*Saccharomyces carlsbergensis* and *cerevisiae*) (Murdock, 1964). The presence of this substance in orange juice is considered an indication of poor sanitary conditions during extraction or concentration processes (Murdock, 1967, 1968). In the present investigation, however, the microbial origin of diacetyl seems to be very improbable: the preparation of juice (product B) was carried out under such conditions (contamination, temperature and time) that growth of microorganisms should have been limited; the residual moisture of the dry product was very low (water activity about 0.12) and would not permit such growth.

Murdock (1964, 1967) suggested that diacetyl could originate from chemical degradation of sugars; the assay with the Voges–Proskauer reaction, however, gave negative results with solutions of sucrose, glucose and citric acid, heated at 120°C for 20 min (pH = 3.5). But El'Ode *et al.* (1965), Scanlan *et al.* (1973) demonstrated the formation of diacetyl in solutions of sugar and amino acids.

These experiments, however, have been performed at high temperatures. It was necessary for us to verify that diacetyl production was also possible under the present experimental conditions.

A model solution containing sucrose, glucose, fructose, ascorbic acid, citric acid and amino acids of the orange juice, was freeze dried. After the water content was raised to 12%, the product was heated at 61°C for 6 and 14 hr.

The analysis of volatiles was carried out as for orange juice. Furfural was observed after 6 hr heating time, diacetyl only after the longer heating time. The measured quantities were larger than in orange juice powder for furfural, but smaller for diacetyl. Thus it seems probable that in orange juice powder other mechanisms also produce diacetyl; oxidation of acetoin might be one of them. Moshonas & Shaw (1972) have detected 3-methyl-2-buten-1-ol in lemon juice. A peak with the same retention time was observed with our model solution; this suggests that this volatile originates from a Maillard reaction. α-terpineol has been reported to be present at high concentrations in orange juice after storage or over-heating by Blair *et al.* (1952), Rymal *et al.* (1968), and Tatum *et al.* (1975). Blair *et al.* (1952) ascribed the origin of this substance to hydration of limonene. The fact that the observed terpineol production increased with water content of the product (Fig. 4) seems to be in accordance with this interpretation.

α-terpineol has been proposed (Askar *et al.* 1973) as an index of prolonged storage or over-heating of orange juice. Furfural has been suggested for the same purpose by Dinsmore & Nagy (1972, 1974) and Nagy & Dinsmore (1974). Furfural does not seem to be very significant as far as flavour change is concerned (Nagy & Randall, 1973). Although the increase of α-terpineol is associated with a bitter taste, this bitterness is not due to this single substance (Swift, 1961).

After heating at 61°C for a time equal to the CTFA, the diacetyl content of the product was estimated to be 0.1 ppm, thus exhibiting a sevenfold increase when compared with the control. The flavour thresholds in various media are in the range of 0.005–0.02 ppm (Siek *et al.*, 1969; Stahl, 1973). It thus seems possible that diacetyl plays a significant part in the early change of flavour of orange juice powder.

Conclusion

The volatiles which exhibit the most significant increase in orange juice powder during storage or heating, before the critical time for flavour alteration is attained, have been found to be furfural and diacetyl. The second of these

compounds, at least, seems to be able to play a significant part in the early changes of flavour of the product. These volatiles are present in small amounts in orange juice; the increase in furfural content during storage or heating of the dry product seems to originate from ascorbic acid degradation; the origin of diacetyl may be also a non-enzymatic browning reaction, and/or oxidation of acetoin. If, however the diacetyl content is high, and furfural content low, the origin of diacetyl may be the microbial activity in the juice before drying.

In orange juice powder with rather high moisture contents (20–40%), the increase of the above two volatiles is less important. On the contrary, α-terpineol and 3-methyl-2-butene-1-ol concentrations show a greater increase. The first one of these compounds can also have a deleterious effect on flavour.

For orange juice powders with a high moisture content α-terpineol may be used as an indicator of over-heating or prolonged storage. For powders of low moisture content furfural and diacetyl may be preferable.

References

Askar, A., Bieling, H.J. & Treptow, H. (1973) *Deutsche Lebensmittel Rundschau 69 Jahrg.* **4**, 162.

Blair, J.S., Godar, E.M., Master, J.E. & Riester, D.W. (1952) *Food Research,* **17**, 235.

Huelin, F.E., Goggiola, I.M., Sidhu, G.S. & Kennett, B.H. (1971) *J. Sci. Fd Agric.* **22**, 540.

Huskins, C.W., Swift, L.J. & Veldhuis, M.K. (1952) *Fd Research,* **17**, 109.

Huskins, C.W. & Swift, L.J. (1953) *Fd Research,* **18**, 360.

Dinsmore, H. & Nagy, S. (1971) *J. Agr. Fd Chem.* **19**, 517.

Dinsmore, H. & Nagy, S. (1972) *J. Fd Sci.* **37**, 768.

Dinsmore H. & Nagy, S. (1974) *J. Ass. off. analyt. Chem.* **57**, 332.

El'Ode, K.E., Dornseifer, T.P., Keith, E.S. & Powers, J.J. (1965) *J. Fd Sci.* **31**, 351.

Moshonas, M.G. & Shaw, P.E. (1972) *J. Agr. Fd Chem.* **20**, 1029.

Murdock, D.I. (1964) *J. Fd Sci.* **30, 354.**

Murdock, D.I. (1967) *Fd Technol., Champaign,* **21**, 643.

Murdock, D.I. (1968) *Fd Technol., Champaign,* **22**, 90.

Nagy, S. & Dinsmore, H. (1974) *J. Fd Sci.* **39**, 1116.

Nagy, S. & Randall, V. (1973) *J. Agr. Fd Chem.* **21**, 272.

Papanicolaou, D. (1975) *Contribution à l'étude des altérations du jus d'orange au cours de séchage et du stockage à l'état déshydraté.* Thèse Docteur-ingénieur, Université de Dijon.

Rymal, K.S., Wolford, R.W., Ahmed, E.M., Dennison, R.A. (1968) *Fd Technol.* **22**, 1592.

Siek, T.J., Albin, I.A., Sather, L.A. & Lindsay, R.C. (1969) *J. Fd Sci.* **34**, 265.

Scanlan, R.A., Kayser, S.G., Libbey, L. & Morgan, M.E. (1973) *J. Agr. Fd Chem.* **21**, 673.

Stahl, W.H. (1973) *Compilation of Odor and Taste Threshold Values Data.* (Ed. by A.S.T.M.) McCormick and Co.

Swift, L.J. (1961) *J. Agr. Fd Chem.* **9**, 298.

Tatum, J.H., Shaw, P.E. & Berry, R.E. (1967) *J. Agr. Fd Chem.* **15**, 773.

Tatum, J.H., Shaw, P.E. & Berry, R.E. (1969) *J. Agr. Fd Chem.* **17**, 38.

Tatum, J.H., Nagy, S. & Berry, R. (1975) *J. Fd Sci.* **40**, 707.

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The effect of iron on botulinal inhibition in perishable canned cured meat

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Summary

Iron is critical to the inhibition of *Cl. botulinum* in perishable canned cured meat. An excess of 'available' iron negates the inhibitory effect of nitrite. A hypothesis for the mechanism of nitrite inhibition which is compatible with existing information is that nitric oxide reacts with an essential iron containing compound within the germinated botulinal cell and prevents outgrowth.

Introduction

The mechanism by which nitrite inhibits botulinal outgrowth in cured meats has been elusive. Attempts have been made to ascribe the inhibitory effect of nitrite to a Perigo type factor. However, the data which have been developed to prove the existence of such a factor in commercially manufactured product have been inconclusive. Formation of the factor described by Perigo, Whiting & Bashford (1967) would require a more severe thermal process than that which is given to perishable cured meats. Despite this, several studies have shown a direct relationship between the level of added nitrite and the degree of botulinal inhibition in perishable cured meat (Christiansen *et al.*, 1973, 1974; Tompkin, Christiansen & Shaparis, 1977).

Perishable canned cured pork is a product which is amenable to study under more controlled conditions than most perishable cured meats. The formulation contains relatively few ingredients. It is given a mild heat treatment which is not believed to be injurious to a botulinal spore inoculum but yet will destroy most non-sporeforming contaminants (Tompkin, Christiansen & Shaparis, 1978b). The product is processed in the container in which it is marketed; so, it is not exposed to post process contamination. Growth of *Bacillus* species which might survive the process has not been observed in the test product manufactured in our laboratory. Excessive levels of thermoduric lactobacilli can

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survive the process and prevent botulinal outgrowth due to their lowering the pH of the product. Such instances seldom occur; however, this possibility must be considered when working with this class of product.

Some variation in the degree of botulinal inhibition with nitrite has been observed under 'controlled' conditions (Tompkin *et al.*, 1977). An extensive series of tests subsequently has been conducted to determine the cause of the variation in inhibition (Tompkin, Christiansen & Shaparis, 1978a, b, c). The ultimate goal of this research has been to learn the mechanism by which nitrite delays botulinal outgrowth. One series of experiments (Tompkin *et al.*, 1978b) demonstrated that the type of meat used in the product can influence botulinal inhibition. There appeared to be an inverse relationship between the amount of muscle pigmentation and the degree of inhibition. Pork and beef hearts showed no inhibition of *Cl. botulinum* even though 156 μg/g sodium nitrite had been added to the product. The rate of nitrite depletion in heart meat was not measurably altered from that of product formulated with pork ham.

Nutritive values for beef hearts, port hearts, beef rump roast, and fresh pork ham show some differences among these meats (Adams, 1975). Heart meat contains about half as much calcium, twice as much iron, and ten times as much riboflavin as rump roast and pork ham. These are approximations since the values listed in USDA Handbook No. 456 are for cooked hearts while the other meats are listed as raw meats.

Experiments described in this paper examined the effect of calcium, iron, and riboflavin content on botulinal inhibition in perishable canned cured meat. The results show iron to be an integral factor in the inhibitory mechanism of nitrite.

Materials and methods

Inoculum

The *Cl. botulinum* inoculum consisted of a mixture of five type A (33A, 36A, 52A, 77A, and 12885A) and five type B (ATCC 7949, 41B, 53B, 213B, and Lamanna B) strains prepared as described by Christiansen *et al.* (1973). The mixed spore suspension was heat shocked at 80°C for 15 min and added to the meat during formulation using a target level of 100 spores/g.

Formulation and processing

Perishable canned comminuted cured meat was formulated with salt, water, and sugar; inoculated; processed; and chilled as previously described (Christiansen *et al.*, 1973). The meat portion consisted of fresh pork ham except in the first experiment where beef liver, hearts, or round were also used. All product was formulated with 156 μg/g of sodium nitrite.

Testing of additives

Riboflavin (U.S.P. grade – Merck & Co., Rahway, N.J.), anhydrous calcium chloride (J. T. Baker Chemical Co., Phillipsburg, N.J.), and purified reduced iron metal powder (Allied Chemical, New York) were added to the ground meat during formulation. The riboflavin and calcium were added to beef round and pork ham to give levels comparable to those reported for heart meat (Adams, 1975). Iron was added to beef round and pork ham at two times the naturally occurring level to retain the calcium-to-iron ratio that reportedly occurs in heart meat (Adams, 1975). The levels used were 4.83 mg riboflavin 207.8 mg $CaCl_2$, and 33.5 mg iron powder per 2250 g of meat.

The effect of different forms of iron and other metal ions were evaluated. The additives included ferrous chloride ($FeCl_2 \cdot 4H_2O$ – Mallinkrodt Chemical Co., St Louis), ferric chloride ($FeCl_3 \cdot 6H_2O$ – J. T. Baker Chemical Co.), magnesium chloride ($MgCl_2 \cdot 6H_2O$ – Allied Chemical, New York), manganese chloride ($MnCl_2 \cdot 4H_2O$ – Matheson Coleman & Bell, Norwood, Ohio), and zinc chloride ($ZnCl_2$ – J. T. Baker Chemical Co.). Each additive was tested at a level of 0.00132 molar to approximate twice the level of iron reported for heart meat (Adams, 1975).

Holding conditions

Twenty-five cans of inoculated product per test variable were placed at 27°C for up to 110 days. Cans were removed from incubation as they swelled.

Microbiological and chemical analyses

Spore levels, toxin assays, and nitrite analyses were determined as described by Christiansen *et al.* (1973). The first five cans to swell from each test variable were tested for botulinal toxin. A total of ninety cans were tested for botulinal toxin; eighty were toxic.

Results

Figure 1 shows the extent of inhibition in control product formulated with pork ham or beef round. Product formulated with beef hearts showed no inhibition. A set of twenty-five cans formulated with beef hearts plus added calcium (data not shown) also showed no inhibition. Product prepared with 50% beef round and 50% beef hearts gave a response intermediate between the responses obtained for beef round and beef hearts (data not shown).

Pork or beef supplemented with riboflavin gave the same degree of inhibition as pork or beef without riboflavin. All variables of pork ham or beef round formulated with iron show a marked loss of inhibition. Addition of calcium or riboflavin along with the iron did not alter this response.

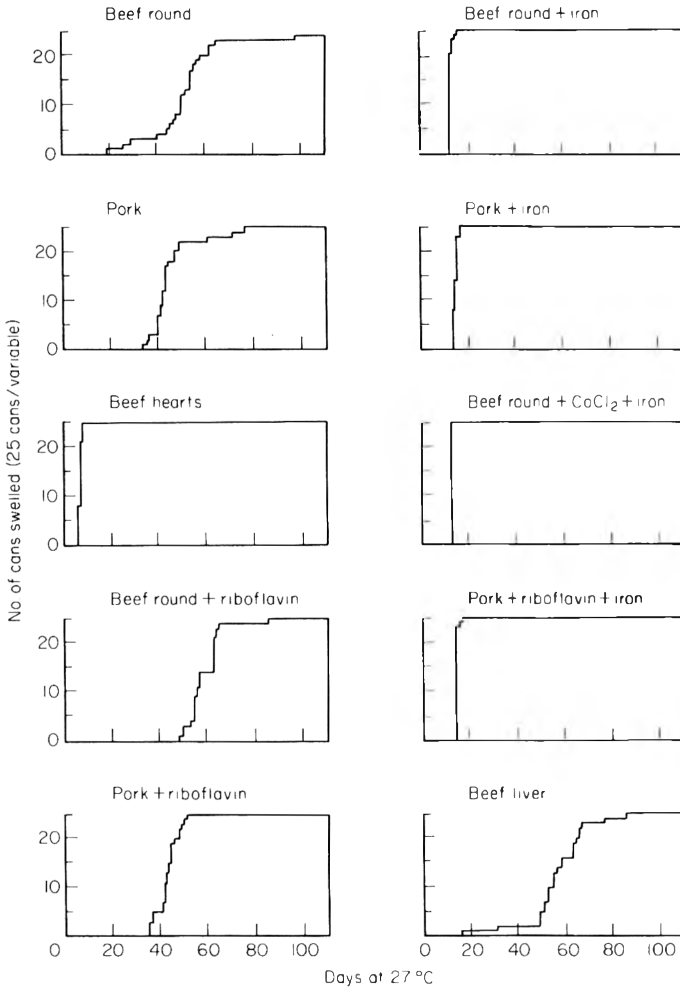

Figure 1. Effect of iron, calcium, and riboflavin on botulinal outgrowth in perishable canned cured meat formulated with 156 μg/g sodium nitrite and held at 27°C.

Product formulated with beef liver gave the same degree of botulinal inhibition as product containing beef round. The data demonstrate that some factor (e.g. iron) in addition to the level of residual nitrite influences the degree or botulinal inhibition in the case of hearts and beef liver. The levels of residual sodium nitrite in product with beef livers, beef hearts, and beef round were 32, 100, and 86 μg/g, respectively, after processing.

The data in Fig. 2 show that adding iron in either the ferrous or ferric state causes a loss of botulinal inhibition. This phenomenon is unique to iron. Magnesium, manganese and zinc did not reduce the inhibitory effect of nitrite. In a subsequent test it has been found that the rate of residual nitrite depletion at 27°C is not altered by the addition of iron.

The levels of iron in product formulated with pork ham, beef round, beef

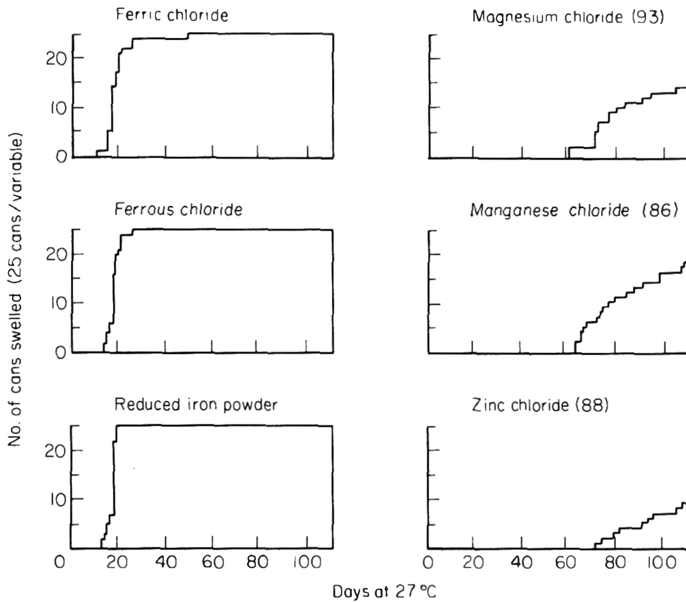

Figure 2. Effect of iron and other metal ions on botulinal outgrowth in perishable canned cured meat formulated with 156 μg/g sodium nitrite and held at 27°C. Values in parentheses are for residual sodium nitrite after processing.

liver, and heart meats were 9–12; 20–27; 49 and 38–53 μg/g, respectively (Table 1). Addition of iron to pork ham resulted in iron levels of 86–113.

Discussion

It was earlier reported that adding EDTA, sodium isoascorbate, sodium ascorbate, or cysteine to perishable canned cured meat enhances the anti-

Table 1. Iron content of laboratory prepared perishable canned cured meat

	No. of samples tested	Level of iron (μg/g)
Pork ham + iron powder	2	86, 87
Pork ham + ferrous chloride	1	88
Pork ham + ferric chloride	1	113
Beef hearts	1	53
Pork hearts	4	38–48
Beef liver	1	49
Beef round	3	20–27
Pork ham	9	9–12

The samples include product prepared for both experiments described in this paper and additional samples from other experiments either completed or in progress.

botulinal effect of nitrite. It was concluded that this effect was due to sequestering a metal ion which is inherent in meat (Tompkin *et al.*, 1978a).

An inverse relationship has been demonstrated between the amount of muscle pigmentation and the degree of botulinal inhibition in perishable canned cured meat (Tompkin *et al.*, 1978b). This effect seemed to be due to the presence of some factor in the meats which strongly influenced nitrite inhibition and botulinal outgrowth. The time for outgrowth to occur in pork or beef hearts was independent of the level of residual nitrite. It is expected, however, that for each type of meat a direct relationship does exist between the level of added nitrite and botulinal outgrowth. An example of this is pork ham (Tompkin *et al.*, 1977).

The present research offers an explanation for the earlier findings. Iron has been found to be a critical factor influencing the degree of botulinal inhibition in perishable canned cured meat. Indeed, an excess of 'available' iron negates the inhibitory effect of residual nitrite. It seems unlikely that the addition of iron merely stimulated outgrowth of *Cl. botulinum*. In the absence of nitrite but in the presence of EDTA (Tompkin *et al.*, 1978a) or isoascorbate (Tompkin *et al.*, 1978c) where iron and other metal ions were chelated, the outgrowth times (time to swell) were the same as in similar variables without the sequestering agents. That is, all non-nitrite variables swelled in approximately 1 week.

Heart meat contains more iron than the skeletal muscles which have been tested (Table 1). This could explain the difference in inhibition obtained with heart meat as opposed to pork ham and beef round. However, beef liver, which has as much iron as heart meat, did not show a loss of inhibition. This indicates that the quantity of iron in the tissue is an incomplete explanation for this effect. A more likely explanation is the availability of the iron in the tissue.

Existing literature offers little information on the role of iron in clostridia other than its involvement in electron transport and energy production. Ferredoxins and flavodoxins are largely responsible for this activity in clostridia. Both are small nonheme iron-sulphur proteins. Yoch & Valentine (1972) listed thirteen clostridial species, including *Cl. sporogenes*, which are known to contain ferredoxin. They also listed eighteen enzymes from fermentative bacteria which are dependent upon ferredoxin for their activity. Most organisms devoid of cytochromes contain ferredoxins (Mortenson & Nakos, 1973). It is predictable that *Cl. botulinum* also contains iron-sulphur proteins which are essential for electron transport, enzyme activity, and energy production.

Residual nitrite dissociates in meats to yield highly reactive nitric oxide which can react with the iron in compounds such as catalase, the peroxidases, cytochromes, cytochrome oxidase, haemoglobin and myoglobin (Bard & Townsend, 1971).

A plausible hypothesis for the inhibitory effect of nitrite upon *Cl. botulinum* is a reaction of nitric oxide with the iron of a compound, such as ferredoxin, within the germinated cell. Such a reaction could interfere with the energy

metabolism of the germinated cell and prevent outgrowth. This would agree with several reports showing that nitrite, at the levels present in commercially cured meats, acts by preventing outgrowth of the germinated spores (Duncan & Foster, 1968; Labbe & Duncan, 1970; Pivnick *et al.*, 1970).

References

Adams, C.F. (1975) *Nutritive Value of American Foods.* Agriculture Handbook No. 456, Agricultural Research Service, United States Department of Agriculture.

Bard, J. & Townsend, W.E. (1971) *The Science of Meat and Meat Products* (Ed. by J.F. Price and B.S. Schweigert) p. 445. W. H. Freeman, San Francisco.

Christiansen, L.N., Johnston, R.W., Kautter, D.A., Howard, J.W. & Aunan, W.J. (1973) *Appl. Microbiol.* **25**, 357 (Erratum, **26**, 653).

Christiansen, L.N., Tompkin, R.B., Shaparis, A.B., Kueper, T.V., Johnston, R.W., Kautter, D.A. & Kolari, O.J. (1974) *Appl. Microbiol.* **27**, 733.

Duncan, C.L. & Foster, E.M. (1968) *Appl. Microbiol.* **16**, 406.

Labbe, R.G. & Duncan, C.L. (1970) *Appl. Microbiol.* **19**, 353.

