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Simulated transit studies on tomatoes; effects of compressive load, container, vibration and maturity on mechanical damage

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Summary

The compressive force-deformation characteristics of tomatoes in the green, turning and red stages were determined with a view to obtaining information on the rupture force for these fruits. This was followed by a simulated transit study which investigated the effects of vibration, compressive load and type of container on mechanically induced damage, using a fractional factorial experimental design. The force-deformation studies showed that ripening leads to a significant decrease in tomato bioyield point, toughness and firmness and an increase in compliance. In the simulated transit study, mechanical damage was significantly affected by the stage of tomato maturity, container type, vibration and compressive load. It was found that the application of uniform moderate pressure on the produce during transportation would reduce the incidence of impact damage. The implications of these findings in the design and general improvement of packaging containers used for conveying perishable agricultural produce in developing countries are discussed.

Introduction

Movement of fruits and vegetables from the farm gate to urban centres in many developing countries is generally accomplished by truck. Commodities are packed in traditional containers such as bamboo baskets, jute bags, or in improvised containers of metal, plastic, paper and wood origin. These improvised containers invariably have been previously used for the packaging of imported or locally manufactured goods and their integrity as physical distribution containers is already in doubt. In Nigeria for instance, the bulk of tomatoes grown under irrigation in the northern parts of the country is shipped to the south, a distance of about 800 km, in V-shaped bamboo baskets of about 23 kg capacity or in wooden or corrugated paperboard boxes of about 9 kg capacity. The majority of trucks used in the transit operations have an interior vertical clearance which allows these baskets to be stacked about four high and the boxes about seven to eight high. Because of the shape of the baskets and the fact that the boxes are of poor integrity, the tomatoes, rather than the containers, generally support the bulk of the load. The above would be true for perishable horticultural produce marketed in several of the developing countries.

As a result of inadequate packaging in the physical distribution system, substantial losses have been reported in the post-harvest transit of perishable horticultural produce in many developing countries (Anon., 1978; Aworh & Olorunda, 1981; Coursey and Booth, 1972; Olorunda & Aboaba, 1978; Olorunda & Aworh, 1983; Pantastico, 1975).

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The majority of these losses are caused by mechanical damage to the produce as a result of static or dynamic stresses during post-harvest transit. Losses are further exacerbated by the prevailing rough surface roads in many of these countries and by rough handling of the containers during loading and unloading.

Most of the reported work on transportation injury associated with the handling of fruits and vegetables was done in the developed countries (Akamine & Goo, 1977; Erickson & Garret, 1977; Fridley & Adrian, 1966; Nelson & Mohsenin, 1968; McColloch, 1962; O'Brien et al., 1963; 1965). In the developing countries, the few relevant works reported are very general in nature (Coursey & Booth, 1972; Olorunda & Aboaba, 1978; Pantastico, 1975). It is only very recently that attempts have been made to look into the effect of packaging containers on mechanical injury of fruits and vegetables in transit (Aworh & Olorunda, 1981; Olorunda & Aworh, 1983). These works have revealed the magnitude of mechanical damage to perishable produce during transit; however, they do not provide information on the nature and cause of this damage. Such information is necessary to formulate guidelines for package improvement and development. In the present work, force-deformation characteristics of tomatoes at different stages of maturity were measured using an Instron testing machine in order to determine the maximum allowable compressive loads for various ripeness levels. This study was followed by another in which an attempt was made to simulate transit conditions comparable to those existing in developing countries, in order to obtain information that could serve as a baseline for package design and development.

Materials and methods

The tomatoes used for the study were the cultivar Reento grown in hydroponic glasshouse culture. They were picked for the fresh market with part of their stem attached. Uniform sized fruits about 5.5 cm diameter were obtained from a wholesale outlet near Vancouver, Canada. Fruits obtained were in the green, turning and red stages of maturity. In the laboratory the fruits were carefully sorted to ensure uniformity and absence of any irregularities.

From the green, turning and red batches, random samples of fruits were obtained for determination of moisture content and Hunterlab colour difference. Moisture content of the tomato fruit was determined by drying cut slices in a vacuum oven at 70°C for 24 hr. Triplicate determinations were made. Hunter colour measurements were carried out using a black adaptor plate fitted to the specimen port of the D-25 optical head. A circular opening 2.70 cm in diameter located in the centre of the black plate allowed a portion of the curved surface of each tomato fruit to be placed against the opening while reading the L, a and b values. The tomato was rotated so that four readings were obtained around the fruit equator and one at the apex. The five readings were averaged to represent the colour of each fruits.

Fruit samples were also randomly obtained from the three batches of tomatoes to be used for the force-deformation studies using a Model 1122 Instron Universal Testing Machine equipped with flat plate fixtures. Just prior to this test, the average diameter of each tomato was taken at the equatorial region by means of vernier calipers. In the compression test, crosshead speed was maintained at 50 mm/min and the chart speed was 500 mm/min. Full scale load varied from 10 to 20 kg. Compression measurements were made until the fruit ruptured while loading the tomatoes in the longitudinal direction with the stylar end facing downward. The force-deformation behaviour
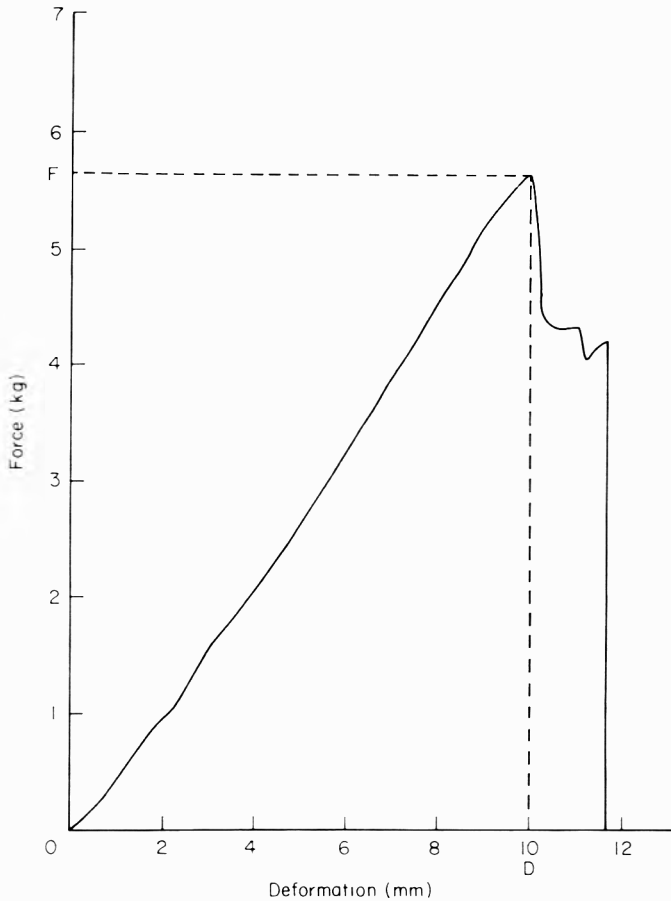
Figure 1. Typical compressive force-deformation curve for flat plate loading of individual tomato. F = Bioyield force; D = Deformation up to bioyield point.

during compression was recorded and from it the bioyield force, fruit deformation, toughness, compliance and firmness values for the tomatoes were derived as shown in Fig. 1. Fruit bioyield values were taken as the peak force (F) just prior to a sudden decrease in force sustained by the fruit due to tissue rupture. Deformation was defined as the distance (D) of crosshead travel from first contact with the tomato surface to the bioyield point. The slope (F/D) of the linear force-deformation curve was considered to be fruit firmness. Toughness was defined as the total energy absorbed up to bioyield as given by the area under the curve. Since the force-deformation curve was linear, the area under the curve was derived from the bioyield force and deformation, 0.5 FD, while compliance (*D/F*) was taken as the reciprocal of fruit firmness. From the bioyield value and the average fruit diameter (*d*) at the equatorial region, the maximum allowable pressure was estimated for the design of the simulated transit studies using the formula $P = 4F/d^2$.

In the simulated transit studies, corrugated paperboard, plexiglass and wooden containers, $15 \times 21 \times 41$ cm (inside dimensions) were used. The corrugated paperboard and the wood would be typical of materials generally used for making packaging containers while the plexiglass was introduced to simulate a smooth rigid surface.

The vibration treatment experienced in trucks was simulated by a single speed linear shaker (Eberbach Corporation, Ann Arbor, MI) operating at 2.7 Hz, moving horizontally with an amplitude of 3.5 cm. This vibration treatment led to movement of the tomatoes in all directions. Preliminary runs indicated that a 1 min shaking time would be adequate for our test conditions, since the damage experienced within this period was comparable to that observed in field studies in Nigeria (Aworh & Olorunda, 1981). On the above premise our test condition could be assumed to be a reasonable representation of what would happen in transit in terms of vibration and displacement of tomatoes. During each test the containers were fitted to the shaker on a platform that could accommodate two containers, therefore each treatment was replicated. The containers were filled with tomatoes weighing 4.55 kg representing approximately forty fruits in number. In order to ensure uniform weight distribution over the tomatoes, bricks of known weight were placed on wooden or plexiglass lids depending on the containers. The lids were slightly smaller than the inside measurement of the containers and were in direct contact with the tomatoes. In the case of the corrugated paperboard containers, corrugated paperboard of the same dimension was placed between the tomatoes and the fitted wooden lid in order to ensure that it was the paperboard and not
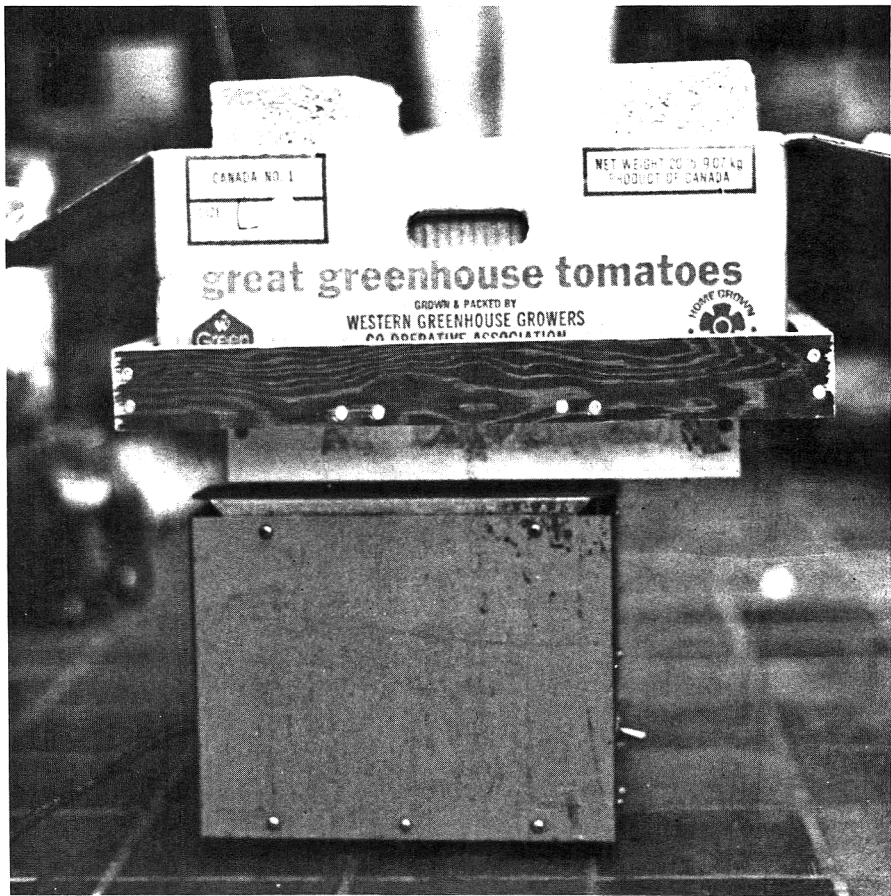

Figure 2. Experimental set-up showing a top-loaded corrugated paperboard box filled with tomatoes. The container was fitted to the linear shaker on a rigid platform that completely supported it thus ensuring uniform pressure distribution.

the wood that was in direct contact with the tomatoes. The compressive loads were 0, 3.63 and 5.45 kg in order to correspond to the three level treatment requirement of the fractional factorial design. Preliminary experiments indicated that lesser loads did not stabilize the tomatoes in the containers against jostling around. These constraining compressive loads of 3.63 and 5.45 kg were about 0.363 and 0.545 kg per tomato assuming that about ten tomatoes sustained the load. Thus, the constraining load was about 3–11% of the bioyield load (Fig. 2).

The variables investigated were (i) container type (corrugated paperboard, plexiglass and wood); (ii) compressive load (0, 3.63 and 5.45 kg); (iii) stage of maturity (green, turning and red); and (iv) vibration (vibration, no vibration). The variables and their combinations results in a total of twenty-seven treatments according to the L^{27} (3^{13}) fractional factorial design by Taguchi (1957). Mechanical damage was evaluated by counting the number of tomatoes that were either pressed out of shape or ruptured in any single test, at the end of each test period. 'Rupture' was used to describe fruits with cuts, cracked or punctured skin, and 'deformation' was used to describe fruits that were permanently pressed out of shape. Mechanical damage in all cases was determined on the basis of the number of tomatoes with each type of fault.

Results and discussion

The average moisture content for the green, turning and red tomatoes used for the study was 94.11, 94.28 and 94.52 and (w, b) respectively. Hunterlab colour difference meter values are given in Table 1. The tomatoes in the three stages of maturity had very different L, a and b values, confirming that the groupings used had distinct colours.

Table 1. Mean values ($n = 6$) for Hunter colour coordinates of tomatoes at different stages of maturity

Stage of maturity	Colour coordinates		
	L	a	b
Green	4.70	– 13.15	– 1.27
Turning	3.91	– 10.48	– 1.56
Red	0.181	– 6.68	– 0.30

Effect of maturity on force-deformation characteristics

The force-deformation parameters were determined for a minimum of 11, 8 and 21 tomatoes at the green, turning and red stages, respectively, and their mean values were used to provide information about the textural characteristics of the tomatoes used for the simulated transit studies. The results are presented in Table 2. The average diameter of tomatoes used in the green, turning and red stages is 5.55 cm.

Ripening leads to a significant decrease in bioyield, toughness and fruit firmness and an increase in compliance. These findings confirm the reports of previous workers (Adegoroye, 1980; Gormley & Keppel, 1976; Hall, 1964; Holt, 1970). The effect of ripening on deformation was not significant while compliance appeared as the most useful parameter for detecting changes in the softness of tomato fruits. From the bioyield values and the calculated cross sectional areas of the tomatoes, the bioyield pressures of 5283, 2852 and 2100 kg/m² were derived for the green, turning and red

Table 2. Mean values for force-deformation characteristics of tomatoes at different stages of maturity

Stage of maturity	Bioyield (kg)	Deformation (mm)	Toughness (kg/mm)	Firmness (kg/mm)	Compliance (mm/kg)
Green (n = 11)	12.78a*	9.79a	64.1a	1.33a	0.824a
Turning (n = 8)	6.90b	9.34a	33.1b	0.753b	1.35b
Red (n = 21)	5.08b	9.30a	24.4b	0.547b	1.90c

*Values in a column with the same letter do not differ at $P = 0.05$ by Duncan's multiple range test.

tomatoes, respectively. These values are an indication of the maximum allowable loads that a tomato can withstand in mechanical handling and storage. It should be realized that in bulk handling the pressure would be distributed among the tomatoes hence they may withstand a pressure higher than the bioyield pressure required to rupture the tomatoes in flat plane loading. However, using these values as a guide, constraining compressive loads of 3.63 and 5.45 kg, as mentioned earlier, were introduced into the simulated transit study in addition to the control in which no weight was introduced. In this experiment, the same weight was used for green, turning and red tomatoes. These constraining compressive loads as mentioned earlier were about 0.363 and 0.545 per tomato assuming ten fruits sustained the load, which represents about 3–11% of the bioyield force.

Effect of simulated transit studies on mechanical damage

The fruits used for the simulated transit studies were examined immediately after the test and following storage at ambient conditions (22–25°C) for 5 days. Preliminary simulated tests indicated that mechanically induced damage which was not too apparent immediately after the test became more pronounced after 5 days. This effect was more evident in fruit at the green and turning stages. Our results were therefore based on measurements taken 5 days after each test.

Signs of mechanical damage such as fruit being pressed out of shape, or fruits with ruptured or punctured skin could be seen in all the treatments, but the severity varied according to the respective treatments. Mechanical damage was also accompanied by characteristic water soaked internal tissue, the magnitude of which varied according to the external symptoms. This observation seems to be in agreement with those of previous workers (Halsey, 1955; McColloch. 1962).

The effects of treatments on mechanical damage (i.e., on the incidence of skin rupture and distorted shapes) are presented in Tables 3–6. As shown in Table 3, skin rupture was significantly affected by the stage of maturity of the tomatoes, vibration of the containers and by the interaction between the compressive load and container type, while the incidence of permanently distorted tomatoes was significantly affected by the compressive load, container type and the stage of maturity of the tomatoes. There were also significant interactions between the compressive load and the stage of maturity as well as the container type and stage of maturity. Table 4 shows that the percentage of tomatoes that were either distorted or ruptured was relatively high at the zero load stage. With increasing load the percentage of both types of damage fell slightly. It would appear that without an applied load there were more opportunities for the tomatoes to move and collide which then resulted in a corresponding rise in the incidence of

Table 3. Treatment and interaction effects on mechanical injury of tomatoes in simulated transit tests

Treatment	d.f.	F ratio	
		Distorted fruit	Ruptured fruit
Compressive load	2	11.62**	0.82
Container	2	44.89**	0.20
Maturity	2	81.96**	13.13**
Vibration	1	2.30	6.24**
Compressive load × Container	4	3.58	3.17*
Compressive load × Maturity	4	11.76**	0.35
Container × Maturity	4	12.66**	0.73

* Denotes significance at $P = 0.05$.
** Denotes significance at $P = 0.01$.

mechanical injury to the tomatoes. By increasing the load to 3.63 kg, the opportunities for movement were considerably curtailed and this seems to have reduced the severity of impacts with a corresponding decrease in mechanical injury. By further increasing the load up to 5.45 kg the incidence of skin puncture and rupture increased, possibly as a result of compression force with more tomatoes now yielding to the applied pressure. The effect of the increased pressure was further exacerbated at this stage because more opportunities were created for skin puncture by the sharp stem ends of the calyx. This force was not sufficient to raise the percentage of fruits permanently pressed out of shape, or distorted, thus suggesting that the fruits could still support more weight before undergoing permanent distortion. Skin puncture by the stem end of the calyx could be minimized by complete removal of calyx from the fruits during harvesting. This practice may, however, create openings for microbial invasion.

The values for mechanically induced injury for the three types of containers and the three stages of maturity were pooled and analysed by Duncan's multiple range test, as shown in Tables 5 and 6, respectively. The analyses revealed no significant differences among the container types in the incidence of fruit rupture, although there seems to be a lower incidence of fruit rupture in the plexiglass containers. In the case of fruits permanently distorted, the analyses showed that fruits packed in plexiglass boxes had the lowest incidence of fruits with permanent distortion. This lower incidence of

Table 4. Percentage of mechanical injuries in tomatoes resulting over a range of compressive loads

Compressive load (kg)	Nature of mechanical injury*	
	Rupture (%)	Distortion (%)
0	17.88	25.44
3.65	13.44	21.33
5.45	21.11	13.44

* Values are the pooled averaged of nine treatments.

Table 5. Effects of container type on mechanically induced injury in tomatoes during simulated transit test

Container type	Nature of mechanical injury*	
	Rupture (%)	Distortion (%)
Wood	18.11a†	31.11a
Corrugated paperboard	18.89a	21.78ab
Plexiglass	15.44a	7.33b

* Values are the pooled average of nine treatments.
† Values in a column with the same letter are not different at $P = 0.05$ by Duncan's multiple range test.

distortion could be possibly due more to the difference in the coefficient of friction than to the difference in the flexibility of the walls of the containers. From all indications it would appear that the smooth surface of the plexiglass box reduced the incidence of mechanical injury in the simulated transit test and hence the incorporation of smooth internal surfaces in the design of containers used for transporting perishable produce may be worth looking into as a way of reducing transit injury due to mechanical causes. With respect to stage of maturity (Table 6) the analyses showed that the stage of maturity at which tomatoes are transported, could have a significant effect on the degree of mechanically induced injury.

Table 6. Effects of stage of maturity on mechanically induced injury in tomatoes during simulated transit test

Stage of maturity	Nature of mechanical injury*	
	Rupture (%)	Distortion (%)
Green	13.56a†	16.00ab
Turning	7.78a	10.00a
Red	31.11b	34.22b

* Values are the pooled average of nine treatments.
† Values in a column with the same letter are not different at $P = 0.05$ by Duncan's multiple range test.

Conclusion

While appreciating the limitations of the test conditions, it is clear from this work that uniform pressure up to a certain limit, applied to the surface of perishable produce in containers, would help significantly to reduce the incidence of mechanical damage during transit. This is thought to be the result of curtailed movement of the produce in the containers which would otherwise have caused impact damage. There were also some positive indications from this work that containers with smooth internal surfaces could cut down on mechanically induced injury of perishable produce in transit,

particularly under the rough road conditions experienced in many developing countries. These findings should have implications in the design and improvement of packaging containers used for the movement of perishable agricultural commodities in developing countries. Returnable plastic containers now being marketed by Mailbox Containers Ltd. Cheshire, England, seem to have this property (i.e. smooth internal surfaces). However, their initial cost may seem prohibitive to the developing countries. With continuous use, the initial investment soon becomes worthwhile. These plastic crates also have the added advantage in that they could help to ensure good sanitary practice. The first author is currently conducting some trial runs with these plastic containers in the Department of Food Technology, University of Ibadan, Nigeria to establish their economic feasibility. Areas of further work should include the design of container covers and the level to which produce should fill the containers in order to curtail movement in transit.

Acknowledgments

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Supplementation of fermented sorghum Kisra bread with legume protein isolates

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Summary

Kisra is a fermented sorghum flour bread which constitutes the staple diet in Sudan. Sorghum, like other cereals, has some limitations due both to its low protein content and to limitations in some essential amino acids particularly lysine. The objective of the present work was to increase the protein content of Kisra and improve the amino acid profile, especially lysine. Protein isolates were prepared from bonavist bean and white bean by alkaline extraction. The protein isolates were used to increase the protein content of the fermented sorghum dough to about 25%. The legume protein isolate supplemented dough was fermented in the traditional method used for making Kisra. The most significant effect of Kisra supplementation was the improvement in protein content (two-fold) as well as the increase in the limiting amino acids. The results indicated a significant increase ($P < 0.01$) in lysine in the protein supplemented doughs and Kisras as compared to the control. The increase in lysine ranged 2–2.6 folds. Sorghum supplementation with legume protein isolates was not accompanied by significant changes in organoleptic properties of the end product.

Introduction

Sorghum (*Sorghum bicolor* (L.) Moench) is the fifth largest cereal crop food in the world and is grown throughout Asia, Africa and North America (Deyoe & Robinson, 1979). It is the most important cereal crop in Sudan. Kisra is a fermented sorghum flour which is baked in a hot plate to form thin sheets of bread having 52–53% moisture when freshly prepared and 60–61% starch, 10–12% protein and 3.8–3.9% crude fibre on dry weight basis (El Tinay, Abdel Gadir & El Hidai, 1979). According to El Hidai (1978) Kisra fermentation was mainly due to lactic acid bacteria *Lactobacillus* sp. and to a lesser degree a yeast, acetic acid and butyric acid fermentations.

Kisra manufacture

Kisra bread is prepared from sorghum flour. The fermented dough, known as 'Ajean', is prepared traditionally by mixing sorghum flour with water in a round earthenware container called a 'Khumara'. A small amount of the previously fermented dough is then added to the mixture of flour and water to act as a starter. After thorough mixing the dough is left to ferment overnight (about 18 hr). The fermented dough is then baked on a hot steel plate (150–160°C) in a process known as 'Aowasa' and is a unique Sudanese art. The flat steel plate is heated with a wood or charcoal fire. When hot (150–160°C) it is cleaned with a piece of cloth containing oil and animal fat. A small amount of the fermented dough (~ 84 g) is spread over the hot plate using a 3'' strip of wood (like a thin ruler). The process of spreading continues till the dough covers the

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plate ($2 \times 2'$) forming a very thin soft sheet of Kisra. The sheet is left on the hot plate for 1–2 sec and is then taken out and is considered ready for eating.

Kisra is the staple food in Sudan and it is estimated that up to 97% of the protein and up to 75% of the calories in the diet of the people residing in Central and Western Sudan is derived from sorghum in the form of Kisra or 'Asida' porridge.

Sorghum, like other cereals has some limitations due to both its low protein content and to limitations in some essential amino acids particularly lysine. Much effort has been focused on methods for producing nutrious foods from plant sources (Bressani, 1975; Horan, 1974; Milner, 1969). Many legumes and oilseeds are high in both protein and lysine. Numerous researchers have reported the preparation and functional properties of protein concentrates and protein isolates from plant, animal and microbial sources (Salterlee, Beribers & Kendrick, 1975; Vananuvat & Kinsella, 1975; Hutton & Campbell, 1977; Ramanatham, Ron & Urs, 1978; Burgess & Kelly, 1979). Such efforts are often aimed at effective utilization of inexpensive proteins for nutritional and functional properties. Protein isolates represent the highest refinement of protein relative to compositional, functional and nutritional properties. Protein isolates are manufactured commercially by alkaline extraction and precipitation at the isoelectric point. The precipitated curd is washed and dried as the isoelectric product or neutralized and dried as the more stable sodium proteinate. Plant protein isolates were produced from faba beans (Bramsnaes & Olsen, 1976), rapeseed (Gilberg & Tornell, 1976) and mung beans (Thompson, 1977). The objective of this work is to increase the protein content of Kisra by addition of legume protein isolates as well as to improve the essential amino acid profile in Kisra bread.

Materials and methods

Bonavist bean (*Dolichus lablab*), white bean (*Phaseolus vulgaris*) and a popular variety: Mayo, were purchased from the local market. The grains were carefully cleaned and freed from foreign material, washed, sun dried and milled into fine powder then passed through 60 mm mesh.

Preparation of legume protein isolates

Preliminary laboratory trials were carried out to establish optimum conditions for producing bonavist bean and white bean protein isolates including extraction of pH, precipitation pH, protein content of the isolate and number of washes. The resulting basic process was used for subsequent large scale preparation of the protein isolates.

The basic laboratory procedure consisted of extracting 5 g of legume flour with 50 ml H_2O adjusting the pH from 1–12 using either 1 NaOH or 3N HCl. The mixture was stirred using a magnetic stirrer during pH adjustment. The extraction mixture was then shaken in an orbital shaker for 15 min at 300 rpm. The pH was readjusted and the mixture was shaken again for 50 min. The extracts were separated by centrifuging at 3000 rpm for 15 min. A liquots from the peptized liquor were taken for nitrogen determination. Protein solubility was plotted against extraction pH and the isoelectric point of the protein determined. Bonavist bean protein was precipitated by adjusting the pH to 4.5 while for white bean protein the pH was adjusted to 4.0. The protein coagulation percentages and the protein content of the precipitate were determined for each extraction pH. The protein precipitates were redispersed with 50 ml H_2O, dissolved at pH 9.0 for bonavist bean and pH 8.0 for white bean and reprecipitated at pH 4.5 and 4.0, respectively. After separation of the protein by centrifuging, it was

given two 50 ml washes at pH 4.5 for bonavist bean and 4.0 for white bean. Each trial was carried out in duplicate and results averaged.

In obtaining protein isolates on a large scale the following factors were taken into account: the pH of highest solubility, the percentage protein coagulation at the iso-electric point and the protein content of the isolates. The basic large scale process for producing protein isolates was based on the laboratory trials. Protein isolates containing 93 and 75% protein were prepared by extracting 1 kg of legume flour with 10 l H_2O at pH 9.0 for bonavist bean and 8.0 for white bean. The protein isolates were washed and drum dried.

Preparation of control dough and Kisra (A)

Fermented dough and Kisra were prepared in the traditional way as used by the housewife. In the control (A) 1 kg of sorghum flour was mixed with 2 l H_2O in a round earthenware container. Previously fermented dough (300 g) was then added to the mixture of flour and H_2O to act as a starter. After thorough mixing samples were taken at 3 hr intervals, in order to follow nutrient changes during the fermentation process since it has been reported that during Ogi (a Nigerian fermented cereal porridge) fermentation there was losses in certain nutrients (Banigo & Muller, 1972), till the end of fermentation which was terminated after 18 hr at room temperature (22–25°C). These samples were designated as A-0 indicating dough at the time of mixing with starter and A-3, A-6, A-9, A-12, A-15 and A-18 indicating samples withdrawn at 3, 6 and 9 hr etc and A-Kisra indicating Kisra from the control dough made at the end of fermentation. These were dried in a vacuum oven at 50°C and finely ground. At the end of the fermentation process the remainder of the dough was baked on a hot plate (150–160°C).

Supplementation of dough with legume protein isolates

Fermentation of legume protein supplemented dough was similar to that of the control sample. Addition of legume protein isolates was adjusted to give a total protein content of about 25% in the fermenting dough.

In formulation B the required amount of white bean protein isolate was added to the mixture of sorghum flour and H_2O and then the starter was added with thorough mixing and left to ferment for 18 hr at room temperature. Samples were withdrawn at the time of addition of starter and this was designated B-0 and at 3 hr intervals till the end of fermentation, designated as B-3, B-6, B-9, B-12, B-15 and B-18. B-Kisra indicates Kisra made from white bean protein isolate supplemented dough at the end of fermentation.

In formulation C the required amount of bonavist bean protein isolate was added to the mixture of sorghum flour and H_2O and then the starter was added with thorough mixing and then left to ferment for 18 hr at room temperature. A sample was withdrawn directly after addition of the starter and was designated as C-0. Samples were then withdrawn at 3 hr intervals till the end of fermentation and were designated as C-3, C-6, C-9, C-12, C-15 and C-18. Kisra made at the end of fermentation of the bonavist bean protein isolate supplemented dough was designated as C-Kisra.

In formulation D the required amount of protein was supplied 50% from white bean protein isolate and 50% from bonavist bean protein isolate which was added to the mixture of sorghum flour and H_2O followed by the addition of the starter with thorough mixing and then left to ferment for 18 hr at room temperature. A sample was withdrawn directly after addition of the starter and was designated as D-0. Samples were then

withdrawn at 3 hr intervals till the end of fermentation and were designated as D-3, D-6, D-9, D-12, D-15 and D-18. Kisra made from the dough at the end of fermentation was designated as D-Kisra.

Chemical analysis

Moisture, crude protein, crude fibre, total and reducing sugars and titratable acidity were determined by the methods of the AOAC (1975). Total soluble solids were determined at 20°C using Abbe refractometer. For amino acid analysis samples were refluxed with 6 M HCl at ambient pressure: sample: volume ratio, 100 mg: 170 ml. Amino acid analysis was carried out on aliquots of the hydrolysate using a JEOL JLC-6AH amino acid analyser programmed for protein hydrolysates. Levels of amino acids were calculated with reference to the response of a standard mixture of amino acids run on the same programme. Peak areas were digitized with an in-line JEOL Model-DK integrator and visualized on a JEOL Model-2 printer.

Organoleptic evaluation

A panel of ten members, composed of adult male and female employees of the Food Research Centre in Khartoum, was used to determine preference of legume protein isolate supplemented Kisra with the unsupplemented control Kisra for colour, 'mouth feel' (texture) and flavour using five points hedonic scale (1 = dislike extremely and 5 = like extremely). Samples tested include freshly prepared sorghum fermented Kisra (control), white bean protein isolate supplemented Kisra, bonavist bean protein isolate supplemented Kisra and 50% white bean protein isolate and 50% bonavist bean protein isolate supplemented Kisra. The order of presentation of the samples was randomized. To determine if the observed differences in judges responses were statistically significant the mean scores were analysed by Duncan's multiple range test (Duncan, 1955).

Results and discussion

Figure 1 shows protein solubility at various pHs. For bonavist bean highest protein extraction occurred at pH 8.0 (90%) while for white bean at pH 12.0 (95%). Protein coagulation was highest (84%) at pH 10.0 for bonavist bean and was highest (87%) at pH 12.0 for white bean (Table 1). However, the protein contents of the isolates were highest at pH 9.0 and amounted to 93% for bonavist bean and at pH 8.0 for white bean and amounted to 75%. The protein content of the isolates was considered the most important factor in protein extraction since high protein content in the isolates would result in minimal addition of ingredients other than protein that might interfere with the sensory quality of Kisra.

