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Contributions and editorial correspondence should be sent to Dr H. Liebmann, c/o Institute of Food Science and Technology (U.K.), 105–111 Euston Street, London NW1 2ED.

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Mycotoxins and the cereals industry—a review

B. G. OSBORNE

Summary

Mycotoxicoses in humans and animals associated with the consumption of mouldy cereals have long been recognized and many are now linked with the occurrence of specific mould metabolites (mycotoxins). Mycotoxins which have been detected in cereals are aflatoxins, zearalenone, ochratoxin A, nivalenol, deoxynivalenol, T-2 toxin and diacetoxyscirpenol and of these only aflatoxin B_1 has so far been shown to exhibit serious toxicity to humans. Surveys have shown that the occurrence of mycotoxins in cereals in the UK and USA is rare except for localized problems with corn in the Southern United States. Also it is clear that aflatoxins are more likely to occur in warm humid climates while the other mycotoxins listed above are more characteristic of temperate climates if there is a wet harvest.

Introduction

Toxic factors of mould origin (mycotoxins) form a branch of study which has attracted much attention over the last 20 years. This has largely been due to the discovery of the carcinogenic properties of the mycotoxin, aflatoxin B_1, and hence the possibility of this substance causing disease and death many years after ingestion of very small amounts (Anon. 1979). The search for analogies to the aflatoxin situation began with the realization that a residue of mould growth, capable of causing severe harm at a time remote from ingestion, could be present in food or animal feed without there being any associated visible mould contamination. This is because the mould could die or be cleaned from the food but the toxin would remain because it diffuses into the food. Knowledge of the widespread occurrence of *Aspergillus flavus,* which produces aflatoxin, began to cause alarm, therefore other common moulds were screened for their ability to produce toxins and many were discovered. Increasingly sophisticated methods

Author's address: Flour Milling and Baking Research Association, Chorleywood, Hertfordshire, WD3 5SH.

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of laboratory analysis have enabled the presence of minute amounts of mycotoxins in food to be demonstrated.

Mycotoxicoses associated with cereals

Evidence of human and animal disease caused by mycotoxins first came from retrospective investigations into outbreaks of diseases of unknown cause which were apparently associated with ingestion of mouldy cereals (Lindsell, 1977). For example, dramatic outbreaks of a disease known as alimentary toxic aleukia (ATA) occurred in the Soviet Union between 1941 and 1947. The enormous war casualties suffered in some areas resulted in the autumn grain harvest being left under the winter snow. Near famine conditions dictated that it be used and over 10% of the population of these areas suffered from ATA. ATA is a severe bone marrow disease with a high mortality and recent research has linked it with the trichothecene group of mycotoxins which are produced at low temperature by *Fusarium* species which grow on moist grain. Similarly in Japan a large amount of mouldy rice was consumed at the end of the Second World War and this gave rise to yellow rice disease. This disease had very similar symptoms to vitamin B_1 deficient beri-beri and isolation of several mycotoxins which could be shown to be the cause of the disease characterized it as a mycotoxicosis. In 1956 and 1963 the wheat harvest of Western Japan was severely attacked by *Fusarium* and the resulting human mycotoxicosis—red mould disease—produced typical gastro-enteritis symptoms. No fatalities were reported and once again the trichothecene group of toxins was implicated.

Trichothecenes are a large group of toxic secondary metabolites produced by fungi imperfecti predominantly of the genera *Fusarium*. *Fusarium* species are active pathogens which are extremely common on cereals and worldwide in distribution. Even perfecly good-looking harvested grain may have internal infection with *Fusarium*. The general structure of trichothecenes is shown in Fig. 1, the common features of all the group being a 9, 10 olefinic double bond and a 12, 13 epoxy group. In addition, most toxins contain a hydroxyl or ester group at C-4, some have additional hydroxyl or ester groups at C-3, C-7, C-15 and an oxo group at C-8.

Toxicological problems in humans and animals associated with the consumption of mouldy grains have long been recognized and those particularly associated with trichothecenes are listed in Table 1. Many of these mycotoxicoses are characterized by common symptoms such as emesis, vomiting, skin necrotization and haemorrhages. It has not been possible so far causally to relate specific trichothecene compounds with specific toxicoses, although toxins have been isolated from actual toxic feed samples in some cases. Deoxynivalenol has been found in corn* samples in the Transkei area of South Africa which is associated with a high incidence of oesophageal cancer and pure T-2 toxin is reported to be carcinogenic to rats (Pathre & Mirocha, 1979). However,

*The term 'corn' in this article is taken to mean maize.

Table 1. Some mycotoxicoses associated with cereals

Toxicosis	Districts and affected species	Symptoms	Fungus
'Taumelgetreide' Toxicosis	Siberia—man, horse, pig, fowl	Headaches, chills, nausea, vomiting	*G. saubinetti*
Alimentary toxic aleukia	USSR—man, horse, pig	Vomiting, diarrhoea skin inflammation, leukopenia, angina	*E. sporotrichiodes*
Stachybotryotoxicosis	Europe—horse	Shock, somatitis dermal necrosis, leukopenia	*Stachybotrys atra*
Bean-hull toxicosis	Japan—horse	Convulsion, cyclic movement	*F. solani et al.* *F. solani et al.*
Dendrodochiotoxicosis	Europe—horse	Skin inflammation	*Dndrochium toxicum*
Mouldy corn toxicosis	USA—pig, cow	Emesis, haemorrhage, refusal of feed	*F. tricinctum*
Red-mold toxicosis	Japan—man, horse, pig, cow	Vomiting, diarrhoea emesis, abortion	*F. graminearum*

long term feeding experiments and long term exposure to skin have produced no evidence of carcinogenicity. Also T-2 toxin and fusarenon-X gave negative mutagenicity results in the Ames test on activation with S9 (Ueno, 1977). At present, therefore, it cannot be concluded that trichothecenes are carcinogenic substances.

The diseases mentioned so far were regarded simply as mould associated toxicoses but the concept of mycotoxins (Martin & Gilman, 1976) was finally developed in the 1960s after an outbreak of a hitherto unknown disease which resulted in the death of 100 000 turkeys. The cause of the disease was traced to Brazilian groundnut meal in the diet from which was isolated a highly toxigenic strain of *Aspergillus flavus*. This particular strain was found to produce under certain circumstances a substance which was called A-FLA-TOXIN and which when fed to turkeys reproduced the disease. Many cases of acute human aflatoxicosis, often fatal, have been recorded in parts of Thailand, India and Africa where the diet is unvaried and predominantly cereal. The symptoms are very similar to those of infectious hepatitis and cereal contaminated with aflatoxin B_1 can cause severe liver damage within a short period. Aflatoxin B_1 (Fig. 1) is the most powerful carcinogenic agent known: 10 millionths of a gram cause liver cancer in rats (Luck & Groll, 1971) and there is epidemiological evidence correlating incidence of human liver cancer in Uganda with dietary levels of aflatoxin B_1 (Alpert *et al.*, 1971).

Apart from trichothecenes, aflatoxin and its precursor, sterigmatocystin, which is also carcinogenic, (Hamasaki & Hatsuda, 1977) there are two other mycotoxins which have been detected in cereals. *Fusarium roseum* growing on cereals in the field can produce zearalenone or F-2 toxin (Fig. 1) which causes

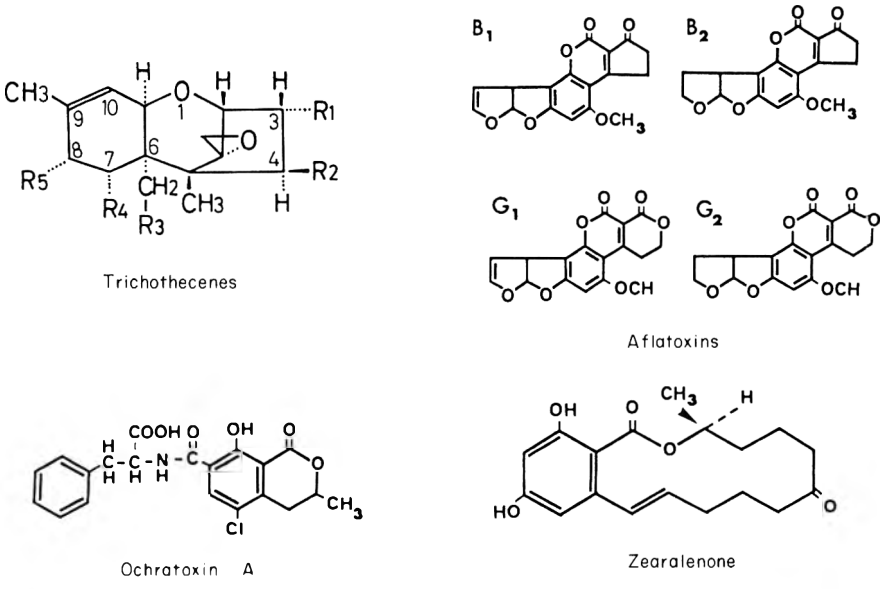

Figure 1. Structures of some mycotoxins which have been detected in cereals.

disease in swine (Bennett & Shotwell, 1979); its human toxicity has not been established. Although hyperoestrogenic effects in swine were reported in the early 1900s a cause-effect relationship between mouldy corn feed and disease was not postulated until 1928. In 1952 the mould species *F. roseum* was causally associated with the disease and later the mycotoxin zearalenone was isolated from it. The natural occurrence of zearalenone in cereal grains has been established and corn has been most often implicated in cases of hyperoestrogenism in swine. Other commodities tested because of adverse effects in animals and found to contain zearalenone were barley, sorghum, sesame and wheat.

Ochratoxin A (Fig. 1), a liver and kidney poison, is produced by *Aspergilli* and *Penicillia,* and, together with another mycotoxin (citrinin) has been shown to be the causative agent in porcine nephropathy in Denmark associated with mouldy barley in the feed (Krough, 1977). Balkan endemic nephropathy, a human disease with similar characteristics to porcine nephropathy, has so far been observed only in certain parts of Bulgaria, Romania and Yugoslavia. Epidemiological evidence has linked the disease with foodborne ochratoxin A (Krough *et al.*, 1977) although the evidence is not as conclusive as for the porcine disease.

Formation of mycotoxins

Moulds are ubiquitous and it is perhaps surprising that the occurrence of their toxins is not more frequent and widespread, until the very precise requirements

for their elaboration are appreciated. The total mould count does not give much information regarding the presence of mycotoxins, neither does identification of the species (*e.g.* only about half of the known strains of *A. flavus* produce aflatoxins). A potential for mycotoxin formation can only be established by the isolation of a toxigenic strain which requires expert and highly specialized knowledge. Even if a toxigenic strain of mould is present in a food the toxic potential is only realized under certain conditions which are different from those required for optimum growth of the mould. This is because mycotoxins are secondary metabolites and are therefore produced in response to environmental stress. This occurs when there is no longer a balanced uptake of nutrients and replicatory growth is no longer possible. The resulting metabolic imbalance is relieved by the initiation of new biosynthetic pathways (Bu'lock, 1965). Thus mycotoxins will not be present in a food unless it has been in environmental conditions which favour mould growth (correct temperature, moisture content etc.), *and* it has been contaminated with a strain of mould capable of producing toxin, *and,* most important of all, a nutrient imbalance has occurred.

Consequently although almost any food can be made to become contaminated with mycotoxins in the laboratory, this indicates no more than a potential for natural occurrence. Also if a food is vigorously growing mould then this is unlikely to be mycotoxin contaminated. The danger foods are those where mould growth has ceased and secondary biosynthesis may have taken over—these will not necessarily *look* very mouldy.

