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Institute of Food Science and Technology

J. Fd Technol. (1977) **12**, 319–324

The measurement of acceptability differences between brands of convenience products sold as dry mixes

JANICE RYLEY, ALISON McKIE, GILLIAN WALKER AND G. GLEW

Summary

Hedonic scale tests using adult member panels to screen brands of three product types for use in schools, indicated that significant acceptability differences attributable to brand occurred with chocolate and strawberry desserts but not with creme caramel. Subsequent investigations in two schools with panels of eighty-four children confirmed the preliminary findings.

The study indicated that important acceptability differences between brands of similar products exist and that hedonic scale testing with small panels on single occasions can be used to screen products by large scale food purchasers for decision making purposes.

Introduction

Over five million school meals are produced daily in the U.K. and a considerable market exists in this area for convenience products. This market will grow if food manufacturers are able to produce attractive, nutritious and low cost dishes.

As part of a project concerned with the nutritional evaluation of convenience foods, it was observed that an increasing number of manufacturers produce similar types of products, which makes selection, on grounds other than cost, a major difficulty for food purchasers in the catering industry.

Hill, Armstrong & Glew (1970) performed taste panel assessments on a variety of frozen, dehydrated, canned and pre-prepared convenience products. The hedonic scale test was used and significant differences between products were observed.

In this paper hedonic scale tests were performed first with small adult panels, and followed by large groups of children, to find the brands with highest preference. This method for distinguishing between apparently similar products

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could be used by food purchasers thereby giving organoleptic information to add to the readily available cost information as a further aid to decision making.

Materials

All the products used were packs prepared for caterers requiring only the addition of water to complete the formulation of the product. The manufacturers' instructions were followed exactly. In most cases the complete pack was processed. If the packs were very large, the pack contents were subdivided and the required quantity of water calculated.

Taste panel method

The method chosen was that described by Amerine, Pangbourne & Roessler (1965). The taster was asked to mark a continuous hedonic preference scale which was scored: 1, like extremely; 2, like very much; 3, like moderately; 4, like slightly; 5, neither like nor dislike; 6, dislike slightly; 7, dislike moderately; 8, dislike very much; 9, dislike extremely.

The twelve member adult panels were asked to taste not more than five items on each occasion in which five brands and five product types were represented. The school panels, drawn from two schools (9–13 years) had forty-two boys and forty-two girls. Each child tasted two items on each occasion in which two product types and two brands were presented. Each product type (two brands of chocolate and strawberry dessert) was presented on two separate occasions.

Statistical analysis

Analysis of variance was applied to the results. If the variance ratio for the difference between brands exceeded the 0.5% level of probability the Student's *t*-test was used to determine significantly preferred brands.

Results and discussion

Table 1 shows the results obtained from the adult panels for chocolate and strawberry desserts and for creme caramel. At the 1% significance level, acceptability differences between brands emerge for the chocolate and strawberry desserts but not for the creme caramel.

Tables 2 and 3 show the mean scores of each brand of chocolate and strawberry dessert. Since the difference in the means (by Student's *t*-test) must be

Table 1. Significance of results of exploratory adult taste panels (Analysis of variance)

Product	Degrees of freedom	Variance ratio	Significance at 1% level
Instant chocolate desserts (four brands)	51	22.7	Yes
Instant strawberry desserts (five brands)	68	5.4	Yes
Creme caramel (three brands)	34	1.1	No

greater or equal to 1.98 to be significant at the 5% level (or $\geqslant 2.63$ at the 1% level), brand A is shown to be significantly more acceptable than brands C and D. Brand B is not significantly different from brands A and C, but B and D are significantly different.

Referring to Tables 4 and 5, since the difference in the means must be greater or equal to 1.25 (by Student's *t*-test) to be significant at the 5% level (or $\geqslant 1.68$ at the 1% level), brand C is shown to be significantly less acceptable than brands A, B, D and E. Brands A, B, D and E are not significantly different one from another.

Since brand A was significantly preferred to brand C for both chocolate and strawberry desserts, brands A and C were chosen for the schools exercise. Table 6 shows the results of the analysis of variance for this experiment. The variance ratio for the difference between brands is highly significant ($P \geqslant 0.001$) with brand A being preferred by boys and girls in both schools.

Table 2. Mean scores from hedonic test for instant chocolate desserts (adult panel)

Brands	Mean hedonic score
A	6.78
B	5.62
C	3.79
D	3.46

Table 3. Significance of mean scores for chocolate desserts (Student's *t*-test for difference between means)

Brands	5% level difference $\geqslant 1.98$	1% level difference $\geqslant 2.63$
A, B	No	No
A, C	Yes	Yes
A, D	Yes	Yes
B, C	No	No
B, D	Yes	No
C, D	No	No

Table 4. Mean scores from the hedonic test for instant strawberry desserts (adult panel)

Brand	Mean hedonic score
A	6.57
B	5.57
C	3.61
D	5.80
E	5.53

Table 5. Significance of mean scores for strawberry desserts (Student's *t*-test for difference between means)

Brands	5% level difference ⩾ 1.25	1% level difference ⩾ 1.68
A, C	Yes	Yes
B, C	Yes	Yes
D, C	Yes	Yes
E, C	Yes	Yes

Note: no other combinations show significantly different means.

For 640 and 1 degree of freedom the *F* value is significant at the 1% level, if the experimental *F* value ⩾6.6 – no other sources of variance in this experiment gave *F* value ⩾6.6.

There were also significant differences between the opinions of boys and girls, and significant interactions between schools and brands and between sex and flavour.

Table 6. Analysis of variance table of hedonic score from an acceptability study, in two schools using: brand A – chocolate dessert and strawberry dessert; brand C – chocolate dessert and strawberry dessert

Source of variation	Degrees of freedom	*F*-value
Schools	1	57
Brands	1	235
Sex	1	31
Flavour	1	1
Replication of samples	1	0.02
Schools/brands	1	22
Sex/flavour	1	25
Residual	640	

Table 7. Mean hedonic scores for an acceptability study in two schools with two brands of chocolate and strawberry desserts

Source of score		Mean score
Brand A	2 schools, 2 flavours	8.01
Brand C		4.84
School 1	2 flavours, 2 brands	7.22
School 2		5.91
Boys	2 schools, 2 flavours, 2 brands	7.07
Girls		6.13
Brand A, School 1	2 flavours	8.39
Brand C, School 1		6.32
Brand A, School 2		7.63
Brand C, School 2		3.35
Strawberry, boys	2 schools, 2 brands	7.62
Chocolate, boys		6.51
Strawberry, girls		5.73
Chocolate, girls		6.52

The mean scores listed in Table 7 show that the boys found the products more acceptable than the girls but both girls and boys preferred the strawberry desserts to the chocolate desserts. The second school gave all the products a lower score than the first school reflecting, perhaps, the less co-operative atmosphere reported by the experimentalist.

Conclusion

It is apparent that important acceptability differences exist between certain types of convenience products which can be attributed to brand. These differences can be detected by the use of a hedonic scale test which does not require the tasters to make direct comparisons between brands and does not require all products to be available on the same occasion.

Preliminary tests indicate that small panels can effectively screen a range of products of a similar type and select those of high or low acceptability. Such a method could be used by large scale food purchasers to give an objective assessment of acceptability and in conjunction with costing information assist in decision making.

Acknowledgments

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A rapid electrochemical test for the assessment of the delamination of lacquered tinplate*

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Summary

External cathodic polarization of lacquered tinplate specimens immersed in solutions of different organic acid anion types and pH was employed to study the delamination produced around a deliberately introduced defect in the coating. Attempts were made to utilize this technique as the basis for a rapid, simple and reproducible method for the evaluation and prediction of the suitability of lacquered tinplate samples exposed to different food media. Results of experiments conducted with three types of lacquers on tinplate in citric, malic, oxalic, tartaric, fumaric, lactic and acetic acids at pH's ranging from 2.5 to 6.0, showed good reproducibility as well as good agreement with results from an accelerated immersion test and with those from actual cases of canned foods.

Introduction

The use by the food canning industry of organic coatings for in-can corrosion protection has increased in recent years and along with it the need for an effective experimental procedure for their evaluation that requires a rapid, simple, inexpensive and reliable method. Several techniques have already been used for this purpose (Wormwell & Brasher, 1949; Pourbaix, 1965; Bird, Jones & Warner, 1971; Wolstenholme, 1973; Kleniewski, 1975; Sherlock, 1976) but little information is available for comparative studies.

Lacquered tinplate cans containing foods very often exhibit interfacial deterioration such as loss of adhesion, underfilm corrosion and film detachment all of which can develop extensively during storage so that the container

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rapidly becomes unsuitable, whereas different food media canned in similar cans are not harmed. The quality of the lacquer itself as well as the composition of the food media both determine whether or not corrosion will occur. Defect free films are very difficult to produce and it is highly improbable that these coatings would remain free of defects throughout their shelf-life. Defects continue to develop as the result of the corrosion of the metal/coated substrate so that rapid localized corrosion results (Koehler, *et al.*, 1959; Leidheiser & Kendig, 1976; Craig & Olson, 1976).

Several authors have assumed that corrosion is initiated by the electrochemical reduction of the oxide film, formed on the metal surface during the industrial process, between metal and lacquer that appear at these site of defects on coatings; its precise role, however, in the corrosion of lacquered samples has not yet been elucidated (Aubrun, 1970; Gonzales, Josephic & Oriani, 1974). The oxide layer may also have a detrimental effect on the adherence of lacquer (Trillat, Tertian & Britton, 1957; Britton, 1965; Craig & Olson, 1976). Differences in the adhesion of lacquers have been associated with the character of the passivation treatment (Carter, 1961), but the precise relationship is still unknown.

The nature of electrochemical reactions at the oxide layer associated with defects in the organic coatings was utilized in the design of a rapid test for predicting corrosion behaviour of three types of organic coating, applied on samples of commercial electrolytic tinplate substrates immersed in various acids at pH values specific to food. This method employs the standard glass vessel so that it is inexpensive and versatile. The results from this experimental method were compared with those from accelerated immersion tests.

Materials and methods

Cell and instrumentation

The electrochemical cell was made from a 50 ml flat top beaker. Electrodes consisted of a large anode of stainless steel and cathode which was the test specimen. The electrodes were held in a vertical position by means of a plexiglass cover. Constant current was supplied by an Elron Galvanostat, Model CHO-1 (Elron Ltd, Haifa, Israel). Currents were measured by a Universal Avometer, Model 7 X MR2 (Avo Ltd, Dover, England).

Materials

Samples of lacquered electrolytic tinplate were supplied by local producers. These included type A: a non-pigmented double coat of epoxyphenolic lacquer (curing temperature 200°C) with a coating weight of 8.4 g m^{-2} on No. 0.50 lb bb^{-1} electrolytic tinplate: type B: a non-pigmented one coat

oleoresinous lacquer (curing temperature 175°C) with a coating weight of 5.5 g m^{-2} on 0.75/0.50 lb bb^{-1} electrolytic tinplate; type C: a non-pigmented two coat lacquer, where one oleoresinous coat was cured at 175°C and the other epoxyphenolic coat was cured at 200°C with a coating weight of 8.9 g m^{-2} on 0.75/0.50 lb bb^{-1} electrolytic tinplate. Adhesion, as determined by the lacquer adhesion 'Scotch Tape' test (Hoare & Britton, 1960), was 95% for types A and C lacquers and 98% for type B lacquer.

Rectangular test specimens with a 2.25 cm^2 area were used throughout this work. Using a fine point, two perpendicular scratches of 1.5 cm long were traced on the lacquer film (see Fig. 1). The metallic back surface and the cut edges were masked with paraffin. These specimens were used for all tests described in this paper.

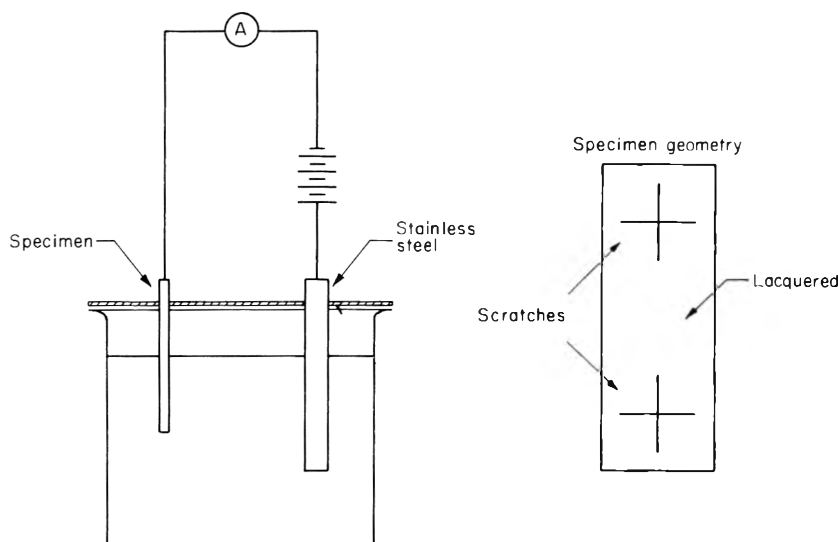


Figure 1. Schematic diagram of experimental apparatus for the assessment of delamination of scratched lacquered tinplate specimens, by cathodic reduction of the tin oxide film between the tinplate and lacquer.

The electrolytes in all tests were 1% aqueous solutions of citric acid, malic acid, lactic acid, oxalic acid, tartaric acid, fumaric acid, acetic acid, as well as 1% sodium salts of the same acids prepared from analytical grade chemicals and distilled water. Model solutions of pH = 2.5, 3.0, 3.5, 4.0, 4.5, 5.0, 5.5, 6.0, were prepared similarly from 1% acid solutions with pH adjustment using the respective sodium salt solutions. These anions and pH values were chosen to correspond to the large range common to canned food products.

An outline of the details of the experiments carried out in this work is given in Table 1.

Table 1. Experimental details

Tinplate		EL Dif. 75/50		EL No. 50	
	Type	Base coat	Top coat	Base coat	Top coat
Lacquer system	A			Epoxyphenolic	Epoxyphenolic
	B	Oleoresinous			
	C	Oleoresinous	Epoxyphenolic		
Total coating weight ($g\ m^{-2}$)	A				8.4
	B		5.5		
	C		8.9		
Total thickness (μm)	A				7
	B		5		
	C		7		
Adhesion (%)	A				95
	B		98		
	C		95		

Acid system:	Citric	Malic	Oxalic	Tartaric	Fumaric	Lactic	Acetic
pK_a	3.13	3.40	1.27	2.89	3.02	3.86	4.76
	4.76	5.26	4.29	4.16	4.38		
	6.40						

Method

Lacquered specimens, prepared as described above, were immersed and held vertically in the electrolyte bath, and were connected to the stainless steel anode placed in the bath some minutes before (Fig. 1). A constant current of 50 mA was applied for 90 sec and then the specimen was removed from the bath, rinsed with distilled water, dried, and assessed for adhesion by the Scotch Tape test. All tests were carried out with two replicates.

All tests were performed in electrolyte solutions in contact with air in the ambient laboratory atmosphere. No attempt was made to control the amount of dissolved oxygen in the bath. pH was measured after each determination. A fresh solution was used for each test.

Results were compared with accelerated immersion tests: scratched lacquered specimens were dipped in model solutions and stored for 90 days at 32–35°C. Assessment included the degree of delamination of the lacquer and visible corrosion of the substrate.

Results and discussion

Results of electrochemical tests performed with three types of organic lacquers

on tinplate in different solutions of several organic acids at various pH values are shown in Table 2. Results are expressed as percent lacquer detachment.

There were differences in behaviour of the lacquered tinplate samples, which varied not only with the organic acid type, but depended as well upon pH in the same acid solution. Generally, at lower pH values (2.5) there was good adhesion in all the acid solutions tested except oxalic acid solutions, in which delamination occurred even at the lowest pH (2.5). Higher pH of all acid solutions resulted in increased delamination of lacquers. In acetic acid solutions there was good adhesion for a larger range of pH: delamination was not observed at or below pH 4.5.

These results are in good agreement with findings in actual practice, i.e., in cans of pickles that contain acetic acid, good adhesion of lacquers is observed throughout the two year shelf-life. However, loss of adhesion usually occurs in cans containing lemon juice which is known to contain a high percentage of oxalic acid. The results of our tests with oxalic acid at pH 2.5 help to explain why in cans containing lemon juice there is loss of adhesion even at that very low pH. Delamination in cans of tomato paste is another case which corresponds well with the results of our tests with citric and malic acids.

Data from Table 2 indicate that there were some differences in delamination behaviour in the lacquers tested. Type B lacquer – an oleoresinous one coat type – exhibited better adhesion at scratches (no delamination at scratches) than type C lacquer – a two coat oleoresinous + epoxyphenolic (both applied on same tinplate specimens) and type A lacquer – a two coat epoxyphenolic lacquer applied on a different tinplate specimen. However, the type B lacquer has many pores on its coating, and corrosion starts at these sites. Type A and C lacquers have much fewer pores but will deliminate more readily at the site of scratches than type B lacquer.

Results of accelerated immersion tests (90 days, 35°C) of scratched lacquered specimens in the same model solutions, expressed in Table 3 as visual assessments of the appearance of visible corrosion, correspond with the conclusions of the electrochemical test results.

The electrochemical test developed in this investigation consists of inducing delamination around a purposely introduced scratch in the coating by external cathodic polarization of a lacquered specimen.

In order to elucidate the mechanism of delamination or corrosion of actual lacquered cans which takes place in the environment of foods, we investigated the role of the electrolyte composition as well, simulated by aqueous mixtures of various chemicals. The importance of defining the extent to which 'environment' affects the development of corrosion or passivation mechanism was reported by Foley (1975).

The effect of organic anion types that generally constitute the main components of food solutions and that play a direct role in the metal dissolution process by the formation of specific metal anion species complexes was first investigated.

The results of different experiments involving cathodic polarizing of scratched

Table 2. Results (% lacquer detachment)* of the tests on scratched lacquered tinplate specimens in various organic acids

Acid system	pH 2.5			pH 3.0			pH 3.5			pH 4.0		pH 4.5		pH 5.0		pH 5.5		pH 6.0	
	Type			Type			Type			Type		Type		Type		Type		Type	
	A	B	C	A	B	C	A	B	C	B	C	B	C	B	C	B	C	B	C
Citric	7	5	9	41	9	33	90	20	41	18	40	20	45	22	36	22	36	31	45
Malic	9	2	5	80	2	8	85	8	9	7	67	11	50	12	56	12	63	12	61
Oxalic	55	18	58	55	11	62	68	18	66	20	58	24	58	25	66	24	66	27	62
Tartaric	9	7	9	64	4		74	18	65	63	70	54	85	31	70	45	68	40	65
Fumaric	9	4	8	55	4	9	98	11	65	11	47	11	65	13	81	13	56	15	54
Lactic	9	4	7	13	4	7	74	6	81	11	63	12	65	12	83	14	68	11	56
Acetic	6	4	6	9	4	8	9	7	9	9	9	50	61	27	74	22	83	25	68

* 10% lacquer detachment is considered by local standards as the maximum limit of acceptability.

Table 3. Visual assessment of delamination and corrosion of type B lacquer coated tinplate after immersion tests for 90 days*

Acid system	pH 2.5	pH 3.0	pH 3.5	pH 4.0	pH4.5	pH 5.0	pH 5.5	pH 6.0
Citric	No corrosion	Small isolated corrosion spots		Delamination of lacquer and corrosion spots →				
Malic	No corrosion →		Small isolated corrosion spots		Delamination of lacquer and corrosion spots			
Oxalic	Delamination of lacquer and corrosion spots →							
Tartaric	No corrosion	Small isolated corrosion spots		Delamination of lacquer and corrosion spots →				
Fumaric	No corrosion	Small isolated corrosion spots		Delamination of lacquer and corrosion spots →				
Lactic	No corrosion →		Small isolated corrosion spots		Delamination of lacquer and corrosion spots			
Acetic	No corrosion →				Delamination of lacquer and corrosion spots			

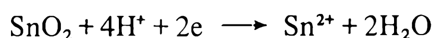
* Results for types A and C lacquers were similar to type B.

lacquered specimens in organic acid solution systems of varying pH together with the consideration of ionization constants of these acids demonstrate the correlation between the appearance of delamination at a certain pH value, and the ability to form complexes of that particular system.

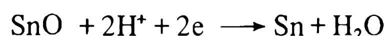
The total fraction of ionized anionic species (pK_a values) increases with a rise in the pH, (maximum at about pH 3.0–3.5 in citric, tartaric and fumaric acids, and about pH 4.0–4.5 in citric, malic, oxalic, tartaric, fumaric and lactic acids) so that there is an increase in the formation of complexes at these pH values. In oxalic acid solutions, the first ionization occurs at a lower pH of 1.5–2, but acetic acid ionizes only at pH 4.0–4.5.

This information suggests that delamination in our experiments occurred as follows: Cathodic polarization of the scratched lacquered tinplate specimen induces the cathodic reduction of the tin oxide film between the lacquer and the tinplate that appears at site of the scratch, the rate of dissolution being increased by the formation of soluble tin complexes with the respective acid anion in the aqueous phase.

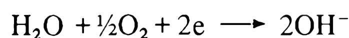
The pH increase in model solution systems of pH 4.0 or higher measured after each test, indicates that cathodic reactions do indeed occur in the delamination region, since all the likely cathodic reactions either consume hydrogen ions or produce hydroxyl ions.



$$SnO_2 + 4H^+ + 2e \longrightarrow Sn^{2+} + 2H_2O$$



$$SnO + 2H^+ + 2e \longrightarrow Sn + H_2O$$



$$H_2O + \tfrac{1}{2}O_2 + 2e \longrightarrow 2OH^-$$

Conclusions

The proposed electrochemical test presented in this investigation offers a useful and rapid means to define and predict ranges of suitability of the lacquered tinplate samples for different environmental conditions common to food.

The basis of this experimental method was drawn from suggestions in the literature that the cathodic reduction of the tin oxide layer between the tinplate and the organic layer is probably the starting point in delamination. With this method, cathodic polarization resulted in the reduction of the tin oxide, as shown also by a pH increase of the solution. However, the pH increase does not necessarily indicate which reaction occurs at the cathode, since the reaction $H_2O + \tfrac{1}{2}O_2 + 2e \rightarrow 2OH^-$, which may also occur can increase the pH as well.

Ionized organic anion species present in model solution in which the test is performed, at various pH values, account for complexing of tin so that lacquer delamination and corrosion continues in these solutions enhanced or slowed down in rate as affected by the respective pK_a value.

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An evaluation of the performance claimed for a chemical time/ temperature integrating device

GILLIAN ARNOLD AND D. J. COOK

Summary

The manufacturers' claim for the performance and accuracy of a chemical time/ temperature integrator for monitoring the storage history of foods at chilling and ambient temperatures has been tested. The claims for linearity of response and a maximum error of 10% at full scale, are not met. Individual devices of the same type were also unacceptably variable one with another throughout their scale.

Introduction

Several devices for integrating the time and temperature conditions used for storing frozen and chilled foods are now available to the food industry (Cook & Goodenough, 1976).

One such device functions by ammonia gas diffusing over a paper strip, impregnated with a suitable dye which changes colour as the gas diffuses along it. The gas is evolved by a suitable compound sealed within a glass vial, from which it is released by breaking the vial, thus activating the device. The gas released is contained within an impermeable transparent plastic tube and diffusion can only take place over the impregnated strip as described.

From the information available it is not possible to comment on the principle on which the device is based. The authors have only tested the manufacturers' published claims for the performance of the device. These are that at constant temperature the colour signal should move at a uniform speed along a scale, superimposed over the dye impregnated strip, and marked in ten equal divisions. Secondly, it is claimed that the time to reach a full scale colour change should not vary by more than 10% from that specified. At different and varying temperatures the signal will move at different speeds and different models of the device are suitable for particular ranges of temperature to be monitored. The manufacturers' claims for two models of the device are shown in Table 1.

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Table 1. Response rate of time/temperature integrator manufacturers' claim)

Model A			
Temperature (°C)	4.4	15.6	26.7
Time for full scale (days)	110	18	3
Time per division (days)	11	1.8	0.3
Model B			
Temperature (°C)	−17.8	−6.5	4.4
Time for full scale (days)	300	69	19
Time per division (days)	30	6.9	1.9

A relationship of the type:

$$\log_e t = \alpha - \beta T$$

where t is the claimed time to reach full scale and T the temperature in °F, was fitted to these claims for each model and estimates of the parameters α and β obtained (8.3 and 0.09 for model A, 5.7 and 0.07 for model B). These relationships were used for estimating the expected time to reach full scale for a temperature within the approved range for the particular model. The fitted line is shown in Fig. 1 for each of the models.

Materials and methods

The devices tested were obtained directly from the manufacturer after providing a specification of the situations to be monitored. They were stored at ambiant temperature until activated, no storage temperature or maximum dura-

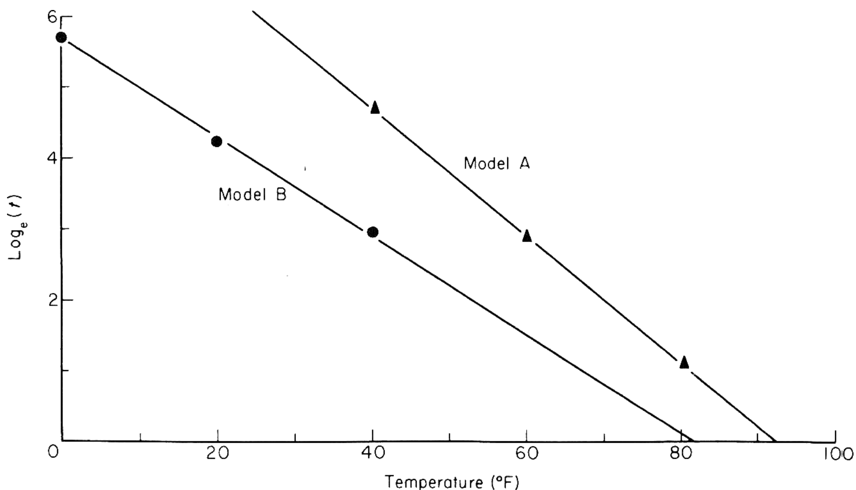

Figure 1. Time to reach full scale reading at different temperatures.

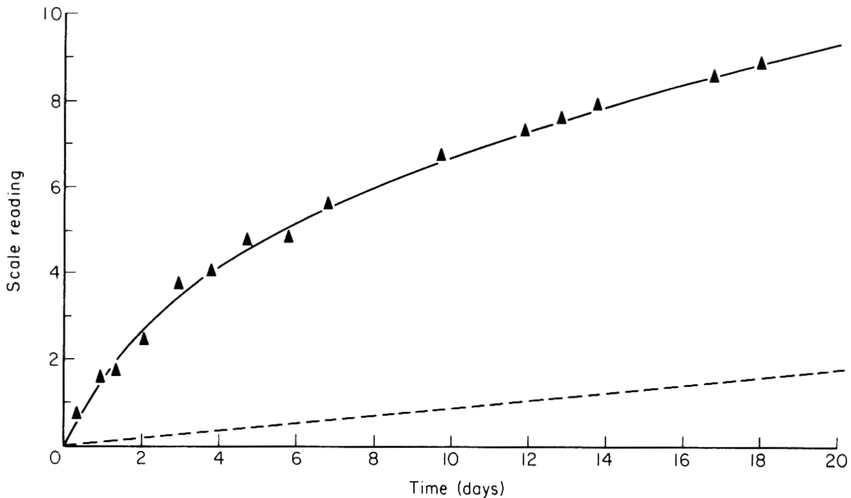

Figure 2. Change of scale reading with time; model A at 4.4°C. ▲, Fitted response; — — —, claimed response.

tion of storage being suggested by the manufacturer. All the devices (300 in all) were used within 4 months of receipt.

Three main experiments were carried out with the devices to look at:

I. The performance of model A at a constant temperature of 4.4°C.
II. The performance of model A at a constant temperature of 25°C.
III. The performance of model B at a constant temperature of 4.4°C.

These temperatures are within the particular temperature regimes of the tested devices, with 4.4°C being common to both devices. In the three tests 150 devices were used; 100 of model A and 50 of B.

Devices were activated by breaking the vial by firm pressure and successful activation was indicated by the colour change of a spot over the vial. After activation devices were placed between layers of blocks of oxyethyl methyl cellulose ('Tylose' grade MH 100*), which had been previously stabilized at the test temperatures.

The devices were examined at intervals appropriate for each device and test temperature. Scale readings (1–10) were recorded.

Results

For each test of performance, the data were analysed for:

(1) Linearity of response with time at a constant temperature, (2) compliance with the claim for a ± 10% accuracy for the expected time to reach full scale, and (3) uniformity of response between replicate devices of one model.

*Tylose blocks made to the specifications in the appropriate British and ISO standards are used as a food substitute for evaluating the performance of domestic freezers.