Mortenson, L.E. & Nakos, G. (1973) *Iron-Sulphur Proteins,* Vol. 1, *Biological Properties* (Ed. by W. Lovenberg), p. 37. Academic Press, London.

Perigo, J.A., Whiting, E. & Bashford, T.E. (1967) *J. Fd Technol,* **2**, 377.

Pivnick, H., Johnston, M.A., Thacker, C. & Loynes, R. (1970) *Can. Inst. Fd Technol. J.* **3**, 103.

Tompkin, R.B., Christiansen, L.N. & Shaparis, A.B. (1977) *J. Fd Sci.* **42**, 1046.

Tompkin, R.B., Christiansen, L.N. & Shaparis, A.B. (1978a) *J. Fd Sci.* **43**, 1368.

Tompkin, R.B., Christiansen, L.N. & Shaparis, A.B. (1978b) *Appl. Environ. Microbiol.* **35**, 886.

Tompkin, R.B., Christiansen, L.N. & Shaparis, A.B. (1978c) *Appl. Environ. Microbiol.* **35**, 59.

Yoch, D.C. & Valentine, R.C. (1972) *Ann. Rev. Microbiol.* **26**, 139.

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The effects on broiler chicken of polyphosphate injection during commercial processing

I. Changes in weight and texture

T. C. GREY, D. ROBINSON AND J. M. JONES

Summary

The process of injection of 5% polyphosphate solution into the breast of broiler chickens under commercial conditions had a variable but insignificant effect on the weight of water absorbed during immersion chilling and did not influence the amount of fluid lost on thawing or cooking. However, it produced a net increase in the weight of the carcase at all stages (chilling, freezing, thawing and cooking) subsequent to injection. After thawing and cooking a significant tenderizing effect in treated birds was observed.

Introduction

The extensive use of polyphosphate to reduce processing and cooking losses during the commercial processing of red meats led to investigations in America of the possible use of polyphosphates to reduce weight losses which occurred during the commercial processing of poultry.

The presence of low amounts (*c.* 2.5%) of polyphosphate in the chill water generally increased the amount of water taken up by carcases during chilling while levels of 5–12% polyphosphate in the chill water tended to reduce the amount of water taken up. During the subsequent storage in ice, treated birds lost less moisture than did untreated carcases, (Klose, Campbell & Hanson, 1962, 1963; Mountney & Arganosa, 1962; Schermerhorn & Stadelman, 1962; Mahon, 1963; Thomson, Kotula & Novotny, 1963). In addition to its effects on moisture uptake, polyphosphate treatment was reported to reduce cooking losses and to improve the tenderness of the meat (Schermerhorn & Stadelman, 1962; Spencer & Smith, 1962; May, Helmer & Saffle, 1962). Klose *et al.* (1962) found, however, that polyphosphates had no effect on meat tenderness.

Despite the claims made for polyphosphate treatment, the process was not widely adopted by American poultry processors because it was not feasible to

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immerse broiler carcases for 6 to 18 hr, the time required to allow adequate penetration of polyphosphates through the skin. To overcome this difficulty, methods for the addition of polyphosphate by injection were described in the United States and the United Kingdom (Schwall, Rogers & Corbin, 1968; Hale, 1977; Albright & Wilson, 1963). In the U.S.A., where processing methods are carefully controlled by Federal inspection, the addition of 3% of the carcase weight by injection techniques is allowed, but we are not aware that the injection of polyphosphate is carried out commercially in the U.S.A.

Hale (1977) found that cooking yields and tenderness were improved after injection of chilled carcases with a solution containing 12% polyphosphate.

In the United Kingdom, where injection procedures have been employed by a number of companies for several years, a total weight of 5% polyphosphate solution equivalent to 4–7% of the weight of the eviscerated carcase, is injected prior to immersion chilling.

This paper reports the results of an investigation into the effect of polyphosphates on the water retention and texture of broiler carcases using the injection and immersion chilling procedure normally employed in United Kingdom processing plants. A second paper reports on the perceived effects of polyphosphates on the meat as measured by consumer and experienced sensory panels (Griffiths & Wilkinson, 1978).

Materials and methods

Reagents

Analytical reagents were purchased from BDH Chemicals Ltd, Poole, Dorset, and were of 'Analar' grade except for ammonium metavanadate. Glass distilled water was used for the preparation of aqueous solutions.

Injection solutions

PURON 6040 concentrated polyphosphate solution was obtained from Albright and Wilson Ltd, Oldbury, Warley, Worcs. The injection solution was prepared by diluting one volume of PURON 6040 (sp. gr. 1.425, 20°C) with ten volumes of water. It was found necessary to stir the solution thoroughly to obtain complete mixing; this was checked by taking the specific gravity (1.040 at 20°C for a 5% w/v solution).

The manufacturer's recommended amount of 5% w/v polyphosphate solution to be injected is equal to 4–7% of the weight of the eviscerated carcase, this level of polyphosphate will be termed the 'normal dose', which was compared with half and twice this amount. Solutions were injected into the breast on either side of the keel bone using a hand-operated pneumatic injector unit (Autarky Machine Co. Ltd, East Grinstead, Sussex.)

Estimation of phosphorus

Phosphorus (as P_2O_5) in the injection solution and breast muscle was determined following the method of Grey, Robinson & Jones, (1977).

Materials

Ross I broiler chickens were grown under commercial rearing conditions. At slaughter the birds were between 50–56 days of age and weighed up to 2.5 kg (live weight). The broilers were part of the daily throughput of commercial plants.

Processing

Broilers were hung on shackles, electrically stunned and killed with an outside neck cut. After bleeding for 2.5 min the birds were scalded at 51–53°C mechanically plucked and automatically eviscerated. The production was sampled by removing carcases sequentially at the end of evisceration and before the final spray wash. The carcases were labelled, weighed, injected with approximately 60 ml of polyphosphate solution, reweighed and rehung on the processing line. Control (uninjected) carcases were given the same treatment but were not injected. To allow for any variation in processing conditions, twenty-five controls followed by twenty-five treated carcases were taken alternately throughout the day. All carcases were chilled in a two-stage through-flow immersion chiller in which the water was agitated by rotating paddles. The operation of the chillers was controlled as described in the AVEC Code of Practice (Anon., 1975). The water temperature in the first stage averaged 13 and 2°C in the second to which ice was added. At the end of chilling the carcases were rehung to drip for approximately 14 min. The carcases were then removed from the shackles reweighed, bagged and immediately frozen in a blast freezer to –20°C.

Four visits involving 8 days of commercial processing were made at intervals over a period of 13 months. Broilers which had been monitored as above were retained from each day's processing, frozen and transported to the laboratory in a refrigerated vehicle and subsequently stored at –20°C until used.

Carcases to be air-chilled were removed from the end of the eviscerating line before spray washing and transported to the laboratory in insulated containers. Treated birds (ten per group) were injected with 5% polyphosphate solution, water or air using the Autarky Injector and placed in an air chiller at 1°C for 2½ hr. The air flow in the chiller was 3.5 m/sec. The carcases were then bagged and blast frozen to –20°C.

Thawing and determination of thaw loss

Batches of five frozen carcases were removed from storage at –20°C, the outside of the bag containing the carcase was dried with a paper towel, the

carcase and wrapper weighed and the carcase allowed to thaw out overnight at constant temperature (18°C). The carcase was hung to drain for 1 hr, dried and weighed. The bag was removed, dried with a paper towel and weighed. After correcting for the bag weight, the difference between the thawed and frozen carcase weight was regarded as thaw loss and expressed as a percentage of the frozen weight.

Cooking and determination of cooking loss

Thawed carcases of known weight were placed in 'Roastabags' (Bacofoil, Silvertown, London E16) and cooked in an oven pre-set at 195°C. Each bird was cooked for 20 min/0.45 kg with an additional 20 min. The cooked birds were drained for 15 min at room temperature, wiped with a paper towel and weighed. The loss in weight, i.e. the cooking loss, was expressed as a percentage of the thawed weight.

Physical determination of texture

The *pectoralis major* breast muscle was removed from the cooled roasted broilers and blocks (1 × 2 × 0.5 cm) of the muscle were prepared. The force required to shear the blocks was measured with a Grunewald tenderometer (Mechanical Laboratory, Technical College, Karlsruhe, W. Germany). Results have been expressed as the force in kg required to shear a 0.5 cm thickness of muscle.

Results

The effect of variation in injection dose of polyphosphate solution in commercial plants

In preliminary experiments an attempt was made to examine the effect of varying the amount of polyphosphate solution injected on the weight changes occurring during chilling, thawing and cooking of carcases. The injection solution contained 5% w/v Puron (equivalent to 3.3 w/w P_2O_5) and the mean weight of solution injected in the 'normal' treatment was equivalent to 4.4% of the weight of the eviscerated carcase.

The results are summarised in Table 1. Injection of half or normal dose polyphosphate resulted in a small increase in the amount of water uptake, while the converse was true in the case of the twice normal dose.

When thaw loss was compared with total weight gain, viz. the percentage increase in weight due to injection plus percentage increase during immersion chilling, there was an increased retention of fluid as the injection dose was increased. However, when the thaw loss was compared only with the water absorbed during chilling in relation to increased polyphosphate dose, the reverse relationship was observed.

Table 1. The effect of variation of injection dose on change in weight during chilling, thawing and cooking. The number of samples, n, is given in parentheses

		5% polyphosphate injection level		
	(Untreated) control	0.5 × normal dose	Normal dose	2 × normal dose
Eviscerated weight (g)	1357 (39)	1458 (33)	1366 (33)	1368 (32)
*Weight of solution injected (%)	–	1.82	4.40	8.34
Concentration of polyphosphate as P_2O_5 of raw breast muscle (wet wt)	0.50 (6)	0.72 (6)	0.96 (6)	1.16 (6)
† Chiller water absorbed (%)	6.26 (39)	6.61 (33)	6.64 (33)	5.63 (32)
Total increase in wt (%)	6.26 (39)	8.43 (33)	11.04 (33)	13.97 (32)
‡ Thaw loss (%)	4.86 (15)	4.97 (10)	5.16 (15)	5.50 (10)
§ Cooking loss (%)	23.59 (10)	25.76 (10)	24.51 (10)	25.76 (10)

All values are the mean of the number of broilers examined.

*Weight of solution injected = injection wt ÷ evisceration wt × 100.

† Chiller water absorbed = wt after chilling – (eviscerated wt + injection wt) ÷ eviscerated wt × 100.

‡ Thaw loss = frozen wt – thawed wt ÷ frozen wt × 100.

§ Cooking loss = thawed wt – cooked wt ÷ thawed wt × 100.

Varying the polyphosphate dosage had little or no effect on cooking loss. The group of control birds lost less weight than any of the treated groups, while the 'normal' dose carcases lost less than the other treated groups.

Weight changes during chilling

The data obtained during 8 days of processing in a typical commercial plant are expressed both in weights and as percentages in Table 2. In order to give an indication of variation in the data from 1019 uninjected and 957 normal dose injected broilers, the overall mean values, the range of means from all the 8 days of processing and the standard deviation found within a day are included. The eviscerated weights of the carcases used for control and injection were similar, and apparent weight of water absorbed during chilling was also similar in both cases but the total weight gained by injected carcases was always approximately 5% higher than that of controls because of the residual polyphosphate solution in the carcase. The range of percentage increases in weight resulting from injection was due to the variation in eviscerated weight in any batch of chickens sampled on a particular day.

The effects of eviscerated weight, injection and day of experiment on the weight of water absorbed during immersion chilling are shown in Table 3. The analyses of variance showed that injection itself had no significant effect but that there was an indication that water absorption varied according to the day of the experiment and that the effect of injection was also variable to some

Table 2. Weight changes during commercial processing

	Uninjected (n = 1019)			Injected (n = 957)		
	Mean	Range of day means	s.d. between birds in one processing day	Mean	Range of day means	s.d. between birds in one processing day
Eviscerated wt (g)	1322	1148–1453	168	1326	1194–1505	162
Injected wt (g)	–	–	–	1388	1259–1569	163
Final wt (g) (after immersion chill)	1423	1243–1556	175	1487	1338–1668	167
Weight of injection (g)	–	–	–	62	58–66	5
Chiller water absorbed (g)	101	91–125	41	99	79–127	42
Total increase in wt (g)	101	91–125	41	161	144–188	42
Injection (%) (of eviscerated wt)	–	–	–	4.8	4.3–5.6	0.7
Chiller water absorbed (%)	7.8	6.9–9.5	3.4	7.6	6.3–9.5	3.5
Total increase in wt (%)	7.8	6.9–9.5	3.4	12.4	10.6–14.0	3.7

Table 3. Analysis of variance of processing data

	Degrees of freedom	Mean square
Days	7	35956***
Injection	1	2820
Eviscerated weight	4	1814
Days × injection	7	4903**
Days × eviscerated weight	28	2387
Injection × weight	4	896
Error	1924	1724

***, **Significant at 0.1% level and the 1.0% level respectively.

extent. The computed mean absorption of water in the chilling system was 7.76% s.e. 0.15 for controls and 7.45% s.e. 0.14 for injected birds.

Weight changes during thawing and cooking

A random sample of frozen broiler chickens obtained from all 8 days of processing mentioned in (a) above was thawed and cooked. A total of forty-eight control and eighty injected chickens, ranging in eviscerated weight from 1.1–1.8 kg, was studied. A summary of the mean percentage weight changes is shown in Table 4. By chance, the amount of chill water absorbed by the injected chickens was higher than that in the uninjected groups in good support of the degree of variation found during these experiments.

On thawing, uninjected carcases lost fluid approximately equal to that absorbed during spray washing and immersion chilling. However, under the same conditions injected carcases lost only 52% of the total weight gained during injection and chilling.

When all these samples were cooked the losses of weight were greater in the injected chickens. Taking all these results together an amount of fluid equivalent to 80% of the liquid injected in the polyphosphate group was retained after cooking.

Table 4. The effect of injection on thaw and cooking losses

	Uninjected		Injected	
	Mean	s.d.	Mean	s.d.
Number of broilers	48		80	
Increase in weight after injection (%)			4.93	0.87
Chiller water absorbed (%)	6.01	2.72	6.71	3.26
Total gain (%)	6.01	2.72	11.64	3.57
Thaw loss (%)	5.89	1.55	6.10	1.62
Cooking loss (%)	24.37	3.67	25.85	3.71

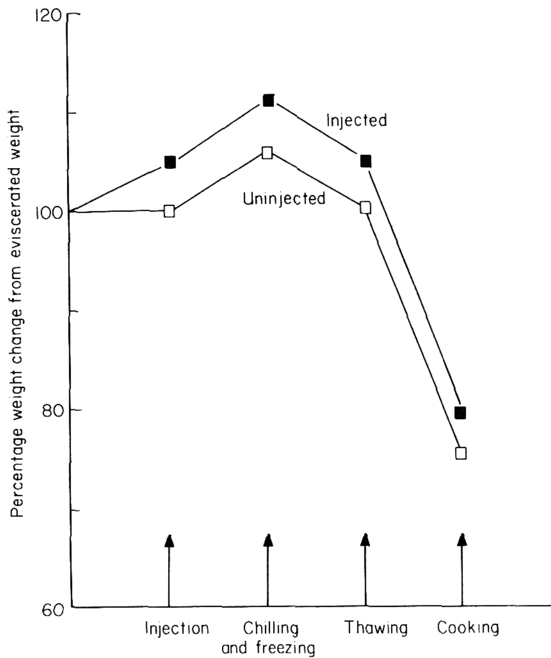

Figure 1. The percentage change from eviscerated weight during processing, thawing and cooking of broiler chickens.

The percentage weight changes starting from eviscerated weight equal to 100% can best be visualized in the form shown in Fig. 1 which clearly demonstrates the effects of injection in increasing the weight of the carcase before freezing and the subsequent effects on thawing and cooking. In commercial practice, however, giblets will be added to the carcase pack prior to freezing and will increase the weight by approximately 110 g. Therefore, the weight of chiller water absorbed will be proportionately smaller in the oven-ready product.

The weight of the giblets has not been included in the calculations of Fig. 1.

The effect of injection on breast muscle texture

The force to shear values for cooked muscle blocks taken from the same random sample of injected and uninjected commercially immersion-chilled broilers are shown in Table 5. It was not possible to examine the effect of injecting different media into carcases under commercial conditions, but this was done by injecting water or air into broilers subsequently air-chilled in the laboratory. These data are also recorded in Table 5. Analysis of variance on all these results showed that the injection of polyphosphate solution significantly affected the texture of the breast muscle and that variation in injection dose, in immersion chilled carcases 0.5 and 2 X normal had a similar effect. The texture of the 0.5 dose, mean shear value 1.25, s.d 0.24 kg, $n = 10$, was not significantly

Table 5. The effect on injection on the physical determination of texture

	Uninjected		Injection media					
			Air		Water		5% polyphosphate	
	Mean	s.d.	Mean	s.d.	Mean	s.d.	Mean	s.d.
Wet chilled (force to shear, kg)	1.99	0.55					1.55**	0.49
	(n = 30)						(n = 30)	
Air chilled (force to shear, kg)	1.91	0.26	1.86	0.20	2.08	0.16	1.51**	0.22
	(n = 5)		(n = 5)		(n = 5)		(n = 6)	

** Significantly different at 1.0% level from the uninjected sample.

different from the normal (shown in Table 5) however 2 × normal dose shear value 0.90 s.d. 0.16 kg, $n = 10$ was significantly more tender than any of the other treatments. There was, however, no significant difference in texture between the uninjected broilers and those injected with air and water. The results are in general agreement with sensory evaluation on a similar batch of immersion chilled broilers (Griffiths & Wilkinson, 1978).

Effect of injection on air-chilled broilers

In order to study the effect of injection on carcases which were not chilled by immersion, twenty broilers killed under commercial conditions were quickly transported to the laboratory and half the group were injected with polyphosphate solution. All the carcases were then chilled in a laboratory air chiller.

There was no significant difference between the groups on chilling or thawing but cooking losses were higher in the polyphosphate injected groups, as shown in Table 6.

Table 6. Effect of injection on air chilled broilers

Treatment	Control	5% polyphosphate injection
Number of broilers	10	10
Increase in weight after injection (%)	–	4.70
Loss in weight after chilling (%)	1.13	0.91
Thaw loss	0.42	0.46
Cooking loss	24.1 (n = 8)	26.1 (n = 8)
Concentration of polyphosphate as % P_2O_5 of breast muscle (wet wt)		
(a) raw	0.53 (n = 2)	0.93 (n = 2)
(b) cooked	0.54 (n = 2)	0.86 (n = 2)

Table 7. Effect of immersion chilling on P_2O_5 of breast muscle (wet wt)

Treatment	Control		5% Polyphosphate injection	
	No chill	Immersion chill	No chill	Immersion chill
Number of birds	10	10	10	10
Weight of solution injected (%)	–	–	4.53	4.75
Chiller water absorbed (%)	–	6.4	–	7.70
Total gain (%)	–	6.4	4.53	12.45
Thaw loss (%)	0.67	3.7	Nil	4.3
Concentration of polyphosphate as P_2O_5 % of breast muscle (wet wt)	0.53	0.53	0.85	0.84

The retention of polyphosphate in the carcase during processing

In the earlier stages of this investigation, analysis of whole breast muscle for polyphosphate (as P_2O_5) indicated that losses were occurring during processing. It was, therefore, necessary to carry out a controlled experiment under factory conditions in order to account for these losses. The results of such an experiment are shown in Table 7. 'No chill' birds (injected or non-injected) were removed from the processing line at the end of evisceration and before the spray wash, bagged and frozen and compared with 'immersion chill' birds which were processed so that all other conditions were as previously described. The loss of polyphosphate (as P_2O_5) during 21–24 min immersion chilling was minimal. However, when the complete breast muscle was analysed, after freezing and thawing, and correction was made for loss of polyphosphate (P_2O_5) found in the drip, it was possible to account for only 70% of the added polyphosphate concentration. Further examination of broilers, immediately after injection, showed that the loss of phosphate solution from the muscle occurred after the initial weighing but before immersion chilling since fluid could be seen seeping from the two injection holes. Analysis of thigh muscle showed that there was no increase in mean P_2O_5 concentration as a result of injection.

Discussion

Results of previous studies in other countries using marinating techniques (Klose *et al.*, 1962, 1963; Mountney & Arganosa, 1962; Schermerhorn & Stadelman, 1962; Spencer & Smith, 1962; Monk, Mountney & Prudent, 1964) and more recently, injection methods (Hale, 1977; Farr & May, 1970; Brotsky, 1976) have not been entirely consistent. In general, addition of polyphosphate

has been claimed to result in increased weight after cooking and a more tender product.

At an early stage in our experiments the effect of variation in injection dose suggested that processing factors other than polyphosphate injection were having a greater influence on water absorption in the chillers. This conclusion was evident from a summary of all the factory processing data and the subsequent analysis of variance. When the weight of chill water absorbed by injected and non-injected carcases was compared, the variation in percentage water absorption became a function of eviscerated weight rather than the amount of polyphosphate injected. It should be noted, however, that the polyphosphate injected carcases will have lost a small but variable quantity of fluid prior to entering the immersion chiller. The actual weight of water absorbed in the chiller will, therefore, be proportionately higher.

Examination of all the results shows that thawing and cooking losses were not influenced by injection with polyphosphate but the cooked weight was always higher (for any eviscerated weight) in the injected chicken because of the retention of an amount equivalent to 80% of the fluid injected. This increase in fluid retained in injected chickens could explain the increase in juiciness found by some people in a consumer test and by the Institute taste panel (Griffiths & Wilkinson, 1978).

Our results also show that the injection of polyphosphates has a tenderizing effect on the breast muscle as measured by the Grunewald tenderometer, and this was in good agreement with the findings of the sensory panel (Griffiths & Wilkinson, 1978).

The increased succulence of the cooked chicken observed by a proportion of the population in our consumer tests is significant; the weight relationships, which are a consequence of the process carried out to achieve this, are well illustrated in Fig. 1.

Finally, it will be of interest to the poultry industry to point out that, when the polyphosphate concentration in chilled and unchilled carcase breasts was measured, it was shown that no significant losses of phosphate were occurring during the mechanical immersion chilling process, but in the short interval between injection and chilling, up to 30% of the P_2O_5 added in the 60 ml of injection solution was lost.