For these reasons the presence of moulds does not prove that toxins are present and the only satisfactory means of establishing this is by chemical analysis. However, moulds have a remarkable ability to synthesise toxic compounds having a wide variety of chemical structures, and because these compounds occur in only minute quantities in complex biological matrices their analysis is quite difficult. At the present time about 200 mycotoxins have been discovered but analytical chemistry and toxicology cannot keep pace with such a rate of discovery. For example, although trichothecene mycotoxins have been implicated on biological evidence as causative agents of a number of toxicoses, only a few authentic reports of trichothecenes found naturally occurring in foods or feeds exist today. This lack of evidence is primarily due to the fact that no adequate practical method for unambiguous detection has hitherto been available.

Occurrence of mycotoxins in cereals

Despite the limitations, increasingly sophisticated methods of analysis have resulted in the detection of very low levels of mycotoxins in foods. By 1974 it was evident that even in countries such as the United States where commodities are produced under the highest standards of harvesting and storage, some crops were regularly affected by contamination with mycotoxins albeit at a very low level and with a low incidence (Shotwell, 1977; Stoloff, 1977). Aflatoxins, for

example, occur only very rarely indeed in small grains (wheat, rye, oats, barley, sorghum, rice, millet); of 3489 samples examined by the US Department of Agriculture from 1968–1975, detectable amounts of aflatoxins were found in only nineteen samples at an average total level of 5 μg/kg. As little as 14–26% of aflatoxin B_1 contamination of wheat was found to survive flour milling and bread baking (L'vova *et al.*, 1977). Since aflatoxin formation occurs most readily in commodities with a high oil content grown in warm and humid climates it is perhaps not surprising that corn is the cereal most likely to be contaminated with this toxin. However still only forty-eight positive results were obtained in surveys in the US involving 2117 samples although a higher incidence has occurred in localized areas (Shotwell *et al.*, 1973). In the wet milling of corn almost all the aflatoxin accumulates in the feed fractions and almost none in the starch (Bennett & Anderson, 1978), nevertheless it has recently been estimated that about 80% of the Americans' exposure to aflatoxin in their diets is attributable to the consumption of corn and its products. The area of major concern in the UK is the carry-over of aflatoxin into milk and dairy products arising from the consumption by cattle of contaminated feedstuffs (Anon. 1980a).

Ochratoxin A was first reported in one sample of corn included in a survey of 283 samples in the USA. Subsequently it has been detected in wheat, oats, barley, rye in Canada, Denmark, France, Poland, Sweden, Yugoslavia and the USA (Anon. 1979). There is no evidence of human disease caused by direct ingestion of ochratoxin A but it is known to carry-over into tissues of animals fed with contaminated feed.

Zearalenone has been encountered as a natural contaminant of cereals, particularly corn, with an incidence as high as 17% of 223 samples when conditions in the Southern States of the USA were conducive to *Fusarium* ear rot (Anon. 1979).

In the UK, the incidence of mycotoxins in cereal products for human consumption has been almost zero. For example, in 1969 ten commercial flours and the ten wheats from which they had been milled were examined for aflatoxins and none detected (Daniels, 1969). Nevertheless, the Ministry of Agriculture, Fisheries and Food Steering Group on Food Surveillance recommended in 1975 that there was a need to monitor commodities for the presence of mycotoxins particularly when the commodity forms an important item of diet (e.g. flour). Accordingly, scientists at the Ministry of Agriculture, Fisheries and Food tested 113 samples from the National Flour Survey for aflatoxins, ochratoxin A, zearalenone and sterigmatocystin but none of these mycotoxins was detected (Anon. 1980a). Further surveillance has been carried out over the last 2 years; 200 samples of commercial flour were collected from throughout the UK and analysed for eleven different mycotoxins and no contamination was detected (Osborne, 1979a). Ochratoxin A has been reported once as a contaminant of mouldy flour which was in a paper sack and had become wet during transportation (Richardson *et al.*, 1978). Mould growth and toxin formation ensued but both were confined to the lumpy portions while the sieved flour was

uncontaminated. It has subsequently been demonstrated that this is not an isolated case and two further cases of ochratoxin A in mouldy flour lumps from bulk silos were discovered but these findings must be viewed in perspective with the results of the 200 sample survey. If present in flour which is subsequently baked, ochratoxin A survives bread baking but two-thirds is lost during biscuit baking due to the presence of bicarbonate in the dough (Osborne, 1979b). Waste bread which may often be mouldy is used as a component of pig feed and in view of the Danish work on ochratoxin A it was necessary to establish whether any mycotoxin contamination exists in mouldy bread. In all, fifty samples were analysed and only one contained a small trace of ochratoxin A, therefore there is no evidence that mouldy bread may be toxic to either pigs or humans (Osborne, 1980).

Table 2. Contamination of cereals with trichothecenes

Commodity	Country	Toxin	Incidence	Level (ppm)	Reference
Corn	USA	T-2	1/1	2.0	Hsu *et al.* (1972)
Corn	USA	T-2?	94/173	0.05–1.0	Eppley *et al.* (1974)
Mixed feed	USA	DAS, T-2, Vomitoxin	9/200	0.04–1.8	Mirocha *et al.* (1976)
Corn	USA	Vomitoxin	24/52	0.5–10	Vesonder *et al.* (1978)
Grain	Finland	T-2	9/230	0.01–0.05	Ylimaki *et al.* (1979)
Corn	Austria	Vomitoxin	3/3	1.3–7.9	Vesonder & Ciegler (1979)
Corn	Canada	Vomitoxin			
Wheat/ barley	Japan	Vomitoxin, nivalenol	?	62.3 71.5	Yashizawa & Morooka (1977)
Barley	Japan	Vomitoxin Vomitoxin	?	4.2	Pathre & Microcha (1977)
Corn	S.Africa	(+F-2)	?	0.25–7.4	Marasas (1978)
Wheat	UK	Vomitoxin	1/35	0.09	Osborne (1981)

The ubiquitous nature of *Fusaria* in freshly harvested cereals is an indication that trichothecenes may be the most important of natural toxins in such commodities. Unfortunately, however, due to the fact that adequate analytical methodology has only recently been developed data on their natural occurrence are few (Table 2). Only four trichothecenes have been found naturally – T-2 toxin, diacetoxyscirpenol, nivalenol and deoxynivalenol (vomitoxin) – and all in cereals. The first discovery of the natural occurrence of trichothecenes was in Wisconsin in 1971 when T-2 was isolated from corn which had caused mouldy corn toxicosis (Hsu *et al.*, 1972). Deoxynivalenol was discovered in a batch of corn that had caused vomiting in pigs in the USA (Vesonder *et al.*, 1973), and in wheat and barley responsible for red mould disease in Japan (Yoshizawa & Morooka, 1977). In both cases the disease was associated with the coincidence of the rainy season with the harvesting of the crops. Deoxynivalenol was detected in wheat in Canada in 1980 (Anon., 1980b) but a survey of thirty-five

samples of home-grown wheat in the UK in the same year only revealed one sample contained a trace of this mycotoxin (0.09 mg/kg) (Osborne, 1981).

It is clear that *Fusaria* are common in cereals in wet temperate climates such as N. Europe, Japan and parts of N. America and that trichothecene contamination does sometimes occur. There are too few data to establish patterns but since analytical techniques are now capable of sensitive detection of these toxins harvest surveys for *Fusarium* toxins may now be carried out.

Conclusions

Mycotoxins are dangerous naturally occurring food contaminants which in no way are caused by man. Their presence in commodities cannot be predicted and no visible mould growth need be present at the time of ingestion or processing of a food for mycotoxin contamination to be present. However, mycotoxins only occur when a specific mould is present and conditions are exactly right so their presence in foods should not be common. There is now a growing body of evidence to show that mycotoxins do not occur in cereals destined for human consumption in the UK except in very rare cases. The limits of detection for mycotoxins are in the parts per billion (μg/kg) range although different toxins have different detection limits. Typical figures are 1 ppb for aflatoxin B_1, 20 ppb for ochratoxin A, vomitoxin, 100 ppb for zearalenone, most trichothecenes and sterigmocystin.

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Lupinseed—a new source of edible oil

J. G. FLEETWOOD AND B. J. F. HUDSON*

Summary

Lupin can be grown as an arable leguminous oilseed crop in temperate climates. Though the oil yield, as a proportion of dry seed, is somewhat low in comparison with soyabeans, the oil is readily extractable and can be refined by conventional processes to yield a pale bland edible oil in good yield.

Owing to the presence of linolenic acid, lupinseed oil, like soyabean oil, has limited stability in ambient storage conditions but its quality compares favourably with that of soyabean oil or rapeseed oil.

Introduction

The United Kingdom, in common with other countries in Northern Europe, is heavily dependent on the importation of tropical and sub-tropical oilseeds, oils and meals to meet domestic requirements. During the three years, 1978–1980, upwards of 1 600 000 tonnes of oilseeds were crushed annually in the UK, of which from 60–80% were soyabeans, yielding on average around 200 000 tonnes of crude oil annually. This was supplemented to a minor extent by imported oil, according to the price of oil and crushing costs.

Rapeseed or 'oilseed rape', as it is known to farmers, has assumed increasing importance as an indigenous crop, both in Northern Europe and in North America in the last two decades. Owing to outstandingly successful breeding programmes new varieties with very low erucic acid and glucosinolate contents have been introduced. Taking the oil and the meal together, however, it is not expected that rapeseed can, in the foreseeable future, compete successfully with soyabeans, especially in respect of its protein component. Since, however, soyabeans cannot be grown reliably in Northern latitudes it remains desirable, in order to reduce our dependence on imported oilseeds, to develop a similar

Authors' address: *Department of Food Science, University of Reading, London Road, Reading RG1 5AQ.

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legume crop more readily adaptable to UK climatic and agronomic conditions, and not competitive with home-grown rapeseed.

Among those plants whose seeds store useful quantities of oil, but which are not currently exploited, the genus Lupinus, with its various species adapted to temperate climates, seemed worthy of consideration. Being a legume, this crop also does not require nitrogen fertilizers, and indeed enriches the soil in which it is cultivated.

The lupin has a long history as a food plant, use being traceable as far back as the civilisation of ancient Egypt, Greece, Rome and South America. Today it is still grown as a good grain crop by peasant farmers in the Sudan, the Mediterranean littoral and in Peru and Chile. In more temperate climates, including parts of the UK, it has been used as green fodder. During the past fifty years, as a result of selective breeding programmes in several countries, the main obstacle to lupin as a food crop, its content of toxic alkaloids, has been removed in several species. On a world-wide basis, increasing interest in the crop is now becoming evident, both as a source of animal feed and for human food.

In an earlier publication (Hudson *et al.*, 1976) we drew attention to the possibilities of lupin as an arable food crop for temperate climates, and briefly noted a range of technically and nutritionally important features. The present communication is concerned exclusively with its potential as a source of edible oil, and with the extraction, refining and quality of the oil itself. The studies reported here were part of a composite programme undertaken in the University of Reading with the support of a grant from the Wolfson Foundation (1975–1980).

Materials and methods

All analytical and processing studies were carried out with whole, dry mature seeds of defined species. Many species of Lupinus are recorded but, for practical purposes, the four which have been studied most intensively, and which are available most readily in small quantities are:

L. albus – widely cultivated and studied, particularly in Southern and Eastern Europe, and in North Africa, where it is often known as *L. termis*.

L. angustifolius – first grown in Europe, but now of special commercial importance in Australia and New Zealand.

L. luteus – of particular interest in Eastern Europe.

L. mutabilis – native to South America, and there under development.

Large-scale studies on extraction, refining and oil stability were carried out in the present study exclusively with *L. albus* (cv Buttercup, grown in South Africa, the only seed available in quantity at the time) using industrial practice and techniques based on those described in standard texts (Bailey, 1964; Andersen, 1962). Very recent trends in refining, e.g. 'physical refining' which employs higher temperatures than previously, were not studied. Acidity and peroxide values were determined by the IUPAC (1973) method.