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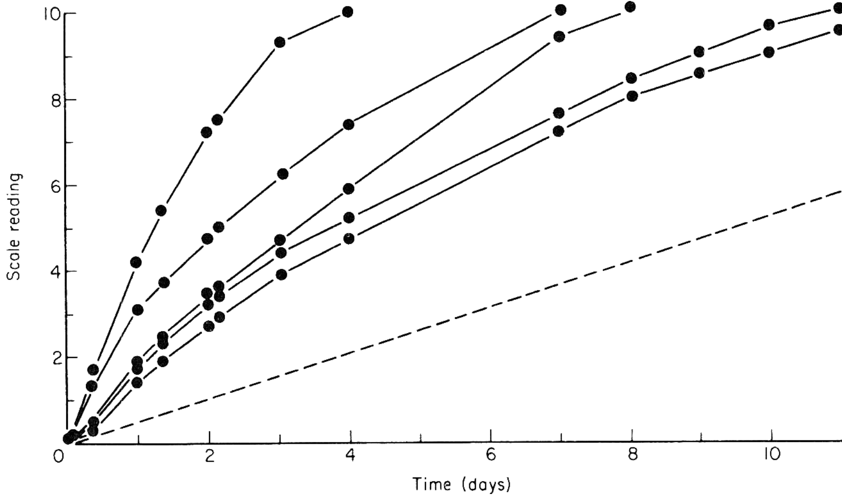

Figure 3. Change of scale reading with time; model B at 4.4°C. – – –, claimed response.

For all three main experiments, the lack of fit for a linear response was significant at the 0.1% level, i.e. the colour signal did not progress uniformly along the scale at a constant temperature. This is clearly shown in Fig. 2 where the means for replicates and the claimed response are plotted for model A at 4.4°C. An empirical curve of the double exponential type was fitted to the data and is also shown; this is given by:

$$y = 13.6 - 11.0 \exp(-0.05x) - 2.6 \exp(-0.52x)$$

where y is the scale reading and x the time in days measured from activation when the reading was taken.

It was not practically possible to record the exact time that each device reached full scale, but an upper limit for that time is given by the first recorded time for which the scale reading was 10. At 4.4°C the claimed time to completion for model A is 110 days but by 18 days 20% of the tested devices had reached full scale. The mean reading for the other devices was 8.6, with a lowest reading of 7.8 at that time. At the same temperature, the claimed time to full scale for model B is 19 days, but after 7 days at this temperature 50% of the tested devices had completed their colour change over the full scale.

The diversity of response by devices of the same type is amply demonstrated by Fig. 3 which shows the response of a representative sample of five devices of type B from those incubated at 4.4°C. The response claimed by the manufacturers is also shown.

Supplementary tests

(i) A set of devices (model A) was placed on top of 'Tylose' blocks instead of between them; these devices were subject to the slight temperature fluctuations of air in the incubator (25°C), which were not experienced by devices insulated

between layers of blocks. These exposed devices showed an average advancement of 0.5 on the scale above those between the blocks. The placement of devices therefore has a measurable effect on the scale readings, even for the slight temperature range found within an incubator.

(ii) The temperature of devices at the time of activation was varied. Some were activated only after stabilizing their temperature at 4.4°C between 'Tylose' blocks; others were kept at ambient temperatures of between 10°C–20°C, up to the time of activation and placement at 4.4°C between 'Tylose' blocks. In the latter case the colour change moved along the scale faster during the first day than it did for the temperature stabilized devices. However, this was not significant when compared with the great variation in signal between devices and compared with the claims made for signal speed.

An attempt was made to follow the response of the devices to sequences of temperature change, that would be found in practical food quality control situations. Individual devices certainly respond slower in colder conditions within their temperature range for working, and speeded up in proportion to increase in temperature, but because of the variation between devices and the discrepancy from the claimed rate of response, no significant conclusions could be drawn from this experiment.

Conclusions

The devices do not meet the claims made by the manufacturer; they did not show a linear response and showed more than a 10% difference in the time to reach a full scale signal from that specified. This was not an effect confined to the batches of both models of the device tested as it reflects the results obtained in independent tests made on the devices in the United States. Devices of the same model were also unacceptably variable compared one with another.

The food processing industry is looking for inexpensive but accurate devices to monitor storage and distribution conditions in order to extend efficient quality control procedures until the food is in the hand of the consumer. Consumer protection groups are aware of devices of the type considered in this paper and expect them to provide further safeguards to the quality of food they purchase.

In this climate of opinion it is important that the purchase of time/temperature monitoring devices has confidence in them. If the manufacturer cannot guarantee such products, it is essential that limitations in performance and guidance for use are provided nationally for the U.K. food processing industry (Cook & Goodenough, 1976).

References

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Protein concentrates from slaughter animal blood

I. Preparation and purification of red blood cell concentrates

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Summary

Purified protein concentrates, containing 95 and 98% protein (dry weight basis), were prepared from centrifugally separated porcine red blood cells by dilution with deionized water followed by either ultrafiltration or diafiltration. Optimal operating conditions, in terms of temperature, pressure and flow rate, for membrane processing of red blood cells in a plate and frame RO/UF plant were established. Permeation rate during ultrafiltration of red blood cells decreased with increasing protein concentration. Protein concentration of up to a maximum of 30% (liquid basis) was achieved by simple ultrafiltration. During diafiltration permeation rate increased with decreasing levels of non-protein material in the retentate. Mass balance equations were developed to allow ultrafiltration and diafiltration process times to be calculated for pre-specified degrees of protein purification. The purification of red blood cells was better achieved by dilution followed by diafiltration at low protein levels. The optimal protein content for diafiltration will depend on the intended post-purification use of the cell concentrate.

Introduction

In recent years, predicted shortages have stimulated interest in all possible sources of protein. Concomitant with the need to increase food production is a related need to conserve both energy and raw materials to offset resource limitation and environmental pollution in industrial societies. This concept has led to the suggestion that wastes be considered as valuable resources and that their recycling should be regarded as an important industrial procedure (Hughes, 1974). In this light it would appear reasonable to examine methods of upgrading to edible quality animal products which are high in protein but which at present, are not processed for human consumption. Abattoir blood constitutes a potential source of large quantities of animal protein which is currently wasted or underutilized (Akers, 1973).

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There is presently, however, interest in the fractionation of whole blood and in the isolation and purification of these fractions for use as human food. Porter & Michaels (1971) have concentrated whole blood and blood plasma by ultrafiltration. Delaney, Donnelly & Bender (1975) have reported on the use of ultrafiltration for the concentration and purification of porcine blood plasma and on the subsequent conversion of the concentrated plasma solutions into powders by spray drying. A later report, (Delaney, 1975), described the nutritive value of blood plasma concentrates prepared in this fashion. Eriksson & von Bockelmann (1975) have reported on the ultrafiltration of animal blood serum prepared from pooled porcine and bovine blood. Young & Lawrie (1974) prepared meat analogues from bovine blood plasma protein by suitably denaturing the protein and applying a wet fibre spinning process.

Centrifugal fractionation of whole blood yields as primary components plasma and cellular fractions. On average the fractionation of whole blood yields *c.* 70% plasma and *c.* 30% red cells. The cellular fraction is composed mainly of erythrocytes which encapsulate the haem protein. The level of haemoglobulin in whole blood ranges from 11 to 17%. Few reports have appeared in the literature on the treatment or use of this red cell fraction. Tybor, Dill and Landmann (1973) reported on a process for the preparation of protein concentrates from both the plasma and red cell fractions. The haemoglobin fraction is subjected to a decolorization process to remove the haem from the protein moiety. A recent report (Tybor, Dill & Landmann, 1975) describes some of the compositional and functional properties of these proteins isolated from bovine blood by a continuous pilot process.

This present work is concerned with the preparation and purification of red blood cell concentrates (RBC) from porcine blood by ultrafiltration and diafiltration.

Materials and methods

Materials

Whole pig blood was obtained from the Mitchelstown Co-operative Society Ltd, Mitchelstown, Co. Cork. The blood was treated and centrifugally separated as described by Delaney *et al.* (1975). Fractionation was effected at *c.* 4°C.

Experimental procedures

Preliminary experiments were designed to establish optimal operating conditions for membrane processing of the red blood cells. Two basic types of experiment were performed.

(1) The performance of the membrane plant at a controlled feed composition was investigated using continuous recycle of permeate and concentrate. Operating parameters such as temperature, pressure, and flow rate were varied and their effects studied.

(2) The effect of feed composition on the performance of the ultrafiltration (UF) plant was investigated. In these tests, red cell concentrates (RBC) were diluted to various solids concentrations with deionized water and pumped through the UF module. The permeate was collected and retentate returned to the feed reservoir.

Purification of red blood cell concentrates (RBC)

A given level of protein purification may be achieved in a number of ways.

(1) *By simple ultrafiltration.* In this case the low molecular contaminants are eliminated at the highest possible concentration and therefore the volumes of permeate that need to be removed are small. However, in many cases the process is of long duration because low permeation rates result from the increase in protein concentration.

(2) *By diafiltration.* In this case the concentration of low molecular weight solutes decreases during the process and consequently large volumes of permeate need to be removed. Protein concentration, which is the most important factor limiting membrane permeability remains constant during the process and permeation rates remain high.

The red cell fraction prepared by centrifugal separation of whole porcine blood typically had a total solids content of 30–32%. Eighty-five to 90% of the dry matter was protein (N × 6.25). This was equivalent to 26–29% protein in the red cell fraction. This is above the generally accepted protein level for membrane processing (Peri & Pompei, 1973). Consequently, the red cell fraction required dilution with deionized water in all cases before purification by simple ultrafiltration or diafiltration could be applied. A practical process to purify the RBC thus involved a dilution step followed either by a simple ultrafiltration step or by a diafiltration step.

The choice of dilution – simple ultrafiltration or dilution – diafiltration should be made on the basis of whichever makes the more effective use of a given membrane area. For the purpose of this present work, a process was defined as optimal if it permitted a given level of protein purification to be achieved with minimum membrane area or for a given membrane plant, in the shortest time.

Equipment

The membrane plant employed for these studies was a De Danske Sukkerfabrikker (DDS) 20 cm diameter plate and frame reverse osmosis/ultrafiltration

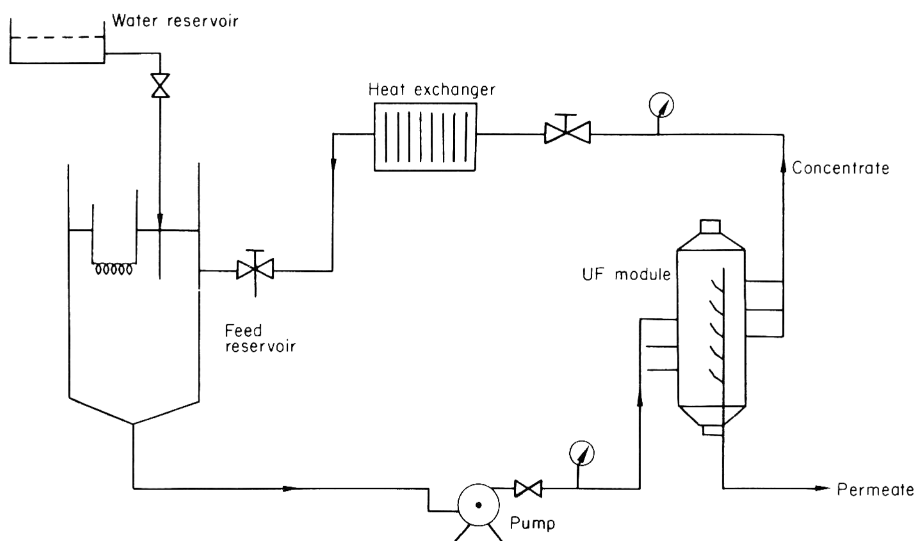

Figure 1. Experimental arrangement for ultrafiltration and/or diafiltration of red blood cells (RBC).

plant. The plant was fitted with DDS type 600 membranes with a nominal 100% rejection of molecules greater than 20 000 daltons. The total membrane area employed was 0.33 m^2. The experimental arrangement used for the ultrafiltration and diafiltration experiments is shown in Fig. 1. All experiments were carried out on 30 litre batches. The water reservoir, which contained deionized water, was used only for diafiltration experiments.

Chemical analysis

Samples of original red cell fraction, diluted and concentrated RBC's and permeates were analysed for total solids by gravimetric procedure, ash by combustion at 550°C and nitrogen and non-protein nitrogen by micro-Kjeldahl procedure (AOAC, 1970). Protein was estimated from micro-Kjeldahl nitrogen values using the factor N × 6.25.

Viscosity measurements. Viscosity determinations were made on red cell feeds and concentrates with a Brookfield Model RVT synchroelectric viscometer.

Results

Temperature

The results in Fig. 2 show that flux rate was linearly related to temperature. The slope of the curve is 0.5 litre m^{-2} hr^{-1} $°C^{-1}$.

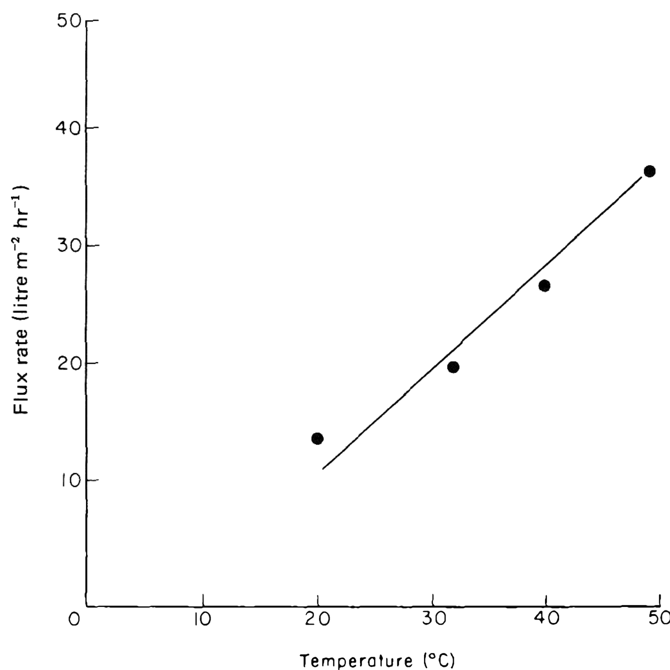

Figure 2. Relation between temperature and flux rate for ultrafiltration of RBC (*c.* 11% total solids in feed).

Pressure and flow rate

The effects of pressure on flux rate for a range of flow rates are illustrated in Fig. 3. Flux rate was practically independent of applied pressure over the range 1–5 kg cm^{-2}. Flux rate was however strongly dependent on flow rate through the module. Permeate flux increased from *c.* 10 litre m^{-2} hr^{-1} at a flow rate of 3.5 litre min^{-1} to *c.* 45 litre m^{-2} hr^{-1} at a flow rate of 14 litre min^{-1}. At very high flow rates (12 to 14 litre min^{-1}) there was a slight increase in flux with increase in pressure.

Feed composition

The relations between volumetric concentration ratio, total solids, protein content and flux rate are illustrated by the data in Figs 4 and 5. The data in Fig. 4 refer to a feed of *c.* 3.46% total solids, 3.2% protein and 0.26% non-protein material (NPM). Total solids content and protein content increase almost linearly with volumetric concentration ratio up to a concentration ratio of 5:1. Permeation flux showed a decline over the same concentration ratio range. The instantaneous flux fell from *c.* 52 litre m^{-2} hr^{-1} to *c.* 28 litre m^{-2} hr^{-1}.

The data in Fig. 5 refer to a feed of *c.* 15.25% total solids, *c.* 13.3% protein and *c.* 1.45% NPM. Total solids content and protein content increased almost linearly with concentration ratio up to a concentration ratio of 3:1. Permea-

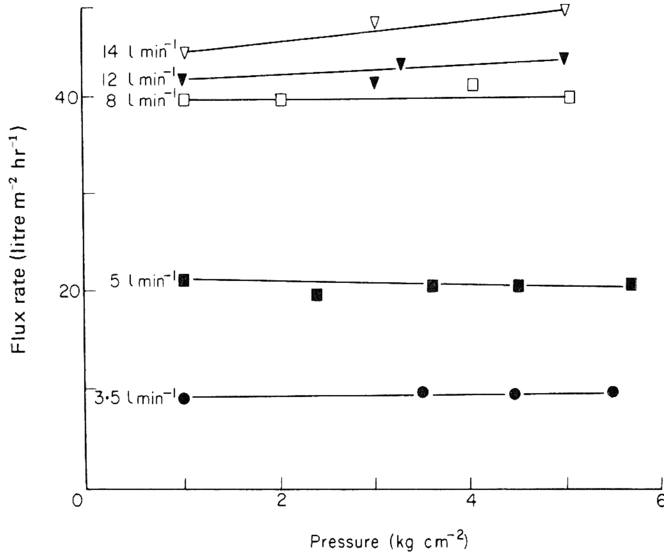

Figure 3. Effect of feed flow rate and hydraulic pressure in flux rate for ultrafiltration of RBC (*c.* 11% total solids in feed).

tion flux showed a decrease from *c.* 28 to *c.* 5 litre m^{-2} hr^{-1} over the same concentration range. The ash content in the concentrate increased from *c.* 0.7% to *c.* 0.9% as the concentration ratio was increased from 1 : 1 to 3 : 1. At a concentration ratio of 3 : 1 the total solids content in the concentrate was *c.* 36.7% and the protein content *c.* 34.9%. This represents the maximum level of protein concentration that could be achieved with the present experimental set-up.

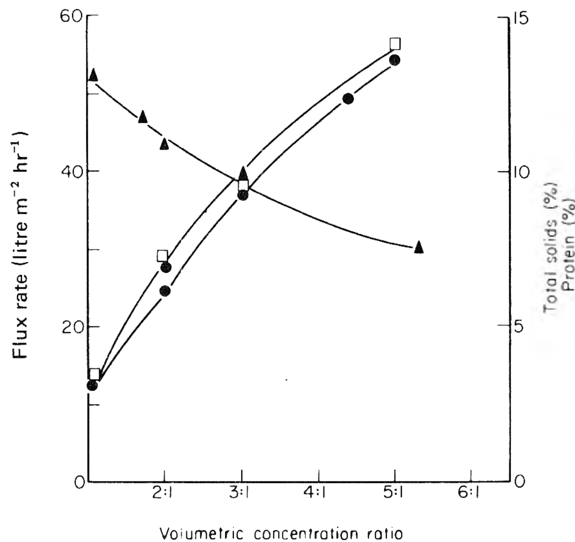

Figure 4. Variation in flux rate (▲), and total solids (□), and protein (●) contents with volumetric concentration ratio for ultrafiltration of RBC (*c.* 3.5% total solids in feed).

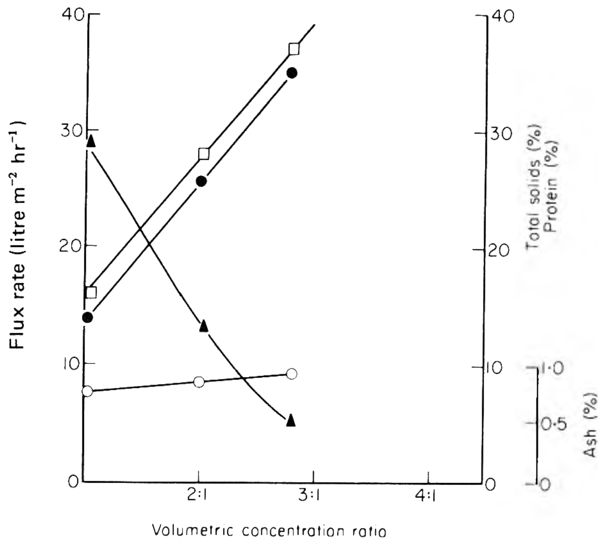

Figure 5. Variation in flux rate (▲), total solids (□), protein (●) and ash (○) contents with volumetric concentration ratio for ultrafiltration of RBC (*c*. 15.5% total solids in feed).

More general data, illustrating the relation between flux rate and protein content of feed during ultrafiltration of red cells are given in Fig. 6. Here, instantaneous flux data are plotted against protein content on a logarithmic scale. The curve was prepared from data from a number of individual runs. Flux rate was found to be inversely proportional to the logarithm of the protein content of the concentrate.

Viscosity and solids content of feed

The relation between viscosity and total solids content in feed is illustrated by the data in Fig. 7. The data were generated at two temperatures 21.5 and 48°C.

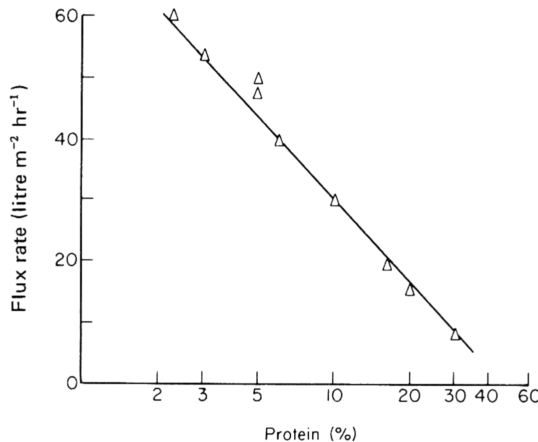

Figure 6. Effect of protein concentration on flux rate for ultrafiltration of RBC.

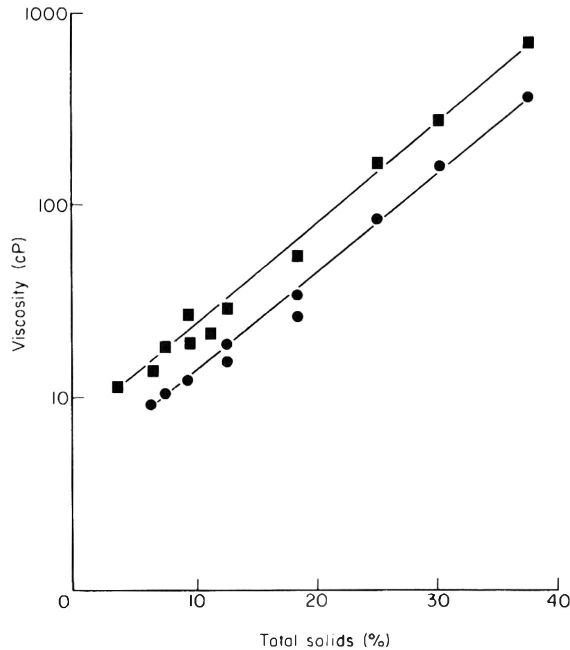

Figure 7. Viscosity of red blood cell concentrate at 48°C (●) and 21.5°C (■).

A direct linear relation was found between the logarithm of viscosity and the percent total solids in the feed. A red cell concentrate of *c.* 10% total solids content had a viscosity of *c.* 30 cP at 21.5°C. The viscosity of RBC at 30% total solids was *c.* 400 cP. At 48°C, RBC's of similar total solids contents had viscosities of *c.* 15 cP and 180 cP respectively.

Purification of RBC

Simple ultrafiltration. In this situation, the reduction in volume of the retentate is accompanied by a concentration and purification of the proteins because of their high retention compared to that of the low molecular weight contaminants. The final concentration of protein in the retentate may be calculated from the following equation.

$$C_f = C_1\left(1 + \frac{V_1 - V_f}{V_f}\bar{R}\right) \qquad (1)$$

where C_f = final concentration of protein, C_1 = initial concentration of protein, V_1 = initial volume of retentate, V_f = final volume of retentate, $\bar{R}$ = average retention of protein.

The relationship described in Equation 1 may be used to calculate the degree of protein purification achieved from any given reduction in retentate volume. The time required to achieve a pre-specified reduction in

retentate volume may be calculated from the following equation

$$t_1 = \int_0^{V_p} \frac{dV_p}{PR} \quad (2)$$

where t_1 = time required to permeate a given volume V_p, PR = variable value for permeation rate.

The integral may be solved analytically for given experimental conditions if the relation between V_p and PR is known. V_p is related to protein content by a simple exponential relation while the relation between protein content and permeation rate depends on the specific operating conditions employed and cannot always be described by a simple equation. It is easier and more accurate to solve the equation by graphical integration based on experimental rate curves. This was the approach used in the present work. Figure 8 contains data on the average flux rates achieved for various volumetric reduction ratios with different RBC feeds. The times required to achieve 95 and 98% protein purification (dry weight basis) of 100 litre batch lots of red cells are given in Table 1. The times were calculated for a plant with an effective membrane area of 1 m^2.

Diafiltration. In diafiltration, one is primarily interested in the time required to reduce the concentration of a low molecular weight non-retained solute in the retentate to a pre-specified value. In this case the proportion of protein : dry matter is increased as the non-protein material (NPM) is removed. Permeation rate curves as a function of NPM concentration for a range of RBC feeds are given in Fig. 9. Permeation rates increased with decreasing NPM content. In

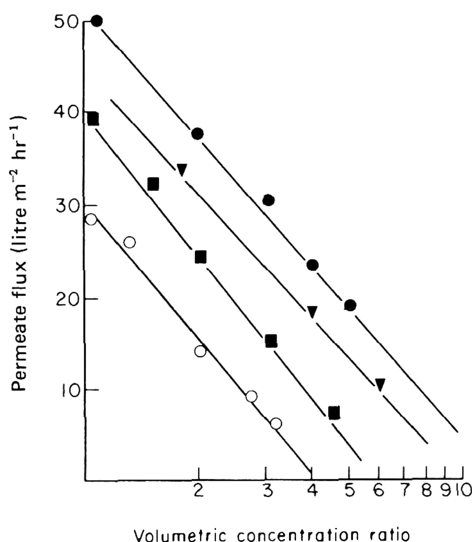

Figure 8. The relation between average permeation flux and concentration ratio for ultrafiltration of RBC with different initial protein levels. ●, 3% protein; ▼, 6% protein; ■, 11% protein; ○, 15% protein.

Table 1. Length of ultrafiltration process for the production of red blood cell concentrates (RBC) having 95 and 98% protein on a dry weight basis from different protein content feeds

Protein purity (%)	Time (hr)			
	Protein in feed (%)			
	3	6	11	15
95	1.35	2.54	8.3	11.1
98	3.6	7.4	not attainable	

other words, permeation rate progressively increased during diafiltration. The following equation is proposed to describe the NPM flux.

$$-V_0\, dC_{NPM} = A\, J_{NPM}\, dt_2 \tag{3}$$

where V_0 = constant volume during diafiltration, C_{NPM} = concentration of NPM in the retentate, J_{NPM} = the permeate flux of NPM, A = effective membrane area, t_2 = the time for diafiltration.

Before Equation 3 can be solved, it is first necessary to obtain J_{NPM} as a function of C_{NPM}. Considering the data in Fig. 9 the following relation can be postulated

$$J_{NPM} = k_1 \frac{1}{C_{NPM}} \tag{4}$$

where k_1 is found from the slope of the curves relating permeation rate and the reciprocal of percent NPM. A general relationship for k_1 in terms of protein

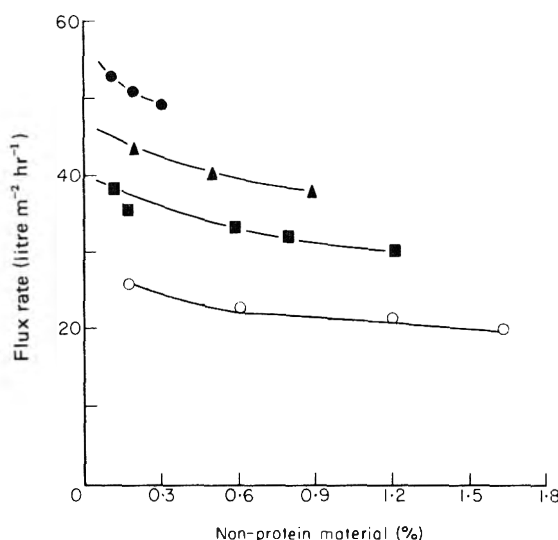

Figure 9. Permeation rate during diafiltration of RBC at different protein concentrations. Symbols as in Fig. 8.

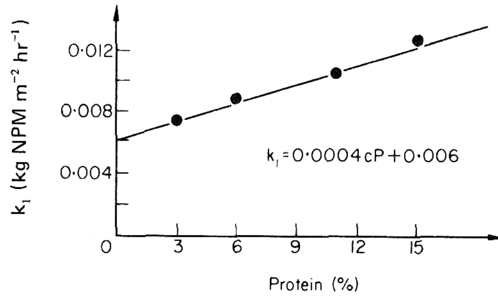

Figure 10. Graphical solution for relation between J_{NPM} flux and protein concentration (k_1) for diafiltration of RBC.

content of feed was obtained from Fig. 10. The relationship found was

$$k_1 = 0.0004\, C_p + 0.006 \quad (5)$$

where C_p is the protein content of RBC feed.

The constant k_1 thus describes J_{NPM} in terms of protein content of feed. Using this relationship NPM flux curves were prepared for a number of feeds with differing protein contents (Fig. 11). Using this approximation for J_{NPM}, Equation 1 may be integrated and the diafiltration time estimated from Equation 6

$$t_2 = \frac{V_0}{Ak_1}\left[\frac{(C_0)^2 - (Ct)^2}{2}\right] \quad (6)$$

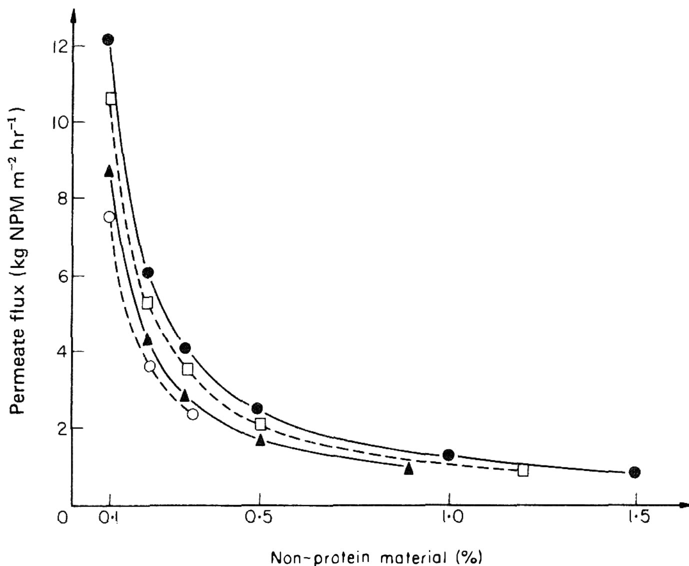

Figure 11. Permeate flux curves for J_{NPM} during diafiltration of RBC at different protein concentrations. ○, 3% protein; ▲, 6% protein; □, 11% protein; ●, 15% protein.

where V_0 = constant volume during diafiltration, A = effective membrane area, $k_1 = 0.0004\, C_p + 0.006$, C_0 = concentration of NPM at start of diafiltration, C_t = concentration of NPM at end of diafiltration.