Table 2 shows changes in pH, titratable acidity, total soluble solids, crude protein, crude fibre and total and reducing sugars during Kisra fermentation. The pH in the unsupplemented dough (control) dropped from 5.28 to 3.76 at the end of fermentation. Au & Fields (1981) reported a pH drop from 6.7 to 3.8 during the first 2 days of sorghum fermentation and gradually levelled off on the third and fourth day. They also reported a concomitant rise in titratable acidity. Acidity increased from 0.32 to 1.08% expressed as lactic acid. In the white bean protein isolate supplemented dough the pH dropped from 4.40 to 3.71 and acidity increased from 1.24 to 2.01%. In the bonavist bean protein isolate supplemented dough the pH dropped from 4.75 to 3.87 and acidity increased from 0.81 to 1.70%. In the legume protein isolates mixture supplemented dough the pH dropped from 4.50 to 3.70 and acidity increased from 1.01 to 1.94%. Total soluble solids
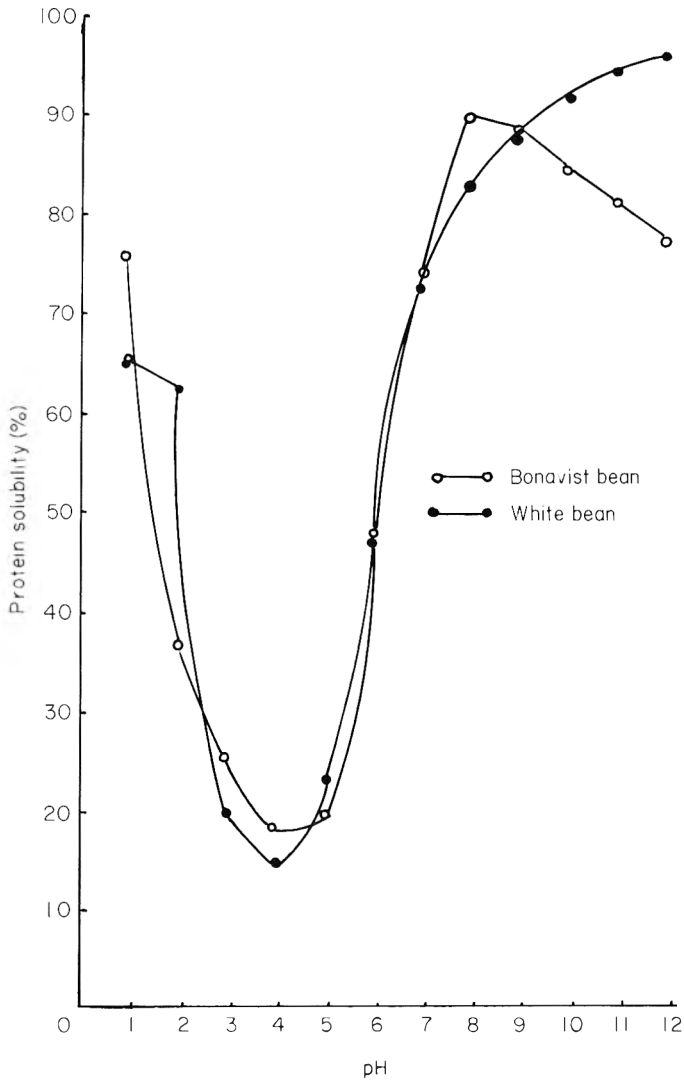
Figure 1. Legume protein solubility at various pH.

were much higher in the protein supplemented dough compared to the control. The general pattern showed an initial increase in soluble solids at the commencement of the fermentation followed by a decrease towards the end of fermentation. Steinkraus (1967) stated that during the fermentation of idli the total soluble solids increased. The crude protein content of the control sample ranged from 12.2 to 13.6%. In the white bean supplemented Kisra the protein ranged from 24.9 to 25.9%; while in the bonavist bean supplemented Kisra the ranges were 24.2–25.8% and 26.5–27.2% in the legume protein mixture supplemented dough. The most significant effect of legume protein isolate supplementation of Kisra on the proximate composition of fermented doughs and Kisras was the improvement in protein content (Table 2). A two-fold increase in protein content was realized. In general there is a slight increase in protein as a result of Kisra fermentation. Total and reducing sugars decreased during Kisra fermentation, while crude fibre fluctuated.

Table 1. Legume protein extraction, coagulation and protein contents of the isolates

pH	Protein extracted (%)		Protein precipitated at the isolectric point (%)		Protein content of precipitate (%)	
	White bean	Bonavist bean	White bean	Bonavist bean	White bean	Bonavist bean
1	65.32	75.53				
2	62.39	36.99				
3	20.25	25.41				
4	14.83	18.39				
5	23.14	19.63				
6	47.43	47.88				
7	72.38	73.98		80.84		90.48
8	82.51	89.53	79.39	82.24	75.05	91.00
9	87.54	87.80	79.89	83.46	72.36	92.75
10	91.57	83.83	82.97	83.92	67.69	88.55
11	94.33	80.89	85.33	83.40	65.84	86.80
12	95.09	77.09	87.30	82.06	61.43	85.75

Table 3 shows the amino acid content of sorghum flour, white bean protein isolate and bonavist bean protein isolate together with amino acid composition of the fermented doughs. The amino acid profile of the legume protein isolates is characteristic of the legume proteins with a high lysine content and low sulphur amino acid content (Pusztai *et al.*, 1979). The lysine content of the sorghum flour is only 41% of reference FAO pattern (World Health Organization, 1973). The lysine level in the white bean protein isolate supplemented Kisra was significantly ($P < 0.01$) increased. The increase was 2.6-fold and represents 77% of the reference pattern. Threonine is significantly ($P < 0.01$) enriched while methionine, leucine and tyrosine dropped significantly ($P < 0.01$). However, the leucine level is adequate compared to the FAO pattern. The lysine content in the bonavist bean supplemented Kisra is significantly ($P < 0.01$) improved compared to the control. The increase in lysine is two-fold and represents 62% of the reference pattern. Cystine, valine, methionine, leucine and tyrosine levels significantly ($P < 0.01$) decreased compared to the control. The leucine and valine levels are however adequate compared to the FAO pattern. The lysine level in the protein mixture supplemented Kisra was significantly ($P < 0.01$) increased. The increase in lysine was 2.5-fold over the control and represents 74% of the reference pattern. Methionine, leucine and tyrosine were significantly ($P < 0.01$) reduced compared to the control. The leucine level however is adequate compared to the FAO pattern. The amino acids isoleucine and phenylalanine showed insignificant ($P < 0.01$) variability between the legume protein supplemented Kisra and the control.

Table 4 shows the mean scores for colour, texture and flavour of the control Kisra together with the legume protein isolate supplemented Kisras. The sensory preference of the supplemented Kisras was to the same extent as that of the control Kisra. This has been achieved with significantly increased protein content and quality over the unsupplemented Kisra (control).

Table 2. Nutrient content of fermented dough and Kisra

Sample	pH	Titratable acidity (% lactic acid)	Total soluble (%)	Crude protein (%)	Crude fibre (%)	Total sugars (%)	Reducing sugars (%)
A-0	5.28	0.32	5.06	12.21	1.66	2.87	2.63
A-3	4.67	0.39	6.33	12.48	1.66	2.62	2.38
A-6	4.33	0.56	8.01	12.39	2.07	2.59	2.55
A-9	4.04	0.70	8.16	12.77	1.82	1.08	1.04
A-12	3.86	0.90	7.96	13.60	1.85	0.94	0.88
A-15	3.81	1.02	6.02	12.67	1.95	0.74	0.67
A-18	3.76	1.08	5.00	12.63	1.83	0.69	0.60
A-Kisra				12.51	2.58	1.00	0.88
B-0	4.40	1.24	10.81	24.90	1.57	3.09	2.86
B-3	4.33	1.18	13.02	24.90	1.96	2.87	2.36
B-6	4.25	1.47	14.02	25.35	1.23	2.68	1.88
B-9	4.13	1.61	13.14	25.25	1.93	2.15	1.32
B-12	4.00	1.71	10.94	25.65	1.49	2.09	1.22
B-15	3.86	1.67	10.54	25.90	1.54	1.05	0.92
B-18	3.71	2.01	10.14	25.55	1.59	1.00	0.85
B-Kisra				25.30	1.57	1.42	1.19
C-0	4.75	0.81	10.01	24.85	1.39	2.24	1.87
C-3	4.70	0.72	10.41	24.20	1.28	1.76	1.31
C-6	4.57	1.00	10.68	25.00	1.67	1.77	1.32
C-9	4.35	1.19	10.01	24.35	1.03	1.68	1.28
C-12	4.12	1.17	9.41	24.65	1.39	1.29	0.77
C-15	4.00	1.76	9.21	25.75	1.38	0.70	0.46
C-18	3.87	1.70	8.34	25.15	1.40	0.96	0.67
C-Kisra				24.95	1.51	1.35	0.96
D-0	4.50	1.01	13.02	26.71	1.34	2.73	2.36
D-3	4.49	1.14	13.22	26.46	1.59	2.62	1.99
D-6	4.34	1.31	12.08	26.94	1.46	2.64	1.67
D-9	4.22	1.50	11.48	25.46	1.59	2.02	1.15
D-12	4.06	1.40	10.61	26.50	1.13	1.90	1.10
D-15	3.89	1.72	10.21	27.18	1.28	1.61	0.72
D-18	3.79	1.94	9.34	27.00	1.48	2.27	1.83
D-Kisra				27.22	1.19	1.43	0.95

A-0: Unsupplemented dough (control) directly after the addition of the starter.

A-3–A-18: Unsupplemented dough (control) at various fermentation times.

A-Kisra: Designates Kisra made from the unsupplemented dough (control) at the end of fermentation.

B-0: Dough supplemented with white bean protein isolate directly after the addition of the starter.

B-3–B-18: Dough supplemented with white bean protein isolate at various fermentation times.

B-Kisra: Designates Kisra made from white bean protein isolate supplemented dough at the end of fermentation.

C-0: Dough supplemented with bonavist bean protein isolate directly after the addition of the starter.

C-3–C-18: Dough supplemented with bonavist bean protein isolate at various fermentation times.

C-Kisra: Designates Kisra made from bonavist bean protein isolate supplemented dough at the end of fermentation.

D-0: Dough supplemented with legume protein isolates mixture directly after the addition of the starter.

D-3–D-18: Dough supplemented with legume protein isolates mixture at various fermentation times.

D-Kisra: Designates Kisra made from legume protein isolates mixture at the end of fermentation.

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Table 3. Amino acid content g/16 g N of sorghum grain, bonavist bean protein isolate, white bean protein isolate, fermented sorghum flour (control), fermented dough supplemented with white bean, bonavist bean and legume protein mixture isolate.

Sample No.	Asp	Thr	Ser	Glu	Pro	Gly	Ala	Cys	Val	Met	Isoleu	Leu	Tyr	Phe	Lys	His	Arg
Sorghum flour	8.88	3.62	5.36	30.53	10.74	4.11	12.13	1.65	7.04	0.91	4.82	17.09	5.55	6.71	2.29	3.46	5.42
Bonavist bean	12.82	3.06	5.10	19.55	3.87	4.05	4.49	0.87	6.35	0.14	4.95	9.44	3.94	5.98	6.06	3.75	7.84
White bean	12.84	3.38	5.31	17.33		3.91	4.27	0.59	6.41	0.62	5.10	8.91	3.92	6.13	5.73	3.56	6.31
A-0	7.94	3.31	4.62	27.77	11.64	3.97	10.67	1.50	6.12	1.11	4.45	16.36	4.74	5.68	1.43	2.99	4.41
A-3	7.91	3.27	4.50	25.44	10.08	3.62	10.89	1.26	6.08	1.24	4.41	16.30	4.71	5.10	1.42	2.82	4.38
A-6	7.73	3.18	4.47	27.91	10.14	3.64	10.97	1.74	6.05	1.30	4.48	16.76	5.04	5.72	1.45	2.8	4.59
A-12	7.73	3.17	4.44	26.46	9.28	3.46	10.25	1.33	5.59	1.23	4.24	15.54	4.84	5.59	1.73	2.83	1.10
A-18	7.68	3.24	4.51	26.86	8.92	3.59	10.53	1.37	5.75	1.10	4.21	15.79	4.76	5.42	1.88	2.70	4.69
A-Kisra	7.07	3.22	4.52	28.08	9.15	3.38	11.33	1.65	5.94	0.77	4.36	15.61	5.04	4.74	1.79	2.61	4.42
Mean	7.8	3.23	4.51	27.1	9.9	3.61	10.64	1.48	5.92	1.13	4.36	16.06	4.86	5.38	1.62	2.8	4.5
S.s.d.	$0.29	$0.05	$0.06	$0.94	$0.91	$0.19	$0.24	$0.17	$0.19	$0.12	$0.10	$0.44	$0.14	$0.35	$0.19	$0.118	$0.116
B-0	10.28	3.56	5.06	20.57	5.31	3.14	6.05	0.85	5.62	0.41	4.35	10.46	3.62	4.46	3.61	2.81	5.64
B-3	10.32	3.60	5.05	19.70	6.41	3.57	5.91	1.50	5.56	0.44	4.32	10.31	3.54	4.42	3.56	2.77	5.76
B-6	10.80	3.57	4.95	19.80	6.51	3.68	6.04	0.75	5.55	0.46	4.34	10.15	3.64	5.28	4.08	2.93	5.49
B-12	10.92	3.71	5.10	19.90	6.61	3.63	5.93	0.83	5.63	0.38	4.26	10.16	3.42	5.05	4.4	2.84	5.52
B-18	11.14	3.68	5.10	20.02	7.03	3.73	6.40	1.01	5.76	0.54	4.43	10.40	3.74	5.42	1.13	3.00	5.43
B-Kisra	12.27	3.95	5.62	21.14	6.84	4.04	6.38	0.92	6.46	0.63	4.83	11.03	3.42	4.36	5.38	3.28	6.41
Mean	10.96	3.68*	5.15	20.2	6.54	3.6	6.12	0.98	5.76	0.48*	4.42	10.42*	3.56*	4.83	4.23*	2.9	5.7
S.s.d.	$0.66	$0.13	$0.22	$0.51	$0.55	$0.27	$0.2	$0.25	$0.32	$0.01	$0.19	$0.30	$0.12	$0.43	$0.62	$0.17	$0.33
C-0	10.31	2.90	4.74	20.62	6.80	3.53	6.27	1.07	5.08	0.28	4.18	11.03	3.86	4.97	2.84	2.82	5.15
C-3	10.45	2.96	4.90	21.30	7.13	3.53	6.38	0.98	5.15	0.33	4.13	11.09	3.82	5.06	2.73	3.01	5.23
C-6	10.20	3.01	4.74	21.00	6.73	3.39	6.34	1.07	5.16	0.42	4.16	10.90	3.82	4.96	3.11	2.78	5.07
C-12	10.60	3.13	4.81	21.93	7.29	3.47	6.53	0.85	5.36	0.37	4.28	11.09	3.99	5.14	3.90	2.99	5.51
C-18	10.24	3.07	4.66	21.64	6.96	3.38	6.22	0.91	5.26	0.46	4.20	10.85	3.94	4.99	3.75	2.78	5.10
C-Kisra	10.49	3.07	4.72	21.30	6.88	3.45	6.17	1.12	5.23	0.34	4.20	10.67	3.84	4.88	4.20	2.85	5.26
Mean	10.4	2.02*	4.76	21.3	6.97	3.46	6.32	1.00*	5.21*	0.37*	4.19	10.94*	3.88*	5.00	3.42*	2.9	5.22
S.s.d.	$0.143	$0.08	$0.08	$0.42	$0.19	$0.06	$0.118	$0.10	$0.09	$0.06	$0.05	$0.15	$0.12	$0.08	$0.56	$0.09	$0.15
D-0	11.09	3.51	5.00	21.05	6.83	3.67	5.65	0.79	5.62	0.51	4.36	10.57	3.61	5.13	3.46	2.84	5.39
D-3	11.20	3.55	5.00	21.02	6.06	3.71	5.71	0.84	5.72	0.48	4.35	10.57	3.58	5.08	3.57	2.83	5.35
D-6	10.43	3.39	4.72	19.89	6.10	3.49	5.53	0.89	5.58	0.49	4.20	10.34	3.73	5.05	3.73	2.70	5.25
D-12	10.90	3.35	5.00	20.01	6.45	3.58	5.61	0.78	5.58	0.47	4.28	10.06	3.84	5.21	4.18	2.87	5.47
D-18	11.12	3.47	5.10	19.93	6.13	3.77	5.92	1.29	5.66	0.33	4.42	9.94	3.91	5.36	4.66	3.10	5.80
D-Kisra	10.69	3.25	5.09	19.66	6.39	3.54	5.88	1.40	5.17	0.41	4.21	10.56	3.95	5.21	4.72	2.91	5.78
Mean	10.91	3.42	4.99	20.4	6.33	3.63	5.72	1.00	5.56	0.45*	4.3	10.34*	3.77*	5.18	4.05*	2.9	5.5
S.s.d.	$0.27	$0.10	$0.13	$0.61	$0.27	$0.10	$0.14	$0.25	$0.18	$0.06	$0.08	$0.26	$0.14	$0.10	$0.50	$0.12	$0.21

*Significantly (P 0.01) different from control.

Table 4. Mean scores Ss.d. for sensory attributes of Kisras

Sample	Flavour	Colour	Texture
Unsupplemented Kisra (control)	4.6S0.49[a]*	3.9S0.30[b]	3.4S0.66[c]
White bean protein isolate supplemented Kisra	3.9S0.54[a]	3.7S0.64[b]	3.6S0.49[c]
Bonavist bean protein isolate supplemented Kisra	4.0S0.45[a]	4.0S0.45[b]	3.9S0.54[c]
Legume protein isolate mixture supplemented Kisra	4.2S0.40[a]	4.1S0.30[b]	3.8S0.40[c]

*Means in a column sharing the same letter are not significantly different (P ~0.05) as determined by Duncan's multiple range test.

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Development of a high energy biscuit for use as a food supplement in disaster relief

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Summary

International aid agencies undertake supplementary feeding programmes following disasters in order to alleviate or prevent protein energy malnutrition. The associated problems include lack of facilities for preparation and distribution of food on site and food hygiene dangers. In response to these factors a biscuit of high calorific value and acceptable organoleptic qualities has been developed. It is currently being used by Oxfam as part of their disaster relief programme in Ethiopia.

Introduction

Food aid is currently supplied to 'less-developed' countries via government departments and the non-governmental aid agencies of Western nations. Much of this food aid is derived from agricultural surpluses produced in the U.S.A. and EEC, a small proportion of which is used in emergency or acute disaster situations, for example the Ethiopian drought in 1984–1985. Once such emergencies have been acknowledged by aid agencies, it is of great importance that food distribution should begin as quickly as possible and that the food is suitable for use during the disruption found in an acute disaster.

It is the policy of aid agencies to provide food supplements with the aim of preventing protein energy malnutrition among groups within the population that are described as 'vulnerable', for example: young children, pregnant and lactating women and the sick (Peel, 1979). Oxfam recommend that a food supplement containing 500 Kcal (2.09 mJ) (8% of which should be supplied by protein), should be eaten each day (Oxfam, 1980).

Wherever possible aid agencies use indigenous foods as supplements to prevent the creation of dependence on imported foods. However, indigenous foods are often in short supply and the use of imported foods then becomes necessary. Bulk supplies of dried milk and blends of cereals are both used during disaster relief operations, often with the addition of edible oils to increase the energy density of the supplement. These foods need to be reconstituted on site, requiring water (which may be contaminated and or in short supply), cooking fuel, and cooking and serving utensils.

The use of biscuits to achieve the required levels of energy and protein supplementation has been proposed by a number of authorities (Buchanan & Townsend, 1969; Chablaix, 1980; Tsen *et al.*, 1973). A review of the composition of biscuits produced recently for use in food aid programmes as shown in Table 1.

These biscuits possess a long shelf life which enables large scale production and storage to take place prior to distribution. The low moisture content enables economical transportation and a reduced potential health hazard from microbial contamination.

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Table 1. Composition of biscuits designed for use as food supplements

Name of biscuit	Energy (KJ)	(Kcal)	Protein (%)	Fat (%)	Carbohydrate (%)	Moisture (%)	References
Australian milk biscuit	1880	449	20	20	50.4	< 3	Buchanan & Townsend. 1969
New Zealand milk biscuit	2002	478	24	24.8	42.3	—	Chapman. 1968
Indian milk biscuit	2259	539	16.6	28.6	57.4	2.6	Bassi & Sukamar. 1973
Dutch biscuit	1765	422	20	11	64.8	2	Anon. 1977
Ugandan peanut biscuit	2058	492	20.9	26	—	—	Clegg. 1960
American biscuit	2079	497	15.1	26	54.2	4.7	Ranhotra. 1980

The protein content of the biscuits described in Table 1 is higher (16–24%) than commercial products available in the U.K. (approximately 4–6%). This was because the dominant body of opinion at the time of their development emphasized lack of protein in the diet as being the major nutritional deficiency. However, it is now realized that adequate supplies of energy are necessary to enable proteins to be used for growth and repair of body tissues (MacLaren, 1974). The high protein content of the biscuits often resulted in a product which was unacceptable in taste to the intended recipients. particularly young children. The strong flavour of the Australian Milk Biscuit (Bolin & Davis. 1970) and the bitter gummy mouth feel of the NDRI Milk Biscuit (Buchanan. 1971) have caused these biscuits to be unacceptable.

In the light of these studies. it is essential that a biscuit intended for use as a food supplement be judged as acceptable for infant consumption. A shelf life of 6 months in varying climatic conditions would benefit distribution and each daily supplement should be 'energy-dense', for example 500 Kcal (2.09 mJ) per 100 g (Lusty, pers. comm.). This paper describes the development of a biscuit which is able to meet the above criteria. at a cost which is sufficiently low to enable economic production.

Materials and methods

Ingredient formulation and baking

The biscuit formulation comprised soya oil. butter oil. wheat flour. castor sugar, dried skimmed milk, whole egg, salt and water, the proportions being varied according to experimental requirements. In preliminary experiments the 'single stage' method of dough preparation was followed using a mixer fitted with a standard 'K' beater. The dry ingredients were blended and the oil and liquid egg added. The whole was then mixed for 40 sec. In subsequent studies the 'multi stage' method of dough preparation was employed (Matz & Matz, 1978). Dried milk and oils were creamed for 30 sec. prior to the addition of the other ingredients.

In both methods the resulting dough was kneaded by hand for 15 sec and then rolled to a uniform thickness of 12 ± 2 mm. The rolled dough was cut by hand to form biscuits which measured $75 \times 30 \times 12$ mm and weighed 35 ± 2.25 g.

The biscuits were baked in a Carter Simon rotary oven, at 4 rpm. Temperature and time of baking were varied according to the experimental requirements.

Analyses

Selected formulations were examined using untrained taste panels consisting of twenty people, in individual booths, under standardized conditions (Amerine, Pangbourne & Roessler, 1965). The panellists were asked to rank one group of six biscuit samples for colour, brittleness, taste and overall acceptability. Statistical significance (5% level) of the rank totals was calculated using standard methods (Amerine *et al.*, 1965).

Analyses for ash, moisture, fat and nitrogen in both ingredients and biscuits were made using standard methods (Egan, Kirk & Sawyer, 1981).

Results and discussion

Butter oil, soya oil and dried skimmed milk powder were included in the biscuit formulations, as they are widely used as food supplements by aid agencies (Ville de Goyet, Seaman & Geiger, 1978). Wheat flour was chosen as the cereal, because to aid agencies it is cheaper than other available cereals, it provides a substantial amount of protein and it possesses desirable baking characteristics.

Preliminary experiments

The aim of the initial experiments was to produce a biscuit of the correct nutritional value. To obtain the correct amounts of energy and protein in the biscuit, the proportions of fat, protein and carbohydrate in the formulation were adjusted to 30%, 9% and 55% respectively. The effect of using soya oil or butter oil and the effect of the addition of liquid (whole egg or water) on the acceptability of the biscuit, were examined.

Table 2. Biscuit formulations used in preliminary experiments

Formulations	1		2		3		4	
	g	%	g	%	g	%	g	%
Soya oil	—	—	160	29	—	—	160	27
Butter oil	160	29	—	—	160	27	—	—
Water	—	—	—	—	20	3.5	20	3.5
Whole egg	—	—	—	—	20	3.5	20	3.5
Castor sugar	62.5	11	62.5	11	62 5	11	62.5	11
Dried milk	75	14	75	14	75	13	75	13
Wheat flour (household plain)	250	36	250	46	250	42.5	250	42.5

Four formulations (Table 2) were prepared using the single stage method of dough preparation (Matz & Matz, 1978). Doughs produced from each formulation possessed poor cohesion and broke easily. The formulations containing no added water and egg (nos. 1 and 2; Table 2) were difficult to roll or cut and broke into fine crumbs. The absence of water or whole egg is likely to have prevented the partial devlopment of the gluten matrix and hence the observed lack of cohesion.

The biscuits which contained added water and egg were found to have a more uniform texture and produced less surface browning. All the biscuit formulations had a uniform surface colour and their interior showed no evidence of non-enzymic browning reactions. Differences in taste, between those biscuits made with soya oil and those

made with butter oil were discernible, but neither taste was thought to be disagreeable by the investigators.

Due to the handling problems encountered with the single stage mixing method, the multi stage method was examined. It produced a dough consisting of large crumbs, which was more adhesive on manual kneading. This method was therefore adopted in subsequent studies.

These preliminary studies resulted in a biscuit of the correct nutritional value, but the effect of other factors including the quantity and type of liquid, the ratio of butter to soya oil and baking conditions were studied to obtain a more acceptable biscuit in terms of taste, texture and appearance.

Effect of time and temperature of baking

Prepared biscuits were baked at various temperatures and times in order to determine the combination required to produce a biscuit of suitable appearance and texture, which contained less than 5% moisture. The shape and dimensions of the biscuit also affect the baking time and biscuit quality. A thinner, flatter biscuit requires a shorter baking time and has more even moisture distribution than a thicker 'finger' biscuit of the same weight. However non-enzymic browning reactions are more likely throughout the thinner biscuit and as lysine, which is the limiting amino acid in wheat flour, is involved in browning, and becomes nutritionally unavailable, it is desirable to limit browning. The dimensions of the biscuit ($7.5 \times 5.0 \times 0.7$ mm) were therefore chosen as a compromise between economy of baking and minimum loss of lysine.

Dough handling qualities

The quantity of water in the recipe, the inclusion of egg and the ratio of butter oil to soya oil were each examined for their effect on dough handling properties and biscuit quality. The composition of the formulations is presented in Table 3.

The results indicated that the ratio of fats had no effect on the consistency of the dough. This only varied with the amount of water added. The formulations which contained more than 40 g water produced greasy doughs, which were fragile and

Table 3. Biscuit formulations examined for their effect on dough handling and biscuit quality

Variables:							
Egg			20			0	
Water		20	40	60	20	40	60
		Formulation numbers					
Soya oil (g)	80	1	4	7	10	13	16
Butter oil (g)	80						
Soya oil (g)	120	2	5	8	11	14	17
Butter oil (g)							
Soya oil (g)	140	3	6	9	12	15	18
Butter oil (g)	20						
Standardized ingredients: (g)							
Wheat flour				250			
Dried skimmed milk				75			
Salt				2			
Castor sugar				62.5			

difficult to roll. It is likely that the additional water in the recipe may have made it difficult for a stable emulsion to form within the dough, thus allowing the oil to partially separate out. In addition these doughs each contained a more adhesive crumb structure, which suggests that they underwent greater gluten development, than those doughs containing less than 40 g water. The formulations which contained 40 g or less of water produced a dry crumbly dough which rolled and cut well. These were therefore used in subsequent studies.

Sensory analysis

The formulations were randomly assigned into three sets to be assessed by three taste panels. The two most acceptable biscuits from each of the three groups were then placed in a fourth group and ranked by a fourth taste panel to determine the most acceptable biscuit.

The results (Table 4) indicate that the acceptability of the biscuits was affected by the quantity of water or egg in the formulation. The formulations which contained 60 g water and 20 g egg (Table 3; nos 7–9) were least preferred for taste and recipes 8 and 9 were least acceptable (Table 4).

Table 4. Acceptability rank totals for the four taste panels

Taste panel number							
1		2		3		4	
Biscuit*	Rank total	Biscuit*	Rank total	Biscuit*	Rank total	Biscuit*	Rank total
14	41†	12	51†	11	44†	11	64
15	58	4	85	10	51†	5	85
6	77	16	67	8	98‡	12	52†
18	82	13	49†	17	60	10	50†
2	72	5	61	3	63	14	83
1	69	7	86	9	105†	13	84

* See Table 3 for biscuit formulation.
† Significantly more acceptable (5% level).
‡ Significantly less acceptable (5% level).

The formulations which contained 40 g water or less and no egg were found to be significantly more acceptable and were therefore selected to be assessed by the fourth taste panel (Table 4). Of these recipes, four were found to be significantly darker in colour and three were significantly more brittle, which suggests that these qualities enhanced the acceptability of the biscuit.

The panellists did not appear to have preferences regarding the ratios of butter oil to soya oil. It is likely that the untrained panel regarded texture as of more importance to acceptibility than taste.

The taste panellists were not however representative of the expected recipients of the biscuits, who would be expected to originate from a variety of cultural backgrounds. Acceptability trials were therefore carried out in nursery schools in England, and India using formulation no. 10.

In England, thirty-six out of thirty-eight Asian and Caribbean children accepted and ate a biscuit. Two children would not accept the biscuit and two children did not finish their first biscuit. They were identified by the head mistress as not being accustomed to eating Western foods in their traditional home diet. More than 50% of the children ate a second biscuit and some a third. No distributed biscuits were left uneaten, indicating a high level of acceptability.

In India, children attending a village nursery school, received 100 g of biscuits daily for a period of 2 weeks. The children were aged between 9 months and 9 years and were generally from lower caste families. Attendance at the school doubled to approximately sixty after the first day of distribution and was sustained for the 2-week trial. Acceptability of the biscuits, as rated by the school supervisors, was consistently high (Mitchell, 1983).

Chemical analyses

The results from the chemical analysis are shown in Table 5. The composition of the biscuit was also calculated from food composition tables (Paul & Southgate, 1978). The energy level which was calculated from the chemical analyses was lower than the figure calculated from food composition tables, probably because the chemical analyses only considered extractable fat and any fat not extracted was ignored in the energy calculation. The protein content of the wheatflour was found to be lower than the literature value.

Table 5. Composition of biscuit formulation '10' and wheat flour

		Results of chemical analyses		Composition according to food composition tables (Paul & South-gate, 1978)	
		Flour	Biscuit	Flour	Biscuit
Water	(%)	13.2	3.5	13.0	6.0
Protein	(%)	7.8	8.4	9.8	9.4
Fat	(%)	ND	27.6	1.2	29.7
Ash	(%)	2.6	2.4	ND	ND
Carbohydrate (by difference)	(%)	ND	57.9	80.1	52.6

ND. No data available.

Conclusions

The final recipe that was adopted was satisfactory in achieving the required nutritional value and palatability, simultaneously with good baking characteristics. The indications of the feeding trial carried out with children from different cultural backgrounds suggest that the biscuit would be suitable for use in a number of countries. Although some children were unaccustomed to eating biscuits in their traditional diet, it is likely that they would be accepted for a short duration following a disaster. The children who ate three biscuits took an extra 2.09 mJ and approximately 8 g protein, which represents a valuable addition to their diet. The biscuit is highly palatable to young children and it

seems unlikely that they would want to consume the same quantity of energy and protein in the same time if the food was in a less energy dense form.

In certain disaster situations, where there is insufficient indigenous food, the biscuit is a useful supplement as it overcomes many of the problems encountered in supplementary feeding programmes, in particular, preparation and distribution problems and dangers associated with poor hygiene during food preparation.

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Pickling of dates at the early khalal stage

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Summary

The possibility of pickling dates at the khalal stage was investigated. The effect of boiling, lye treatment, cutting and brine solution were studied. The results of this preliminary study suggest that a good date pickle could be obtained using a brine solution of 40 salometer and adding acetic acid. Cutting and lye treatment of fruits were not necessary, while boiling of fruits for 5 min was helpful to produce an acceptable product.

Introduction

Dates (*Phoenix dactylifera*) are considered a major fruit crop in Saudi Arabia with an annual production of about 400000 tons. Date fruits pass through five growth and developmental stages, i.e. hababauk, kimri, khalal, rutab and tamar (Dowson & Aten, 1962). Khalal stage is marked by the turning of the colour of the skin from green to yellow, pink or scarlet according to variety. The high nutritive value of dates was reported by Dowson & Aten (1962), and Yousif *et al.* (1982). In addition, the substantial quantities of dates produced in Saudi Arabia and their popularity among the whole population justify their processing and utilization in pickle preparation.

Pickles as flavourful products represent a palatable and safe way to preserve foods. The skilful blending of spices, sugar and vinegar with fruits and vegetables give crisp, firm texture and pungent sweet-sour flavour. Pickles may be classified as relishes, fresh pack pickles, brined pickles and fruit pickles (USDA, 1973). The preparation ranges from prolonged fermentation period for brined pickles, through very limited fermentation in fresh pack pickles, to no fermentation in fruit pickles (McWilliams & Paine, 1977).

Green olives are pickled after harvesting the fully sized unripe fruits. At this point, the fruits contain a bitter alkaloid, oleurepen, which must be controlled. The bitterness is removed by treating the olives with a 2% sodium hydroxide solution at room temperature. In Spain a brine of 44 salometer is used for pickling of olives, whereas a 50–54 salometer brine is used in California (Cruess, 1958).

Recently, Alodaidi, Albaradei & Abdelmasih (1982) studied the possibility of pickling Iraqui Zahdi dates at the kimri stage. Factors affecting product quality from the view point of full, segmented and pricked fruits, type of pickling solution (NaCl 15%, NaCl 15% +2% acetic acid and NaCl 15% + some green pepper) and pickling period were studied. They reported that a satisfactory product was obtained after a 6-week pickling period for each kimri preparation treated with NaCl 15% +2% acetic acid.

The present study was undertaken to study the possibility of pickling dates at the khalal stage. The effect of some variables such as boiling, lye treatment, cutting and brine solution concentration on the quality of pickled dates were studied.

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Materials and methods

Date fruits of Um-Rhaim variety in the early khalal stage were procured in July 1984 from Al-Shaawan Farm, Al-Hasa. A bunch weighing about 30 kg was used in the pickling experiments. Fruits were separated, cleaned and prepared for pickling. The fruits were divided into four portions of 7.5 kg each. Each portion was treated with boiling, lye treatment, cutting or brine solution.

Estimation of some physico-chemical properties of the date fruit before pickling

Fruit weight, length and diameter. Triplicate samples each of ten fruits were taken randomly for measurement of weight, length and diameter. Each fruit was weighed to the nearest 0.1 g and measured for length and diameter to the nearest 0.1 cm.

Moisture. Triplicate 10 g destoned and cut date fruit samples were weighed in aluminium dishes and placed in an electrical oven at $100 \pm 1°C$ for 16 hr (AOAC, 1975).

Total soluble solids. Total soluble solids (°Brix) were determined using a refractometer.

Pickling of date fruits

The effect of four variables (brine solution concentration, lye treatment, boiling and cutting) on the pickling of date fruits were taken into consideration in this experiment.

Brine solution concentration. Three treatments of brine solution (40, 40+2% acetic acid and 60 salometer*) were used in this study. Fifty date fruits (about 250 g) were filled in 250 ml glass jars and covered with the brine solution at ratio of 1:1 w/v. After 5 weeks the pickled dates were evaluated organoleptically.

Lye treatment. Due to bitterness in date fruits in the kimri and khalal stages which is associated with the presence of tannins and other polyphenolic compounds, lye treatment was carried out to effect the waxy skin of the fruit aiding the release of polyphenolic compounds from the fruits. Date fruits were soaked in 1.0% and 0.5% sodium hydroxide solution for either 8 or 16 hr. The dates were then taken out, washed four times with water, filled in glass jars, covered with 40+2% acetic acid salometer brine solution and kept for 5 weeks and then evaluated organoleptically.