Experimental

Oil extraction

Whole mature seed, of 5–7% moisture content, was ground in a Christie & Norris 8″ laboratory cross beater mill fitted with 0.75 mm screen, or for small quantities in a coffee mill. Approximately 80% of the flour passed a 300 μm screen. The oil was extracted with hexane (b.p. 60–80°) either in 5.5 kg quantities in a QVF glass liquid/solid extraction plant in which the temperature of the solvent in contact with the seed was 35–40°C, or in smaller quantities in a Soxhlet apparatus. The extraction time was 20–24 hr over a 3 day period for the larger quantity, or 2 hr for smaller batches. The solvent was removed by evaporation under vacuum at a temperature of about 35°C, yielding a deep orange crude oil.

Precooking of the flour, when applicable (i.e. before solvent extraction), was with live steam at atmospheric pressure for 15 min, during which the moisture content increased from about 6 to 10–11%, and then at 14 lbs pressure (120°C) for a further 30 min. The cooked flour was air dried for 24 hr at room temperature and finally in a vacuum oven at 80° for 30 min.

For example, using *L. albus* (cv Kievsky Mutant), in small scale extractions, dried milled seed (220 g) was extracted with light petroleum (b.p. 60–80°C, 750 ml). Yield of oil: 24.15 g (12.1%). In large scale extractions, 5.5 kg of seed was extracted with light petroleum (b.p. 60–80°C) (21 l). Yield of oil: 617 g (11.2%).

Oil refining

(1) Degumming. The lupin oil from *L. albus,* cv Buttercup (400 g, acidity 0.82%) was warmed to 50° and hot water (10 ml) slowly added with stirring. The stirring was continued for 30 min and the aqueous gum allowed to settle for approximately 4 hr. The oil layer was carefully decanted, re-warmed at 50°C and neutralized by the addition of 2 N NaOH (10.25 ml ≡ 75% excess) as described in the following paragraph. Recovery of oil: 377.2 g (94.3%).

The introduction of a degumming stage did not improve the yield or alleviate the problems of emulsion formation during neutralization, which normally accompany laboratory refining procedures.

(2) Neutralization. Batches of non-degummed oil varied in acidity between 0.6 and 3.0% (as oleic acid). In an experiment with an oil of typical acidity, lupin oil (400.0 g, acidity 0.72%) was warmed to 50°C. Sodium hydroxide solution (9.0 ml of 2 N, 75% excess) was added slowly with stirring over a period of 10 min. The temperature was maintained with stirring for a further 30 min, and the oil was then cooled to room temperature. After settling for about 2 hr the oil was carefully decanted from the dark tarry sediment and washed repeatedly with hot water until the washings were below pH 7.0. Sodium chloride was added to the first few washes to minimise emulsion formation. Residual water was removed under vacuum at 40°C, and the vacuum released with nitrogen. Recovery 380.6 g (95%).

(3) Bleaching. Neutralized oil (380.0 g) was heated to 60°C with stirring in a closed reaction flask and Fullers Earth (30.5 g) added. The flask was flushed with nitrogen and the heating continued gently until the temperature reached 106°C after approximately 35 minutes. The flask was connected to a vacuum pump at 12 mm Hg pressure for 5 minutes to remove any water remaining in the oil and the vacuum released with nitrogen. The oil was allowed to cool to 80°C under nitrogen, when the absorbent was removed by filtration to yield a clear yellow oil. Recovery 351 g (92.4%).

(4) Deodorization. The bleached oil (347.1 g) was heated in a multinecked reaction flask to 105°C at 12 mmHg pressure. Water was introduced slowly through a fine capillary tube reaching to the bottom of the flask to generate a continuous vigorous supply of steam. The temperature was gradually raised to 175–180°C and maintained at this level for 30 min. The water supply was then stopped, and after a few minutes, no more steam being produced, the oil was allowed to cool to 80–90°C before breaking the vacuum with nitrogen. The refined oil was stored in completely filled glass containers at 2–3°C until required. Recovery 346.2 g (99.7%).

The refined oil had an acidity of 0.09%, peroxide value 0.07 and a primrose-yellow colour (Lovibond Y 12.0, R 0.7 in a 25 mm cell). As we have shown in an earlier publication, the distinctive yellow colour is due to the presence in all lupinseed oils of carotenoids (El-Difrawi & Hudson, 1979). Accordingly, a heat-bleaching step was investigated as an alternative to conventional deodorization.

(5) Combined heat bleaching-deodorization. Earth-bleached oil (344.1 g) in a multinecked reaction flask fitted with fine capillary tube for water inlet, was heated under vacuum to 225°C during 25 min, but without the introduction of water. The temperature was maintained for a further 30 min, and then allowed to cool to 175°C. A slow stream of water was introduced to generate a vigorous supply of steam, and continued for 45 min during which time the temperature was gradually raised to 225°C. The water supply was stopped and the oil allowed to cool to less than 100°C under vacuum before the vacuum was broken with nitrogen. The pale straw coloured oil (Lovibond Y 1.4, R 0.6 in a 25 mm cell) was stored at 2–3°C in completely filled glass containers. Recovery 343.1 g (99.7%).

Oil composition and stability

Component fatty acids were determined as in our earlier publication (Hudson *et al.*, 1976). Oil stability was assessed from peroxide value determination (IUPAC, 1973) and induction periods using the FIRA-Astell apparatus (Meara & Weir, 1976).

Results and discussion

Extraction of lupinseed oil from seed, and refining techniques

From the foregoing, and with the reservation that studies have been in glass equipment on a laboratory scale, it can be concluded that the extraction and refining of lupinseed oil does not present any insuperable difficulties; and that refining yields are acceptable. The oil, when refined by conventional methods has a bright yellow colour, owing to the presence of carotenoids, of which β-carotene and zeaxanthin are the main components (El-Difrawi & Hudson, 1979). Like palm oil, therefore, it can be decolourized by a heat bleaching process, and, probably, by 'physical' refining involving steam-treatment at much higher temperatures than are used in deodorization.

The oil content of seed (see Table 1) is at present unacceptably low, since if one is to take the 18% level of oil in soyabeans (*Glycine max*) as a minimum standard, even the best seed, *L. mutabilis*, would not prove economic to extract. Some strains of both *L. mutabilis* (up to 17%) and *L. albus* (up to 11%) have however, been found to contain significantly higher levels of oil than the mean figures quoted in Table 1 and this has been found sufficiently encouraging to make breeding programmes for improved oil yield realistic (Williams, 1978; Fleetwood & Hudson, in preparation).

Oil composition

Crude lupinseed oil contains, in addition to triglycerides and small amounts of free fatty acids (0.6 to 3.0%), small amounts of phospholipids.

These are insufficiently significant to demand a specific degumming step in the refining sequence. Such a step can be included but offers little, if any,

Table 1. Mean yields and component fatty acids of lupinseed oils (standard deviations)

Species (no. of samples)	Oil (%)	Component fatty acids (%)–C atoms: double bonds					
		16:0	18:0	18:1	18:2	18:3	Other
L. albus (65)	9.2 (1.85)	7.7	1.8	53.1	18.0	9.0	10.4
L. angustifolius (15)	4.1 (1.08)	12.0	6.0	34.8	39.1	4.7	3.4
L. luteus (18)	3.9 (0.78)	7.1	2.6	26.6	47.4	6.4	9.9
L. mutabilis (74)	13.1 (3.51)	12.4	7.5	47.2	28.1	2.3	1.7
Glycine max	18	12	4	24	51	9	—
*Brassica napus**	44	5.3	1.5	58.8	22.4	10.5	1.5

*Cultivar Jet Neuf, at present grown widely in the UK.

advantage. Lupinseed oil contains 1.0 to 1.5% of non-saponifiable matter, which includes in addition to the carotenoids already mentioned, tocopherols, sterols and triterpene alcohols. The detailed composition of this material will be the subject of a separate communication.

Many analyses of lupinseed oil for component fatty acids have been carried out. Mean figures are given in Table 1, which also includes, for comparison, data for soyabean oil (Gunstone & Podley, 1965). Apart from the five main fatty acids, small quantities of 16:1, 20:0, 20:1, 22:0, 22:1 and 24:0 can also be detected, especially in *L. albus* and *L. luteus*. 22:1 may be erucic acid, but this has not yet been confirmed: it is absent in *L. mutabilis*. It will be seen that, in general, the fatty acid compositions of lupinseed and soyabean oils are similar, but that there is marked inter-specific variation in the case of lupinseed oil. More detailed information on this topic, including statistical treatment, will be published separately.

Oil stability

As might be expected from its content of linolenic acid, which is comparable with that of soyabean and rapeseed oils, lupinseed oil, though completely acceptable when freshly refined, has indifferent stability under ambient storage conditions. FIRA-Astell induction periods at 100°C compared well with soyabean oil, but were inferior to fully refined groundnut oil or palm oil, which are free from linolenic acid:

Lupinseed oil (*L. albus*)	– 7– 8 hr
Soyabean oil	– 6– 8 hr
Rapeseed oil	– 8–10 hr
Groundnut oil	– 20–24 hr
Palm oil	– 40–50 hr

In view of the relatively high content of 18:3 in *L. albus* (see Table 1) it may well be that oil from the other species, which are significantly lower in 18:3, would prove more stable.

Conclusions

Lupinseed oil can be extracted from seed and refined to an acceptable standard for edible use by conventional processes. The principal component fatty acids are oleic and linoleic, though linolenic is also present in all species so far examined. As a result, the oil has limited stability, but in this respect it is comparable with soyabean and rapeseed oils. The principal factor discouraging the use of lupin as a temperate oilseed crop is its comparatively low oil content. Breeding programmes may succeed in raising oil yields to acceptable levels.

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Chemical and physical characteristics, fatty acid composition and toxicity of buffalo gourd oil, *Cucurbita foetidissima*

N. N. KHOURY,* S. DAGHER AND W. SAWAYA

Summary

In this study, *Cucurbita foetidissima* (Buffalo gourd) was investigated as a source of edible oil for human consumption. The seeds are rich in oil (28%) as well as protein (29%). General chemical tests such as the iodine number, saponification number, acid value and others were favourably compared to values of other edible oils. The analysis of the crude oil by means of gas liquid chromatography showed that it is rich in linoleic acid (63%) and has a high unsaturated to saturated fatty acid ratio. The high percentage of linoleic acid and the extremely low percentage of linolenic acid indicate that the oil could be a valuable edible oil, with a relatively good stability. Chicken feeding experiments showed that the oil did not contain any toxic substances causing death or interfering with growth rate and feed conversion.

Introduction

Cucurbita foetidissima, also known as Buffalo gourd, (BG), is a wild xerophilous gourd that belongs to the Cucurbitaceae family. It is indigenous to both arid and temperate regions, and grows wild in many parts of the USA and Northern Mexico (Bolley, McCormack & Curtis, 1950; Shahani *et al.*, 1951). It is also planted in Lebanon, India, Pakistan, Iran, Kuwait and Jordan (Havener, 1974).

Bemis *et al.* (1967) found that the fatty acid composition of *C. foetidissima* and other cucurbit seeds indicated that they could be valuable sources of edible oil. Indeed, pumpkin seed oil was more than 99% digested by rats (Asenjo & Goyco, 1951), and several cucurbit seed oils were used for cooking in many

Authors' addresses: Lebanese National Council for Scientific Research, Beirut, Lebanon and Department of Food Science, American University of Beirut, Beirut, Lebanon.
*To whom correspondence should be addressed at: Crush International (USA) Inc. P.O. Box 113–6154, Beirut, Lebanon.