Equation 6 was used to calculate the time required to produce 95 and 98% protein concentrates by diafiltration of 100 litre batches of different RBC feeds. The results are given in Table 2.

Table 2. Length of diafiltration process for the production of red blood cell concentrates (RBC) having 95 and 98% protein on a dry weight basis from different protein content feeds

Protein purity (%)	Time (hr)			
	Protein in feed (%)			
	3	6	11	15
95	0.02	0.15	0.57	0.66
98	0.06	0.20	0.69	0.89

Discussion

General performance of membrane plant

Temperature. Flux rate was found to be linearly related to ultrafiltration temperature. The slope of the curve was 0.5 litre $m^{-2}\ hr^{-1}\ {}^{\circ}C^{-1}$ (Fig. 2). This is in agreement with the strong temperature dependence of ultrafiltration membranes generally found (Donnelly & Delaney, 1974). This is higher than the value of 0.32 litre $m^{-2}\ hr^{-1}\ {}^{\circ}C^{-1}$ obtained for the ultrafiltration of cheese whey using PCI type T5A cellulose acetate membranes (Donnelly & Delaney, 1974). Eriksson & von Brockelman (1975) reported an average batch flux *v.* temperature of 0.2 litre $m^{-2}\ hr^{-1}\ {}^{\circ}C^{-1}$ for pooled animal blood serum. Delaney *et al.* (1975) found a similar flux *v.* temperature relationship for porcine blood plasma. Thus, for ultrafiltration of red blood cells, an operating temperature in the range of 45–50°C would appear appropriate if the general conditions governing membrane processing and operating temperature are fulfilled. The principal among these conditions are (i) damage to either membranes or products is avoided (ii) microbial growth is prevented or curtailed to a minimum, and (iii) flux rate is maximized.

Pressure and flow rate

The effect of pressure and flow rate on permeation flux is illustrated in Fig. 3. The results are similar to those obtained previously with whey systems (Donnelly & Delaney, 1974) and blood plasma systems (Delaney *et al.*, 1975)

and are consistent with membrane polarization theory (Blatt *et al.*, 1970). As flux rate was practically independent of pressure over the range 1 to 5 kg cm^{-2}, a pressure of *c.* 4.0 kg cm^{-2} was used for all ultrafiltration experiments. A flow rate of 8 litre min^{-1} (which is the recommended flow rate for the DDS 20 cm diameter plant) was adopted for most experiments.

Feed composition

The protein concentration levels achieved during ultrafiltration of RBC (Figs 4 and 5) were close to the theoretical predictions assuming zero retention of non-protein material (NPM) and complete retention of protein for the DDS 600 membrane. For a feed protein content of *c.* 3.5%, the actual protein concentration effects achieved for 3 : 1 and 5 : 1 volumetric reductions were 2.94 and 4.2 respectively (Fig. 3). The actual protein concentration effect achieved diverged from the theoretical at high volumetric concentration ratios (> 3 : 1) indicating higher retention of NPM at higher levels of protein. The general inverse linear relationship between flux rate and logarithm of protein content (Fig. 6) agrees with theoretical predictions (Blatt *et al.*, 1970). Similar relations were found for other systems (Donnelly & Delaney, 1974; Delaney *et al.*, 1975). Thirty percent protein in the liquid concentrate was the maximum protein concentration that could realistically be achieved during ultrafiltration of red cells under the conditions described in this work. At a protein feed concentration of 30%, the instantaneous ultrafiltration flux was *c.* 7 litre m^{-2} hr^{-1}. This is considerably higher than the generally accepted upper limit for protein concentration of 22 to 24% (Peri & Pompei, 1973).

Viscosity and total solids in feed

The increases in viscosity which accompany increases in the total solids of feed are one of the major factors causing the decline in flux experienced at high volumetric concentration ratios. This is because, for microporous ultrafiltration membranes, solvent flow is dependent solely on solvent viscosity. That this effect is present in the ultrafiltration of red cell concentrates is supported by the data in Fig. 7. A linear relationship was found between total solids content of feed and the logarithm of viscosity. The differences in the slopes of the viscosity *v.* total solids curves at 21.5 and 48°C explains the strong permeation flux–temperature dependence found (Fig. 2).

Purification of red cell concentrates

Simple ultrafiltration. The times required to achieve cell concentrates of differing purities with various protein content feeds are given in Table 1. The

time required to achieve a pre-specified degree of protein purification was found to increase with increasing protein content of feed. This is reasonable because the amount of NPM required to be removed is higher with higher total solids feeds and also greater retention of NPM takes place at higher protein concentrations (Figs 4 and 5) and lower average flux rates occur at high protein contents (Fig. 8). It was not practically possible to achieve 98% purity protein concentrates with feeds containing 11 or 15% protein by simple ultrafiltration. This was because the volumetric concentration ratios required to achieve this degree of protein purification resulted in protein contents in the liquid concentrates in excess of 30% which is above the upper practical operating limit for the membrane system. The results in Table 1 refer to the times required to treat 100 litre batches of red cells with an effective membrane area of 1 m^2. The total times required to treat 100-litre batches of red cells (*c.* 30% total solids) as centrifugally separated are given in Table 3. The advantage, in terms of shortest processing time of diluting the red cells to *c.* 3 to 6% protein before purifying by simple ultrafiltration is obvious.

Table 3. Comparison of equivalent times required to purify 30 kg lots of centrifugally separated red cells by ultrafiltration or diafiltration

	Protein purity (%)	Time (hr)			
		Protein in feed (%)			
		3	6	11	15
Ultrafiltration	95	13.5	12.8	22.6	22.2
	98	36.0	37.0	not attainable	
Diafiltration	95	0.2	0.8	1.6	1.32
	98	0.6	1.0	1.9	1.8

Diafiltration. In contrast to simple ultrafiltration where permeation rate decreased with increases in protein content (Fig. 6), the permeation rate during diafiltration of red cells at any specific protein level increased with decreasing NPM contents. The behaviour of red cells during diafiltration is dissimilar to that of cheese whey but similar to that of skim milk (Peri & Pompei, 1973). In the case of whey, permeation rates decrease during the diafiltration process. The reasons advanced for the decrease in permeation rate during diafiltration of whey is that progressive plugging of membranes take place with the high content of small molecular weight non-protein nitrogen material in whey (Peri & Pompei, 1973). The non-protein nitrogen fractions (proteoses and peptones, etc.) contain molecules with dimensions similar to those of the membrane pores and these tend to penetrate into the membrane structure and plug it. No pore plugging occurs during diafiltration of red cells. The main mechanism of permeation reduction in the case of red cells would appear to be concentration polarization at the solution–membrane interface. However, because of the particular nature of the protein in the red cell concentrates, concentration

polarization would not appear as important a consideration for membrane processing of red cells as it is for blood plasma (Delaney *et al.*, 1975). The dilution of the red cell retentate which occurs during diafiltration results in a sharp decrease in viscosity and density and has a marked positive effect on permeation rate.

The times required to purify red cells concentrates containing various protein levels, by diafiltration, are given in Table 2. Again, the time required to achieve a pre-determined protein purification increases with increasing protein content of feed. The times required are in all cases dramatically lower than those required for purification by simple ultrafiltration (Table 1). Table 3 contains equivalent times required to purify 30 kg lots of centrifugally separated red blood cells by ultrafiltration and diafiltration. Diafiltration is clearly superior to ultrafiltration as a process for purifying red blood cell concentrates in terms of the markedly shorter processing times required. For purification by simple ultrafiltration, prior dilution of separated red cells to 6% protein, appears optimal if a 95% protein concentrate is required and dilution to 3% protein if a 98% protein concentrate is required (Table 3). In the case of diafiltration, purification times decrease with decreasing levels of protein in feed. Thus the lowest protein level which is practical is the optimal level for diafiltration. This would appear to be *c.* 3% protein. The adoption of ultrafiltration or diafiltration as the specific process of choice for the purification of red blood cells, will depend to a large degree on the intended use of the purified retentates. This choice will be especially critical if additional concentration of the cells prior to either drying or freezing is desired.

Acknowledgments

Appreciation is extended to Ms Noirin Hurley and Mr Robert Kennedy for skilled technical assistance. The author is particularly grateful to Ms Ane-Grethe Olesen-Delaney for advice with material balance calculations. Thanks are expressed to Mitchelstown Co-Operative Society Ltd, Co. Cork for providing separated red blood cells.

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Protein concentrates from slaughter animal blood

II. Composition and properties of spray dried red blood cell concentrates

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Summary

Purified red blood cell concentrates (RBC), prepared by ultrafiltration and diafiltration (Delaney, 1977) were spray dried at air inlet temperatures of 185°C. The spray dried powders contained *c.* 95% 'true' protein, *c.* 0.62% non-protein nitrogen, *c.* 1.7% fat, *c.* 1.6% minerals and *c.* 2% moisture. The purification processes effected a reduction of *c.* 60% in the content of non-protein material in the red cells. Red cell protein contained adequate levels of the essential amino acids, threonine, tryptophan, valine, leucine, phenylalanine and lysine. Isoleucine and methionine plus cyst(e)ine were the limiting amino acids. RBC powders exhibited protein solubilities in water of 75–95% over the pH range 2–10. Protein solubility was at a minimum at pH 7.2. Ionic background had a very marked effect on protein solubility in water at pH 3.0 (solubility fell from *c.* 95% to *c.* 3% in 4.0 M NaCl) but little effect at pH 8.0. Coagulation of red cell concentrate proteins occurred at temperatures in the range of 47–55°C. The degree and rate of coagulation was dependent upon the concentration of proteins present. At pH values of 4 and 10 no coagulation occurred with a 5% RBC dispersion during heating at 55°C for upwards to 150 min. Ultrafiltered/diafiltered RBC powder products contained a higher proportion of particles in the 106 to < 45 μm size range than did controls. Adsorption and desorption isotherms for RBC powders were determined. The BET monolayer value for RBC powders was 6.5–6.7%.

Introduction

At the present time there is a growing world shortage of animal proteins suitable for human consumption. In spite of this, considerable quantities of slaughter animal blood are wasted annually (Akers, 1973). Because blood protein represents a good source of high biological value protein (Young *et al.*, 1973; Tybor, Dill & Landmann, 1975; Delaney, 1975; Wilson, 1974) there is much current interest in the fractionation of whole blood and in upgrading the

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different fractions for use as human food (Tybor, Dill & Landmann, 1973; Delaney, Donnelly & Bender, 1975; Eriksson & von Bockelman, 1975). Most of this work has been concerned with the treatment and use of the blood plasma fraction. Delaney (1977) has reported on the preparation and purification of red blood cell concentrates (RBC) by ultrafiltration and diafiltration. This present paper describes the composition and some of the properties of such red blood cell concentrates.

Materials

Red blood cell concentrates were prepared and purified as described by Delaney (1977). The concentrates were either spray dried directly or concentrated to *c.* 30% total solids by vacuum evaporation and then spray dried. A sample of red cell concentrate was spray dried directly after centrifugal separation to act as a control.

Evaporation

Bulked samples of several individual ultrafiltration/diafiltration runs were evaporated to *c.* 30% total solids in an Anhydro falling film vacuum evaporator (Anhydro AS, Copenhagen). Bulk product temperature was maintained below 40°C.

Spray drying

Cell concentrates were dried on an Anhydro No. 3 pilot-scale spray dryer (Anhydro AS, Copenhagen). Air inlet temperature was 185°C and air outlet temperature 85°C. Atomization was by centrifugal disc using speeds of 35 000 r.p.m.

Methods

Moisture, ash, fat, nitrogen and non-protein nitrogen (NPN) were determined in accordance with standard AOAC procedures (1970). Protein was estimated from Kjeldahl nitrogen values using the factor N × 6.25.

Specific mineral analysis

Calcium, magnesium, sodium and iron were estimated on a Perkin-Elmer Techtron AA6D atomic absorption spectrophotometer. Potassium was determined by flame photometry on the same instrument.

Chloride. An adaptation of the method for the estimation of chlorides in waste water was employed (Taras *et al.*, 1971). A pH meter (pHM28 Radiometer, Copenhagen) was operated as a milli-voltmeter in the range −200 to +800 mV. The titrating agent used was 0.1 M silver nitrate.

Phosphorus. The total phosphorus content, expressed as percent by weight, was determined by the IDF procedure (1967) on fluid and reconstituted red blood cell concentrates.

Amino acid analysis

After acid hydrolysis (6 N HCl, 105°C, 20 hr) duplicate samples were analysed by the Spackman, Stein & Moore (1958) procedure of ion-exchange chromatography using a Jeol amino acid analyser. Cyst(e)ine and methionine were estimated from the hydrolysates of performic acid oxidized samples. Tryptophan was determined on separate samples after alkaline hydrolysis by the method of Slump & Schreuder (1967).

Bulk density

Bulk density was determined on 50-g powder samples in accordance with the ADMI procedure (1965). The results were expressed in g/ml.

Powder solubility

Solubility of powder samples was determined by the ADMI (1965) procedure.

Particle size analysis

Particle size distribution of powder samples was determined by sieving through British Standard–410 sieves (British Standards, 1963).

Viscosity

Viscosity determinations on rehydrated powder samples were made with a Brookfield RVT synchroelectric viscometer.

Protein solubility

Protein solubility was estimated by a method based on Nitrogen Solubility Index (AOCS, 1969) as described by De Wit & De Boer (1975).

Heat stability/coagulation of RBC proteins

The method adopted was based on that reported by Toohill (1975). RBC dispersions at different total solids levels were prepared using an ADMI (1965) solubility mixer. Fifteen-ml aliquots of each RBC dispersion were dispensed into McArtney bottles (35 ml capacity, 2.5 cm diameter) fitted with screw caps. Samples were placed in a controlled temperature water bath. A control sample fitted with a 0–110°C Mg in glass thermometer was included to determine when samples reached the temperature of the test. The samples were heated over a range of temperatures from 40 to 55°C. Temperature stability of the samples was assessed on the basis of two parameters. By tipping and slowly rotating the McArtney bottles (without removing them from the water bath) at specified time intervals (1–2 min) the first signs of precipitation were noted. This was identified as 'flecking' on the side of the glass bottle as it was slowly rotated. The time at which flecking was observed was recorded. The first visually observed sign of setting or clotting of undisturbed RBC dispersions was also recorded. This was the 'clotting' or coagulation time. The coagulation times of RBC dispersions at different pH values were measured. The pH of the RBC dispersions was adjusted by 0.1 N HCl or by 0.1 N NaOH, as appropriate.

Water adsorption

Water sorption and desorption isotherms were determined for different powder samples by allowing the powders to reach equilibrium in confined atmospheres over saturated salt solutions (Rockland, 1960). The experiments were carried out in sealed desiccators at a temperature of 22.5°C. The data for the composition of different relative humidity atmospheres were obtained from water vapour tables of Perry (1950) and lithium chloride data of Young (1967).

Results and discussion

Gross composition of spray dried concentrates

General compositional data of one of the diafiltered (DF) spray dried concentrates are given in Table 1. Data on a control product are included for comparison. The main differences in the products, lay in their respective contents of protein, NPN and minerals. The DF product contained 95.08% 'true' protein compared to 85.9% in the control product. The NPN content in the DF concentrate was 0.62% and that in the control was 1.43%. The ash content was reduced from 3.3% in the control to 1.6% in the DF concentrate. The diafiltration process, thus effectively increased the true protein content at the expense of NPN and lower molecular weight material. The lipid content of the red blood cell concentrate was increased during diafiltration from 1.3 to

Table 1. Composition of spray dried diafiltered and control red blood cell concentrates

	DF RBC (%)	Control RBC (%)
Crude protein (N × 6.25)	95.7	87.3
Non-protein nitrogen (NPN)	0.62	1.43
True protein (TN − NPN) × 6.25	95.08	85.9
Fat	1.7	1.3
Moisture	2.0	3.2
Ash	1.6	3.1
Na	0.2	0.6
K	0.25	0.9
P	0.16	0.16
Ca	0.11	0.12
Fe	0.20	0.14
Cl	0.12	0.4
Mg (ppm)	110	90

1.7%. This was as expected. Ultrafiltration membranes generally exhibit the same rejection characteristics for lipids as for proteins (Donnelly & Delaney, 1974).

The chief cations present in the control red cell concentrate, in order of decreasing concentration, were potassium, sodium, calcium and magnesium. The major anions present were chlorides and phosphates. The potassium, sodium and chloride contents of the red blood cell concentrate were reduced during diafiltration in roughly the same ratio as the total ash content. Some selective retention of calcium, magnesium, phosphate and iron appeared to occur during diafiltration. These minerals may not be present in the red cells in a form which freely permeates the ultrafiltration membrane and are consequently retained. Additionally, some minerals may be wholly or partly protein bound and are selectively concentrated with proteins during diafiltration.

Amino acid composition of red blood cell concentrates

The detailed amino acid profile for DF RBC is given in Table 2. Data on the amino acid profiles of an ultrafiltered blood plasma concentrate and whole freeze dried blood are also tabulated. A comparison of the essential amino acids of the RBC protein with an 'ideal' protein, the FAO (1965) whole egg reference protein revealed that RBC values fell below egg values for threonine, isoleucine, tyrosine, tryptophan and methionine plus cyst(e)ine. The most serious limitation appears to be in the content of isoleucine, which is *c.* 6% of the egg value. Isoleucine is also the first limiting amino acid in whole blood and blood plasma (Table 2). Isoleucine is generally accepted as the amino acid limiting the biological value of blood proteins (Delaney, 1975). RBC protein is

Table 2. Amino acid composition of diafiltered red blood cell concentrate (DF RBC) (g amino acid/16 g N)

Amino acid	DF RBC	UF blood* plasma concentrate	Whole freeze† dried blood	Whole egg FAO reference protein
Asp	10.4	10.1	9.9	
Thr	4.7	8.5	5.2	5.1
Ser	3.3	6.5	5.4	
Glu	7.7	14.0	8.8	
Pro	3.4	5.9	4.0	
Gly	3.8	3.3	3.9	
Ala	8.4	5.1	7.7	
Val	8.0	5.8	9.1	7.3
Ile	0.4	3.2	0.9	6.6
Leu	13.6	9.2	12.4	8.8
Tyr	2.6	4.7	3.3	4.2
Phe	8.0	4.5	7.0	5.8
Lys	10.3	9.4	9.2	6.4
His	7.2	3.2	5.6	
Arg	3.6	5.1	3.8	
Trp§	1.4	1.3	1.4	1.6
Met‡	1.8	1.1	1.3	3.1
Cys‡	0.3	2.1	–	2.4
Total amino acids	98.9	103.0	98.9	
Total EAA	51.1	49.8	49.8	51.3
Chemical score	6.1	49.0	13.6	–
EAAI	25.4	70.1	38.6	–

* Data after Delaney (1975); † data after Wilson (1974); ‡ results taken from performic acid oxidized samples; § tryptophan determined by separate colorimetric analysis.

a particularly valuable source of valine, leucine, phenylalanine and lysine. The amino acid profile for diafiltered RBC is similar to that reported by Tybor *et al.* (1975) for decolorized globin protein concentrate.

The chemical score (CS) for the RBC, based on the method of Mitchell (1954) was 6.1. Whole blood had a CS of 13.6 (Table 2). In both cases, isoleucine was the first limiting amino acid. A CS of 5 was calculated for the globin isolate prepared by Tybor *et al.* (1975) based on the amino acid composition reported. The essential amino acid index (EAAI) (Oser, 1951) for RBC proteins was 25.4. Whole blood proteins had an EAAI of 38.6. Blood plasma concentrate has a CS of 49 and an EAAI of 70.1 (Table 2) and was shown to be a nutritionally adequate source of protein similar to casein (Delaney, 1975). The diafiltered RBC would not constitute an adequate sole source of dietary protein but would require supplementation with isoleucine and possibly methionine plus cyst(e)ine. This contention is supported by the work of Albanese *et al.* (1951) who found that isoleucine supplementation was necessary when feeding a human haemoglobin hydrolysate. Young *et al.* (1973)

reported a Protein Efficiency Ratio (PER) of −1.93 ± 0.12 for a decolorized globin preparation. The globin preparation failed to support life in rats without isoleucine supplementation.

Protein solubility of red blood cell concentrates

The protein solubility of one diafiltered RBC powder is plotted against pH in Fig. 1. The DF RBC exhibited good solubility over the range 2–10. Minimum solubility of *c.* 78% was observed at pH 7.2. The control RBC exhibited a similar solubility pattern. The overall solubility of the control product was lower than that of the DF RBC. The minimum solubility of the control RBC was 59%. Tybor *et al.* (1975) reported a different solubility/pH relationship for a globin isolate. In this case minimum solubility was observed at pH values ranging from 7.0–7.5. However minimum solubility values were only *c.* 15%. All of the globin isolates displayed maximum solubilities at values ⩽ pH 6.0. The differences in the solubility patterns of the DF RBC and that of the globin isolate may be due to changes induced in the globin protein during the isolation and decolorizing procedures.

The relation between ionic strength and protein solubility for the red cell concentrates is illustrated in Fig. 2. Ionic strength had little effect on solubility at pH 8.0 but had a marked effect at pH 3.0. At pH 3.0 the protein solubility of the DF RBC fell from *c.* 86% to *c.* 5% as the ionic concentration was increased from 0 to 4.0 M NaCl. The control product behaved in a similar fashion. The solubility behaviour of RBC proteins in different pH/ionic environments was similar to that of blood plasma proteins (Delaney *et al.*, 1975).

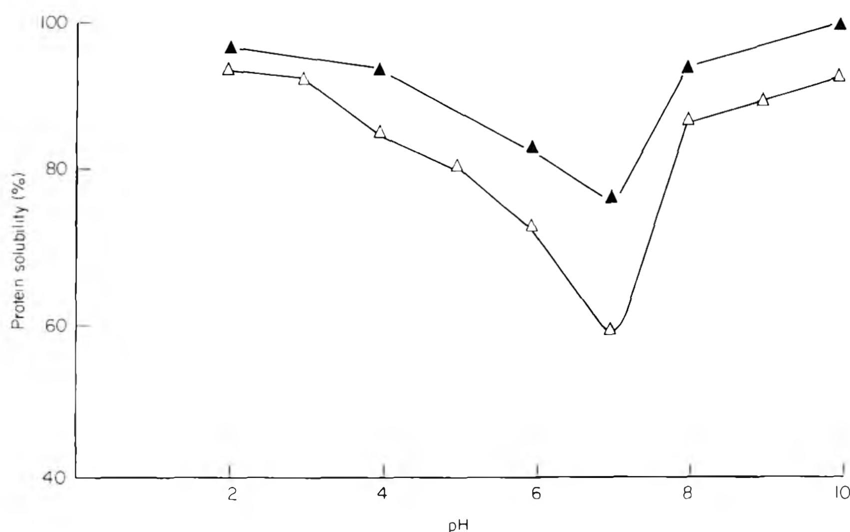

Figure 1. Protein solubility of red blood cell concentrates as a function of pH. ▲ DF RBC; △ control RBC.

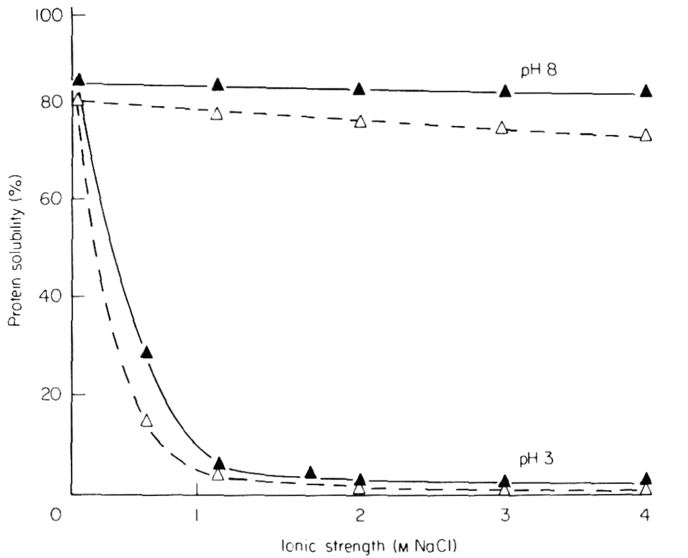

Figure 2. Protein solubility of red blood cell concentrates as a function of ionic strength and pH. ▲ DF RBC; △ control RBC.

ADMI solubility of RBC powders

Spray dried diafiltered red cell concentrates gave lower ADMI solubility indices (i.e. exhibited higher solubilities) than control RBC. Diafiltered RBC powders typically had a solubility index of 0.4–0.8 ml while control RBC gave values of 1.0–1.5 ml. Commercial spray dried red blood cell powders had ADMI solubility values of 1.0–2.0 ml. The results for ADMI solubility are in general agreement with those for specific protein solubility; namely that the purification process improves the solubility of red cell concentrate preparations. The solubility procedure however really provides an index of insolubility and was designed specifically for milk powders. It is an empirical procedure and has not been optimized for RBC powders. The results reported, should therefore be interpreted with caution. The solubility index values for red cell concentrate powders are higher than those usually obtained for milk powders. The method gave good reproducibility for spray dried blood plasma concentrate powders (R.A.M. Delaney, unpublished observations) and gives an index of overall solubility of the protein concentrate powders which is generally useful. The method has severe limitations when applied to protein concentrate powders of poor wettability (R.A.M. Delaney, unpublished observations).

Heat stability of red blood cell concentrates

The results of experiments on heat stability of RBC dispersions are given in Table 3. These results indicate that visually discernible heat coagulation of red

Table 3. Heat stability of spray dried diafiltered red blood cell concentrate dispersions

RBC conc. percentage total solids (wt/vol)	Temperature (°C)		
	40	47	55
5	NC	NC	Flecking 5 min Soft clot 11 min Firm clot 13 min
10	NC	NC	Flecking 3 min Soft clot 8 min Firm clot 10 min
15	–	Flecking 55 min	Flecking 1.5 min Firm clot 3 min

NC = no coagulation.

cell protein took place at temperatures between 47 and 55°C. Coagulation time at 55°C decreased with increasing solids content in dispersions. At a total solids content of 5% it required 13 min to form a firm clot at 55°C. At a total solids content of 15% a firm clot was formed in 3 min. The data in Table 4 indicate the effect of pH on the heat stability of red cell proteins. Heat coagulation of RBC protein occurred more quickly at pH values of 6.0 to 7.5 than at other pH values. At pH 4.0, the RBC proteins did not coagulate on heating at 55°C for up to 120 min. A similar effect was observed at pH 10. An explanantion for the high heat sensitivity of red cell proteins at pH values of 6–7.5, may be found in the data on specific solubility of red cell proteins at different pH values (Fig. 1). Red cell proteins exhibited minimum solubility at pH values of around 7.0. The isoelectric point for red cell proteins would appear to be around this value. At high and low pH RBC protein exhibited maximum solubilities and this coincides with the pH values at which decreased heat sensitivities were observed. The protective effect of pH on heat denaturation of protein has been reported for other systems including whey protein (Guy, Vettel & Pallansch,

Table 4. Heat stability of spray dried diafiltered red blood cell concentrate dispersions at different pH values

pH	Time (min)		
	Flecking	Soft clot	Firm clot
4	No clot after 150 min		
6	5	7	10
7.5	3	5	8
10	No clot after 120 min		

Temperature 55°C. RBC 5% total solids.

1966). The results reported in this present study, while they must be regarded as preliminary, provide worthwhile information on the heat stability of red cell proteins. Haemoglobin, which is the principal protein present in the red cell concentrate is reported to denature spectacularly at about 57°C (Rossi Fanelli, Antonini & Caputo, 1964). This agrees well with the temperature of 55°C reported here as critical for red cell proteins.

Viscosity of RBC dispersions

The viscosity of rehydrated diafiltered red cell concentrates and control concentrates are given in Fig. 3. The viscosity of DF RBC dispersions increased from about 10 cP at *c.* 5% total solids to *c.* 600 cP at 40% total solids. These results agree well with viscosity data on diafiltered liquid red cell concentrates reported earlier (Delaney, 1977). The viscosities of control RBC dispersions were always lower than those of DF RBC dispersions at equivalent total solids concentrations. Presumably this was because at any given solids concentration, the diafiltered red cell concentrate contained a higher level of protein.

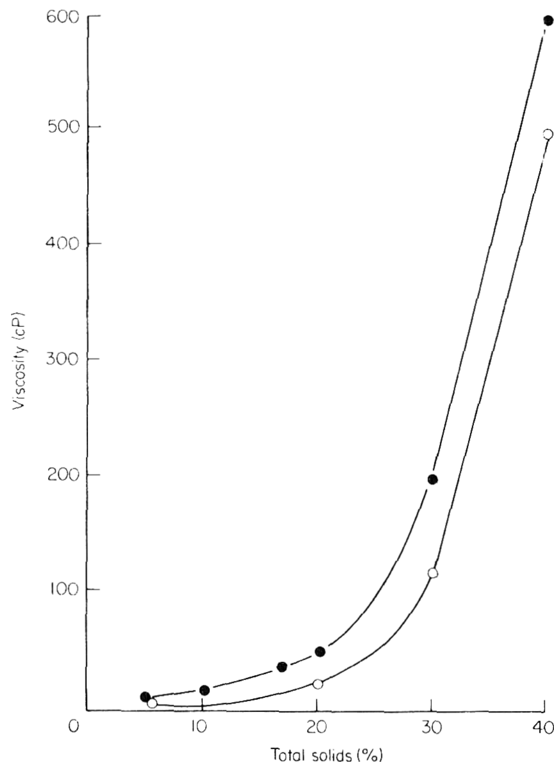

Figure 3. Viscosity of red blood cell concentrate dispersions. ● DF/UF RBC; ○ control RBC.