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References

Albright & Wilson (MFG) (1963) British Pat 935,413.
Anon. (1975) AVEC Code of Practice for the continuous immersion chilling of chickens. British Poultry Meat Association Ltd, London.
Brotsky, E. (1976) *Poultry Sci.,* **55**, 653.
Farr, A.J. & May, K.N. (1970) *Poultry Sci.,* **49**, 268.
Grey, T.C., Robinson, D. & Jones, J.M. (1977) *J. Sci. Fd Agric.* **28**, 822.
Griffiths, N.M. & Wilkinson, C.C.L. (1978) *J. Fd Technol.,* **13**, 541
Hale, K.K. Jnr (1977) *Poultry Sci.,* **56**, 865.
Klose, A.A., Campbell, A.A. & Hanson, H.L. (1962) *Poultry Sci.,* **41**, 1655.
Klose, A.A., Campbell, A.A. & Hanson, H.L. (1963) *Poultry Sci.,* **42**, 743.
Mahon, J.H. (1963) U.S. Pat 3,104,170.
May, K.N., Helmer, R.L. & Saffle, R.L. (1962) *Poultry Sci.,* **41**, 1665.
May, K.N., Helmer, R.L. & Saffle, R.L. (1963) *Poultry Sci.,* **42**, 24.
Monk, J.A., Mountney, G.J. & Prudent, I. (1964) *Fd Technol., Champaign,* **18**, 226.
Mountney, G.J. & Arganosa, F.C. (1962) *Poultry Sci.,* **41**, 1668.
Schermerhorn, E.P. & Stadelman, W.J. (1962) *Poultry Sci.,* **41**, 1680.
Schwall, D.V., Rogers, A.B. & Corbin, D. (1968) U.S. Pat 3,399,063.
Spencer, J.V. & Smith, L.E. (1962) *Poultry Sci.,* **41**, 1685.
Thomson, J.E., Kotula, A.W. & Novotny, J.F. (1963) U.S.D.A. Marketing Research Report No. 605, Washington D.C.

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The effects on broiler chicken of polyphosphate injection during commercial processing

II. Sensory assessment by consumers and an experienced panel

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Summary

Chickens injected with a polyphosphate solution and control samples which had not been injected were submitted to a consumer panel of 389 households. Preference test and laboratory sensory assessments were also made on similar birds. In all tests polyphosphated birds were significantly more tender and juicy, but 32–45% of consumers did not differentiate between the treatments when eaten as part of a meal. In preference tests 265 people divided approximately equally in preferences between birds injected with polyphosphate and control birds.

Introduction

It has been claimed in the patent literature (Albright & Wilson (MFG), 1963; Schwall, Rogers & Corbin, 1968) that the treatment of poultry meat with polyphosphate solution, both inhibits the deterioration of flavour, odour, taste and colour due to storage, and improves flavour, tenderness and juiciness ratings.

These claims have been substantiated in relation to texture, on chickens treated by a marination procedure, using small laboratory panels (May, Helmer & Saffle, 1962; Landes, 1972) and by physical methods (Peterson, 1977). Work has been reported using the injection technique on parts of birds followed by physical measurements (Farr & May, 1970) and laboratory assessment (Brotsky, 1976), and also on whole carcases using tenderometer measurements (Hale, 1977). In all but the last study the work has been carried out on small numbers of birds bought in, or on specially reared birds.

The injection technique is of particular interest since it has been used commercially in the United Kingdom since the early 1970s. The present work was designed to investigate the reaction of consumers to the quality (flavour, texture and juiciness) of broiler chickens injected with polyphosphate com-

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pared with control birds taken from normal production lines in a processing plant. Assessment of birds from the same production runs were carried out by an experienced laboratory sensory panel, and physical and chemical tests on birds from the same batches are reported in the preceding paper (Grey, Robinson & Jones, 1978).

Experimental

Production of broilers

Chickens were injected with polyphosphate solution and immersion chilled as reported in Part I (Grey *et al.*, 1978), non-injected chickens were used as controls, the birds were stored at $-20°C$ until tested. All birds were within the weight range 1.4–2.0 kg.

Laboratory panel assessment

Three experiments were carried out. The preliminary investigation compared non-injected chickens with samples injected with three different weights of polyphosphate solution (Puron 6040, Albright & Wilson), at half, equal and double the normal commercial weight (i.e. 62 g, *c.* 4.5% body weight). Subsequent experiments (Trials I and II) used only the normal commercial weight.

Birds were thawed for 16 hr at 18°C, drained for 1 hr and roasted in 'Roastabags' (Bacofoil, Silvertown, London E16) at 195°C for 20 min/0.45 kg + 20 min; the final temperature in the deep breast muscle was 92–97°C. Four test birds (some injected and some non-injected) and a labelled control (non-injected) were cooked for each session.

Immediately after cooking the *pectoralis major* and *minor* muscles were removed from one half of each bird for flavour assessment. The hot meat was cut into pieces approximately $2 \times 2 \times 1$ cm and placed in warmed (40°C) coded pots. A panel of fourteen experienced assessors compared the flavour of each test sample with the labelled control, and rated the size of the flavour difference as shown in Table 1. Panel members were also asked to characterize the difference.

When cold the *pectoralis major* from the other half of each bird was cut into pieces $1 \times 1 \times 0.5$ cm. These samples were judged for texture and juiciness by fourteen experienced assessors at a separate panel session. The attributes were rated on the eight point scales in Table 1.

All laboratory assessments were completed within 6 weeks of slaughter. Results were analysed by analyses of variance followed by Students t-test.

Consumer assessments – in home trials

Selection of consumer panel. The panel was drawn from the City of Norwich (population 120 000) and its immediate suburbs (population 51 000). For Trial

Table 1. Scales used in laboratory sensory assessment

Flavour difference		Texture		Juiciness	
No difference	(0)*	Extremely tender	(1)	Extremely juicy	(1)
Very slight difference	(1)	Very tender	(2)	Very juicy	(2)
Slight difference	(2)	Moderately tender	(3)	Moderately juicy	(3)
Moderate difference	(3)	Slightly tender	(4)	Slightly juicy	(4)
Large difference	(4)	Slightly tough	(5)	Slightly dry	(5)
		Moderately tough	(6)	Moderately dry	(6)
		Very tough	(7)	Very dry	(7)
		Extremely tough	(8)	Extremely dry	(8)

*Numbers in parentheses were not on the panel sheets, but were assigned subsequently for analysis of results.

I (April 1976) every fortieth entry in the electoral register for four wards was drawn, and for Trial II (September 1976) every forty-ninth entry from a further four wards. If the entry indicated a single person household or an Institution, it was rejected and the next entry on the register was selected.

A letter was sent to the female member of each household, explaining the work of the Food Research Institute, and asking for their help in assessing the quality of two frozen chickens to be eaten as part of a meal. A prepaid reply form was enclosed for particulars of all members of the household over 12 years of age, and for possible interview times. Those responding positively were personally interviewed, when details of the questionnaire were explained,

Table 2. Formation and Structure of consumer panels

Formation	No. electors in 4 wards	No. households selected	No. accepting and taking part
Trial I	20619	504	198
Trial II	24621	503	191

Household composition

No. of tasters:	1	2	3	4	5	6	7	8
Trial I	2	109	41	33	6	5	1	1
Trial II	1	98	44	34	9	5	–	–

Tasters by sex and age

	Male		Female		
	Adult	12–18	Adult	12–18	Total
Trial I	233	32	247	38	550
Trial II	231	46	235	28	540

general questions about method of cooking and frequency of consumption of chicken were asked, and times for delivery of the birds arranged. The structure of the two consumer panels is shown in Table 2.

Distribution of chickens

The first birds were distributed to households not more than 3 weeks after the interviews had been carried out; instructions and questionnaires (one per person) were left at the same time. The second birds and questionnaires were delivered 1 week later. Birds were randomly allocated to households, half of which received the non-injected chicken first, the other half the polyphosphated birds.

Instructions and questionnaires

The cook was asked to roast the bird in the way normally used by the household, and serve it as part of a meal. The cook was further asked to assess the appearance of the raw meat as excellent (1), good (2), poor (3) or unacceptable (4); to say whether the cooked bird carved more (1), less (3) or as easily as usual (2). The cook, and each member of the household in the survey, was asked to eat the part of the chicken they normally ate (i.e. light meat – breast and/or dark meat – leg) and independently rate for flavour, juiciness and texture (Table 3).

Table 3. Scales used on questionnaires for all members of household in consumer trials

Flavour		Texture		Juiciness		Overall quality (Trial II only)	
Excellent	(1)*	Very tender	(1)	Very juicy	(1)	Excellent	(1)
Good	(2)	Moderately tender	(2)	Moderately juicy	(2)	Good	(2)
Poor	(3)	Slightly tender	(3)	Slightly juicy	(3)	Fair	(3)
Unacceptable	(4)	Slightly tough	(4)	Dry	(4)	Poor	(4)
		Moderately tough	(5)			Unacceptable	(5)
		Very tough	(6)				

*Numbers in parentheses were not on the questionnaires, but were assigned subsequently for analysis of results.

In Trial II the cook was also asked to record the time and temperature of cooking and whether the chicken was stuffed. Each respondent was asked to rate each bird for overall quality (Table 3). A supplementary questionnaire was delivered with the second bird, to gauge whether the household found the first bird better or worse than the second. All assessments were completed within 5 weeks of slaughter. The results for each attribute was analysed by the χ^2 test.

Consumer preference trials

Preference trials were carried out at three Evening Institutes in Norwich and district using birds processed for Trial II. In all, sixteen non-injected and sixteen polyphosphated birds were cooked, using the same technique as for the laboratory panel assessment. When cold the breast muscle (*pectoralis major* and *minor*) was carved into slices approximately 0.5 cm thick, cut into pieces approximately 3 X 4 cm and placed in coded waxpaper cups. People attending the evening classes were invited to eat a piece from each treatment and record their preference, giving their own reasons. 265 assessors (136 male, 129 female) participated in the tests.

Results and discussion

Laboratory assessment

The mean value for the laboratory sensory assessment, together with the significance of the polyphosphate effect are shown in Table 4.

The level of significance of the flavour difference varied between the experiments from highly significant in the preliminary trial to no significant difference in Trial II. Over the three experiments 76% of the polyphosphated birds

Table 4. Laboratory assessment of polyphosphate and non-injected chickens for flavour, texture and juiciness (panel of fourteen)

		Preliminary	Trial I	Trial II
Flavour difference				
Non-injected	Mean	0.6 (9)†	0.8 (10)	0.7 (10)
	Range	0.4–1.2	0.5–1.4	0.3–1.5
Polyphosphated (62 g)	Mean	1.5 (8)***	1.3 (10)**	1.1 (10)
	Range	1.2–1.9	1.0–1.5	0.6–2.1
Texture				
Non-injected	Mean	4.2 (8)	4.2 (16)	4.7 (10)
	Range	3.4–5.7	2.5–5.9	3.8–6.5
Polyphosphated (62 g)	Mean	2.7 (8)***	2.8 (16)***	2.7 (10)***
	Range	2.0–3.5	1.8–6.0	2.3–3.4
Juiciness				
Non-injected	Mean	5.1 (8)	4.7 (16)	4.5 (10)
	Range	4.6–5.8	4.0–5.4	4.1–5.0
Polyphosphated (62 g)	Mean	3.6 (8)***	3.5 (16)***	4.0 (10)**
	Range	2.7–4.0	2.8–4.0	3.2–4.6

* 5% probability level.
** 1% probability level.
*** 0.1% probability level.
† Numbers in parentheses are number of chickens assessed.

were perceived as only very slightly different from the labelled non-injected control, this difference was not large enough for assessors to characterize adequately; the remaining 24% had a low level of fishy or musty flavour. The values for the non-injected birds are a measure of the usual variation between birds.

In all three investigations the polyphosphate injected chickens were significantly more tender and more juicy than the non-injected. The texture values for all the polyphosphated birds fell within the range very tender to moderately/slightly tender (2.0–3.5), whereas all but three of the thirty-four non-injected birds were tougher, moderately/slightly tender to moderately/very tough (3.5–6.5). Similarly, for juiciness, thirty-one out of the thirty-four polyphosphated birds were between very/moderately juicy and slightly juicy (2.7–4.1) but all the non-injected birds were less juicy.

The birds treated with half the commercial dose of polyphosphate were slightly more tender (mean 3.3) and juicier (mean 4.7) than the non-injected but little different in flavour (mean 0.8). Those given double the commercial amount were all significantly (0.1% level) more tender (mean 1.8) and juicier (mean 2.8), being described by the panel as like 'chicken paste'; the flavour (mean 2.4) was salty and less strong.

The experienced assessors generally agreed on their assessment of individual birds; for example, in the texture rating for Trial I, all the panel agreed that one of the non-injected birds was very tender (mean 2.5) and that one of the polyphosphated samples was moderately tough (mean 6.0). The texture, juiciness and flavour ranges shown by this panel are therefore largely a reflection of bird to bird variation.

Consumer assessment – in home

The χ^2 results demonstrate no statistical difference in the distribution of observations over the flavour scales for light or dark meat, or for the appear-

Table 5. In home consumer assessment of polyphosphated and non-injected chickens, mean value for atrributes

	Trial I		Trial II	
	Non-injected	Polyphosphated	Non-injected	Polyphosphated
Flavour light*	1.9	1.9	1.9	1.9
Flavour dark*	1.9	1.9	1.9	1.9
Texture light	1.9	1.6	1.8	1.5
Texture dark	1.9	1.6	1.8	1.5
Juiciness light	2.2	2.0	2.3	1.9
Juiciness dark	2.2	2.0	2.1	2.1
Appearance raw	1.7	1.7	1.7	1.6
Carvability	1.8	1.7	1.9	1.7
Overall quality	–	–	2.1	1.9

* Refers to light meat (breast) or dark meat (leg).

ance of the raw meat. The differences in distribution are highly significant (0.1% probability level) for texture and juiciness and for overall quality, and significant at the 5% level for carvability. The actual mean differences (Table 5) are very small in relation to the scales. However, when the differences in ratings between polyphosphated and non-injected samples for individuals, were plotted as histograms (Fig. 1), it became clear that, although over 30% of individuals recorded no difference between the birds, a larger proportion of the others found the polyphosphated samples more tender and juicy than found

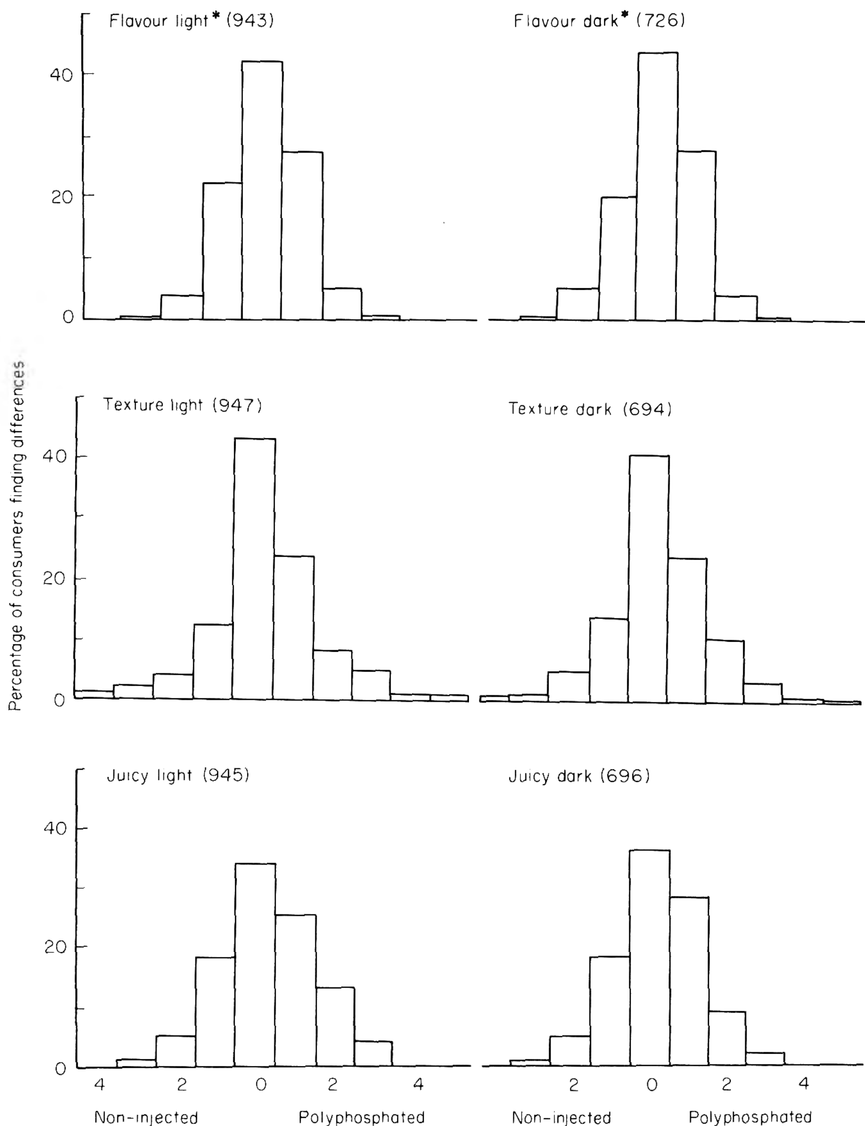

Figure 1. Distribution of consumer scores (polyphosphate minus non-injected), Trials I and II combined. The number of scores is shown in parentheses, * refers to light meat (breast) or dark meat (leg).

them less so. The histograms for texture and juiciness are not normally distributed but significantly skewed; the flavour histogram is symmetrical.

The texture differences between the two treatments in the dark meat are inexplicable as Grey *et al.* (1978) have shown no migration of polyphosphate into the thigh. However, of the 603 consumers who assessed the texture of both breast and leg meat, 37% found the same difference between treatments for both meats, possibly suggesting that their assessments were not independent.

No significant difference was shown between men and women in their assessments, there was no effect of method of cooking, how often the family normally ate chicken or time of day of processing the bird at the plant.

The high percentage of individual assessors giving the same score for specific attributes for non-injected and polyphosphated chicken was not reflected in the results of the supplementary question on the household preference in Trial II. Fifty-four percent of households preferred the polyphosphated chicken; 36% the non-injected and 10% had no preference; the order of presentation of the birds had no significant effect on the results. The difference in these results could have occurred because the latter was a consensus of opinion. Chickens from both treatments were generally acceptable. Only three verbal complaints were received on the 778 birds distributed. No bird was rated unacceptable by any person.

Consumer preferences

In the side-by-side preference tests 41% preferred the polyphosphated chicken, 47% the non-injected and 12% had no preference. Over half (55%) of those preferring the polyphosphated bird found the sample more tender and juicy, whereas 63% preferred the non-injected bird for flavour (Table 6). This side-by-side preference test showed a smaller percentage of consumers preferred the polyphosphated bird than in the 'in-home' test, which relied on memory of one sample over a week.

General discussion

The suburbs of Norwich were included in the consumer trials to counteract the high percentage of municipal property in the City: in both trials there was a

Table 6. Reasons given for preferring sample in preference tests (% of responses)

	Polyphosphate	Non-injected
More/better flavour	38	63
More tender	22	8
More juicy	33	7
Drier/firmer	2	15
Better texture	4	3
No reason	1	4

selection of types of housing reflecting a wide cross-section of income. The willingness of households to participate in the work (39% and 38%) was slightly lower than the average for consumer trials, but nevertheless a large number of individuals (1090) did take part. Because the results from the experienced laboratory panel and the consumer panels have been obtained using different scales and applying different statistical tests, a detailed comparison of the results and of the two approaches is unjustified. However, in this series of trials both panels found that the polyphosphate injected birds were more tender and juicy than the non-injected, but individuals differed in their preference. What this work cannot forecast is which type of chicken a significant proportion of consumers would choose.

Acknowledgments

Mr H. J. Charie and Dr W. B. Hall of Unilever Research Ltd, Colworth House and Dr D. N. Rhodes, ARC Meat Research Institute are thanked for advice on the consumer trials; Mrs S. M. Ring and Mr R. Stansfield for analysis of the data; Mrs C. E. Thurston for technical assistance and the panels at Food Research Institute and in the Norwich area for their cooperation.

References

Albright & Wilson (MFG) (1963) British Patent 935,413.
Brotsky, E. (1976) *Poultry Sci.* **55**, 653.
Farr, A.J. & May, K.N. (1970) *Poultry Sci.* **49**, 268.
Grey, T.C., Robinson, D. & Jones, J.M. (1978) *J. Fd Technol.* **13**, 529.
Hale, K.K. Jr (1977) *Poultry Sci.* **56**, 859.
Landes, D.R. (1972) *Poultry Sci.* **51**, 641.
May, K.N., Helmer, R.L. & Saffle, R.L. (1962) *Poultry Sci.* **41**, 1665.
Peterson, D.W. (1977) *J. Fd Sci.* **42**, 100.
Schwall, D.V., Rogers, A.B. & Corbin, D. (1968) U.S. Patent 3, 399,063.

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'Collapse', a structural transition in freeze dried carbohydrates

I. Evaluation of analytical methods

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Summary

A number of techniques used to study 'collapse' in freeze dried carbohydrates were described. These include heating of samples in sealed ampoules, DTA, TMA, and various methods involving heating under an optical microscope. Accuracy and reproducibility of 'collapse' temperature (T_c) measurements were evaluated. It was shown that glass transitions occur near T_c in a number of freeze dried carbohydrates. 'Collapse' was found to occur over a range of temperatures up to 40°C. The extent of 'collapse' was quantitatively measured and found to be linear with temperature. T_c was found to be independent of heating rate.

Introduction

Carbohydrate materials which have been prepared from solution by freeze drying are generally found to be in a metastable state, in that non-first order structural transitions can occur. These transitions have been described by a variety of labels, but in most cases involve a shrinkage of the matrix and onset of a glassy appearance. Crystallization of the amorphous material is often a result of these structural transitions. Some of the properties of these transitions, which have been called 'collapse', have been described by Tsourouflis, Flink & Karel (1976), while a number of authors have evaluated the recrystallization of the amorphous matrix (Makower & Dye, 1956; Karel, 1975; Silver, 1976). In addition, related transitions in the frozen state or during freeze drying have been described by Luyet (1960), Ito (1970), MacKenzie (1966), and Bellows & King (1973).

In earlier work from this laboratory, we have described a method for evaluating 'collapse' temperatures (hereafter denoted T_c) in freeze dried carbohydrates

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based on heating the samples in sealed ampoules (Tsourouflis *et al.*, 1976). In this paper we will describe and evaluate a number of methods which have been used for the continuation of our studies on the 'collapse' phenomenon. We also demonstrate the range of information obtainable with the different methods. In the accompanying papers (To & Flink, 1978a, b) we present the results of studies in which these methods have been used.