Cutting. Date fruits were cut vertically on two sides and then put in three glass jars, covered with the three brine solutions described above and kept for 5 weeks, after which they were evaluated organoleptically. (Three other glass jars contained uncut dates and covered with the various brine solutions were used as a control.)

Boiling. The effect of boiling the fruits for 5, 10 and 15 min on the quality of pickled dates were also studied. Three jars each containing fifty fruits and covered with one of the three brine solutions described above, for each duration, were prepared. The pickled dates were evaluated organoleptically after 5 weeks.

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*Percent salt in solution ×4 = degree salometer.

Organoleptic evaluation of the pickled dates

The pickled dates were evaluated organoleptically by judges at the Date Palm Research Centre. Each test was carried out twice during 1 week using the special test sheet as described by Larmond (1970). Results were tabulated and analysed statistically using analysis of variance. Different tests (Larmond, 1970) were used for the organoleptic evaluation of the pickled dates. The paired comparison preference test was used to study the importance of cutting and lye treatment in the date pickle making. In addition, the effect of lye treatment on the pickle acceptability, as well as the effect of boiling, was also evaluated by using multiple comparison difference test. Furthermore, the effect of brine solution concentration on the pickle quality was studied using the ranking preference test.

Results and discussions

The approximate physico-chemical analysis of the date fruits in the early khalal stage are presented in Table 1. Results of Table 1 indicate that the fresh khalal dates contain a higher moisture percentage (80%) compared with those of olives (70%) (Desrosier & Desrosier, 1977). On the other hand, Alogaidi *et al.* (1982) reported a value of 84% of moisture for Iraqi dates used in pickling at the kimri stage. It is also clear from Table 1 that the khalal dates used contain a comparatively high total soluble solids values (Brix) which are mostly sugars.

Table 1. Approximate physico-chemical analysis of Um-Rhaim date fruit in the early khalal stage

Fruit weight (g)	Fruit diameter (cm)	Fruit length (cm)	Brix (degrees)	Moisture (%)
4.8–5.0	1.65–2.0	2.2–2.6	13–17	80

The effect of brine solution concentration on the quality of the produced pickled dates was determined organoleptically. The analysis of variance for the results of the ranking test and the significance of the mean scores for the tested date pickle samples are tabulated in Tables 2 and 3.

From the results of the ranking tests it can be seen that the panellists preferred sample B which was pickled in brine solution of 40 salometer and containing added acetic acid since nine of the thirteen panellists ranked it as the first. The results of Tables 2 and 3 also revealed that sample B which contained the added acetic acid differed significantly ($P = 0.10$) from sample C which was pickled in the high brine solution (60

Table 2. Analysis of variance for three samples of pickled dates. A, B and C prepared in 40, 40 + 2% acetic acid and 60 salometer respectively

Source of difference	(df) Degrees of freedom	(SS) Sum of square	(MS) Mean of square	(F) value (calculated)	(F) value (tabulated)
Samples	2	7.12	3.56	7.12	3.5 (5%)
Panellist	12	0.00	0.00		
Error	24	11.86	0.50		5.61 (1%)
Total	38	18.98	0.50		

Table 3. Significance of mean scores for three date pickle samples, A, B and C pickled in 40, 40+2% acetic acid and 60 salometer

Samples	5% Significance level difference is more than 0.80	1% Significance level difference is more than 0.83
A, B	No	No
B, C	Yes	Yes
A, C	No	No

salometer). These results are in agreement with those obtained by Alogaidi *et al.* (1982). It could be concluded that good quality date pickles could be obtained by using a brine solution of 40 salometer containing added acetic acid.

The effect of cutting on the quality of the pickled dates is given in Table 4. It is clear that sample D (with cuts) did not differ significantly from sample E (without cuts). This means that cutting may not be necessary in the processing of pickled dates.

Table 4. Results of paired comparison preference test conducted on two date pickle samples, D and E in which D had two vertical cuts while E had none

Total number of panellists	Number of panellists preferring sample D	Number of panellists preferring sample E	Number of preferences for one sample to have significant differences
11	5	6	10

Table 5. Results of paired comparison preference test carried out on two date pickle samples, F and G which had been lye treated*

Total number of panellists	Number of panellists preferring sample F	Number of panellists preferring sample G	Number of preferences for one sample to have significant differences
11	5	6	10

*F: This date pickle sample was soaked in 1% NaOH for 16 hr.
G: This date pickle sample was soaked in 1% NaOH for 8 hr.

Table 6. Results of paired comparison preference test carried out on two date pickle samples, H and I which had been lye treated*

Total number of panellists	Number of panellists preferring sample H	Number of panellists preferring sample I	Number of preferences for one sample to have significant differences
11	5	6	10

*H: This date pickle sample was soaked in 0.5% NaOH for 8 hr.
I: This date pickle sample was soaked in 1% NaOH for 8 hr.

Table 7. The multiple comparison test mean scores and the analysis of variance for two date pickle samples, M and N which have been lye treated (in 0.5% and 1.0% NaOH solution respectively) and compared with a control (untreated sample)

| | | Sample No. | | |
	Panellist	(M)	(N)	Total
Total	12	66	61	127
Mean		5.5	5.08	

Analysis of variance

Source of difference	df	SS	MS	F value (calculated)	F value (tabulated)
Sample	1	1.04	1.04		
Panellist	11	34.46	3.13		
Error	11	37.46	3.40	0.30	4.75 (0.05)
Total	23	72.96	3.17		9.33 (0.01)

Table 8. The sensory evaluation mean scores and the analysis of variance for three date pickle samples, O, P and Q, which have been pickled in a brine solution of 40 salometer + 2% acetic acid and boiled for 5, 10 and 15 min

| | | Sample No. | | | |
	Panellist	O	P	Q	Total
Total	9	39	49	49	137
Mean		4.33	5.44	5.44	

Analysis of variance

Source of difference	df	SS	MS	F value (calculated)	F value (tabulated)
Sample	2	7.41	3.70	1.33	3.63 (5%)
Panellist	8	51.86			
Error	16	44.59	2.78		6.23 (1%)
Total	26	103.86			

The effects of the duration and concentration of lye treatment on the quality of the produced date pickle are presented in Tables 5 and 6. Here also, no significant difference could be noticed either in the effect of duration of the lye treatment (8 or 16 hr) or the concentration of the lye solution (using 0.5% or 1% NaOH) on the quality of the produced date pickle. Moreover, it is obvious from results of Table 7 that lye treatment did not affect significantly the acceptability of the product.

The effect of boiling of date fruits for 5, 10 and 15 min on the quality of the produced pickles are shown in Table 8. Forty salometer and added acetic acid brine solution was

used in preparing the pickles. Results of Table 8 reveal that no significant differences could be observed between the three samples boiled for 5, 10 and 15 min, since the calculated F value (1.33) was less than the tabulated one (3.63). Furthermore, the results of the multiple comparison difference test used in comparing the boiled samples (5, 10 and 15 min) with a control (unboiled sample) (Table 8), showed that pre-boiled produce for 5 min was preferred to the control (unboiled). Therefore, it is suggested that in date pickling, boiling fruits for 5 mins enough. Further studies involving physico-chemical changes as well as fermentation will be carried out to substantiate the result of this preliminary work.

Acknowledgment

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Anthocyanin colourants from elderberry (*Sambucus nigra* L.). 1. Process considerations for production of the liquid extract

KIRSTEN BRØNNUM-HANSEN*, F. JACOBSEN† AND J. M. FLINK

Summary

Elderberry is shown to be a good source of anthocyanins (Acy), having between 2–10 mg Acy/g berry. Anthocyanin concentration is very dependent on variety and maturity. Pressing of the berries gives a significant difference between weight distribution (pomace = 25–40% of total weight) and anthocyanin distribution (Acy in pomace = 75–98% of total Acy). Freezing of the berries prior to pressing gives much less pomace weight, with only a small reduction of anthocyanin distribution in the pomace. Tests of various extraction media (HCl acidified alcohols, aqueous HCl and aqueous citric acid) showed the highest anthocyanin recovery for aqueous HCl (0.1 M). Multiple extraction tests show that most of the anthocyanin is extracted from the pomace within two to three steps. The influence of extraction time and extraction temperature is discussed.

Introduction

Interest in natural colouring agents from various fruit and vegetable materials has increased in the recent past, as questions regarding the safety of artificial colours have been raised. Included among the natural colouring agents which have been investigated are anthocyanins from many fruits, and in several review articles, the anthocyanin compositions of these materials have been presented (Timberlake, 1980; Francis, 1977; Shrikhande, 1976). The most frequently investigated raw materials have been cranberry and grape, as the solids remaining after juice/wine production can be a good, relatively inexpensive source of anthocyanins. In Denmark, elderberry is cultivated for production of various food products, including juice and soup. In these cases there will be solids remaining which contain a large amount of anthocyanins.

To obtain anthocyanins from the solids remaining from juice production, it is necessary to conduct an extraction. Several solvent systems have been investigated as extraction media for various raw materials; most studies have settled on acidified methanol or ethanol solvents. For example, Fuleki & Francis (1968a) used 95% EtOH-0.1 N HCl and 95% EtOH-1.5 N HCl blends to extract anthocyanin from cranberry, while Chiriboga & Francis (1970; 1973) recommend MeOH with 0.03% HCl for cranberry pomace. Metivier, Francis & Clydesdale (1980) indicated that HCl

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acidified MeOH is 20% more effective than the corresponding acidified EtOH for the extraction of wine pomace. They also investigated HCl in water, and a number of organic acids in water or EtOH. With HCl acidification. MeOH was found to be 73% more effective than water. Citric acid was the most effective acid with EtOH, and acetic acid the most effective with water. Other solvent systems investigated have included: MeOH-tartaric acid for grape pomace (Philip, 1974), SO_2/water for grape pomace (Palamidis & Markakis, 1975), and for blueberry and red cabbage (Shewfeldt & Ahmed, 1977; 1978) and a variety of buffers for sunflowers (Pifferi & Vaccari, 1983).

In addition to choice of solvent, other factors such as solids-solvent ratio, extraction time, and sample freeze-thaw have been investigated (Pifferi & Vaccari, 1983; Metivier *et al.*, 1980; Chiriboga & Francis, 1973; Sapers, Jones & Maher, 1983).

In Denmark, elderberry is cultivated for production of juice and other products. As elderberry has a very intense, dark colour, indicative of a high anthocyanin content, the current study was undertaken to investigate the extraction behaviour of elderberry pomace as the first step for the production of a dried anthocyanin colouring material.

Materials and methods

Raw material

Elderberries were obtained from the research orchards (Pometet) at The Royal Veterinary and Agricultural University's Research Farm at Tåstrup, Denmark. Harvesting was generally conducted in September to October. As the studies reported here were conducted over several years (between 1977 and 1984), climatic based variation of raw material composition is unavoidable. In one study twenty-six different research varieties were investigated. Generally, a limited number of varieties were used. The elderberries were removed from their stems by hand and those elderberries not used for studies with fresh material were frozen and stored at $-25°C$. Frozen material was thawed by placing at room temperature overnight.

Standard pressing of elderberry

Fresh or frozen/thawed elderberries were pressed in a laboratory hydraulic press. After a study on the influence of pressing conditions on anthocyanin yield, a standard pressing procedure was established. In this standard pressing procedure, 100 or 200 g of elderberries (depending on the amount of pomace required for extraction) were subjected to a pressure of 100 atm for 10 sec. The pressure was then allowed to fall for 2 min (generally falling close to 0), after which it was released.

Standard extraction of elderberry pomace

In the initial phase of this study, an EtOH-HCl extraction (Fuleki & Francis, 1968a) was used to evaluate anthocyanin concentrations. Following the investigations of extraction solvent effectiveness, a standard extraction procedure was developed, which was used in the remaining phases of the study. In the standard extraction procedure, a 5 g sample of pomace is macerated for 2 min in a blender with 100 ml of 0.1 M aqueous HCl. The disintegrated pomace is then extracted for between 2–30 min with stirring, after which it is filtered on a fritted glass filter. (In some early studies, the samples were also centrifuged for 10 min prior to filtering). The residue was re-extracted twice with 50 ml of 0.1 M aqueous HCl and filtered. For total anthocyanin contents, the three extracts were pooled prior to measurement.

Anthocyanin concentration

Total anthocyanin concentration and Degradation Index (DI) values were measured using the spectrophotometric pH differential method (Fuleki & Francis, 1968b; Wrolstad, 1976) and expressed as cyanidin-3-glucoside, the major anthocyanin compound in elderberry (Brønnum-Hansen & Hansen, 1983).

Organoleptic evaluation

As a part of the evaluation of twenty-six elderberry varieties, the juices of the most anthocyanin rich varieties were ranked for intensity of elderberry aroma and taste by an untrained panel.

Results and discussion

In the 'Results and discussion' section, and in the Tables, the abbreviation Acy is used for anthocyanins (as in mg Acy/g berries). As a part of the initial investigations, the weight and moisture distributions of the various components of a fresh elderberry sample (12.7% solids) was measured. For the pressing conditions used, there were about equal amounts of juice (51%) and pomace (49%). the juice having 6.0% solids. The pomace was 55% skins (16.6% solids) and 45% seeds (24.0% solids). Skins comprised about 27% of the elderberry.

Studies on maturity and variety

The influence of elderberry maturity on pressability and on anthocyanin concentration and distribution is shown in Table 1 for two research varieties harvested in 1979 and 1980. (Variety 17 is also known as Sambu). In all cases, it is seen that anthocyanin concentration increases markedly with increasing maturity, while pressability (expressed as percent pomace obtained for the standard pressing procedure) remains unchanged, as does the percentage of total anthocyanins which is found in the pomace. The results show a strong similarity between the 2 years. It is obvious that harvesting date must be carefully chosen, if maximal anthocyanin production is to be achieved.

Varietal differences in pressability, anthocyanin concentration and anthocyanin distribution are also noted in Table 1. In another experiment conducted in 1979, the same characteristics were evaluated for twenty-six research varieties. Pressability of frozen elderberry (measured as % pomace) varied from 39.3 to 22.3% (mean: $29.3 \pm 4.1\%$) pomace. Anthocyanin concentration was also quite variable, ranging from 2.3 to 9.8 mg (mean: 5.7 ± 2.0 mg) Acy/g elderberry, while the anthocyanin distribution in the pomace (% Acy in pomace) ranged from 78 to 96% (mean: $89.3 \pm 0.5\%$) with most samples being 90% or above. For production of an anthocyanin extract, it is desirable to have as high an anthocyanin concentration and percent anthocyanin in the pomace as possible. A low percent pomace is also desirable. as this reduces the amount of material to be extracted, as well as indicating a higher elderberry juice productivity.

If an anthocyanin colour-extract is to be the primary product from the elderberries, then the characteristic elderberry aroma and taste should be as low as possible. Eight varieties having the highest anthocyanin concentrations were evaluated organoleptically for elderberry aroma and taste. As an untrained panel was used, the samples were ranked on the basis of aroma and taste intensity. Two varieties (13 and 17) were chosen for use in the remainder of this study. Variety 13 was chosen because it had a combination of high anthocyanin content and distribution (% Acy in pomace) and relatively low organoleptic ranking. Variety 17, named Sambu, was chosen because it is

Table 1. Influence of maturity on elderberry anthocyanin content and on mass and anthocyanin distribution in the pomace (frozen berries)

	Variety 13–65			Variety 17–65		
	% Pomace	% Acy in pomace	mg Acy /g Berries	% Pomace	% Acy in pomace	mg Acy /g Berries
Sept. 1979						
6	38.3	98	3.44	28.8	92	1.35
13	37.9	98	4.52	28.8	94	3.43
20	38.1	98	7.08	28.5	94	3.79
27	35.1	98	8.01	27.0	94	6.81
Sept. 1980						
4	34.1	98	4.61	29.5	94	2.26
11	34.5	98	5.14	28.0	96	3.63
18	34.1	98	8.03	28.3	95	4.99
25	33.0	98	8.27	27.1	94	6.95

considered to have a good commercial potential for juice production (Groven & Kaack, 1977), which could be combined with a colour-extract production to give total berry utilization. It can also be noted here that anthocyanin concentration in elderberry (2–10 mg Acy/g elderberry in this study) is much higher than reported for the anthocyanin sources most frequently investigated (grape and cranberries having about 0.6–0.8 mg Acy/g fruit; Sapers *et al.*, 1983; Metivier *et al.*, 1980).

Berry handling prior to extraction

To improve production efficiency, it will often be an advantage after harvest to store the cleaned berries frozen prior to final processing. Several tests were conducted to evaluate the influence of freezing on colour extract production. Results in Table 2 show that while freezing gives a significantly lower percent pomace (i.e. a higher juice yield), the decrease in % Acy in the pomace is relatively small. The differences in pressability for 1977 and 1980 results from use of different pressing conditions. (In one test series, it appeared that freezing could give a reduction in total anthocyanin content, but subsequent experiments could not confirm this.) It is seen that a freezing step gives less pomace for extraction without significantly reducing the anthocyanin available in the

Table 2. Influence on mass and anthocyanin distribution in pomace

	Fresh		Frozen	
Variety	% Pomace	% Acy in pomace	% Pomace	% Acy in pomace
1977				
13–65	56.2	98.9	46.8	96.1
6–65	55.3	99.1	44.3	95.2
9–62	56.4	97.7	47.4	92.6
1980				
13–65	48.3	97.7	27.1	93.2
17–65	49.5	98.5	27.8	89.1

Table 3. Influence of pressing conditions on mass and anthocyanin distribution in pomace

Amount of berries (g)	Pressing pressure (atm)	% Pomace	% of anthocyanin in pomace	Total anthocyanin content (mg Acy/g berries)*
100	50	22	77	4.61
	100	20	75	4.32
	200	18	70	5.40
200	50	20	71	4.74
	100	20	67	4.17
	200	13	53	3.48
300	50	32	69	4 27
	200	20	56	3 58

*Sum of anthocyanins in juice and two extractions of the pomace with EtOH-0.1% HCl.

pomace. This reduction in the amount of pomace gives a corresponding increase in juice amount, which makes a freezing step doubly advantageous when colour-extract production is combined with juice production. Besides production capacity considerations, pressing conditions will also influence the percent pomace and the % Acy in the pomace, both of which are important for subsequent colour-extract production. A study of pressing conditions was conducted in 1978 using frozen Sambu elderberry. Three amounts of elderberry per batch (100, 200 and 300 g, giving bed thicknesses of 10, 20 and 30 mm) were pressed at three pressing pressures (50, 100 and 200 atm). From the results (Table 3), it is seen that percent pomace is relatively constant (about 20%), except for the low pressure pressing of the largest berry amount (50 atm, 300 g). With one exception the % Acy in the pomace appears to decrease as the amount of berries per batch increases, and as the pressing pressure increases. The total anthocyanin contents (mg Acy/g berries) and % Acy in pomace are somewhat lower than measured in the other years, which can be due to climatic variation, harvesting prior to full maturity, or use of the EtOH-HCl extraction procedure in this phase of the study. (See later for extraction's influence). Degradation Index measurements were made on all juices and extracts. No differences were observed between juices and extracts, nor between the different pressing conditions. All values were low (1.10–1.13), indicating little anthocyanin degradation.

On the basis of the higher % Acy in the pomace, pressing of 100 g elderberry at 100 atm was chosen as the standard pressing procedure for the subsequent extraction studies. In the later freeze drying studies, where much more pomace was required for preparing extract, 200 g of elderberry were pressed per batch. In a commercial facility, where for example, costs associated with loading and unloading the press must also be considered, other conditions may be preferred.

Test of extraction conditions

Several studies on extraction of anthocyanins have been conducted in the period 1977 to 1984. As raw material differences will give varying anthocyanin concentrations in the elderberry, comparisons of extraction efficiency on the basis of extract concentration or amount anthocyanin extracted must be limited to a single experiment series.

Table 4. Anthocyanin yield with multiple extractions of elderberry pomace

Extraction step no.	1977			1980			1984		
	Extr. conc. (mg Acy/l)	Acy cont. (mg Acy/g pomace)	% Yield	Extr. conc. (mg Acy/l)	Acy cont. (mg Acy/g pomace)	% Yield	Extr. conc. (mg Acy/l)	Acy cont. (mg Acy/g pomace)	% Yield
1	1190	11.9	61.7	1440	11.5	81.6	503	9.2	54
2	250	2.5	74.7	433	3.5	94.0	348	6.0	89
3	236	2.4	86.9	139	1.1	97.9	78	1.5	98
4	167	1.7	95.6	72	0.6	99.4	17	0.5	100
5	86	0.8	100	29	0.2	100	—	—	—
Pressing pressure (atm)		200			100			100	
Extraction steps	100 ml 99% EtOH-0.1 HCl			First 100 ml 0.1 M HCl Rest 50 ml 0.1 M HCl			100 ml 0.1 M HCl		
Total Acy (mg/g pomace)		19.3			16.9			17.2	

Comparisons between experiments can only be made on the basis of percentage of the total anthocyanin present in the raw material which is extracted under the test conditions.

Multiple extractions of elderberry pomace are conducted to determine total anthocyanin content in the pomace, this being necessary for evaluation of extraction efficiency. With respect to further processing, it is desirable that as much anthocyanin is extracted into as little solvent as possible. In Table 4 are the results of three multiple extraction tests. As expected, the largest extraction occurs in the first extraction step. Most of the anthocyanin present in the pomace is extracted in the first three extraction steps, with the incremental yield thereafter falling sharply. It was noted that after two extractions, subsequent extracts have an increasing opalescence. This extraction behaviour gives relatively low anthocyanin concentrations in the extract already after two extractions, and in practice, it is not likely that more than one or two extractions of the pomace would be conducted.

In the literature, several solvents have been used for extraction of anthocyanins from plant tissue. These have generally been alcoholic solutions acidified with HCl, so that the pH is near 1. In this study (conducted in 1978), several of these acidified, alcoholic solutions were compared for their effectiveness in extracting elderberry anthocyanins. Deionized water and 0.1 M HCl were also included, as a non-alcoholic extraction media could be advantageous by simplifying later processing steps. In this phase of the study, 5 g of elderberry pomace (45% solids) was blended with 100 ml of solvent. The blend, together with 50 ml solvent which was used to wash the blender, were allowed to stand overnight at 5°C, after which the blend was vacuum filtered and the filter cake then washed with 50 ml additional solvent, vacuum still being applied. The total volume of solvent obtained and anthocyanin content were then measured. Each extraction was conducted in duplicate, with good agreement.

Table 5. Influence of extraction solvent on anthocyanin yields from elderberry pomace[*]

Extraction solvent	pH	% HCl[†]	Anthocyanin conc. mg/l	Volume re- covered (ml)	Anthocyanin yield mg/g pomace	DI[‡]
96% EtOH: 1.5 M HCl (85:15 v/v)	1.15	0.7	761.4	150	22.8	1.12
96% EtOH to pH = 1 with 1.5 M HCl	1.0	0.9	686.9	160	22.0	1.13
99.9% EtOH, 0.1% HCl	ca.0.3[§]	0.1	620.7	152	18.8	1.14
99.9% MeOH, 0.1% HCl	ca.0.7[§]	0.1	736.5	144	21.1	1.13
0.1 M HCl	1.0	0.8	662.0	186	24.6	1.11
H_2O (deionized)	5.5	0	269.0	165	8.7	1.13

[*] Average of two extractions.
[†] ml 37% HCl/100 ml solution.
[‡] Degradation Index.
[§] pH in pure alcohol not necessarily a precise measure of H^+ concentration.

The results of these extraction studies are given in Table 5. It can be seen that the alcoholic solvents generally give higher anthocyanin concentrations in the extract than the aqueous based media, but due to lower solvent recovery, the anthocyanin yield is actually slightly higher with the aqueous HCl solvent. The lower solvent recovery could

Table 6. Extraction of elderberry anthocyanins with citric acid solutions

Extraction solvent	Solution cost ($/1000 l)*	Acy conc. (mg/l)	Total solids in extract (g/l)
Citric acid 0.01%	0.17	297.1	0.1
Citric acid 0.1%	1.70	386.3	1
Citric acid 1%	17.00	552.6	10
Citric acid 10%	170.00	680.0	100
Citric acid 20%	340.00	768.1	200
HCl 0.1 M	1.00	785.7	3.6

*1984: Citric acid = 17 Dk.kr/kg, HCl = 2.75 Dk.kr/kg. $1 = 10 Dk.kr.

be due to a higher retention of alcoholic solvent in the filter cake, though it is more likely that evaporation of alcohol during the vacuum filtration is responsible. That deionized water alone is not effective as an extractant was not unexpected, as anthocyanins have a different structure at higher pH values. Degradation Index values are almost the same for all extraction solvents.

In another test (conducted in 1980), extraction with aqueous citric acid was examined, as it would be expected to be more taste-compatible with the food systems where anthocyanin extracts would be ultimately used than the HCl based extracts. From the results in Table 6, it is seen that anthocyanin extraction increases as citric acid concentration increases, with a 20% citric acid solution giving the same extract concentration as found for the 0.1 M HCl. Also given in Table 6 are the solvent costs, based on industrial-scale purchases (1984, citric acid $1.70/kg; HCl, $0.25/kg). It is obvious that citric acid extraction is much more costly if extraction levels similar to those obtained with HCl are desired. It can also be noted that this extract will contain much more total solute than the HCl extract (200 *versus* 3.6 g/l). It appears that both the alcoholic HCl and aqueous HCl solvents would be suitable for extracting elderberry anthocyanins. Other factors, such as solvent cost and the related requirement for solvent recovery, the technological need for alcohol removal prior to subsequent processing, and especially for ethanols sake, the non-technical problem associated with requirements for strict controls to satisfy tax authorities, must also be considered. When all factors are evaluated, it would seem that aqueous 0.1 M HCl is the best choice as extraction solvent for elderberry anthocyanins. The subsequent extraction studies, and extract preparations for the freeze-drying studies were conducted with 0.1 M HCL.

One process parameter of importance is how fast anthocyanins can be extracted from the elderberry pomace. Several times in the course of this study, time dependence for degree of extraction has been investigated. Typical results for extraction of extensively crushed pomace with 0.1 M HCl were as follows: time in min, (% of total anthocyanin in first extraction), 2 (71), 5 (66), 10 (90), 30 (89) and 60 (90). The extraction process proceeds rapidly and reaches equilibrium already after 10 min of contact, with 90% of the total anthocyanin present in the first extract. This rapid extraction behaviour has been observed in all tests conducted. Tests with extraction times longer than 60 min do not show increased yield. Extractions conducted at 20 and 40°C did not show any differences, which in part can possibly be related to the high degree of extractability noted for extraction at 20°C.

Conclusions

Elderberry is shown to be a good source of anthocyanins (Acy), having between 2–10 mg Acy/g berry, depending on variety. After pressing the berries to obtain the juice, the remaining pomace comprises about 25–40% of total weight and 75–98% of total Acy. Freezing of the berries prior to pressing results in only a small change of anthocyanin distribution between pomace and juice. Aqueous HCl (0.1 M) was preferred as extraction media, as it gave the highest anthocyanin recovery and entails fewer technical problems. To obtain most of the anthocyanin, 2–3 room temperature extractions of the pomace of 10 min each is adequate.

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Anthocyanin colourants from elderberry (*Sambucus nigra* L.). 2. Process considerations for production of a freeze dried product

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Summary

Freeze drying of elderberry anthocyanin extract under the proper conditions produces an intensely coloured powder with retention of both anthocyanins and physical structure. The various conditions examined included extract concentration, stabilizer concentration, pH and freeze drying conditions. It was found that minimum anthocyanin degradation and product structure alteration occur, when the undiluted extract is adjusted to pH = 3, has DE 20 maltodextrin structure stabilizer added to a 2.5% level and is freeze dried at a maximum platen temperature of 75°C and maximum product temperature of 60°C. Under these conditions, the recovery of anthocyanin is 94% and physical structure and solubility is preserved.

Introduction

When considering the use of natural pigments as food colourants, colour stability is of major concern. Colour stability is greatly affected by the presence of water, this being an important factor in the degradation of anthocyanins. When the water activity is reduced, anthocyanin stability increases, which might be due to reduced mobility of reactants and limited oxygen solubility. Most of the literature in this area deals with the stability of anthocyanins in solution under the influence of different chemical and physical factors (Markakis, 1982), with but few studies reported on stability during drying or of the dry material. Erlandson & Wrolstad (1972) prepared freeze dried strawberry puree for use in studies on storage stability of dry anthocyanins. Freeze drying was conducted at 49°C and a pressure of 300–900 mTorr. As they do not give a measure for anthocyanin content before freeze drying, calculation of anthocyanin loss is not possible.

Chiriboga & Francis (1973) diluted a purified alcoholic anthocyanin concentrate from cranberry with water so the material had a moisture content of 65%, before freeze drying in a pilot plant freeze dryer. The found a loss of anthocyanin of 4.7%. In the dried pigment powder, a total of 17% was anthocyanin, the remaining components were suggested to be organic acids, polyphenolic substances and carbohydrates, this giving a very hygroscopic powder. To produce a satisfactory product Calvi & Francis (1978) incorporated low dextrose equivalent corn syrup solids as support for freeze drying, using the same conditions as Chiriboga & Francis (1973). Palamidis & Markakis

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(1975) used a freeze dried grape product in an investigation of a colour fortified carbonated beverage. The freeze drying conditions were not described.

Roselle (*Hibiscus sabdariffa* L.) from the West Indies, which contains 1.5% anthocyanin on a dry weight basis, has been used as a concentrate for a freeze dried product (Esselen & Sammy, 1975). The concentrate had 9% soluble solids content and a pH of 2.7. In a pilot plant freeze dryer, the platen temperature reached 24°C, the condenser temperature was −45°C and vacuum was 10 mTorr. While the product was hygroscopic, it was quite stable during storage, even at 20°C.

Whole sour cherries were treated with selected chemicals (sodium bisulphite, sucrose, ascorbic acid or calcium chloride) and then after quick freezing, freeze dehydrated for 14 h at a platen temperature of 57°C. Anthocyanin content dropped from 13.2 down to 9.3 mg/100 g cherries, with the best retention of colour being found in the sodium bisulphite treated sample (Do *et al.*, 1975; 1976).

Soukup & Maing (1977) noted that dry natural colours, i.e. anthocyanins, derived from aqueous extracts or juices are hygroscopic, though this can be overcome by a co-drying with dextrins or gums. They noted that this solution increases the bulk volume of the powders, which might create incompatibility problems for certain product systems.

Shewfeldt & Ahmed (1977; 1978) have compared freeze dried products derived from red cabbage or blueberries, after extraction with either SO_2 water, or acidified methanol. These extracts were foamed with 8% (dry weight basis) carboxymethylcellulose before freeze drying for 36 hours at 30°C. They noted that the methanol preparations were clearly superior to the SO_2 preparations and that, while both anthocyanin sources were adequate, red cabbage was the cheaper. Sulc *et al.* (1980) studied degradation of colour during the drying of raspberry pulp concentrates. Both freeze and spray drying at 50–60°C were conducted, with colour losses of 11.4% and 15.8% respectively being found. When drying was conducted at higher temperatures, a major degradation of anthocyanins resulted in a colour loss of up to 70%.

Main, Clydesdale & Francis (1978) developed an extraction procedure which was used in a number of studies by Francis and co-workers on the spray drying of extracts of different plant materials (Main *et al.*, 1978; Clydesdale *et al.*, 1978; 1979a, b and c). A maltodextrin, Morrex 1918 (10–13 DE) was added to the extract/concentrate as a spray drying carrier agent to produce 30% total solids mixture. They observed that the outlet air temperature should not be greater than 100°C if one is to avoid a considerable anthocyanin degradation, as measured by the degradation index.

A potential source of anthocyanins for food colouring is elderberry (*Sambucus nigra* L.), a small tree-shrub growing freely in the northern hemisphere with fruits which are an extremely good source of anthocyanins of a simple composition and with few other phenolic substances. In our first paper in this series (Brønnum-Hansen, Jacobsen & Flink, 1985), we reported results of studies on the extraction of anthocyanins from elderberry pomace. In the current paper, the results of investigations of the influence of freeze drying conditions on the freeze dried anthocyanin product, prepared by the previously reported extraction procedure, are presented.

Materials and methods

Raw material

The elderberries (varieties 13–65) were grown in 1980 at Pometet, The Royal Veterinary and Agricultural University, Tåstrup, Denmark, harvested in early October

and frozen at $-25°C$. The anthocyanin extracts were prepared by extracting 5 g samples of elderberry pomace with 200 ml of 0.1 M hydrochloric acid as described previously (Brønnum-Hansen, *et al.*, 1985). The anthocyanin content of this extract was determined by the spectrophotometric pH differential method described by Fuleki & Francis (1968) and was found to be 750 mg/l calculated as cyanidin-3-glucoside.

Treatments of extract

Prior to freeze drying, the individual samples were prepared by modifying the extract's composition. Anthocyanin concentration was either 100% or 20% (5× dilution) of that in the original extract. pH levels of 1–5 were achieved by adjusting the original extract with anhydrous sodium carbonate, (E. Merck, G.F.R.), and a structure stabilizer (20 DE malto-dextrin: Maltrin M-200, Grain Products Co., U.S.A.) was added at levels between 0 and 5% on a solution basis. Five ml of these modified extracts were placed in small plastic beakers (giving a 6–7 mm thick layer) and frozen in a blast freezer at $-50°C$.

Freeze drying

Freeze drying was conducted on a computer controlled system (Heto, Birkerød, Denmark) in which heater temperatures, product temperatures (measured at four different locations with thermocouples), product weight, process pressure, and energy usage are monitored and also used for feedback process control. Completion of drying is determined by an evaluation based on both product temperature and product weight loss. In this study, freeze drying was conducted at five combinations of initial heater set point temperature (50–125°C) and maximum allowed product temperature (30–90°C). After the product reaches the maximum allowed product temperature, heater set point temperature decreases in order to maintain the product at its maximum allowed temperature. In the remaining text, freeze drying conditions will be given in terms of these two initial set points, e.g. 75/60 would mean an initial heater set point of 75°C and a maximum allowed product temperature of 60°C. For all freeze drying experiments, condenser temperature was $-55°C$ and chamber pressure was between 50–100 mTorr. At the completion of drying, dry nitrogen gas was filled into the chamber.

Analyses

Freshly prepared, frozen and freeze dried extract, as well as extract held refrigerated for the duration of the freeze drying process, were analysed by one or more of the following methods. (Freeze dried product was redissolved in a volume of 0.1 M hydrochloric acid corresponding to the weight loss under freeze drying).