Bulk density of RBC powders

Data on the bulk density of diafiltered and control RBC powders are given in Table 5. The bulk density of DF RBC powder was typically *c.* 18.4% higher than that of the control product. This was when samples were spray dried at comparable feed solids contents (*c.* 30%). Commercial spray dried blood powders usually had bulk densities of about 0.6 g ml^{-1}.

Table 5. Bulk density of spray dried diafiltered red blood cell concentrate (DFH RBC)

	Bulk density (g ml^{-1})	
	loose	packed
DF RBC	0.71	0.76
Control RBC	0.60	0.63

Particle size distribution in RBC powders

Data on the particle size distribution in diafiltered and control RBC powders are given in Fig. 4. The particles in the control powder fell within two main size ranges; 300–125 μm and 106–<45 μm. Approximately 50% of the particles were in either group. In contrast, the diafiltered RBC powder contained a higher proportion of smaller particles. Approximately 80% of the particles in the DF RBC powder were in the 106–<45 μm size range. The reason for the

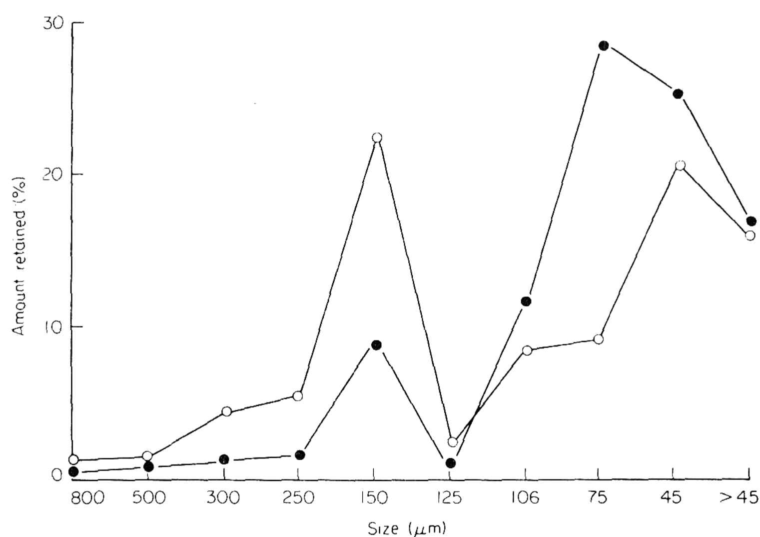

Figure 4. Particle size distribution in red cell concentrate powders. ● DF RBC; ○ control RBC.

higher proportion of smaller particles in the diafiltered RBC powder may be due to breakdown of red cell structure caused by lysis of the erythrocytes induced during dilution and purification.

The differences in the particle size distributions in the two powders helps to explain the higher bulk densities observed for diafiltered RBC powders. It might be expected that the bulk density of a powder would increase as the size distribution range of particles widens with an appreciable proportion of smaller particles present. The smaller particles are probably able to pack into the interstices between the larger ones.

Water adsorption on RBC powders

Figure 5 contains data on the water adsorption and water desorption isotherms for spray dried diafiltered red cell concentrate powders at 22.5°C. The adsorption isotherm follows a smooth continuous sigmoid curve and corresponds in shape to a type I isotherm according to the classification of Brunauer, Emmet & Teller (1938). The desorption isotherm also described a smooth continuous sigmoid curve and shows a typical hysteresis effect. There appears to be some adsorbed water which is irreversibly bound and which cannot be removed during desorption (Fig. 5). BET isotherms were constructed over the activity range 0.1–0.5 and monolayer values calculated (Brunauer *et al.*, 1938). The monolayer value for a range of spray dried diafiltered RBC powders was 6.5–6.7%. As shown by Salwin (1959) the BET monolayer value appears to be the most stable moisture content for storage of foods.

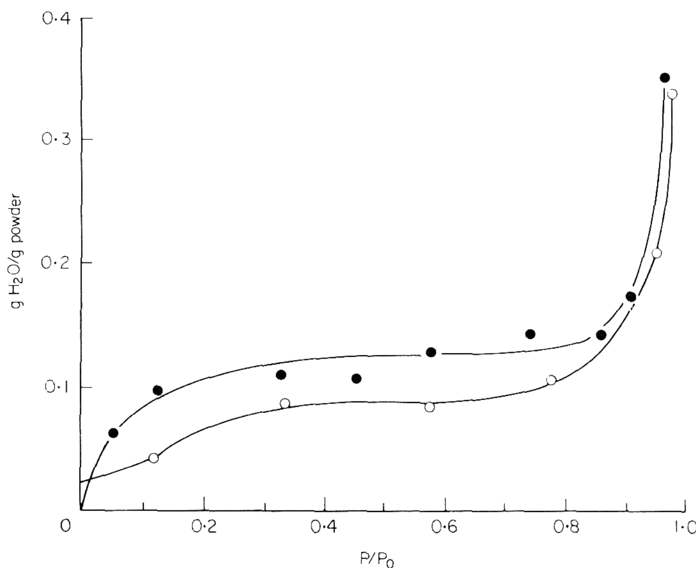

Figure 5. Sorption isotherms for diafiltered red cell concentrate powder. ● Adsorption; ○ desorption.

Conclusions

Porcine red blood cell concentrates were successfully purified by ultrafiltration and/or diafiltration prior to spray drying. The resultant powders have a significantly reduced ash and non-protein nitrogen content compared with non-diafiltered powders. The removal of non-protein material from the red cells appears to improve functionality of the red cell concentrates, which have higher protein solubilities and total powder solubilities than control products. Membrane processed RBC powders have a higher distribution of smaller size particles and as a consequence higher bulk densities than control products. The red cell concentrate proteins were valuable sources of a number of essential amino acids in particular valine, leucine, phenylalanine and lysine. On the basis of essential amino acid contents red cell concentrate proteins should complement wheat, corn, soy or other vegetable proteins in the diet. The low level of isoleucine in red cell proteins rules out its use as the sole source of dietary protein.

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Coliform counts on blood meals and characterization of *Escherichia coli* isolated from them*

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Summary

A total of fifty blood meal samples were prepared; twenty-five by the incorporation of calcium oxide in the fresh blood at a ratio of 1 : 8 and the remaining samples without adding calcium oxide. The most probable number of coliforms and *Escherichia coli* were recorded to be 3×10^2 and 2.3×10^2 g^{-1} respectively in the untreated samples, whereas calcium oxide treated samples did not reveal the presence of coliforms. Of the eleven *E. coli* isolates, five serogroups (O_{79}, O_{30}, O_{25}, O_{17} and O_{11}) were identified. The significance of the isolates from the view-point of human and livestock health have been discussed.

Introduction

The losses incurred in the form of non- and under-utilization of blood and other slaughterhouse byproducts in India are of high magnitude as is evident from the fact that about 64% of the blood spilt in the slaughterhouses is thrown away in the drains because of lack of facilities for its collection and for want of technical know-how for its utilization, thus resulting in the loss of significant amount of protein and other useful substances (M. S. Swaminathan, personal communication).

The available literature concerning the microbiology of blood meal is scanty, however, workers have tried to evaluate its nutritive value and recommended blood meal as livestock and poultry feed supplement (Fratzer & Green, 1957; Ali & Momin, 1964). Commercially prepared animal feeds have been reported to be an important source of *Salmonella* and other infections (Griffin, 1952; Morehouse & Wedman, 1961). By employing fluorescent antibody technique Laramore & Moritz (1969) reported the detection of salmonellae in animal feed and feed ingredients.

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Keeping in view the importance of blood meal in livestock and poultry feed supplement, the present study was undertaken to determine the most probable number of coliforms and *Escherichia coli* in blood meal samples and also to characterize *E. coli* isolates.

Materials and methods

The fresh blood, collected from two to six goats at a time, was pooled in a clean circular pan made of galvanized sheet as described by Mann (1967). The goats were slaughtered at the local slaughterhouse using the 'Halal' (Mohammedan) method of slaughtering. The pooled blood was processed immediately after the collection. The following two methods were adopted for the preparation of blood meal.

Untreated. The pooled blood was transferred to an iron pan and heated with constant stirring on an electric heater until it coagulated to a dark coloured semisolid mass.

Treated. Calcium oxide (BDH) was added in the fresh blood at a ratio of 1:8 (1 part CaO and 8 parts fresh blood). It was thoroughly mixed with blood and processed as described with the untreated method.

The processed blood was uniformly spread over in a clean galvanized tin tray with raised edges. The tray was then placed in the open air for sun-drying at a height of about 60 cm from the ground level in order to reduce dust-borne contamination. The drying process was considered as complete when the cooked blood masses turned hard and brittle. Usually it took 15 hr.

The blood meal samples were collected in clean polythene bags and brought to the laboratory for further studies. The samples were powdered with the help of an electrically operated grinder. The powdered blood meal samples were then stored at room temperature in separate stoppered glass jars.

In all fifty blood meal samples were prepared of which twenty-five were prepared by adding CaO in the fresh blood (treated samples) and the remaining twenty-five samples without adding CaO (untreated samples).

The resuscitation of sublethally impaired cells was done by employing standard procedures using MacConkey's lactose bile broth (MLBB) tubes (North, 1961; Mossel & Ratto, 1970). In addition, samples were examined after storing them from seven to fifteen days and providing sufficient aeration. This procedure has been found to allow a reasonable degree of restoration of damaged cells (Mossel & Ratto, 1970).

Determination of most probable number (MPN) of coliforms and E. coli

The MPN of coliforms and *E. coli* in the blood meal samples were determined by using a three tube technique. One gram of powdered blood meal sample was dissolved in 100 ml of sterile 1 N saline solution. Samples of 10,

1 and 0.1 ml of diluted suspensions were poured in MLBB tubes containing 10, 5 and 5 ml broth, respectively. Two sets were prepared for each sample, one was incubated at 37°C for 48 hr for determination of MPN of coliforms and the other set at 44°C for 48 hr for MPN of *E. coli* organisms. The tubes showing gas production were recorded as positive and MPN calculated with the help of the MPN table (Jacobs & Gerstein, 1960).

Isolation and identification of E. coli

The positive tubes from MLBB inoculated and 44°C incubated set were selected for the isolation of *E. coli.* The morphological, cultural and biochemical characteristics of *E. coli* isolates were carried out according to the methods described by Edwards & Ewing (1972). The serological typing of the *E. coli* isolates was done through the courtesy of National *Salmonella* and *Escherichia* Centre, Kasauli, India.

Effect of calcium oxide on E. coli

During the microbiological studies on blood meal samples, it was observed that CaO treated samples, in general, showed a lower microbial load than the untreated samples. An experiment was, therefore, planned to see the inhibitory effect of CaO, if any, on *E. coli* isolates.

A sterile cotton swab saturated in 24 hr old broth culture of *E. coli* was streaked on nutrient agar plate. The discs were made from Whatman filter paper no. 40, sterilized and soaked in different dilutions of CaO (1:4, 1:8, 1:16 and 1:32) in sterile distilled water. The discs of each dilution were placed over an inoculated plate. The plate was incubated at 37°C for 48 hr and the zone of inhibition recorded.

Results

The coliform organisms in the untreated blood meal samples ranged from nil to 1100 with an average of 3×10^2 g^{-1}. Like coliforms, *E. coli* counts also ranged from nil to 1100 but with an average of 2.3×10^2 g^{-1}. Out of the total of twenty-five untreated samples examined, sixteen (64%) were found to be positive for coliforms and eleven (44%) for *E. coli.* None of the twenty-five CaO treated samples resulted in the isolation of coliform organisms (Table 1).

All the eleven *E. coli* isolates were found to be Gram negative, short, motile rods. The biochemical reactions of the isolates are in agreement with those described by Edwards & Ewing (1972). Five *E. coli* serogroups encountered in order of frequency were: O_{79} (three strains), O_{30} (two), O_{25} (two), O_{17} (one) and O_{11} (one) and two rough strains.

Calcium oxide was found to have an inhibitory effect on *E. coli* organisms. It

Table 1. Recovery of coliforms from blood meal samples

Type of sample	Samples examined	No. of samples positive		Average count per gram	
		Coliforms	*E. coli*	Coliforms	*E. coli*
Untreated	25	16 (64%)	11 (44%)	3×10^2	2.3×10^2
Calcium oxide treated	25	nil	nil	nil	nil

was particularly more effective in 1 : 4 and 1 : 8 dilutions as the zone of inhibition was greater. The inhibitory zone was shorter in 1 : 16 dilution and no inhibition was observed in 1 : 32 dilution (Fig. 1).

Discussion

Coliform counts are frequently used as indicators of faecal contamination, plant cleanliness, post cooking contamination and contamination during handling

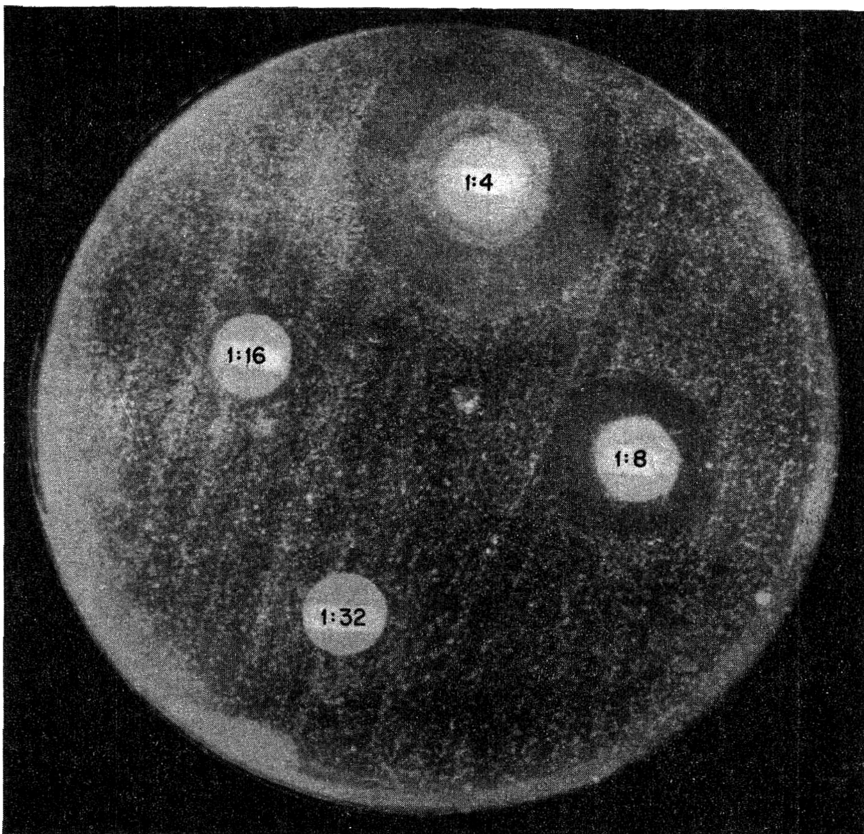

Figure 1. Effect of calcium oxide on *E. coli;* note the zone of inhibition in 1 : 4 and 1 : 8 dilutions.

and processing (Hobbs & Gilbert, 1970). A coliform count of 300 g^{-1} and *E. coli* count of 230 g^{-1} were recorded in the untreated blood meal samples. Thus *E. coli*: coliform ratio was found to be 1 to 1.3. Coliforms could not be observed in any of the CaO treated blood meal samples. This might probably be due to the inhibitory effect of CaO on coliforms which has also been proved experimentally and is depicted in Fig. 1. Incorporation of CaO in fresh blood was reported to limit microbial growth (Mann, 1967). It is, therefore, recommended that CaO be incorporated for commercial blood meal production.

Of the five *E. coli* 'O' serogroups encountered from the blood meal samples of goats, O_{11} and O_{17} have earlier been recorded from calves (Sakazaki & Namioka, 1956; Glantz, Rothenbocher & Hokanson, 1968; Verma & Adlakha, 1970), poultry (Sojka & Carnaghan, 1961; Gupta & Singh, 1969), fish (Abdulrashid, 1976) and from human beings (Cooke, 1968); O_{25} from calves (Sakazaki & Namioka, 1956), dogs (Wesselinoff & Dimow, 1961), poultry (Takahashi, 1966; Arunachalam, Jaganaman & Balaprokasam, 1974), fish (Abdulrashid, 1976) and from infantile diarrhoea and urinary tract infections in human beings (Wesselinoff & Dimow, 1961; Taylor, 1961) and O_{30} from cattle (Glantz *et al.*, 1968). Recently O_{30} serogroup has also been isolated from chickens suffering from septicaemia, enteritis and egg peritonitis (Sharma *et al.*, 1977) and human urine and infantile diarrhoea cases in Tarai region of Uttar Pradesh (Pandey, 1975). Serogroup O_{79} has been reported from pigs (Sojka, 1965). None of the serogroups recovered in this study have so far been recorded in literature to have any significant enteropathogenic effect in human beings although there appears to be no definite relationship between enteropathogenicity and serogrouping.

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Continuous measurements of the pH of beef muscle in intact beef carcases

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Summary

A method is described for making continuous measurements of the pH of anaerobic muscle. The technique is shown to be applicable to measurement in an intact beef carcase post-mortem. This technique is compared with conventional measurements upon homogenates prepared from samples of metabolically inhibited muscle and the results are seen to be closely comparable. The continuous measurement of tissue pH has the advantage that relatively rapid events may be followed with ease.

Introduction

It is customary to measure the pH of muscle tissue by homogenizing a sample in a medium containing iodoacetate to inhibit the enzyme glyceraldehyde-3-phosphate dehydrogenase and thus to arrest glycolysis and the consequent pH fall which occurs in anaerobic tissue. This technique suffers from the disadvantage that it destroys the sample, causes major changes in the concentration of the glycolytic intermediates and is also discontinuous, since successive samples have to be prepared to measure successive instantaneous pH values. In addition, uncertainties arise concerning dilution effects and the changes in the pK values of the intracellular buffers during the preparation of the tissue homogenate from the sample (see Bendall (1973) for a recent review of the problem). Consequently, it was decided to evolve a continuous, non-destructive monitor of tissue pH which could be employed in a wide variety of circumstances and which would also be convenient for measurement of the kinetics of pH changes which occur relatively rapidly (on a time scale of minutes).

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Materials and methods

Preliminary considerations

Various non-destructive methods of measuring tissue pH might be employed, some of which could be adapted for continuous monitoring. In view of the relative robustness and simplicity of manipulation of the pH-responsive glass membrane, it was decided to adopt the conventional electrometric measurement of the e.m.f. of a cell consisting of a glass electrode in contact with the tissue and an appropriate reference electrode consisting of a half cell of standard potential connected electrically to the tissue via a suitable salt bridge and a liquid/liquid junction with the tissue fluid. The aim was to produce electrometric equipment capable of recording tissue pH in a wide variety of experimental situations over the temperature range 5–40°C and in circumstances where the electrical connections to the tissue, other than those provided by the pH measuring equipment, would be rather unpredictable and subject to change.

Unlike a small object on the laboratory bench, an object the size of a beef carcase is likely to be connected to the environment electrically by important pathways other than the pH meter. In addition, it is desirable for practical purposes, when connecting the system to other recording equipment, to provide an electrical output from the electrometric equipment which, unlike that from a conventional pH meter, is not electrically floating but is referred to ground potential. This output was arranged for convenience in the present case to be 1 V per pH unit change in potential at the input and was provided by the output voltage of an operational amplifier. This was monitored with a digital

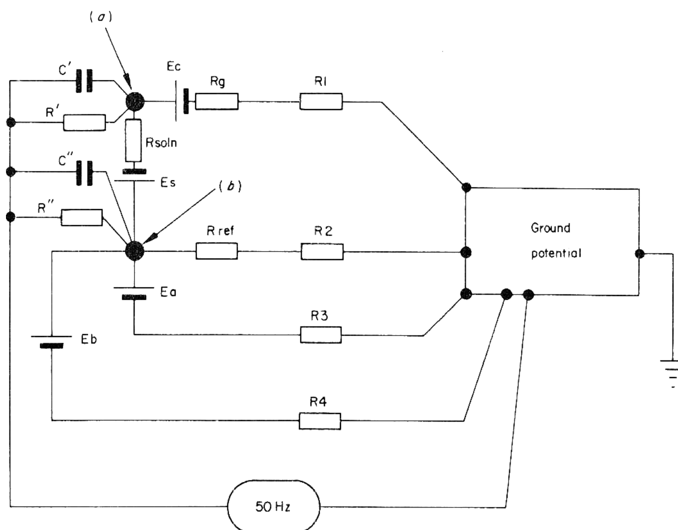

Figure 1. Schematic diagram of the electrical arrangements of the recording system. See text for explanation.

voltmeter (Solartron 7040) and a chart recorder. Figure 1 indicates, schematically, the various electrical connections to the pH measuring cell. The points (*a*) and (*b*) in the figure are the points of contact within the tissue, of the glass and reference electrodes respectively. The overall e.m.f. of the pH measuring cell, lumped together for convenience as Ec, is monitored via R1, the total resistance presented by the potential-measuring equipment, between the glass electrode (resistance Rg) and ground and via R2, the total resistance between the reference electrode (resistance Rref) and ground. The experimental object is also connected to ground by other pathways. Other electrodes inserted into the carcase may provide such routes and, if the carcase is suspended from an overhead gantry by a metal hook, this provides another possible electrical connection. These paths are generalized in Fig. 1, as batteries Ea and Eb, in series with resistances R3 and R4 respectively. In addition, at low frequencies, an appreciable signal may be generated by the local electric field caused by adjacent power lines (at 50 Hz and its harmonics). This is injected through various resistances and capacitances generalized as R′, R″, C′, and C″. In addition, if the glass and reference electrodes are physically separated, then the intervening solution and biological material may influence the observed potential of the cell as represented by a resistance, Rsoln and a battery, Es in series between A and B.

In order to monitor the glass electrode potential faithfully, R1 must be large by comparison with Rg, which is itself generally of the order of $10^8\,\Omega$ at room temperature. Rref by comparison, is relatively small, but R2 should still be made as large as possible in order to limit the currents flowing through the reference electrode via those other paths which connect the experimental object to ground (through batteries Ea and Eb and resistances R3 and R4 in Fig. 1). These currents, circulating through the reference electrode, tend to polarize it and thus tend to modify the potential established by that electrode and hence the observed value of Ec.

Equipment employed

The pH measuring electrometer system which was employed (see Fig. 2) departs from conventional practice by providing a symmetrical differential input stage (amplifiers 1 and 2). The system was arranged as a small pre-amplifier (A in Fig. 1) encased in a diecast box 89 × 35 × 30 mm, which could be hung from the carcase, although electrically isolated from it within a polythene bag. The glass electrode (Radiometer type G 122c) was connected to the pre-amplifier by a low noise screened cable and BNC plug and socket. The pre-amplifier was connected to the main amplifier (B) by a suitable length of screened four-core cable which also carried the necessary power supply for the pre-amplifier.

The necessary isolation of the reference electrode from ground was provided by the differential input stage (amplifiers 1 and 2). The pre-amplifier A

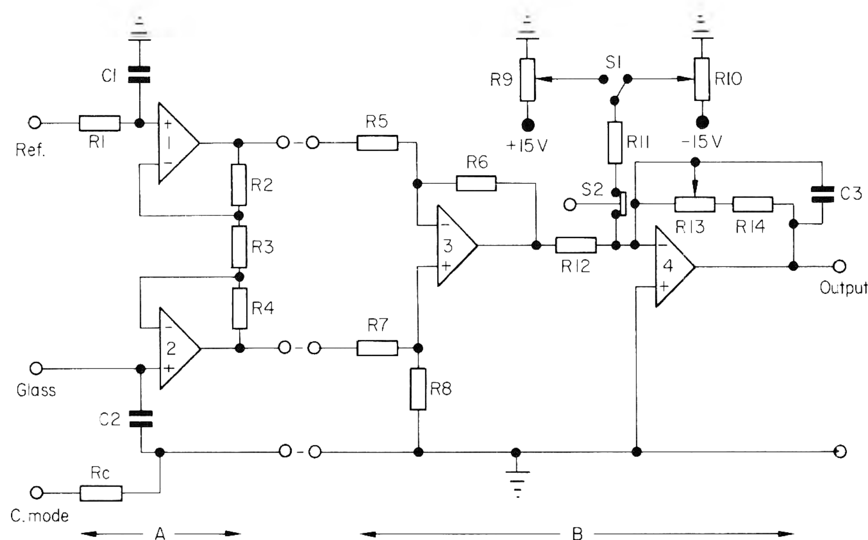

Figure 2. Diagram of the pH recording apparatus. C1, C2, 1 nF polystyrene; C3, 1 μF polyester; R1, $10^8\,\Omega$ (Welwyn Resistors Ltd); R2, R4 150 kΩ; R3, 33 kΩ; R5, R6, R7, R8, 33 kΩ; R9, R10, 10 kΩ wirewound, 20 turn; R11, 150 kΩ; R12, 22 kΩ; R13, 10 kΩ wirewound, 20 turn; R14, 33 kΩ; Rc, 50 kΩ. Operational amplifiers, 1 and 2 with FET input, bias current 3 pA at 20°C; 3 and 4, silicon monolithic 741 type, all from R.S. Components Ltd. All resistors 0.5 W, thick film ±2% unless otherwise stated. Glass-electrode, type G122c (Radiometer Ltd) of 4 mm diameter and with an Ag/AgCl internal reference element.

provided a differential gain of 10 and amplifier 3 acted as a unity gain converter to convert this differential signal into a single ended form. Amplifier 4, in inverting mode, provided voltage offset and the extra voltage gain necessary to produce an output of 1 V per pH unit change in e.m.f. at the input. The offset arrangements were sufficient both to null the signal from Ec for purposes of recording small variations of pH using a chart recorder and to provide an output of (e.g.) +7 V at a known pH of 7 when calibrating the electrode system for pH measurements. Switch S2 permitted the momentary removal of the internally derived offset signal.

The common mode voltages developed by the electrode system were established and stabilized by the third electrode, a substantial silver, silver chloride element labelled 'C.mode' in Fig. 2, inserted directly into the musculature near the pH measuring cell and connected to ground via a resistance (Rc) of 50 kΩ. The common mode rejection of the amplifying system was made as high as possible by carefully selecting and matching the values of the relevant gain setting resistors from a large stock of nominally the same value. The input impedance to the reference and the glass electrode terminals was approximately balanced by the insertion of R1 ($10^8\,\Omega$) in series with the reference electrode, whilst C1 and C2 approximately balanced the two input capacitances and restricted the bandwidth. The input time constant was about 0.1 sec. In this

way, spurious signals such as 50 Hz interference were rendered similar at each input to the instrumentation amplifier system and therefore were largely eliminated by subtraction at the input to amplifier 3.

Oxygen tension (PO_2)

Measurements of PO_2 adjacent to the glass electrode bulb were made polarographically with an oxygen electrode (Cater & Silver, 1961). The cathode was provided by a 25 μm diameter wire sealed through the end of a pyrex tube of 0.311 mm bore. The cathode was held at -0.6 V with respect to a remote silver, silver chloride reference electrode inserted into the musculature, and the diffusion current was measured with an electrometer operational amplifier in the inverting mode, connected to a potentiometric recorder. The electrode provided a current of about 3×10^{-8} A at atmospheric PO_2 and the diffusion current was a linear function of PO_2 in gas mixtures of nitrogen and oxygen of various known composition.

Measurement of muscle pH by discontinuous sampling

The pH of tissue samples was measured by removing a 0.5 g sample of muscle from about 1 cm below the surface, chopping it finely with scissors, and homogenizing it in 5 ml of a solution of 150 mM KCl and 5 mM iodoacetate brought to pH 7 with KOH. The pH of the homogenate was then measured with a Radiometer combination electrode.