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countries (Curtis, 1946), and the seeds themselves were used as a food by the American Indian (Shahani *et al.*, 1951).

The oil was also analyzed by Curtis (1946) and Bemis *et al.* (1967) who found that it has a linoleic acid content of 61% with a ratio of 4.45 of unsaturated to saturated fatty acids. The composition of this oil calculated by Shahani *et al.* (1951) from spectrophotometric absorption and methyl ester distillation data showed that linolein and olein were the main glycerides.

Materials and methods

Materials

Fatty acid methyl esters were purchased from Sigma Chemical Company, St Louis, Missouri. Gas liquid chromatography packing material was purchased from Applied Scientific Laboratories, Inc., State College, Pennsylvania. Casein, vitamins, minerals, cellulose, glucose and amino acids were all purchased from Nutritional Biochemicals Corporation (NBC), Cleveland, Ohio. All other reagents were obtained commercially and were of analytical grade.

Cucurbita foetidissima seeds used in this investigation were harvested from experimental plantings grown in Tel Amara agricultural research station in the northeast of Lebanon. Another batch of seeds obtained from the same source but kept for 6 months at room temperature and for two years at 5°C were also used for comparison.

Extraction of the oil

The cleaned seeds were ground in a Wiley Mill (Model No. 2, 20 mesh) grinder and the oil from each batch of 700–800 g was extracted in a large soxhlet extraction apparatus using circulating hot hexane at 45°C. The solvent was circulated three times and then removed from the oil by means of a rotary evaporator. The recovered oil was greenish light-brown in colour.

Chemical analysis of the seeds and oil

The proximate analysis of the seeds including moisture, protein, fat, fibre and ash as well as the chemical properties of the crude oil such as the saponification value, iodine value, percentage soluble and insoluble acids and unsaponifiable matter were determined according to the AOAC (1965) standard methods. The acid value was determined according to Devine & Williams (1961) and the refractive index was measured using an Abbe Refractometer.

Fatty acid composition

Fatty acid composition of the oil samples were tentatively identified by gas liquid chromatography of the free volatile fatty acids (C1-C8) and of the methyl esters of long chain fatty acids using a Perkin-Elmer model 900 chromatograph equipped with a flame ionization detector. For the preparation of the methyl esters, the oil was hydrolyzed with methanolic sodium hydroxide for 5–10 min, and then methylated with 14% boron trifluoride in methanol, using the AOAC (1970) standard method. The gas chromatographic analysis of the methyl esters was carried out on a 183×0.32 cm coiled glass column, packed with 20% diethylene glycol succinate (DEGS) on 80–100 mesh Chromosorb W. The injector and manifold temperatures were 225°C and 250 °C respectively. A temperature programme was used for the column as follows: 100°C for 2 min, 3°C/min to 185°C, hold at 185°C for 6 min. Nitrogen was the carrier gas, with a flow rate of 50 ml/min. The volatile fatty acids were isolated from the oil by steam distillation and collected as their non-volatile sodium salts according to Karleskind, Valmalle & Wolff (1970). The salts were then converted to their free state just prior to chromatographic analysis. In this case the 183×0.32 cm coiled glass column was packed with 15% diethylene glycol succinate plus 2.5% phosphoric acid on 80–100 mesh Chromosorb W. The temperature of both the injector and manifold was 200°C. A temperature program was used for the column as follows: 115°C for 4 min, 16°C/min to 130°C for 8 min. Nitrogen was the carrier gas, with a flow rate of 50 ml/min. The identities of peaks were established by comparison with retention times of standard methyl esters. Concentrations of each component were calculated from peak area following the area normalization and the internal standardization methods and expressed as percentages of the total. The internal standards used in the analysis of long chain fatty acids and volatile free fatty acids were heptadecanoic acid methyl ester and caprylic acid, respectively.

Feeding trials

The feeding experiment consisted of dividing one day old mixed sex broiler chicks that were maintained on commercial feed for 2 days into groups of six in such a way that the average weight of each group was close to the other. Four treatments of two replicates each were used. The first treatment consisted of feeding commercial feed only while the second one consisted of mixing the commercial feed with 10% refined corn oil.

The other two treatments were exactly the same as the second one except that in one 10% of Buffalo gourd oil (BGO) was used and in the other 10% of refined corn oil treated with hexane was used. Taking into consideration that *C. foetidissima* oil was extracted by hexane, the traces of which might still be present in the oil, the refined corn oil was mixed with 250 ml hexane and the solvent was removed by the rotary evaporator. Thus the conditions were the same for both *C. foetidissima* oil and refined corn oil.

Results and discussion

Chemical analysis of Buffalo gourd (BG) seed and oil

Proximate analysis of the *Cucurbita foetidissima* seeds (Table 1) showed selectively high value of crude protein and crude oil indicating that *C. foetidissima* could be used as a dual purpose seed similar to soybean and other oil seed crops. However, due to the high ash and crude fibre content, the seeds would have to be decorticated prior to extraction or the meal processed further to make it suitable as a stock feed.

Table 1. Proximate analysis of the seeds and characterization of the oil of *C. foetidissima*

	Seed	Oil
Protein % (F = 6.25)	29.5	
Oil %	28.5	
Fibre %	26.7	
Ash %	3.0	
Refractive Index at 25°C		1.471
Iodine value		144
Saponification value		201
Free fatty acids, as percent oleic		2.39
Insoluble acids, Hehner number		36.25
Soluble acids (%)		16.42
Unsaponifiable matter (%)		0.94

As for the chemical analysis of the oil, it showed a relatively high iodine value (144) thus reflecting a high proportion of unsaturated fatty acid glycerides, a low acid value expressed as percent oleic acid indicating that no particular problem would be encountered upon refining in order to get a bland oil. The saponification value (201) on the other hand indicated a mean molecular weight of the glycerides comprising the oil of 837 g.

Fatty acid composition

Gas liquid chromatographic analysis of the long chain fatty acids of the *C. foetidissima* seed oil is presented in Table 2 which compares the composition of

Table 2. Fatty acid methyl esters composition of old and fresh Buffalo gourd oil expressed as percent of the total fatty acid methyl esters

Type of oil	Myristic C_{14}	Palmitic C_{16}	Stearic C_{18}	Oleic $C_{18:1}$	Linoleic $C_{18:1}$	Linolenic $C_{18:3}$
Fresh BGO	T[a]	9.3	2.1	25.0	63.6	T
Old BGO	T	8.2	2.6	22.7	66.5	T

[a]T = Traces

the oil extracted from fresh seeds with that extracted from the old stock of seeds.

The present composition of palmitic, stearic, oleic, and linoleic acids of the oils extracted from fresh and old seeds were quite similar thus indicating the stability of the oil when whole seeds are stored. The presence of high levels of linoleic acid indicates that the BG seeds are a valuable source of edible oil since linoleic acid is the precursor of arachidonic acid, and both fatty acids are essential for the human body. Actually the *C. foetidissima* oil ranks second in its linoleic acid content behind safflower oil (75.0% linoleic acid) and comes before corn oil and cotton seed oil (54.0% and 40.0% linoleic, respectively). The oxidative stability of the oil is expected to be satisfactory since only trace amounts of linolenic acid are present. This acid is usually prone to quick oxidation which leads to flavour reversion and polymerization. An additional advantage is the relatively low oleic acid content (25.0%) which was considered by Lowry & Tinsley (1966) to have the inhibitory effect on the conversion of linoleic to arachidonic acid.

Table 3. Short chain fatty acids of fresh and old Buffalo gourd oil expressed as mg per 5 g oil

Type of oil	Butyric C_4	Caproic C_6	Heptanoic C_7	Caprylic C_8
Fresh BGO	T[a]	0.078	0.017	T
Old BGO	T	0.11	0.021	T

[a]T = Traces

The chromatographic analysis of the volatile free fatty acids revealed the presence of caproic (C6:0) and heptanoic acids in measurable amounts. Caprylic and butyric acids were detected in trace amounts only, a reason for which caprylic acid was used as an internal standard. The different values are presented in Table 3, and the corresponding chromatogram is shown in Fig. 1. The oil samples also contained several unidentified volatile compounds that were eluted at a temperature of 60°C with very short retention time. Such components were not investigated further, and the values for the Hehner number and soluble acids calculated from the chromatographic data remained different from those obtained experimentally and shown in Table 1. No clear explanation could be found for this inconsistency. The appearance of new peaks in the chromatogram of the BG oil extracted from the old stock of seeds revealed the possible formation of new components as products of an oxidation taking place in these seeds.

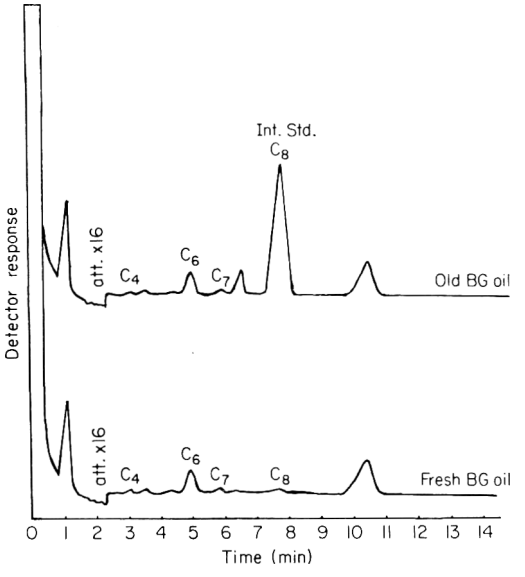

Figure 1. Gas liquid chromatograms of volatile fatty acids found in fresh and old BG oil (*C. foetidissima*). The number on each peak of the top tracing represents the chain length. The identification was by comparison of the retention time to authentic standards and internal standard.

Feeding experiment

The feeding experiment was carried out to investigate the presence of toxic factors in the oil that may interfere with its edibility. Meal of Buffalo gourd after oil extraction has been shown to contain toxic compounds (Baghdadi, 1975; Daghir, Mahmoud & El-Zein, 1980) and alkaloids were identified to be present in the seeds of other species of the Cucurbitaceae family (Watt & Breyer-Brandwijk, 1962). Data on the composition of the diets used in the feeding experiment are shown in Table 4, and on the average body weight, feed efficiency (feed/gain) and feed consumption per chick are shown in Table 5.

Table 4. Composition of the diets used in the feeding experiment

Ingredient (%)	Treatment 1	Treatment 2	Treatment 3	Treatment 4
Commercial feed	100	85	85	85
Casein	—	5	5	5
Purified corn oil	—	10	—	—
Purified corn oil treated with hexane	—	—	10	—
Buffalo gourd oil treated with hexane	—	—	—	10

Table 5. Average body weight, average feed consumption and average feed conversion of chicks maintained on diets of different oil content

	Average body weight (g)				Average feed consumption (g/chick/week)			Average feed conversion feed/gain		
Treatment	Initial	1st wk	2nd wk	3rd wk	1st wk	2nd wk	3rd wk	1st wk	2nd wk	3rd wk
I.	69±0.4*	104±0.2	221±0.6^{a}†	405±3.8^{A}	43.5±1.13	190.7±2.21	320.2±1.39	1.26±0.03^{A}	1.63±0.02^{A}	1.74±0.04^{A}
II.	77±2.4	116±3.8	275±4.9^{ab}	487±9.9^{B}	80.5±0.82	205.1±9.54	313.8±8.02	2.08±0.08^{B}	1.29±0.02^{B}	1.48±0.005^{B}
III.	76±1.5	116±2.6	278±6.6^{b}	507±3.9^{BC}	82.1±9.03	210.6±5.0	329.8±9.26	2.06±0.02^{B}	1.30±0.005^{B}	1.44±0.06^{BC}
IV.	80±1.8	121±4.2	278±6.8^{b}	522±4.9^{C}	84.9±4.63	211.9±2.12	339±6.19	2.08±0.04^{B}	1.35±0.06^{B}	1.39±0.025^{C}

*Means ±s.e. mean.