Total anthocyanin content was determined by the spectrophotometric pH differential method of Fuleki & Francis (1968). For some experiments a HPLC-gradient method (Brønnum-Hansen & Hansen, 1983) was also used. This HPLC method was also used to follow changes in the distribution of the individual anthocyanin components and to investigate the formation of degradation products. The degradation index was calculated according to the method of Fuleki & Frances (1968). The moisture content of the freeze dried product was evaluated gravimetrically by storing the samples over silica gel for 1 week. The colour values of the freeze dried products were measured with a Vipden 501 Densitometer, (Italy), and a Lovibond tintometer, (GB). The physical structure of the freeze dried products was evaluated visually.

Description of experimental series

To elucidate the stability of the anthocyanins during freeze drying processes, three series of experiments were conducted. In the first series, undiluted and diluted elderberry extracts with 0% and 5% maltodextrin stabilizer added were adjusted to five pH levels and freeze dried at 75/60 and 50/30. In the second series, stabilizer was added to the extract (adjusted to pH 2) at levels of 0–5%, and freeze dried at 75/60 and 50/30. In the third series, extract at pH 3 and a stabilizer concentration of 2.5% was freeze dried under four conditions (75/60, 100/60, 125/60, 125/90).

Results and discussion

Results of the first series

Visual observation showed that samples without stabilizer puffed up and were partly lost during the process, while samples with 5% stabilizer retained their original geometry.

Table 1 shows the influence of pH and presence of stabilizer on retention of the total anthocyanin content after a freeze drying process conducted at 75/60. For comparison, the anthocyanin content of the refrigerated extracts is shown. Anthocyanin contents were measured by the pH differential method. The same experiment was carried out with diluted extract and with freeze drying at 50/30, but as these conditions showed similar results to those in Table 1, they are not presented.

Table 1. Retained total anthocyanin content after freeze drying: (%) of raw extract determined by the pH differential method

Stabilizer concentration (%)	pH				
	1	2	3	4	5
0	71	74	80	78	77
5	94	97	96	92	89
Extract kept in refrigerator	98	97	92	72	68

F.D. condition: 75/60.
Stabilizer: Maltrin M-200.

Retention of physical structure was noted to be important for sample solubilization and the low measured retention of anthocyanins with the stabilizer free extracts in Table 1 were judged to be due to reduced solubility of these samples. Thus, the effect of maltodextrin is to prevent collapse during freeze drying of the anthocyanins, this giving a completely soluble product. As expected, the pH level affects the anthocyanin content, with decreasing retention for increasing pH. It is also noted in Table 1 that the freeze dried product is better preserved than the refrigerated aqueous extracts, the only exception being at pH 1. This can be explained by the considerable lowering of pH during freezing and freeze drying (Van den Berg, 1966; Williams-Smith *et al.*, 1977) which apparently gives a higher stability to the anthocyanins as long as the H^+ concentration increase does not result in acid-catalysed degradation reactions (Flink, 1983) as seems to have occurred in pH 1 sample.
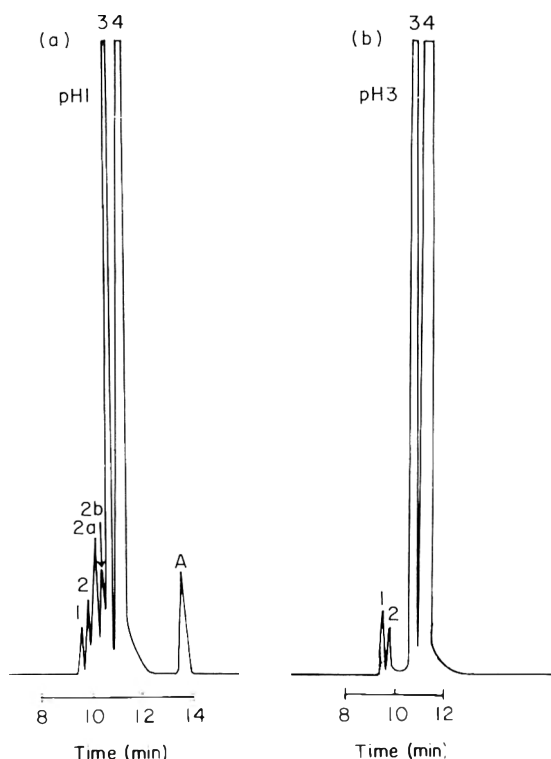
Figure 1. Chromatograms of freeze-dried elderberry extract, adjusted to (a) pH 1 and (b) pH 3 before freeze drying. Support: Nucleosil 5 μ C 18, 150×4.6 mm. Mobile phase: Tetrahydrofuran in 0.05 M phosphoric acid (pH 1.8) going from 1–40% in 15 min. Flow rate: 1.2 ml/min. Detection wavelength: 510 nm.

Peak no.	Compound
1	Cyanidin-3-sambubioside-5-glucoside
2	Cyanidin-3-glucoside-5-glucoside
3	Cyanidin-3-sambubioside
4	Cyanidin-3-glucoside
A	Cyanidin
2a	Unidentified
2b	Unidentified

The variation of the anthocyanin composition of the elderberry extracts at the 5 pH levels was studied by HPLC. Figure 1 shows chromatograms at 510 nm for the pH 1 and pH 3 freeze dried product. In the pH 3 sample, only the four anthocyanins found in the original extract are present, while in the pH 1 sample, alterations in the composition of the anthocyanins occur, partly a hydrolysis to the aglycone (peak A) as well as the appearance of two unidentified compounds (peaks 2a, 2b) absorbing light at 510 nm. From Table 2, which gives the distribution between the various anthocyanins, it is obvious that an alteration occurs in the composition of anthocyanins for the pH 1 and pH 2 freeze dried products, though the original composition is much better retained in the pH 2 sample. From pH 3 to pH 5 there is no change from the original distribution, but as shown in Table 3, there is a significant important decrease in total anthocyanin

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Table 2. The anthocyanins in elder-
berry; (%) distribution of the vari-
ous anthocyanins after freeze drying

Peak no.	pH		
	1	2	3, 4, 5
1	1	1	1
2	1	1	1
3	24	31	33
4	67	66	65
A	2	0.5	—
2a	3	0.2	—
2b	2	—	—

F.D. condition: 75/60.
Stabilizer concentration: 5%
Maltrin M-200 w/v.
For identification of peak num-
ber, see Fig. 1.

content, most pronounced for the two small 3,5-diglycoside peaks (1, 2) but also noted
for the two 3-glycoside peaks (3, 4). For the pH 1 sample an apparent discrepancy is
noted between the total anthocyanin content measured spectophotometrically by the
pH differential method (Table 1) and the 'total anthocyanin' expressed as a weighted
average of HPLC peaks 1–4 (Table 3). In part, this is due to omission of the aglycone
(peak A) and unidentified peaks (2a, 2b) in HPLC calculations. In addition some
observations have indicated that the reconstituted pH 1 sample has a darker cast. The
extent to which this can influence the difference in measurement is not known.

Results of the second series
    The influence of stabilizer concentration at pH 2 is shown in Table 4. At stabilizer
concentrations below 2%, the original structure is not retained during freeze drying and
the samples' solubility is incomplete. For stabilizer concentrations of 3% or higher

Table 3. Anthocyanins in elderberry; retention of
the original anthocyanins after freeze drying

Peak no.	pH				
	1	2	3	4	5
1	67	83	92	86	73
2	80	89	96	86	73
3	57	97	97	95	94
4	80	98	95	93	91
Weighted average*	78	97	96	92	91

F.D. condition: 75/60.
Stabilizer concentration: 5% Maltrin M-200
w/v.

$$\overline{R_i} = \frac{\Sigma\ R_i\,wt\%_i}{\Sigma\ wt\%_i}.$$

Table 4. Influence of stabilizer concentration on the structure of the freeze dried product

Stabilizer concentration (%)	Anthocyanin retention (%)	Structure*	Solubility[+]
0	74	p	nc/ppt
0.5	96	p	nc/ppt
1	96	c	nc/ppt
2	97	c	c
3	97	o	c
4	97	o	c
5	97	o	c

Conditions as in Table 1, pH 2.

*Structure: p = puffed, fluffy; c = cratered surface; o = original structure maintained.

[+]Solubility: nc = not completely soluble; ppt =white precipitate observed; c = completely soluble.

sample structure and solubility is completely retained. For the 2% sample, solubility was complete even though a minor surface cratering was noted. The degree of insolubility was markedly larger in the stabilizer free sample than in the two other samples with low stabilizer concentration, where the presence of white precipitate was much less noticeable. This difference is reflected in the total anthocyanin retention, near the original level, as measured by the pH differential method.

Results of the third series

In the third series, the pH level and stabilizer concentration were chosen according to the results of the first and second series. To avoid any alteration in the composition and the least decrease in total content of anthocyanins, pH 3 was chosen. The stabilizer concentration was set to 2.5%, the lowest possible concentration where original structure and solubility are retained. At this stabilizer concentration, anthocyanin extract comprised approximately 30% of the final dry product.

Various aspects of the course of the freeze drying process are given in Fig. 2 and Table 5. Figure 2 gives the weight loss curves for the four anthocyanin products. As expected, the drying rate increases with increasing plate temperature, so that the overall drying time decreases. A higher maximum allowed product temperature did not have any effect on freeze drying time, which also has been observed in other studies (Flink & Moledina, 1982). Table 5 gives further information on the heating stress that the anthocyanin product has been subjected to under the various freeze drying processes. It should be noted that measurement of sample temperatures can be rather sensitive to thermocouple placement in the sample, and thus some small deviations between freeze drying experiments can be expected. As noted from Fig. 2, it is seen in Table 5 that the total freeze drying time decreases with increasing heating plate temperature. It is further noted that the time for the surface to achieve the maximum allowed product temperature is shorter for the 100/60 and 125/60 experiments than for the 75/60 test. The difference between the 100 and 125°C runs undoubtedly results from slightly differing placement of the surface thermocouple, but in any case, for both there is only a short period until the surface reached 60°C and the heater temperature starts to
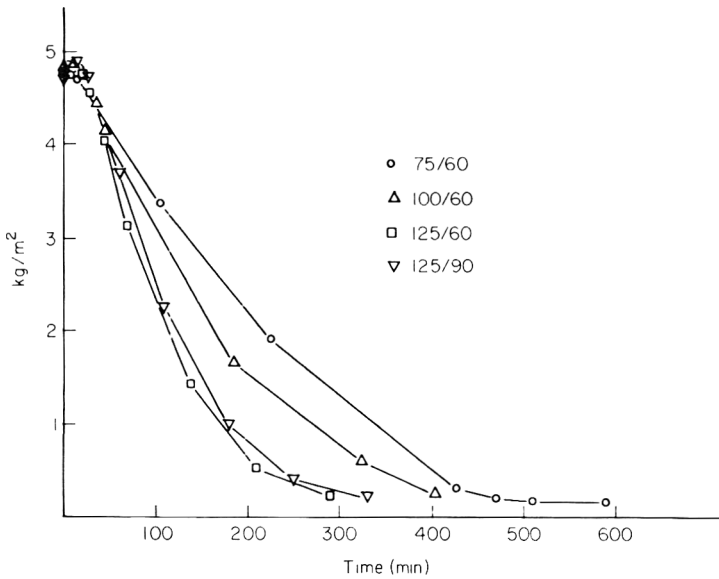
Figure 2. Weight loss curves for the four different freeze drying conditions, with an elderberry extract at pH 3 and stabilizer concentration of 2.5%.

fall. The next two columns of Table 5 present the best measure of the heating stress under freeze drying. Here are given the time that the sample surface remains at the maximum allowed temperature, and the maximum temperature reached in the sample interior (bulk). It is seen that the combination of heating temperature and maximum allowed product temperature act to give a sizable variation in the heating stress. For example, run 75/60 takes so long that even though the surface is at 60°C for only 80 min, the bulk manages to reach 55°C before the drying is completed. For the 125/60 test, the sample bulk attains 60°C before the drying is completed. It is obvious that the 125/90 sample has had the highest heating stress, as the sample surface has been at 90°C for 130 min, and the bulk achieves a temperature of 85°C at the completion of drying. In the last column are given the respective electrical energy usages by the heating plates. These indicate that more energy is required to conduct the drying when the heating plate temperature and/or maximum allowed product temperature are increased, even though the drying time is shorter.

Table 5. The parameters during the four freeze drying processes

F.D. condition	Total F.D. time	Time for surface to attain max. allowed temperature	Time with surface at max. allowed temperature	Max. temperature in product interior	Energy usage kW/sec
75/60	590	430	80	55	1326
100/60	405	45	280	50	1723
125/60	290	70	140	60	1870
125/90	330	120*	130	85	2496

*Note that max. allowed surface temperature = 90°C for the sample.

It can be summarized that as the heating temperature and/or maximum product temperature are increased, the samples have undergone a higher heating stress, the freeze-drying time is decreased and the energy usage has increased.

Table 6. Influence of freeze drying condition on the structure and colour of the freeze dried product

F.D. condition	Surface	Structure[*]	Anthocyanin retention (%)	Hunter values[+]		
				L	a	b
75/60	glossy	o	94	35.1	46.3	−11.9
100/60	mat	c	94	29.9	36.4	−7.9
125/60	glossy	o	94	24.8	32.5	−7.4
125/90	glossy	o	94	23.1	30.3	−6.1

[*] Abbreviations, see Table 4.
[+] Reflectance from powder.

Table 6 shows what influence the different freeze drying heating stresses have had on the structure and colour of the freeze dried anthocyanin products. It can be seen that the different freeze drying conditions have essentially not affected the sample structure. The differences noted for 100/60 are slight, especially when compared to the structural changes noted earlier in series 1 and 2. Regarding sample colour, it can be seen that anthocyanin retention is quite high, and not dependent on the freeze drying heating stress. When the Hunter values are examined, some differences are observed, which seem to be related to the heating stress during freeze drying. As the heating stress increases, the samples become darker (decreasing L value) and there is a shift to lower redness and blueness (decreasing a and increasing b values, respectively). As the anthocyanin contents are equal for all samples, these changes are not related to loss of anthocyanin, and thus there must be another cause for the measured darkening. Non-enzymatic browning is one possibility. Measurement of absorption at 440 nm in the sample solution showed a slight increase, but the degradation index remain unchanged in the four anthocyanin products, indicating only a minor darkening in solution. Another possibility relates to structural change during freeze drying. It has been shown for freeze dried coffee that micro-scale collapse (i.e. where collapse is not observable by the naked eye) can result in a darkened surface (Flink, 1975; Ettrup-Petersen, Lorenzen & Fosbøl, 1970). That this is a physical surface phenomenon was demonstrated by rehydrating the dry samples after which all gave the same colour in solutions. This behaviour is similar to that observed in the current study.

Conclusions

Anthocyanin extracts of elderberries (*Sambucus nigra* L.) were freeze dried under various conditions, after variation of extract concentration, pH and stabilizer concentration. The samples were analysed for anthocyanin content and composition spectrophotometrically and by high performance liquid chromatography, and surface colour was recorded in Hunters L, a, b values. Visual evaluation was made of physical structure and appearance, and the solubility was examined.

Similar results for the evaluation of the freeze dried diluted and undiluted extract indicated no advantage of diluting the material, as the starting amount just becomes larger.

When the extract pH was varied between 1 and 5, freeze drying caused an alteration in the anthocyanin composition in the extracts with low pH, partly because of a hydrolysis and partly by another reaction indicated by the appearance of two un-identified compounds with intermediate retention times. This is presumably due to a considerable increase in hydrogen ion concentration under the freezing and freeze drying process. It has been indicated by Van den Berg (1966) and Williams-Smith *et al.* (1977), that the pH of a solution can change by up to 3 pH units during freezing, this promoting hydrolysis. Flink (1983) has shown that these freezing effects are intensified by subsequent freeze-drying. At higher pH, a significant important decrease in total anthocyanin content is observed, a natural consequence of the well known instability of anthocyanins in non-acidic media (Brouillard, 1982).

Addition of a maltodextrin structure stabilizer at concentrations above 2% not only prevented collapse of the physical structure, but also provided the freeze dried product with good solubility qualities, these factors giving good anthocyanin retention and avoidance of the formation of a white precipitate.

After establishment of the desirable extract conditions (pH value = 3, stabilizer concentration = 2.5%), various freeze drying conditions were applied to undiluted elderberry extract to find out how much heat stress the product could tolerate without anthocyanin loss and remain a usable product. The anthocyanin retention is very high, the 6% anthocyanin loss being comparable with the findings of Chiriboga & Francis (1973), and much better than the results from freeze drying whole sour cherries, where the loss of anthocyanin was approximately 30% (Do *et al.*, 1976). According to the Hunter values, there is a slight change for the dry material from a brighter and lighter colour to a darker value when the maximum temperature of the plate and/or product are raised, but in solution it has no significance. This darkening together with higher energy usage when freeze drying at higher heating temperatures resulted in choosing 75/60 as the condition for freeze drying for our further studies on storage stability (Brφnnum Hansen & Flink, 1985).

Acknowledgment

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Anthocyanin colourants from elderberry (*Sambucus nigra* L.). 3. Storage stability of the freeze dried product

KIRSTEN BRØNNUM-HANSEN* AND J. M. FLINK

Summary

Storage of freeze dried anthocyanin extract from elderberry was conducted under various conditions of water activity (a_w), temperature, atmosphere composition and presence or absence of light. It was found that at $a_w \leq 0.31$, water uptake has essentially no effect on the anthocyanin product. At $a_w \geq 0.5$, a significant increase in anthocyanin degradation rate is observed. The BET monolayer value for water sorption (a_w = approximately 0.4) coincide with the a_w interval ($0.31 \leq a_w \leq 0.5$) over which anthocyanin degradation shows a marked change in behaviour.

Temperature during storage is of prime importance to anthocyanin stability with high temperature in combination with high water activity having the most pronounced effect on the stability of anthocyanins. At conditions of 50°C, 0.5 a_w, the anthocyanin half life was found to be approximately 2 months.

The two factors, light intensity and presence of oxygen, were found to have no significant effect on anthocyanin stability, indicating that anthocyanin products with limited access to water are quite inert systems. When the dry anthocyanin extract was stored at ambient temperature and low a_w (≤ 0.3), the degradation rate was found to be so low (anthocyanin half life > 5 years) that the use of the powder in low moisture food products is evident.

Introduction

When producing a freeze dried anthocyanin product it is important to have information on the product stability, not only during the freeze drying process, but also during subsequent storage, where the product will be exposed to different environmental stresses. Of these stresses, variation of available moisture is especially significant as the product's water activity will influence the rate of many chemical and physical changes. For example, reduced water activity could result in reduced mobility of reactants and limited oxygen solubility which influence the stability of anthocyanins.

Only a few studies have dealt with relationship between water activity (a_w) and anthocyanin stability. Erlandson & Wrolstad (1972) examined freeze dried strawberry puree after storage under air or nitrogen at 37°C in the dark at a_w of 1.0, 0.75, 0.57, 0.32, 0.11, < 0.01. They found a pronounced effect of a_w on the rate of anthocyanin degradation, and suggested that the sorption isotherm could be used to assess storage stability of anthocyanin pigments in low moisture systems. In particular anthocyanin

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degradation first began at $a_w > 0.11$ and increased in rate with increasing water activity.

A part of the studies by Francis and co-workers on spray drying of anthocyanin extracts (Clydesdale *et al.*, 1978; 1979a, b and c) dealt with storage of the dried powders under various conditions of temperature, water activity and atmosphere composition. They found that two food products which had anthocyanin added as a natural pigment had greater hygroscopicity when compared to products with added synthetic colourants. They reported caking of the anthocyanin added strawberry gelatin mix and cherry beverage mix at water activities between 0.2 and 0.3, when the temperature during storage was kept at 38°C. The storage was conducted over a period of 16 weeks and showed considerable pigment breakdown after 12 weeks of storage, most pronounced at high temperatures. Their observation of caking (water uptake) does not correspond well with the estimated shelf lives reported.

In the current study, freeze dried anthocyanin powder prepared by the methods described in the second article in this series (Brønnum-Hansen & Flink, 1985) was investigated for product stability when stored under different conditions of water activity, temperature, atmosphere composition and presence of light.

Materials and methods

Preparation of freeze dried extract

Details regarding preparation of the freeze dried product used for this storage were presented in the second paper of this series (Brønnum-Hansen & Flink, 1985). To an undiluted aqueous HCl extract of elderberry with pH adjusted to 3 was added a structure stabilizer (Maltodextrin DE = 20) at a concentration of 2.5%. Small plastic beakers containing 5 ml (about 7 mm in thickness corresponding to a dry weight of approximately 180 mg) were freeze dried with an initial heater setpoint of 75°C and a maximum allowed product temperature of 60°C.

In one study, the undiluted extract (with 5% structure stabilizer) was adjusted to five pH levels between 1 and 5 before freeze drying at the same conditions.

Description of the experimental series

To elucidate the storage stability of the anthocyanins in the freeze dried samples, four series of experiments were conducted. In the first series, all samples were stored at room temperature, sample water activity (a_w) was varied from 0 to 0.75, half of the samples being stored in darkness, the other half being placed in light. In the second series, samples were stored in darkness at high and low water activity (0, 0.51) at three different temperatures (5, 20, 55°C). In the third series, samples were stored in darkness at high and low water activity (0, 0.51), with the absence or presence of oxygen. In the fourth series, samples with an initial variation of pH (before freeze drying) from 1 to 5 were stored in darkness, at room temperature and $a_w = 0$.

Storage conditions

To control sample water activity, the small plastic beakers were placed in desiccators containing the following saturated salt solutions (Rockland, 1960): 0, silica gel; 0.11, lithium chloride; 0.22, potassium acetate; 0.31, magnesium chloride; 0.51, magnesium nitrate; 0.75, sodium chloride. The desiccators were then evacuated and the samples allowed to equilibrate with the constant humidity environment overnight, after which the desired atmosphere was re-established.

Table 1. Half lives for dried anthocyanin samples as a function of water activity (a_w) at room temperature for storage in light and darkness. The colour and structure are described after 1 year of storage

a_w	Colour*	Structure*	$T_{1/2}$+ (light) (days)	$T_{1/2}$+ (darkness) (days)
0	o	o	4000[a]	4500[a]
0.11	o	o	3200[a,b]	3200[a,b]
0.22	o	o	2300[b]	2500[b]
0.31	o	ms	1900[b]	2100[b]
0.51	d	md	1200[c]	1300[c]
0.75	b	cd, c	370[d]	430[d]

*o = original colour/structure maintained; d = darkened; b = black; ms = minor shrinking; md = minor deliquescence; c = caking; cd = complete deliquescence.

+Value obtained from regression lines; treatment having different letters are different on the 95% level of significance.

The desiccators containing samples to be exposed to light were placed in a southern window, which had a film curtain in place which only allowed 12.5% transmission of the uv light. The uv light stress on the samples was estimated to be 25% of the stress they would have received had they been placed in a northern window, (standard condition) (P.E. Pedersen, 1984, pers. comm.). The three temperatures used in the storage studies were 5°C (refrigerator), approximately 20°C (room temperature), and 55°C (thermostated oven). For storage in the absence of oxygen, the desiccators were evacuated periodically. In the first three series, samples were removed for evaluation five times during a 1 year period. In the fourth series, sampling was performed three times during a 2 year period.

Analyses

Total anthocyanin content and degradation index was determined spectrophotometrically, according to the method by Fuleki & Francis (1968). In addition, a HPLC gradient method (Brønnum-Hansen & Hansen, 1983) was utilized. Details of these analyses are described in the second article in this series (Brønnum-Hansen & Flink, 1985). For a major part of this study, the HPLC gradient method was directly transferred to a Water Associates automatic HPLC system.

Results and discussion

Results of the first series

Visual observation of the samples stored at the different water activities gave indications already after 1 week that changes were occurring in the samples stored at the highest humidity. After 1 year of storage, there were little additional changes (Table 1), and it was noted that storage at the highest humidity results in major sample changes, such as fully destroyed structure, complete loss of red colour and greatly reduced solubility. For water activities below 0.31, the samples are unchanged in all respects. At a_w = 0.31, a minor shrinking is observed, while at a_w = 0.51, both colour and structure are affected to a minor extent. All samples stored at $a_w < 0.75$ were fully soluble. Generally, no difference is noted between the samples stored in light and darkness,

though the two highest a_w levels, the samples stored in darkness were observed to have retained red colour at the surface better than the samples stored in light. This visual difference was only recognized after the second half year of storage, and did not give significant differences for the measured retention of anthocyanins.

The anthocyanin contents in the various samples were measured by spectro-photometry, and the half lives for anthocyanin loss (listed in Table 1) were calculated on the basis that the degradation of anthocyanins follows first order kinetics, as plots of the log of anthocyanin concentration *versus* time were found to be linear. As seen in Table 1, the half lives are decreasing with increasing water activity.

When examining the statistical significance (at the 95% level) between the different treatments, it is obvious that the degradation rate is not really influenced until a_w reaches 0.51. Samples at $a_w = 0$ are significantly different from $a_w = 0.22$ and 0.31; $a_w = 0.11$ lies between and is not significantly different from either. There is a signifi-cant difference between the two highest water activities (0.51, 0.75), as well as between these and the samples with lower a_w. In contrast Erlandson & Wrolstad (1972) found that anthocyanin degradation occur to a major extent with $a_w > 0.11$, whereas in this study an $a_w > 0.51$ first resulted in sizable anthocyanin degradation. These differences are presumably due to difference in sample composition (strawberry puree *versus* elderberry anthocyanin extract), inclusive inherent pH differences, as well as storage temperature effects (38 *versus* 20°C).
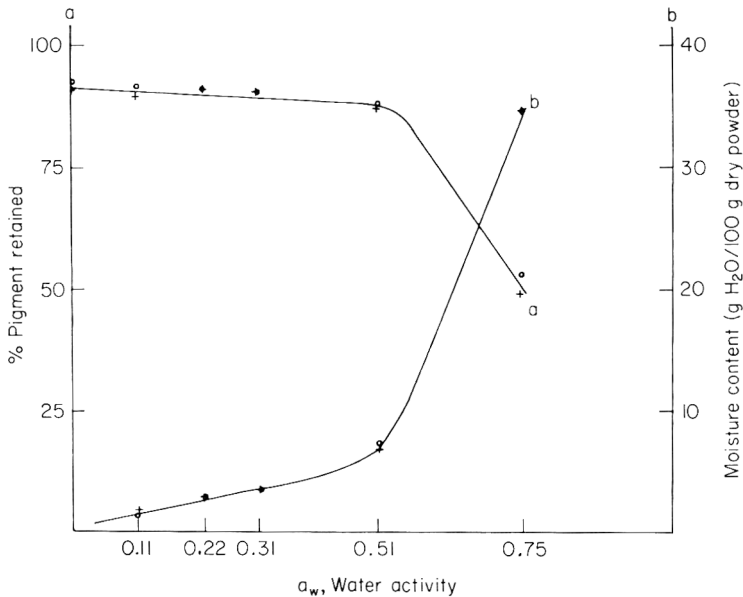
Figure 1. Effect of water activity (a_w) of freeze dried elderberry extract on pigment retention (a) and water sorption (b) after 1 year of storage at ambient temperature in light (+) and in dark (o). Moisture content $= g\ H_2O/100\ g$ dry powder.

In Fig. 1, the anthocyanin retention after 1 year of storage at various water activities is shown together with the water sorption isotherm for the freeze dried product. It can be seen that the marked fall in anthocyanin retention is associated with the sharp rise of the available water. The BET monolayer value for the product was calculated from the water sorption data and found to be 3.7 g/100 g dry powder. Caurie (1981) presented a

revised evaluation of the water sorption isotherm with respect to predicting product stability. He noted that chemical reactions are very active only when moisture levels are two to four times the monolayer value, as calculated by the Caurie equation. In this study, the Caurie monolayer is 4.3 g/100 g dry powder.

According to the literature relating BET analysis with product stability, the highest sample stability is generally associated with sample moisture contents below the monolayer value, and physical changes and chemical degradaticn do not occur until the moisture exceeds the monolayer value. In this study, the calculated monolayer values (BET and Caurie) are similar and correspond to a water activity of 0.35–0.40. Thus, the samples stored at 0.31 and below should have the highest stability, which is in agreement with $T_{1/2}$ values given in Table 1 (and the significant differences found) where there is essentially no loss of anthocyanins for water contents below the monolayer value. At water activities above the monolayer value, degradation proceeds more rapidly due to the presence of multilayer and/or free water. According to the above-mentioned evaluation by Caurie (1981), this agrees with the results noted for storage at 0.51 and 0.75, where the degradation rate increases dramatically only at $a_w = 0.75$. It can be mentioned that as the 0.75 sample is completely collapsed, the state of water is uncertain, and that the water content for this sample is much higher than normally observed for uncollapsed materials, and for the water cortent predicted by Caurie equation for this a_w value. In Table 2, the retentions of the individual anthocyanin components (determined by HPLC), as well as the overall retentions determined by

Table 2. Retention (%) of the original anthocyanins after 6 and 12 months of storage in light at six different levels of relative humidity (a_W). The original content just after freeze drying was measured to be 697 mz/l and is set to 100%.

a_w	Storage time (months)	HPLC peak no.				Overall Acy retention (%)	
		1	2	3	4	HPLC*	spect
—	0	100	100	100	100	100	100
0	6	93	98	95	92	93	94
	12	94	95	95	91	92	92
0.11	6	98	89	93	89	91	92
	12	90	92	91	89	90	91
0.22	6	98	93	95	90	92	93
	12	90	91	92	88	90	92
0.31	6	89	93	92	88	89	91
	12	86	89	90	87	88	90
0.51	6	84	89	92	87	89	90
	12	78	75	89	82	84	87
0.75	6	76	69	79	67	71	70
	12	54	45	59	45	50	50
> 0.99+	6	42	39	27	4	12	17
	12	—	—	3	—	—	1

*HPLC retention determined by weighted average of peaks.
+Extract solution stored in refrigerator.

spectrophotometry and HPLC are given. The tabulated values are for storage in light; results for storage in darkness are similar, generally being 1–2% higher. As noted from the half lives in Table 1, anthocyanin retention is not affected by water activity up to $a_w = 0.51$. In Table 2, it is also seen that HPLC peaks 1 and 3 (corresponding to cyanidin-3-sambubioside-5-glucoside and cyanidin-3-sambubioside, respectively) are generally less affected by humidity than peaks 2 and 4 (cyanidin-3-glucose-5-glucoside and cyanidin-3-glucoside, respectively), the effect being most pronounced at the high water activities. Further, it is seen that when the anthocyanin extract is stored in the presence of large excess of water (i.e. in solution), the degradation of anthocyanins is much more rapid than even in the freeze dried samples stored at the highest a_w.

Results of the second series

The effect of temperature on anthocyanin loss and sample structure was dependent on sample water activity. When the water activity was low ($a_w = 0$), there was no effect of temperature on sample structure, or visual colour, while at $a_w = 0.51$, all samples were altered, with the sample stored at 5°C even showing minor shrinking. Visual sample colour was also affected at $a_w = 0.51$ for storage at 20 and 55°C, with the most pronounced effect at the higher temperature (Table 3).

Table 3. Half lives for dried anthocyanin samples as a function of temperature at high and low water activity when stored in darkness. The colour and structure are described after 1 year of storage

Temperature (°C)	$T_{1/2}$[†]($a_w = 0$) (days)	Colour[*]	Structure[*]	$T_{1/2}$[†]($a_w = 0$) (days)	Colour	Structure
5	4800[a]	o	o	1900[b]	o	ms
20	4400[a]	o	o	1200[c]	d	md
55	800[c]	o	o	70[d]	b	cd, c

[*]Abbreviations see Table 1.
[†]See Table 1.

A statistical examination of the half lives for anthocyanin loss at $a_w = 0$ show no difference for 5 and 20°C, while storage at 55°C is significantly different. Storage at 55°C, $a_w = 0$ (55/0) gives a shorter half life than at 5/0.51 and 20/0.51, though the difference between 55/0 and 20/0.51 is not significant. As expected, the shortest half life (approximately 2 months) occurs for storage with the combination of a high a_w and temperature. This degradation is the most rapid one observed in the entire study.

Examination of anthocyanin loss kinetics by the Arrhenius equation (Table 4) show

Table 4. Observed reaction rates and activation energies for anthocyanin degradation in freeze dried powder, stored at low and high water activity

a_w	Temp. (°C)	k (per day)	E_a (kJ/mol)
0	5	1.44×10^{-4}	
	20	1.58×10^{-4}	28.6
	55	8.60×10^{-4}	
0.51	5	3.65×10^{-4}	
	20	5.46×10^{-4}	52.2
	55	9.97×10^{-3}	

that the average activation energy for the disappearance of anthocyanins in the freeze dried powder is twice as high for storage at $a_w = 0.51$, than for storage in the dry state. The activation energy values are relatively low with respect to values normally associated with chemical reactions, and thus may reflect for the storage conditions examined that the rates of degradation reactions are limited by physical structural transformations which must occur first.

On the basis of visual observation of structure change and sizable rise in degradation rate it is likely that at 55°C and $a_w = 0.51$ the physical structural transformation is no longer limiting. To fully elucidate physical *versus* chemical limitations to anthocyanin degradation requires more storage temperatures than used in this study.

Results of the third series

The study conducted with and without presence of oxygen for the two water activities investigated (0; 0.51) showed absolutely no difference in the half lives for anthocyanin degradation ($T_{1/2}$ ($a_w = 0$) = 4200 days; $T_{1/2}$ ($a_w = 0.51$) = 1200 days). There was a slight difference in visual appearance for the samples stored in air at a water activity of 0.51, where the surface colour was darkened and a beginning deliquescence was observed. Spectrophotometric measurements, however, did not show any unusual deviations from the degradation tendencies already observed. These results are in good agreement with those by Erlandson & Wrolstad (1972). where oxygen was found to have only little effect on anthocyanin degradation in freeze dried strawberry puree. Storage in air, however, was found to have an influence on the rate of browning. In our study, where degradation index is used as a measure of browning, only a slight increase was observed after one year of storage. The findings by Clydesdale *et al.* (1978; 1979a, b and c), where storage in nitrogen greatly increased the stability of products containing spray dried powders of grape and cranberry pigments, do not correspond with our observations.