Reference electrode arrangement

A very high resistance reference electrode could be employed because of the exceedingly high impedance offered by both the glass and reference electrode inputs of the differential pre-amplifier configuration. Accordingly, a junction between 3 M KCl and the tissue fluid was employed, but it was so constricted that essentially no outflow of the reference electrolyte occurred. A flexible plastic tube of 1 mm bore was filled with 3 M KCl blocked at one end with a tapered glass stopper. The film of reference electrolyte thus trapped between the inner wall of the tube and the stopper, provided a liquid to liquid junction with a resistance between 5 and $14 \times 10^5\ \Omega$ when immersed in 100 mM KCl, and control experiments indicated that this junction developed a constant low potential (< 3 mV) when held in contact with muscle tissue for many hours. Uncertainties about liquid junction potentials were largely eliminated by this means. 3 M KCl was employed in preference to saturated KCl since when the temperature of a saturated salt solution is altered, an appreciable time must elapse before an equilibrium state of saturation is re-established. Figure 3

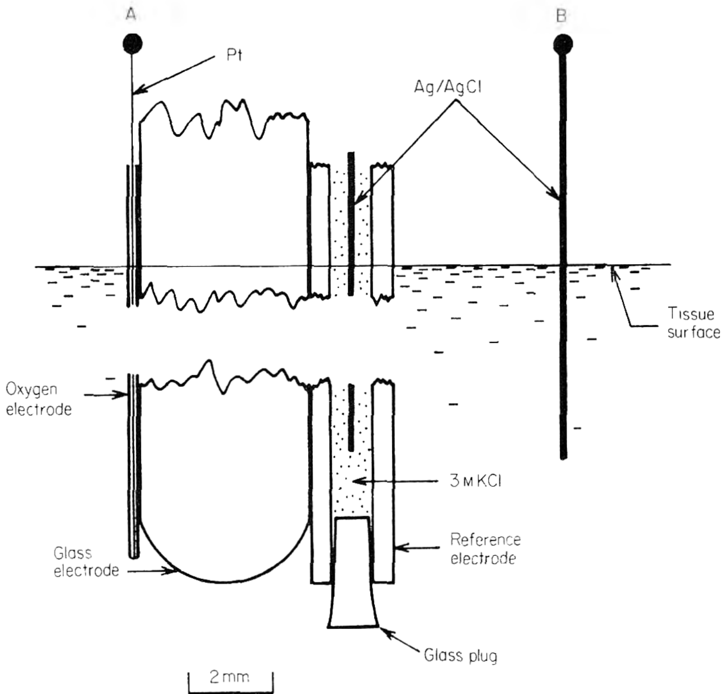

Figure 3. Diagram of the electrode arrangement used. Point A was held at virtual ground potential by the electrometer amplifier used, in the inverting configuration, to measure the diffusion current flowing from the platinum cathode. Point B was held either near ground potential (via a resistor of 50 kΩ) when the polarographic electrode was absent, or at + 0.6 V with respect to ground when the polarographic electrode was connected. The Ag/AgCl electrode serving as the anode and connected to point B, was about 2 cm away from the polarographic cathode.

summarizes the arrangements of the several electrodes used for the measurement of pH and PO_2 with equipment of the type II design. The glass electrode membrane and the liquid junction with the reference electrode were held in close proximity to minimize the possibility that some part of the membrane potential of adjacent muscle cells might be superimposed upon the observed potential.

Temperature compensation

The amplifier system was maintained at a constant temperature for these measurements and all offset voltages due to the amplifiers themselves had been nulled previously at this temperature by the use of circuits which are not depicted in Fig. 2. The changes in amplifier offset voltage that would have been induced by the maximum temperature changes in these experiments are, however, small by comparison with the temperature-induced voltage changes encountered in the pH measuring cell itself.

Temperature compensation of the pH measuring cell was performed as indicated below.

Variation of R13 altered the overall voltage gain of the system in order to compensate for such variations in the slope of the relationship between the glass electrode potential and pH as are produced by temperature variations over the range 5–40°C. This compensation, which is provided on commercial pH meters, is of limited value when the temperature is continually varying, and it was employed only for measurements conducted at one particular constant temperature at which the system was calibrated using a standard buffer solution. The overall potential (Ec) of the pH-measuring cell is in fact the algebraic sum of a number of potential differences with different temperature coefficients. The absolute magnitude of these potential differences cannot be measured individually. If, however, each potential difference were contributed by a process obeying the Nernst relationship for a univalent cation or anion then, from the magnitude of the overall cell potential Ec at a particular pH, the temperature coefficient of the cell e.m.f. could be calculated as a function of pH. Neither the asymmetry potential across the glass membrane, nor the standard potential of the silver–silver chloride reference elements obey this relationship with temperature (see the detailed discussion in Dole, 1941) so an empirical correction was applied to the present measurements.

The pH of a standard buffer solution (Robinson & Stokes, 1959) held at 20°C, was measured with the electrometric equipment set to produce a 1 V change at the output for a 58.17 mV (1 pH unit at 20°C) change at the input. All components, of the pH measuring electrochemical cell were completely immersed in the buffer solution. The output voltage was then set to indicate the known pH of a standard buffer (in units of volts). The temperature of the pH measuring cell and of the standard buffer was then altered and the indicated pH (1 pH unit at 20°C ≡ 1 volt at the output), was noted as a function of temperature over the working range. These data, together with the known temperature dependence of the pH of the standard buffer (Robinson & Stokes, 1959) enabled a calibration curve to be constructed relating the error in the indicated pH to the temperature of the cell. This procedure was repeated for several values of pH (20°C) across the working range using various standard buffer solutions. Linear relationships were obtained in each case, and the slopes of these relationships plotted as a function of pH (20°C) produced a straight line with a slope of about 0.2 mV (or 0.0034 pH units at 20°C) per °C per pH unit and with an intercept on the pH axis near the pH at which this particular pH-measuring cell developed no overall e.m.f.

The Nernst relationship, describing the potential difference produced by an activity difference of the univalent cation or anion, predicts that for a potential difference produced by a tenfold activity difference (≡ 1 pH unit), the observed potential should increase by 0.198 mV for an increase in temperature of 1°C. Thus for the pH-measuring electrochemical cells employed in these experiments, it appears that the temperature coefficient of the cell e.m.f. can be predicted from this limiting assumption, probably because the contributions

from those potentials with a temperature dependence different from this (in particular, the standard potentials of the Ag/AgCl electrodes) cancel out in the overall e.m.f., Ec. It is evident that, in practical terms, since the temperature coefficient of the overall cell e.m.f. increases linearly with the magnitude of that e.m.f., the cell e.m.f. should be made as small as possible for minimum coefficient.

Results and discussion

Preliminary observations indicated that a conventional glass-plus-calomel combination electrode pair produced erratic results when inserted into muscle tissue.

An excised beef sternomandibularis muscle (3 hr post-mortem) was held under liquid paraffin which was flushed continually with nitrogen to maintain anaerobiosis. A Radiometer type GK 2301 C glass-plus-calomel combination electrode was inserted obliquely into the muscle to a depth of 2 cm. A remote saturated calomel electrode was also arranged in electrical contact with the surface of the muscle via a strip of filter paper 1 cm wide and soaked in saturated KCl. A Radiometer PM 62 pH meter together with a recorder were used for pH measurement and could be connected via a switch either to the remote reference electrode or to the local saturated calomel reference electrode combined in the shank of the glass electrode itself. The latter electrode made contact with the tissue via the usual porous ceramic plug. Figure 4 displays a recording obtained. During the initial part of the trace, the local reference electrode was employed, at *a*, the remote reference was selected instead, and successively at *b*, *c*, *d* and *e* the local, remote, local and remote electrodes respectively, were switched into the circuit. It is evident that use of the local reference electrode caused the pH apparently to decline much more rapidly

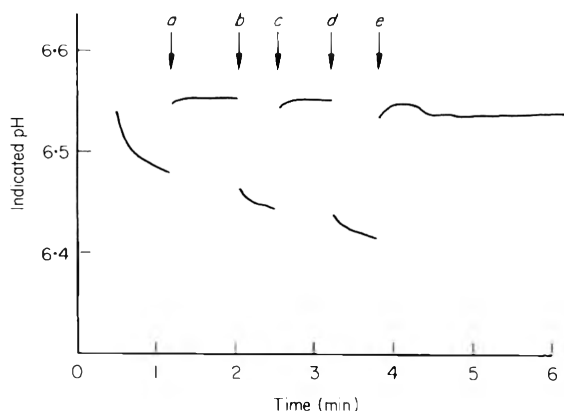

Figure 4. Record of pH measurements in an isolated beef sternomandibularis muscle. The effect of different reference electrode systems; temperature 20°C. See text for further explanation.

than did the remote electrode which presented a large area for liquid/liquid contact with the muscle through the filter paper strip. Other experiments (not shown) indicated that when a liquid junction is made between muscle tissue and saturated KCl in a small fibre or ceramic plug, then the resistance of the plug gradually rises and the liquid junction potential in the plug increases. (The saturated KCl side becomes increasingly negative.) It appears that the junction develops cation selective properties probably due to the precipitation of protein, derived from the muscle, within the pores of the plug. Very low resistance junctions of large area were found to be free from this defect.

Difficulties arise, however, if appreciable amounts of KCl, such as are lost from a very low resistance junction with a saturated KCl solution, come into contact with the muscle tissue. The results of Table 1 illustrate the acceleration of glycolysis (implied by the acceleration of the rate of pH fall) observed when KCl, rather than NaCl bathed the muscle in the vicinity of the glass electrode. This effect may be attributed to the depolarization of the muscle cell membrane by K^+ and the consequent calcium-mediated activation of the contractile apparatus and hence of metabolism (Hodgkin & Horowicz, 1960a,b; Scopes, 1974). The presence of EGTA, to chelate free calcium ions, produced a small decrease in the rate of pH fall by comparison with that observed in 0.1 M NaCl alone. Calcium released from intracellular stores within those fibres damaged by the initial insertion of the measuring electrodes, might be expected to accelerate glycolysis in the damaged fibres (Bendall, 1973). It seems possible that the retardation caused by the presence of EGTA reflects the removal of this activating calcium previously released by slight damage to the muscle during insertion of the electrodes.

Table 1. Rate of pH fall in anaerobic beef sternomandibularis muscle exposed to various saline solutions

Saline	Rate of pH fall (pH hr^{-1}) (mean ± s.e.m.)	No. of measurements
0.1 M NaCl	0.10 (±0.01)	5
0.1 M NaCl + 0.5 mM Na EGTA	0.08 (±0.02)	5
1.0 M NaCl	0.12 (±0.02)	5
0.1 M KCl	0.39 (±0.03)	5
1.0 M KCl	0.41 (±0.03)	5

An isolated beef sternomandibularis muscle was held at 22°C under paraffin oil which was continually bubbled with nitrogen. It was impaled obliquely to a depth of 2 cm with the glass electrode and a 3 M KCl-containing reference electrode tube (see Fig. 3). The region around the glass electrode bulb was irrigated with 0.1 ml of the appropriate saline, rendered anaerobic by previous bubbling with nitrogen. The pH of the saline was permitted to equilibrate with that of the tissue fluid for 10 min and the pH fall was then recorded for either 10 min (KCl-containing salines) or 30 min (NaCl-containing salines). In no case did the pH fall below 6.8.

Abbreviation: Na EGTA, ethyleneglycolbis (aminoethyl ether) -N,N′-tetraacetic acid, brought to pH 7 with NaOH.

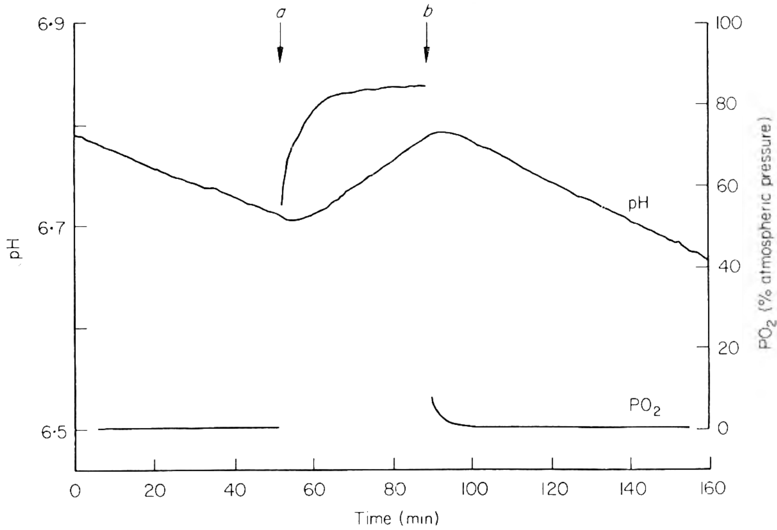

Figure 5. Record of pH and PO_2 in an isolated beef sternomandibularis muscle; temperature 22°C. For further explanation, see text.

In order to render the method generally useful for recording pH in anaerobic muscles, it was necessary to ensure that the introduction of the glass and reference electrodes did not render the interior of the muscle aerobic. This was assessed with the oxygen electrode. In Fig. 5 is recorded an experiment in which an excised beef sternomandibularis muscle, held in air, was impaled obliquely to a depth of 3 cm by three adjacent electrodes, with their tips at the same level, the glass and reference electrodes and the polarographic oxygen electrode cathode. So long as care was taken in making the initial incision, necessary to enable the electrodes to enter the tissue, it was found that the resilience of the muscle caused it to seal around the electrodes and the oxygen tension at the level of the glass electrode bulb, fell to less than 0.2 atmospheric in less than 1 min and to undetectable levels 5 min after the insertion of the electrodes. In the experiment of Fig. 5, a fine glass capillary was inserted adjacent and parallel to the electrodes until its tip was 0.5 cm above the ends of the electrodes. This capillary tube was connected to a source of oxygen. At *a*, a very slow flow of oxygen was started, causing the PO_2 near the glass electrode to rise almost to atmospheric whilst at *b*, the flow was stopped and respiratory activity in the muscle soon consumed the residual oxygen. The simultaneous record of muscle pH shows that the steady fall of pH was interrupted by the aerobic phase and converted into a rise (as lactic acid was consumed). This rise in pH ceased and a fall in pH was initiated when the tissue again became anaerobic.

With the validity of the technique apparently established, experiments were conducted to measure the pH decline in muscles of intact beef sides in the slaughter house. Figure 6 records data obtained from the *longissimus dorsi* muscle of a beef side. A glass-plus-reference electrode assembly was inserted 4 cm into the muscle together with a copper-constantan thermocouple junction.

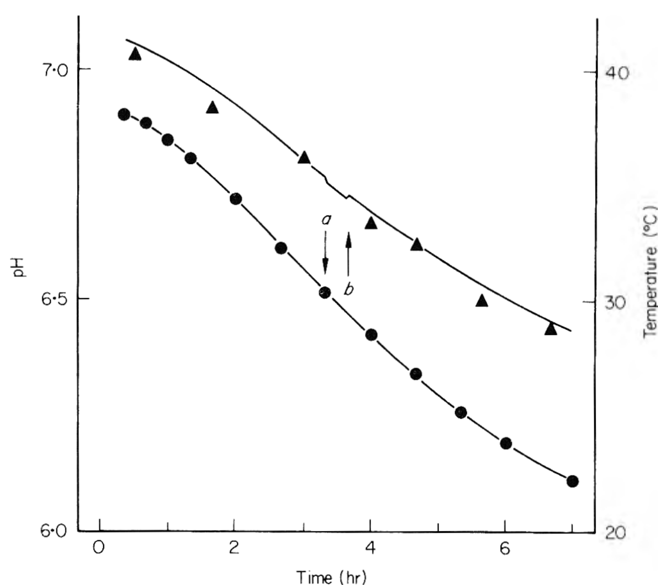

Figure 6. Record of pH fall in the longissimus dorsi muscle of a beef side cooling towards an ambient temperature of 15°C. The pH trace (upper curve) has been corrected for the observed temperature changes as described in the text. ●, temperature measured at the glass electrode with a copper-constantan thermocouple junction; ▲, pH of tissue samples homogenized in KCl plus iodoacetate.

The electrode assembly was lagged with a glass wool and aluminium foil so that the temperature of the electrode shank, remote from the glass membrane (measured with another thermocouple) was never more than 3°C below that measured simultaneously at the glass electrode bulb. For temperature correction of the pH measurements, this latter temperature was used. The pH drop in the musculature was also followed by removing muscle samples from around the electrodes. The fall in pH recorded by the two methods is seen to be quite similar.

At *a*, a potential difference of 9 V was inserted between the common-mode electrode and ground; at *b* it was removed. From the offset in the measured pH produced by this procedure, a common-mode rejection ratio of 84 dB may be calculated for the electrometric system used.

Various small systematic differences might have been anticipated between the discontinuous sampling measurement and the continuous monitor with the glass electrode. Poisoning with iodoacetate is expected to produce a rise in the pH of the homogenate due to the redistribution of glycolytic intermediates. All measurements on homogenates were made at 20°C; the pH of the muscle or of the homogenate would be lower at higher temperature. These effects would tend to produce a higher pH value by sampling (see Bendall (1973) for a detailed discussion). On the other hand, the sampling method is largely a measure of intracellular pH whilst the glass electrode samples extracellular pH. The two different techniques might be expected to yield different results

reflecting the different pH of the two different compartments. It is difficult, *a priori,* to estimate the pH difference between extra- and intracellular compartments in these particular experiments, although the decline in extracellular pH might be expected to lag behind the pH decline intracellularly due to the exceedingly low proton permeability of the muscle cell membrane (Aickin & Thomas, 1975). pH changes measured extracellularly with a relatively large glass electrode might lag behing the intracellular events causing them by as much as several minutes. This possibility is under study.

Conclusions

It appears that the use of the glass electrode for continuous measurements of muscle pH is practicable. The difficulties sometimes encountered with a conventional measuring system are due to inadequacies of the reference electrode and poor common-mode rejection. These difficulties are largely overcome by the present design which provides two highly desirable characteristics, very high common-mode rejection and the ability to use reference electrode systems of very high impedance. The range of common-mode voltages which can be tolerated by such a differential amplifier system is, however, limited (to about ± 12 V in the present case). If greater common-mode voltages were to occur, then a totally different solution, such as an optical link between the input and output circuits, would have to be adopted.

Acknowledgments

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Measurement of internal pressures and tensions in meat during freezing, frozen storage and thawing

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Summary

The stresses developed in the centre of cylindrical samples of beef and pork muscle were measured with a miniature pressure transducer during freezing, storage and thawing. During freezing, internal compression developed at a rate that increased as freezing progressed and most of the pressure was developed after the centre had commenced to freeze. Generally the circumferential tension in the outer surface of the muscle reached breaking point and a shallow crack formed along the length of the muscle, or the surface yielded causing a bulge. The internal stress then fell rapidly and subsequently recovered. The investigation showed that the stresses developed in meat during freezing can reach much higher values than hitherto recorded, a maximum stress of almost 60 bars being obtained in one particular sample. When the frozen muscles were stored at the temperature at which they were frozen the axial compressive stress declined exponentially, while the radial stress, after a brief period of initial decline, generally increased. When the samples were thawed in ambient air the radial and axial stress decreased initially, generally becoming tensile. This was followed by a recovery of the compressive stress which subsequently gradually declined as the temperature of the centre increased.

Introduction

There has been a trend in commerce to freeze foods at increasingly faster rates, in order to increase the throughput of refrigeration plant. The rate at which the temperature can be lowered is governed not only by the rate at which heat is conducted, but also by the mechanical strength of the partially frozen sample because volume changes can cause stresses to build up during freezing, large enough to distort the shape of the product and to crack the surface, effects which render the product less saleable. For example, Lorentzen (1964) found that the freezing of whole sprats in liquid nitrogen gave an unacceptable

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product because the fish burst, with the extrusion of parts of their intestines. Severe and unacceptable cracking also occurred when larger fish were frozen in this way (Lorentzen, 1964; Anon., 1963). Pritchard (1974) reported that his company had found it necessary to restrict their rate of freezing of cuts of meat, due to the splitting of the product when too high a rate was employed.

The first measurements of the pressures developed during the freezing of meat were made by Griffiths & Awbery in 1927, by a method that involved balancing the pressure produced in the meat on one side of a diaphragm with hydrostatic pressure applied on the other side. In freezing a piece of beef of unspecified size at an unspecified temperature, they obtained a typical pressure build up of about 13 bar in 15 hr, before being released by cracking. Lorentzen (1964) using a similar device, measured the pressure development in the thick section of a beef hind during freezing in air at about −36°C. Partial release of the pressure occurred from time to time due to cracking, peak pressures of up to 12 bar being reached.

Kagan & Ukrainets (1970), using a pressure transducer system, measured the pressure at the centre of meat 4–5 cm thick, during freezing in solid carbon dioxide. The pressure was observed to build up rapidly as the centre commenced to freeze, reaching a maximum of about 4 bar at −30 to −35°C and thereafter remaining constant. No cracking was observed. They also measured pressures during blast freezing at −112 to −120°C of hindquarters of beef and sides of beef and pork, measurements being made at depths of 2–8 cm in the region of the gluteus muscles of the hip. Pressures were observed to be greater nearer the surface and at 2 cm depth reached a peak value of 5.5 bar at −25 to −30°C in 1 hr and thereafter remained constant. Coarse cracks occurred in the surface, although no associated releases in pressure were recorded.

Questions that these studies have left unanswered are:

(1) Why is it that high cooling rates cause cracking while slow rates do not?
(2) What happens to the stresses during storage?
(3) How are the stresses affected by storage conditions?
(4) How are the stresses released during thawing?

The object of this investigation was to study the factors influencing the stresses developed during freezing of meat and to determine the changes in the stress pattern during subsequent storage and thawing; simple geometrical shapes were utilized, namely the approximately cylindrical muscles, *M. semitendinosus* and *M. rectus femoris* of pork and beef.

Method

A miniature pressure transducer (Kulite Sensors Ltd, type CQMS–125–1000) of range 0–70 bar and diameter 3 mm was mounted at the end of an insulated stainless steel tube (Fig. 1) and calibrated, using a Budenberg Dead-weight Pressure Tester, in 1 bar steps up to about 60 bar and at 10–15°C

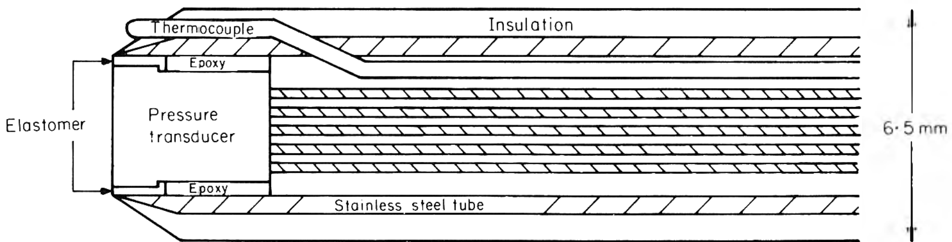

Figure 1. The pressure probe.

intervals in constant temperature coldrooms between 0 and −40°C. The transducer output was found to be slightly temperature dependent and slightly non-linear with pressure. At a constant pressure the maximum change in output over the temperature range concerned was equivalent to 1 bar and the maximum departure from linearity with pressure was 3 bar. Since the transducer zero (output for zero pressure) varied with temperature and time within the range equivalent to ± 2 bar, it was determined for each freezing–storage–thawing cycle from its initial and final values and its pre-determined variation with temperature and time. The total error in pressure measurement including reproducibility, calibration accuracy and uncertainty in the transducer zero was estimated to be about ± 0.5 bar.

Muscles were trimmed of surface fat, the tapered end pieces removed, and stored in a refrigerator at about 5°C. The roughly cylindrical samples ranged from 8 to 20 cm in length and from 3 to 11 cm in diameter (Table 1). The pressure probe was inserted either axially or radially such that the transducer was at the centre of the muscle. The muscle was then wrapped in PVC 'Cling Film' and immersed in a well-stirred constant temperature refrigerated bath containing silicone fluid at the appropriate freezing temperature (−10, −20, −30, −40 or −50°C). Samples were first frozen to the bath temperature and held for 1 to 4 days, before thawing in undisturbed air at ambient temperature (20 ± 5°C). Pressure and temperature variations were recorded on a data logger throughout the freezing–storage–thawing cycle.

Results

Freezing

After an initial period of calm the internal pressure rose at a rate that increased as freezing progressed. Most of the pressure developed after the centre had commenced to freeze (plateau regions of Figs 2 and 3). Generally after a time the pressure was released suddenly due to cracking or bulging of the surface either axially or radially. Cracking occurred along the length of the muscle surface, roughly parallel to the fibres. The time course of the pressure development, the magnitude of the observed stresses and duration of the stress

Table 1. Axial stresses during freezing

Expt no.	Freezing temp. (°C)	Muscle*	Dimensions		Stress at −1°C (bar)	Stress release			Freezing Time† (hr)	Final stress‡	
			Diam. (cm)	Length (cm)		Amount (bar)	Duration (min)	Temperature (°C)		(bar)	Time (hr)
1	−50	P St	6	15	9.3	40.8 to 32.6 = 8.2	3.2	−18.4 to −32.6	0.6	36.6	1.1
2		P St	6	15	7.0	12.7 to 4.4 = 8.3	7.3	− 3.2 to −30.6	0.6	7.6	1.3
3		P Rf	7	10	6.1	14.5 to 14.3 = 0.2	0.8	−12.5 to −15.3	0.8	31.5	1.8
4		P Rf	6.5	10	4.3	16.9 to 15.4 = 1.5	2.1	−18.7 to −25.9	0.5	20.7	1.3
5		P Rf	7	10	8.4	58.8 to 45.0 = 13.8	3.4	−13.5 to −30.9	0.7	50.2	1.3
6		P St	6	15	7.8	13.0 to 8.1 = 4.9	4.5	− 3.6 to −24.2	0.5	12.4	1.2
7		P St	5	15	6.0	19.0 to 15.5 = 3.5	2.2	− 9.2 to −24.6	0.5	21.5	1.2
8		C St	5.5	15	9.5	27.4 to 22.6 = 4.8	3.2	− 8.8 to −28.8	0.5	29.0	1.0
9		C St	3.5	11	1.8	24.1 to 21.5 = 2.6	1.3	− 8.7 to −23.4	0.3	28.5	0.6
10		B St	11	20	1.5	0	—	—	1.5	32.1	3.5
11	−40	C St	4.5	15	5.3	0	—	—	0.5	28.5	0.8
12		C Rf	6.5	10	7.8	36.7 to 26.7 = 10.0	6.5	4.7 to 29.6	0.8	28.0	1.3
13		P St	5.5	15	3.5	12.2 to 11.6 = 0.6	3	−10.9 to −22.2	0.6	16.2	1.1
14		P Rf	7	10	6.4	17.4 to 10.7 = 6.7	5.5	− 4.7 to −23.9	0.8	14.4	1.3
15		C Rf	4.5	8	2.4	3.6 to −1.3 = 4.9	3.9	− 2.8 to −14.5	0.3	8.9	0.7
16		B St	9	20	9.6	(25.2 to 25.0 = 0.2	2.3	− 5.5 to − 8.6)	1.4	28.9	2.2
						(26.9 to 26.0 = 0.9	3.5	−18.1 to −24.3)			

17	−30	P Rf	6.5	10	0.9	8.6 to 5.4 = 3.2	4.5	−13.2 to −18.0	0.8	12.8	1.4
18		P St	5.5	12	0.8		—	—	0.7	16.9	1.2
19		B St	8	20	3.8	(11.1 to 10.8 = 0.3	2.3	− 9.5 to −11.6)	1.6	12.9	2.5
						(11.1 to 11.0 = 0.1	1	−15.1 to −16.1)			
20		P St	5.5	15	1.0	12.9 to 10.7 = 2.2	3	−11.5 to −17.4	0.7	15.7	1.2
21		P Rf	7	11	2.6	10.4 to 8.4 = 2.0	4.4	− 3.3 to −14.6	1.1	11.3	1.7
22		C Rf	4.5	8	2.0	9.7 to 6.8 = 2.9	4.4	− 7.1 to −21.7	0.4	7.1	0.6
23	−20	P Rf	7	10	0.5	3.5 to −0.5 = 4.0	10	− 4.1 to −10.2	1.5	3.6	2.5
24		P St	5.5	14	0.4	0	—	—	1.1	9.2	1.5
25		P Rf	6.5	9	0.6	11.4 to 9.6 = 1.8	1.7	− 3.9 to −6.9	1.4	12.8	1.8
26		P St	5	13	0.2	0	—	—	1.0	7.8	1.4
27		C Rf	5	10	0.3	5.6 to 2.4 = 3.2	14.7	− 5.1 to −14.5	0.9	4.7	1.4
28		B Rf	10	18	1.5	9.9 to 9.5 = 0.4	5.1	− 5.8 to −8.0	3.0	10.6	3.6
29	−10	P Rf	6.5	10	0.1	2.4 to 2.2 = 0.2	6	− 1.9 to −3.7		2.3	3.2
30		P St	5	14	0.0	0	—	—		2.3	2.9
31		P Rf	7	10	0.1	0	—	—		3.9	3.5
32		P St	5.5	13	0.1	0	—	—		2.0	2.6
33		C St	3	11	0.0	0	—	—		2.8	1.4
34		B St	9	20	0.2	0	—	—		2.8	7.5

*P, indicates pork; B, beef; C, calf; St, *M. semitendinosus*; Rf, *M. rectus femoris*.
† Time taken for centre to reach −10°C.
‡ Centre temperature within 1°C of freezing temperature.

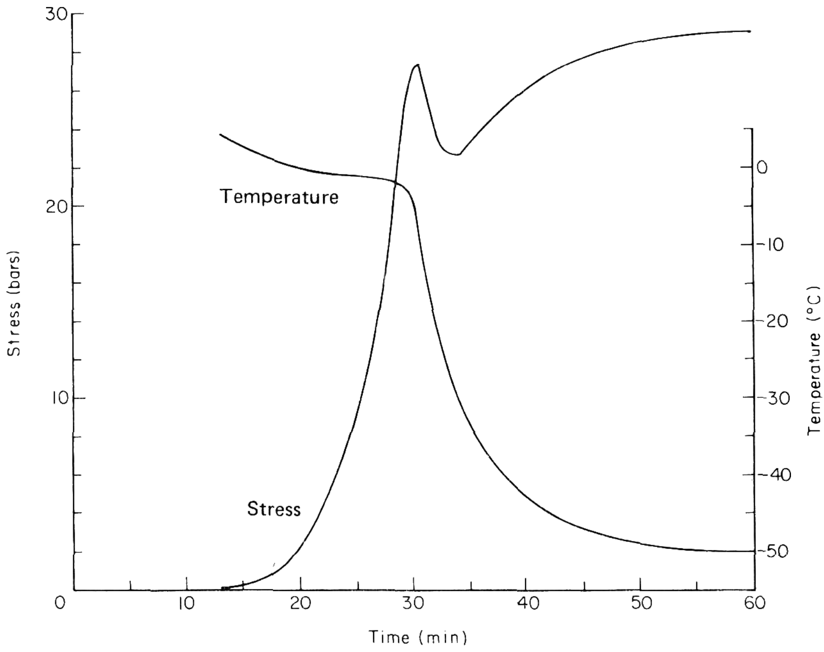

Figure 2. Axial stress during freezing, experiment 8.