†Figures followed by capital letters are significantly different at 1% level – otherwise smaller letters indicate significance at 5% level of probability.

Statistical analysis of the average feed conversion data showed that treatments II, III and IV receiving diets in which 10% corn oil or Buffalo gourd oil were incorporated behaved similarly during the first and second weeks, but were significantly different at 1% level from treatment I. Additional third-week results showed that the chicks fed BG oil had a similar performance for the average body weight and the average feed conversion as those receiving 10% corn oil. Such results give positive indications about the edibility of BGO. Furthermore, they may lend support to the comparison of the nutritional value of BGO with that of corn oil and diminish the possibility of the presence of toxic factors.

Conclusion

Work so far carried out on the characterization of the BGO showed that it is rich in linoleic acid (63%) and has a high unsaturated to saturated fatty acids ratio. Heating of the oil did not show any significant decrease in the unsaturated fatty acids thus showing that BGO is relatively stable mainly due to absence of linolenic acid which is most prone to oxidation, and possibly causing flavour reversion. Feeding trials as well have shown that apparently BGO does not contain toxic substances interfering with the growth rate of chicks. So from these results, BGO seems to be an edible oil comparable to other currently used vegetable oils and having satisfactory nutritional value. Additional investigation should be carried out on the adaptation of present extraction and refining technology to large scale production of this oil.

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Simulation of moisture movement during drying a starchy food product—cassava

J. C. IGBEKA

Summary

A mathematical model of the moisture transfer in cassava during drying was developed. The model took into account the dependence of moisture diffusivity on moisture content and temperature and was therefore not made a constant. The solution of the model was achieved by an IBM 360 digital computer which utilized the statement oriented 360 CSMP (Continuous Systems Modelling Program). The predicted moisture profiles were compared with experimental data. The correlation coefficients between the predicted and experimental values ranged between 0.97 and 0.99. The mechanism of moisture transfer was analysed.

Introduction

Air drying of solids involves vaporization of water contained by the solid, and removal of the vapour in a stream of air. The heat requirement is large because of the latent heat of vaporization. The phenomenon is one of diffusion and mechanical mixing, diffusional resistance being mainly in an air 'film' just above the liquid surface, (Sherwood, 1936). This diffusional resistance is the sum of the resistance of a surface laminar layer through which moisture is transferred by molecular diffusion and the resistance of an eddy layer in which the transfer is by eddy motion or mixing. The above is what goes on at the surface. But within the product, water molecules are released by diffusion to levels of lower water concentration. Many researchers have solved the diffusion equation, assuming a constant diffusion coefficient. It has been shown (Igbeka *et al.*, 1976) that the diffusion coefficient for many food products is not constant but is a function of concentration. Other investigators, Hougen *et al.* (1940), Van Arsdel (1947), also noted that the diffusion coefficient was a function of concentration but did not try to obtain a solution of the diffusion equation under these conditions.

Author's address: Department of Agricultural Engineering, University of Ibadan, Ibadan, Nigeria

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This study takes the variability of the diffusion coefficient into account. A mathematical model was developed for predicting the moisture movement during the drying of cassava. Experimental dryings were performed at different conditions and the moisture profiles determined. These were compared with the predicted profiles. In the analysis, other modes of moisture transfer were considered. These were compared with the experimental results.

Review of the literature

Crank (1975) derived the fundamental equation of diffusion in an isotropic medium from Fick's first law.

$$\frac{\partial c}{\partial t} = \frac{\partial}{\partial x} D\frac{\partial c}{\partial x} + \frac{\partial}{\partial y} D\frac{\partial c}{\partial y} + \frac{\partial}{\partial z} D\frac{\partial c}{\partial z} \tag{1}$$

where c = concentration
t = time
x, y and z = co-ordinates

Sherwood (1936) noted that in certain slow-drying solids no constant rate period appeared, and internal liquid diffusion controlled from the start. He therefore described the relation between drying time, t, and average moisture content, M, for an infinite slab by solving the diffusion equation (1) for the boundary conditions of a step change in environmental moisture from initial to equilibrium, and a uniform controlled moisture ratio. The solution was:

$$\frac{M-M_e}{M_o-M_e} = \frac{8}{\pi^2} \sum_{n=1}^{\infty} \frac{1}{(2n-1)^2} \exp\left[-(\pi/2)^2 \frac{Kt}{(2n-1)^2 L^2}\right] \tag{2}$$

where M = Moisture at any time, M_e = Equilibrium moisture
M_o = Initial moisture, K = liquid diffusivity
L = half the slab thickness
t = time elapsed.

Many different mathematical equations have been used by researchers (Lewis, 1921; Henderson & Perry, 1961) to describe moisture movement from a fully exposed object. Their models assumed that all the resistance to moisture flow was concentrated in a layer at the surface of the material.

On the other hand, if it is assumed that the resistance to moisture flow is uniformly distributed throughout the interior of the material, then the fundamental diffusion equation would be an appropriate model. Wang & Hall (1961) stated that if the temperature distribution within the medium is uniform, the diffusion equation with concentration as the driving force is adequate in describing moisture movement from the medium. Van Arsdel (1947), suggested that the potential could be either concentration or vapour pressure difference and

the appropriate choice for expressing the diffusion equation is determined by the experimental procedure. Hamdy & Johnson (1968) used the method of finite difference to solve the unidirectional diffusion equation on a Pace RT-48 analog computer for a coefficient that was an arbitrary function of both position and concentration. Igbeka *et al.* (1976) found that in drying cassava and potato, no constant rate period was present. The drying proceeded in the falling rate period. Also they investigated a method of measuring variable moisture diffusivity experimentally. In a later work, Igbeka, (1976) presented a mathematical representation of variable diffusivity of moisture during the drying of cassava and potato.

Often the data for diffusion of gases in solids are not given as diffusivities but as permeabilities *Pm*, in cm^3 of gas at NTP (0°C and 1 atm pressure) diffusing per second per cm^2 cross-sectional area through a solid, 1.0 mm thick, under a pressure difference of 100 mm Hg. Cassava being a porous solid with capillary pores could exhibit capillary flow. The process can then be defined by Poiseuille's law. The mechanism of vapour diffusion is similar to liquid diffusion; the two could be described by the same equation. The main difference is in the temperature dependence of diffusion coefficient, *D* (Geankoplis, 1972).

Mathematical modelling of moisture transfer in a slab

If moisture diffused as a vapour or liquid, the diffusion equation would be represented as

$$\frac{dM}{dt} = \frac{d}{dx}\left(Dm\,\frac{dM}{dx}\right) \tag{3}$$

where $dM = f(M)$
the boundary conditions would be

$M = Me \quad X = 0 \quad t > 0$
$M = Mo \quad x > 0 \quad t = 0$
$M = Mo \quad x = L \quad t > 0$

The model assumed an isothermal condition within the product. The product initial moisture concentration was assumed uniform and also the moisture content at the bottom was equal to the initial moisture content at all times. It was further assumed that there was no film layer just above the surface of the product to impede moisture flow from the product surface, so internal resistance was controlling. Because the above model could not be solved by any classical method, an IBM 360 digital computer which utilized the statement oriented 360 CSMP (Continuous System Modelling Program) was used. This system with its limitation had only one independent variable, time, and could not solve problems with two independent variables like this model. The space co-ordinate

was eliminated as a variable (Hamdy & Barre, 1969), by dividing the slab into ten equal slices and assuming that the moisture diffusivity in each slice was constant.

The nodes started from the bottom to the top (Fig. 1), for the purpose of this computer analysis. This was to conform with the direction of moisture movement and eliminate sign problems. Also non-dimensional moisture, C, and thickness, δ were used. They were defined as follows:

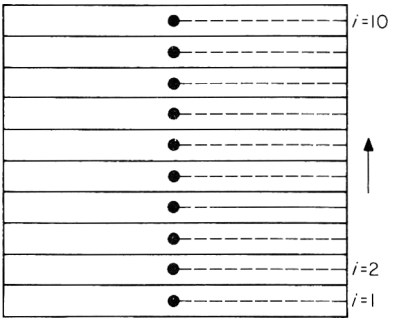

Figure 1. Nodes of the ten in sample.

$$C = \frac{M-Me}{Mo-Me} \text{ and } \delta = \frac{x}{L} \tag{4}$$

the sets of modelling equations used were the same as those derived by Agrawal *et al.* (1975) for bologna slab with a modification in the diffusivity, *Dm*. The final equations were:

$$C_1 = \frac{100}{L} \int_0^T D_{m2}(C_2 - C_1)\, dt \tag{5}$$

$$C_i(2\text{–}9) = \frac{100}{L^2} \int_0^T Dmi(C_i + 1 - 2C_i + C_i - 1)\, dt \tag{6}$$

$$C_{10} = \frac{400}{3L^2} \int_0^T D_{m10}(-3C_{10} - C_a)\, dt \tag{7}$$

where T is total time.

Dm is a function of both moisture content and temperature. Three temperature levels were used: 55, 65 and 75°C. The mathematical relationships between *Dm* and moisture content, temperature as suggested by Igbeka (1976), for cassava

and potato were used in the program. The general equation for cassava was

$$D = -0.0274 - 5.74 \times 10^{-6}M + 5.98 \times 10^{-6}e^{M} + 0.0275_{e} - 1/T + 2.23 \times 10^{-6}XR \quad (8)$$

where M is moisture content
T is absolute temperature
XR is the relative humidity indicator
It is 0 if r.h. = 60% and 1 if r.h. = 10%
The program was made to print and at the same time plot the concentration or moisture versus time at each node by using the Computer language *PRTPLT*. Also the diffusivity D_m was plotted against the concentration.

Experimental

To ascertain the validity of the model, an experimental drying was carried out under the conditions stipulated in the model. A wind tunnel or an experimental dryer developed and built in the Agricultural Engineering Department of Ohio State University, Columbus, was used for the drying experiments. It comprised a fan, a heating section, steam injection tower, drying column and a test section. The fan blew air through the heating section, which contained three 3 KVA heating elements. The heaters could be used separately or together. Temperatures (drybulb and wetbulb) were controlled with thermostatic recorder controllers to within 1.5°C. The humidity of the drying air could be varied by injecting steam into the dryer. Flow straighteners were inserted at the bends to reduce transverse flow, and flow mixers in the form of screens ensured a uniform distribution of air velocity across the test section. The dryer was usable with continuous recirculation, direct flow-through or proportions of the two by means of shutters at appropriate places. Cassava slabs, 5.8 cm in diameter and 1.0 cm thick, were used. The sample was placed on a solid metal shelf before placing it in the drying chamber. Air of known relative humidity and temperature and at a considerable high velocity (2.26 m/sec) was blown past the surface of the sample to effect moisture removal from the surface. The high air velocity was used to minimize the effect of film resistance at the surface (Whitaker *et al.*, 1969); therefore the film resistance was assumed to be zero. The sides of the sample were coated with a special high vacuum grease to eliminate moisture loss from the sides and assure that the moisture loss was purely unidirectional from the upper surface of the sample. At the end of the drying experiment, a core was removed from the centre and cut into slices of approximately 0.1 cm thickness using a microtome. The moisture content of the slices was determined by drying in an air oven. An identical sample of the same size and weight placed also in the test section was used to monitor the temperatures at three places (0.2, 0.5, 0.8 cm from the surface). This was to verify the assumption that there was no significant temperature gradient within the product during the experiment. The surface temperature was also measured

during the drying period for use in the analysis of moisture movement mechanism. Experiments were conducted at 55°, 65° and 75°C at 10% and 60% r.h. at eight different times. Two replications of each experiment were made. Tables 1 and 2 show data of two such experiments.