Results of the fourth series

The effect of extract pH prior to freeze drying on the subsequent storage stability of the freeze dried powder, was investigated for a very mild storage condition (darkness, ambient temperature and $a_w = 0$). The visual colour and structure was unchanged after 2 years of storage. Anthocyanin retention was measured by both HPCL and spectro-photometric analysis at 0, 3, 12 and 24 months. As anthocyanin retention based on weighted averages of the HPLC peaks corresponds very well with retention measured by spectrophotometric analysis, only results for spectrophotometric analysis are given in Table 5. It is noted that differences in anthocyanin degradation between the samples is minimal, though degradation is more rapid at the highest pH value. The relative degradation behaviour of the four individual anthocyanins, as measured by HPCL, did not change during the storage period. Results for samples stored 24 months are given in Table 5. It can be seen that the differences in retention of the four individual antho-cyanins do not follow the same degradation pattern as seen in the first series, where the water activity was varied. In the first series, peak no. 3 (cyanidin-3-sambubioside) was found to be the most resistant of the four peaks, whereas in this series it appears to be the least resistant. The choice of such mild storage conditions resulted in degradation half lives which are so long that the observed differences in degradation rate have little practical significance. The extent to which sample pH differences will have more influence on storage stability under harder storage conditions is therefore not neces-sarily predictable from the above results.

Table 5. Retention (%) of the original anthocyanins after 3, 12 and 24 months of storage in the dark at room temperature, of freeze dried extract with varied initial pH

	Months	pH 1	pH 2	pH 3	pH 4	pH 5
Overall anthocyanin retention (%)[*]	0	100	100	100	100	100
	3	98	98	98	96	93
	12	95	96	95	89	89
	24	93	92	89	87	84
$T_{1/2}$ (months)		156	143	147	134	107
Retention of individual anthocyanin components at 24 months	Peak[†]					
	1	90	90	90	91	90
	2	88	88	86	88	85
	3	89	88	86	85	80
	4	93	91	91	87	83

The original anthocyanin content after freeze drying was measured to be 651 mg/l and is set to 100%. For influence of freeze drying on the extracts, see Table 3 in Brønnum-Hansen and Flink (1985).

[*]Measured spectrophotometrically.

[†]Degradation peaks not present in original extract are not included.

Conclusions

A freeze dried anthocyanin extract from elderberry was stored under different conditions of water activity, temperature, atmosphere composition and presence of light and investigated for product stability. Water activity influences the stability of the anthocyanins, when the a_w exceeds 0.5. Below this value, the samples are almost unchanged after 1 year of storage. Storage in light and darkness gives no significant differences in stability, the only difference observed is a slight visual darkening of the surface on the samples stored in light. Measurement of anthocyanin content did not indicate related changes.

It was found that calculated monolayer values (BET and Caurie) of approximately 4 g H_2 per 100 g dry powder corresponded to a water activity of 0.35–0.4. When examining the correlation between moisture content and sample stability, this study shows good agreement with the general tendency cited in the literature, that the highest sample stability is generally associated with moisture content below the monolayer value. In contrast, Erlandson & Wrolstad (1972) found a gradually increasing anthocyanin degradation with increasing a_w, degradation already being of major importance at $a_w \geq 0.11$.

When following the individual anthocyanins, differences in degradation rate were observed, with the anthocyanins containing the disaccharide, sambubiose, seeming to be more resistant to degradation than the two other anthocyanins, which contain only glucose.

High temperature in combination with high water activity had the most pronounced effect on the stability of anthocyanins. The highest degradation rate in the entire storage study was found when $a_w = 0.51$ and temperature was 55°C; at the same time structure and colour were lost and the solubility was greatly impaired.

Oxygen is often shown to have a marked effect on the stability of anthocyanins in solution (Markakis, 1982), but this study shows that in environments where moisture contents are low, the presence or absence of oxygen is without influence. Additionally, presence or absence of oxygen did not have any effect on browning.

When a dry powder is kept under mild storage conditions at ambient temperature. the half lives found are in the range of 5–10 years. This is so slow that the possible applicability of the freeze dried powder as colorant in food products of low moisture content is evident.

Acknowledgment

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Effect of freezing rate on the denaturation of myofibrillar proteins

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Summary

Freezing of bovine muscle has a denaturating effect on myofibrillar proteins, Differential Scanning Calorimetry (DSC) studies of fresh and frozen muscle at different freezing rates show a decrease on denaturation enthalpies; the lower the freezing rate the greater the loss. When measuring the specific areas (ratio between each partial area in cm^2, and the dry weight of the sample, in mg) of the DSC thermograms, it can be observed that the area ascribed to myosin decreases with freezing, while the area corresponding to actin is not affected. These results are in agreement with the ATPase activity decreases as a consequence of freezing observing higher losses at lower freezing rates. The denaturation observed could be a result of a partial unfolding of the myosin head being more pronounced at low freezing rate.

Introduction

Freezing and frozen storage are important methods for meat preservation. However, these treatments carry along chemical and microstructural changes that modify the final product quality.

One of the most remarkable effects of freezing is the decrease in the muscle water holding capacity after thawing. Such a decrease is associated with the fact that during freezing, water–protein associations are replaced by protein–protein associations or other interactions (Fennema, 1977; Hamm, 1975). In fish, the changes observed in texture as well as in the muscle water holding capacity have been related to myofibrillar protein denaturation during freezing and frozen storage (Fennema, Powrie & Marth, 1973; Matsumoto, 1980; Shenouda, 1980). As regards bovine muscle few data have been obtained on the effects of storage of frozen muscle on protein structure and the influence of different freezing rates. (Awad, Powrie & Fennema, 1968; Rahelić, Pribis & Skenderović, 1974; Petrović & Rahelić, 1981.)

In the present study we investigated both by DSC and by enzyme measurements the denaturation of myofibrillar proteins in meat samples frozen at different freezing rates.

Materials and methods

Meat sample preparation

Bovine semitendinosus muscle from steers, 48 hr post-mortem, fresh and frozen at high, intermediate and low rate was used.

The freezing method used was described in previous studies (Bevilacqua, Zaritzky

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& Calvelo, 1979; Añón & Calvelo, 1980). Meat cylinders (5 cm diameter and 8–10 cm long) were cut with their longitudinal axes parallel to the fibres. They were frozen by placing one of their bases on a metallic plate at $-40°C$; obtaining in this way an unidirectional heat flow. Different surface heat transfer resistances were achieved by placing acrylic slabs of different thickness and known thermal conductivity between the heat exchanger and the meat cylinder to be frozen. The thermal history was followed through copper-constant thermocouples disposed along the longitudinal axis of the cylinder and connected to a datalogger (Fluke-model 2240 B). Once the whole meat cylinder reached $-25°C$, 0.5 cm slices were cut. Local freezing rate of each slice was characterized by its characteristic freezing time, t_c, defined as the period in which the meat temperature changes from -1 to $-7°C$ (Bevilacqua *et al.*, 1979; Añón & Calvelo, 1980). Meat slices were grouped in accordance to their t_c as follows: high ($t_c < 5$ min), intermediate (20 min $< t_c < 25$ min) and low ($t_c > 60$ min) freezing rates.

Thawing was carried out by placing frozen meat slices (5 cm diameter, 0.5 cm thickness) in a 4°C room overnight.

Myofibrils preparations

Muscle myofibrils were prepared by the method described by Coll & Robson (1967) from 2 g of ground muscle. The purified myofibrils were suspended in 0.15 M KCl-0.03 M Tris-HCl solution (pH 7.6). Experiments were performed with fresh and frozen samples.

Protein determination

Protein concentration was determined using the Biuret method described by Gornall, Bardawil & David (1949) and modified by Robson, Goll & Temple (1968). The Lowry method (Lowry *et al.*, 1951) was used as an alternative for some samples. These results were confirmed by determining total nitrogen content using Kjeldahl method (converting factors: 6.00 for myofibrils and 6.25 for total proteins).

0.6 M KCl Soluble total proteins and myofibrillar proteins

Protein solubility was assayed on 2 g of ground muscle in 20 ml of 0.6 M KCl-30 mM Tris-HCl solution at pH 7.6. This was homogenized in an Omni-Mixer (Sorvall-model 17106) (three times, 15 sec with 45 sec intervals) and gently stirred overnight at 4°C. The suspension was centrifuged at $16000 \times \mathbf{g}$ for 1 hr, then protein concentration was determined in the supernatant.

Myofibrillar proteins were extracted from isolated myofibrils with 0.6 M KCl-30 mM Tris-HCl solution at pH 7.6. Preparations were gently stirred overnight at 4°C, then were centrifuged at $17000 \times \mathbf{g}$ for 15 min; myofibrillar proteins were assayed in the supernatant.

ATPase activity measurement

Myofibrillar ATPase activity in presence of Ca^{2+} and Mg^{2+} ions as activators was determined using the procedure described by Hay, Currie & Wolfe (1973). The reaction medium was 0.02 M KCl-20 mM Tris-Acetate pH 7.0, 1 mM $MgCl_2$ (or $CaCl_2$) and 1 mM ATP with 0.4–1.0 mg protein/ml at 25°C. Results are expressed as μmol Pi produced/min/mg protein.

Differential scanning calorimetry (DSC)

DSC runs were performed with a 910 Dupont equipment on the following samples: (i) whole muscle; (ii) muscle depleted of connective tissue and sarcoplasmic proteins; and (iii) myofibrils. In all cases assayed samples were prepared from fresh and frozen muscle.

Samples of (ii) were obtained from ground muscle treated with Ringer solution (0.15 M NaCl, 3 mM KCl and 3 mM $CaCl_2$) stirring overnight at 4°C. The pH was adjusted to the desired value with 1 N NaOH or 1 N HCl solutions. The connective tissue was manually removed from the softer myofibrillar tissue with a scalpel (Stabursvik & Martens, 1980). Myofibrils sample (iii) were obtained from myofibrils suspension by centrifugation at $2500 \times$ **g**, for 15 min. Sedimented myofibrils were washed three times with Ringer solution at a determined pH value.

Temperature calibration was performed using Indium and water melting points as standards. The cell constant E was determined from Indium DSC thermograms. Wet samples of 15–25 mg were hermetically sealed in aluminium pans. Dry weight was determined after the run, by drying punctured pans at 105°C for 24 hr. Denaturation enthalpies (ΔHd) were estimated by measuring the area (expressed as cm^2) under the DSC transition curve (a baseline was constructed as a straight line from the beginning to the end of the endotherm). Freezing rate effects on muscle proteins denaturation were also studied calculating the specific areas (partial area/total mg of dry sample) for each peak.
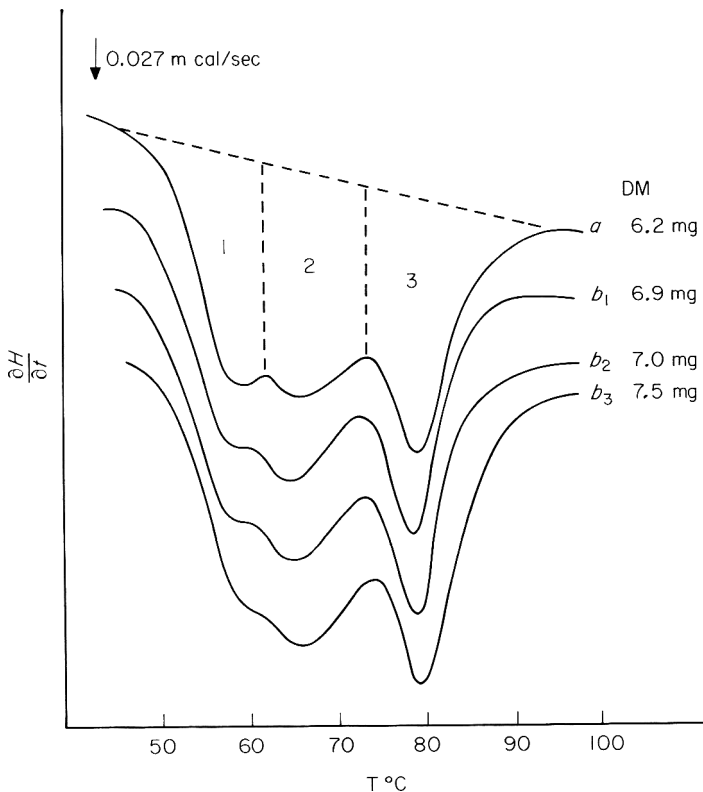
Figure 1. DSC thermograms of whole bovine muscle at pH 5.6, heating rate $\beta = 10°C/min$. (a) Fresh muscle; (b) frozen muscle. b_1, b_2 and b_3 indicate the freezing rate as follows: b_1: $t_c < 5$ min (rapid freezing); b_2: $t_c = 20$–25 min (intermediate freezing); b_3: $t_c > 60$ min (slow freezing); DM: dry matter weight. Unfrozen muscle.

Statistical methods

Variance analysis (ANAVA) at a 95% confidence level was used for statistical evaluations of ATPase activity, apparent enthalpies of denaturation and specific areas. Six to eleven values for each sample were included in the calculation. The least significant difference (l.s.d.) was calculated.

Results and discussion

Differential scanning calorimetry study

DSC thermograms of fresh and frozen whole bovine muscle (pH 5.6 ± 0.1) show three peaks, peak 1 corresponds to myosin, peak 3 to actin and peak 2 is attributed to the additive effect of myosin, sarcoplasmic proteins and connective tissue denaturation (Fig. 1) (Wright, Leach & Wilding, 1977; Stabursvik & Martens, 1980; Martens, Stabursvik & Martens, 1982). The main difference between the thermograms is located in the region corresponding to peak 1. This peak diminished considerably after slow freezing and thawing but a smaller extent after rapid freezing.

Intending to determine if one or more myofibrillar proteins are affected by freezing, DSC thermograms of whole muscle were divided into three partial areas (corresponding to peaks 1, 2 and 3) as shown in Fig. 1. Specific areas are expressed as the ratio
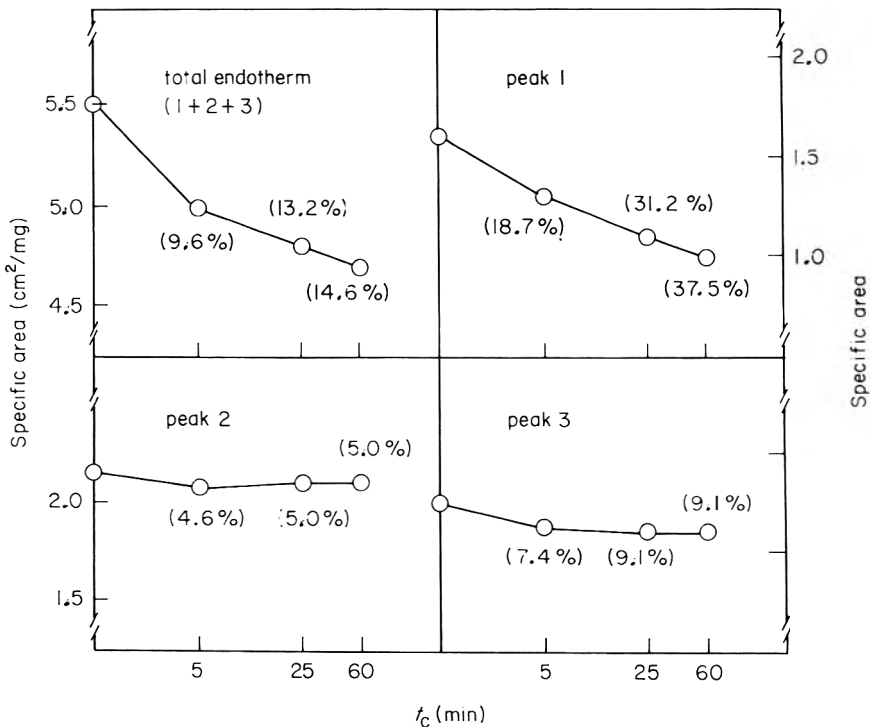
Figure 2. Effect of freezing rate on the specific areas (cm^2/mg) of the DSC peaks of whole bovine muscle. The abcissa is a logarithmic scale. Heating rate $\beta = 10°C/min$. The least significant difference at a 95% confidence level (l.s.d.$_{0.05}$) values calculated for each area were as follows: total endotherm, 0.24; peak 1, 0.18; peak 2, 0.20 and peak 3, 0.25. Percentages values indicate specific area losses between frozen and unfrozen muscle. Minimum number of assays: six.
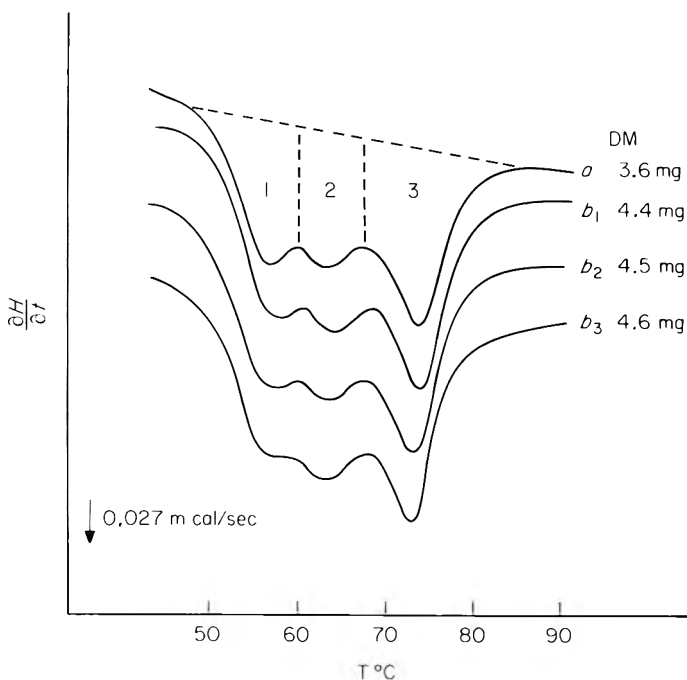
Figure 3. DSC thermograms of bovine muscle treated with Ringer solution at pH 5.6; heating rate β = 10°C/min. (a) Fresh muscle depleted of sarcoplasmic proteins and connective tissue; (b) frozen muscle depleted of sarcoplasmic proteins and connective tissue. b_1, b_2 and b_3 indicate the freezing rate as follows: b_1: t_c < 5 min (rapid freezing); b_2: t_c = 20–25 min (intermediate freezing); b_3: t_c > 60 min (slow freezing); DM: dry matter weight.

between each partial area (in cm^2) and the dry weight of the sample (in mg) used in each run. Figure 2 shows significant differences between fresh and frozen muscle. The same figure also shows the tendency of specific areas corresponding to peaks 2 and 3 to remain constant, while those corresponding to peak 1 and the total endotherm tend to decrease with freezing. These results indicate that myosin is the protein affected by the freezing process, especially the region of the molecule of myosin that contributes to peak 1. Previous investigations (Goodno, Harris & Swenson, 1976; Wright *et al.*, 1977; Stabursvik & Martens, 1980) have shown that myosin presents a complex pattern in its denaturation behaviour. The multiple thermal transitions observed could be related to different regions of the molecule being affected by the pH and ionic strength of the environment (Wright & Wilding, 1984). Under the conditions used in the present study (pH 5.6; T = 0.16) peak 1 was interpreted as representing denaturation of heavy meromyosin S-1 (HMM S-1).

Figure 3 shows DSC thermograms of fresh and frozen muscle (pH 5.6±0.1) depleted of sarcoplasmic proteins (muscle treated with Ringer solution at pH 5.6) and connective tissue. These endotherms also show three peaks which may be mainly attributed to thermal transition of myofibrillar proteins. Then, peaks 1 and 2 would correspond to myosin and peak 3 to actin. However, it is important to consider that, although myosin and actin are the major myofibrillar proteins, other minor proteins would contribute to the total thermogram. According to results obtained in our laboratory (Wagner & Añón, 1985) peaks 1 and 2 correspond to thick filament (myosin) and peak 3 to thin filament (actin plus minor proteins). As in whole muscle,

Table 1. Effect of freezing rate on the specific areas of the DSC peaks of bovine muscle treated with Ringer Solution at pH 5.6, and heating rate $\beta = 10°C/min$

Freezing rate (min)	Specific area (cm^2/mg)*			
	Total endotherm	Peak 1	Peak 2	Peak 3
Unfrozen	5.78 ± 0.27	2.20 ± 0.22	1.42 ± 0.27	2.16 ± 0.16
High ($t_c < 5$)	5.13 ± 0.19	1.77 ± 0.14	1.40 ± 0.20	1.96 ± 0.11
Intermediate				
($t_c = 20–25$)	4.81 ± 0.28	1.59 ± 0.17	1.37 ± 0.11	1.85 ± 0.12
Low ($t_c > 60$)	4.87 ± 0.17	1.50 ± 0.15	1.46 ± 0.16	1.91 ± 0.11
L.s.d.$_{0.05}$	0.26	0.21	0.23	0.21

*Values shown are the means with standard deviations of a minimum of six samples.
L.s.d.$_{0.05}$ Least significant difference at a 95% confidence level.

the thermograms of muscle depleted of sarcoplasmic proteins and connective tissue show that the area of peak 1 decreases as a consequence of freezing, showing higher decrease after slow freezing and thawing. In this case, specific areas corresponding to peaks 1, 2 and 3 were also calculated. Table 1 shows that specific areas corresponding to peaks 2 and 3 remain constant, while those corresponding to peak 1 and the total endotherm decrease with freezing. The results indicate that the area corresponding to peak 1 is the most affected, similarly to the results obtained with whole muscle. DSC thermograms of myofibrils from fresh muscle depict the same peaks (results not shown).

Comparison of thermograms corresponding to whole muscle, muscle depleted of sarcoplasmic proteins and connective tissue and isolated myofibrils shows a decrease in the T_{max} values (Table 2). These results show the importance of the chemical environment on the thermal stability of proteins (see (i) and (ii) values of Table 2). Sarcoplasmic proteins and other soluble compounds removal and the addition of Ca^{2+}, Na^+, K^+ and Cl^- ions provided by Ringer solution seem to leave myofibrillar proteins unprotected and more labile to heat. In addition, the minor values of T_{max} for isolated

Table 2. T_{max} of the thermograms obtained for fresh bovine muscle (i) whole muscle; (ii) muscle depleted of sarcoplasmic proteins and connective tissue; (iii) isolated myofibrils

Sample	T_{max} (°C)		
	Peak 1	Peak 2	Peak 3
(i)	58.9 ± 0.4	67.2 ± 1.3	80.2 ± 0.7
(ii)	57.7 ± 0.5	63.9 ± 0.7	74.0 ± 0.4
(iii)	54.8 ± 0.5	60.6 ± 0.5	67.5 ± 0.5

DSC running conditions: pH 5.6; $\Gamma = 0.16$; heating rate $\beta = 10°C/min$ dry weight = 3–8 mg for samples (i) and (ii); 1.6–3.0 mg for sample (iii).
T_{max}, peak maximum temperature.
Values shown are the means with standard deviations of a minimum of six samples.
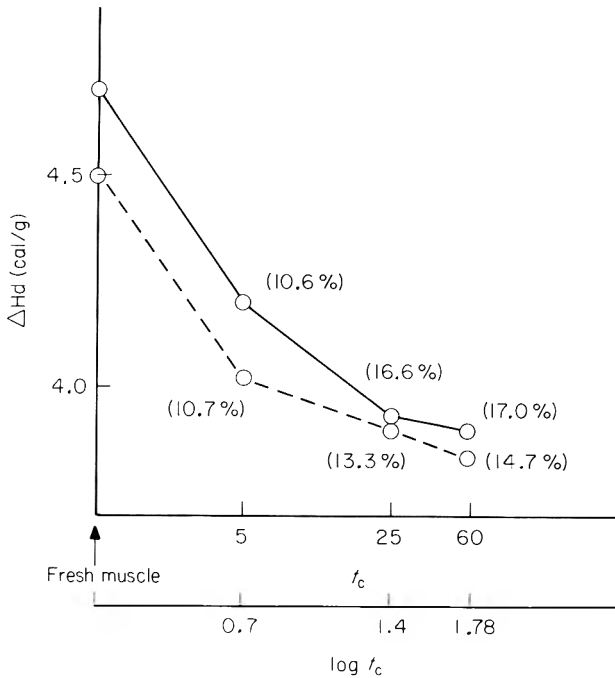
Figure 4. Enthalpy of denaturation of whole bovine muscle (pH 5.6) as a function of the freezing rate. Percentage values indicate enthalpy losses between frozen and fresh muscle. Minimum number of assays: six. — — — Whole bovine muscle; ——— bovine muscle depleted of sarcoplasmic proteins and connective tissue.

myofibrils could be due to the changes of the environment and disruption of the cellular structure during myofibril preparations. As regards to the influence of freezing no significant differences between the frozen and unfrozen muscle T_{max} were observed (Figs 1 and 3).

Figure 4 shows the influence of the freezing rate on total denaturation enthalpies (ΔHd). ΔHd for fresh whole muscle resulted in a value of 4.50 ± 0.15 cal/g, being 4.10 ± 0.10, 3.90 ± 0.09 and 3.84 ± 0.10 cal/g for muscle frozen at high, intermediate and low freezing rates, respectively. These values are statistically different from those

Table 3. Denaturation enthalpy (ΔHd) of isolated myofibrils from fresh and frozen muscle

Myofibrils of bovine muscle, freezing rate (min)	ΔHd (cal/gr dry weight)	% oss
Fresh	5.51 ± 0.10	—
High ($t_c < 5$)	5.33 ± 0.06	3.27
Intermediate ($t_c = 20-25$)	5.06 ± 0.05	8.17
Low ($t_c > 60$)	5.01 ± 0.05	9.07

DSC running conditions: pH 5.6; $\Gamma = 0.16$; heating rate $\beta = 10°C/min$; dry weight 1.6–3.0 mg.

Values shown are the means with standard deviations of a minimum of six samples.
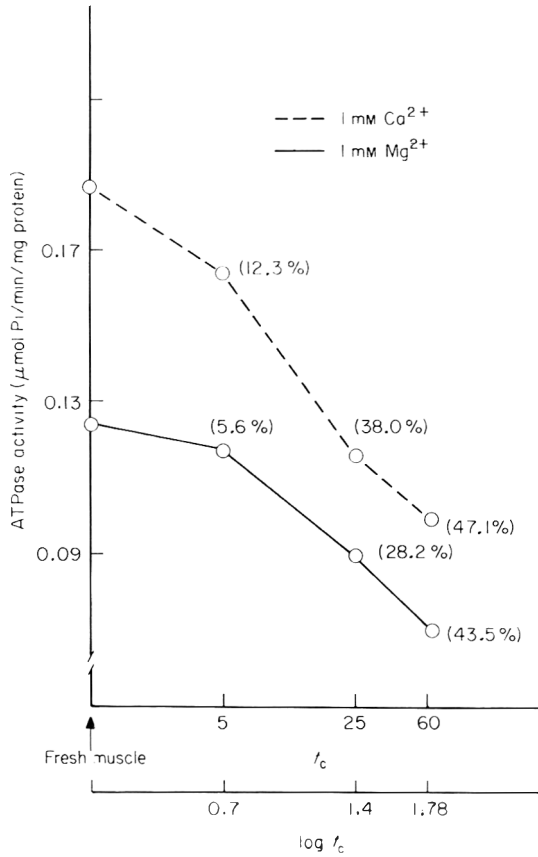
Figure 5. ATPase activity of myofibrils isolated from bovine muscle as a function of the freezing rate. Percentage values indicate ATPase activity losses respect to myofibril from fresh muscle. Minimum number of assays: eight.

corresponding to fresh muscle and show a tendency to give lower ΔHd values for low freezing rates with respect to high freezing rates ($l.s.d._{0+05} = 0.24$). Muscle depleted of sarcoplasmic proteins and connective tissue shows the same behaviour (Fig. 4). In order to assess which proteins are involved in the decrease of enthalpy observed, the ΔHd values of myofibrillar proteins were calculated (Table 3). Statistical analysis shows that ΔHd values of frozen muscle myofibrils are lower than those corresponding to fresh muscle myofibrils showing a higher decrease as the freezing t_c value increases ($l.s.d._{0+05} = 0.33$).

These results indicate that freezing has a denaturing effect on myofibrillar proteins independent of freezing rate and that this effect has a tendency to be more pronounced for slow freezing. The denaturing effect would lead to a protein unfolding resulting in a lower enthalpy value compared to the native protein state. These results also suggest bond disruption during the freezing process.

Studies on ATPase activity

Figure 5 depicts ATPase activity values of isolated myofibrils from fresh and frozen muscle *versus* $\log t_c$ using Ca^{2+} and Mg^{2+} ions as enzymes activators. These results show that ATPase activity decreases as a consequence of freezing observing higher losses at

higher t_c values. ATPase activity values for fresh muscle myofibrils are 0.124 ± 0.025 and 0.187 ± 0.010 μmol Pi/min/mg when Mg^{2+} and Ca^{2+} ions are used as activators respectively, and decrease to 0.117 ± 0.012 and 0.164 ± 0.005 μmol Pi/min/mg for rapid freezing. Statistical analysis does not show significant differences for those values ($l.s.d._{0.05} = 0.032$) suggesting that rapid freezing does not affect myofibril ATPase activity.

At intermediate freezing rates, ATPase activities decrease to 0.089 ± 0.014 and 0.116 ± 0.004 μmol Pi/min/mg respectively. Slow freezing shows lower values, 0.070 ± 0.010 and 0.099 ± 0.020 μmol Pi/min/mg respectively. These values are significantly different ($l.s.d._{0.05} = 0.022$) from those corresponding to myofibrils from fresh and rapid frozen muscle indicating a deteriorative effect upon freezing.

Table 4. Soluble total proteins and soluble myofibrils in 0.6 M KCl solution at pH 7.6 for fresh and frozen muscle

Muscle freezing rate (mm)	% Ts	% Ms
Fresh	15.2 ± 1.1	81.0 ± 5.8
High ($t_c < 5$)	15.3 ± 1.2	79.6 ± 10.5
Intermediate ($t_c = 20-25$)	15.1 ± 1.8	n.d.
Low ($t_c > 60$)	15.2 ± 1.3	79.1 ± 8.0

% Ts, Percentage values of soluble total proteins respect to wet whole muscle.
% Ms, Percentage values of myofibril proteins respect to isolated myofibrils.
Values shown are the means with standard deviations of a minimum of nine samples.

The decrease of myofibril ATPase activity when Mg^{2+} ion is used as activator could be due to a decrease of the myosin affinity for actin and/or to an alteration of the enzymic properties of actomyosin (or myosin). Otherwise, the decrease of ATPase activity when Ca^{2+} is used as activator could indicate a change in the free myosin structure. Then ATPase activity decrease would imply a change produced by freezing on the myosin head which contains both the active site of the enzyme and the actin interaction site (Ockerman, 1977). There is a good agreement between results on ATPase activity and the interpretation that the decrease of ΔHd of peak 1 is due to denaturation of the myosin head (HMM S-1).

The denaturation observed could be a result of a partial unfolding of the molecule with exposure of hydrophobic groups. This unfolding would induce protein aggregation during frozen storage as suggested by the fact that total and myofibrillar protein solubility does not change by freezing (Table 4) but decreases during frozen storage (Awad *et al.*, 1968; Matsumoto, 1980). Unfolding would be a result of the local increase of ionic strength as a consequence of freezing and water migration from the myofibrillar space or dehydration of myofibrillar protein. The results obtained indicate that denaturation is more pronounced at low freezing rate.

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Protein digestibility of pigskin and bovine tendon in rats

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Summary

The protein nutritional value of pigskins and bovine tendons, with collagen contents of 75 and 95% respectively, was evaluated using amino acid analyses and nitrogen balance experiments on rats. Pigskins contain somewhat higher amounts of tryptophan, methionine, cystine and tyrosine than tendons. The amounts of several essential amino acids were low. The true digestibilities were for young (4 month old), unscalded pigskin 96.1%, old (5 year old), unscalded pigskin 92.5%, calf tendon 97.3% and cow tendon 92.4%. Small but statistically significant differences due to the animal age were observed. Insoluble collagen, extracted from bovine tendon, had a digestibility of 95.2%. Wet heat treatment of old pigskin and cow tendon samples at 74°C for 30 min significantly increased the digestibilities to 96.3 and 97.3% respectively. The results indicate that prior denaturation of collagen, as obtained by, for example, heat treatment, is not necessary for the digestion of pigskin and tendon in rats.

Introduction

Muscle proteins are considered to be more rapidly and easily digested than collagen (Brüggeman *et al.*, 1964; Davidson *et al.*, 1979) and many plant proteins (Snook, 1973). Native collagen is considered more or less indigestible (Loewit, 1970; Cheftel, 1977, 1979; Kies, 1981; Ashgar & Henrickson, 1982) but heating and the conversion to gelatin makes it susceptible to enzymes and thus digestible (Paul, 1972; Rogowski, 1980; Ashgar & Henrickson, 1982). Gelatin has a digestibility of 90% *in vivo* (Mauron, 1973). Harkness, Harkness & Venn (1978) showed that native rat tail tendon was about 100% absorbed when given to rats. However, few *in vivo* investigations on the digestibility of food collagen have been performed, and these have been evaluated as apparent digestibility in rat assay. The collagenous materials used in these studies, however, cannot be considered native since they were either limed or partly heated. Thus Whitmore *et al.* (1975) found limed cattle hide to be 90% digestible. Happich *et al.* (1975) showed a digestibility of 88% for partially defatted chopped beef containing 50% collagen and heated to about 50°C. Raw, probably scalded, pigs ears with high level of collagen were 88% digestible (Vaughn *et al.*, 1979).

In a previous study (Laser Reuterswärd *et al.*, 1982) it was shown that the true digestibility of a mixture of raw pigskin and beef containing 50% collagen was 99%. The pig used was 6 months old and the carcass was scalded according to normal practice. Scalding may denature some of the soluble collagen in immature pigs (Johns & Courts,

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1977). It is known that insoluble collagen from a mature animal shows an increasing resistance to solubilizing agents, including pepsin, due to the stabilization of inter-molecular crosslinks (Weiss, 1976; Sims & Bailey, 1981). No studies of the *in vivo* digestibility of collagen from mature animals are available in the literature. However, Harkness *et al.* (1978) found that the digestibility of rat tail tendon, evaluated in a rat assay, was drastically reduced after the introduction of artificial crosslinks.

The aim of the present study was to evaluate the true digestibility of highly colla-genous materials such as pigskin and bovine tendon in a rat assay. The effects of the age as well as scalding of carcasses and the cooking of pigskin and tendon samples were investigated.