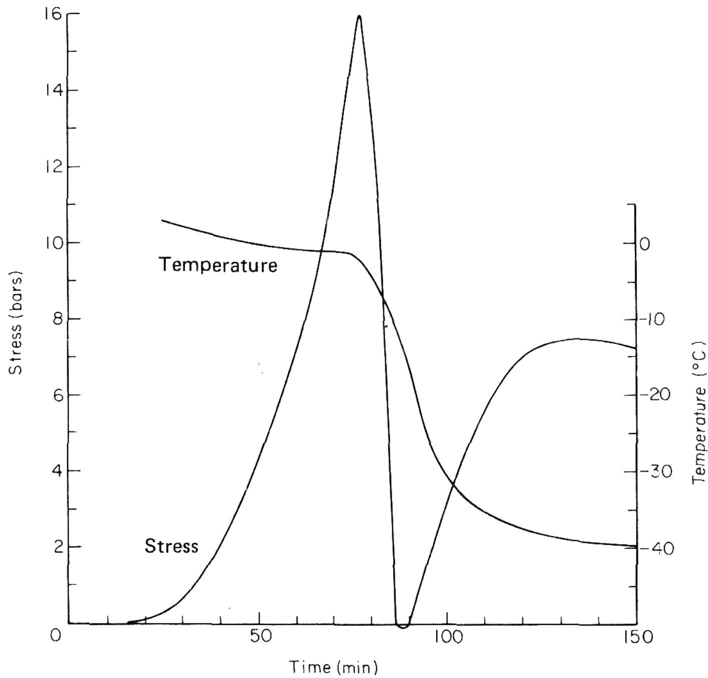

Figure 3. Radial stress during freezing, experiment 38.

Table 2. Radial stresses during freezing of beef semitendinosus samples of length 18 cm and diameter 9–10 cm

Expt no.	Freezing temperature (°C)	Stress at −1°C (bar)	Stress release			Freezing time (hr)	Final stress	
			Amount (bar)	Duration (min)	Temperature (°C)		(bar)	Time (hr)
35	−50	2.4	6.3 to −13.3 = 19.6	18.4	−7.1 to −19.5	0.9	1.7	2.4
36		0.9	2.2 to − 0.4 = 2.6	33.2	−2.5 to −28.3	1.2	13.4	2.8
37	−40	2.2	5.3 to − 0.5 = 5.8	7.3	−3.7 to − 7.8	1.8	−0.6	3.3
38		6.5	15.9 to 0 = 15.9	11	−2.4 to −12.4	1.4	7.4	2.3
39		7.6	24.5 to 10.0 = 14.5	7.5	−4.6 to −14.4	1.4	28.2	2.6
40		2.1	4.7 to 0.3 = 4.4	19.7	−1.8 to −18.7	1.3	6.8	2.3
41	−30	2.1	8.2 to 2.3 = 5.9	77	−4.0 to −29.3	2.1	2.3	3.2
42		0.4	(0.4 to − 0.3 = 0.7	22.2)	−0.9 to − 5.4	1.8	−0.5	4.5
			(0.1 to − 0.5 = 0.6	49)	−17.4 to −23.8			
43		1.2	(6.0 to 0.1 = 5.9	14.7)	− 4.9 to −14.7	1.5	−1.1	2.8
			(0.5 to − 1.1 = 1.6	73)	−18.8 to −29.4			

releases varied considerably between apparently similar samples frozen under identical conditions (Tables 1 and 2). Axial stress release was observed in only one of the samples frozen at −10°C, although it was found in the majority of the samples frozen at the lower temperatures. On the whole, the magnitude of the internal stresses was greater the lower the freezing temperature, as was the degree of surface cracking and distortion and resulting release in stress (Table 3). There appeared to be no correlation between the dimensions of the samples and the degree of cracking. Although the degree of distortion could not readily

Table 3. Stresses and degree of cracking during freezing

Freezing temperature (°C)	Stress releases				Maximum stresses				Degree of cracking	
	Axially		Radially		Axially		Radially		Total length (cm)	Depth (mm)
	mean	S.D.	mean	S.D.	mean	S.D.	mean	S.D.		
−10	0	0.1	—	—	2.7	0.7	—	—	0	0
−20	1.6	1.7	—	—	8.3	3.3	—	—	0	0
−30	1.8	1.3	4.9	3.2	13.2	2.7	4.9	4.0	0–2	0–2
−40	3.9	4.0	10.2	5.9	22.8	10.3	14.0	10.5	0–5	0–15
−50	4.8	4.4	11.1	12.0	28.9	13.7	9.9	5.0	0–11	0–5

be put into numerical terms, it was obviously greatest at the lower temperatures i.e. −40 and −50°C. The maximum internal stress occurred either as the temperature approached the freezing temperature, or just prior to a large stress release, after which the stress recovered only partially. Generally, stress releases were higher radially than axially and greater stresses were attained axially than radially.

Storage

During storage axial stress decreased, becoming exponential generally within 0–6 hr. Generally, observations were continued until the stress became steady or nearly so; if the latter, the asymptote was obtained by the method of Mangelsdorf (1959). Results are tabulated in Table 4. It can be seen that the higher the freezing temperature the lower the decay constant, i.e. the faster the decay. Also decay tended to be slower the longer the muscle, although the correlation was low. An Arrhenius plot of the decay constants showed that the decay had an activation energy of 26.9 ± 4.8 kJ mole^{-1}. The correlation coefficient was 0.73.

Radial stresses varied in a different way (Fig. 4, Table 5). At the commencement of storage, radial stress tended to be much lower than axial stress. It

Table 4. Axial stresses during storage

Expt no.	Freezing temperature (°C)	Initial stress (bar)	Decay constant (hr)		Asymptote (bar)
5	−50	50.2	25.8		25.6
6		12.4	22.9		− 7.1
7		21.5	120	mean = 40.6	− 4.1
8		29.0	21.2	S.D. = 40.3	−22.5
9		28.5	10.6		15.6
10		32.1	42.8		− 2.9
11	−40	28.5	23.8		4.3
12		28.0	18.3		0.1
13		16.2	14.5	mean = 15.2	6.0
14		14.4	11.1	S.D. = 5.9	− 1.9
15		8.9	6.7		− 0.9
16		28.9	16.6		−25
17	−30	12.8	7.5		6.6
18		16.9	7.8		7.0
19		12.9	12.2	mean = 7.5	4.2
20		15.7	7.9	S.D. = 2.9	6.0
21		11.3	3.4		7.3
22		7.1	6.2		0.3
23	−20	3.6	13.0		2.8
24		9.2	6.3		4.8
25		12.8	5.8	mean = 9.9	6.5
26		7.8	11.2	S.D. = 7.5	3.3
27		4.7	0.8		3.9
28		10.6	22.4		5.0
29	−10	2.3	0.7		1.5
30		2.3	2.0		0.7
31		3.9	6.4	mean = 3.5	1.3
32		2.0	2.0	S.D. = 2.2	0.6
33		2.8	4.6		0.6
34		2.8	5.0		0.5

decreased initially, i.e. became less positive (compressive) or more negative (tensile), reached a minimum value and then, after a time, generally increased, approaching equilibrium exponentially, with a time constant similar to that for axial stress decay.

Thawing

During thawing, axial and radial stresses varied in a similar manner (Figs 5 and 6, Tables 6 and 7). Initially the stresses decreased, generally becoming

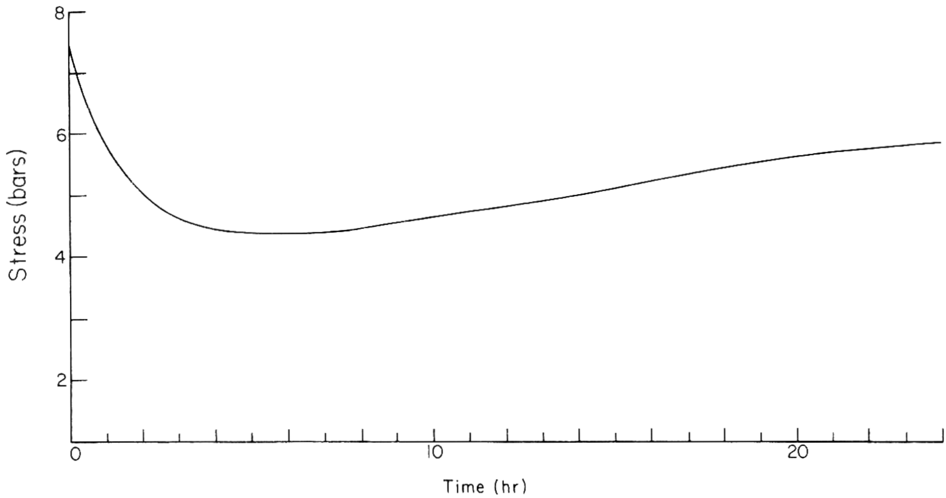

Figure 4. Radial stress during storage, experiment 38.

tensile, then they increased becoming compressive, and finally decreased to zero on the completion of thawing.

Discussion

During the initial stages of freezing the shell of frozen tissue formed at the surface of the muscle grew inwards compressing the unfrozen core and causing the internal pressure to rise; this in turn pushed the frozen shell outwards stretching it circumferentially. Consequently circumferential tension developed in the outer surface of the frozen shell while near the inner boundary it was in

Table 5. Radial stresses during storage

Expt no.	Freezing temperature (°C)	Initial stress (bar)	Minimum stress		Increasing stress period	
			(bar)	Duration (hr)	Time constant (hr)	Asymptote (bar)
35	−50	1.7	0.9	2.5 to 5	25.1	3.0
36		13.4	6.9	8.5 to 11	16.9	11.9
37	−40	−0.6	−1.0	2.5 to 3.5	13.5	−0.1
38		7.4	4.5	3.5 to 8	24.0	7.4
39		28.2	27.8	1.5 to 3	18.9	32.0
40		6.8	4.3	14 to >45	—	—
41	−30	2.3	1.0	12 to >60	—	—
42		−0.5	−0.5	0 to 1.5	15.7	0.3
43		−1.1	−1.4	11 to >90	—	—

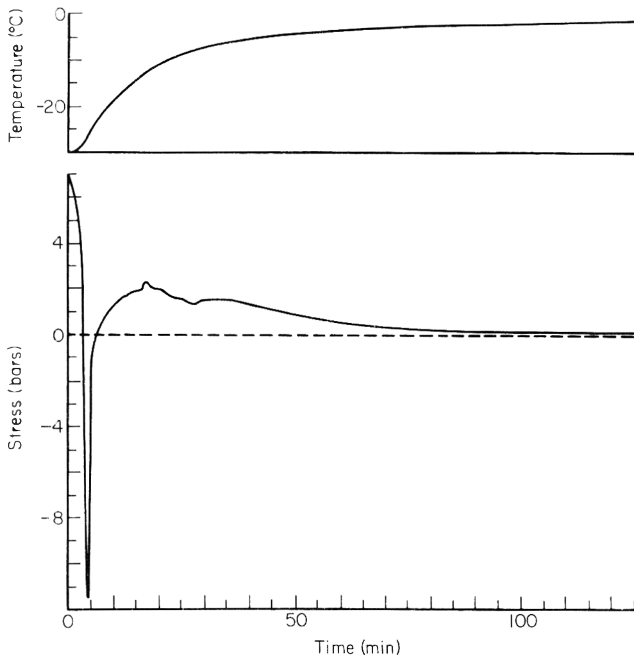

Figure 5. Axial stress during thawing, experiment 18.

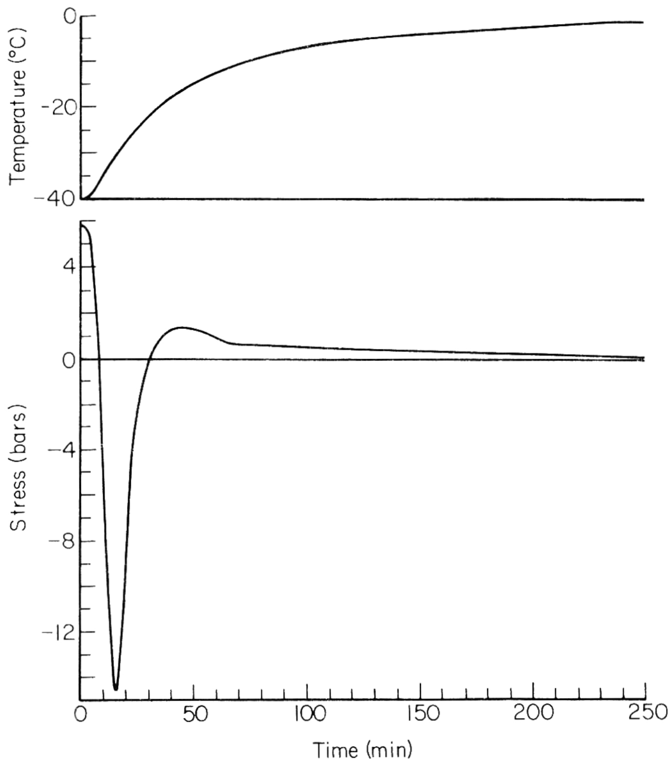

Figure 6. Radial stress during thawing, experiment 38.

Table 6. Axial stresses during thawing

Expt no.	Initial Temp. (°C)	Initial Stress (bar)	Minimum stress (bar)	Minimum stress Time (min)	Minimum stress Temp. (°C)	Maximum stress (bar)	Maximum stress Time (min)	Maximum stress Temp. (°C)
1	−50	34.6	5.8	10	−32.0	11.7	16	−22.1
2		7.6	−9.2	7	−42.0	1.2	37	−11.7
3		31.5	−6.2	12.5	−27.9	2.2	29	−12.1
4		20.7	1.6	15.5	−27.1	3.9	23	−18.8
5		27.2	−1.3	9.5	−36.2	11.9	19.5	−20.3
6		−0.6	−3.1	3.5	−46.7	2.3	25.5	−11.9
7		7.7	−8.9	1.5	−49.2	2.7	21	−11.8
8		−1.4	−2.1	1.5	−50.0	5.2	22.5	−14.6
9		17.0	−0.5	7.5	−29.5	2.9	10	−20.6
10		11.1	−19.5	8	−47.9	6.3	64	−16.5
11	−40	4.4	−24.6	3.5	−35.4	3.0	12.5	−19.2
12		1.2	−13.4	8	−31.7	6.8	20	−18.3
13		6.2	−8.3	9	27.1	0.6	34	−7.7
14		0.2	−22	7	−33.3	8.8	20	−17.0
15		−0.9	−6.5	5.5	−30.4	1.1	14	−13.9
16		−25	−33.1	3.5	−39.8	9.2	47.5	−15.5
17	−30	6.8	0	17	−16.1	0.1	54	−5.3
18		7.0	−11.7	4.5	−25.7	2.3	16.5	−12.9
19		5.3	−2.1	12	−23.8	2.5	27.5	−15.8
20		6.6	−4.6	7	−23.7	3.7	26	−9.8
21		7.3	1.2	11	−21.3	2.3	28	−11.9
22		0.3	−3.9	3	−27.8	2.3	9	−16.6
23	−20	2.8	1.7	3	−19.9	3.3	8	−16.5
24		4.9	−0.1	34	−5.8	0.2	55	−3.9
25		6.6	5.0	3.5	−19.8	5.6	5.5	−18.4
26		3.3	0	6.5	−16.0	0.4	20	−8.8
27		3.9	−0.6	22	−8.1	0.2	32	−2.1
28		5.0	2.4	5.5	−20.0	6.5	29	−13.7
29	−10	1.5	1.1	1.5	−9.7	1.5	4.5	−9.5
30		0.7	−0.5	12	−7.0	−0.2	67	−1.3
31		1.3	1.3	2	−10.1	2.4	6.5	−9.6
32		0.6	0	7.5	−8.6	0.2	15	−7.2
33		0.6	−0.8	7.5	−7.4	0	80	−1.7
34		0.9	0.7	3	−9.3	1.0	10	−9.0

a state of circumferential compression caused by the expansion of the water as it froze. To maintain mechanical equilibrium the radial compressive stress in the shell increased inwards from its surface value of zero (to be in equilibrium with the environment) to an internal value equal to the internal pressure (for equilibrium with the core). The stress therefore varied from point to point, was anisotropic and could be resolved into two components, one representing a

Table 7. Radial stresses during thawing

Expt no.	Initial		Minimum stress			Maximum stress		
	Temp. (°C)	Pressure (bar)	(bar)	Time (min)	Temp. (°C)	(bar)	Time (min)	Temp. (°C)
35	−50	2.3	−0.3	4.5	−47.1	1.4	13	−37.6
36		11.0	−11.5	11.5	−41.2	1.2	52	−15.4
37	−40	0	−1.5	9.5	−36.5	0	18	−30.6
38		5.8	−14.5	15.5	−30.8	1.4	42.5	−17.3
39		30.2	−4.4	24	−23.9	3.9	50.5	−13.5
40		4.2	−9.7	14	−28.3	2.1	65.5	−7.9
41	−30	1.0*	1.8	20	−21.9	3.0	46.5	−13.8
42		0	−0.5	9	−25.5	0.4	40	−13.3
43		−1.5	−6.7	7	−27.3	0	15	−20.4

*Increased to 2.7 bar at −26.0°C during initial 11.5 min.

hydrostatic pressure, the other representing the shearing stresses (cf. King & Fletcher's (1973) analysis of a freezing water drop).

The growth of internal pressure depended on the ratio of the volume of ice formed to the total volume of the unfrozen core and on the resistance of the frozen shell to stretching. Consequently the growth of pressure was not appreciable until a substantial amount of the muscle was frozen. Once established, the stress at the centre rose at a rate which increased as the freezing progressed, (e.g. Figs 2 and 3) the unfrozen core growing smaller, the frozen shell thicker. Eventually the circumferential tension in the outer surface reached breaking point and the surface cracked along a line normal to the direction of maximum stress or yielded causing a bulge in the surface. Since the circumferential stress was tensile on the surface while compressive at the centre there must have been a region of zero circumferential stress at some radial position. Consequently, cracks were superficial and did not penetrate deeply into the muscle (Table 3).

While the internal stresses were being created they were also tending to relax. The relaxation was measured directly at the end of the freezing process when muscles were held at constant temperature over a period of days. Apparently similar samples, frozen and stored under identical conditions, showed considerable variation in their relaxation times (Table 4). Nevertheless there was a strong temperature dependence with mean relaxation times ranging from around 40 hr at −50°C to about 4 hr at −10°C. During freezing the relaxation must have been faster than the corresponding storage relaxation because of the higher temperature effective during the freezing process.

If the freezing took place within a period of time much shorter than the characteristic time of the relaxation, then little relaxation could occur during the course of freezing and large internal stresses could develop. Relaxation

occurred subsequently during storage. On the other hand, if the characteristic time of the relaxation was comparable with the time for freezing then an appreciable relaxation occurred during the course of the freezing process itself and the observed stress was less than that occurring at higher freezing rates. Similarly if the characteristic time of the relaxation was much shorter than the time taken to freeze, the observed stress was small (see Fig. 7).

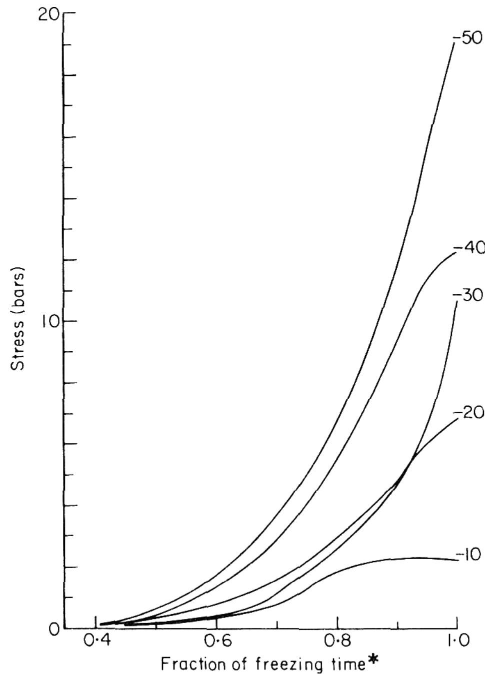

Figure 7. Initial stress development in pork semitendinosus during freezing, experiments 7, 13, 20, 24, 30. *Time for centre to reach -10°C.

Hence the development of large internal stresses could be inhibited by either freezing at a higher temperature or reducing the freezing rate by freezing in stages, both resulting in greater stress relaxation and consequently reduced stress development and risk of cracking.

It is emphasized that there was considerable variability in the time constants for the stress relaxation of apparently similar samples frozen under identical conditions, and the cause of the decay is not fully understood. The experiments gave some clues.

(a) The time constants for the decline in axial stress and radial increase were similar under comparable conditions and it is possible that a single process caused both.

(b) The activation energy of the axial decay, 26.9 ± 4.8 kJ mole^{-1} contrasts with that for the creep of ice, 50–85 kJ mole^{-1} at similar pressures and temperatures (Barnes, Tabor & Walker, 1971) but equals, within the experimental uncertainty, that of the viscosity of supercooled water, 28.4 kJ mole^{-1},

calculated from Hallett's (1963) measurements between −10 and −24°C. The activation energy of the diffusion coefficient, D_2, of water in frozen fish muscle at low levels of hydration, 37.3 ± 2.1 kJ mole^{-1} (Kent & Jason, 1975) is higher than that of the axial stress decay.

(c) The path lengths for the diffusion of water that correspond with the mean time constants of Table 4, range from 0.2 to 0.4 mm, typical of the dimensions of ice crystals and their separation (calculations made for linear diffusion using Kent & Jason's (1975) data for D_2 in fish muscle).

(d) The gradual accretion of ice that was observed in frozen fish muscle by Kent (1975) and Kent & Jason (1975) occurred at a much slower rate than the relaxations of mechanical stress reported in this paper. It therefore follows that if the accretion of ice in mammalian muscle occurs at the same rate as observed in fish muscle then the accretion of ice was neither produced by nor caused the relaxation of internal stress.

The rapid fall in the central compressive stress during the initial stages of thawing occurred very rapidly, too quickly to be caused by the significant transfer of heat into the interior of the muscle (see for example Figs 5 and 6, Tables 6 and 7) and the cause must have been the heating of the surface. The time course of the stress release and its subsequent partial recovery is very similar to that observed when the surface cracked or yielded suddenly during freezing (compare the sudden releases of pressure in Figs 2 and 3 with those shown in Figs 5 and 6) and it is probably a similar catastrophic cracking or yielding of the surface under the action of the partial melting of a proportion of ice in the outer tissue that caused the rapid decline and subsequent partial recovery of internal compressive stress. The final decline of the residual stress mirrored the increase in centre temperature (Figs 5 and 6), the consequent reduction in size of the frozen core and with it the ability to withstand internal compression.

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Storage studies of freeze dried lemon crystals

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Summary

The practical shelf life and storage stability of freeze dried pure lemon crystals was studied with a view to possible application of the results in freeze drying of lemon products. The crystals were extremely hygroscopic and exhibited a high tendency to caking on storage at ambient temperature, while under refrigeration they preserved their free flow property and dissolved promptly both in hot and in cold water. With regard to browning deterioration, it was found that low temperature is a decisive factor in its prevention, while low water activity is not enough in itself.

Contrary to the effects of temperature and water activity upon the browning deterioration, almost no effect was observed due to inert atmosphere packaging.

Browning deterioration at adverse conditions was proceeded by a steep rate of carbon dioxide evolution, thus suggesting that in dehydrated food products, carbon dioxide evolution rates at the lag period could be used as an early indicator for the length of the lag period and the intensity of browning yet to be developed in the course of storage.

Introduction

The shelf life of citrus powders is reduced by water absorption, caking and non-enzymatic browning. The first two effects are favoured by the amorphous structure of the sugars, resulting from the dehydration process, and are mainly controlled by composition, storage temperature and water activity (Makower & Dye, 1956; Notter, Taylor & Downes, 1959). The third effect is associated with the Maillard reaction between reducing sugars and amino compounds, acid caramelization of the sugars, and degradation of ascorbic acid (Eskin, Henderson & Townsend, 1971). These deterioration reactions were observed in dehydrated model systems and dried citrus products even at low water activity (Karel & Nickerson, 1964; Karel & Labuza, 1968; Shaw *et al.*, 1970) corre-

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sponding to a water content below the monolayer. In addition, the browning of citrus products is accompanied by development of off flavour and evolution of carbon dioxide and similarly to caking, it is strongly affected by storage temperature (Shaw *et al.*, 1970) and water activity.

Domestic use of lemons and lemon juice is mainly as flavouring additives, and as such they are consumed in small quantities the year round. In these circumstances they can be conveniently and economically replaced by a dehydrated product. However, while extensive research has been devoted in this respect to orange and grapefruit juice* there is only scanty information on lemon juice, following the unsuccessful attempt to freeze-dry it during World War II (Harper & Tappel, 1957). The present study was undertaken accordingly on freeze-dried pure lemon crystals, with a view to possible application of the results in freeze drying of lemon products.

Materials and methods

Materials

Lemon concentrate. Freshly made frozen concentrate from local industry (30° Bx, 22% w/w acidity expressed as anhydrous citric acid).

Constant water activity. Conditions were obtained by using the following saturated solution (Rockland, 1960): $0.0\,a_w$ – P_2O_5 (powder); $0.06\,a_w$ – NaOH; $0.11\,a_w$ – LiCl; $0.22\,a_w$ – KAc.

Methods

Preparation of lemon crystals. The concentrate was diluted with distilled water to 20° Bx, stored overnight at –20°C in a deep-freeze cabinet, subcooled with liquid nitrogen, granulated and screened (2–5 mm), and evenly spread ($5\,kg\,m^{-2}$) on the trays of a $2.5\,m^2$ Grenco RU-25 pilot freeze drier. Freeze drying was carried out by two sided radiation plates (kept at 45°C) for 10 hr under 0.1–0.01 torr with the condenser temperature being kept at 45–50°C below zero); the product was further dried over P_2O_5 under vacuum at 15°C for 48 hr.

Experimental procedure

The samples were prepared in a low humidity room (20°C, 30% R.H.) to minimize water absorption.

*Vacuum puff drying (Strashun & Talburt, 1954), foam drying (Bissett *et al.*, 1963) and freeze drying (Monzini & Maltini, 1969) were found suitable for this purpose, but industrial application is still limited at present, mainly because of high production costs, low bulk density of the products and storage stability problems.

(*a*) *Sorption isotherms.* The sorption pattern was determined gravimetrically by a Model RG Cahn Gram Electrobalance (Ventron Corp., Paramount, U.S.A.), installed in a vacuum system. A constant water-activity level (range 0.02–0.6) was obtained and maintained by connecting the evacuated system (10^{-3} mm Hg) to a thermostatically controlled (±0.05°C) air free flask containing distilled water. The weight gain of thoroughly dehydrated samples (0.1–0.2 g) was continuously monitored to equilibrium.

(*b*) *Browning.* Five-gram samples in small open glass jars were placed in air-tight desiccators (one half of them evacuated thrice and flushed with pure nitrogen; flushing was repeated following withdrawal of a sample) over the constant water-activity medium. The desiccators were stored in thermostatically controlled walk-in storage rooms (at 4, 25 and 35°C respectively), and samples were removed periodically for examination. Each sample was dissolved in distilled water to a final volume of 100 ml, and browning was determined in a clarified juice extract at 420 nm (Curl, 1949).

(*c*) *Headspace composition.* Fifty-gram samples were placed in No. 2 cans, equilibrated at the selected constant water activity levels; machine sealed and stored at 4, 25 and 35°C. The machine sealed cans were equipped with short copper tubing (10 mm x ¼ in. I.D.), carrying a rubber septum (Hamilton ¼ in. x 8 mm) and soldered to the inside of the previously centre punched cover. (In the case of nitrogen packing the sealed cans were evacuated and nitrogen flushed by means of a syringe needle inserted through the septum). Gas samples of 0.5 ml were withdrawn with a gas tight Hamilton syringe and injected into a Unigraph TC-406 gas chromatograph (Becker, Delft, Holland) equipped with dual thermal conductivity detectors. Copper columns (8 ft x ⅛ in.) filled with

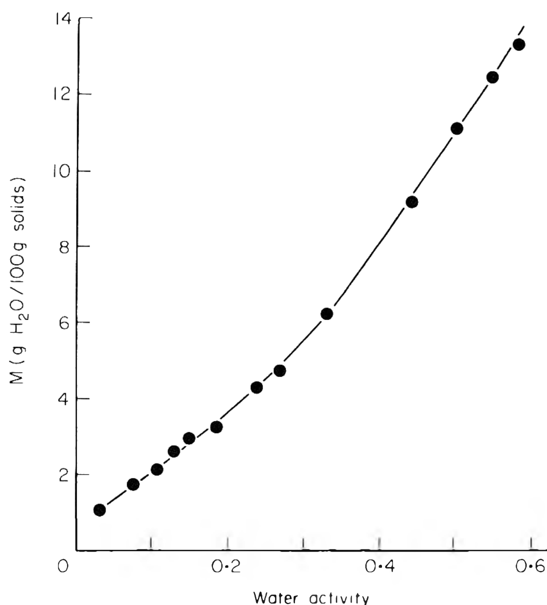

Figure 1. Water sorption isotherm of freeze dried lemon crystals at 25°C.