Results and discussion

During the experiment the temperature gradient within the product at any time was negligible (0.3 to 0.5°C). The reduction in thickness (longitudinal shrinkage) was insignificant (2%) and therefore did not affect the data analysis.

The moisture contents as determined experimentally are given in Tables 1 and 2. The Tables show that the moisture content at the bottom was the initial

Table 1. Experimental moisture profiles of cassava at 10%RH

Xcm	55°C, M_0 = 2.14 g/g M, g/g d.b.		65°C, M_0 = 1.86 g/g M, g/g d.b.		75°C, M_0 = 2.14 g/g M, g/g d.b.	
	Rep No. 1	Rep No. 2	Rep No. 1	Rep No. 2	Rep No. 1	Rep No. 2
0.05	0.81	0.79	0.38	0.37	0.55	0.56
0.15	1.24	1.25	0.99	0.99	1.02	0.99
0.25	1.50	1.50	1.28	1.28	1.40	1.42
0.35	1.68	1.70	1.46	1.44	1.67	1.69
0.45	1.28	1.81	1.59	1.60	1.84	1.82
0.55	1.93	1.94	1.69	1.67	1.94	1.95
0.65	2.01	2.00	1.74	1.76	2.06	2.07
0.75	2.08	2.06	1.80	1.83	2.11	2.11
0.85	2.12	2.14	1.85	1.86	2.14	2.12
0.95	2.14	2.14	1.86	1.86	2.14	2.14

Table 2. Experimental moisture profiles of cassava at 60% r.h.

Xcm	55°C, M_0 = 1.71 g/g M, g/g d.b.		65°C, M_0 = 1.67 g/g M, g/g d.b.		75°C, M_0 = 2.14 g/g M, g/g d.b.	
	Rep No. 1	Rep No. 2	Rep No. 1	Rep No. 2	Rep No. 1	Rep No. 2
0.05	0.76	0.74	0.78	0.75	0.41	0.41
0.15	1.10	1.09	1.06	1.08	0.83	0.80
0.25	1.29	1.28	1.22	1.20	1.16	1.16
0.35	1.42	1.43	1.31	1.30	1.44	1.43
0.45	1.52	1.52	1.37	1.39	1.65	1.66
0.55	1.61	1.63	1.40	1.43	1.84	1.84
0.65	1.66	1.65	1.46	1.45	1.96	1.93
0.75	1.69	1.69	1.58	1.45	2.06	2.06
0.85	1.71	1.70	1.62	1.66	2.13	2.14
0.95	1.71	1.71	1.67	1.67	2.14	2.14

moisture content, thereby satisfying the third boundary condition of the model. To determine the validity of the mathematical model adopted in this study, the results of the simulation were compared with the results of the experimental drying tests.

A statistical analysis showed that the probability that the actual measurements of moisture profiles matched the simulated values ranged between 0.975 and 0.998 as determined by the χ^2 test of goodness of fit. The mean value of the errors between simulated and measured moisture content was 2.64% (0.0264 g/g) dry basis, with a standard deviation of 10.44% (0.1044 g/g) dry basis. A linear regression applied between measured and simulated moisture contents gave a correlation coefficient of 0.978. Figures 2 and 3 show the graphical comparison between the simulated and experimental moisture profiles. In general, the simulated and experimental results show acceptable agreement.

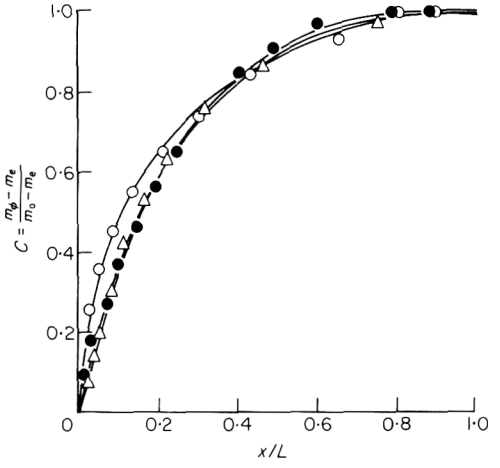

Figure 2. Comparison of experimental and predicted moisture profiles at 10% r.h. O, 55°C; △, 65°C; ●, 75°C; ———, fitted.

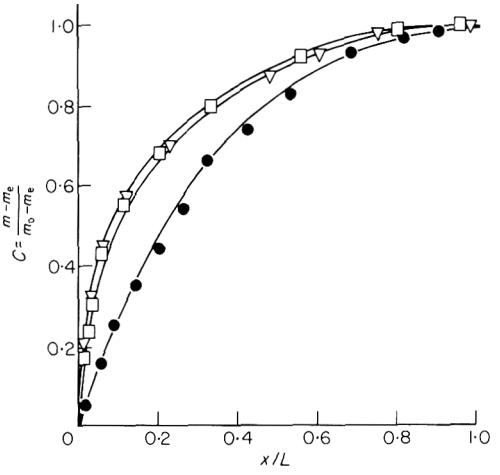

Figure 3. Comparison of experimental and predicted moisture profiles at 60% r.h. □, 55°C; △, 65°C; ●, 75°C; ———, fitted.

Mechanism of moisture transfer

The validity of the model is evidence that the controlling mechanism of moisture transfer was liquid diffusion. Figure 4 shows the result of the temperature history at the surface of the product. It showed that the surface temperature rose continuously and approached the dry bulb temperature. This allowed the deduction that there was no constant drying period, since the surface temperature was never constant. This confirmed the findings of Igbeka *et al.* (1976). From the above, it was concluded that the internal water movement was the controlling mechanism from the beginning of the drying process.

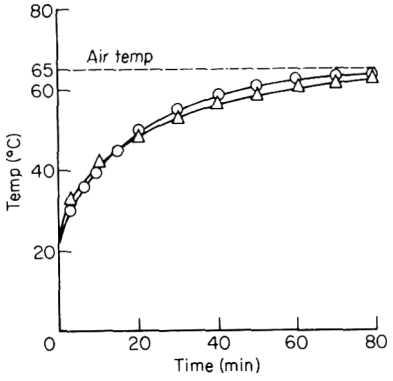

Figure 4. Typical surface temperature of sample at 65°C air temperature O, 10% r.h.; △, 60% r.h.

It might be possible that mechanisms other than liquid diffusion could be responsible for moisture movement. These mechanisms were analysed to see how well they fitted the experimental results.

Capillary flow

The flow of gases in a capillarity system is similar to that encountered in diffusion through a solid and could be characterized by a permeability coefficient, *Pm*. According to Poiseuille's law, in a capillary of radius *r* and length *L*,

$$N_A = \frac{r^2 g_c \text{ pave}}{8\mu^{LR} g^T} (P_1 - P_2) \tag{9}$$

Where N_A = rate of flow or flux, g/(sec)$^{cm^2}$
P_1, P_2 = pressure, dynes/cm^2
Rg = Universal gas constant
T = Absolute temperature, °K
μ = 1 Viscosity, g/cm (sec)
pave = $(P_1+P_2)/2$

If the rate of flow is measured in terms of gas or liquid volume, V, at the average pressure flowing per unit time per unit cross-section of the solid, then equation (9) becomes

$$\text{pave } V = \frac{Pm \text{ pave } (p_1 - p_2)}{Z} \tag{10}$$

where V is given as $N_A RT/\text{pave}$
Z = solid thickness
Pm = permeability, which has the same units as diffusion coefficient.
Equation (9) shows that Pm, varies inversely as the gas viscosity, which in turn increases with temperature. Then Pm, varies with $1/T$ and not with $\exp(-1/T)$ as found experimentally. Therefore, the possibility of Poiseuille flow in cassava must be excluded.

Vapour diffusion

From kinetic theory of gaseous diffusion, the relationship between diffusion coefficient, D, the mean free path, l and the mean velocity $\bar{u}$ is given as

$$D = 1/3\bar{u}l \tag{11}$$

But $\bar{u}$ is proportional to $\sqrt{T}$ (Geankoplis, 1972) hence the temperature dependence of D will be $T^{3/2}$. This is again different from the experimental data.

Knudsen flow

If the mean free path, l of the gas molecules is very large compared to the pore diameter d, in cassava, then the flow will be Knudsen type, (Geankoplis, 1972). The Knudsen diffusivity, D_k is independent of pressure and is given as

$$D_k = 9.7(10^3)\bar{r}\sqrt{\frac{T°K}{M_w}} \tag{12}$$

where r is the radius, in cm, of the pore
M_w is the molecular weight, g mass/g mole
From the above equation, D_k, varies with $\sqrt{T}$, which is not the temperature dependence found experimentally.

On the basis of the above discussions and analysis, it is concluded that liquid water diffusion is the most appropriate mechanism to describe the drying behaviour of cassava.

With the mechanism of moisture movement established, it will be possible to predict the drying time of cassava, yam or starchy tuber food crops. It may be possible to apply the results of this research in the reverse process of sorption during storage, when fully exposed from one side.

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Moisture isotherms of a processed meat product—Bologna

J. C. IGBEKA* AND J. L. BLAISDELL

Summary

A study was carried out to obtain moisture isotherms of an intermediate-moisture processed meat product at a refrigerated and three ambient temperatures. Results showed that the isotherms were sigmoidal in shape. The Henderson equation was applied and analysed. It was found that the storage stability of the product is maintained at 5°C. Heats of desorption were presented.

Introduction

The equilibrium relative humidity or water activity has been used as a basis to understand microbial growth (Labuza, Tannenbaum & Karel, 1970; Rockland, 1969; Macara, 1943) and to determine the extent of enzymic reaction in food products. Some researchers (Rockland, 1969; Stitt, 1958) define the equilibrium relative humidity as the water available for enzymic reactions, while others (Labuza, Tannenbaum & Karel, 1970; Macara, 1943) assert that the relationship between relative humidity and moisture is that contributed by water in its several states. This second definition provides a basis for delimiting the regions of activity of particular spoilage mechanisms.

In this study, the product used was in the fresh condition at an average initial moisture content of 1.2 dry basis. It is therefore possible that at high relative humidity levels, the product will sorb moisture and at low relative humidity levels, moisture will be desorbed. This is likely to introduce some error in the isotherm plots due to hysteresis effect. Hysteresis exists for some biological products as is documented by several researchers. This effect is neglected in this study, and moisture isotherm data were confined to the desorption process.

Authors' address: Department of Agricultural Engineering, Ohio State University, Columbus, Ohio, U.S.A.

*Present address and to whom correspondence should be directed: Department of Agricultural Engineering, University of Ibadan, Ibadan, Nigeria.

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This study was carried out primarily to obtain moisture isotherms of intermediate-moisture processed meat product—bologna, at a refrigerated and three ambient temperatures. From the moisture isotherms, the heat of desorption or sorption was determined. This will be useful in designing drying processes and analyzing storage processes of the product. Also the pore-size distribution was calculated.

Previous work

Much work has been done on the equilibrium relative humidity of cereals and vegetables. Henderson (1952) developed an empirical equation to represent conventional equilibrium moisture data. The isotherm equation derived by Day & Nelson (1963) was a modification of Henderson's. Chung & Pfost (1966) obtained heat and free energy changes of absorption and desorption of corn hull and its products (corn gluten, corn germ and corn starch). They also developed a general isotherm equation by adopting the potential theory. Rockland (1957) presented a new treatment of hygroscopic equilibria. He introduced the concept of local isotherms, which he said represented the specific type of water binding. Igbeka, *et al.* (1975) presented data for equilibrium moisture content of cassava and white potato. They obtained the heats of desorption and the pore size distribution of both products. Also, they reported an isotherm equation based on Rockland's approach.