Materials and methods

The methods which have been used to study the 'collapse' phenomenon include a modification of Tsourouflis *et al.* (1976) ampoule method, differential thermal analysis (DTA), thermal mechanical analysis (TMA), and various techniques based on optical microscopy. These are listed in Table 1 together with some specific properties and applications.

Table 1. Methods developed for the study of collapse

Method	Temperature range (°C)	Sample size (mg)	Specific applications
Ampoules	−20 to 250	25 to 1000	T_c of dry and wet samples
Microscope heater	25 to 250	< 1	Small quantities of dry samples, recrystallization temperatures
DTA	−100 to 500	10 to 50	T_g of dry samples, volatile loss, recrystallization
TMA	−100 to 200	5 to 20	Complementary to DTA in T_g, semi-quantitative 'collapse'
Photomicroscopy	25 to 180	< 1	Quantitative measure of the degree of 'collapse'

Ampoules

The ampoule method was modified from that of Tsourouflis *et al.* (1976). A single heating bath with improved temperature control allowed the temperature intervals used for 'collapse' determination to be reduced. This eliminated the need for the interpolation procedure by Tsourouflis *et al.* (1976). Three different constant temperature baths were used to cover the range of T_c from −20 to 250°C. For temperatures between −20 to +30°C, ampoules were immersed in denatured alcohol in a wide-mouth Dewar flask (15 cm diameter). Cooling was achieved by circulation of coolant (alcohol at −78°C) in a brass cooling coil, while heating was accomplished with a 250 W immersion heater. Temperature of the bath was monitored by a copper-constantan thermocouple

and controlled by a Love Temperature Controller (Wheeling, Illinois), which starts or stops the flow of coolant into the coil. Vigorous stirring was maintained with an electric stirrer. For temperatures between ambient and about 55°C, a stirred water bath was used (Exatherm P5 el, Juchhein Labortecknik, Schwartzwald, West Germany). For most samples studied, T_c were between 40 and 180°C and a mineral oil bath (50 × 30 × 12 cm) with two Haake Temperature Controllers (Type E51, Berlin, West Germany) and an electric stirrer was used. Samples with T_c above 180°C were heated in oil over a stirring hot plate (Corning PC 351, New York). The oil was in a 1 litre glass beaker, insulated with asbestos cloth. The heater was controlled by a Love Temperature Controller. All temperatures were measured with mercury-in-glass thermometers except in sub-ambient cases where an alcohol-in-glass thermometer was used. In a typical run, samples were placed into the corresponding baths at the lowest bath temperature. The temperature was raised 2°C every 15 min until 'collapse' has occurred in all samples. It generally required less than 3 min for the bath to attain a 2°C rise in temperature. At the end of each 15 min period, all samples were inspected visually for 'collapse', the 'collapsed' ones noted, and the controllers set to a temperature 2°C higher.

Since flame sealed ampoules offered a completely closed system, this was the only method capable of determining T_c of moist samples without changes in their moisture contents occurring during heating. Rehumidification of freeze dried samples was done according to Tsourouflis *et al.* (1976) and moisture contents (expressed as a percentage of dry weight) were determined gravimetrically.

Differential thermal analysis (DTA)

Differential Thermal Analyzer (model 900, Du Pont de Nemours and Co. Inc., Wilmington, Delaware) was used to measure transition phenomena in freeze dried samples. Thermograms were run in air at atmospheric pressure with glass beads in the reference tube. The instrument settings were generally 20°C/in (0.8 mV/in) for the temperature scale, 0.5°C/in (0.2 mV/in) for the differential temperature scale, and 20°C/min for the heating rate. The sample was rapidly crushed to a powder in a manner aimed at minimizing water uptake. In order to better match the thermal properties of the sample and reference tubes, as well as to minimize boiling and bubbling of the sample at high temperatures, the powdered sample (about 2 mg) was packed into a 4 mm glass macro tube between two layers of glass beads approximately 0.5 mm in thickness. The macro tube was selected because the larger sample size increased the sensitivity of the method for detecting small ΔT changes associated with second order transitions. Prior to the start of each thermogram, the silver heating block was cooled over liquid nitrogen vapours to bring its temperature down to around −20°C. This was necessary to prevent the uneven temperature distribution within the heating block at the start of heating from interfering with those transition signals which appeared close to ambient temperatures, e.g. those of freeze dried sucrose.

Thermal mechanical analysis (TMA)

TMA was performed using a Du Pont 940 Thermal Mechanical Analyzer, which was a plug-in module of the Du Pont 900 DTA. It measured the temperature at which a material started to soften or flow while subjected to a constant load. The vertical displacement of the sample was sensed by a movable core differential transducer (LVDT) capable of detecting a displacement of 2×10^{-5} in (5×10^{-4} mm) at its highest sensitivity setting of 0.004 mV/in. The sample to be measured was first crushed between two glass slides and a piece of 1 mm or less in height selected. This was sandwiched between two aluminium foil discs of 6 mm diameter and placed into the sample tube. The aluminium foil prevented sticking of the sample to the probe and sample tube, thus facilitating cleaning after a run. The instrument settings were generally 20°C/in (0.8 mV/in) on the temperature scale. 0.4 mV/in on the ΔT scale and 10°C/min heating rate. The load on the sample was 5 g. All runs were started at least 20°C below the expected transition temperatures to allow the heating conditions to stabilize prior to structure transition. Limitations of the TMA apparatus were the relatively low maximum operating temperature (200°C or below) and the maximum heating rates achievable (10°C/min below 100°C, 5°C/min above 100°C).

Optical microscopy

All optical microscopic examinations were made on an Olympus FH microscope equipped with trinocular head and a set of polarizing filters. Incorporation of a heating stage on the microscope permitted viewing of structural transitions on a microscale and thus evaluation of T_c. Control of the sample temperature and heating rate was achieved using a custom-built temperature programmer. By photographing the sample at various stages during the heating procedure, changes in the sample size (in terms of area) was measured and the degree of 'collapse' evaluated as a function of temperature. In fact, it was through this method that 'collapse' was observed to occur over a wide range of temperatures (see Results and Discussions).

The heating system consisted essentially of three parts: a 25×25 mm glass coverslip having a transparent, electrically resistive coating, a programmable heating control unit, and a temperature monitoring system. The coverslip heater was fabricated by heating the 25 mm square cover glass (Corning No. 1, New York) to about 400°C on an aluminium block over a Bunsen flame. A mixture consisting of 17 g stannic chloride pentahydrate, 34 g of acetone and 0.3 g of antimony pentachloride was sprayed onto the glass through an air brush (Paasche H 3") at 20 psig dry nitrogen. About 20 ml of the mixture were needed for each batch of six cover glasses. Proper resistance was about 100 ohms when measured with point electrodes placed near opposite edges of the coverslip. A well-sprayed coverslip would have a blue-grey tinge. Power to the cover glass was supplied through thin wires attached to the tin oxide side by conductive epoxy cement (Dynaloy 340, Hanover, New Jersey). For ease of

handling under the microscope, the coverslip heater was taped, non-conducting side up, to a 75 × 25 mm glass microscope slide. A custom-built temperature programmer was used to regulate the DC voltage input to the coverslip heater (Chen, 1977). Temperature of the coverslip was monitored by a fine-wire copper-constantan thermocouple of 0.002 in diameter with a bead diameter of 0.006 in (Omega Engineering, Stamford, Conn.). Eleven discrete heating rates between 2 and 40°C/min were available and the upper limit of heating was variable from 30 to approximately 190°C. At any point during the heating program, the programmer could be manually switched to operate in the isothermal mode, after which heating could be resumed at the previous rate or at a new rate if desired. The fine-wire thermocouple was also connected to a recorder equipped with an event marker (Sargent DSRG) so that transitions observed in the microscope could be entered onto the temperature recording. A schematic of the system is given in Fig. 1.

In a typical determination, flakes of freeze dried sample were broken off with a needle and placed on the coverslip heater. Three to five drops of silicone oil (Dow Corning 200 Fluid, 500 cSt viscosity) were added, the controlling thermocouple placed in the centre of the sample and a 12 × 12 mm cover glass added to sandwich the sample flakes and thermocouple (Fig. 1). The silicone oil was necessary to give good thermal contact between the sample and the heater. The top cover glass held the sample and thermocouple in place and also prevented exposure of the sample to air due to flow of the silicone oil at high temperatures. After both sample and thermocouple bead were brought into focus (generally at × 150 magnification) the heating programme was commenced. Particular flakes were chosen for examination and photographed at various

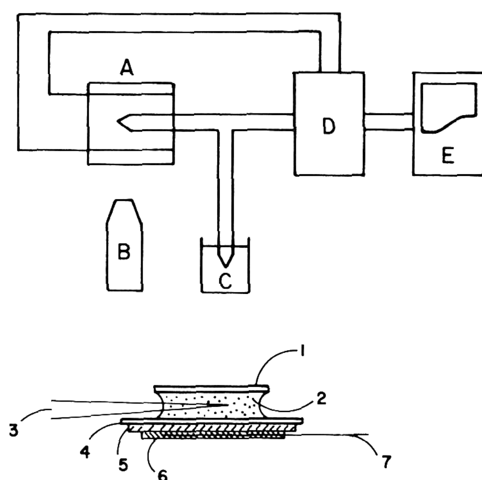

Figure 1. Schematic of the microscope heating stage. A Slide heater, B microscope, C ice reference, D temperature programmer, E recorder; 1 coverslip (12 × 12 mm), 2 sample-oil mixture, 3 thermocouple, 4 coverslip (25 × 25 mm), 5 tin oxide coating, 6 conductive epoxy, 7 leadout wire.

stages in the heating process. The area of the flake was determined gravimetrically by cutting the flake from the photograph and weighing the paper (so-called 'cut and weigh' technique). The extent of 'collapse' was defined in terms of the change in flake area. Changes in flake area were expressed by either of two methods. The first method required carrying the 'collapse' treatment until the flake had become a round structureless 'blob' and no further change in area was observed. In this case fractional 'collapse' was defined as $(A_i - A)/(A_i - A_f)$, where A_i and A_f denoted initial and final areas respectively. The second method was to measure 'collapse' as percent of initial area, i.e. $(A_i - A)/A_i$, thus eliminating the need to determine the final area.

Results and discussion

Evaluation of ampoule method for determining T_c

The ampoule method is the only means by which effect of moisture on T_c can be studied quantitatively. It has been found that moisture plays a very important role in 'collapse', and a 1% difference in moisture is enough to change T_c by 5°C in most samples studied. Residual moisture after freeze drying therefore can drastically alter T_c of the 'dry' substance. When ampoules containing different amounts of sucrose solution (20% w/w solids) are freeze dried for 48 hr and their T_c determined, it is found that ampoules containing more solution initially have lower T_c (Fig. 2, curve A). If the set of sucrose

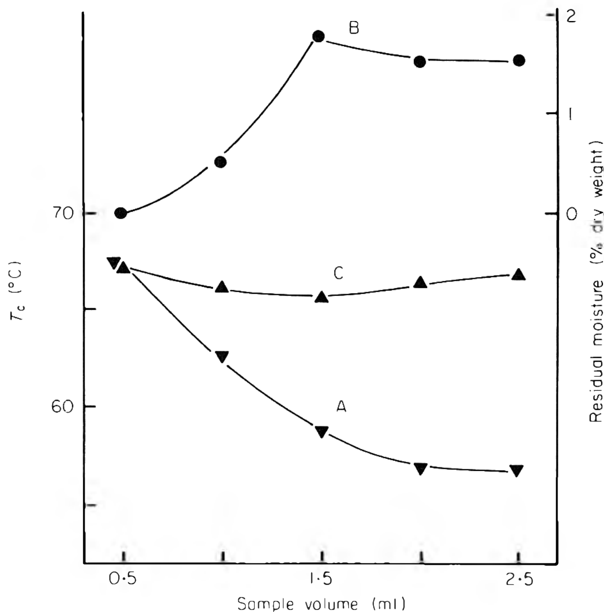

Figure 2. Effect of sample volume on residual moisture and T_c of freeze dried sucrose. Curve A, freeze dried; curve B, residual moisture after freeze drying; curve C, freeze dried followed by vacuum oven drying.

samples are subjected to additional vacuum over drying at 45°C for 12 hr and then their T_c measured, the dependence on sample volume is eliminated. In every case the T_c of samples additionally dried in the vacuum oven are higher than those which are merely freeze dried at ambient temperature conditions (Fig. 2, curve C). The reason for the lower T_c in samples containing larger volumes of solution is that residual moisture in larger volume samples is higher (Fig. 2, curve B). Presumably this is due to the greater sample thickness, since the diameter of the ampoules is constant. Figure 2 shows that in order to obtain as low a moisture content as possible in the freeze dried samples without collapsing them, it will be necessary to further dry them in a vacuum oven at a temperature slightly below T_c. All subsequent T_c determinations with ampoules were done with prior vacuum oven drying.

Evaluation of optical microscope method for determining T_c

The T_c of any sample could be found by heating it at a fixed rate (usually 40°C/min) and noting the temperature when sample shrinkage was first observed. This gave a quick and simple method for T_c measurements making it comparable to routine melting point determinations. This microscope method was evaluated for accuracy and sensitivity by measuring the melting points of crystalline compounds having known melting points between 40 and 180°C.

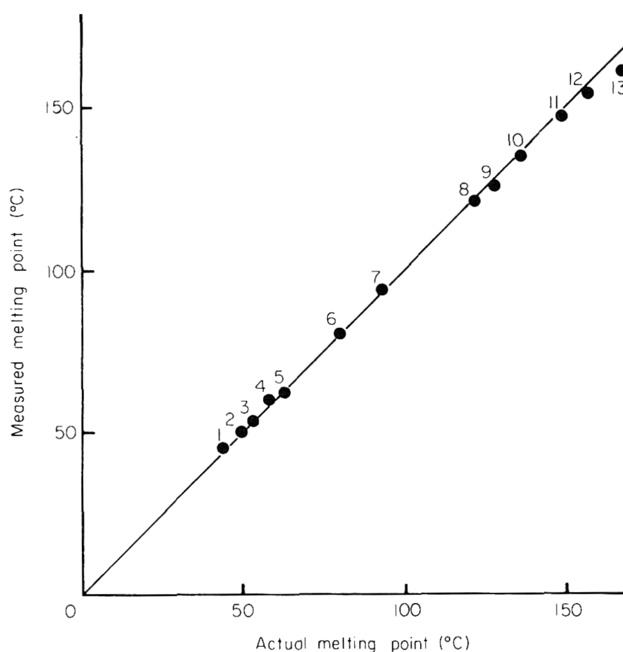

Figure 3. Melting points of crystalline materials determined by the microscope heating stage. 1 Lauric acid, 2 Thymol, 3 α-tristearin, 4 sodium acetate $3H_2O$, 5 tripalmitin, 6 naphthalene, 7 α-naphthol, 8 benzoic acid, 9 fructose, 10 malonic acid, 11 cholesterol, 12 glucose, 13 mannitol.

The procedures were exactly the same as in T_c measurements. Figure 3 shows a very good correlation between measured and known melting points.

To evaluate the reproducibility of the microscope method for T_c determinations, measurements were made on five different batches of freeze dried maltose which have been stored over $CaCl_2$ since preparation. Five T_c determinations were made on each batch and the results given in Table 2. Samples A and B had been given an additional 24 hr of vacuum oven drying at 80°C prior to T_c determination. It can be seen that within each batch, the standard deviations are all less than 2%. Analysis of variance shows that samples A and B differ significantly ($P < 0.01$) from C, D and E, but that there is no difference within each group (Table 3). This difference between A and B, and C, D and E, is due to the lower moisture content for the samples which were vacuum dried just prior to analysis. The between-day variation in T_c of maltose was also investigated over a 10-day period and no variation was found once the moisture content of the sample stabilized.

Evaluation of DTA and TMA methods for measuring T_c

DTA and TMA methods of T_c determinations will reduce the influence of operator variability which exists with the visual microscope or ampoule methods. Performance of the DTA was first evaluated with melting endotherms of a number of crystalline carbohydrates. The melting points were within 2 to 3°C of the true values and ΔT of melting were routinely between 1 to 3°C. Figure 4 shows DTA traces for freeze dried sucrose, maltose and their 1 : 1 mixture. All samples were prepared from solutions containing 20% solids by

Table 2. Microscope T_c for five samples of freeze dried maltose

Sample	T_c(°C)					$\bar{T}_c$	s.d
A	102	101	104	103	103	102.6	1.14
B	102	99	101	102	102	101.2	1.30
C	95	95	95	96	93	94.8	1.10
D	96	94	96	98	94	94.6	1.67
E	99	95	96	95	97	96.4	1.67

Table 3. One-way analysis of variance of T_c measurements of maltose

Groups	d.f.*	F	Tabulated F values		Significance
			95%	99%	
A, B, C, D, E	4, 20	31.8	2.87	4.43	$P < 0.01$
A, B	1, 8	3.27	5.32	11.3	NS
C, D, E	2, 12	1.41	3.89	6.93	NS

* Degrees of freedom.

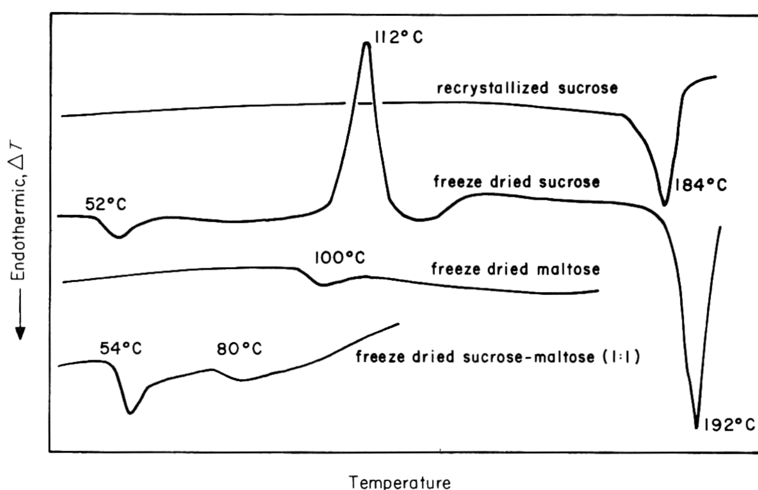

Figure 4. DTA of freeze dried sucrose, maltose and their mixture.

weight and freeze dried for 48 hr at ambient temperature with a pressure of < 50 millitorr. The DTA of freeze dried sucrose shows a second order transition at 52°C, a recrystallization exotherm at 112°C, and a melting endotherm associated with the now crystalline sample at 192°C. On cooling this melted sample of sucrose to ambient temperature and then reheating, no DTA features other than the melting endotherm can be seen. This type of DTA behaviour for freeze dried sucrose has previously been noted by Simatos & Blond (1975). Figure 4 also shows the DTA of freeze dried maltose which indicates a second order transition at 100°C, the T_c of the sample. When a solution of 10% sucrose and 10% maltose was freeze dried, the DTA of the resulting matrix shows transitions at 54 and 80°C, the latter temperature being close to the T_c for this mixture as determined microscopically. The data show that second order type

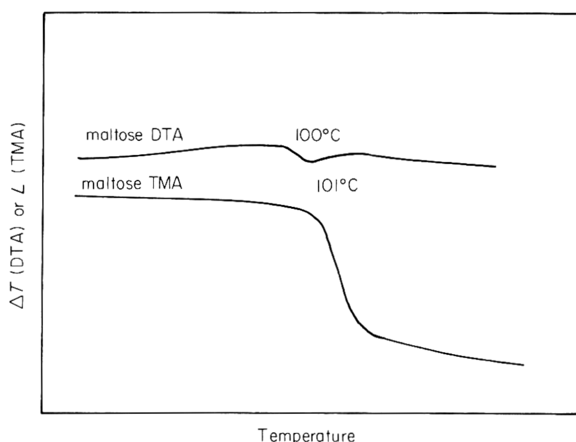

Figure 5. Transition temperatures of freeze dried maltose determined by DTA and TMA.

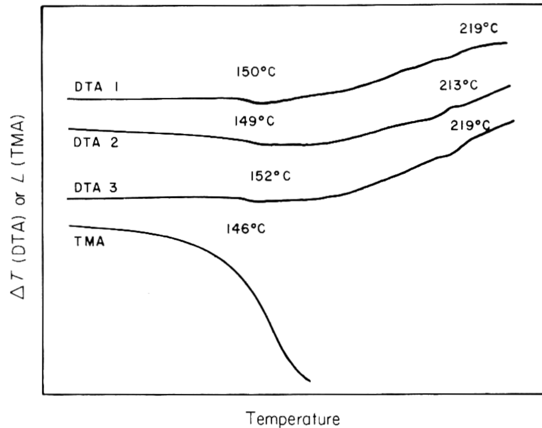

Figure 6. Transition temperatures of freeze dried maltodextrin (Maltrin M250) determined by DTA and TMA.

glass transitions occur at or slightly below the T_c for freeze dried carbohydrates.

The DTA and TMA modes use the same *x*-*y* recorder and accompanying electronics. Thus both the DTA and TMA traces can be put on the same thermogram, sharing the common *x*-axis temperature scale. In general, the TMA curve shows only one transition. This occurs at the T_c of the freeze dried sample. In freeze dried maltose, both DTA and TMA show a transition at around 100°C (Fig. 5). In a few instances, T_c cannot be readily determined from the DTA trace alone. An example is freeze dried maltodextrin M250 whose DTA shows only faint indications of transitions at 150 and 219°C (Fig. 6–DTA 1, 2 and 3 are replicates for the same M250 sample). By overlaying the TMA curve onto the DTA, it becomes clear that T_c of this sample is at 146–150°C. The suspected DTA transition at higher temperature cannot be confirmed by TMA since it is outside the operating range of the Du Pont 940 instrument. From these examples it can be seen that DTA and TMA complement each other in providing information on structural changes of freeze dried carbohydrates.