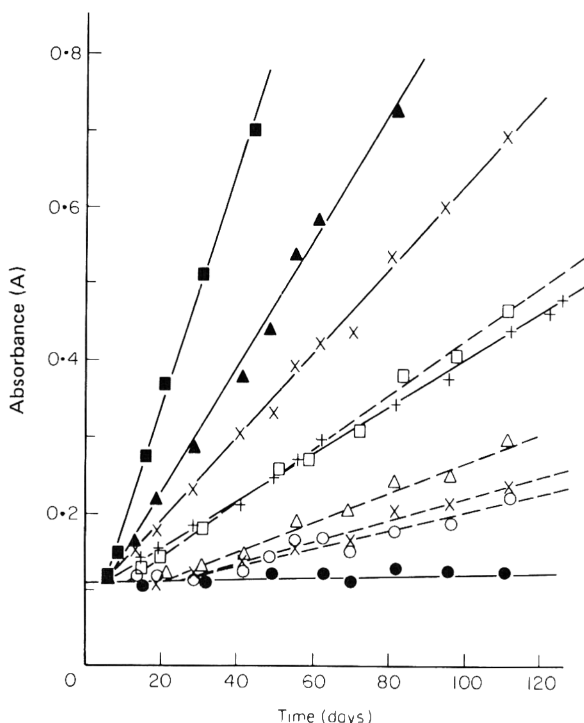

Figure 2. Effect of storage temperature and water activity on browning development in air packed lemon crystals. ●——● 4°C, a_w = 0–0.22; ○– – –○ 25°C, a_w ~ 0; X– – –X 25°C. a_w = 0.06; △– – –△ 25°C, a_w = 0.11; □– – –□ 25°C, a_w = 0.22; +——+ 35°C, a_w ~ 0; X——X 35°C, a_w = 0.06; ▲——▲ 35°C, a_w = 0.11; ■——■ 35°C, a_w = 0.22.

Porapak Q80/100 mesh ('Waters' Inc., U.S.A.) were used for carbon dioxide separation. The carrier gas was helium with flow rate 20 ml/min; temperature of columns and detectors, 35°C; filament current, 200 mA.

Results and discussion

Hygroscopicity, caking and sorption properties

The crystals were obtained as uniform, free flowing particles with a low bulk density of about 0.2 g cm^{-3}. The crystals were extremely hygroscopic and tended to stick even after short exposure to ambient conditions (25°C and 60% R.H.). Fresh crystals dissolved promptly both in hot and in cold water. On storage, caking was observed in all samples at 25 and 35°C, even at the lowest (near zero) water activity.

The sorption isotherm at 25°C is given in Fig. 1. It may be noted that even at < 0.2 water activity the moisture content has an equilibrium value of 3%, which favours caking. The isotherm does not follow the B.E.T. equation and is

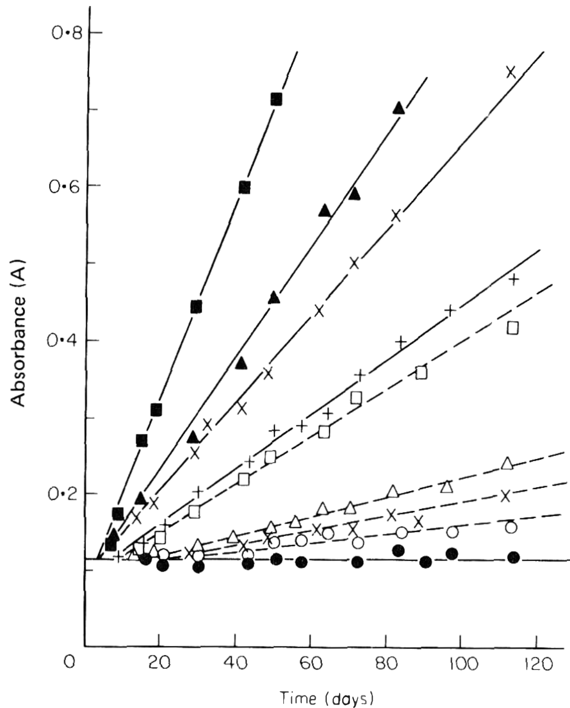

Figure 3. Effect of storage temperature and water activity on browning development in nitrogen packed lemon. Key as in Fig. 2.

similar, in this sense, to dehydrated foods rich in low molecular weight sugars and acids (Salwin, 1962).

Browning

The browning *v.* time curves (Figs 2 and 3) show a similar pattern in all versions, namely a lag interval with no appreciable increase in absorbance, followed by change at a linear rate. Similar patterns were also reported for other citrus products, such as dehydrated orange crystals (Karel & Nickerson, 1964; Meydav, 1975).

Storage temperature was found to have a pronounced effect on both the lag time and rate of change. At 4°C no browning occurred over 4 months, irrespective of the water activity level. At the higher temperatures (35 and 25°C) the lag time was reduced to a few days and several weeks respectively, the ratio being approximately 4–5 for all water activity levels and atmospheres. The effect on the rate of change was similar, with ratios of 3–4 and 5–6 for the air and nitrogen packed crystals respectively.

Table 1 shows that both the lag time and rate of change are also affected by the water activity level, the former decreasing and the latter increasing with it.

Table 1. Effect of temperature and water activity on lag interval and browning rates in air packed lemon crystals

Water activity	Lag time (days)		Browning rate (A/day × 10^3)	
	25°C	35°C	25°C	35°C
0	30	6	1.17	3.2
0.06	25	5	1.42	5.5
0.11	20	5	1.90	8.2
0.22	10	4	3.50	15.2

Table 2. Effect of temperature and water activity on lag interval and browning rates in nitrogen packed lemon crystals

Water activity	Lag time (days)		Browning rate (A/day × 10^3)	
	25°C	35°C	25°C	35°C
0	30	7	0.62	3.2
0.06	25	5	1.00	5.7
0.11	20	5	1.25	7.3
0.22	10	4	2.75	12.5

Significantly, a low level by itself does not prevent browning unless temperature conditions are also favourable. Contrary to the effects of temperature and water activity upon the browning deterioration, almost no effect was observed due to inert atmosphere packaging (Table 2).

Carbon dioxide evolution

Non-enzymatic browning reactions are accompanied by carbon dioxide evolution, either due to Strecker degradation of amino compounds or due to deterioration of ascorbic acid (Eskin, Henderson & Townsend, 1971). Therefore an attempt was made in this investigation to characterize the time pattern of the browning deterioration of dry lemon crystals by the carbon dioxide evolution during storage (Figs 4 and 5). It can be seen that these two time curves are similar in pattern, namely a relatively high evolution rate at the initial period, gradually decreasing to a lower steady rate thereafter. Generally speaking, the carbon dioxide content in the headspace is in correlation with browning intensity and increases steeply under conditions normally favouring rapid browning (high temperature and/or high water activity); this steep increase can be detected already during the early browning period, and apparently can be used as an early predictor for the length of the lag period and the intensity of browning yet to be developed in the course of storage.

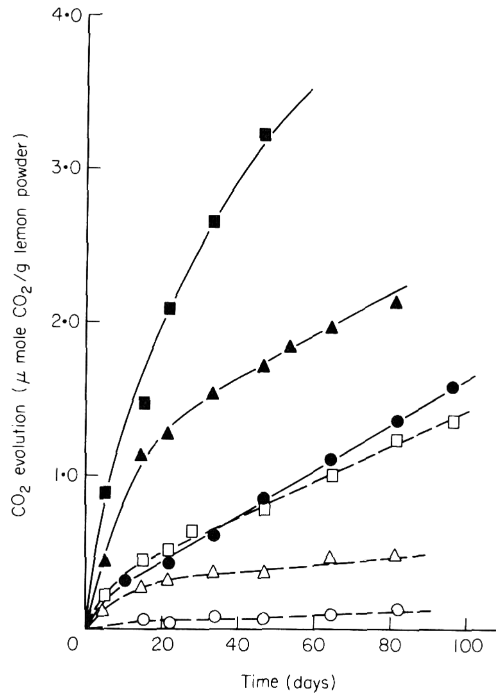

Figure 4. Carbon dioxide evolution in air packed lemon crystals. ○– – –○ 25°C, $a_w \sim 0$; △– – –△ 25°C, $a_w = 0.11$; □– – –□ 25°C, $a_w = 0.22$; ●——● 35°C, $a_w \sim 0$; ▲——▲ 35°C, $a_w = 0.11$; ■——■ 35°C, $a_w = 0.22$.

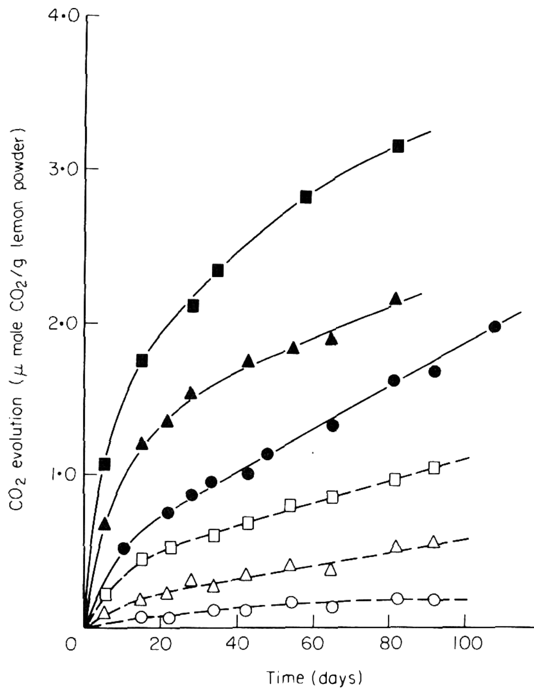

Figure 5. Carbon dioxide evolution in N_2-packed lemon crystals. Key as in Fig. 4.

Further studies applying this approach for the prediction of browning in dry food systems are at present being undertaken.

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Time/temperature relationship for thermal inactivation of pectinesterase in mandarin orange (*Citrus reticulata* Blanco) juice

NIRANKAR NATH AND S. RANGANNA

Summary

The values for thermal inactivation of pectinesterase in mandarin orange juice were $F_{197.5}^{19.4} = 1.00$ and $D_{197.5}^{20.6} = 0.54$ min at pH 3.6 and $F_{201.5}^{17} = 1.00$ and $D_{201.5}^{18.2} = 0.438$ min at pH 4.0. The *F* values were equivalent to 1.85 *D* at pH 3.6 and 2.28 *D* at pH 4.0. A 2 *D* process at pH 3.6 and 2.5 *D* process at pH 4.0 are recommended in the commercial pasteurization of juice.

A viscosity procedure described is more sensitive than the jelly test for determining the thermal inactivation of pectinesterase.

Introduction

Mandarin oranges are mostly processed in the form of juice, concentrate and segments. Generally, the pH of orange is lower than 4.0, and spoilage is caused by non-spore forming bacteria, e.g. *lactobacilli*, or by yeasts whose thermal resistance is lower than the enzyme pectinesterase (PE) naturally present (Evans, King & Bartholomew, 1949; Murdock, Troy & Folinazzo, 1953; Mannheim & Ziv, 1962). Hence, the adequacy of pasteurization is dependent on the inactivation of PE.

The heat treatment necessary to inactivate the PE in the juice varies with variety, pH, etc. (Rouse & Atkins, 1952, 1953; Atkins & Rouse, 1953; Kew *et al.*, 1957; Rothschild, van Vliet & Karsenty, 1975). Heating times and temperatures reported are the commercial pasteurization conditions of the juice and not the actual thermal inactivation time (TIT) of the enzyme which could be used in process evaluation. Eagerman & Rouse (1976) determined the time/temperature relationship and also the time required for two decimal reductions of the PE activity at different temperatures. Investigations carried out to determine the TIT and the decimal reduction time of PE in mandarin orange juice form the subject matter of this paper.

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Materials and methods

Preparation of orange juice

Mandarin oranges purchased from the local market were peeled and segments separated. A cold lye peeling procedure was adopted to peel the segments. The segments were soaked in 1.5% HCl for 60 min at 80° F (26.7°C), washed in water, soaked thereafter in 1% NaOH for 25 min and rewashed thoroughly. After removing the seeds, the segments were blended in a Waring blender. The total soluble solids (TSS) of the juice were adjusted to 12% by addition of sugar and the pH of different sub-samples were adjusted to 3.6 and 4.0. The pulp content of the juice was 16%. Prepared samples were filled into polyethylene bags, quick frozen in a plate freezer at −40° F (−40°C) and stored at 0° F (−20° C). The samples were thawed immediately prior to use.

Thermal inactivation time of pectinesterase

The technique used was similar to that of Bigelow & Esty (1920) for determination of the thermal death time of bacteria. Pyrex glass tubes of 13.5 cm length, 1.6 cm OD and 1.4 cm ID were used. Ten ml of the sample were pipetted into each of the tubes, heated in a thermostatic water bath for known times and cooled immediately by plunging the tubes into chilled water. Two tubes were used for each time and temperature, and the experiment was performed in triplicate.

The come-up time was determined using a 10.5 cm long needle type thermocouple fitted with spacers to position the thermocouple in the centre of the tube. The temperature at various time-intervals was noted with a manually operated Leeds and Northrup potentiometer calibrated in terms of degrees Fahrenheit. This was done in triplicate for each temperature.

Test for pectinesterase activity

The sensitized jelly test procedure of Rothschild *et al.* (1975) was further modified to determine the time required to inactivate the PE. Ten ml of 0.5% pectin (6% methoxyl) solution was added to 10 ml of the sample, the pH adjusted to 7.0 with 0.1 N NaOH, and the volume made to 60 ml with distilled water. To this, 1 ml of 1 N calcium chloride solution plus a few drops of toluene were added, mixed and then transferred to a 100 ml stoppered conical flask and incubated at 93.2° F (34°C) for 48 hr.

The control sample having no enzyme activity was prepared in the same way using a sample of juice heated in a TIT tube for 10 min in boiling water.

Viscosity was measured at a temperature of 80° F (26.7°C) using a Brookfield Synchrolectric viscometer with No. 1 spindle and a speed of 60 rpm. Any

increase in the viscosity of the sample after incubation as compared to the value prior to incubation or to that of the control sample incubated similarly indicated the presence of the active enzyme. The minimum time required to give no activity in the sample was taken as the TIT for that temperature.

Decimal reduction time (D) *of pectinesterase*

Ten ml of the juice were heated in TIT tubes for known times at different temperatures as in the TIT determination. The heating times selected at each temperature were above the come-up time but well below the TIT of the enzyme. The initial and the residual PE activities were determined by the method of MacDonnel, Jensen & Lineweaver (1945) as modified by Rouse & Atkins (1952) and expressed as PE units $\times 10^{-4}$ ml^{-1}. The D value was calculated using the formula suggested by Stumbo (1973) for bacteria

$$D = \frac{U}{\log a - \log b}$$

Where a is the initial activity and b is the activity which survived the heating time of U in minutes. The D values calculated for different heating times at one temperature were averaged.

Correction for heating lag during come-up time

The extent of inactivation attained during the time lag in heating and cooling was calculated by applying the general method of Bigelow *et al.* (1920) for process calculation as described by Nath & Ranganna (1977).

Thermal inactivation time and thermal resistance curves

These curves were plotted on a semilog paper by applying the analysis of least squares (Brownlee, 1960).

Results and discussion

Acidity, pH, TSS and PE activity in orange varied considerably (Table 1). The pH of fruit varied from 3.0 in early rainy season crops of June–September to 4.0 in late main summer crops of November–February. Generally, the summer crop is processed when the pH ranges from 3.6 to 4.0. Hence, TIT studies were carried out at pH values of 3.6 and 4.0, the lowest and the highest likely to be found. The TSS of 12% simulate the canned juice. Rouse (1953)

Table 1. Total soluble solids, acidity, pH and PE activity in orange and in the preparation used for thermal inactivation studies

Particulars	Edible portion of orange*	Orange juice used for inactivation studies†	
TSS (%)	7–12	12.0	
Acidity as anhydrous citric acid (%)	0.59–1.15	0.638	0.58
pH	3.0–4.0	3.6	4.0
Pectinesterase activity (PE units × 10^{-} ml^{-1})	20.6–52.0	37–52	

Prepared by *hand peeling; †lye peeling of segments and blending thereafter.

reported the PE activity in raw juices of different varieties of orange to vary from 1 to 4×10^{-2} PE units/°Brix. The juice used in the present study had an activity ranging from 3.08 to 4.33×10^{-2} PE units/°Brix. In the processing plants, the juice is extracted from mandarin oranges in a Taglith juice extractor which results in a juice of much lower pulp content than 16% in the present study. Consequently, the PE activity, would be much less. The PE activity in the juice used in the present study is the highest that is likely to be found for the product in question.

Test for the inactivation of pectinesterase

Rothschild *et al.* (1975), in their studies, considered pasteurization to be adequate, if after three days of incubation, the reaction mixture remained free flowing (–) or insufficient, if the jelly had formed (+). In the present study, an increase in the viscosity was observed in some of the incubated samples which had received heat treatment sufficient enough to render the sample free flowing. Hence, viscosity was determined in samples which had not jellied to determine the time required at a particular temperature for thermal inactivation of PE. An incubation period of 48 hr at 93.2°F (34°C) was found sufficient. A typical set of results obtained by the above two sets is shown in Table 2. At pH 3.6 and 4.0, the time required to inactivate the PE at 190°F (87.8°C) was 4 and 5 min respectively, by the jelly-test, and 5 and 8 min respectively, by the viscosity method. The TIT mentioned above include the come-up time. These results show that the viscosity method is sensitive to the residual PE activity at levels which the jelly-test cannot detect.

To determine the *F* value (the time required at a particular temperature to completely inactive the enzyme) or the *D* value, 10 ml of the sample were required which necessitated the use of TIT tubes of a larger diameter (1.4 cm) than the tubes (0.7 cm) used in the thermal death time (TDT) studies of

Table 2. Comparison of sensitized jelly-test and viscosity method for determining thermal inactivation time of PE in orange juice

pH	Temperature (°F)	Uncorrected heating time (min)	Jelly-test	Viscosity (cp)
3.6	190	0	Good jelly	N.D.
		3	Soft jelly	45.0
		4	Free flowing	8.5
		5	Free flowing	5.0
		6	Free flowing	5.0
		7	Free flowing	5.0
4.0	190	0	Good jelly	N.D.
		4	Soft jelly	42.0
		5	Free flowing	18.0
		6	Free flowing	8.0
		7	Free flowing	8.0
		8	Free flowing	5.0
		9	Free flowing	5.0
Boiling water		10	Free flowing	5.0

N.D. = not determined.

bacterial spores. The come-up time was, therefore, longer. Since its effectiveness (percentage of the come-up time having lethal effect on the enzyme) was found and the correction applied, the accuracy of the F and D values was not impaired.

Correction for heating lag

The corrections necessary for heating lag in the determination of the thermal death time of bacteria have been found by Ball *et al.* (1937) and Sognefest & Benjamin (1944) but not in the determination of TIT of enzymes. Earlier workers (Kaplan, Esselen & Fellers, 1949; Nebesky *et al.*, 1950; Dastur, Weckel & von Elbe, 1968; Nanjundaswamy, Saroja & Ranganna, 1973) used either the correction found for bacteria or assumed an arbitrary value. Eagerman & Rouse (1976) used the flask method wherein the concentrated enzyme preparation was added to the juice preheated to the temperature and vigorously stirred, which obviated the need for a lag correction. The general method adopted by the present authors provides a basis for determination of the correction to be applied for the heating lag in the determination of the TIT of the enzymes.

Data relating to the correction to be applied for the heating lag during the come-up time is given in Table 3. The effectiveness decreased slightly as the pH was increased – 44.65% at pH 3.6 and 41.54% at pH 4.0.

Table 3. Corrections for come-up time in the determination of thermal inactivation and decimal reduction times of pectinesterase in orange juice

TSS (%)	pH	Experimental TIT*	Temp. (°F)	Come-up time (min)	Effectiveness† (%)	Corrections to be subtracted (min)
12	3.6	$F_{180}^{26\cdot4} = 11.0$	180	6.6	44.63	3.65
			185	6.6	43.85	3.71
			190	6.6	46.02	3.56
			195	6.6	44.12	3.69
	Mean			6.6	44.65	3.65
	4.0	$F_{180}^{26\cdot3} = 18.6$	180	6.6	42.42	3.8
			185	6.6	40.37	3.94
			190	6.6	43.33	3.74
			195	6.6	40.14	3.95
	Mean			6.6	41.54	3.86

* TIT uncorrected for heating lag used for calculating the effectiveness.
† Percentage of the come-up time having lethal effect on the enzyme.

F, D *and* z *values*

The semilog plot of thermal inactivation time *v.* temperature, which gives the thermal inactivation time curve analogous to the thermal death time curve of bacteria, is shown in Fig. 1. The F value of PE in mandarin orange juice (Table 4) was higher than the heating times and temperatures found sufficient for

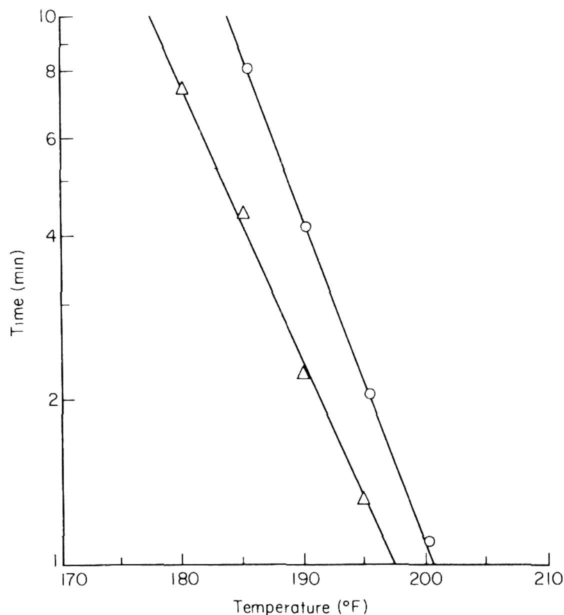

Figure 1. TIT curves of pectinesterase in orange juice. ○ pH 4.0; △ pH 3.6.

Table 4. *F, D* and *z* values of PE in orange juice

pH	*F* and *z* values from TIT curve	*D* and *z* values from thermal-resistance curve	Process equivalence *F/D*	*F* values for commercial pasteurization
3.6	$F_{197\cdot5}^{19\cdot4} = 1.00$	$D_{197\cdot5}^{20\cdot6} = 0.54$	1.85	$F_{197\cdot5} = 1.00$
			2.0	$= 1.09$
			2.5	$= 1.36$
4.0	$F_{201\cdot5}^{17} = 1.00$	$D_{201\cdot5}^{18\cdot2} = 0.438$	2.28	$F_{201\cdot5} = 1.00$
			2.5	$= 1.10$
			3.0	$= 1.32$

inactivation under commercial pasteurization conditions (Rouse & Atkins, 1952, 1953; Atkins & Rouse, 1953, 1954; Kew *et al.*, 1957; Rothschild *et al.*, 1975) or the TIT found by Eagerman & Rouse (1976) using juice from other varieties of orange. The TIT increased with pH.

The enzyme activity in mandarin orange varies considerably (Table 1), and if the enzyme activity in the juice being pasteurized is higher than that used for the TIT studies, the *F* value found may prove inadequate for complete inactivation. Kaplan *et al.* (1949) and Nebesky *et al.* (1950) have made similar observations in other canned products. As in the case of microorganisms, the rate of inactivation of the enzyme is logarithmic and is independent of the initial activity (Zoueil & Esselen, 1959). Hence, the time required to reduce the enzyme activity by one log cycle (*D* value) at different temperatures was

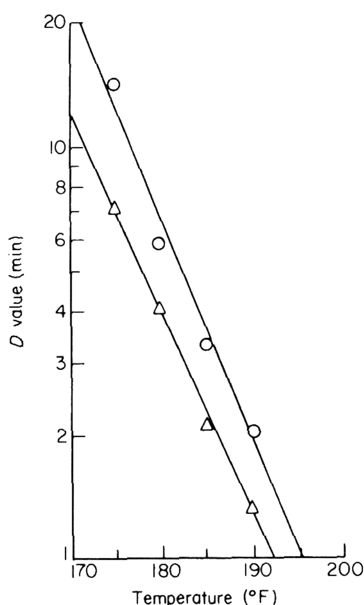

Figure 2. Thermal resistance curves of pectinesterase in orange juice. ○ pH 4.0; △ pH 3.6.

determined. The semilog plot of the *D* value *v.* temperature which gives the thermal resistance curve, analogous to the 'phantom' thermal death time curve (also called thermal resistance curve) of bacteria, is shown in Fig. 2. The *D* value was found to increase as the pH increased (Table 4).

The *z* value (°F required for the slope of the curve to traverse one log cycle) from the thermal resistance curve was slightly higher than the value from the TIT curve (Table 4) which might be attributed to the different procedures used for the determination of *F* and *D* values.

At pH values of 3.6 and 4.0, the *F* value of PE was 1.00 at 197.5°F (91.94°C) and 201.5°F (93.61°C) respectively. The *F* value was equal to 1.85 *D* at pH 3.6 and 2.28 *D* at pH 4.0. A slight increase in the decimal reduction process would ensure not only inactivation of the enzyme but also other variables encountered under commercial pasteurization conditions. Hence, a 2 *D* process at pH 3.6 and a 2.5 *D* process at pH 4.0 are recommended. The corresponding increase in time is very small (Table 4).

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Home frozen strawberries

III. Factors affecting sensory assessment

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Summary

Treatments were devised to improve the quality of home frozen Cambridge Favourite strawberries, stored for 6 months at −18°C. When sugared berries were frozen quickly on trays and packaged the following day, rather than being packaged in sugar before freezing, the appearance of sliced berries and colour of whole berries were improved. If fruit was frozen without sugar and sweetened before tasting, the rate of freezing did not influence quality. Colour and appearance were usually better if sugar was added before freezing. For slow freezing, 60% syrup was a better medium than dry sugar, and texture (whole berries) and flavour (sliced berries) were improved. Sliced berries had a better flavour (syrup treatment) but poorer appearance (sugar treatment) than whole berries. Berries crushed in sugar before freezing had poorer appearance, colour and texture marks, but better flavour scores, than the whole berries frozen in sugar or syrup. Ascorbic acid failed to improve quality.

Introduction

Two previous papers (Hudson *et al.*, 1975a, b) described ways of improving the quality of home frozen strawberries and mentioned most of the relevant literature. Most tests used 60% syrup as the freezing medium, as early work showed it was one of the best freezing agents (Leach *et al.*, 1970). At home, however, dry sugar is usually preferred as it requires no preparation. This paper describes attempts to improve the quality of berries frozen with dry sugar, but includes a few syrup treatments for comparison. Occasionally housewives open-freeze berries on trays to obtain faster freezing rates. Stoll (1966), Gutschmidt (1969) and Durif (1971) obtained better texture and flavour by rapid freezing. However MacArthur & Johnston (1947) and Hudson *et al.* (1975a) found that faster freezing may not necessarily improve quality, presumably because their freezing speeds were not fast enough to be effective.

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The effects of freezing fresh plain berries and adding sugar before tasting have also been investigated in this paper. Dawson, Harris & Alexander (1952) found that adding sugar after freezing gave better flavour, as the fruit was not too sweet. MacArthur & Johnston (1947) also obtained good results using this method.

We also tested whole, sliced and crushed berries, with and without added ascorbic acid. Fieger, DuBois & Kalogereas (1946) obtained better flavour with sliced than whole berries and they found sugar penetration and retention of flavour to be correlated. Hohl (1947) reported better texture and appearance after slicing. Crushing (MacArthur & Johnston, 1947) enhanced most qualities and especially flavour. Usually quality has not been improved by using ascorbic acid (Hohl, 1947; Crivelli & Rosati, 1973; Hudson *et al.*, 1975b).

Materials and methods

Medium sized Cambridge Favourite strawberries were hulled and weighed into 350 g batches. For most treatments berries were left whole, or cut lengthwise into halves, and placed in plastic cartons for slow freezing, or on plastic trays for faster quick freezing. Berries were then (a) sprinkled with 90 g sugar, (b) sprinkled with 90 g sugar mixed with 200 mg ascorbic acid, (c) covered with 350 ml chilled (4°C) 60% (w/w) syrup, or (d) untreated. In other experiments berries were crushed with 90 g sugar, or 90 g sugar and 200 mg ascorbic acid, and were placed in plastic cartons for slow freezing. All material was frozen overnight in a cold room at −20°C and the following morning berries on trays were packaged in cartons. After 6 months storage at −18°C, material was thawed slowly overnight at 4°C, and sensory evaluations carried out as soon as possible after the preparations. For tasting, 90 g sugar were added to the plain berries.

Three experiments were made, each with six treatments and three replicates. An experienced panel of twelve members assessed appearance, colour, texture and flavour on a seven-point hedonic preference scale, 1 being objectionable, 2 poor, 3 below average, 4 average, 5 above average, 6 good and 7 excellent.

Results

Whole berries frozen packed or on trays

Table 1 shows that ascorbic acid did not improve the quality of whole berries and decreased texture scores for slowly frozen fruit ($P = 0.05$). If sugar is added before freezing, significantly better ($P = 0.01$) colour scores result from faster freezing on trays. There was slight evidence that faster freezing also improved appearance.