The equilibrium moisture data for air-dried, pre-cooked beef and pork was reported by Gane (1943). Macara (1943) investigated the effect of different environmental relative humidity on the growth of moulds on dried beef. Hamm (1965) found that sorption and desorption studies on freeze-dried excised muscle tissue or protein extracts could provide vapour pressure data, from which the enthalpy of vapourization at a particular moisture content could be inferred. These data, he noted, could provide clues to the extent of water binding with the protein.

Little information is available on the equilibrium moisture content for individual proteins or muscle tissue at intermediate to high moisture. This might be due to the resultant rapid microbial activity when low moisture products are placed in an environment with a water activity greater than 0.07.

Gur-Arieh *et al.* (1965a, b) reviewed available methods for determining equilibrium moisture content. From an experimental point of view, they used a pressure membrane cell for determining the water activity of flour. Rockland (1957) reported increasing the equilibration rate by 10 to 30 times by using a magnetically driven fan to recirculate air within a closed chamber. Wink (1946) utilized a constant relative humidity desiccator. This method is subject to errors because the product has to be removed from the desiccator each time to be weighed. Bosin & Easthouse (1970) developed a rapid means, simple in operation and design, for determining the equilibrium relative humidity of various materials. This is similar to the technique employed by Rockland (1957).

Experimental

The method of Bosin and Easthouse, with minor modifications, was used in this study for the low and medium moisture levels. The apparatus (Fig. 1) consisted essentially of a magnetic stirrer unit, equilibration chamber(s) in a constant temperature bath and a sensitive weighing balance. The same apparatus was used by Igbeka *et al.* (1975) to determine equilibrium moisture content of cassava and potato.

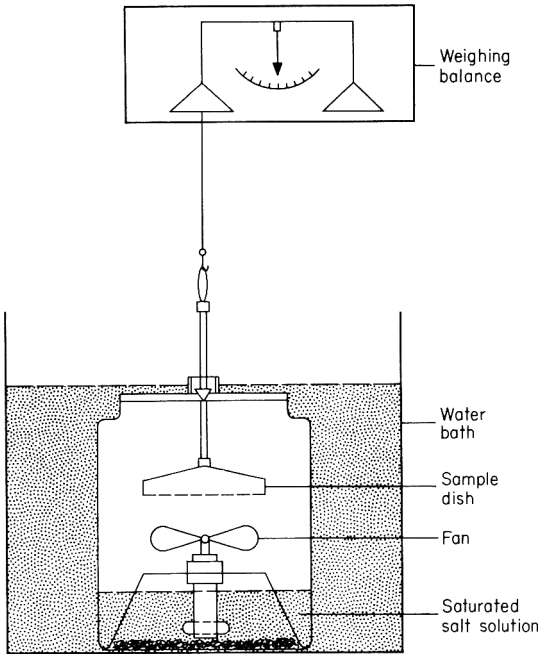

Figure 1. Diagram of the apparatus.

In each equilibration chamber is a stainless steel tripod which supports a magnetically driven aluminium fan. The shaft to which the fan and the teflon-coated spinbar magnet are attached passes through a teflon bushing. The sample dish is suspended above the fan.

A Voland 2200 model weighing balance, of the knife edge suspension type, with two loading pans and chain-slider mechanism, was modified to allow under-pan weighing.

Core samples of meat, 1.3–2.0 mm in thickness and 25 mm in diameter, were placed in the sample dishes with wire mesh bottoms and weighed. The stirrer was started to provide rotation to the fan and agitation to the salt solution. Each sample was weighed at intervals, the frequency of weighing being short initially, and longer as the product approached a constant weight. The fan did not produce any significant buoyancy effect on the specimen pan during weighing. Therefore, the fan was not turned off during weighing. It was assumed

to reach its equilibrium moisture content when there was no significant change in weight, that is, when the change in weight per 4 hr was less than 0.001 g. The equilibration time ranged from 18 to 48 hr. Each sample was then dried in a vacuum oven for 8 hr to determine the bone-dry weight, from which the moisture content at any time, including the equilibrium moisture could be calculated. The experiments were performed at 5°C (refrigeration), 21°C, 38°C (ambient) and 55°C (processing) and at relative humidities of 11, 28, 32, 43, 61, 75 and 80%. Two replications of each determination were made.

At higher moisture levels, the Beckman hygrometer probe was used. This is a self-balancing measuring bridge which is coupled to a sensor for measuring humidity. The sensor is arranged in such a way that there is a very thin air layer between the product and the sensor element. The product conditions this air layer and consequently the sensor element; that is, the water vapour pressure of the product either moistens or dries the air layer, and, in turn, the element, until all three are in equilibrium.

Samples were pre-dried at 21°C in a constant low relative humidity chamber, placed in plastic bags and refrigerated at 4°C for 48 hr to ensure a uniform moisture distribution. They were equilibrated with the calibrated Beckman humidity probe at a known temperature, and weighed. The bone-dry weights were determined by drying in a vacuum oven and the moisture contents calculated. Equilibration time ranged from 10 min to 1.5 hr. Two replications of each run were made and experiments were performed at 5, 21, 38 and 55°C.

Test materials

The meat product used in this research was all-meat bologna with smoke flavouring added, produced by P. Eckrich and Sons (U.S.A.) The composition was beef, pork, water, dextrose, cornsyrup, salt, sodium ascorbate, flavouring and sodium nitrate. The average initial moisture content was 1.2 dry basis.

The following salts were used to attain the necessary relative humidities: lithium chloride (11%), potassium acetate (22%), magnesium chloride (32%), potassium carbonate (43%), sodium nitrite (61%), sodium chloride (75%), ammonium sulphate (80%), potassium nitrate (88%) and potassium sulphate (96%). The relative humidities are values at 21°C (Hall, 1957).

Results and discussion

The set of moisture isotherms are presented in Fig. 2. They are sigmoidal in shape and can be said to be of the Type 2 (Rockland, 1969). The equilibrium moisture contents at low relative humidities (11–43%) are seen to be almost constant, resulting in an almost horizontal line. But the slope of the line at this range increases with decrease in temperature.

The behaviour of the isotherms in this relative humidity range can be interpreted to mean that the product is most stable in this range. An increase in

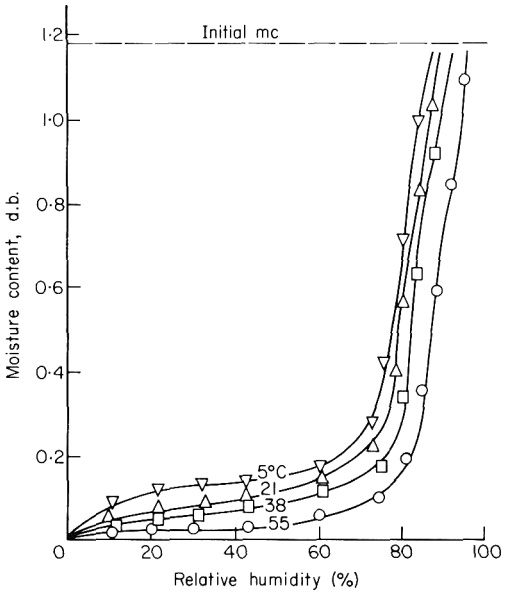

Figure 2. Desorption isotherms of Bologna meat.

the environmental relative humidity in this range will only have a slight effect on the stability of the product.

There is a consistent shift to the right due to increase in temperature at a given moisture content. This shift plays an important role in stability of stored food at different temperatures and a given relative humidity. At all temperatures, the storage stability of the product becomes critical at relative humidities above 70%.

The isotherms were analyzed using the Henderson equation to establish a general equation relating moisture content and equilibrium relative humidity, which might be used to predict and isotherm.

$$1 - a = \exp(-kM^n) \tag{1}$$

where a = relative humidity in decimal
k = constant depending on material
n = constant
M = moisture content as percentage dry basis.

This equation can be written as:

$$\log[-\ln(1-a)] = n \log M + \log k \tag{2}$$

Figure 3 shows a plot of $\log[-\ln(1-a)]$ versus log m at all temperatures. As was expected, more than one straight line could be fitted. This was in agreement with the hypothesis of Rockland (1957). Two straight lines were fitted. These lines intersected at moisture contents of 20.9% and 10% at 5 and 55°C, respectively. The constants n_1, n_2, and k_1, k_2 are listed in Table 1.

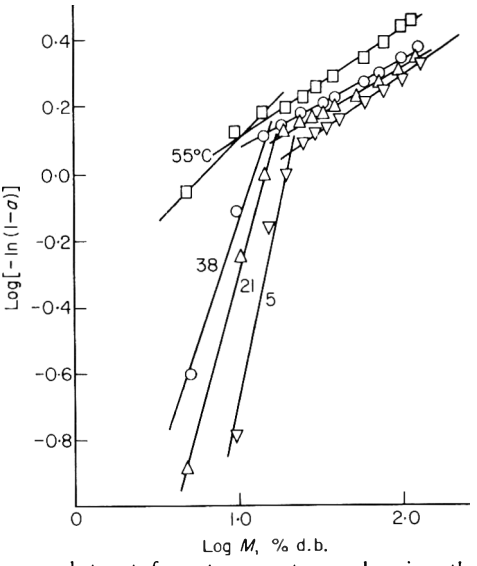

Figure 3. Henderson plots at four temperatures showing the relation between moisture content, M, and water activity, a_W

Table 1. Constants n and k in Henderson plots

Temp. (°C)	n_1	k_1	n_2	k_2	mc break	of in db	p_1*	p_2*
5	2.25	0.001	0.35	0.395	0.2	4	0.985	0.993
21	1.89	0.006	0.28	0.565	0.1	7	0.995	0.974
38	1.51	0.023	0.29	0.586	0.1	5	0.997	0.980
55	0.52	0.383	0.34	0.561	0.1	0	0.985	0.995

*p_1, p_2, are correlation coefficients.

The figures show a significant shift to the left with increase in temperature. The moisture content at which the breaks occur decreases with increase in temperature. This shows that moisture binding is a function of temperature. The break indicates a change in type of water binding. This break occurred at a relative humidity of about 72% at both temperatures of 5 and 55°C.

Above this relative humidity the isotherms increase in the same trend. This is indicated in the slope of the second part of the Henderson plots; the slopes are almost the same (2.97 and 2.86) at the two temperatures, 5 and 55°C, respectively.

Most gram-negative rods (Salmonella, Pseudomonas, etc.) and some yeasts are inhibited at a water activity (a_w) of 0.95 but for the inhibition of most gram-positive bacteria like Bacilli, Lactobacilli and Clostridia an a_w of 0.91 is required. *Staphylococcus aureus* is an exception and will grow down to an a_w in the range of 0.86–0.83. This means that the shelf life will be maintained at 5°C but will deteriorate at 55°C if stored at a moisture content of 1.2 g/g d.b.

The heat of desorption or sorption, Q_s, was determined by using the Clausius–Clapeyron equation. This equation gives the effect of temperature on

vapour pressure ratio or activity. The differential form of the equation is given as:

$$\frac{d \log a}{d\, 1/T} = \frac{-Q_s}{2.303R} \tag{3}$$

where Q_s = heat of sorption or desorption
R = gas constant
T = temperature in absolute.