Evaluation of methods for measuring degree of 'collapse'

The discovery that 'collapse' occurs to varying extent at different temperatures was first noted on a series of freeze dried sucrose and maltose mixtures. These mixtures were subjected to vacuum oven drying at 80°C and their T_c determined by TMA. Those samples with T_c higher than 80°C prior to oven drying had their T_c slightly elevated due to moisture removal. However, samples with T_c lower than 80°C prior to drying underwent varying degrees of 'collapse' during drying. When their T_c were measured by TMA after drying, they were all about 80°C, the temperature at which the 'collapse' in drying took place (Fig. 7). Thus by merely heating a freeze dried sample to a temperature slightly higher than its original T_c, it is possible to then cool it and obtain a

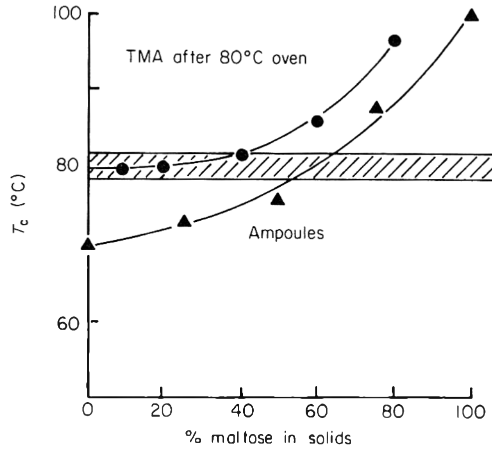

Figure 7. T_c of freeze dried sucrose–maltose mixtures. Triangles, T_c determined by the ampoule method after freeze drying. Circles, T_c determined by TMA after freeze drying and exposure to 80°C.

new T_c equal to the highest temperature previously reached. This was further investigated by heating a sample of freeze dried maltose on the microscope heater. The instant the sample was observed to shrink (i.e. 'collapse'), it was cooled by turning off the heater. The T_c was noted and the cooled sample reheated until 'collapse' was again observed. By plotting the T_c so obtained, against the respective maximum temperature reached previously, a linear relationship resulted (Fig. 8). Using TMA to determine the T_c, the same behaviour was observed. A sample of freeze dried sucrose–glucose (9 : 1 w/w) was

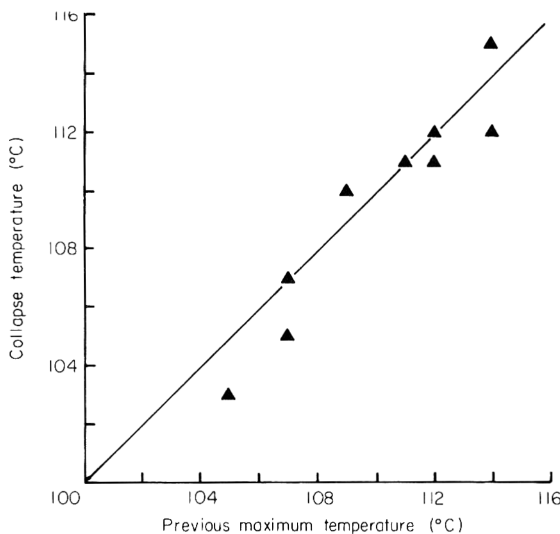

Figure 8. Collapse of freeze dried maltose as a function of previous temperature history.

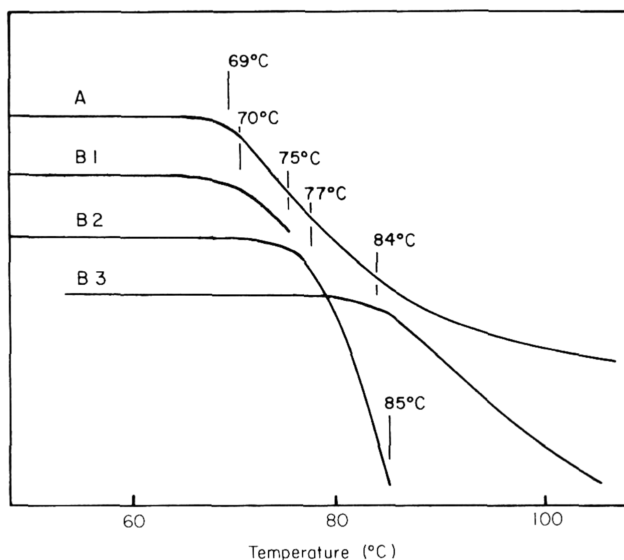

Figure 9. T_c of freeze dried glucose–sucrose mixture (2%: 18% w/w) determined by TMA. Curve A, continuous heating above 100°C. Curve B1, a second sample heated to 75°C and immediately cooled, then reheated to 85°C (curve B2), cooled and heated past 100°C (curve B3). Curves are displaced vertically to facilitate comparison.

heated continuously past 100°C. T_c was found to be around 70°C (Fig. 9, curve A). Another sample was heated to 75°C (Fig. 9, curve B1) just slightly above the T_c noted in the curve A. The 5 g load was removed and the sample rapidly cooled by lowering the heater surrounding it. The same sample was reheated to a higher temperature (curve B2), cooled and heated up a third time to a still higher temperature (curve B3). It can be seen that after each cooling, renewed heating will not give 'collapse' until the previous maximum temperature is reached. This means that 'collapse' is not an all-or-nothing phenomenon; rather, once started, it can be stopped partway or allowed to proceed to a higher degree, depending on the temperature. The use of photomicroscopy shows this behaviour clearly. A flake of freeze dried maltose was heated from ambient to 140°C and photographs taken at various temperatures during heating. The outline of the flake at each temperature is given in Fig. 10 and the fractional 'collapse' $(A_i - A)/(A_i - A_f)$ is plotted against temperature in Fig. 11. The degree of 'collapse' increased linearly from 100 to 120°C and then started to plateau. Flake area did not stop shrinking until about 140°C, giving a range of 40°C in which 'collapse' of maltose was occurring. It is apparent that by measuring the flake area at a number of temperatures and extrapolating to 'zero collapse', one can objectively find the T_c of a sample as it is usually defined. Extrapolation of Fig. 11 to 'zero collapse' gives a T_c of 100°C, identical to results obtained by other methods.

The influence of experimental variables on 'collapse' behaviour was studied using flake area as a measure of degree of 'collapse'. The effect of heating rate

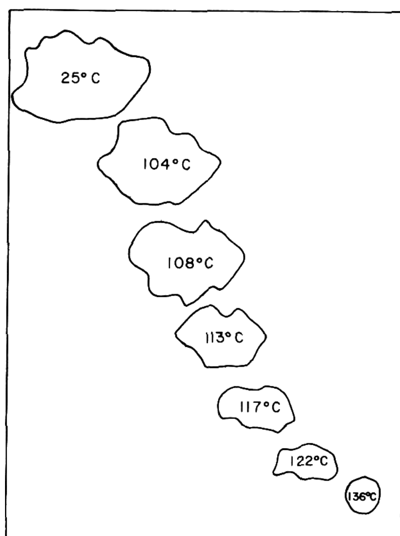

Figure 10. Area of a freeze dried maltose flake at various stages of collapse (magnification ×150).

on T_c of freeze dried maltose was studied. Heating rates of 2, 10, 20, 30 and 40°C/min were used to heat the sample. While slight differences were noted in degree of 'collapse' for heating at 2°C/min and 10–40°C/min, extrapolated T_c was found to be independent of heating rate (Fig. 12). The effect of holding a freeze dried sample of maltose at various temperatures above T_c was investigated. A sample flake was heated from ambient to 140°C. At 107, 115, 125

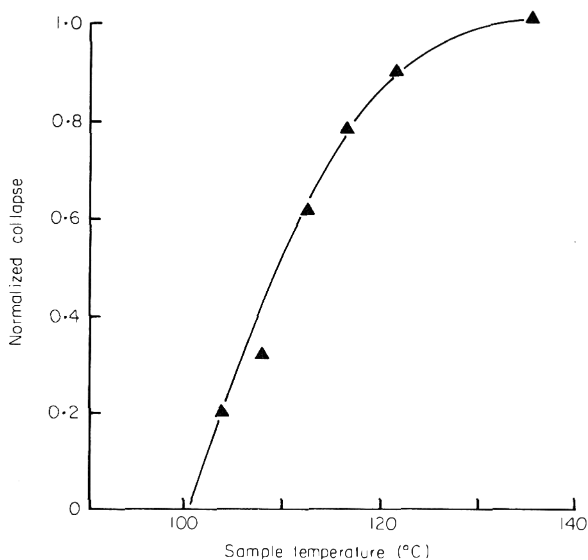

Figure 11. Normalized degree of collapse of freeze dried maltose at different temperatures.

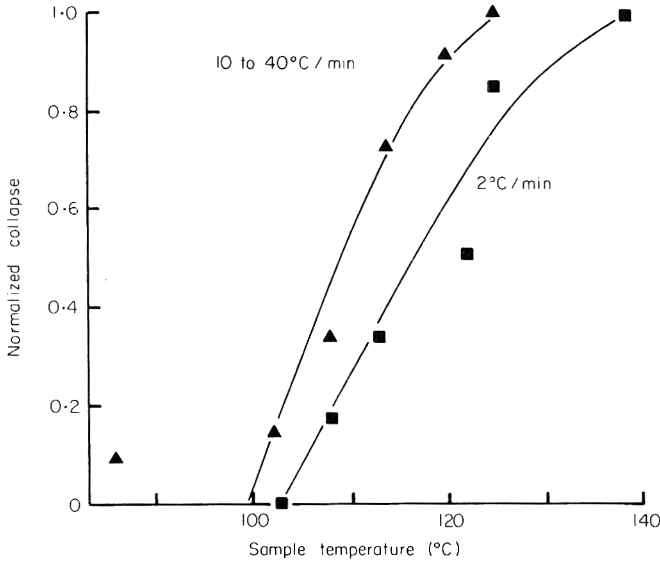

Figure 12. Effect of heating rate on measured T_c of freeze dried maltose.

and 136°C, the temperature programmer was manually switched to the isothermal mode to hold the sample at constant temperature for 5 min. At 0, 2 and 5 min holding time, photographs of the flake were taken. After 5 min the same sample was heated to the next higher temperature (Fig. 13). It can be seen that during each holding period, very little 'collapse' occurred after the first 2 min. Thus degree of 'collapse' is not a time dependent phenomenon, but

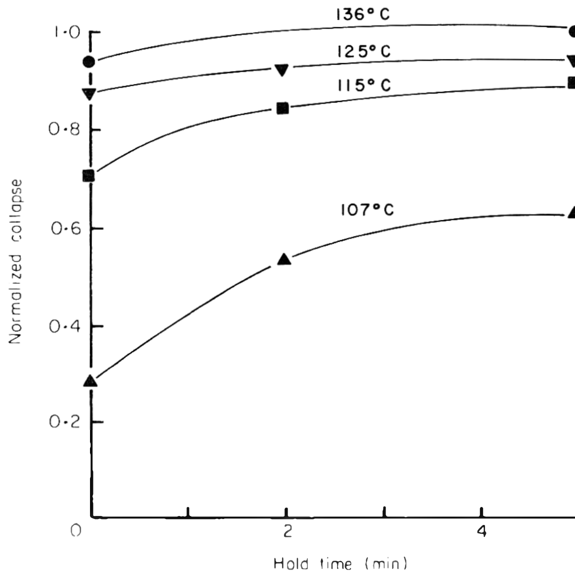

Figure 13. Effect of holding at different temperatures above T_c on the degree of collapse in freeze dried maltose.

rather is solely dictated by the temperature reached in the sample. This type of 'collapse' behaviour is found not only in freeze dried maltose, but in other freeze dried carbohydrates and their mixtures. In most cases the range of temperature over which 'collapse' can be observed is 20–40°C, with degree of 'collapse' varying linearly with temperature for the first 80% of 'collapse' in most systems studied.

Several reports have been noted on the importance of considering intermediate levels of 'collapse' for describing the retention behaviour of volatile flavour compounds during freeze drying (Bellows & King, 1973) and loss of entrapped flavour from previously freeze dried materials (Flink & Karel, 1972; Chirife & Karel, 1974). With the techniques noted above, quantitative determinations of the degree of 'collapse' are now possible. Use of these techniques for studying quantitatively the relationship between 'collapse' and flavour loss are presented in Part III of this series of papers on 'collapse' (To & Flink, 1978b).

Acknowledgments

We thank Professor Robert Cohen of the Chemical Engineering Department for his kind permission to use the DTA and TMA instruments in his laboratory. This work was supported by Grant FD 00713-02 from the Public Health Service, Department of Health, Education and Welfare.

References

Bellows, R.J. & King, C.J. (1973) *AIChE Symposium Series 69 (132)*, 33.

Chen, H.H. (1977) *A Differential Thermal Analysis System for the Optical Microscope.* S.B. thesis, Massachusetts Institute of Technology.

Chirife, J. & Karel, M. (1974) *J. Fd Technol.* **9**, 13.

Flink, J.M. & Karel, M. (1972) *J. Fd Technol.* **7**, 199.

Ito, K. (1970) *Chem. Pharm. Bull.* **18**, 1509.

Karel, M. (1975) In: *Principles of Food Science. Part II. Physical Principles of Food Preservation* (Ed. by M. Karel, O.R. Fennema and D.B. Lund) ch. 8. Marcel Dekker, New York.

Luyet, B.J. (1960) *Ann. N.Y. Acad. Sci.* **85**, 549.

MacKenzie, A.P. (1965) *Bull. Parenteral Drug Assn.* **20**, 101.

Makower, B. & Dye, W.B. (1956) *J. Agr. Fd Chem.* **4**, 72.

Silver, M. (1977) *The Behavior of Invertase in Model Systems at Low Moisture Contents.* Ph.D. thesis, Massachusetts Institute of Technology.

Simatos, D. & Blond, G. (1975) In: *Freeze Drying and Advanced Food Technology* (Ed. by S. A. Goldblith, L.B. Rey and W. W. Rothmayr), p. 401. Academic Press, London.

To, E.C. & Flink, J.M. (1978a) *J. Fd Technol.* **13**, 567.

To, E.C. & Flink, J.M. (1978b) *J. Fd Technol.* **13**, 583.

Tsourouflis, S., Flink, J.M. & Karel, M. (1976) *J. Sci. Fd Agric.* **27**, 509.

(Received 24 February 1978)

J. Fd Technol. (1978) **13**, 567–581

'Collapse', a structural transition in freeze dried carbohydrates

II. Effect of solute composition

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Summary

The influence of moisture, component molecular weight or binary mixture composition on T_c of freeze dried matrices was evaluated. It was shown that $\log(T_c)$ is linearly related to moisture content, with a break in the curve at approximately the BET monolayer. The collapse temperature, T_c, was inversely proportional to molecular weight for a homologous series of malto-oligosaccharides. An expression giving the change in T_c for compositional changes in a binary mixture, with both components remaining amorphous, was shown to apply. Knowing the relationship between T_c and degree of polymerization enabled the prediction of T_c of any multi-component mixture of malto-oligosaccharides based on the general expression $1/T_c = \Sigma\, W_i/T_{ci}$. Large increases in T_c in the presence of a crystalline phase was shown for freeze dried inositol-containing systems at high inositol contents. These results are discussed on the basis of structure in the freeze dried matrix.

Introduction

Among methods commonly used by the food industry for drying, freeze drying is unique in that water is removed by sublimation of ice instead of evaporation of liquid water. Substances which have been freeze dried possess a set of properties that is characteristics of this method of drying. If the initial solids concentration of the starting solution is greater than 1% (weight basis), the freeze dried solid will retain the shape of the container in which it is frozen, thus forming a 'cake'. Other methods of drying produce powders, as in spray drying, or sheets, as in drum drying. Only in freeze drying is it possible to obtain an extensive gross structure of low bulk density. Microscopic examination of freeze dried substances shows layers of needle-like void spaces

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previously occupied by the ice crystals which formed during freezing (Gejl-Hansen & Flink, 1976). When the freeze dried cake is subjected to heating, at a certain temperature a change in structure known as 'collapse' generally occurs (Tsourouflis, Flink & Karel, 1976). This 'collapse' phenomenon is most noticeable as a radial shrinkage of the cake. The cause of this shrinkage has generally been attributed to reduction in the viscosity of the matrix to the point where it becomes too low to support its own weight resulting in flow of the matrix (Bellows & King, 1973). The temperature at which this occurs is a function of the moisture content, as well as the type of solute (Tsourouflis *et al.*, 1976). They found that 'collapse' temperature (T_c) decreases as moisture content of the freeze dried solid increases. It was shown that low molecular weight solutes have lower T_c values than higher molecular weight solutes, and further that addition of high molecular weight gums or starches to solutions of low molecular weight sugars, such as orange juice will increase the T_c of the freeze dried juice.

The phenomenon of 'collapse' is not limited to dried materials. During freeze drying, when the material subjected to drying consists of an ice core, a moist layer with a moisture gradient and a dry layer, part of the moist layer can undergo 'collapse' if the temperature is increased beyond certain critical levels. Using microscopic techniques, MacKenzie (1965) has determined the T_c of a number of solutes during freeze drying. These temperatures also show a dependence on the solutes' molecular weight. Starches and proteins have T_c close to $-10°C$, while glucose and fructose are found to collapse around $-40°C$. Bellows & King (1973) studied the collapse phenomenon by visually observing the temperature at which the freeze drying sample started to show puffing. Their amorphous viscosity theory proposed that during freezing, formation of ice results in development of a concentrated amorphous solute phase (CAS), and that collapse occurs during drying when the viscosity of the CAS phase is below a critical level of 10^7 to 10^{10} cP.

Other examples of structural changes in the 'dry' state due to the combined effects of temperature and moisture can be cited. In a spray drying operation, if the operating temperature or humidity in the chamber are too high for the particular food material, the dried powder will stick to the dryer walls (Brennan, Herrera & Jowitt, 1971). One way to reduce this sticking is by adding less sensitive materials as carriers (Mizrahi, Berk & Cogan, 1967). The process of agglomeration, in which dry powders are carefully re-wetted to cause them to stick together in clumps which are then re-dried, is related to the same 'collapse' (Masters & Stoltze, 1973). Caking of dry powders following exposure to humid air or fluctuating temperatures is also a form of 'collapse' (Peleg & Mannheim, 1977). In all these cases, the dry powders exist initially in an amorphous solid state. 'Collapse' is the visual result of the amorphous solid undergoing viscous flow, when it takes on a liquid-like property. As described in all the above examples 'collapse' appears comparable to second order glass transitions of amorphous polymers in which the transition temperature, T_g is dependent on the diluent content in much the same way that moisture affects 'collapse'.

'Collapse' also appears to be an important factor relative to retaining organo-

leptic quality of dry powders during storage. It is well known that freeze drying and spray drying processes have a high ability to encapsulate flavours and volatile compounds. For freeze drying, flavour volatiles so entrapped have been found to be confined to very minute regions in the matrix and neither grinding nor vacuum packaging can bring about the removal of the entrapped volatiles (Flink, 1975). However, loss of volatiles does occur when the samples are heated or exposed to moisture. At increasing moisture levels in the sample above a critical value more volatile is lost (Chirife, Karel & Flink, 1973; Flink & Karel, 1972). When heat is applied, loss of volatiles does not occur until T_c is exceeded (Chirife & Karel, 1974). Recently Gejl-Hansen & Flink (1977) noted that if the surface-oil layer of carbohydrate emulsions is removed by hexane extraction the dry emulsions do not undergo oxidation even though microscopic examinations showed the presence of entrapped oil. However, when the sample is 'collapsed' by the addition of water, oxidation commences immediately.

It appears that the metastable amorphous state obtained by freeze drying has many exploitable properties such as flavour entrapment and protection of emulsified fat against oxidation, but that retention of these properties depends on maintenance of the metastable, non-collapsed state. Previous studies have focused attention on the moisture and temperature effects of 'collapse' (Tsourouflis *et al.*, 1976). In this paper we present evidence of the existence of second order transitions in freeze dried carbohydrates and give quantitative relationships between moisture and T_c, molecular weight and T_c, and solids composition in mixtures and T_c. The form of these quantitative relationships for the 'collapse' phenomena is similar to ones used in describing glass transition behaviour of polymers. The relationship of 'collapse' to the amorphous and/or crystalline nature of the freeze dried sample is discussed.

Materials and methods

Methods used to determine T_c are described in the preceding paper (To & Flink, 1978). Freeze dried samples which have not been rehumidified were crushed and T_c found by heating under the microscope, by DTA or TMA. The ampoule method was used to study the effect of moisture on T_c. Microscopic observation under cross polarizers was used to test for crystallinity.

In order to establish a quantitative relationship between molecular weight and T_c, gel filtration chromatography on Sephadex G25 was used to fractionate a commercial maltodextrin (Maltrin M250, Grain Processing, Muscatine, Iowa) into the component oligosaccharides. Maltrin M250 was the material chosen for T_c evaluation because its elution profile shows an even distribution of solids in all fractions. T_c was determined on the freeze dried pooled fractions of three gel filtration runs. A 55 × 4.3 cm column was used with a flow rate of 100 ml/hr. The elution time was 8 hr with fractions collected at 6 min intervals. The oligosaccharide concentration in each fraction was determined by the phenol-

sulphuric acid assay (Dubois *et al.*, 1956). This information was used to calculate the amount of solids in each fraction so that all fractions could be rehydrated to 2% solids prior to a final freezing in liquid nitrogen and freeze dried. T_c of each dried fraction was determined microscopically.

Results and discussions

Effect of moisture on T_c

The addition of moisture to freeze dried solids lowers their T_c. In fact the exposure of any dry powder which is water soluble to humidity at room temperature will eventually cause their T_c to be lowered below the ambient temperature and 'collapse' or sticking will result. Tsourouflis *et al.* (1976) found decreases in T_c for increasing moisture content for freeze dried sucrose and maltose as well as for freeze dried maltodextrins of higher molecular weight. (As an aside, it can be noted that the effect of moisture on T_c of two freeze dried proteins, gelatin and bovine serum albumin, showed similar behaviour to that seen with the carbohydrates though the proteins could tolerate a higher level of moisture than the carbohydrates without collapsing.)

A quantitative relationship between moisture and T_c has been presented by Tsourouflis *et al.* (1976). They proposed a linear relationship between log(mc) and T_c or $1/T_c$ where mc is the moisture content on a dry weight basis. Using this relationship they indicated that the T_c during freeze drying could be found by extrapolation of data obtained with dry and rehumidified samples. They also noted that literature values for the sticky point during spray drying and moisture-temperature relationships for agglomeration follow the log(mc) versus T_c or $1/T_c$ plots. This was interpreted to support the amorphous viscosity theory of collapse with the log(mc) versus T_c curves giving the interdependence of moisture and temperature for the critical viscosity of about 10^7 to 10^{10} cP.