Adding sugar after, rather than before, freezing only influenced quality by lowering the score for colour ($P = 0.05$) following a fast freeze on trays. When

Table 1. Mean scores for whole berries with sugar added before freezing (SF) or before tasting (ST)

Treatment	Freezing rate	Appearance	Colour	Texture	Flavour
SF	Fast	4.40 ab	4.24 bc	3.88 b	3.37 ab
SF	Slow	4.33 ab	3.81 a	3.91 b	3.72 b
SF and AA	Fast	4.44 ab	4.41 c	3.90 b	3.29 a
SF and AA	Slow	4.20 a	4.00 ab	3.46 a	3.64 ab
ST	Fast	4.68 b	3.90 a	3.66 ab	3.54 ab
ST	Slow	4.30 ab	3.73 a	3.85 b	3.51 ab
S.E.		0.150	0.131	0.147	0.150

Mean scores followed by the same letter do not differ significantly from one another at the 5% level. S.E., standard error; AA, ascorbic acid.

Table 2. Mean scores for sliced berries with sugar added before freezing (SF) or before tasting (ST)

Treatment	Freezing rate	Appearance	Colour	Texture	Flavour
SF	Fast	4.53 d	4.29 c	3.95 a	3.58 a
SF	Slow	4.13 bc	4.02 abc	3.87 a	3.84 a
SF and AA	Fast	4.27 cd	4.21 bc	3.81 a	3.67 a
SF and AA	Slow	3.85 ab	4.11 bc	3.85 a	3.75 a
ST	Fast	3.83 ab	3.72 a	3.64 a	3.78 a
ST	Slow	3.75 a	3.90 ab	3.74 a	3.71 a
S.E.		0.128	0.130	0.123	0.133

Mean scores followed by the same letter do not differ significantly from one another at the 5% level. S.E., standard error; AA, ascorbic acid.

berries were frozen without sugar, the rate of freezing had no influence on quality.

Sliced berries frozen packed or on trays

Table 2 shows that adding ascorbic acid did not affect quality. If sugar is added before freezing, the appearance of the samples is improved by faster freezing on trays ($P = 0.01$) whether or not ascorbic acid is added.

When berries were frozen plain, and sugar was added for tasting, scores for appearance (quickly and slowly frozen berries) and colour (quickly frozen

berries) were lower than for corresponding berries frozen with sugar. Quality of these sliced unsugared berries was not improved by faster freezing on trays.

Whole, sliced and crushed berries frozen slowly in syrup and sugar

Crushing berries in sugar lowered ($P = 0.01$) scores for appearance, colour and texture (Table 3) but increased ($P = 0.01$) flavour scores. Adding ascorbic acid to crushed berries tended to lower all mean scores, but not significantly.

Whole berries frozen in sugar had significantly better ($P = 0.001$) appearance scores than the sliced berries frozen in the same medium. On the other hand, in the syrup treatments the sliced berries had a better flavour ($P = 0.05$) than the whole ones.

In comparing the sugar and syrup treatments, the latter gave significantly higher scores for texture of whole berries ($P = 0.001$) and for flavour of sliced berries ($P = 0.01$).

Table 3. Mean scores for whole, sliced and crushed berries frozen slowly in sugar or syrup

Treatment	Appearance	Colour	Texture	Flavour
Whole berries in sugar	4.93 d	4.27 b	3.56 bc	3.22 a
Whole berries in syrup	4.66 cd	4.21 b	4.13 d	3.60 ab
Sliced berries in sugar	4.10 b	4.15 b	3.75 c	3.52 ab
Sliced berries in syrup	4.38 bc	4.19 b	3.85 cd	4.09 c
Crushed berries in sugar	2.25 a	3.38 a	3.29 ab	4.15 c
Crushed berries in sugar + AA	1.96 a	3.17 a	3.23 a	3.90 bc
S.E.	0.120	0.119	0.118	0.154

Mean scores followed by the same letter do not differ significantly from one another at the level. S.E., standard error. AA, ascorbic acid.

Discussion

In the home berries can be (a) packed and frozen or (b) spread out on a tray, frozen, and packed the following day. The latter method gives the faster freezing rate. If sugar was added before cooling, the faster freeze improved colour of whole berries and appearance of sliced berries. Similar quality improvement was not attained when berries were frozen plain and sugar was added for tasting. This suggests that the improvements are due, not to the smaller ice crystal size that normally accompanies really fast freezing, but to the shorter time for which fruit is immersed in the sweetening agent before freezing. Presumably colour and appearance are favoured by a shorter soaking time as there is less time for the sugar to cause plasmolysis, cellular damage and loss of cell sap. However slower freezing tended to improve flavour indicating that a longer soak in sugar before freezing increases penetration. Similar results have

been obtained for berries frozen in syrup (Hudson *et al.*, 1975a). Plain berries to which sugar was added for tasting had the poorest colour. Whether to recommend whole, sliced or crushed fruits clearly depends on the use to which the fruit is to be put. Whereas slicing and crushing improve flavour, by allowing greater penetration of sugar, they tend to spoil appearance. Crushing had an adverse effect on colour, appearance and texture, but it improved flavour, so it is valuable if the fruit is to be used in the puréed form and is the most economical of freezer space.

The results confirmed that for slow freezing syrup is a marginally better freezing agent than dry sugar as it improves texture (whole berries) and flavour (sliced berries). In the home a syrup freeze is always slow. Unfortunately we did not directly compare this method with a quick freeze for sugared berries on trays, and the same treatments in different experiments did not give the same mean scores, so it is not possible to make a rigorous comparison. However, standardizing scores between experiments suggests that a slow freeze in syrup gives a better flavour (whole and sliced berries) and texture (whole berries), but a poorer colour (whole berries) than a quick freeze in sugar. For syrup treatments we showed it is better to use sliced berries (improved flavour) whereas for dry sugar methods whole berries are best (improved appearance). In comparing a syrup freeze for sliced berries and a quick freeze in sugar for whole berries, we find fairly strong evidence for improved flavour in the syrup treatment, and no marked differences in other parameters. The syrup treatments do, therefore, seem the best. One additional disadvantage of quick freezing on trays is that unsightly lumps of sucrose hydrates tend to form during storage. These dissolve only slowly during thawing, and often spoil the appearance of the thawed product. In the current experiments these hydrates were ignored by the tasting panel, as its members had been requested to assess the appearance of the fruit and not the syrup.

Ascorbic acid had no effect on the quality of the fruit. As it is used mainly to prevent browning of white fruits it is perhaps hardly surprising that it has always been found to be ineffective with strawberries.

Conclusions

(1) Freezing slowly in a 60% (w/w) chilled syrup gives the best overall quality to frozen strawberries. Slicing the berries improves their flavour.

(2) Freezing in dry sugar is most convenient and quick freezing will improve colour and appearance. Adding sugar after freezing does little to improve quality. Whole berries had a better appearance than those which had been sliced.

(3) Ascorbic acid had no effect on quality.

(4) Berries crushed with sugar had the most flavour and used least freezer space, but they can only be used as a purée.

Acknowledgments

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Quality of home frozen vegetables

III. Effects of salt treatments and boil-in-the-bag methods on retention of chlorophyll in home frozen perpetual spinach

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AND MARGARET E. HOLGATE

Summary

This paper records changes in total chlorophyll and chlorophylls *a* and *b* in perpetual spinach after blanching, storage at −18°C and cooking. In soft water total chlorophyll decreased during blanching but less so when salt (1.2%) was added; salting increased the loss of chlorophyll during storage but decreased losses during cooking. In hard water less chlorophyll was lost and salting had less effect.

Conventional cooking methods were better than 'boil-in-the-bag' cooking which much reduced chlorophyll. Adding salt during cooking had no effect on total chlorophyll retention other than protecting it when cooking spinach blanched in soft water without salt.

Chlorophyll *a* also survived least well when spinach was cooked in the bag. It seemed to be made less stable after using salty (3%) cooling water but survived slightly better in hard than in soft water. Although chlorophyll *b* was more stable, losses were greatest with boil-in-the-bag methods.

In soft water areas it is recommended that salt (1.2%) is added to the blanching water. This is not necessary in hard water regions. Boil-in-the-bag methods are considered unsatisfactory for spinach.

Introduction

The effects of high pH and of a salt blanch in preventing conversion of chlorophyll to phaeophytin in blanched green vegetables were mentioned by Hudson *et al.* (1974a, b). Later Thomopoulos (1975) reported that 1–3% sodium chloride had no effect on the blanching process, but 10% increased the time needed to inactivate catalase. Good correlations between colour and chlorophyll conversion have often been found (Walker, 1964; Eheart & Gott, 1965; Buckle & Edwards, 1970a). This loss in colour is attributed mainly to degradation of

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chlorophyll *a* (Sweeney & Martin, 1961; Schneider, 1967). Chlorophyll *b* occurs in smaller amounts and is the more stable (Aronoff, 1953; Sweeney & Martin, 1958, 1961; Tan & Francis, 1962; Walker, 1964; Eheart & Gott, 1965; Eheart, 1967; Buckle & Edwards, 1970b). More chlorophyll is lost at low pH and Sweeney & Martin (1961) found a pH of between 6 and 7 to be critical for chlorophyll retention. Gupte & Francis (1964) found that blanching at a high pH maintained a good colour in spinach purée stored at room temperature for 18 months.

Much chlorophyll is lost during blanching and more the longer the blanching time (Mackinney & Weast, 1940; Dietrich *et al.*, 1959; Tan & Francis, 1962). Chlorophyll may also decrease during storage (Dietrich *et al.*, 1957, 1960; Walker, 1964; Schneider, 1967; Eheart, 1969) though in runner beans Schneider (1967) found phaeophytin levels were unchanged after 3 months storage at −20°C. Wagenknecht, Lee & Boyle (1952) found no chlorophyll loss in peas stored at −18°C.

Chlorophyll is also lost in a number of vegetables during cooking (Mackinney & Weast, 1940; Sweeney & Martin, 1961; Eheart & Gott, 1965; Eheart 1970). Eheart (1967) reported a lowering of chlorophyll, total acids and pH when frozen broccoli was cooked, but retention of both chlorophyll *a* and *b* was associated with the pH of the vegetable and not with total acids.

Eheart (1970) found that boiling-in-the-bag increased total acid content and losses of total chlorophyll and chlorophyll *b*, but had little effect on visual colour; it retained Vitamin C better than conventional cooking. Pickford (1975) also found that Vitamin C was retained well but reported that heating took 2.5 times longer than the conventional method, and involved a greater loss of colour and flavour.

The volume of water used for blanching and cooking also appears to affect chlorophyll retention. Eheart & Gott (1965) found less conversion of chlorophyll with large quantities of cooking water and concluded this resulted from a greater dilution of the plant acids.

This paper describes further work on the use of salt in blanching and cooling water. Total chlorophyll and chlorophylls *a* and *b* have been estimated in perpetual spinach after blanching and cooling, storage and cooking. Both hard and soft waters were used for blanching and cooling, with and without added salt. Cooking was done conventionally and in the bag, with and without added salt. Boiling in bags is attractive because nutrients would be retained, several vegetables could be cooked in bags together and saucepan cleaning is simpler. We used small flat bags in the hope that the contents would heat quickly and so suffer little loss of quality.

Materials and methods

Samples (150 g) of freshly picked perpetual spinach leaves were placed in muslin bags and each blanched 3 min in 2.1 litre plain or salted (1.2% NaCl)

water. The 'hard' blanching water (pH approximately 7.6) had a total hardness as $CaCO_3$ of 200 ppm and the 'soft' water (pH approximately 6.0) was made up with 1 part tap water and 9 parts distilled water. After blanching, each sample was cooled 3 min in 1.4 litre of chilled 'soft' or 'hard' water, either with or without 3% NaCl. Blanching was done in small aluminium saucepans. After draining the material was packaged in polythene bags or in boil-in-the-bag containers which were frozen and stored for 6 months at −18°C. For conventional cooking samples were removed from their polythene bags and simmered for 10 min in 125 ml unsalted or salted (1.2% NaCl) *tap* water. The immersible bags were placed in boiling water for 25 min. Both cooking procedures raised the temperature of the spinach to approximately 85°C.

Chlorophyll assays were made using Vernon's (1960) method and his equations 13, 1 and 2 were used to calculate percentage retentions of total chlorophyll and chlorophylls *a* and *b* respectively.

Results

Total chlorophyll

Differences in retention of total chlorophyll between hard and soft water treatments were evident (Table 1) and confirmed that chlorophyll is more stable in alkaline (hard) water. Blanching resulted in significant losses of total chlorophyll. Salt blanches tended to decrease loss of total chlorophyll during scalding, but only with soft water samples was the difference significant ($P = 0.01$). Again (Hudson *et al.*, 1974a) there was no advantage in adding 3% salt to the cooling water, indicating that salt is ineffective in cold water.

Total chlorophyll diminished slightly during storage, significantly in material blanched in soft water and most ($P = 0.001$) in salt blanched spinach. Conventional cooking restored the significant differences between the material blanched in soft water with and without salt. Adding salt to cooking water had no effect on chlorophyll retention, apart from improving ($P = 0.05$) its level in material blanched in soft water without salt. None of these differences was significant in spinach treated in the hard water.

Boil-in-the-bag methods were only investigated using samples blanched in hard water. They showed losses of total chlorophyll which were approximately 10% greater than with conventional cooking. Boiling-in-the-bag gave a significantly better ($P = 0.05$) chlorophyll retention in material blanched with salt, but despite this conventional cooking was still best.

Chlorophyll a

Changes in chlorophyll *a* followed those for total chlorophyll, but because the standard error of the results was greater, there were fewer significant differences. All material lost a significant amount of chlorophyll during blanching.

Table 1. Percentage of chlorophylls in perpetual spinach before and after blanching, storing at $-18^{\circ}C$; and cooking

Chlorophyll	Treatments	Moderately hard blanching and cooling water			Soft blanching and cooling water	
		No salt in blanching or cooling water	1.2% NaCl in blanching water	3% NaCl in cooling water	No salt in blanching or cooling water	1.2% NaCl in blanching water
Total	Unblanched, fresh	96.1 a	96.1 a	96.1 a	96.1 a	96.1 a
	Blanched, fresh	85.5 bc	89.2 b	84.8 bc	75.3 d	83.9 bc
	Blanched, frozen 6 months					
	Uncooked	83.8 bc	86.3 bc	83.6 bc	68.2 e	67.6 e
	Cooked without salt	81.6 c	84.3 bc	81.8 c	46.5 h	61.0 fg
	Cooked with 1.2% salt	83.1 c	82.9 c	81.6 c	55.1 g	64.4 ef
	Cooked-in-bag	67.8 e	74.9 d	65.4 ef	–	–
a	Unblanched, fresh	108.1 a	108.1 a	108.1 a	108.1 a	108.1 a
	Blanched, fresh	87.9 bcde	89.2 bcde	87.4 bcde	82.5 cdef	85.2 bcde
	Blanched, frozen 6 months					
	Uncooked	91.3 bc	92.7 bc	85.8 bcde	70.8 ghj	69.5 ghj
	Cooked without salt	82.2 cdef	96.3 b	79.7 defg	52.6 k	59.6 jk
	Cooked with 1.2% salt	91.1 bc	90.5 bcd	78.7 efg	52.9 k	60.9 jk
	Cooked-in-bag	66.5 hj	73.5 fgh	73.7 fgh	–	–
b	Unblanched, fresh	63.5 ab	63.5 ab	63.5 ab	63.5 ab	63.5 ab
	Blanched, fresh	60.8 abc	63.0 ab	61.6 abc	57.7 abcd	60.4 abc
	Blanched, frozen 6 months					
	Uncooked	48.5 de	54.4 abcde	52.5 cde	51.6 cde	51.3 cde
	Cooked without salt	64.2 a	57.7 abcd	56.2 abcd	44.3 e	53.2 bcde
	Cooked with 1.2% salt	61.0 abc	56.6 abcd	54.0 abcde	48.1 de	52.3 cde
	Cooked in-bag	49.9 de	49.7 de	52.2 cde	–	

Standard errors: total chlorophyll = 2.14; chlorophyll *a* = 4.00; chlorophyll *b* = 3.71. Mean scores followed by the same letter do not differ significantly from one another at the 5% level.

After cooling there were no significant differences between the hard and soft water treatments. However the spinach blanched in soft water lost significantly greater amounts of chlorophyll *a* during storage than the material blanched in hard water. The resistant hard water blanched spinach suffered no chlorophyll loss during conventional cooking. Losses during cooking occurred in material cooked in the bag and in the soft water treatment blanched without salt. Adding salt to cooking water did not affect the retention of the pigment.

Chlorophyll b

Chlorophyll *b* was more stable than chlorophyll *a* and was not significantly decreased by any of the blanching treatments, although some data showed smaller chlorophyll *b* concentrations after blanching. Generally no significant loss of pigment occurred during storage, though concentrations again tended to decrease. Significant losses during storage occurred only in material blanched and cooled in hard water without salt. This treatment was also exceptional in that chlorophyll *b* increased during conventional cooking. Material cooked in the bag generally had less chlorophyll *b* than was found in freshly blanched spinach. Adding salt to cooking water did not affect chlorophyll *b* content. Chlorophyll *b* was generally lower in spinach, blanched in soft water and cooked, than in the fresh material.

Discussion

This work confirmed previous findings that a high pH stabilizes chlorophyll molecules during blanching, cooking, and to a lesser extent during storage at −18°C.

Although the pH of the water is of great importance, the pH of the vegetables themselves is also known to be critical (Schneider, 1967; Sweeney & Martin, 1961). We now know that salt added to soft blanching water may also be beneficial in stabilizing chlorophyll. The effect is less pronounced, or non-existent, with hard water, indicating that the ionic composition of the water is of importance. Addition of salt does not affect pH and it is thus probable that the salt blanch prevents leaching of the magnesium ions during blanching and cooking. Higher levels (3%) of salt may have an even greater protective effect in soft water areas (Hudson *et al.*, 1974a). The results indicate that although 1.2% salt may help to prevent chlorophyll conversion, it cannot entirely prevent chlorophyll loss in acidic media. It is not as effective in stabilizing chlorophyll as an increase in pH, which implies that a carbonate salt would be more effective. The disadvantage of using such a salt in the home would be to know the quantity to add without increasing the pH to such an extent that both Vitamin C and texture would be adversely affected.

Perpetual spinach is not heated to any great extent during cooking, and this

would explain why chlorophyll losses during cooking were not greater. In hard water regions the chlorophyll levels of spinach are not affected by cooking and salt added for cooking could not improve colour. In soft water regions, however, salt added for cooking improves chlorophyll retention if unsalted water is used for blanching. Results showed that it is better to stabilize the chlorophyll with salt during blanching, than to rely on it improving colour when added to cooking water.

Boil-in-the-bag methods were disappointing because of the longer cooking time and the increase in chlorophyll loss. The greater breakdown of chlorophyll is no doubt due to retention of volatile acids and lack of neutralization of the cell sap with tap water.

Vernon's (1960) method for chlorophyll determinations presented certain anomalies. Our percentage values of chlorophyll *b* in raw material seemed very low, but Eheart & Gott (1965) and Eheart (1969, 1970) found low levels in other vegetables. Another anomaly was the increase in chlorophyll *b* found during cooking. Gupte & Francis (1964) and Buckle & Edwards (1969) found increases of this pigment in purées stored at room temperature. Tan & Francis (1962) found more phaeophytin than could be accounted for by loss of chlorophyll in spinach purée; they suggested the formation of another substance with higher absorption coefficients. Eheart discovered increases in chlorophyll *a* and total chlorophyll in frozen broccoli stored for 6 months (1967), and an increase in chlorophyll *b* in broccoli stored at −15°C for 5 months (1970). There is a general tendency in these findings for the chlorophyll to build up during the first few months of storage and then to decline so that, after a year, there is less than in the blanched material. Presumably the anomalies will be explained by the formation of other chlorophyll degradation products as suggested by Buckle & Edwards (1969, 1970a, 1970b).

Conclusions

In perpetual spinach losses in total chlorophyll during blanching, frozen storage and cooking were found to be influenced by the type of water used for blanching. With soft water, chlorophyll is relatively unstable and is lost progressively during blanching, storage and cooking. To improve stability it is recommended that 1.2% sodium chloride is added to the blanching water. Hard water stabilizes chlorophyll molecules and there is no advantage in using salt for blanching in regions where this water is found.

Large chlorophyll losses occur when material is cooked in the bag. The method is thus unsatisfactory but, if used, then 1.2% salt should be added to the blanching water.

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Technical note: Improvement of texture of frozen vegetables by stepwise blanching treatments. II

E. STEINBUCH

In continuation of experiments published earlier in this journal (Steinbuch, 1976) the effect of stepwise blanching treatments on the quality of frozen vegetables has been studied further. As has been explained already it would be advantageous to achieve a firmer texture in some of the products. The application of a pre-heat treatment at a moderate temperature prior to a normal blanching process for the purpose of enzyme inactivation can contribute to a considerable hardening of certain vegetable tissues.

The application of an additional pretreatment at temperatures of 70–90°C may lead to undesirable changes. Prolonged exposure of vegetables to these temperatures produces a striking increase of hardness of the product as measured by tenderometer, and may be judged as too tough by quality panels. In addition some green vegetables develop a somewhat dull olive-like colour due to the effect of the heat process on the chlorophyl.

In order to avoid this undesirable colour change and toughness the experiments were repeated using alternative time and temperature conditions. The effect of the blanching time at 70°C on the texture of both whole and sliced beans is shown in Fig. 1.

The firmness of the beans is indicated by tenderometer value (TM), and an assessment of the sensory properties of the beans is shown also. The hardening effect of prolonged heating times is confirmed. This is accompanied by a decrease of the bright green colour and a rise of olive brown appearance. A short treatment at 70°C results in a firm texture and a retention of the green colour.

Figure 2 indicates that the texture of whole beans is firmer than that of sliced beans. The application of higher temperatures during the pre-heat treatment also leads to increasing firmness of the beans without the occurrence of toughness. In contrast with the effects of lower temperatures the green colour of whole and sliced beans is well preserved by pretreatments at elevated temperatures prior to the normal blanching process.

Some experimental work has indicated also that the texture of frozen cauliflower and bean sprouts may also be improved by the stepwise blanching treatments.

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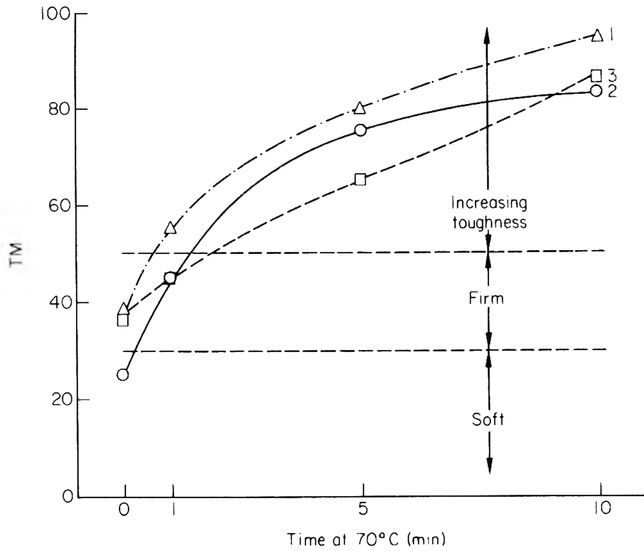

Figure 1. The effect of a pretreatment at 70°C prior to normal blanching conditions (3 min, 98°C) on the texture of frozen green beans. (1) Unsliced beans; (2) beans, sliced after the first treatment; (3) beans, sliced after the second treatment.

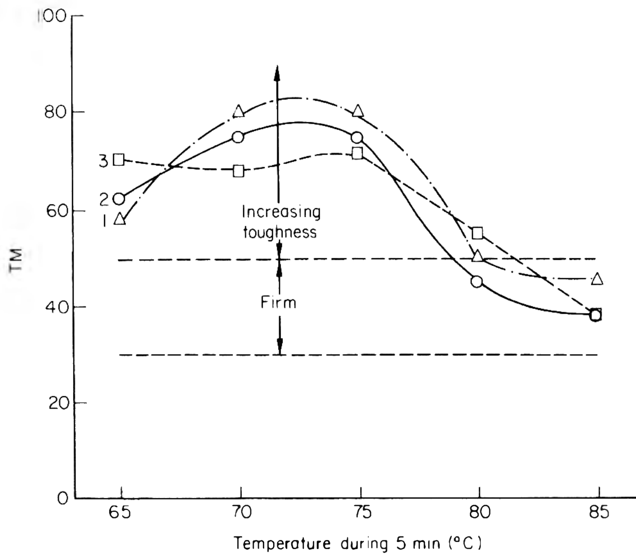

Figure 2. The effect of pretreatments at 65–85°C, prior to normal blanching conditions (3 min, 98°C) on the texture of frozen green beans. Legend as in Fig. 1.

Reference

Steinbuch, E. (1976) *J. Fd Technol.* **11**, 313.

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Technical note: Some observations on the solubility of lentil bean protein in moderately acidic solutions

C. G. ANDERSON, C. R. ROMO AND N. DE PABLO

In a recent article dealing with legumes (Anderson & Romo, 1976) we reported that the solubility of protein in a Chilean variety of lentil bean (*Lens culinaris*) appeared to be a sensitive function of extraction time in the pH region below a pH of 2.5. Furthermore, we established that the solubility of the proteins from Chilean grown lentil exhibited general acid solubility, which was in direct contrast to results obtained for *Lens culinaris* protein solubility reported by Fan & Sosulski, 1974. These authors reported that lentil protein demonstrated general acid insolubility.

The only major difference between Fan & Sosulski's method for determination of protein solubility and ours was that in their method an extraction time of 2 hr was employed, while in our method, the sample was extracted for only 20 min. We reasoned that the conflicting results were presumably due to this difference in extraction times. The possibility existed that as the extraction time increased, irreversible denaturation of the proteins was occurring, thus, an extraction time of 2 hr might yield results indicating a low acid solubility when compared to results obtained using a 20 min extraction time.

We have now quantified the relationship between lentil protein solubility and extraction time in the pH range of 2.6 to 1.35 and found that contrary to our hypothesis, the lentil protein studied by us demonstrates high solubility utilizing an extraction time of 2 hr. The results of the study are shown in Table 1.

Fan & Sosulski (1974) reported an NSI at pH 2.0 of approximately 56% with a decrease in NSI to a value of approximately 35% at pH 2.5 utilizing an extraction time of 2 hr for the protein extraction process. In contrast, as shown in Table 1, we found using an extraction time of 2 hr that the protein in Chilean grown lentil exhibits a solubility maximum in the acidic pH range of 89.3% NSI at pH 2.6 (Sample 20, Table 1) decreasing to a value of 72.6% NSI at pH 1.95 (Sample 12, Table 1).

It can also be seen from Table 1 that increasing the extraction time at all pH values studied from 20 min to 2 hr does produce a slight decrease in solubility as we hypothesized (compare Samples 3 and 4; 7 and 8; 11 and 12; 15 and 16;

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Table 1. Nitrogen solubility index (protein solubility) as a function of extraction time at various pH values for lentil protein*

Sample	pH	Extraction time (min)	NSI (%)†
1	1.35	5	67.1
2	1.35	10	70.3
3	1.40	20	75.7
4	1.40	120	74.6
5	1.70	5	65.0
6	1.70	10	70.6
7	1.70	20	77.2
8	1.65	120	76.9
9	1.90	5	67.6
10	1.90	10	72.0
11	1.90	20	75.6
12	1.95	120	72.6
13	2.1	5	70.9
14	2.1	10	75.8
15	2.1	20	78.0
16	2.1	120	75.7
17	2.6	5	88.8
18	2.6	10	89.0
19	2.6	20	91.5
20	2.6	120	89.3

* Dry basis. † Average of duplicate samples.

19 and 20 in Table 1), but not nearly of the magnitude expected. The data also indicate that as the extraction time is increased from 5 min to 20 min that a general, albeit small, increase in solubility occurs, with the degree of the increase becoming less pronounced as the pH is increased to a value of 2.6.

It can now be concluded, based upon the data presented above, that the differences observed for lentil protein solubility by Fan & Sosulski and us in the acidic region are probably due to a true difference in the nature of the proteins found in the two different samples of lentil bean.

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London: Elek Books, 1976. Pp. x + iii. £2.95 (Paperback).

The book assesses the value of pesticides to the community and explores ways of determining what restrictions and controls are necessary for their safe use.

Food Legume Processing and Utilization. By A. Siegel & B. Fawcett.
Ottawa: International Development Research Centre, 1976. Pp. 88 (Paperback).

This volume has been written with special emphasis on application in developing countries and covers traditional processes as well as processes on an industrial scale.

Proceedings of the Third International Biodegradation Symposium. Ed. by J. M. Sharpley & A. M. Kaplan.
London: Applied Science Publishers, 1976. Pp. vi + 126. £7.00.

Contains papers on biodegradation of agricultural products and on post-harvest deterioration of myotoxins.

1976. Pp. i + 46. £3.00 (Paperback).

Papers presented at the session on immobilized enzymes for degradation.

Black Currant Juice Processing Technology. By V. S. Charley.
Braunschweig: Verlag Günter Hempel, 1977. Pp. 126. DM36.00.

An authoritative monograph on all aspects of the production of black currant juice.

Inhibition and Inactivation of Vegetative Microbes. Ed by F. A. Skinner & W. B. Hugo.
London: Academic Press, 1977. Pp. xiii + 378. £12.00.

Papers presented at a symposium held in Nottingham in 1975.

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

SI UNITS

gram	g	Joule	J
kilogram	$kg = 10^3 g$	Newton	N
milligram	$mg = 10^{-3} g$	Watt	W
metre	m	Centigrade	°C
millimetre	$mm = 10^{-3} m$	hour	hr
micrometre	$\mu m = 10^{-6} m$	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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