Materials and methods

Sample preparation

Pigskins. Skins were removed immediately after slaughter from a 4 month old pig and a 5 year old sow and manually shaved with a razor. Another pigskin was taken from a 6 month old pig which had been scalded according to slaughterhouse procedures in water at 60°C for 10 min. All the pigskins were freed of meaty parts and visible fat. The samples were finely cut in a Moulinex blender. Half of each sample was vacuum packed in a plastic bag less than 1 cm thick, heated in a water bath at 74 ± 1°C for 30 min and immediately chilled. The heated samples were finely recut in a Moulinex blender. The unheated and heated samples were lyophilized. The unheated samples were minced in a knife homogenizer (Janke & Kunkel, West Germany) and the heated samples in a Moulinex blender. The unscalded young and old pigskins, when surveyed under a low magnification microscope, appeared similar and were porous and crystalline-like having a tight structure (Laser Reuterswärd, 1985). Except for the unscalded young and old pigskins, the other pigskin samples were mixed with a portion of maize starch and ground in a hammermill with a sieve of 0.8 mm before being mixed into the diets.

Tendons. Achilles tendons were collected from forty 7 month old calves and from forty cows with an average age of 6.6 years (range 4–12.5). The calf tendons were collected 24 h and the cow tendons 1–6 days after slaughter. The tendons were freed of meaty parts and visible fat and frozen for not more than 2 months. After thawing, the tendons were cut into smaller pieces. The wet calf and cow samples were ground to pass a 3 mm sieve and immediately chilled. The temperature of the calf sample reached 32°C and the cow sample 44°C during grinding. Half of the cow sample was packed and heated as described for the pigskin samples above and cut in a Moulinex blender. The unheated and heated samples were lyophilized and homogenized in a hammermill. When surveyed under low magnification the unheated hammermilled calf and cow samples appeared similar with a porous and crystalline-like structure. The heated hammermilled cow sample showed tighter particles (Laser Reuterswärd, 1985).

Insoluble collagen from bovine Achilles tendon, prepared according to Einbinder & Schubert (1951), was purchased from Sigma Chemical Co., U.S.A. The tendon samples were mixed into the diets without any further preparation.

Chemical analyses

Nitrogen determined by the Kjeldahl method and hydroxyproline analysed on a

Technicon Auto-Analyser according to Stegemann (1958) as modified by Weber (1973). Calculations of collagen and protein contents were made according to Laser Reutersward *et al.* (1982).

Amino acid analyses were performed on duplicate samples after acid hydrolisys in 6 N HCl at 110°C for 24 hr. Cystine and methionine were determined after performic acid oxidation. Separation was performed on a Durrum D-500 Analyser. Tryptophan was hydrolysed with 3 M mercaptoethanesulfonic acid at 110°C for 24 hr and analysed on a Beckman 120B Amino Acid Analyser, at the Central Amino Acid Laboratory, Biochemical Centre, Uppsala, Sweden.

Nitrogen balance experiments

Nitrogen balance studies were performed on growing rats, male Sprague-Dawley, Anticimex, Sweden. Each diet was tested on five rats. The animals were kept individually in metabolic cages according to Eggum (1973) at 20°C and 55% r.h. After 4 days of acclimatization the urine and faeces were collected for 5 days. The diet was composed according to Eggum (1973) with modified contents of vitamins and minerals according to Forsum, Hambraeus & Siddiqi (1973). The composition of the diet was as follows (% of dry matter): protein corresponding to 1.5% nitrogen, maize oil 5%, cellulose powder 5%, mineral mixture 4.8%, vitamin mixture 0.8%, choline chloride 0.2%, saccharose 10% and maize starch up to 100%.

The diets were supplemented with L-methionine (Biochemische Zwecke, Merck), L-tryptophan (chromatographically homogeneous), L-isoleucine and L-histidine HCl (BDH Chemicals Ltd., U.K.). The levels of these four amino acids in the diets (% of dry matter) were for pigskins 0.20, 0.06, 0.04 and 0.06% and for tendons 0.15, 0.10, 0.10 and 0.10%, respectively. A diet containing ANRC Reference Protein High Nitrogen Casein (Sheffield Chemicals, U.S.A.) supplemented with 0.22% methionine (dry matter) was used as a reference.

The nitrogen contents of the feed, urine and faeces were analysed by the Kjeldahl method. Calculations of true digestibility, biological value and net protein utilization were performed with the Thomas-Mitchell equations as described by Eggum (1973). Values for endogenous and metabolic nitrogen were obtained from experiments with a diet containing fat extracted, lyophilized, whole hens egg corresponding to 0.72% nitrogen (dry basis). Values obtained during the 5 day balance period were for faeces, 60.5 mg and urine 76.2 mg.

Statistical evaluation

Means and standard deviations were calculated and analysed by Student's *t*-test (Bailey, 1981).

Results and discussion

Amino acid composition

The amino acid composition as well as the collagen and protein content are shown in Table 1. The protein in the tendon samples contained more collagen than the protein in pigskins, the value for pigskin agrees with the results reported by Johns (1977). The variations in total protein content were mainly due to the varying amounts of fat.

The amino acid composition of the different pigskins was similar (Table 1). No data on the complete amino acid composition of intact pigskin is available in the literature.

Table 1. Amino acid compositions, protein and collagen content of pigskin and tendon samples from animals of different ages

	Pigskin						Tendon			
	4 month unscalded	4 month heated	6 month scalded	6 month scalded/ heated	5 year unscalded	5 year heated	Calf raw	Cow raw	Cow heated	Insoluble collagen
Aspartic acid	6.8	6.9	7.3	6.6	6.9	7.2	6.1	5.9	6.3	6.3
Threonine	2.4	2.4	2.4	2.3	2.3	2.4	2.1	1.9	2.1	2.0
Serine	4.2	4.3	4.4	4.1	4.4	4.6	3.5	3.4	3.6	3.6
Glutamatic acid	11.2	11.4	12.0	10.8	11.4	12.0	10.2	9.8	10.5	10.6
Proline	12.5	12.9	14.4	13.7	13.0	14.7	12.5	12.4	13.0	13.9
Glycine	21.4	21.6	24.0	22.1	21.2	22.9	21.7	21.7	22.9	25.4
Alanine	8.8	9.2	10.1	9.1	9.2	10.0	9.3	9.2	9.7	10.4
Cystine	0.57	0.76	0.38	0.25	0.48	0.52	tr	0.18	0.20	tr
Valine	3.1	3.1	3.2	2.9	3.0	3.1	2.6	2.5	2.7	2.6
Methionine	1.5	1.7	1.6	1.2	1.3	1.1	0.82	0.78	0.89	0.97
Isoleucine	1.9	1.8	1.8	1.6	1.8	1.8	1.7	1.6	1.7	1.6
Leucine	4.1	4.1	4.1	3.7	3.9	4.0	3.6	3.4	3.6	3.3
Tyrosine	1.3	1.3	1.3	1.1	1.2	1.2	0.81	0.78	0.72	0.69
Phenylalanine	2.4	2.5	2.6	2.4	2.4	2.5	2.1	2.0	2.1	2.1
Histidine	0.93	1.0	1.1	0.98	0.95	0.98	0.77	0.70	0.75	0.76
Lysine	3.9	4.2	4.4	4.1	4.1	4.3	3.2	3.1	3.4	2.9
Hydroxylysine	0.86	0.78	0.89	0.93	0.71	0.72	2.2	1.7	1.8	2.6
Arginine	8.2	8.7	9.3	8.5	8.7	9.3	8.5	8.2	8.9	9.0
Ammonia	0.97	0.90	0.95	0.84	0.93	0.91	0.77	0.81	0.78	0.80
Hydroxyproline	10.1	10.4	10.4	10.7	11.0	11.2	13.2	13.7	13.5	13.6
Tryptophan	0.14	0.09	0.15	0.16	0.18	0.17	0.07	tr	tr	0.00
Total	107.2	110.0	116.6	108.0	109.1	115.3	105.6	103.8	109.2	113.1
Protein/dry weight (%)	47.4	52.1	58.1	63.5	70.2	70.8	93.5	99.7	97.2	102.2
Collagen as % of protein	71.8	71.7	71.3	73.7	76.1	77.6	92.5	96.7	94.8	96.4

Amino acids expressed in g/18 g N, corresponding to 100 g protein.
Heat treatment was performed on wet material at 74°C for 30 min.

However, when compared to results obtained by Eastoe (1967) on pure gelatin the contents of glycine, proline and hydroxyproline were lower in pigskin. In contrast considerably higher amounts of cystine, methionine and tyrosine was found in the intact pigskin. The tryptophan content was about 0.15 g/100 g protein in pigskins but this amino acid is lacking in gelatin.

The amino acid composition of the four tendon samples (Table 1) was similar and in good agreement with the pattern reported for cattle Achilles tendon by Eastoe (1967).

In the tendons, the content of lysine was considerably lower and that of hydroxy-lysine higher than the corresponding values in pigskins. However, the sum of lysine and hydroxylysine was fairly constant, in pigskins 4.8–5.3 and in tendons 4.8 to 5.5 g/18 g N, similar to the findings of Eastoe (1967).

An approximate 28% loss of methionine was found for the scalded/heated pigskin when compared to the scalded one. Loss of methionine during heat treatment of pork has been reported previously by Donoso *et al.* (1962).

The chemical scores for several amino acids in pigskins and tendons were very low when calculated in relation to the need of the rat according to the Food and Nutrition Board (1974) as cited by Steinke (1979). For pigskins and tendons the chemical scores were for tryptophan 11 and zero, respectively and for isoleucine, methionine/cystine and histidine the values were all below 31 and 33. Supplementation with these amino acids was performed to provide a better amino acid balance and thus ensure a more reliable determination of digestibility. The amounts of amino acids supplemented could be considered negligible compared to the total protein content and would not influence the evaluation of digestibility.

Table 2. Mean values of true digestibility for pigskin and tendon samples from animals of different ages

	True digestibility (%)	Significance [1]									
Pigskin		1	2	3	4	5	6				
1. Young (4 month) unscalded	96.1 (1.2)[‡]	—	ns	ns	ns	**	ns				
2. Young (4 month) heated	97.4 (1.5)		—	ns	ns	**	ns				
3. Young (6 month) scalded	97.5 (1.9)			—	ns	**	ns				
4. Young (6 month) scalded/heated	97.7 (1.3)				—	***	ns				
5. Old (5 year) unscalded	92.5 (1.7)					—	*				
6. Old (5 year) heated	96.3 (2.5)						—				
Bovine achilles tendon								7	8	9	10
7. Calf (7 month) raw	97.3 (1.0)							—	**	ns	ns
8. Cow (6.6 year) raw	92.4 (2.2)								—	**	ns
9. Cow (6.6 year) heated	97.8 (2.0)									—	ns
10. Insoluble collagen	95.2 (2.2)										—
Reference casein (pigskin)	99.9 (1.8)										
Reference casein (tendon)	99.1 (0.8)										

[‡]ns = not significant.
[‡]$n = 4$.
* = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$.

$n = 5$ and standard deviations within brackets. Wet heat treatment of sample nos 4, 6 and 9 was performed at 74°C for 30 min.

Nitrogen balance experiments

Table 2 shows results of true protein digestibility. All pigskins and tendon samples were digested to at least 92%. A significant difference in digestibility was found between young and old pigskins as well as between calf and cow tendon ($P < 0.01$).

Heat treatment of the old pigskin and the cow tendon samples resulted in a significant increase in digestibility ($P < 0.05$; $P < 0.01$, respectively). However, for the two young pigskin samples no significant effect of heat treatment or scalding was found. The digestibilities of the heated old pigskin and cow tendon samples were not significantly different from the corresponding unheated young pigskin and calf tendon (i.e., the amount of uneaten food expressed as a percentage of the total food offered).

The feed residues (i.e., the amount of uneaten food expressed as a percentage of the total food offered) were for the pigskin samples between 21–45%, the highest value 9–14% and with casein 1%. According to Eggum (1973), animals with feed residues tended to give lower digestibility values than animals without feed residues, but the difference was not statistically significant. Althought the feed residues in the present study were in some cases quite large, this would not have caused overestimation of the digestibility. Some of the samples used in the present study were previously studied in an *in vitro* system evaluated as nitrogen solubility. Incubation was performed at 37°C with pepsin at pH 1.5 and pancreatin at pH 6.8 followed by precipitation with trichloroacetic acid (TCA). With the young and old unscalded pigskins the TCA solubility values were 94 and 75% respectively. With the four tendon samples the solubilities were all about 92% (Laser Reuterswärd, 1985).

The values obtained *in vitro* are thus in the same range as the *in vivo* values except for the old pigskin sample where a much lower *in vitro* value was obtained. A high degree of solubilization *in vitro* could therefore be considered as an indication of high digestibility *in vivo* for these kinds of samples.

It is known that as animals age collagen fibres become more stable as assessed by tensile strength, isometric tension, swelling and solubility in hot water and acid. This is due to the stabilization of the intermolecular crosslinks (Weiss, 1976; Sims & Bailey, 1981; Bailey, 1982). Increased resistance to digestion by, for example, pepsin in mature animals has also been reported (Weiss, 1976) resulting in decreased solubility in pepsin/acetic acid for insoluble collagen from old animals (Etherington, 1977). The present *in vivo* study indicates that despite an increase in age, high digestibilities were obtained under conditions occurring in the gastrointestinal tract of the rat. In contrast, when artificial crosslinks were introduced, the *in vivo* digestibility of rat tail tendon decreased from 102 to 39% (Harkness *et al.*, 1978).

The present results also show that the sample of extracted insoluble collagen from tendon had a digestibility of 95.2%, a value between those of calf and cow tendons but not significantly different from either of them. Mucopolysaccharides and soluble proteins from the collagen tissue matrix were extracted in this sample. Etherington (1977) pointed out that proteoglycans and mucopolysaccharides may have a stabilizing role complementary to the intermolecular crosslinks of collagen and that these carbo-hydrates may restrict access of an enzyme to the non-helical telopeptide region. However, the present findings indicate that the presence of the tissue matrix of pigskin and tendon did not limit digestion *in vivo*.

In skin and tendons the collagen fibres are (about 90%) insoluble (Johns, 1977; Carmichael & Lawrie, 1967) and insoluble collagen denatures at a temperature of about 65°C when heated wet (Bailey, 1968). The temperatures obtained during grinding of the wet calf and cow samples were thus well below the denaturation temperature.

The present results show that the unheated samples as well as the heated ones were highly digestible. This indicates that prior heat treatment is not necessary since solubilization and gelatinization of the insoluble collagen obviously occur during digestion *in vivo*. This is discussed in more detail elsewhere (Laser Reuterswärd, 1985; Laser Reuterswärd & Fabiansson, 1984).

The biological values after supplementation for all pigskin and tendon diets were between 44.3 and 52.2%. No significant differences were found between pigskin samples or between tendon samples. Since the true digestibility values were quite high the net protein utilization values were found to be close to that of the biological values, between 41.4 and 50.6%.

It could be concluded that protein in pigskin and bovine tendon was digested to at least 92% *in vivo* irrespective of animal age and prior heat treatment. Young pigskin and calf tendon were somewhat more digestible than old pigskin and cow tendon. Prior heat treatment of tissues from old animals increased the digestibilities to the same level as seen in young animals.

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Effect of hot water spray washing on the appearance of lamb carcasses of different weights

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Summary

Lamb carcasses of > 22 kg weight were not adversely affected by spray washing, but carcasses of < 22 kg weight were affected to some extent. Characteristics of carcasses significantly affected included the appearance of the fat in the crutch region and on the back, and the degree of flaccidity and dampness of the abdominal region. These faults were alleviated somewhat during refrigerated storage and consequently the faults detected in the experiments were probably not of commercial significance since, under pilot market tests in Europe, buyers did not find any faults in spray washed carcasses.

Introduction

Traditionally, wiping cloths have been used to clean carcasses and while they removed visible dirt to some extent these cloths became highly contaminated with microorganisms (Bryce-Jones, 1969). The use of these cloths is now banned by veterinary regulations such as those of the European Economic Community (Anon., 1964) so that alternative methods of cleaning carcasses have to be developed.

Spray washing at the end of the processing line is one such method which can be readily employed in commercial practice. While it has been shown to improve the microbiological quality of carcasses (Patterson, 1968; Bailey, 1971; Kelly, Dempster & McLoughlin, 1974; Kotula *et al.*, 1974; Bailey & Roberts, 1976; Kelly, 1978) the meat industry in Ireland was reluctant to adopt the method for lamb carcasses since it was indicated that appearance was adversely affected by spray washing.

Previous studies have shown that high pressure and cold water systems maximize damage to the carcass surface (Patterson, 1972; Bailey, 1972) consequently only relatively low line pressure and hot water systems were examined in this study.

The purpose of this study was to establish and quantify how the appearance of different sizes of lamb carcasses were affected by spray washing. The term 'bloom' is used in the meat industry to indicate the freshness and keeping quality of carcasses and is based mainly on the appearance of the surface but it has never been adequately defined. Thus it was necessary in the experiments to develop a method of assessment sufficiently accurate to evaluate the effects of spray washing on carcass appearance.

Materials and methods

Carcass selection

Lamb carcasses of the required weight were removed from the processing line

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immediately after evisceration at one of the major lamb carcass exporting plants and hung from a detention rail adjacent to the main factory line. Throughout the experiments carcasses of similar appearance were selected on the basis of visual assessment by staff of both An Foras Taluntais and the meat plant.

Spray washing procedure

Carcasses, suspended by the hind legs, were washed using a Mono pump Type SH 22 A5 (Mono Pumps Ltd., Dublin 11) with a fan type spray nozzle (Delavan FJ 080) held at a distance of approximately 15 cm from the carcass. Washing was carried out so that the complete surface of the carcass was washed once in each 30 sec cycle of the wash time. It was possible to vary the line pressure of the water from 3.5 to 7.7 kg/cm^2. The volume of water delivered at these pressures was 5.7 and 9.1 l/min respectively. Water was heated by steam injection until it reached the required temperature upon impact with the carcass. The required concentration of chlorine was obtained by adding a solution of sodium hypochlorite ('Chloros' I.C.I. Agricultural Grade) which contained about 12% active chlorine. The concentration of chlorine in solution was determined by an iodometic method (Vogel, 1961). In experiments 1 and 2 half the carcasses selected were retained as unwashed controls for evaluation of carcass appearance. In experiment 3 only one side of each carcass was subjected to a washing treatment; the other side was protected by plastic sheeting during washing and was subsequently used as a control in the evaluation of carcass appearance.

After washing treatments carcasses were allowed to drain for 15 min at ambient temperature (12±2.5°C). Carcasses were then stored under the following refrigeration conditions: mean temperature (°C) 1.5±0.5, fluctuation up to 6° (max. 3/hr); relative humidity 84–94%; air speed (m S^{-1}) at entry 4.8, below/above carcasses 1.0–3.0, between carcasses 0.0–0.2, within abdominal cavity < 0.1.

Assessment of carcass appearance

To simulate market conditions carcasses were removed from the refrigerated storage area and hung for 1 hr at ambient temperature before the assessment of appearance commenced. Two parameters were used to assess appearance; (i) cleanliness, (ii) appearance of surface tissue. Each parameter was evaluated as follows.

(i) *Cleanliness.* A panel of three judges inspected carcasses for cleanliness. This panel consisted of a member of the Meat Industry, the Veterinary Profession and the Meat Research Department of An Foras Taluntais. Carcasses were grouped in pairs, each pair consisting of a carcass which had received an experimental washing treatment and an unwashed control carcass which had received the usual factory dressing. After 1 and 3 days post mortem the panel was instructed to evaluate which carcass, if any, was the cleaner of the pair.

(ii) *Appearance of the surface tissues.* (a) In experiments 1 and 2 a panel of seven judges inspected carcasses after 1 and 3 days post mortem to evaluate the *initial appearance* of the surface tissues. Carcasses were grouped in pairs as outlined above for the assessment of cleanliness. Each member of the panel examined each pair in turn and selected the carcass, if any, which they considered to have superior appearance. They then examined each pair in turn again and assigned a grade for appearance to each carcass based on a five point scale: 'poor, fair, good, very good, excellent'.

(b) In experiment 3 where only one side of each carcass was subjected to a washing

treatment, a panel of three judges assessed *appearance in detail* after 1 and 7 days. These judges evaluated different characteristics of carcasses to be either normal (= 3.0), slightly abnormal (= 2.0) or abnormal (= 1.0). The effect of spray washing on carcass appearance was estimated by subtracting the mean of the scores assigned to the unwashed (control) side from the mean of the scores assigned to the washed side. They were asked to examine each side of the carcass with respect to the quality of the following characteristics:

(a) appearance of subcutaneous fat in the crutch region and on the back of the carcass;

(b) appearance of the leg and neck tissue;

(c) appearance of the abdominal region, whether damp, exhibiting an unusual colour or an unusual degree of flaccidity.

(iii) *European market assessment.* A selection of the European customers of some Irish lamb exporting plants were asked to examine batches of carcasses which had been transported to Europe in refrigerated containers. Each batch consisted of eighty carcasses of different weights subjected to an experimental washing treatment and unwashed controls. The customers were asked to point out unsatisfactory carcasses and to explain the reason for their dissatisfaction.

Statistical analysis

Comparisons of carcass appearance scores were made using analysis of variance or *t*-tests.

Results

Investigations were carried out to set the parameters to be examined. Initial observation indicated that carcass classification based on weight (set at 22 kg) gave a reasonable division with respect to the problems encountered. Thus two experiments (1 and 2) were set up to evaluate both cleanliness and overall appearance. In experiment 1 carcasses of weight greater than 22 kg were treated and in experiment 2 those of weight less than 22 kg were treated. Carcasses were subjected to the spray washing treatments outlined in Table 1. Results from the investigations on cleanliness are presented first followed by the investigations on overall appearance.

(i) *Cleanliness*

All carcasses treated by spray washing in experiment 1 were compared with unwashed carcasses for cleanliness. In 78% of the comparisons the washed carcasses were judged to be visually cleaner (Table 2). In 7% of the comparisons the judges detected no difference while in 15% of the comparisons the unwashed carcasses were selected as cleaner. The data from each judge was analysed separately thus the standard error reflects the degree of variation existing between the judges. The results of this experiment also indicated that cleanliness of carcasses was not greatly influenced by the temperature of the water (Table 2). Similar results were obtained in trials in experiment 2. Furthermore the results of experiment 3 indicated that there was little difference in cleanliness between carcasses washed with water at 37°C or 65°C at pressures of 3.5, 5.6, 7.7 kg/cm^2 (results not shown). Thus carcass type had little or no significant effect on the efficiency of the spray washing technique with respect to cleanliness.

Table 1. Treatment used in experiments 1–3 to examine the effect of spray washing on carcass appearance

Experiment 1 (carcass weight > 22 kg)			Experiment 2 (carcass weight < 22 kg)		Experiment 3 (carcass weight < 22 kg)		
					(a) Age > 16 weeks	(b) Age < 16 weeks	
Temp. (°C)	Chlorine (μg/ml)	Duration (sec)	Temp. (°C)	Chlorine (μg/ml)	Temp. (°C)	Temp. (°C)	Pressure (kg/cm²)
37	0	30					
	0	120	50	0, 30, 95			
	450	30					
	450	120					
			65	0, 30, 95	37	37	3.5, 5.6, 7.7
80	0	30					
	0	120					
	450	30	80	0, 30, 95	65	65	3.5, 5.6, 7.7
	450	120					
Pressure (kg/cm)		3.5	3.5		3.5	v	
Duration (sec)		v	120		60/half carcass	60/half carcass	
Chlorine (μg/ml)		v	v		0	0	
No. of carcass/treatment		8*	6*		8	6	
Total number of carcasses		64 (+64 control carcasses)	54 (+54 control carcasses)		16	36	

v = Variable details shown in upper part of table.
* = The same number of unwashed carcasses were held as controls.

(ii) Effect of spray washing on overall appearance

Experiment 1: Carcass of > 22 kg weight. (Heavy autumn lambs). These carcasses had a good covering of subcutaneous fat. The results show that spray washed carcasses were judged to have as good an appearance as unwashed ones (results not shown). It was noted that the surface of carcasses spray washed with water at 80°C became slightly white in appearance immediately after treatment. However, after 24 hr refrigerated

Table 2. Influence of spray washing on the cleanliness of carcasses

(a) Comparison of the cleanliness of spray washed carcasses with unwashed controls in experiment 1	Mean (%) ± s.e.
Number of times* that washed carcasses were judged cleaner	78 ± 9.2
Number of times* that unwashed carcasses were judged cleaner	15 ± 10.0
Number of times* that there was no difference between washed and unwashed carcasses	7 ± 2.0

(b) Effect of temperature of spray washing on the cleanliness of carcasses in experiment 1. Number of carcasses judged cleaner than unwashed controls* Temperature (°C)	Mean (%) ± s.e.
37	81.25 ± 21.8
80	84.4 ± 18.75

*Expressed as a percentage of the total judgments made.

storage this was no longer evident and the judges did not detect any significant difference between carcasses spray washed with water at 37°C or 80°C. Similarly there was no significant difference between carcasses spray washed for 30 or 120 sec nor between those washed with unchlorinated water or water containing chlorine (450 μg/ml). After 24 hr storage no odour of chlorine was detected from carcasses treated with chlorinated water.

Experiment 2: Carcasses of < 22 kg weight. (Spring lambs). Initial studies showed a significant effect between washed and unwashed carcasses (results not shown). Consequently a greater range of temperature and chlorine concentration was examined in this experiment than those in experiment 1.

These carcasses had a light covering of subcutaneous fat. The spray washed carcasses were not considered to have as good an appearance as unwashed ones (Table 3). The numerical scores assigned to unwashed carcasses were significantly higher than those assigned to washed carcasses judged after 24 hr ($P < 0.05$) and after 72 hr ($P < 0.001$). However, the deleterious effect of spray washing was not significantly influenced by the temperature of the water used (50°, 65°, 80°C) or by the presence or absence of chlorine in the water and indeed subsequent experiments (experiment 3) also showed that variation in line pressure (3.5, 5.6, 7.7 kg/cm^2 were tested) did not exert any effect on the faults observed.

Table 3. Comparison of spray washed and unwashed carcasses in experiment 2 (< 22 kg weight)

	After 24 hr		After 72 hr	
	Washed	Unwashed	Washed	Unwashed
Total number of preference judgments	96	159	70	142
Mean numerical score*	3.35	3.55	2.80	3.26
Standard error of difference (d.f. = 47)		±0.09		±0.11
F-test (effect of spray washing)		*		***
Difference between scores at 24 and 72 hr		0.55		0.29
Standard error of difference (d.f. = 47)		±0.08		±0.07
F-test significance of difference		***		***

*Scores based on a five point scale (5 = excellent, 4 = very good, 3 = good, 2 = fair, 1 = poor).

(iii) *Major characteristics of carcasses (< 22 kg weight) significantly affected by spray washing.*

In these experiments only one side of each carcass was spray washed. The other side then served as a within-carcass control for the evaluation of appearance. Carcasses selected for these experiments were < 22 kg in weight and were divided into 2 types; (a) > 16 weeks, (b) < 16 weeks in age.

Experiment 3a: Carcasses from mature lambs (> 16 weeks in age). Characteristics of the carcass significantly affected by spray washing are shown in Table 4. The results showed that when assessed after a day's refrigerated storage spray washing had a deleterious effect on:

(i) the appearance of the fat in the crutch and on the back (fat or normal appearance

had an oily consistency, while fat with a dry, powdery and flaky appearance was considered abnormal);

(ii) the degree of flaccidity and dampness of the abdominal region (after slaughter the abdominal region is flaccid, but normally becomes increasingly rigid during chill storage);

(iii) the colour of the abdominal region which was judged to have been paler than that on unwashed carcasses.

However, when assessed after 7 days refrigerated storage the abdominal region of spray washed carcasses was no longer considered to have an abnormal degree of flaccidity. It was also evident that the faults in the appearance of the fat and colour of the abdominal region were not as pronounced as after 1 day's storage (Table 4). No differences were reported for other characteristics of the carcass (i.e. legs, neck).

The results also showed that these deleterious effects of spray washing on different carcass characteristics were not significantly different when the water used for spray washing was at 37°C or 65°C.

Experiment 3b: Carcasses from lambs < 16 weeks in age. These carcasses were from lambs aged 10 to 16 weeks. These 'spring lambs' had been born in the first few months of the year. These carcasses were less susceptible to damage from spray washing than carcasses from the more mature animals used in experiment 3a (Table 5). The main difference was that the appearance of fat on the back and the degree of flaccidity of the abdominal region on carcasses in this experiment were not affected by spray washing. However, other characteristics of these carcasses affected (Table 5) were:

(i) the appearance of the fat in the crutch region after 1 and 7 days;

(ii) the dampness and colour of the abdominal region after 1 day, but not after 7 days refrigerated storage.

There was no significant difference in these deleterious effects on appearance of spray washing carcasses with water at 50°C compared with 65°C or 80°C or with water delivered at pressures of 3.5 kg/cm^2 compared with 5.6 or 7.7 kg/cm^2 (results not shown). Thus these faults were not significantly influenced by these modifications to the washing procedure. This probably indicates that the dominant effect noted on these carcasses resulted from contact with water rather than physical effects resulting from the temperature or pressure of the spray water used. Indeed the effect noted between carcasses of greater than and less than 22 kg probably reflects the reaction of each type to water rather than damage due to the variables (e.g. temperature, pressures etc.) associated with the spraying technique.

(iv) *European market assessment*

Commercial significance of the faults detected. To establish if the faults due to spray washing detected in experiment 2 and 3 were distinguishable under normal commercial market conditions a pilot market evaluation was carried out. Four consignments of carcasses were transported to European markets for evaluation by selected customers. By agreement with the Department of Agriculture forty carcasses (< 22 kg) in each consignment were not spray washed. Instead blood and dirt were removed from these control carcasses with sterile disposable cloths. A further forty carcasses (< 22 kg) in each consignment were spray washed with unchlorinated water at 3.5 kg/cm^2 for 120 sec at either 45°C or 70°C. The carcasses were transported along with the plant's normal consignment of 800 carcasses in a refrigerated container and judged by buyers (both retail and wholesale) on arrival in Paris and Brussels. In no instance did any customer

Table 4. Effect of spray washing on carcass appearance scores* in experiment 3a (> 16 weeks)

	After 1 day			After 7 days			Difference between 1 and 7 days		
	Mean score difference†	s.e.	Level of significance†	Mean score difference	s.e.	Level of significance†	Mean score difference	s.e.	Level of significance
Crutch fat appearance	−0.58	±0.16	**	−0.37	±0.13	*	−0.21	±0.10	*
Back fat appearance	−0.42	±0.08	**	−0.17	±0.07	*	−0.25	±0.10	*
Abdominal region:									
(i) Flaccidity	−0.32	±0.13	*	−0.05	±0.03	NS	−0.27	±0.07	**
(ii) Dampness	−0.45	±0.18	*	0.00	NT§	NT		NT	
(iii) Colour	−0.38	±0.12	*	−0.17	±0.07	*	−0.21	±0.09	*

* Scores based on a three point scale (3 = normal, 2 = slightly abnormal, 1 = abnormal).
† Score (washed side)—score (unwashed side) = score difference.
‡ One tailed *t*-test was employed (d.f. = 22).
§NT = Not tested; NS = Not significant.

Table 5. Effect of spray washing on carcass appearance scores in experiment 3b (< 16 weeks)*

	After 1 day			After 7 days			Difference between 1 and 7 days		
	Mean score difference	s.e.	Level of significance	Mean score difference	s.e.	Level of significance	Mean score difference	s.e.	Level of significance
Crutch fat appearance	−0.19	±0.06	*	−0.20	±0.06	*	0.01	±0.04	NS
Abdominal region:									
(i) Dampness	−0.16	±0.04	**	0.00	NT	NT	NT		
(ii) Colour	−0.13	±0.05	*	−0.02	±0.06	NS	−0.11	±0.05	*

* See footnote to Table 4.

find any fault in carcasses which had been spray washed or indeed any difference between spray washed and unwashed carcasses. Thus indicating that with the superficial examination carried out under normal commercial conditions these faults (reported above) were not identified but this does not exclude the possibility that these faults may have some commercial significance (e.g. under shorter periods of storage).

Discussion

Previous authors have shown that spray washing can improve the microbiological quality of lamb carcasses but less attention has been given to the effect of spray washing on the appearance of different types of carcasses (Patterson, 1968: Bailey, 1971; Kelly *et al.*, 1974; Kelly *et al.*, 1980; Kelly, Dempster & McLoughlin, 1981; Kelly, Lynch & McLoughlin, 1982).

This study shows the effect of spray washing on carcass appearance was greatly influenced by the type of carcass treated, e.g. carcasses of > 22 kg (experiment 1) were not affected by spray washing, possibly because of their good covering of subcutaneous fat whereas those of < 22 kg were affected. It was evident that damage to carcass appearance was not influenced by the temperature. The fat covering possibly confers some hydrophobic properties which offer greater protection to water damage (up to 80°C), chlorine content (up to 450 μg/ml) or pressure of the water (up to 7.7 kg/cm^2) used but it is possible that higher temperature, chlorine content or pressure could have resulted in increased damage. Patterson (1972) in studying the effect of spray washing sheep carcasses with pressure of up to 24.5 kg/cm^2 found that serious damage to carcasses could result if the jet was too penetrating. The nature of the damage (e.g. flaccidity) suggests that faults arose due to contact with the water rather than mechanical damage (e.g. tearing as a consequence of jet pressure).

Some of the faults due to spray washing (i.e. flaccidity and dampness of the abdominal region) were no longer evident after 7 days storage and other faults (e.g. appearance of the fat) were less evident after 7 days than after 1 day's storage. Indeed it is possible these faults were due to the presence of the water used for spray washing on or within the surface tissues of the carcass, and that alleviation of the faults during refrigerated storage could have been due to the evaporation of excess water from the surface tissues (Hicks, Scott & Vickery, 1956) or to the reestablishment of some biochemical equilibrium that was upset during washing.

Since the faults in carcass appearance due to spray washing were alleviated to some extent during refrigerated storage and since customers in European markets did not find any faults in carcass which had been washed, spray washing can be recommended to the meat industry as a suitable method of producing lamb carcasses with a high standard of cleanliness and microbiological quality. However greater attention should be directed at the spray washing techniques in commercial situations where the period of refrigerated storage is short and in all cases damage must be balanced against the benefit in terms of reduced contamination and extended storage life.