In the present study when collapse of freeze dried maltose was examined at high moistures and subzero T_c, it was found that the plot of log(mc) versus T_c or $1/T_c$ is not linear. (It should be noted that the form of the equation does not provide a T_c for samples at zero moisture since log of zero is undefined.) Thus, while the Tsourouflis, *et al.* (1976) relationship had its practical value, it will be shown below that more meaningful interpretations can be obtained by relating log T_c and mc. Such a plot for maltose shows a straight line with a change in slope around 7% moisture (Fig. 1). Nissan (1976) studied the effects of hydrogen bond breaking on the mechanical strength of cellulosics and found the following linear relationship between log of the normalized Young's modulus and moisture content:

$$\ln (E/E_0) = -\mathrm{mc} \qquad mc < \mathrm{BET} \qquad (1)$$

$$\ln (E/E_0) = C_1 - C_2 (mc) \qquad mc \geqslant \mathrm{BET} \qquad (2)$$

where E is the Young's modulus and is related to the number of hydrogen

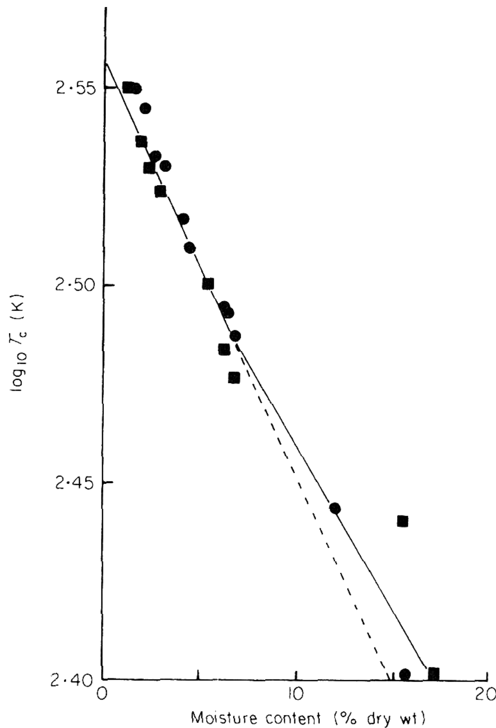

Figure 1. Log(T_c) versus moisture content for freeze dried maltose. ●, Slow frozen, ■, fast frozen.

bonds per unit volume, E_0 is Young's modulus at zero moisture, C_1 and C_2 are constants, and BET is the moisture content at the monolayer value. Equations (1) and (2) represent two straight lines of different slopes and intercepts. According to Nissan (1976) the first equation represents the situation when the moisture content is less than the BET value and the addition of one molecule of H_2O leads to the breaking of one H-bond. The second equation is applicable when the moisture content is greater than the BET value and sorption of an additional H_2O molecule leads to multiple H-bond breakage. It is noted that in a similar manner the log(T_c) of maltose is linearly related to moisture content with a change in slope at 7% moisture, which is near the BET for freeze dried maltose (Flink & Karel, 1972). This similarity leads us to believe that both T_c and E measure an equivalent structural force which is hydrogen bonding. Thus there is a theoretical basis for the log(T_c) versus *mc* equation; in addition this form also permits the assignment of T_c for samples of zero moisture as well as the possibility for extrapolation of a T_c value for freeze drying if the moisture content of the concentrated amorphous solute is known (MacKenzie, 1965; Aguilera & Flink, 1974). From data of Tsourouflis *et al.* (1976) and Maltini (1977), a plot of log(T_c) versus *mc* for freeze dried sucrose also was found to be linear with a break at 10% moisture. Further, when data from Karivaya & Hoeve (1975) showing the effect of moisture on the glass point of elastin was

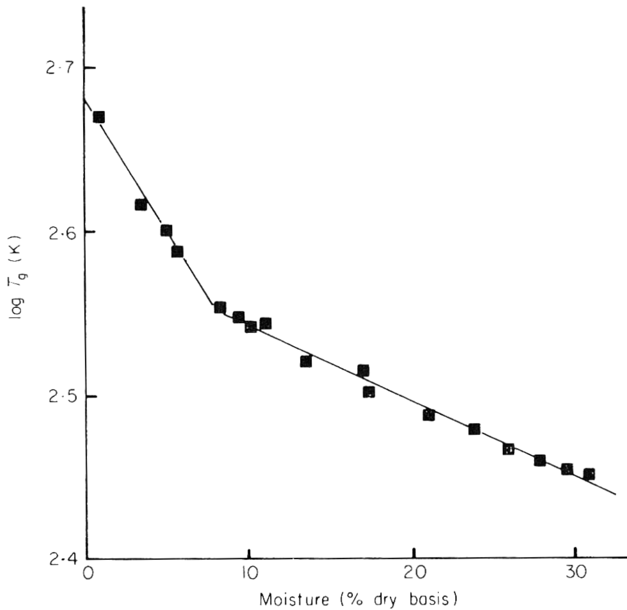

Figure 2. Log(T_g) versus moisture content for oven dried elastin (data of Karivaya & Hoeve, 1975).

plotted as log(T_g) versus *mc*, two straight line segments were obtained with a break occurring at 8% moisture (Fig. 2). This suggests that collapse in freeze dried carbohydrates and glass transition of an oven dried protein, elastin, may be related phenomena associated with the breaking of H-bonds.

Effect of molecular weight on T_c

The effect of molecular weight on T_c has been qualitatively studied by Tsouroufiis *et al.* (1976). They found that within a series of maltodextrins having dextrose equivalents (DE) of 10, 15, 20 and 25, the T_c increased as the DE values decreased. The DE value is an index of the degree of hydrolysis undergone by a starch, with higher DE values corresponding to greater extents of hydrolysis (i.e. lower average molecular weight). Thus, mixtures containing low molecular weight solutes (i.e. high DE values) gave low T_c and vice versa. In addition, T_c of a homologous series of pure compounds are also found to increase with molecular weight. For example starch has a higher T_c than the sugars maltotroise, maltose or glucose. A quantitative relationship between T_c and molecular weight has not been established.

In the present study, a DE 25 maltodextrin (Maltrin M250) was fractionated and the T_c of all fractions determined. An average molecular weight for each fraction was calculated from the elution volume using the method of Schmidt & Enevoldsen (1976). They showed that the degree of polymerization for a malto-oligosaccharide series is linearly related to $-\log(K)$, where K is defined as $(V_e - V_0)/(V_t - V_0)$. V_e is the elution volume, V_0 is the void volume, and V_t

is the total volume. T_c measurements on maltose and maltodextrin series were used to develop a relationship between degree of polymerization and T_c. Since unfractionated maltodextrins were used, an average degree of polymerization based on the dextrose equivalent value had to be used. The validity of this was tested by using this relationship and the T_c measurements of the M250 fractions noted above to determine the degree of polymerization versus $-\log(K)$ behaviour for the M250 fractions. As predicted by Schmidt & Enevoldsen (1976) a straight line was obtained. From this, a plot of degree of polymerization versus T_c for the fractionated M250 was made (Fig. 3) and by assuming that only malto-oligosaccharides are present, an average molecular weight calculated. In Fig. 4 it is seen that T_c of the fractions versus the reciprocal of their molecular weight gives a straight line for degrees of polymerization greater than or equal to three (i.e. glucose and maltose deviate from the line). Included in Fig. 4 is the molecular weight dependence of the glass transition temperature (T_g) of polystyrene taken from the literature (Meares, 1965). This curve shows that T_g of polystyrene is related to its molecular weight by the expression

$$T_g = T_g^{\infty} + K/\mathrm{MW} \tag{3}$$

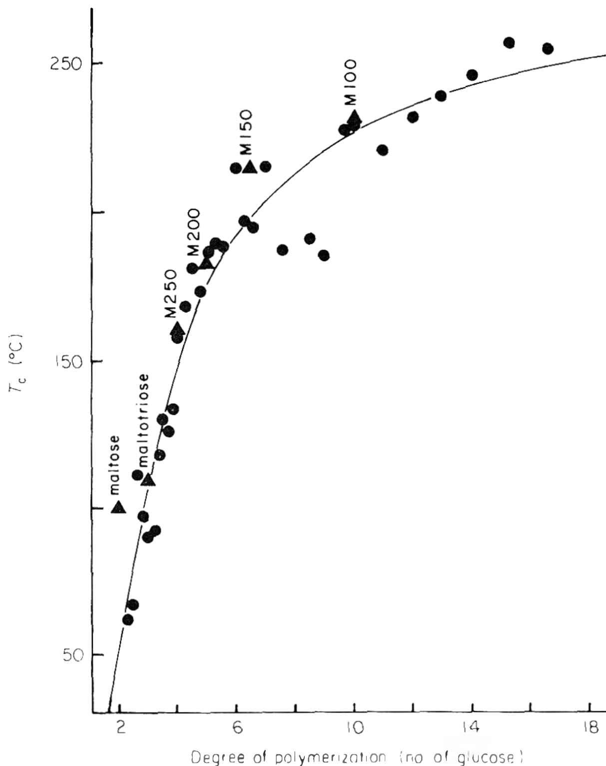

Figure 3. T_c of chromatographed fractions of Maltrin M250 on G25 versus degree of polymerization. ●, chromatographed fractions, ▲, reference compounds, not chromatographed.

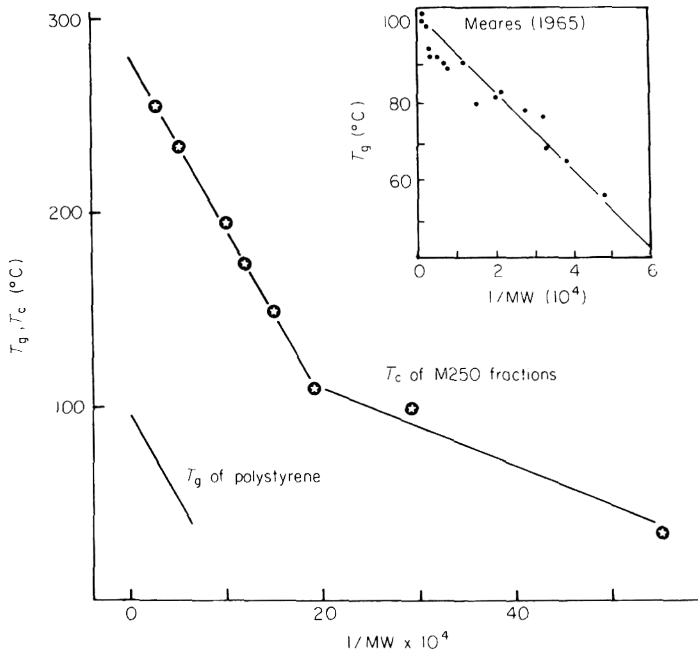

Figure 4. Effect of molecular weight on T_c of malto-oligosaccharides and on T_g of polystyrene.

where T_g^{∞} is the glass transition temperature at infinite molecular weight, K is a constant related to the physical properties of the polymer and MW is the molecular weight. T_c of malto-oligosaccharides, which follow the same relationship (Fig. 4) have an extrapolated T_c^{∞} of around 280°C. This higher value of T_c^{∞} as compared to T_g^{∞} of polystyrene (about 100°C) may be due to the higher bond energies in H-bonds in the freeze dried carbohydrate network as compared to Van der Waals' forces in the polystyrene network.

Prediction of T_c *in freeze dried mixtures of known composition*

The results of Tsourouflis *et al.* (1976) have shown that freeze dried solids containing a mixture of two solutes have a T_c intermediate between the T_c of each of the pure components. A smooth curve could generally connect the T_c values for all mixtures of a given binary solute system, with the exception of some food gums where addition of small quantities of gum to a low T_c solute such as orange juice powder resulted in a discontinuous jump in T_c. In the present study, microscopic evaluation showed the T_c of a number of dry binary solute systems to be quantitatively related to the weight fraction and T_c of each pure component in the following manner:

$$1/T_c = (W_1/T_{c1} + W_2 B/T_{c2})/(W_1 + W_2 B) \qquad (4)$$

where T_c are in degrees absolute, W is the weight fraction, B is a constant, and

the subscripts 1 and 2 denote the two solute components in the mixture. For T_g of ideal copolymers the B value is obtainable from free volume theory (Meares, 1965) but in practice its value is hard to predict. Knowing the T_c of the pure solutes and one mixture, eqn (4) is used to solve for the constant B. Once the B for a system is known, the T_c of mixtures of the two solutes in any ratio can be predicted. Presumably the same type equation will also be valid for humidified systems. Equally valuable, eqn (4) permits the prediction of T_c of pure solutes which are very hard to freeze dry due to the low 'collapse' temperature during freeze drying (e.g. fructose and glucose). In this case, B is found from T_c values for two or three mixtures of the low T_c component with a high T_c solute such as maltodextrin M150. The fit between predicted and measured values of T_c for mixtures containing maltose or M150 is shown in Fig. 5, together with the B values for each of the binary solute systems. In the strictest sense the term binary should not be used in mixtures containing maltodextrins (e.g. Maltrin M150), since even though freeze dried maltodextrins can be characterized by a single T_c, they in fact contain solutes having a wide range of degree of polymerization (DP). As noted in Fig. 5, however, maltodextrins do act as a single component in mixtures. Using the relationship between T_c and DP given in Fig. 3, together with malto-oligosaccharide distri-

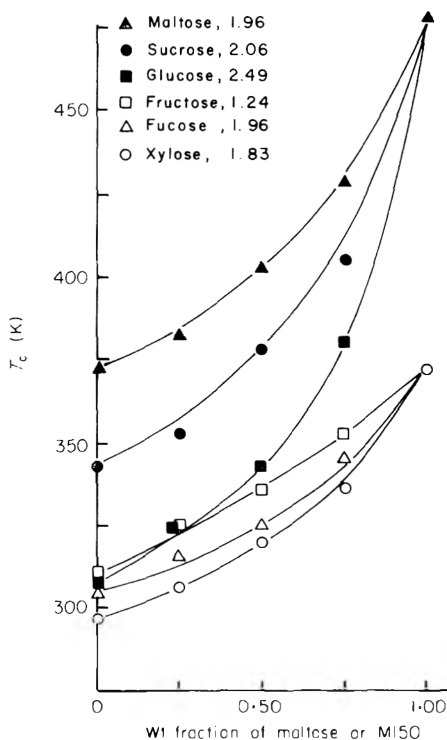

Figure 5. T_c of mixtures containing Maltrin M150 (solid symbols) or maltose (open symbols). Symbols are measured values, lines follow T_c as predicted by eqn (4) in text; the numbers indicate B values for that mixture.

bution data obtainable by gel filtration chromatography, the T_c of any multi-solute matrix composed of the homologous series of malto-oligosaccharides can be predicted using a generalized form of eqn (4):

$$1/T_c = \Sigma W_i/T_{ci}. \tag{5}$$

This relationship has been tested with maltodextrins having DE values of 10, 15, 20 and 25 as well as various mixtures of Maltrin M150 (DE 15) with glucose and maltose. The predicted T_c, based on composition, are reasonably close to actual values measured experimentally. Table 1 shows the composition of

Table 1. Fractional weight percentages and T_c used for predictions of T_c of malto-oligosaccharide mixtures

		Weight of maltrin fractions (%)			
DP	T_c(°C)	M250	M200	M150	M100
1	35	4.50	2.04	1.04	0.31
2	100	9.40	6.05	3.96	1.34
3	120	9.50	8.53	6.32	3.44
4	145	7.60	6.39	5.25	2.98
5	175	6.60	6.11	4.75	2.64
6	184	9.80	12.00	8.79	5.35
7	205	8.60	10.38	8.98	6.41
8	215	4.90	4.29	4.78	4.20
9	220	3.10	2.71	3.26	2.41
10	225	2.30	2.09	2.57	2.41
11	230	1.90	1.77	2.07	2.12
12	235	1.70	1.73	1.94	1.85
13	240	1.60	1.43	1.74	1.73
14	242	1.50	1.21	1.48	1.62
15	245	1.00	1.19	1.34	1.47
16	248	1.10	0.86	1.18	1.17
17	250	1.10	0.83	0.95	1.10
20	255	23.30	30.59	39.49	56.78
	Predicted T_c	150°C	171°C	190°C	217°C

different maltodextrins together with their predicted T_c, based on T_c values for each DP given in the second column. Using this composition data, it is possible to calculate the weight average and number average molecular weights of the four maltodextrins. The results in Fig. 3 are based on a relationship between T_c and DE values. Since DE values are indices of number average molecular weights, Fig. 4 therefore shows a relationship between T_c and number average molecular weight of malto-oligosaccharides. However, the fact that T_c of the four maltodextrins lie on the T_c versus DP curve for chromatographed fractions (Fig. 3) clearly indicates that molecular weight dispersion of the mixture has little effect on its resulting T_c.

Crystallinity in freeze dried solids

Almost all water-soluble carbohydrates freeze dry to an amorphous powder. While X-ray diffraction studies of freeze dried sucrose have indicated a largely unorganized condition, electron diffraction studies have shown that minute regions of crystallinity exist in these samples. Such regions are not found in the amorphous state obtained by rapid quenching of molten sucrose (Simatos & Blond, 1975). These regions are too small to be observed under polarized light with the optical microscope. In the present study, crystallinity is defined in terms of a sample being able to refract polarized light and having a melting temperature at which this ability is lost.

The importance of the amorphous or crystalline state in determining T_c of freeze dried substances is shown with binary solute systems containing the hexitol, inositol. Binary solute solutions containing various weight fractions of inositol and a second solute were prepared at a total solids content of 20% (weight basis). These solutions are freeze dried and their T_c determined by the ampoule method. Results show that freeze dried inositol has a 'T_c' close to 220°C, comparable to that of freeze dried Maltrin M150 (Fig. 6). However, T_c of binary solute mixtures containing inositol do not follow values predicted by eqn (4). Addition of inositol to maltose resulted in the lowering of T_c to below

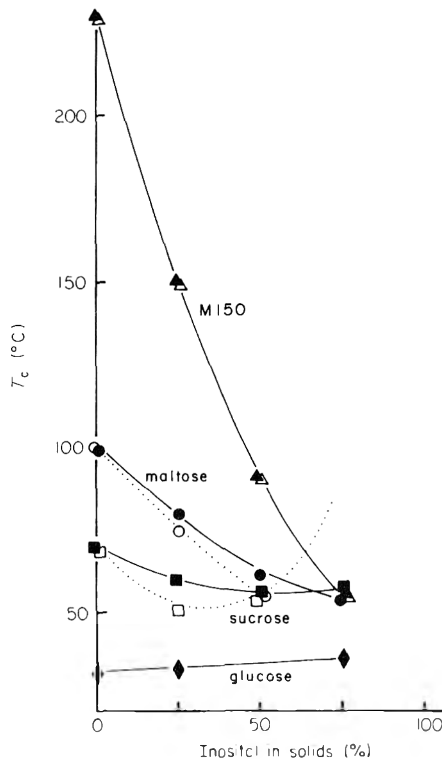

Figure 6. T_c of mixtures containing inositol. Initial solution concentrations are 20% w/w (open symbols) and 10% w/w (filled symbols).

100°C instead of an increase toward higher temperatures. The fact that addition of inositol lower the T_c of Maltrin M150, maltose and sucrose (T_c in the pure state 220, 100 and 70°C respectively) but does not affect the T_c of glucose indicates that it behaves as if it has a 'T_c' of 35°C. Microscopic investigations indicate that the anomalous behaviour of inositol in the freeze dried state is due to the formation of a crystalline matrix when pure inositol is freeze dried, but not when it is freeze dried in binary mixtures. Further investigations were conducted at total solids contents of 2, 10, 15 and 20% (weight basis) using maltose as the second solute. Two freezing rates were obtained by either immersion in liquid nitrogen (fast frozen) or placing in still air at −20°C (slow frozen). After freeze drying, T_c of the mixtures were determined by the ampoule method (Fig. 7). Results show that the T_c of the maltose-inositol system depends on the total solute concentration, solute ratio in the mixture and the freezing rate. At either freezing rate, as total solids in the starting solution decrease from 20 to 2%, the inversion from amorphous to crystalline matrix occurs at a higher and higher percentage of inositol in the solids. For samples having 20% solids with fast freezing, inversion occurs above 50% inositol in the solids whereas in the slow frozen sample, inversion to the crystalline state occur at 40% inositol. As the total solids content decreases, freezing rate has much less effect on the composition giving inversion of the inositol structure. The addition of small amounts of maltose to inositol does not cause the matrix to become amorphous. Rather it lowers the melting point of the crystalline inositol as would be expected from the melting point lowering effect of impurities. Microscopic examination of this system reveals that

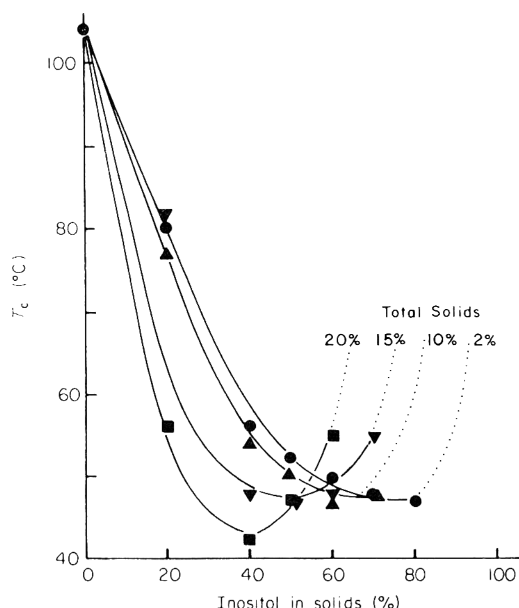

Figure 7. T_c of maltose-inositol mixtures having different total solids contents in the initial solution.

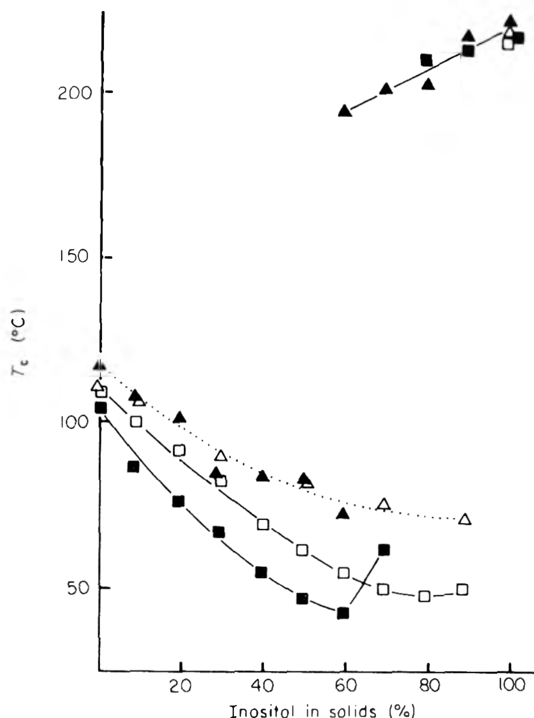

Figure 8. T_c of maltose-inositol mixtures having 10% solids in the starting solution. Open symbols, fast frozen; solid symbols, slow frozen. Triangles, T_c determined by ampoules; squares, T_c determined by microscope.

morphological behaviour can be divided into three parts, *based on the percentage of inositol in the total solids.* Thus in a sample prepared from a solution of 10% total solids, the matrix is totally amorphous when the inositol is from 0 to 30% of the total solids, partially amorphous from 30 to 80% inositol, and totally crystalline above 80% inositol (Fig. 8). The demarcation between the three regions is not sharp but depends on the freezing rate, and probably on the initial solids content as well. From DTA thermograms, the existence of crystallinity was shown for samples with 30% inositol in the solids. The melting endotherm for the crystalline inositol is shifted to lower temperatures as the percentage of maltose in the solids is increased. Recrystallization exotherms at 89–94°C were also observed in samples with 50 and 70% inositol in the solids.

Conclusions

Quantitative relationships between T_c and the two main factors which affect it, moisture content and molecular weight, have been established. In addition, an equation was developed for predicting T_c of binary solute mixtures from known values of T_c and weight fractions of any three combinations of the solutes including pure systems. For a homologous series of malto-oligosac-

charides, this relationship was extended to predict T_c of any multi-component mixture based on its composition.

The relationship between T_c and moisture is based on a theory which assumes hydrogen bonding as the main structural force. The relationship between T_c and molecular weight is identical to the equation for T_g of mixed polymers, which is based on the free volume theory of glass transition in polymeric materials. It is clear that 'collapse' and glass transition are phenomenologically similar events. However, while glass transitions in polymeric materials are generally reversible, 'collapse' behaviour of freeze dried matrices are irreversible. Furthermore, just as amorphous polymers can have regions of crystallinity, amorphous freeze dried matrices can also be partially crystalline. When the crystalline component of the mixture is present in sufficient concentration to form the supporting matrix after freeze drying, the 'T_c' is markedly increased. Only on a microscopic scale can the difference between 'collapse' of the amorphous phase and melting of the crystalline phase be distinguished.

Acknowledgments

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References

Aguilera, J.M. & Flink, J.M. (1974) *J. Fd Technol.* **9**, 391.
Bellows, R.J. & King, C.J. (1973) *AIChE Symposium Series 69* (132), 33.
Brennan, J.G., Herrera, J. & Jowitt, R. (1971) *J. Fd Technol.* **6**, 295.
Chirife, J., Karel, M. & Flink, J. (1973) *J. Fd Sci.* **38**, 671.
Chirife, J. & Karel, M. (1974) *J. Fd Technol.* **9**, 13.
Dubois, M., Gilles, K.A., Hamilton, J.K., Rebers, P.A. & Smith, F. (1956) *Anal. Chem.* **28** (3), 350.
Flink, J.M. & Karel, M. (1972) *J. Fd Technol.* **7**, 199.
Flink, J.M. (1975) In: *Freeze Drying and Advanced Food Technology*, (Ed. by S.A. Goldblith, L. Rey and W.W. Rothmayr) p. 351. Academic Press, London.
Gejl-Hansen, F. & Flink, J.M. (1976) *J. Fd Sci.* **41**, 483.
Gejl-Hansen, F. & Flink, J.M. (1977) *J. Fd Sci.* **42**, 1049.
Karivaya, S.R. & Hoeve, C.A.J. (1975) *Proc. Nat. Acad. Sci.* **72** (4), 3505.
MacKenzie, A.P. (1965) *Ann. N.Y. Acad. Sci.* **125**, Art 2, 522.
Maltini, E. (1977) In: *Freezing, Frozen Storage and Freeze Drying of Biological Materials and Foodstuffs.* Report by IIF-IIR Commissions C1 and C2 on Karlsruhe, G.D.R., 1977-1, pp. 43–51.
Masters, K. & Stoltze, A. (1973) *Fd Eng.* **45** (2), 72.
Meares, P. (1965) *Polymer Structure and Bulk Properties*, p. 264. Van Nostrand, London.
Mizrahi, S., Berk, Z. & Cogan, V. (1967) *Cereal Sci. Today*, **12** (8), 1050.

Nissan, A.H. (1976) *Macromolecules,* **9**, 840.
Peleg, Y. & Mannheim, C.H. (1977) *J. Fd Proc. Preser.* **1** (1), 13.
Schmidt, F. & Enevoldsen, B.S. (1976) *Carlsberg Res. Commun.* **41** (2), 92.
Simatos, D. & Blond, G. (1975) In: *Freeze Drying and Advanced Food Technology* (Ed. by S.A. Goldblith, L.R. Rey and W.W. Rothmayr), p. 401. Academic Press, London.
To, E.C. & Flink, J.M. (1978) *J. Fd Technol.* **13**, 551.
Tsourouflis, S., Flink, J.M. & Karel, M. (1976) *J. Sci. Fd Agric.* **27**, 509.

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'Collapse', a structural transition in freeze dried carbohydrates

III. Prerequisite of recrystallization

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Summary

A quantitative correlation between loss of entrapped volatiles and 'collapse' was established using methods which permitted the degree of 'collapse' to be measured. The chronological relationship between 'collapse', recrystallization and volatile loss was studied in sucrose–acetone systems using DTA. The structure of sucrose–acetone systems after freeze drying (i.e. a crystalline or amorphous matrix) depended on the composition of the starting solution rather than on conditions during freeze drying. Oxidation behaviour of freeze dried linoleic acid–maltodextrin–glucose emulsions with different 'collapse' temperatures was explained based on recrystallization of the matrices. In the absence of deliberate rehumidification, recrystallization of some freeze dried carbohydrates was found to occur by heating past 'collapse' and holding at these elevated temperatures. There were indications that loss of volatiles and oxidation of entrapped fat is more rapid after the structure turned crystalline than when it was merely in the 'collapsed' condition.

Introduction

The stability of many dry food products depends very much on the storage conditions. Three parameters most commonly controlled in an effort to prolong storage life of dry foodstuffs are water activity, temperature, and oxygen availability. Of these three parameters, moisture and temperature have been shown to markedly influence the structure of dry food substances (Tsourouflis, Flink & Karel, 1976; To & Flink, 1978b). In particular, freeze dried carbohydrates can be made to undergo a structural transition called 'collapse' when exposed to high temperatures and/or high levels of moisture. After the onset of 'collapse', the product may become sticky and cake together if it is a powder,

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or if the pieces are large, some of the pieces will fuse at the surfaces, as in boiled sweets (White & Cakebread, 1966). In addition to the loss of structure, other detrimental consequences can occur in the product after 'collapse', such as the loss of flavour volatiles. Flavour retention in freeze dried substances has been studied in much detail by a number of investigators (see Flink, 1975a, b; Thijssen, 1975 for reviews). It is generally believed that entrapment of small molecules which have vapour pressures higher than water during freeze drying is due to the selective diffusion of water out of the freeze drying matrix (Rulkens & Thijssen, 1972). On a macromolecular scale, Flink & Karel (1970, 1972) found that flavour volatiles are trapped in discrete micro-regions in the freeze dried matrix. This evidence tends to imply that the entrapment of volatiles starts with freezing of the solution, and that the volatiles are already locked into the matrix of supporting solutes prior to sublimation of ice (Flink & Gejl-Hansen, 1972). In addition to flavour retention, freeze dried substances have been found to encapsulate fats and prevent their oxidation (Gejl-Hansen, 1977). The ability to encapsulate either volatiles or fat is dependent on the integrity of the freeze dried structure. Flink & Karel (1972) and Chirife, Karel & Flink (1973) found that exposure of freeze dried substances containing trapped volatiles to various levels of relative humidity will give decreased volatile content, which is lower as the relative humidity is increased. Chirife & Karel (1974) also showed that exposure to methanol vapours resulted in volatile loss, while ethanol vapours had no effect. In the case of encapsulated fat, Gejl-Hansen (1977) found that the extent of oxidation in freeze dried maltose–linoleic acid emulsions whose surface fat had been removed by Soxhlet extraction with hexane increases with relative humidity up to 75% and then decreases slightly at higher relative humidities. He postulated that the loss of encapsulation ability is related to the loss of structure.

Chirife & Karel (1974) further showed that at a constant moisture content in the freeze dried solid, the loss of volatiles will not occur unless some critical temperature is exceeded, which in the case of maltose–propanol systems is found to be 100°C, the 'collapse' temperature (T_c) of maltose (Tsourouflis *et al.*, 1976; To & Flink, 1978b). These authors have implicated 'collapse' (i.e. loss of structure) as the determining factor in the release of encapsulated materials. In addition, Bellows & King (1973) indicated a relationship between visually observed puffing and retention of ethyl acetate during freeze drying. However, no objective quantitative correlation has been established between the two phenomena. More significantly, the state of the freeze dried materials after 'collapse' has not been studied in detail since from a practical standpoint, any dry product would be considered undesirable once it has 'collapsed'. Yet, some of the desirable properties of the freeze dried structure, such as entrapment of flavours, are only partially lost after 'collapse'. In fact, samples which usually 'collapse' during freeze drying, such as glucose are found to retain substantial volatiles. Hence, it is probably not 'collapse' *per se* which causes the loss of entrapment ability but rather some additional changes in the structure of the material. When foods containing sugars or other carbohydrates are dried

from aqueous solutions, dry solids with an amorphous structure can be obtained regardless of whether they are dried by drum drying, spray drying or freeze drying. The ability of amorphous substances to trap other small molecules is a consequence of the existence of 'free volume' in these solids. The amorphous state is not a defined state like the crystalline state, where each molecule has a fixed relationship to other molecules in the structure. Rather, it may be defined as the amount of 'free volume' existing in the system, which varies with temperature (Williams, Landel & Ferry, 1955). The crystalline state because of its limited 'free volume' is unable to accommodate 'impurities' in its structure. It is well known that one of the best ways of purifying a substance is by repeated crystallization. The importance of the amorphous state in retention of volatiles has been shown by Flink & Karel (1972) who presented data on the effect of rehumidification on the level of retained propanol in freeze dried lactose. When kept at 61% relative humidity, the lactose initially picked up water and the level of propanol dropped slightly after a moisture content of around 8% dry weight was reached. However, after attaining a moisture level of 12% the lactose started to recrystallize, as evidenced by the expulsion of water, and the level of propanol dropped sharply thereafter.

In this paper, the relationship between 'collapse' and recrystallization behaviour is investigated and the results used to explain the loss of encapsulated volatiles or oxidation of entrapped lipids which occurs when freeze dried materials are humidified or heated above critical levels.

Materials and methods

The T_c of freeze dried samples were determined on a microscope heating system. By photographing a particular freeze dried flake at various temperatures and measuring its area (gravimetrically by cut and weight), the degree of 'collapse' can be found. Thermograms of the sucrose–acetone systems were measured on a Du Pont 900 DTA. Details of these techniques have been given by To & Flink (1978a). All freeze dried carbohydrates containing volatiles were prepared by dissolving the specified amount of solute in distilled water, adding the appropriate volatile, and freeze drying 1 ml aliquots which have been frozen in liquid nitrogen. Freeze drying was conducted for 48 hr in a Virtis laboratory freeze dryer (Model 10-100, Gardiner, New York) with ambient chamber temperature and a vacuum of less than 50 millitorr. Freeze dried emulsions were prepared using mixtures containing glucose and a maltodextrin with DE of 15 (Maltrin M150) as the carbohydrate solute, linoleic acid as the oil phase and a system of Tween 80 and Span 80 as the emulsifiers. The amounts of the various components/100 g emulsion (prior to freeze drying) were carbohydrate solute, 15 g; oil, 1.5 g; Tween 80, 0.09 g in the aqueous phase; and Span 80, 0.45 g in the oil phase. Emulsification was achieved by maximum speed mixing in a Sovall Omnimixer for 10 min. Freeze dried emulsions were sealed in aluminium foil, crushed to a powder and stored in

evacuated desiccators over P_2O_5. The free fat on the surface of the crushed flakes was removed by extraction with hexane at ambient temperature. Completeness of removal of surface fat was demonstrated by the absence of staining after 6 hr exposure to osmic acid vapours. Oxidation of the oil phase was evaluated by measuring oxygen uptake with Warburg manometers using Apiezon B oil as manometer fluid (Gejl-Hansen, 1977).

Results and discussion

Correlation between loss of volatiles and 'collapse'

Data in the literature suggest a relationship between 'collapse' and loss of trapped volatiles. However, since 'collapse' has not been measured quantitatively, this relationship has only been expressed in terms of factors causing the 'collapse', such as the relative humidity to which the sample is exposed, or the temperature. In the first paper of this series To & Flink (1978a) described a method to measure the degree of 'collapse'. They noted that 'collapse' occurred to various degrees depending on the temperature to which the sample was heated. Presumably, a similar relation between 'collapse' and the moisture content of the sample could be found, especially considering the results of studies on humidification and volatile loss (Flink, 1975b). However, such a relationship is difficult to determine directly since the minute size of the sample flakes prohibits accurate measurement of the moisture content.

The system used to correlate volatile loss and 'collapse' was freeze dried lactose–hexanol. The initial solution was 20% lactose and 0.5% hexanol on a weight basis. Both heat and moisture have been used to cause 'collapse'. The T_c of freeze dried lactose was close to 100°C and the moisture content necessary to 'collapse' lactose at ambient temperature was about 8% (Tsourouflis, 1975). Photographs of an individual freeze dried flake were taken at ×600 magnification under an optical microscope. At this magnification, the entrapped volatile was clearly visible as droplets and their concentration can be expressed by counting the number of droplets present. Rehumidification of the flake was achieved by directing a stream of moist air (bubbled through saturated K_2CO_3) at the sample flake. Photos were taken at various intervals up to 375 min. 'Collapse' by heat was conducted on the microscope heater as described by To & Flink (1978a) and photos were taken at various temperatures above T_c up to 140°C. Figure 1 shows a linear relationship between loss of volatile and degree of 'collapse'. This observation explains the results of Chirife *et al.* (1973) in which the loss of volatiles was shown to attain different final levels at different relative humidities. In their study, different levels of 'collapse' had occurred at the equilibrium moisture levels corresponding to the various humidities used.

The difference in slope between the two curves in Fig. 1 may be due to onset of recrystallization in the lactose sample 'collapsed' by moisture. While the moisture 'collapsed' sample has not been observed in polarized light, Flink &

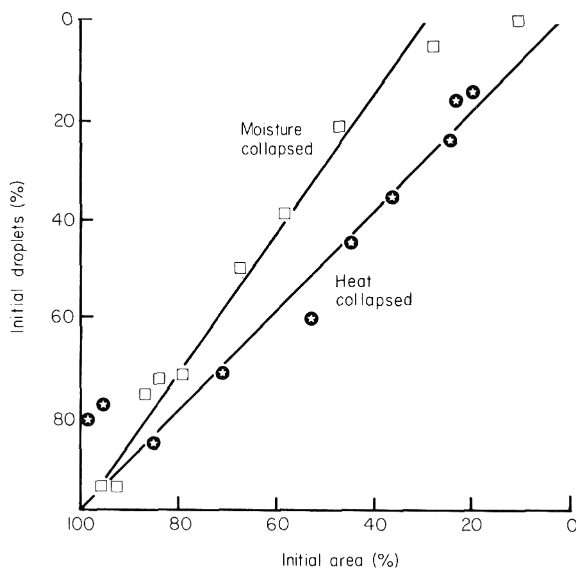

Figure 1. Correlation between degree of 'collapse' and volatile loss in freeze dried lactose–hexanol systems.

Karel (1972) showed that freeze dried lactose will undergo recrystallization after about 6 hr of exposure to 61% r.h. and that total loss of volatiles generally accompanies recrystallization of freeze dried matrices. In our studies, freeze dried lactose has not undergone recrystallization following 'collapse' by heating. It is also possible that in the case of the heat 'collapse' study (Fig. 1) loss of hexanol was hindered by the silicone oil heating medium in which the lactose was immersed.

Influence of recrystallization on volatile loss

The system of freeze dried sucrose–acetone was selected to illustrate the effect of structure on volatile retention and loss. This system took on special interest when it was discovered that certain concentrations of acetone in the initial sucrose solution resulted in a crystalline structure after freeze drying. This phenomenon seems quite unusual and to our knowledge it has not been reported before that sucrose can be freeze dried to the crystalline state. However, some investigators (Shallenberger & Birch, 1975) have indicated that addition of small amounts of acetone can greatly facilitate the crystallization of some sugars such as stachyose from solution. Table 1 gives the effect of sucrose and acetone concentration on the structure of the matrix after freeze drying. Two sets of conditions have been used to freeze dry the samples: the ones in the main chamber of the dryer experience less mass transfer resistance than the samples in the side arm and are less apt to 'collapse' during freeze drying. 'Collapse' during drying can be noticed by the presence of puffed surfaces on the samples. Table 1 shows that at 2% sucrose, none of the samples showed

Table 1. Effect of solids and acetone concentration on structure of freeze dried sucrose

Sucrose concentration	Acetone concentrations (weight basis)							
	Chamber samples				Side arm samples			
	5%	1%	0.5%	0.25%	5%	1%	0.5%	0.25%
Collapse during freeze drying								
30%	+	–	–	–	+	+	+	+
20%	+	–	–	–	+	+	+	+
10%	–	–	–	–	+	+	–	–
2%	–	–	–	–	+	+	–	–
Crystallinity after freeze drying								
30%	+	+	–	–	+	+	+	+
20%	+	+	–	–	+	+	–	–
10%	+	±	–	–	+	+	+	–
2%	–	–	–	–	–	–	–	–

any crystallinity or were 'collapsed', while at 30% sucrose, all collapsed' samples were also crystalline. The interaction between sucrose and acetone concentrations and its effect on the crystallinity of the freeze dried matrix is shown in Fig. 2. At high sucrose concentration only small amounts of acetone are required to bring about crystallization, while at low sucrose concentrations, the curve appears to asymptotically approach an infinite acetone concentration.

Each of the sixteen freeze dried sucrose–acetone samples in Table 1 was analysed in the DTA, and as expected, crystalline samples showed no features other than a melting endotherm associated with the crystalline state. When the DTA of the amorphous samples were examined (Fig. 3), four distinct features

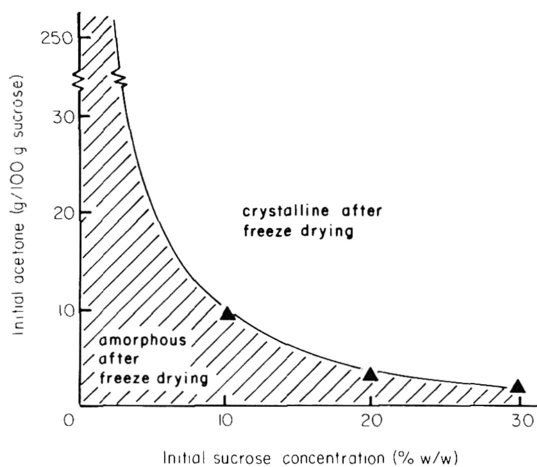

Figure 2. Effect of initial sucrose and acetone concentrations on crystallinity of sucrose after freeze drying.

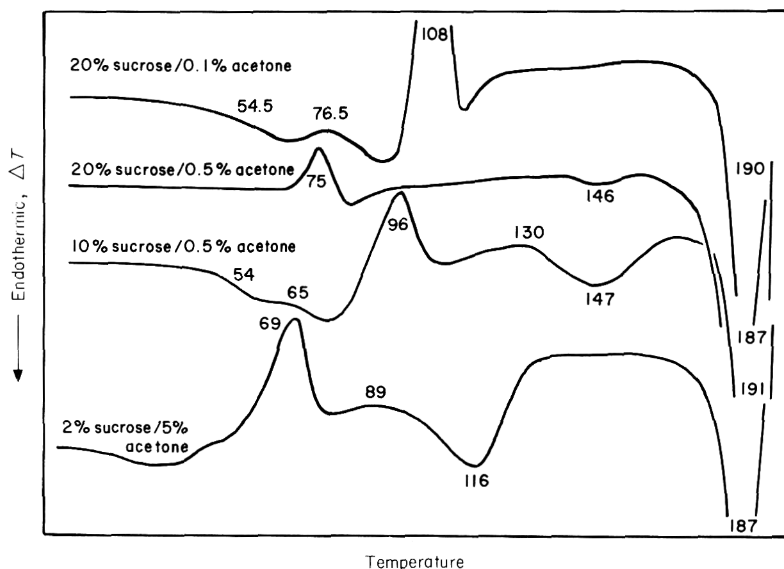

Figure 3. DTA thermograms of sucrose-acetone mixtures remaining amorphous after freeze drying.

were observed: a second order transition at 55°C which was slightly lower than the T_C of freeze dried sucrose; an exotherm at 70–108°C corresponding to the recrystallization of freeze dried sucrose (To & Flink, 1978a; Simatos & Blond, 1975); an endotherm at 116–146°C which can be attributed to the vaporization of the now free acetone; and the endotherm at 190°C caused by the melting of the now crystalline sample. The appearance of a second order transition slightly below the visual T_C was reported in a previous paper (To & Flink, 1978a). It is significant to note the order in which these events occur. The second order transition at 55°C is followed by recrystallization, then volatile loss, and finally melting. The loss of acetone does not take place after 'collapse', only after recrystallization. However, since DTA is run at a heating rate of 20°C/min, the results shown in Fig. 3 do not exclude the possibility that volatile loss may also occur if the sample is held at a temperature between 'collapse' and recrystallization. On the other hand it is also possible that in the study of maltose–propanol by Chirife & Karel (1974), the holding of maltose above its T_C may have resulted in recrystallization of the maltose to the extent that some of the trapped propanol is lost. Crystallinity in the maltose was not checked in that study.