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Comparison of the extrusion cooking of a soya isolate and a soya flour

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Summary

The effects of barrel temperature, die temperature and moisture content on soya extrusion were investigated using response surface analysis. Seven variables were measured, and a model derived for the effect of temperature and moisture content on pressure. Two samples were investigated, a soya flour (with a high protein dispersibility index) and a commercial soya isolate consisting of denatured protein. Differences between the samples are discussed. Changes along the length of the barrel, were investigated using a 'dead stop technique' and differential scanning calorimetry (DSC) revealed the points at which the major protein components in soya flour were denatured.

Introduction

The ability of plant proteins to be texturized into structured bodies having a fibre-like nature, by extrusion, has been exploited since the 1960's. The ingredients most commonly used are defatted soya flour or soya grits, having a protein content of about 50% and 30% carbohydrate. Although it has been assumed that the structure of soya extrudate is a result of alterations in the protein fraction, the molecular changes that occur within the extruder are poorly understood.

Soya extrusion has been investigated by a number of authors (Aguilera & Kosikowski, 1976; Maurice & Stanley, 1978; Frazier *et al.*, 1983), using response surface analysis. Graphical representations have been made in two or three dimensions, showing the effect of process variables on extrusion variables and product characteristics. There has been little or no attempt in this work to explain the results in terms of the physico-chemical changes occurring in the extruder. In addition, each investigation has been concerned with one material only, so it is not known how general are the conclusions drawn from these experiments.

It has been suggested that the carbohydrates may play a role in soya extrusion (Taranto *et al.*, 1978a and b; 1981; Sheard, Mitchell & Ledward, 1984). In the work reported here, the extrusion behaviour of soya flour and a commercial soya isolate were investigated using response surface analysis. A viscosity model was developed, using this data, for the two samples. In addition, differential scanning calorimetry (DSC) measurements were made along the length of the barrel on samples obtained using a 'dead stop technique'.

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Materials and methods

Materials

Defatted soya flour (product code 200/70) and soya isolate (product code 610) were obtained from McCauley Edwards Ltd and approximate compositions are shown in Table 1.

Table 1. Manufacturers analyses of soya flour and isolated soya protein

	Defatted soya flour (product code 200/70)	Isolated soya protein (product code 610)
Protein (N × 6.25)*	50.0	91.0
Fat	1.3	0.8
Fibre	3.5	0.1
Ash	6.5	3.8

The non-determined % was assumed to be carbohydrate.

*Commercial processors of soyabeans continue to use 6.25 as a nitrogen conversion factor, though there is general agreement that a smaller factor would be more appropriate (Kellor, 1974).

Extrusion

A Brabender laboratory extruder (model, DN) was used, powered by a Docorder drive, enabling torque to be continuously measured. The grooved barrel had a L/D ratio of 20:1. Pressure and temperatures were measured with a Dynisco pressure transducer (model no. PT480-20M-6) and iron/constantan thermocouples. These were inserted through half inch × 20 UNF tap dies so that the tips were flush with the surface of the extruder at the positions shown in Fig. 1. For all experiments a screw with a 2:1 compression ratio was used, at a speed of 250 rpm. The feed section was kept at 120°C, and the feed hopper at 120 rpm (close to the maximum available). The stainless steel die was hardened and had a diameter of 3 mm and a length of 40 mm. The required amount of distilled water was mixed with the soya using a Kenwood mixer, before equilibrating the moistened feed overnight at 2–3°C.
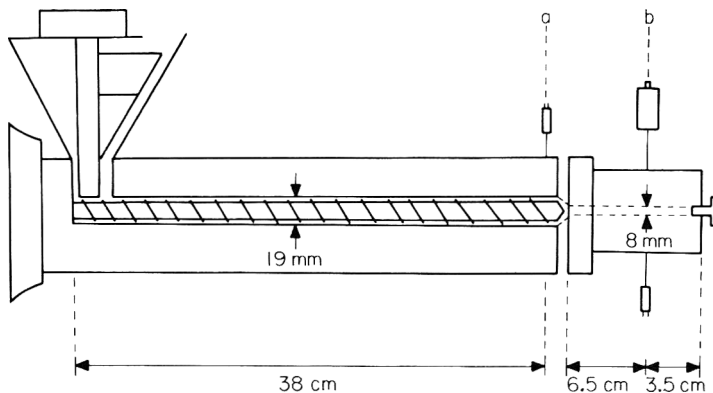
Figure 1. Diagram showing position of pressure transducer and thermocouples.

Experimental design

A three factor, three level experimental design was used with five replicates at the centre point (Table 2). The dependent variables measured were torque, pressure and product temperature (at points a and b in Fig. 1), flow rate, product diameter, OTMS texture and protein solubility after retorting in water. Measurements of the process variables were made and samples of extrudate taken, at steady state conditions.

The results were analysed, using a general statistical package (Genstat) developed at Rothamsted experimental station, allowing up to second order terms and their interactions. Analysis of variance (AOV) was used to show which of the independent variables (linear, quadratic and interactive terms) significantly ($P < 5.1\%$) affected the measured parameters. Estimates of these significant coefficients were made using multiple regression.

Table 2. Experimental design for response surface experiment

Variable	Level	-1	0	$+1$
Moisture content (% d.s.b.)		30	35	40
Barrel temperature (°C)		120	150	180
Die temperature (°C)		120	150	180

OTMS texture

The texture of the retorted product was evaluated by a method similar to that described by Frazier *et al.* (1983). 175 g of distilled water was added to 25 g of material in a can (7 cm × 11 cm) and allowed to stand for 4 hr before retorting at 120°C for 1 hr. The cooled cans were left to stand for a further 16 hr before placing the entire can contents into an OTMS cell having a nine wire grid and a base area of 30 cm². The peak force was recorded using an Instron (model 1140) at a crosshead speed of 50 mm/min and a chart speed of 100 mm/min.

Protein solubility

Solubility measurements were used in connection with the response surface experiment. Samples of extrudate obtained from the designed experiment were ground in a hammer mill and 2.0 g of the powdered material was added to 98 ml of distilled water in a can, which was then retorted at 120°C for 60 min. The cooled contents were filtered through Whatman no. 4 filter paper before determining the protein content, in duplicate, by a micro-kjeldahl technique using 2 ml of filtrate. Protein solubilities were calculated, on a moisture free basis, as a percentage of the protein in the original material using a nitrogen to protein conversion factor of 6.25.

Viscosity determination

The rheology of extrusion doughs can be described by a power law model (Clark, 1978) relating shear stress (τ) and shear rate ($\dot{\gamma}$):

$$\tau = m\dot{\gamma}^n.$$

The effects of temperature, T, and moisture, M, on the consistency index, m, have been described in terms of the relationship given below:

$$m = m_0 e^{A/T} e^{BM}.$$

50

If end effects are neglected, the pressure drop across the die (P) will be given by:

$$P = m_o \left(\frac{3n+1}{n\pi R^3} \right)^n \frac{2L}{R} \, e^{A/T} e^{BM} Q^n.$$

The constants A, B, m and n can be determined, following a logarithmic transformation, using multiple regression. This approach has been used by Levine (1982) on an uncooked flour dough.

To obtain a satisfactory estimate of n, it is necessary to vary the flow rate over a fairly wide range. In our case this was not achieved since for soya flour, in particular, the flow rate was only weakly dependent on moisture content and temperature. Therefore, A, B and m have been calculated assuming various values of n in the range 0.2–0.6. This range is typical of that reported for extrusion doughs (Harper, 1981).

Dead stop technique

At steady state, the extruder was stopped and rapidly disassembled. The screw was removed within 90–120 sec, and samples were taken from it as quickly as possible. The cooled samples were stored in sealed containers at 2–3°C until required. The temperature of the feed, barrel and die sections were 120°C. Feed moisture was 40% (on a dry solids basis). Differential scanning calorimetry was used, as described below, to locate the points at which the major globulins in soya denature.

Differential scanning calorimetry

Samples taken off the extruder were rehydrated to 66% (wet solids basis) and equilibrated for 16 hr at 2–3°C before recording the thermogram. Thermograms were recorded with a Perkin-Elmer DSC 2 using aluminium pans at a heating rate of 5°K/min and a sensitivity of 0.2 mcal/sec.

Results

Response surface

Tables 3 and 4 display the regression equations and summarize the range of the measured parameters found for the two products. Compared with the flour, the isolate had a higher average pressure, a lower average flow rate, greater expansion and had a narrower range of textures. For both materials, OTMS texture correlated negatively with protein solubility (Fig. 2).

Whilst extrusion was stable for soya isolate over the whole set of conditions, the flour ranged from a stable expanded product to a range of rapidly ejected, unexpanded pellets. Figure 3 shows differences between the products obtained at different temperatures. The flow was particularly unstable at high die temperatures, and this may account for the poor fit of the regression equation for flow rate, in the case of the flour.

The parameters m, A and B for different values of n are shown in Table 5. A and B are almost independent of n, and are higher for the flour compared with the isolate. This shows that the viscosity of the flour is more dependent on temperature and moisture than the isolate, and this goes some way to explain the narrower range of expansion ratios, texture and stability for the latter.

Physico-chemical changes along the barrel

The points at which the major soya globulins denatured (for the flour) were at flights 9 and 18 for the 7S and 11S globulins, respectively. Subjective assessment indicated

Table 3. Regression equations and statistical information for soya flour

Temp. (position A) $= 159.1 - 2.6M^* + 15.7B^{***} + 4.7D2^* - 4.3BD^{**} - 6.6BD2^{**}$
% Variance accounted for $= 91.9\%$
% Sum of squares for B was 73%
Mean temp. $= 162.2°C$ (range 142.2–178.8°C)

Temp. (position B) $= 146.1 - 1.6M^{**} - 0.6B^{***} + 11.7D^{***} - 5.8D2^{***} - 1.1MD^* + 6.2BD^{***}$
$+ 4.3B2D^{**} + 8.5BD2^{***} + 2.2BD2D2^* + 1.3MBD^*$
% Variance accounted for $= 99.3\%$
% Sum of squares for D was 72%
Mean temp. $= 143.2$ (range 123.6–173.3)

Torque $= 16.5 - 0.4M^* - 0.9M2^{**} - 7.3B^{***} + 5.2B2^{***} - 0.1D^{**} + 0.4D2^{***} + 0.6MB^* + 1.0M2B^{**} + 0.4MD^*$
$- 1.0M2D^* + 0.5BD^* + 1.8BD2^{**} - 3.5B2D2^{***} - 1.8MBD^{***}$
% Variance accounted for $= 97.3\%$
% Sum of squares for B was 76%
Mean torque $= 18.0$ Nm (range 11.5–28.6)

Pressure $= 1211 - 265M^{***} - 491B^{***} + 150B2^{**} - 149D^{***} - 80D2^* + 156MB2^{**} - 39M2B2^{**} - 34BD^{**} - 93B2D^*$
$+ 91BD2^* - 184MBD^{***} + 143M2BD^{**} + 67MB2D^{**}$
% Variance accounted for $= 83.6\%$
% Sum of squares for B was 58%
Mean pressure $= 1241$ psi (range 458–2299 psi)

Flow rate $= 1.06 + 0.03M^* + 0.05D^{***} - 0.002BD2^*$
% Variance accounted for $= 44.7\%$
% Sum of squares for M, D and BD2 were 17, 34 and 10
Mean flow rate $= 1.06$ ml/sec (range 0.95–1.19)

Product diameter $= 4.03 - 0.14M^{**} - 0.17M2^* + 0.57B^{***} + 0.23B2^{**} + 0.04MB^* + 0.01M2B^* + 0.14MD^*$
$- 0.11M2D^{**} + 0.04BD^* + 0.24B2D^* - 0.28MBD^{***} + 0.19M2BD^{**}$
$- 0.37MB2D^{**} + 0.23MBD2^*$
% Variance accounted for $= 82.2\%$
% Sum of squares for B was 56%
Mean diameter $= 4.0$ mm (range 3.4–6.0 mm)

OTMS texture $= 153.1 - 16.9M^{***} + 19.6M2^{**} + 82.8B^{***} - 5.2B2^* - 54.6D^{***} - 5.2D2^* + 17.0M2B^{**}$
$+ 7.8MD^* + 19.6M2D^{**} - 23.8BD^* - 19.2B2D^{***} - 12.7BD2^* - 7.1MBD^* + 25.9M2BD^{***}$
% Variance accounted for $= 97.2\%$
% Sum of squares for B was 83%
Mean peak force $= 140$ N (range 34–280 N)

Protein solubility $= 43.7 - 0.2M^{**} - 2.3B^{**} + 3.2D^{**} + 2.1D2^{**} + 0.3MB^{**} - 1.3MD^* - 2.0M2D^* - 1.2MD2^*$
$+ 1.4MB2D^*$
% Variance accounted for $= 83.6\%$
% Sum of squares for B and D were 37 and 22%
Mean solubility $= 45\%$ (range 40–50%)

*Significant at 5.0%; **significant at 1.0%; ***significant at 0.1%
M = moisture; B = barrel temperature; D = die temperature.
Quadratic terms are written as M2 and interactive terms as MB.

that the material changed from a granular appearance to a homogeneous plasticized mass at about flight 16 for the flour, and also for the isolate.

Discussion and conclusions

In order to avoid the complication of a very different particle size, soya flour was used rather than soya grits for this study. The problem of feeding fine particles is well known

Table 4. Statistical data and regression equations for soya isolate

Temp. (position A) $= 161.3 - 2.5M^* + 15.8B^{***} + 5.4D2^{**} - 2.5BD^* - 8.0BD2^{**}$
% Variance accounted for $= 94.2\%$
% Sum of squares for B was 73%
Mean temp. $= 164.9°C$ (range $142.7-178.8°C$)

Temp. (position B) $= 146.2 - 2.4M^{***} - 4.9B^{***} + 2.1B2^{***} + 15.0D^{***} - 5.8D2^{***} + 1.6MB^{***}$
$- 3.1MD^{***} + 6.3BD^{***} + 3.8B2D^{***} + 12.1BD2^{***} + 1.2MBD^{**} + 1.5M32D^{**}$
% Variance accounted for $= 99.6\%$
% Sum of squares for D was 79%
Mean temp. $= 143.8°C$ (range $122.4-176.0°C$)

Torque $= 16.5 - 1.7M^{**} - 1.5M2^* - 5.2B^{***} + 1.3B2^* + 1.2D^{**} + 2.3MB^{**} - 1.6MD^{**} + 2.3BD2^*$
$- 0.6B2D2^* - 0.8M2BD^* - 1.5MBD2^*$
% Variance accounted for $= 88.6\%$
% Sum of squares for B was 53%
Mean torque $= 18.0$ Nm (range $11.5-28.6$)

Pressure $= 1723 - 151M^{***} - 71M2^* - 546B^{***} + 100B2^* - 23D^{**} + 155MB^{**} - 156MD^{***} - 72M2D^*$
$+ 152BD^* + 192BD2^{***} - 58B2D2^* + 40MBD^* - 152M2BD^* + 84MB2D^{**} - 126MED2^{**}$
% Variance accounted for $= 95.3\%$
% Sum of squares for B was 73%
Mean pressure $= 1717$ psi (range $1057-2646$ psi)

Flow rate $= 0.82 - 0.12B^* + 0.12D^{***} - 0.07BD^{**} + 0.12BD2^*$
% Variance accounted for $= 70.2\%$
% Sum of squares for D, BD and BD2 were 48, 12 and 10%
Mean flow rate $= 0.82$ ml/sec (range $0.57-1.06$)

Product diameter $= 5.46 - 0.88M^{***} + 0.64B^{***} - 0.22MB^* + 0.27MB2^* - 0.06M2B2^* + 0.13M2D2^* - 0.24B2D2^*$
% Variance accounted for $= 82.2\%$
% Sum of squares for M and B was 44 and 36%
Mean diameter $= 5.4$ mm (range $4.3-7.0$ mm)

OTMS texture $= 202.4 - 28.6M^{***} - 17.7M2^{**} + 34.0B^{**} + 20.6B2^{***} - 20.6D^{***} - 25.6MB^{***}$
$- 16.4M2B^{**} - 11.8M2D^* + 34.7BD^{**} - 13.8BD2^* + 11.0B2D2^{**} - 27.4M2BD^{**}$
% Variance accounted for $= 75.7\%$
% Sum of squares for M, D and MB were 15, 15 and 16%
Mean peak force $= 179$ N (range $110-290$ N)

Protein solubility $= 48.7 + 0.9M^{***} - 4.5B^{***} + 2.8D^{***} + 1.9M2B^{**} + 0.7MB2^* + 0.7M2B2^*$
$- 1.1B2D^{**} - 1.3BD2^* + 0.6B2D2^*$
% Variance accounted for $= 90.0\%$
% Sum of squares for B and D were 58 and 26%
Mean solubility $= 43\%$ (range $44-56\%$)

*Significant at 5.0%; **significant at 1.0%; ***significant at 0.1%.
M = moisture; B = barrel temperature; D = die temperature.
Quadratic terms are written as M2 and interactive terms as MB.

(Coulson & Richardson, 1980) and it is a subject which will be addressed in a subsequent publication.

Our results with flour show, qualitatively, some of the conclusions drawn from the extensive response surface analysis experiment (with soya grits) reported by Frazier *et al.* (1983). For example, a high OTMS texture was favoured by a high barrel temperature and a low die temperature, in both pieces of work.

We found that with soya flour unstable extrusion resulted when the die pressure was low (i.e. at high die temperatures and moisture contents), also in agreement with
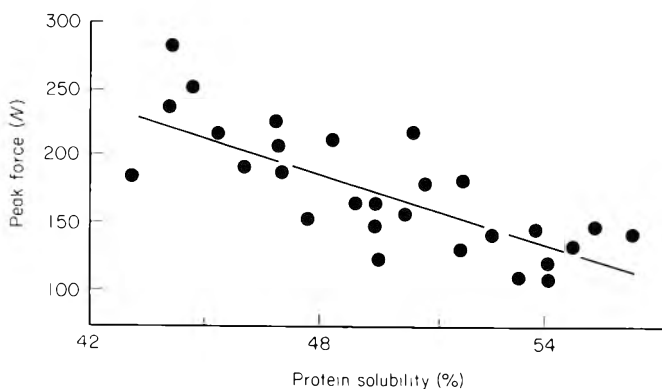
Figure 2. Correlation between OTMS texture and protein solubility for soya isolate.
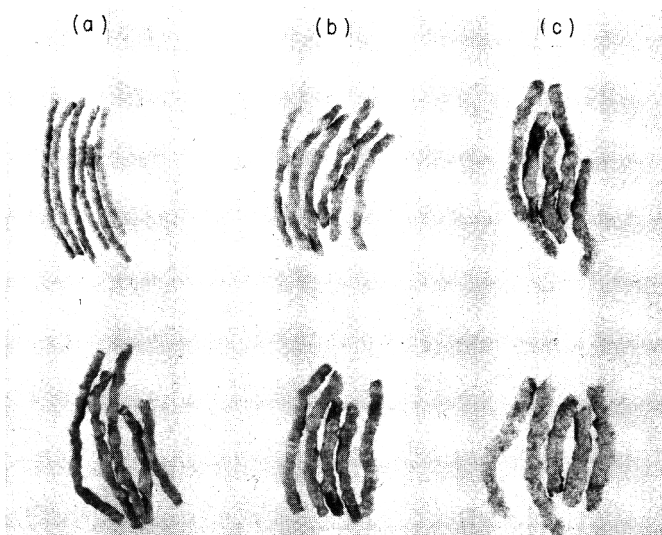
Figure 3. Structure of soya flour (upper) and soya isolate (lower) extruded at 30% moisture with barrel and die temperatures set at (a) 120°C, (b) 150°C and (c) 180°C.

Table 5. Constants of rheological models obtained from extrusion

Flow index	Consistency index, m (Pa.s)	Temp. constant, A (K)	Moisture constant, B	Viscosity $\gamma = 50/s$ $T = 150C$ $M = 35\%$)	Correlation coefficient
Soya flour					
0.2	2.15	4900	−0.041	2404	0.82
0.4	0.76	4854	−0.043	1554	0.81
0.6	0.27	4808	−0.044	1045	0.81
Soya isolate					
0.2	51.7	3504	−0.027	3479	0.87
0.4	22.6	3376	−0.027	2457	0.81
0.6	10.0	3248	−0.028	1696	0.81

Frazier *et al.* (1983). We believe that this is due to water flashing off as steam before the die exit, due to the low pressure. This results in the material being extruded in the form of a series of rapidly ejected, unexpanded pellets.

Quantitative agreement between the two pieces of work is less satisfactory. At the conditions corresponding to the centre point of our design (with a die diameter of 4 mm, rather than the 3 mm die used in this work), the regression equations of Frazier *et al.* (1983) predict a negative OTMS texture (-7.4N) and a product diameter less than 4 mm, whereas, for flour, we found an OTMS texture of 153N and an expansion ratio (product diameter/die diameter) of 1.34. This may reflect a difference between soya flour and grits (perhaps due to particle size) and also illustrates the relatively poor fit that might be expected from a second order polynomial at the edges of the experimental design.

The viscosity of the extrudate was represented by a power law model including exponential terms to represent temperature and moisture dependence. As can be seen from the data in Table 5, the constants A and B (representing the temperature and moisture dependence) were almost independent of n justifying one of the conclusions of this work that isolate viscosity was less dependent on temperature and moisture than the flour. Clearly it would have been desirable to evaluate n by making measurements at different shear rates, achieved by altering the flow rate. There are, however, difficulties in analysing the data from such an approach, since viscosity will also depend on the retention time (Jasberg, Mustakas & Bagley, 1982).

Clearly, neglecting end effects will to some extent invalidate the use of this procedure to obtain absolute rheological parameters. However, the previously mentioned retention time effects (Jasberg *et al.*, 1982) makes such corrections difficult to apply even if measurements were made with a range of dies of different L/D ratios. The alternative approach of determining the pressure drop between two transducers located in the cylindrical part of the die section may have given more satisfactory results. However, we do not believe that the conclusions regarding the moisture and temperature dependence of viscosity would have been substantially altered.

The product temperature was measured at the end of the screw and at the die. Very little difference was found between the soya flour and isolate. In general, we consider that such measurements should be treated with caution, partly because it is not clear to what extent the sensor is actually determining the temperature of the material rather than the surrounding metal, but also because it is to be expected that a substantial temperature gradient will exist in the melt. This is likely to be more pronounced at the die entrance where there is no mixing due to the action of the screw. In previous work (Berrington *et al.*, 1984), we have found (at constant heater temperature) that the product temperature measured at the end of the screw did reflect differences in the properties of the material and this was correlated with the torque. This is to be expected since most of the mechanical energy applied to the extruder is dissipated as heat.

Mean torque and temperature, measured at points A and B (Fig. 1), were similar for the flour and isolate, whilst the pressure and flow rate measurements indicate that the isolate had a substantially higher viscosity at the die. This suggests that the differences in the rheology of the two systems only manifests itself near the die end of the extruder.

Some idea of the changes that take place to the material along the screw can be obtained from the dead stop technique. The DSC results suggest that unfolding is specific at two points in the extruder, corresponding to the denaturation of the 7S and 11S globulins. From the relationship between temperature and moisture content for soya flour (Sheard *et al.*, 1985), the product temperature at flights 9 and 18 must be in

excess of 105°C and 130°C, respectively. Thus, the change from a granular material to a homogeneous plastic mass (at about flight 16), must have occurred at a temperature intermediate between 105°C and 130°C.

Protein denatured during processing, in the isolate, may well be partially aggregated (or partially aligned) prior to extrusion and therefore less flexible, and thus its extrusion behaviour is not unexpectedly different.

The fact that the protein is undenatured in the flour allows for greater conformational change during extrusion, which may be one of the reasons for the wider range of textures, and may account for the greater dependence on moisture and temperature. Soya flour, however, contains about 30% carbohydrate (Table 1). This fraction can be demonstrated microscopically in the textured product (Frazier Crawshaw, 1984), and it may be appropriate to regard the 'melt' as a two phase system, with a continuous phase of protein and a dispersed carbohydrate phase.

It is not clear to what extent the carbohydrate will affect the rheology of the melt or the texture of the final product. Nor is it clear to what extent the differences between soya flour and soya isolate found here can be explained in terms of protein alone. A study of the extrusion behaviour of different soya isolates is now in progress.

Acknowledgments

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Application of Ross' equation for prediction of water activity in intermediate moisture food systems containing a non-solute solid

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Summary

This work investigates the use of the Ross (1975) equation for the prediction of water activity (a_w) in aqueous electrolyte or non-electrolyte solutions to which a non-solute (casein) was added. Water activity of the ternary mixtures (casein-water-solute) was in the range of $a_w \approx 0.85$–0.90 which is of interest for the development of intermediate moisture foods for human consumption. It was found that the use of the Ross equation coupled with a correction for the water strongly bound to casein gives good predictions of a_w.

Introduction

Several studies have been reported in the literature regarding the prediction of water activity (a_w) in aqueous solutions in connection with intermediate moisture foods (IMF). These studies included single electrolyte and non-electrolyte solutions, mixed electrolytes or non-electrolyte solutions, and mixed electrolyte non-electrolyte solutions (Ross, 1975; Benmergui, Ferro Fontán & Chirife, 1979; Chirife, Ferro Fontán & Benmergui, 1980; van der Berg & Bruin, 1981; Teng & Seow, 1981). However, intermediate moisture foods may contain non-solute materials (i.e. proteins, cellulose, starch) in addition to the dissolved substances. It is well known that there is a given ratio of non-solute solids/moisture below which the non-solute material needs not to be taken into account to estimate the a_w of an IMF, since its a_w-lowering effect is negligible (Chirife, 1978). This has been shown to be the case for various water-rich IMF foods such as moist salted fish (Lupin, Boeri & Moschiar, 1981); soft cheeses (Marcos *et al.*, 1981) and tomato concentrates (Chirife, Ferro Fontán & Boquet, 1981). Under certain circumstances, however, non-solute materials contained in IMF may also produce a relatively small (as compared to the lowering due to solute/s but significant contribution to depression of a_w. The influence of non-solute materials in the prediction of a_w of aqueous mixtures in the IM range, has been less frequently studied (Ross, 1975; Chirife, 1978; Lang & Steinberg, 1981). The purpose of the present work was to investigate the use of the Ross (1975) equation in aqueous electrolyte or non-electrolyte solutions relevant to IMF, to which a non-solute (casein) was added.

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Materials and methods

Materials

Casein (vitamin-free). Fluka AG (Buchs SG, Switzerland); monopotassium 1-glutamate and 1-proline were obtained from Sigma Chem. Co. (St Louis, U.S.A.); sodium chloride and $D(-)$ fructose were from Merck (Darmstadt, West Germany); sucrose, sorbitol, maltose and sodium citrate were from Mallinckrodt (St Louis, U.S.A.); potassium chloride was from Carlo Erba (Italy), and $D(+)$ xylose from B.D.H. Chem. Div. (Poole, England). All reagents were thoroughly dried in a vacuum oven at 37°C over magnesium perchlorate for 48 hr before sample preparations.

Sample composition and preparation

Prediction of a_w was studied in ternary systems consisting of casein (non-solute)-water-solute; the ratio casein/water was fixed at 3.0 or 3.5 g casein/g water and different solutes were investigated. Electrolytes were KCl, NaCl and potassium-1-glutamate; non-electrolytes were xylose, fructose, sucrose, maltose, sorbitol and 1-proline. They were chosen in order to provide a wide range of possible interactions between solute and casein (Lang & Steinberg, 1981; Gal, 1975). The conventional a_w range of IMF is about 0.70–0.85 (Kaplow, 1970); however, it is known that the solute/s concentrations needed to achieve the above a_w's, produce serious flavour problems in IMF for human consumption. In fact, present efforts to develop IMF for human consumption are directed to microbial control at a_w around 0.90 or even higher (Fox & Loncin, 1982; Leistner, Rödel & Kriespien, 1981). For this reason, prediction of a_w in the present model systems was restricted to a_w values close to 0.9.

Ternary sample systems (water-solute-casein) or casein-water mixtures were first hand mixed in a beaker and then transferred to a mixer to assure optimum component (non-solute) dispersion. The samples were put into hermetically sealed containers and allowed to equilibrate at 25°C for 24–48 hr before a_w measurements. It was experi-

Table 1. Composition of ternary mixtures consisting of casein, water and solute

	Electrolytes (g)				
System	Casein	Water	NaCl	KCl	K-1-glutamate
E-1	15	5	0.5870	—	—
E-2	17.5	5	0.8173	—	—
E-3	17.5	5	0.5935	—	—
E-4	17.5	5	—	1.1425	—
E-5	17.5	5	—	0.8146	—
E-6	15	5	—	—	1.9430

	Non-electrolytes (g)						
	Casein	Water	Fructose	Sorbitol	Sucrose	Xylose	Maltose	1-Proline
NE-1	15	5	3.3330	—	—	—	—	—
NE-2	15	5	—	3.6200	—	—	—	—
NE-3	17.5	5	—	—	5.2040	—	—	—
NE-4	17.5	5	—	—	—	2.3522	—	—
NE-5	17.5	5	—	—	—	—	4.9815	—
NE-6	17.5	5	—	—	—	—	—	2.2790

mentally determined that this time was enough to achieve internal a_w equilibration in the samples. Table 1 gives the composition of the systems studied.

Determination of water activity

The water activity of the model system was determined using an electronic hygrometer, Humicap HM 14 manufactured by Vaisala, Helsinki, Finland. A statistical evaluation of a_w measurements obtained with this hygrometer has been published elsewhere (Favetto *et al.*, 1983). The hygrometer was carefully checked against different standard saturated salt solutions in the a_w range of interest in the present study and a calibration curve was obtained. The calibration curve was always checked immediately after every set of a_w measurements to ensure reliability of the data. Water activity values at 25°C for the standard saturated salt solutions were as follows: (Chirife *et al.*, 1983): $(NH_4)_2SO_4$ 0.802; KCl 0.843; $BaCl_2$ 0.902; KNO_3 0.925; K_2SO_4 0.974. Four replicates were made for each a_w measurement (and the average was used) at $25 \pm 0.1°C$ in an air-circulating constant temperature cabinet. It has been shown (Favetto *et al.*, 1983) that three or four replicates (depending on a_w level) are sufficient to give a confidence interval of ± 0.005 a_w with this hygrometer.

Results and discussion

Adsorption isotherm of casein

The adsorption isotherm of casein at 25°C was determined in the range of a_w of interest (about 0.84–0.97). The water sorption isotherm in the above range was described using the Smith (1947) equation

$$m = A + B \ln(1 - a_w) \tag{1}$$

where m is moisture content, and A and B are constants to be determined. A plot of m versus $\ln(1 - a_w)$ should be a straight line from which the parameters A and B may be calculated. A least squares analysis was used to obtain the values of the parameters B
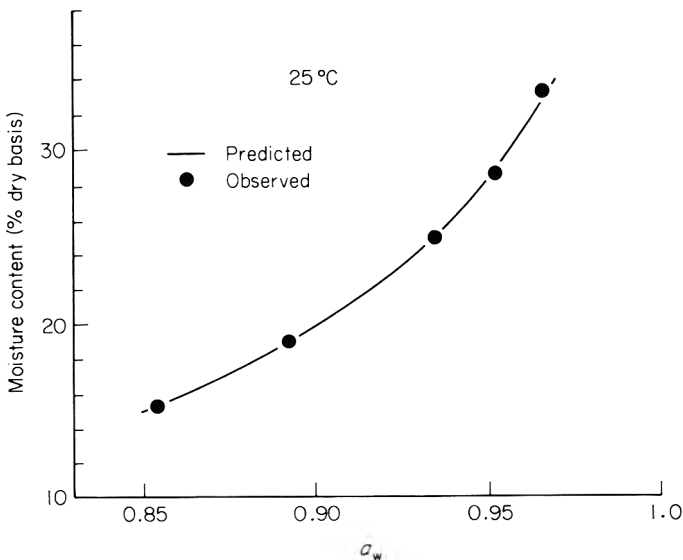
Figure 1. Sorption isotherm of casein at 25°C (predicted curve using equation (1)).

and A which were found to be -0.08801 and -0.28657, respectively. Figure 1 shows a comparison of experimental isotherm and predicted values using equation (1); the agreement may be considered very good (average percentage error in m in the a_w range studied was only 0.84%).

Ross' (1975) equation

Ross' (1975) equation for estimating the a_w of complex aqueous mixtures $((a_w)_M)$ may be written as

$$(a_w)_M = \pi_s (a_w^\circ)_s \tag{2}$$

where a_w° is the a_w of each component at its molality in the mixture. Ross (1975) suggested that in the presence of non-solute materials an 'a_w°' factor may be also assigned to each non-solute on the basis of its sorption isotherm, considering that all the water is sorbed in ech non-solute separately. For the present work, the 'a_w°' for casein is obtained from its adsorption isotherm (shown in Fig. 1) and the a_w° for the different solutes was obtained from literature as described later. Following Benmergui *et al.* (1979), and Chirife *et al.* (1980), experimental data for the a_w of single solutions of NaCl, KCl, sucrose, xylose, maltose, fructose and sorbitol were taken from the compilations of Teng & Lenzi (1974); Robinson & Stokes (1965) and Pitzer & Mayorga (1973). The data for K-1-glutamate and for 1-proline were obtained from Chirife, Alzamora & Ferro Fontán (1983) and Chirife, Ferro Fontán & Scorza (1980), respectively. It is noteworthy that a_w° values for all solutes to be used in equation (2), were also checked experimentally and excellent agreement was always obtained with the above mentioned literature sources.

Table 2 illustrates on the application of equation (2) for the prediction of a_w of ternary mixtures of casein-water-solute. The deviation between observed and predicted a_w values of the mixtures is expressed on a $(1-a_w)$ basis rather than a_w itself. This is because the error in the quantity $1-a_w$ is more significant than the error in a_w, since it

Table 2. Comparison of predicted (Ross' equation) and measured a_w values of ternary mixtures

System*	$(a_w^\circ)_s$†	$(a_w^\circ)_c$‡	$[(a_w)_M]_{exp}$	$[(a_w)_M]_{predicted}$	% Error§
E-1 (NaCl)	0.931	0.966	0.883	0.899	−13.7
E-2 (NaCl)	0.902	0.950	0.847	0.857	−6.5
E-3 (NaCl)	0.930	0.950	0.867	0.884	−12.8
E-4 (KCl)	0.902	0.950	0.845	0.857	−7.7
E-5 (KCl)	0.931	0.950	0.869	0.885	−12.2
E-6 (K-1-Glut.)	0.934	0.966	0.885	0.902	−14.8
NE-1 (Fructose)	0.929	0.966	0.884	0.897	−11.2
NE-2 (Sorbitol)	0.926	0.966	0.879	0.895	−13.2
NE-3 (Sucrose)	0.932	0.950	0.857	0.885	−19.6
NE-4 (Xylose)	0.942	0.950	0.885	0.895	−8.7
NE-5 (1-Proline)	0.917	0.950	0.864	0.871	−5.1
NE-6 (Maltose)	0.942	0.950	0.880	0.895	−12.5

*See Table 1.
† Water activity of each solute at its molality in the mixture.
‡ Water activity of casein at its moisure content in the mixture.
§ % Error $= \left[\dfrac{(a_w)_{exp.} - (a_w)_{pred.}}{1 = (a_w)_{exp.}} \right] 100$.

gives directly the error in the a_w lowering (Chireife, Ferro Fontán & Benmergui, 1980). For example, an error of 3% in the prediction of a_w (at $a_w = 0.90$) may look very reasonable. However, it represents an absolute difference (Δa_w) of almost 0.03 and this difference may not be acceptable for many purposes. Examination of Table 2 shows that for all systems studied the predicted a_w values of the mixtures are close to, but always above, the experimental ones. Differences are in the range $\Delta a_w = 0.01$–0.028 for samples containing all kinds of electrolytes or non-electrolytes. This result may be interpreted on the assumption that a small fraction of water is strongly sorbed in the casein and is not available to dissolve the solute. Thus, the (a_w^o) for each solute should be calculated taking this correction into account. Lüscher-Mattli & Rüegg (1982) proposed a hydration model for biopolymers on the basis of a literature review of various aspects of biopolymer-water systems. Through the analysis of water sorption isotherms of casein using the B.E.T. model and the Hailwood–Horrobin model (1946), they calculated that there is a fraction of strongly bound water in casein which amounts to 5.0–6.0% (dry basis). Similar results were reported by Rüegg & Häni (1975) who measured the infra-red spectra of casein at various water activities, and found that the strongest hydrogen bonds between polar sites and water molecules correspond to a water content close to 5% (dry basis). It is interesting to note that Hailwood–Horrobin's equation is mathematically equivalent to the well known G.A.B. sorption model (Bizot, 1983) as shown by Boquet, Chirife & Iglesias, (1980).

We may now correct our predictions of a_w in ternary mixtures of casein-water solute by simply considering that 5.5 g water per 100 g of casein are not available to dissolve the solute (the figure 5.5 was an average between the aforementioned values for the strongly bound water to casein). This was done and the results are shown on Table 3 which compares measured a_w values with those predicted using Ross' equation but correcting for the strongly bound water. It can be seen that the agreement is now considerably better than before. For instance, the average error in the a_w prediction for all systems is about 3.6% as compared to 11.5% obtained without correcting for strongly bound water (Table 2). It should be noted, however, that in reality the

Table 3. Improved prediction of the a_w of ternary mixtures using the Ross' equation and taking into account the strongly bound water in casein

System	$[(a_w)_M]_{exp.}$	$[(a_w)_M]_{predicted}$	% Error*
E-1	0.883	0.885	− 1.7
E-2	0.847	0.836	+ 7.2
E-3	0.867	0.867	0.0
E-4	0.845	0.834	+ 7.1
E-5	0.869	0.867	+ 1.5
E-6	0.885	0.889	− 3.5
NE-1	0.884	0.884	0.0
NE-2	0.879	0.881	− 1.7
NE-3	0.857	0.867	− 7.0
NE-4	0.885	0.882	+ 2.6
NE-5	0.864	0.850	+ 10.0
NE-6	0.880	0.881	− 0.8

$$* \% \text{ Error} = \left[\frac{(a_w)_{exp.} - (a_w)_{pred.}}{1 - (a_w)_{exp.}} \right] 100.$$

windows only allowed moisture content in processing would encompass, in some cases, a wider range in a_w than the error found using the uncorrected Ross' equation.

Ross's equation also assumes that there are no interactions between solute and non-solute. Various authors, including Gal (1975) and Hardy & Steinberg (1984) have shown that NaCl may interact with casein, the degree of the interaction depending on the salt to protein ratio, the moisture content and the a_w. However, in the present work, NaCl binding does not need to be taken into account to make a reasonable accurate prediction of a_w. The same applies for the other solutes studied (electrolytes as well as non-electrolytes), which were intentionally chosen to provide a wide range of possible interactions with casein. In all cases the Ross' equation performed satisfactorily.

The present results are, of course, only valid for casein/solutes mixtures and not necessarily for actual food mixtures. Thus, similar studies should be performed on more complex food systems.

Acknowledgments

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Technical note: Oxygen permeability at sub-zero temperatures of plastic films used for vacuum packaging of meat

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Introduction

The storage life of vacuum packaged meat is inversely related to the oxygen permeability of the film used for packaging (Newton & Rigg, 1979). Vacuum packaged meats are usually stored at temperatures close to 0°C while the meat exposes the packaging films to conditions of high humidity (Rigg, 1979). Reported oxygen permeabilities of packaging films are usually those measured at ambient temperatures and moderate humidities, but both temperature and humidity can affect the rates at which gases are transmitted through films. Eustace (1981) commented upon the need to determine the oxygen transmission rates of films under conditions that the films would experience in commercial use. He observed that oxygen transmission through nylon based films increased markedly at high humidity and that oxygen transmission rates for polyvinyl alcohol, polyvinylidene chloride and nylon based barrier films at 3.5°C were only 10–15% of their rates at 25°C. Although it seems reasonable to assume that application of the Arrhenius relationship would allow these results to be extrapolated to the temperatures used for long term storage of vacuum packaged meat, +1 to −1°C, this assumption requires verification. We therefore examined the oxygen transmission rates of two films in common commercial usage for vacuum packaging of meat, under conditions of high humidity and at temperatures encompassing the range in which vacuum packaged meats are usually stored.

Materials and methods

The films tested for oxygen transmission were a nylon-polyethylene laminate stated to have an oxygen transmission rate of > 100 ml/m^2/24 hr/atm at 23°C and 90% r.h. (Film 9502. Wrightcel, Feilding, New Zealand) and an ethylene/vinyl acetate copolymer–polyvinylidene chloride laminate stated to have an oxygen transmission rate of 30 to 40 ml/m^2/24 hr/atm at 25°C and 75% r.h. (Cryovac. W.R. Grace, Porirua, New Zealand).

Oxygen transmission rates were measured by the concentration-increase method of Landrock & Proctor (1952) using an apparatus similar to that of Davis & Huntington (1977). The upper compartment (2100 ml capacity) was separated from the lower, calibrated compartment (11.81 ml capacity) by a sheet (177 cm^2) of the film being tested. The apparatus was enclosed in an insulated cabinet fitted with an internal cooling coil through which isopropyl alcohol was passed from a refrigerated/heated circulator with temperature control to ±0.05°C (Julabo, Seelbach, DDR). The temperature was continuously recorded from a thermopile that was calibrated by means of

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a quartz thermometer before it was sealed into the upper compartment. The apparatus was operated in a room where ambient temperature was controlled at 10°C.

The upper compartment was filled to a gauge pressure 100 kPa above that of the lower compartment with oxygen saturated with water vapour at the ambient room temperature. The lower compartment was purged and then filled with oxygen-free dry nitrogen. Using a gas-tight syringe, replicate samples (500 μl) of gas were withdrawn from the lower compartment at suitable intervals for determination of oxygen concentrations. Averaged measurements for oxygen concentration at five times were plotted to obtain the rate of oxygen transfer at each temperature for each film.

A gas chromatograph (Model 3700. Varian, Palo Alto, CA, U.S.A.) fitted with two 1800 × 3 mm O.D. stainless steel columns, the first containing Chromosorb 102 80/100 mesh and the second Molecular Sieve 5A 60/80 mesh, was used for gas analysis. Helium at a flow rate of 23 ml/min was used as the carrier gas and detection was by a thermal conductivity detector. The apparatus was calibrated using a standard gas mixture (Scotty II. Supelco, Bellefonte, PA, U.S.A.). Peak areas were quantified by an electronic integrator (Supergrator III. Columbia Scientific Industries, Austin, TX, U.S.A.). The nitrogen peak was used as an internal standard in determining the oxygen concentrations in tested gas samples. This allowed account to be taken of the reductions of gas pressure in the lower chamber that resulted from sample removal.

Results and discussion

For both films, Arrhenius plots of oxygen transmission data showed a sharp break around 0°C (Fig. 1). Extrapolation of the plots obtained from data for temperatures
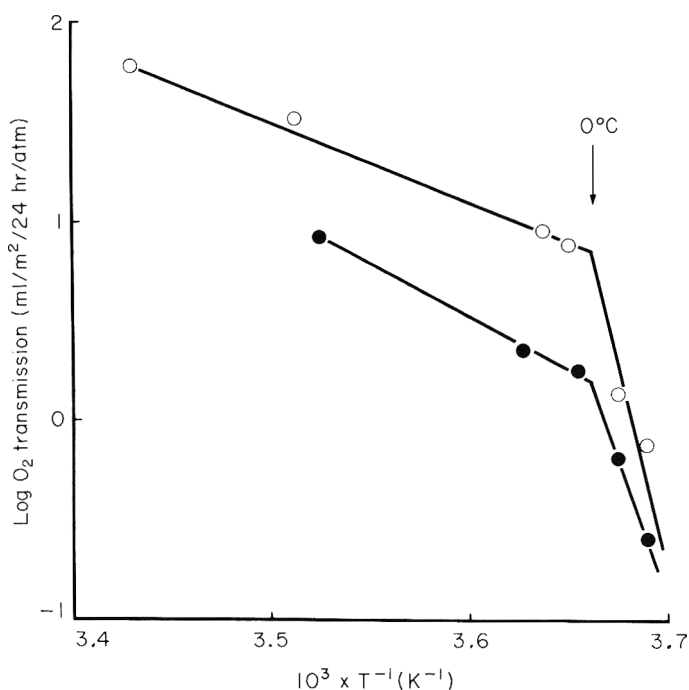
Figure 1. Arrhenius plot of oxygen transmission rate against temperature for nylon-($\varnothing$) and vinylidene-($\bullet$) based laminated films used for vacuum packaging of meat.

above 0°C would give respective transmission rates for the nylon and vinylidene films at $-1°C$ of 5.9 and 1.3 ml $O_2/m^2/24$ hr/atm whereas, from sub-zero data, the respective transmission rates at $-1°C$ were 2.0 and 0.6 ml $O_2/m^2/24$ hr/atm. The plots for temperatures above zero diverged with decreasing temperature because the vinylidene film had a proportionally greater decrease in oxygen transmission with decreasing temperature than the nylon film. In contrast, at sub-zero temperatures the plots converged to intersect at about $-5°C$. However, oxygen transmission rates were immeasurable at $-5°C$, being less than 0.05 ml $O_2/m^2/24$ hr/atm.

Because the change in permeability characteristics occurred close to 0°C, it seems reasonable to suggest that the phenomenon is due to freezing of water associated with the film. This can only be established by extensive work on the behaviour of water associated with plastic films. However, it is clear that film permeabilities at even marginally sub-zero temperatures cannot, with any assurance, be predicted from data obtained at higher temperatures. For products like chilled meat that are stored in a temperature range that encompasses the freezing temperature, a small decrease in storage temperature to below 0°C can greatly alter the absolute and relative permeabilities of packaging films. Moreover, it seems possible that other films may be essentially impermeable to oxygen at temperatures used for frozen food storage whatever their permeability characteristics at temperatures above zero. The dominant effect that temperature can have on film permeabilities should be recognized when films are evaluated for use with products that may be stored at sub-zero temperatures.

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Book Reviews

Food Engineering Fundamentals. By J. Clair Batty and Steven L. Folkman.
New York: John Wiley, 1983. Pp. xi + 300. ISBN 0 471 05694 4. £29.40.

Several engineering books for students of food technology have appeared in the last 2 years. This book is the latest and is based on material developed at Utah State University which aims to provide "food science students with quatitative problem-solving ability rather than just a superficial survey of engineering sciences".

The book starts with five chapters which provide an introduction to some of the basic concepts of engineering (dimensions, systems, equations of state etc.). A further five chapters follow which cover elements of work, heat, energy, entropy and ideal gas mixtures. After this, consideration is given to the more applied aspects of the subject. Chapters follow on Refrigeration, Fluid Flow, Steady-state Heat Transfer, Transient Heat Transfer, Evaporation Techniques and Drying of Food Products. Numerous worked examples (the majority in S.I. units) are given in the text with each chapter ending with further problems (to which no answers are given). The presentation is very clear although the worked examples often tend to interrupt the flow of the text, making it difficult to find a particular topic quickly. Diagrams are given which, in addition to displaying the authors' humour, provide a valuable guide to some of the difficult concepts.

Each topic is clearly developed from the basic fundamental principles, as the title implies, and then applied these to food situations. With the level of initial background knowledge required being low for this book it is inevitable that some of the more applied aspects are dealt with rather briefly. Thus in this case the text is primarily an introduction to the engineering calculations which confirms the authors' statement given above. The specific development of these fundamentals to food processing is covered although, as with a number of these books, an indication of the complexity of problems associated with food processing, arising from the very varied composition and structure of foods, is lacking.

With other books on the subject having recently appeared a comparison is inevitable. In particular, *Unit Operations in Food Processing* by R. L. Earle, must be considered an alternative to the current volume. The book by Earle spends less time on the basic principles and more on the applied aspects of the calculations. One example will demonstrate: whilst this book uses the log mean temperature difference for heat transfer calculations with no apparent justification, the book by Earle derives it by integration.

Will I be recommending this book for food technology students? I think not, although it will be a useful book to have available for those occasions when a slightly different approach is required to provide an insight, which might be difficult to achieve from other texts.

<div align="right">*D. J. Jukes*</div>

Polymer Chemistry: The Basic Concepts. By Paul C. Hiemenz.
New York: Marcel Dekker, 1984. Pp. xi + 738. ISBN 0 8247 7082 X. SFr. 97.00

I welcomed the opportunity to review this book from an author with an excellent textbook on Colloid and Surface Chemistry. This text is pertinent at the present time as there is a recent upsurge in interest in Polymer Chemistry. The emphasis is on the physical chemistry of polymers with one section dealing with the organic chemistry of polymerization reactions. The first chapter is an overview of the types of chain linkage and various definitions, number average molecular weight etc., used in polymer terminology. The next section of three chapters is concerned with the mechanical behaviour of bulk polymers and the coverage embraces the viscous state, the elastic and viscoelastic states and the glassy and crystalline state. This is followed by a section on classes of polymers and polymerization reactions: the three chapters in this section are headed 'Condensation or step-growth Polymerization', 'Addition or chain-growth, Polymerization' and 'Polymers with Micro-structure', and in these chapters the organic chemical concepts are presented. The final section of the book discusses the physical properties of polymers in solution; chapters are devoted to the thermodynamic properties, frictional properties and light scattering properties of molecules in solution.

In the preface the author asserts that he has tried to write a polymer textbook that belongs in the realm of physical chemistry i.e. fundamental, molecular and interesting. In this he has succeeded. The book assumes little prior knowledge of polymer chemistry and takes the reader to a stage where research literature can be tackled. The primary emphasis of the book is on the structure, energetics and dynamics of molecules. To me the text succeeded in being interesting. The presentation and lay-out is clear and visually stimulating, no easy achievement with the presentation of so many mathematical formulae. There are few typographical errors. To help the reader over fifty worked examples are given in the text and problems, based on data from the literature, are included at the end of each chapter. Summaries of physical theory necessary for the development of a topic are included and theoretical models are treated in sufficient detail to be readily comprehensible to the novice.

Polymer chemistry embraces an enormous literature and obviously in a book of this size some topics are omitted and others dealt with very briefly. For example discussion of copolymers formed by the step-growth mechanism and the use of Ziegler-Natta catalysts to regulate geometrical isomerism have been omitted. Treatment of anionic and cationic polymerization is superficial, although adequate coverage is given to free radical polymerization in solutions and in emulsions.

The book is suitable as a main or reference text for honours students. The introductory chapter contains information which is used throughout, but after that the book is divided into three essentially independent parts. Engineering and physics students will find chapters 2–4, dealing with the bulk properties of polymers, relevant, whereas for a polymer course, biased towards organic chemistry, chapters 5–7 are suitable. The final section of chapters 8–10, encompassing the solution properties of polymers, is tailored to the needs of students of biochemistry and molecular biology. The whole text forms an excellent basis for a course of postgraduate lectures or an M.Sc. course. and is a coherent introduction to the subject for the research worker whose research interests are veering towards the realms of polymer chemistry. Well done and thank you Paul Hiemenz.

Thelma M. Hardman

Nutrition: An Integrated Approach, 3rd edn. By Ruth L. Pike and Myrtle L. Brown. New York: John Wiley, 1984. Pp. xiv + 1068. ISBN 0 471 80625 0. £13.95.

Since the last edition was published in 1973, the subject of nutrition has advanced considerably. To cope with this increase in knowledge. Ruth Pike and Myrtle Brown have co-opted twelve other authors to produce the new edition, while retaining overall editorial responsibility. Therefore, although this is a compendium of work by different authors, names are not attached to particular chapters and there is no readily detectable change in style from one chapter to another.

The aim of writing the book has been to integrate, as far as possible, the contributions of related scientific disciplines to the study of nutrition. This approach is a unique one, which has made earlier editions of this textbook a standard text in many nutrition courses at the university level. Throughout the book an attempt is made to foster a questioning attitude and to emphasize the depth and limitations of present knowledge by showing historical development. Although in the preface the intended readership is not specified, it is obviously written for the student of medicine, nutrition or related areas.

The approach used in the book is clear and well thoughtout, without the dry layout and excessive detail found in comparable medical texts. It is divided into five parts, which are: The Nutrients, Physiological Aspects of Nutrition, The Cell, Specialized Cells and The Complex Organism. There is very up-to-date and detailed description of digestion and absorption of nutrients. Biochemistry receives excellent treatment, with a good balance of historical background. One example is the treatment of folacin (folic acid). The mode of action of this is complex, but in the book it is presented in a readily understandable form for anyone with a basic science training. For those interested in the mechanisms by which sodium raises blood pressure, there is a clearly described section indicating the hormones responsible. Current research is dealt with well in, for example, a section on the brown fat cell. The implications of this tissue in dietary-induced thermogenesis is reviewed and useful references given.

The reader might be inclined to think that the book covers the whole of nutrition, but this is not the case. The more applied aspects of the subject are not covered, e.g. vegetarianism, diabetes, heart disease, infant feeding. However, among some of the more nutritionally orientated chapters, I found particularly useful Determination of Nutrient Needs: Vitamins, Dietary Standards and Nutrition Surveys. The book is clearly illustrated with line drawings and diagrams of biochemical pathways and has some well chosen photographs and photomicrographs. There is an extensive bibliography (179 pages) in which are listed all the references to key research papers and reviews given throughout the text. In this bibliography titles of journal articles are given which makes them more useful, although it does take up a lot of space.

Generally, the book is easy to read and recommended for those who wish to refresh their memories on the scientific development and background to a particular nutritional topic. Therefore, as well as being useful to the student, it makes a good reference book for the researcher or teacher in nutrition to have on the bookshelf.

Ann Walker

The Revival of Injured Microbes. Edited by M. H. E. Andrew and A. D. Russell.
Society for Applied Bacteriology Symposium Series No. 12.
London: Academic Press, 1984. Pp. xiii + 394. ISBN 0 12 058520 0. £29.50.

For some time now microbiologists working in quality assurance in the food industry
have been aware of the problems which may be involved in attempts to detect in
preserved and processed foods the presence of micro-organisms which may be sub-
lethally damaged. Normally particular populations (e.g. coliforms, salmonellae,
Staphylococcus aureus) are detected and/or counted by the use of selective isolation
media. These media usually rely on the incorporation of inhibitory agents to which
however the microorganisms being sought are only *relatively* more resistant than other
microorganisms. The use of such media may sound the death knell for the target
microorganisms if these be injured, unless resuscitation techniques are first used to
permit the microorganisms to repair the damage. Although a number of monographs
concerned with inhibition, death and survival of microbes (e.g. Society for Applied
Bacteriology Symposium, 5: *Inhibition and Inactivation of Vegetative Microbes*, and
Society for General Microbiology Symposium, 26: *The Survival of Vegetative Microbes*)
have touched upon the repair of microbial injury, I believe that this is the first
monograph to concern itself specifically with the nature of injury to microorganisms,
and the repair mechanisms and hence resuscitation techniques required to permit
detection of the injured organisms.

The contributors to this symposium discuss: potential sites of damage in micro-
organisms exposed to chemical or physical agents; the stress of unbalanced growth and
starvation in microorganisms; the injury caused by refrigeration, freezing, freeze
drying, heating, chemical inhibitors, and (in the case of anaerobes) exposure to oxygen.
Repair mechanisms and the choice of detection media are also discussed, together with
the possible harmful effects of selective media and possible remedies.

This volume is thoroughly recommended to all microbiologists who are concerned
with and about the choice of media and isolation techniques appropriate for the
selection of microorganisms in foods subjected to preservation techniques.

W. F. Harrigan

Food Additives Tables. Updated edition for Classes IX–XIII. Edited by M. Fondu,
H. van Gindertael-Zegers de Beyl, G. Bronkers, A. Stein and P. Carton. Amsterdam:
Elsevier, 1984. Pp. x + 224. ISBN 0 444 42286 2. US$166.75, Dfl. 450.00.

This volume is concerned with four classes of foodstuffs: (i) Milk and the great variety of
derivatives therefrom (Class IX); (ii) eggs and related products (Class X); (iii) fats and
oils including butter and margarine (Class XI); and (iv) drinks of all kinds, both
alcoholic and 'soft' (Class XII), and what may or may not be added to them for
manufacturing, nutritional, appearance and textural, or preservative purposes in
nineteen developed countries but excluding the Communist Block. The choice of
countries is one which takes account of the sophistication and efficacy of the food laws
which obtain in them but which is also angled specifically to include those which have
substantial food import requirements so that would-be exporters can become aware of
the legal 'additive' constraints imposed on particular products and classes of product.

The book is aimed at food manufacturers who are looking for export markets, at

suppliers of food additives (including flavour houses) and at advertizing agents and all those concerned with packaging (including labelling) of the would-be exports to ensure that they waste the minimum of time and effort in effecting such product changes as may be necessary to make the final packaged product conform to the food laws of the country or countries in which the product is proposed to be sold.

This is in no sense a readable book; initially it stands or falls by the ease of access to the required information and the accuracy of the information provided. Regarding ease of access it is difficult to see how in all the circumstances and with the enormous body of information to be dealt with it would be possible significantly to improve access. Furthermore, with such a body of information it is not possible to carry out anything other than spot checks on accuracy. Such checks carried out during a 6-week period when guidance was sought on the possibility of using several additives in countries with as diverse laws as Japan, U.S.A., Portugal and France proved to be correct in essence though they could in some instances have been more helpful if more detail had been provided.

Another aspect of accuracy concerns the recency of the data, in this type of publication a very major concern as food laws are constantly being revised and changed. This book is up-dated to the first quarter of 1984, so that it would be necessary to have recourse to the author for confirmation of 'no change' before any major decisions were to be made on the data provided. In any case the effective and useful shelf life of the book is limited to a very few years when it is proposed that it will be replaced by an up-dated volume. In the meantime it can be said that presentation, layout and design are well up to the standard expected from Elsevier and that no typographical errors have been encountered.

My principal criticism is not of the book itself but of it as a data source which needs, but does not and, from its non-looseleaf binding, cannot receive continual up-dating and of the fact that, for most potential users, probably less than 10% of the contents of the volume will ever be utilized.

In the preface it is mentioned that the information is computerized, which means that there is a data base already established which is up-dated and modified as changes arise, so that when another edition is called for it amounts to an edited print-out.

It will be a fact that almost all firms or organization needing the kind of data presented in this book will be computer users and will have some means of drawing on established data bases or banks. How very much better then to be able to access the required specific information in as much or as little detail as is required in the full knowledge that it is complete and accurate up to, say, 28 days previously. Access charges could be quite as remunerative as what is bound to be a somewhat limited sale of a relatively short lived volume costing around £150. This comment, of course, raises the difficult question of what relatively ephemeral information should be enshrined in a hardback publication; in this case any failure in being 'up to the minute' in accuracy could have massive financial implications and clearly any information gleaned from it could only be regarded as generally indicative and subject to a great deal of checking. There is also the interpretative element which comes in which, in my experience, is quite a major factor and which is not and cannot be dealt with either in this publication or for that matter in a data base.

So whilst the volume can be generally helpful to the potential purchasers previously mentioned, my view is that an accessible databank on the subject would be much preferable.

R. G. Booth

Microbiological Methods for Environmental Biotechnology. Edited by John M. Grainger and J. M. Lynch. London: Academic Press, 1984. Pp. xvii + 421. ISBN 0 12 295040 2. £45.00.

The nineteenth in the Technical Series of the Society for Applied Bacteriology is, as usual in this series, the collected contributions presented at Society's Demonstration Meeting in 1982. There are twenty-three chapters each concerned with a method or methods used in an aspect of biotechnology. The subject matter covers an extremely wide range as would be expected from the title and topic of the meeting and there are a total of sixty contributors. Since the intention was to cover environmental biotechnology (whatever it is!) in the broadest possible way, the Society is to be congratulated on achieving its objective.

The editors' introduction explains the diversity of topics, microorganisms, microbial activities, substrates, applications, industrial processes. Mathematical modelling of cellulose decomposition, rearing insects in the laboratory (with reference to virus disease control of insects), computer control of a photobioreactor for algal growth and preparation of mollusc tissue (for use in monitoring bacteria in water) are some of the techniques which illustrates the diversity.

Biotechnology, and perhaps especially environmental biotechnology, is by its very nature and diversity, of interest to many who are readers occupied in a wide range of industries, occupations and activities. Many aspects are of inter-disciplinary interest but most of the methods described would of necessity be undertaken by specialists.

By being successful in attracting the large number and wide range of contributors, the relatively large publication of over 400 pages deserves to attract a very large readership. However, with relatively few exceptions, individual readers will be concerned with a few and, at best, a minority of the subjects discussed and may regard personal purchase at a cost of £45 as unjustified.

In common with other publications which are conference proceedings or collected papers presented at meetings, it is a matter of regret that particular contributions or groups of publications cannot be made available separately and therefore to a much larger number of potential purchasers. This is especially true where, as in this instance, the contents are largely concerned with methods and techniques which may not appear in such detail in research publications or elsewhere.

The book will find a home in libraries of many kinds, for use by teachers, researchers and industrialists and in specialist areas such as agriculture, food processing, waste treatment, water supply, chemical and biological engineering. The standard of presentation of text, plates and figure is high and a degree of uniformity amongst contributions has been maintained, presumably by editorial effort and the cooperation of contributors.

S. Baines

Impedance Microbiology. By Ruth and Gideon Eden. Letchworth: Research Studies Press Ltd., 1984. Pp. xiii + 170. ISBN 0 86380 020 3. £24.50.

The use of electrical methods in microbiology, principally for detecting and enumerating microorganisms, has been one of the fastest developing methodologies in recent years particularly within the food, pharmaceutical and water testing industries. For

some time now this methodology has needed a reference book to detail the principles behind impedance microbiology and to assess the current status of its applications. The Edens' book certainly fills this gap and will I am sure be regarded as the standard text on this subject for some time to come.

The book is not a critical comparison of impedance methods with other rapid methods but is nontheless valuable for that. It adopts the tome of an advanced users' workshop covering both theoretical and practical aspects of using the technique. Chapters are included to cover the background to automated methods, the history and theory of impedance microbiology, mathematical analysis of impedance methods, considerations in the development of specific impedance methods, the use of impedance for enumeration of microorganisms, non-counting applications, instrumentation and statistical analysis of scattergrams. Uses of impedance in clinical microbiology are covered but, one suspects, not the same depth as the food applications which is where the authors' expertise really lies. I found the sections on electrodes and surface effects particularly valuable as they raised my level of understanding of the theory behind the method.

The least effective chapter is the last on instrumentation where the authors, who work for one manufacturer, have allowed their major competitor space to describe his own instrument. This probably seemed a fair and just idea at the time but the end result reads like a re-printing of the sales brochure and is of little help to the uncommitted user.

The text is remarkably error free although the language is strange in places, for example the development of impedance proceedings (sic) instead of procedures, and the use of the 'bioburdens'. Nevertheless this is a book that has fulfilled a requirement and I am sure will find favour amongst all users of impedance methodology. It's a book for the laboratory rather than the library, which I imagine is what the authors intended.

C. S. Gutteridge

NATIONAL COLLEGE PROFESSORSHIP
OF FOOD TECHNOLOGY

Applications are invited for the National College Professorship of Food Technology which will become vacant on 1 October 1986.

The University is seeking to appoint a Professor whose principal interests lie within food processing and preservation and who is able to provide academic leadership in that area in particular. Applications from candidates who have experience in, or well-established links with, the food industry will be welcome.

Further information may be obtained from the Registrar, Room 212, Whiteknights House, The University, P.O. Box 217, Whiteknights, Reading, RG6 2AH.

The closing date for applications is 31 January 1986.

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Biophysical Methods in Food Research

Critical Reports on Applied Chemistry, Volume 5

Edited by H. W-S. Chan MA, PhD, *Head of the Chemistry and Biochemistry Division, Food Research Institute, Norwich*

This book reviews the latest major developments in the application of biophysical methods of studying molecular structure and function to food systems. In most cases the techniques have been used only recently in food research, and the discussion therefore takes the form of an introduction to a technique, followed by examples of its application to food science where this is possible. A chief aim of these reports is to generate interest in the techniques so as to widen the scope of their application, and the reader is given an indication of the potential and likely future development of these methods.

Contents: Thermoanalytical methods in food research *D. J. Wright*; Optical methods as applied to biopolymer solutions *V. J. Morris*; Spectroscopic methods: nuclear magnetic resonance and photoacoustic spectroscopy *P. S. Belton*; Rheological methods *S. B. Ross-Murphy*

1984. 200 pages, 75 illustrations. £19.50
0 632 01212 9

Science Writing for Beginners

A.D. Farr BA, PhD, FIMLS, Editor, *Medical Laboratory Sciences*

Scientists need to write frequently throughout their professional careers, yet few have had any training in writing technique. This book is intended primarily for beginners at writing, and covers American as well as English usage. Written in a discursive rather than prescriptive style, the suggestions and comments made in the book are applicable to all aspects of science writing. The author has included a unique chapter on writing with the use of a word processor.

Contents: Introduction: why must scientists write?; Writing good English; Writing essays; Writing science papers for publication 1: the body of the paper; Writing science papers for publication 2: the 'peripherals'; Some other kinds of writing; How to prepare and submit your paper, and deal with editors and printers; How to write project reports, dissertations and theses; So you fancy writing a book?; What about word processors?

1985. 128 pages, 5 illustrations. Paper, £5.80
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JOURNAL OF

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JOURNAL OF FOOD TECHNOLOGY: NOTICE TO CONTRIBUTORS

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Typescripts (three complete copies) should be sent to the Editor, *Journal of Food Technology*, c/o Institute of Food Science and Technology (U.K.), 20 Queensberry Place, London SW7 2DR. Papers should be type-written on one side of the paper only, with a 1½ inch margin, and the lines should be doubled-spaced. In addition to the title of the paper there should be a 'running title' (for page headings) of not more than 45 letters (including spaces). The paper should bear the name of the author(s) and of the laboratory or research institute where the work has been carried out. The full postal address of the principal author should be given as a footnote. (The proofs will be sent to this author and address unless otherwise indicated.) The Editor reserves the right to make literary corrections.

Arrangement. Papers should normally be divided into: (a) Summary, brief, self-contained and embodying the main conclusions; (b) Introduction; (c) Materials and methods; (d) Results, as concise as possible (both tables and figures illustrating the same data will rarely be permitted); (e) Discussion and conclusions; (f) Acknowledgments; (g) References. Authors should consult the current issue in order to ensure that their manuscript conforms to the Journal conventions on such things as subheadings, layout of tables, etc.

References. Only papers closely related to the author's work should be included; exhaustive lists should be avoided. References in the text should be made by giving the author's surname, with the year of publication in parentheses. When reference is made to a work by three authors all names should be given when cited for the first time, and thereafter only the first name, adding *et al.*, e.g. Smith *et al.* (1958). The '*et al.*' form should always be used for works by four or more authors. If several papers by the same author and from the same year are cited, a, b, c, etc. should be put after the year of publication, e.g. Smith *et al.* (1958a). All references should be brought together at the end of the paper in alphabetical order. References to articles and papers should mention (a) name(s) of the author(s) (b) year of publication in parentheses; (c) title of paper; (d) the title of the journal given in full and not abbreviated, set in italics (underlined once in typescript); (e) the volume number; (f) the first and last page numbers of the paper—e.g.

Steiner, E. H. (1966). Sequential procedures for triangular and paired comparison tasting tests. *Journal of Food Technology*, **1**, 41–53.

References to books and monographs should include (a) name(s) and initials of author(s) or editor(s); year of publication in parentheses; (b) title, underlined; (c) edition; (d) page referred to; (e) place of publication and publisher—e.g.

Lawrie, R. A. (1979). *Meat Science*, 3rd edition. Oxford: Pergamon Press.

In the case of edited multi-author monographs, the editor(s) should be indicated in parentheses after the book title—e.g.

Hawthorn, J. (1980). Scientific basis of food control. In *Food Control in Action* (edited by P. O. Dennis, J. R. Blanchfield & A. G. Ward). Pp. 17–33. Barking, Essex: Applied Science.

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

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	$\mu = 10^{-6}$ m	minute	min
nanometre	nm = 10^{-9} m	second	sec
litre	l = 10^{-3} m³		

Chemical formulae and solutions. The correct molecular formula should be used indicating whether the substance is anhydrous or hydrated. Solutions should be given in terms of molarity. The arithmetic basis of other expressions of concentration must be clearly defined.

Species names. In addition to common or trivial names of plants, animals or microorganisms, the species should normally also be indicated by use of the latin binomial at least once. This should be given in full and italicized (underlined once in typescript). In subsequent use, the generic name may be abbreviated to a single letter provided that this does not result in ambiguity.

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

Tables. There should be as few tables as possible and these should include only essential data; the data should not be crowded together. The main heading should be in bold with an Arabic number, e.g. **Table 2.** Each table must have a caption in small letters. Vertical lines should not be used.

Offprints. Fifty offprints will be issued free with each paper but additional copies may be purchased if ordered on the printed card which will be sent to the senior author with the proofs.

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