Influence of recrystallization on fat oxidation

The effect of 'collapse' on the oxidation of encapsulated oil was studied using three emulsions having different T_C values, obtained by varying the ratio of glucose to Maltrin M150 in the matrix-forming solute. Samples with 90, 75

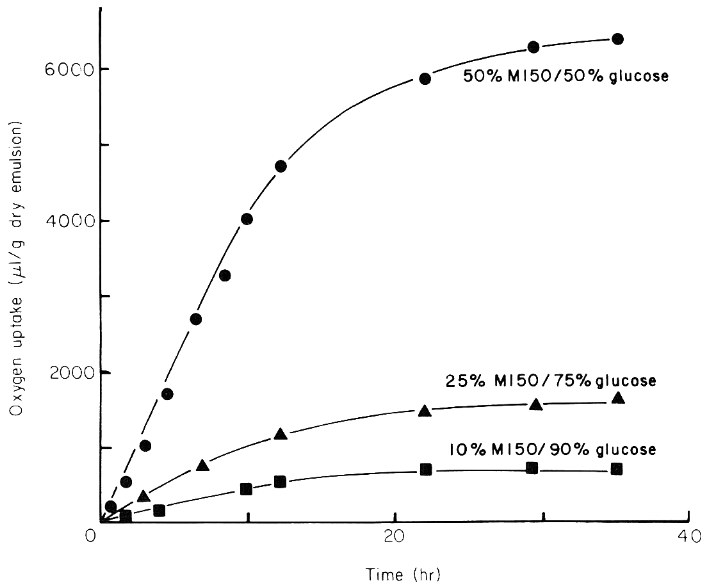

Figure 4. Oxidation of unwashed freeze dried emulsions at 52.5°C.

and 50% glucose have T_c of 46, 55 and 81°C respectively, as determined by the microscope method (To & Flink, 1978a). When the oxidation of the unwashed freeze dried emulsions were followed, the levels of oxidation were found to be related to the T_c of the emulsion regardless of the temperature at which the oxidation was followed. Figure 4 shows a set of representative oxidation curves

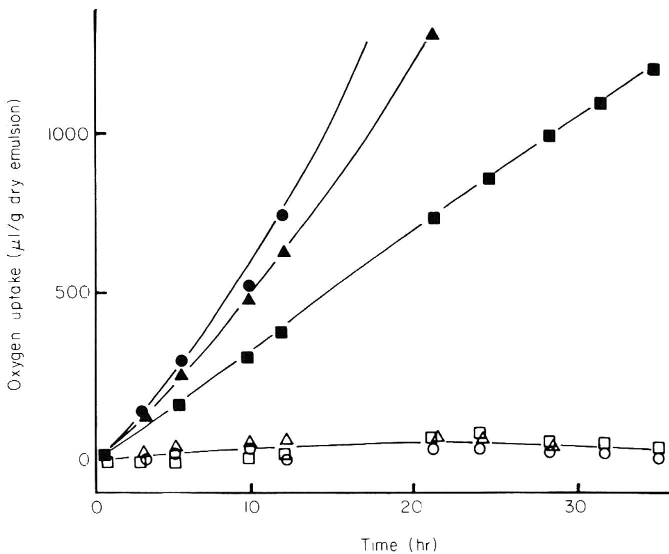

Figure 5. Oxidation of washed and unwashed freeze dried emulsions at 35°C. □, 90% glucose, washed; ■, 90%, unwashed; △, 75% glucose, washed; ▲, 75%, unwashed; ○, 50% glucose, washed; ●, 50% unwashed.

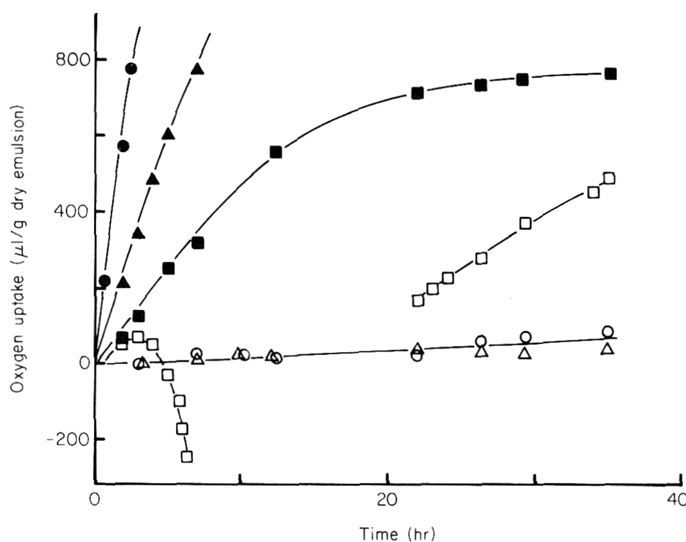

Figure 6. Oxidation of washed and unwashed freeze dried emulsions at 52.5°C (see Fig. 5 legend).

at 52.5°C. It can be seen that the highest oxidation level is attained by the sample with 50% glucose and hence the highest T_c (81°C). This tends to indicate that the amount of encapsulated oil is highest in the sample with the lowest T_c, which is in agreement with data of Gejl-Hansen (1977) showing that maltose, with a T_c of 100°C has a higher oil encapsulation level than Maltrin M150, whose T_c is 220°C.

The oxidation studies were carried out at temperatures of 35, 52.5 and 65°C, chosen so that zero, one, or two emulsions would be 'collapsed' respectively. Figure 5 shows the oxidation at 35°C, where none of the T_c are exceeded. As expected, only the emulsions containing free surface fat show uptake of gas indicative of oxidation. All three emulsions which have been washed free of surface fat show no oxidation. Figure 6 shows the oxidation at 52.5°C and in this case, the washed emulsion whose T_c is below the oxidation temperature, shows oxidation after about 20 hr. The other two washed emulsions whose T_c are above 52.5°C show no signs of oxygen uptake. The oxidized washed emulsion can be seen to behave unpredictably at first by its release of gas instead of uptake. (This will be discussed later after further data are presented.) In Fig. 7, the oxidation was conducted at 65°C and the drop in the curve can be observed in three cases, i.e. washed samples with 90 and 75% glucose, and the unwashed sample with 90% glucose. At 65°C, the two samples with the lower T_c are expected to 'collapse' and as a result release their entrapped fat and show oxidation. Only one of the two samples showed oxidation, and only after what appeared to be a release of some gas. When the samples were examined after the oxidation run under polarized light in the optical microscope, the four samples whose T_c have been exceeded were found to

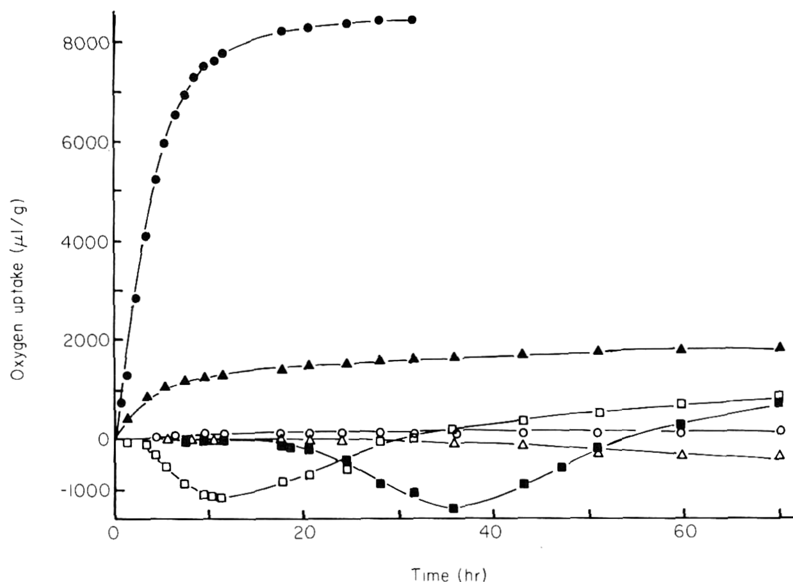

Figure 7. Oxidation of washed and unwashed freeze dried emulsions at 65°C (see Fig. 5 legend).

be crystalline (Table 2) while the two whose T_c was above 65°C remained amorphous. On this basis it seems likely that the dip in the oxidation curve was caused by the release of water as the samples recrystallized following 'collapse'. The moisture being expelled could be the normal residual water which has been trapped in the sample during freeze drying or it could have been picked up from the air during transfer of the sample to the Warburg apparatus. The significant point, however, is that release of trapped oil, like release of acetone from freeze dried sucrose, did not occur after 'collapse' but rather after re-crystallization when the structure can no longer accommodate other molecules. It should also be pointed out that the Warburg manometer curves really do not measure oxygen absorption specifically but rather the net change in the gas

Table 2. Presence (+) and absence (–) of crystallinity in emulsions and after oxidation runs at three temperatures

Percentage glucose	Oxidation temperature (°C)		
	65	52.5	35
90	+	+	±
90W*	+	+	–
75	±	–	–
75W*	+	–	–
50	–	–	–
50W*	–	–	–

*Washed with hexane after freeze drying.

volume in the manometer. Thus if oxidation occurs at the same time as the release of water due to recrystallization a flat curve may result. In the case of the 75% glucose emulsion at 65°C the rates of oxidation and recrystallization could be offsetting so that no net change in the gas phase was noted.

Conclusions

Linear relationships between an objectively measured, quantitative extent of 'collapse' and volatile loss has been established in a freeze dried lactose–hexanol system exposed to moisture or high temperatures. At a given extent of 'collapse', more volatiles were lost when 'collapse' was achieved by moisture sorption than by heating in the dry state. A possible cause could be the recrystallization of lactose after being 'collapsed' by moisture. Loss of acetone from a freeze dried sucrose–acetone system was detected by DTA after 'collapse' and recrystallization of sucrose. Release of entrapped linoleic acid and its subsequent oxidation was determined by manometric techniques to occur after initiation of matrix recrystallization following 'collapse' of freeze dried emulsions. In freeze dried carbohydrates, the transformation of the amorphous structure to a crystalline one was preceeded by 'collapse'. Recrystallization of matrix solutes was found to be a major cause of loss of entrapment ability in the dry state.

Acknowledgments

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References

Bellows, R.J. & King, C.J. (1973) *AIChE Symposium Series* **69** (132), 33.
Chirife, J., Karel, M. & Flink, J. (1973) *J. Fd Sci.* **38**, 671.
Chirife, J. & Karel, M. (1974) *J. Fd Technol.* **9**, 13.
Flink, J.M. & Gejl-Hansen, F. (1972) *J. Agr. Fd Chem.* **20** (3), 691.
Flink, J.M. & Karel, M. (1970) *J. Agr. Fd Chem.* **18**, 295.
Flink, J.M. & Karel, M. (1972) *J. Fd Technol.* **7**, 199.
Flink, J.M. (1975a) In: *Freeze Drying and Advanced Food Technology,* (Ed. by S.A. Goldblith, L.R. Rey and W.W. Rothmayr) p. 351. Academic Press, London.
Flink, J.M. (1975b) *J. Agr. Fd Chem.* **23** (6), 1019.
Gejl-Hansen, F. (1977) *Microstructure and stability of freeze dried solute containing oil-in-water emulsions.* Sc.D. thesis, Massachusetts Institute of Technology.
Rulkens, W.H. & Thijssen, H.A.C. (1972) *J. Fd Technol.* **7**, 79.

Shallenberger, R. & Birch, G.G. (1975) *Sugar Chemistry*, Avi, Westport, Conn.
Simatos, D. & Blond, G. (1975) In: *Freeze Drying and Advanced Food Technology* (Ed. by S.A. Goldblith, L.R. Rey and W.W. Rothmayr), p. 401. Academic Press, London.
Thijssen, H.A.C. (1975) In: *Freeze Drying and Advanced Food Technology* (Ed. by S.A. Goldblith, L.R. Rey and W.W. Rothmayr) p. 373. Academic Press, London.
To, E.C. & Flink, J.M. (1978a) *J. Fd Technol.* **13**, 551.
To. E.C. & Flink, J.M. (1978b) *J. Fd Technol.* **13**, 567.
Tsourouflis, S. (1975) *Collapse temperature of freeze dried carbohydrate solutions – effect of composition and moisture content*. S.M. thesis, Massachusetts Institute of Technolcgy.
Tsourouflis, S., Flink, J.M. & Karel, M. (1976) *J. Sci. Fd Agric.* **27**, 509.
White, G.W. & Cakebread, S.H. (1966) *J. Fd Technol.* **1**, 73.
Williams, M.L., Landel, R.F. & Ferry, J.D. (1955) *J. Am. chem. Soc.* **71**, 3701.

(Received 24 February 1978)

J. Fd Technol. (1978) **13**, 595–598

Book reviews

Food Quality and Nutrition (Research Priorities for Thermal Processing). Ed. by W. K. Downey.
London: Applied Science Publishers Ltd., 1978. Pp. xx + 712. £25.00.

This book which is printed directly from the typescript has been produced remarkably quickly. It details the papers given at the conference held in Dublin in November 1977, organized by COST (European Co-operation Scientific Technical Research) as an industrial food technology seminar. COST is the intergovernmental framework proposed by the European Community and adopted by nineteen European states in 1971 to promote co-operation in the field of science and technology. A number of other projects have been developed and the industrial food technology project was suggested in 1974 with the food committee of the organisation proposing food quality and nutrition – thermal processing as the first seminar. The objectives were to familiarize research directors and those involved with research and development with projects in the area and to define specific aspects which would require further research and development.

The initial five plenary papers at the symposium set out the current focus of research and development for the different food commodities. M. Jul deals with meat processing and distribution, W. K. Downey and P. F. Fox deal with milk processing, J. J. Connell deals with fish and fish products, C. Mercier and J. Delort-Laval deal with cereals and finally C. Cantarelli deals with the thermal processing of fruit and vegetables. These five addresses, taking up some 140 pages of the book, are interesting to read and are well referenced. Also included are the opening address by the Minister for Industry, Commerce and Energy for Ireland and the keynote address by E. von Sydow dealing with the arrangements for COST. This paper by Dr. E. von Sydow is a useful reference for the historical development of the project.

Following the five plenary lectures the invited lectures given at the five sessions are then printed in full. The first session dealt with pasteurization and blanching procedures. T. Ohlsson dealt with meat, fish and convenience food products, an interesting paper with a useful list of references. This was followed by a paper by J. Foley and J. Buckley dealing with milk and the blanching of fruit and vegetables. It is a pity here to see such terms as 'thermization' gaining international use. The next session dealt with sterilization and here F. Wirth dealt with the modern heat preservation of canned meat products. He was followed by H. Burton dealing with quality aspects of thermal sterilization processors. This was an interesting paper to read and sets forth very clearly the aims and needs of the milk and other industries. H. A. Leniger and

S. Bruin dealt with the present state of the art of food dehydration in the third session and they were followed by F. Escher and B. Blanc dealing with the quality and nutritional aspects of food dehydration. W. Vyncke dealt with aspects of chilling, freezing and thawing on fish quality and J. Munoz-Delgado spoke on the effects of freezing, storage and distribution on quality and nutritive attributes of foods in particular fruit and vegetables in the fourth session. In the final session of invited papers. R. Zacharias dealt with the effects of domestic and large scale cooking on quality and nutritive value of vegetables and fruits and following her, A. E. Bender dealt with the effect of heat on protein rich foods.

At this stage in the book we have the invited address given at the dinner by J. Bernstein dealing with the nutritional needs and research priorities as viewed from the U.S. National Academy of Sciences. An interesting and provocative paper and well worthy of study by those of us based on this side of the Atlantic. What has happened in the last few years in the United States could well come to us before long. Malnutrition, intervention and research priorities are all something that will have to be considered by the research and development directors of the food industry in Europe as well as academic and governmental representatives.

The final 250 pages of the book are occupied by the brief papers presented by invited experts on problems of specific quality, nutritional, safety and other aspects of thermal processes which in their eyes need further research and development. These papers, the majority of which are only three or four pages in length, some with and some without references, are very interesting to read and clearly set before the reader what has to be done in the future. Among the invited speakers we have papers from D. H. Buss, G. Brubacher, A. Dahlquist and co-authors followed by G. Tomassi, J. W. G. Porter, F. Fidanza and W. Seibel all dealing with nutritional problems as applied to the different commodity groups under discussion. These are followed by a series of papers dealing with dairy products including B. Blanc, J. F. A. Rook, M. Naudts, R. Negri, G. C. Cheeseman, M. Caric, H. G. Kessler and M. Heikonen and P. Linko. In the next section dealing with meat products there are papers from O. Kvaale and H. Martens, K. Ostlund, C. Bailey, L. Leistner and A. W. Holmes. Cereals are discussed by D. A. Southgate, G. Fabrian, J. Olkku and P. Linko, W. Seibel, D. Schlettwein-Gsell and finally P. F. Fottrell and co-authors. The next group dealt with fruit and vegetables and we have papers by M. Woods, V. Wenner, S. D. Holdsworth, J. Solms and F. Escher, J. Ryley and G. Glew and finally K. Gierschner. In the final group, fish products were discussed by G. Londahl, F. Bramsnaes, P. Strom, M. de la Higuera, J. C. O'Connor and W. Vyncke.

The last thirty-four pages of the book are taken up by the concluding reports from the five different commodity panels and the nutrition co-ordination group. Clearly these reports are of immense value to anyone who has to plan research for the next 5–10 years in his or her particular area. These papers should be automatic reading for anyone responsible for research and

development. The book ends with an alphabetical list of the participants for the symposium together with their full addresses.

An interesting book which should be browsed through and various pages read as interest is aroused. Clearly, this volume may only be of interest and help for the next 5–10 years and after that, it is hoped that all the problems outlined, will be solved. The publishers and the editor are to be congratulated on producing the volume so quickly, within 9 months of the seminar and although typescript is not quite so easy to read as the normal letterpress, a layout from double spacing typing offers no difficulty to the reader.

I. D. Morton

How Ready are Ready-to-serve Foods? Ed. by K. Paulus.
Basle: S. Karger AG, 1978. Pp. viii + 336. US$74.00.

This book records the proceedings of a conference which took place in Karlsruhe in August, 1977. The meeting was organized by IUFOST and the Commission International des Industries Agricoles et Alimentaires. The complete absence of any paper from the U.K. indicates how badly the conference was advertised in this country. Indeed from nearly 200 delegates there were only five people from Britain.

The book is divided into six main sections. The first section presents definitions of ready-to-serve foods which are extremely useful and could replace the older wider term 'convenience foods'. The author of the first paper suggests that we need to differentiate between different degrees of 'readiness'. Three other papers then summarize the market situation in Europe and North America. The next section, which is the largest, deals with preparation and production technology. There are twelve papers of uneven quality; some reiterate old material, others present refreshing new material. In the latter category there are papers on microwave sterilization, rigid tray-pack foods of A10 can size suitable for catering outlets and meat patty frying. Another paper deals with nutritional comparisons of ready-to-eat with traditional foods. There is a useful review section on packaging and storage of these products followed by a section containing three papers on regeneration and reheating. Microbiology and hygiene are well covered in the next section which is followed by two papers on economic considerations. One paper deals with energy use in cold stores, weight losses and changes in sensory quality. In the final paper of this section some of the economic considerations related to the use of pouch sterilized foods in institutional feeding are explored.

It is a difficult task to invite people to present papers at a meeting of this kind and expect them to necessarily fall into a pattern. Dr Paulus has organized the material as usefully as possible and in his final remarks summarizes the problems to be faced by both food manufacturers and caterers. The book is expensive but does contain some very useful material.

G. Glew

Books received

Bibliography of Selected References on Beef. By Z. A. Holmes.
Chicago: The American Dietetic Association, 1978. Pp. 148. US$9.50.

Includes 276 annotated and 1063 unannotated references to research papers (1930–1977).

Food, Health and Farming. Ed. by C. J. Robbins.
Reading: Centre for Agricultural Strategy, 1978. Pp. 119. £2.20.

This volume contains reports of three panels of the Centre for Agricultural Strategy dealing with fats, cereals, fruits and vegetables and sweeteners.

The Prevention of Food Poisoning. By J. Trickett.
Cheltenham: Stanley Thornes Ltd, 1978. Pp. x + 114. £3.95.

An introduction for students of catering and public health courses.

Meal Management. Concepts and Applications. By G. G. Fonosch and E. F. Kvitka.
San Francisco: Harper & Row, 1978. Pp. x + 395 US$11.95.

A first university textbook for home economics students.

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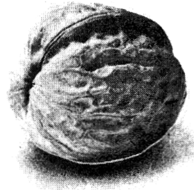

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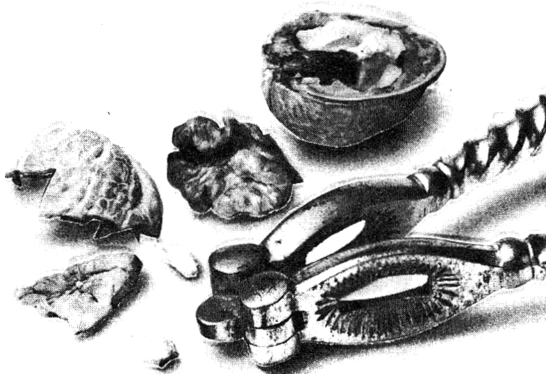

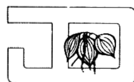

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OCCUPATIONAL ASTHMA STUDY BY INDUSTRIAL INJURIES ADVISORY COUNCIL

The Industrial Injuries Advisory Council has been asked by the Secretary of State for Social Services to consider whether occupationally induced asthma, caused by exposure to substances encountered at work, should be prescribed as an industrial disease.

A disease may be prescribed if it can properly be regarded as a risk of occupation and not a risk common to everyone, and if the occupational link can be established in particular cases or presumed with reasonable certainty. In the course of its study, the Council will be concerned to identify occupations, and substances used in those occupations, which may give rise to occupational asthma; and to consider the medical aspects, including the problem of distinguishing occupationally caused asthma from non-occupationally caused asthma.

A number of substances which are commonly encountered at work are believed to be associated with asthma including isocyanates, penicillin, grain dust, flour, wood dusts and biological washing powders. Some of these substances are also widely met outside the working environment.

The Council wishes to receive evidence from any interested persons or organisations and will supply a short explanatory memorandum on request. Evidence and communications should be sent to the Secretary, Industrial Injuries Advisory Council, Room 511 (A17), Keysign House, 429 Oxford Street, London W1R 2HT as soon as possible and not later than 31 January 1979.

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The Journal of General Microbiology

Volume 108, Part 2, October 1978

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P. B. RODGERS and C. J. KNOWLES Cyanide Production and Degradation During Growth of *Chromobacterium violaceum*.

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M. E. WARD, P. R. LAMBDEN, J. E. HECKELS and P. J. WATT The Surface Properties of *Neisseria gonorrhoeae*: Determinants of Susceptibility to Antibody Complement Killing.

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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

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