A semi-log plot of a, versus $1/T$ (Fig. 4) at different moisture levels produced a linear relation. This is in agreement with the Claudius–Clapeyron relationship. The slope of each line $\frac{Q_s}{2.303R}$ was found by linear regression and the heat of desorption calculated. Figure 5 shows a plot of the heat of desorption versus moisture content. The heat of desorption decreases with increase in moisture content and approaches a constant value. This is in agreement with the findings for cassava and potato (Igbeka, *et al.*, 1975), and for corn and corn products (Chung & Pfost, 1966).

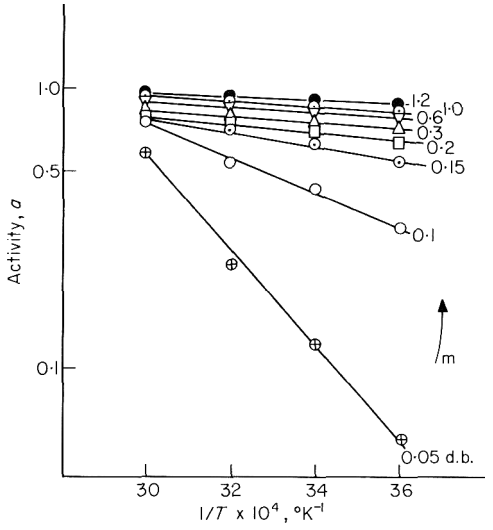

Figure 4. Semi-log plot of a versus $1/T$ at different moisture contents.

Figure 5 shows that quite a substantial amount of energy must be supplied to desorb the last traces of water from bologna. This energy decreases rapidly until a moisture content of 0.15 g/g d.b. An almost constant energy input (10.33 cal/mol) equivalent to that required to evaporate pure water is needed to desorb moisture from bologna beyond moisture content of 0.4 g/g d.b.

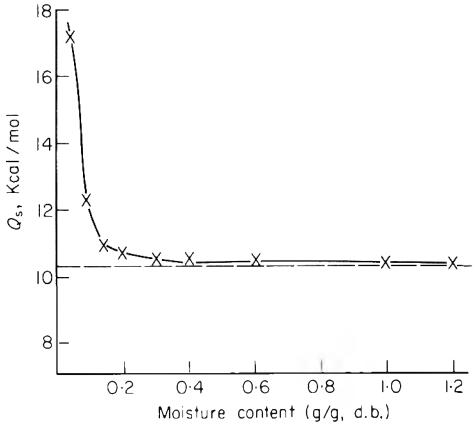

Figure 5. Plot of heat of desorption *v.* moisture content.

The average pore size at any given moisture was determined by combining the Kelvin & Halsey (1948) equations. The Kelvin equation is:

$$r_K = \frac{2\,\sigma\,\overline{V}}{R_g T \ln a} \tag{4}$$

where r_k = Kelvin radius
σ = surface tension
$\overline{V}$ = molal volume of sorbate in its bulk liquid condition
R_g = universal gas constant
T = absolute temperature.

This equation applies primarily in the condensation region of the isotherm. The Halsey equation is:

$$t(A) = 32\,\frac{1.75^{1/3}}{\ln a} \tag{5}$$

where t = multilayer adsorption in angstrŏms.

The effective average pore size, R, is therefore:

$$R = r_K + t \tag{6}$$

Table 2 shows the average pore size distribution at 5 and 55°C. The data indicates that the pore radius increases with increases in moisture content and temperature.

Table 2. Calculated average pore-size (in angströms) distribution at different temperatures

	Temperature (°C)			
m (d.b.)	5	21	38	55
0.05	2.6	3.2	3.7	6.6
0.10	3.4	5.2	6.2	9.2
0.15	5.9	7.3	8.9	10.3
0.20	7.6	9.4	9.6	11.0
0.25	8.8	9.8	10.4	11.7
0.30	9.3	10.3	10.9	12.8
0.40	10.4	10.6	11.9	13.9
0.60	11.0	11.6	12.9	16.3
0.80	11.8	13.0	14.4	20.2
1.00	13.0	15.0	16.1	24.5
1.20	15.6	17.9	20.4	27.7

Conclusions

In the light of the above results and discussions, it is concluded that moisture desorption isotherms of this bologna meat mixture are sigmoidal and can be described by the Henderson equation. The heat of sorption decreases with increase in moisture content up to a moisture content of 0.4 g/g d.b., and remains approximately constant with further increase in moisture content. The storage stability of bologna meat will be maintained at 5°C but at 55°C microbial deterioration will be enhanced.

Acknowledgment

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Moisture sorption isotherms of potato slices

G. MAZZA

Summary

Water vapour sorption isotherms of Norchip, Russet Burbank and Norland potatoes were determined by the salt solution technique at 10, 25 and 40°C. The adsorption isotherms of potatoes were of sigmoid shape and were affected by drying method, temperature and sugars addition. The freeze dried products adsorbed more water vapour than the vacuum dried materials. Addition of glucose, sucrose or lactose to the potatoes caused the equilibrium moisture content to decrease in the low and intermediate water activity ranges. The sorption was temperature independent in the a_w range 0.11–0.35 and showed a hysteresis loop at intermediate moisture levels.

Introduction

Although many different potato cultivars are grown commercially in North America each year, only a few cultivars account for most of the production. These differ in time of maturity, yield, appearance, disease resistance and market and processing quality. Processing quality is affected by specific gravity or dry matter content, starch content, sugar content, degree of maturity, size, shape, peeling loss and other characteristics. Fully mature potatoes are highly desirable for drying. Use of mature tubers results in a higher quality processed product, increase in yield of processed product, and increase in food value per unit weight of raw stock, as well as a higher yield of product per acre. Usually, as potatoes become more mature, they increase in dry matter content. This, in turn, affects the factors mentioned above. Optimum maturity can best be obtained by early planting, late harvesting and slow killing of potato vines (Talburt & Smith, 1975. However, planting and harvesting dates are very often determined by the prevailing temperatures in any given area and by the moisture content and other characteristics of the soil. Also, when rapid methods of vine killing are employed there is little or no opportunity for transfer of food

Author's address: Alberta Horticultural Research Center, Brooks, Alberta TOJ 0J0, Canada.

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from tops to tubers. As a result, the tubers are often of lower dry matter and of different chemical composition, usually less starch and higher sugars. There are also important differences between varieties in cell size, cell wall thickness and size distribution patterns of their starch granules (Reeve, 1967; Reeve, Timm & Weaver, 1973). Also, increase in starch granule size occurs during growth while gelatinization temperature of the starch granules decreases (Geddes, Greenwood & Mackenzie, 1965). Thus, even during the same growing season, the potato dehydrator is often processing a raw material which has different physico-chemical characteristics.

Equilibrium moisture content of potatoes, an important characteristic which influences several aspects of drying and storage, has been studied by numerous investigators (Makower & Dehority, 1943; Gane, 1950; Görling, 1958; Taylor, 1961; Saravacos & Stinchfield, 1965). However, no rigorous investigation of the effects of drying method, sugars addition, temperature and cultivar on the equilibrium has yet been reported. In a preceding paper (Mazza, 1980a), the water vapour desorption isotherms of blanched and sulphited Norchip and Norland potato slices were determined for the temperature range $10 \leq T \leq 40°C$ and the water activity, $0.11 \leq a_w \leq 0.87$. An attempt to describe the experimental data using existing theories was also made. In the present work, the moisture adsorption isotherms of freeze dried and vacuum dried sliced Norchip, Russet Burbank and Norland potatoes, were determined over a water activity range from 0.11 to 0.87 at 10, 25 and 40°C. The influence of the addition of sucrose, lactose and glucose on the equilibrium moisture content of Norchip potatoes was also studied.

Materials and methods

Potatoes

The cultivars of potatoes examined were an early (Norland), a medium early (Norchip) and a late maturing (Russet Burbank). They were planted in southern Alberta on May 29, 1979 and harvested on September 19. Between harvest and use, the tubers were stored at 5°C and 95% relative humidity.

Sample preparation

Samples of the three potato cultivars were prepared by cutting slices 1.3×1.3 cm $\times$ 3 mm. These were blanched for 1 min in boiling water, cooled in ice water and dipped in 1% sodium bisulphite solution for 1 min prior to sugar treatment, drying or freeze drying and determination of the equilibrium moisture content. To the sugar treated samples, sucrose, glucose or lactose was added by soaking approximately 500 g of potato slices/L of 15% glucose, lactose or sucrose solution for 2 hr just prior to drying. Drying was carried out in a vacuum oven at

70°C and 48.8 mmHg vacuum for 24 hr. Freeze drying was carried out in a Virtis freeze drier, Model 25 SRC 3, at a condenser temperature of −60°C, a pressure less than 50 μ and a shelf temperature of 30°C. Blanching and sulphiting the potato slices were carried out to duplicate commercial dehydration practices and to reduce the danger of mould growth in samples exposed to high humdities.

Isotherms

The moisture sorption isotherms were determined gravimetrically by placing fresh, vacuum oven or freeze dried samples in vacuum desiccators containing saturated salt solutions which give different constant relative humidities (Rockland, 1960). The desiccators were kept in a constant temperature cabinet (± 0.5°C) until equilibrium was reached. The time required for the samples to reach equilibrium varied with the relative humidity and the temperature. The moisture content of the equilibrated samples was determined by drying the samples in a vacuum oven at 70°C and 48.8 mmHg vacuum for 24 hr. Equilibrium moisture contents were determined over the range 11–87% relative humidity at 10, 25 and 40°C. Triplicate and, in some cases, quadruplicate determinations were made on the equilibrium moisture content of each sample at each temperature. The sorption isotherms were obtained by plotting the moisture content of the samples, expressed as kg H_2O/kg of dry matter (DM) versus water activity (a_w).

Results and discussion

The adsorption isotherms at 25°C of freeze dried and vacuum dried potatoes are shown in Fig. 1. Freeze dried samples adsorbed more water vapour at low relative humidities than the vacuum oven dried samples. At higher relative humidities the difference in adsorption diminished and the isotherms tended to coincide. The two curves have the typical sigmoid shape of type II isotherms as indicated by the BET method (Labuza, 1968). Consequently, they were subjected to the analysis by the BET method. Least squares analysis was used to obtain the slopes and the intercepts of the BET plots from which the monolayer was calculated. The calculated values of the monolayer water contents (X_m) were 4.20 for the vacuum dried samples and 6.34 kg H_2O/100 kg DM for the freeze dried samples.

The higher adsorptive capacity of the freeze dried potato was probably caused by the highly porous structure of this product. The fact that this adsorptive capacity was more affected by the porous structure at low moisture region suggests that the high amount of sugars, which adsorb more water at high relative humidities, affected the shape of the isotherms.

Figure 2 shows the effect of cultivar and sucrose, glucose and lactose addition on the adsorption isotherms of freeze dried potatoes. As can be observed, cultivar had no effect on the water vapour sorption of freeze dried potato slices.

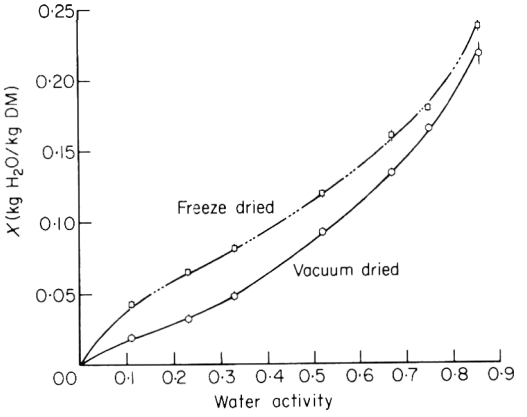

Figure 1. Adsorption isotherms at 25°C of freeze dried (□) and vacuum dried (O) Norchip potatoes.

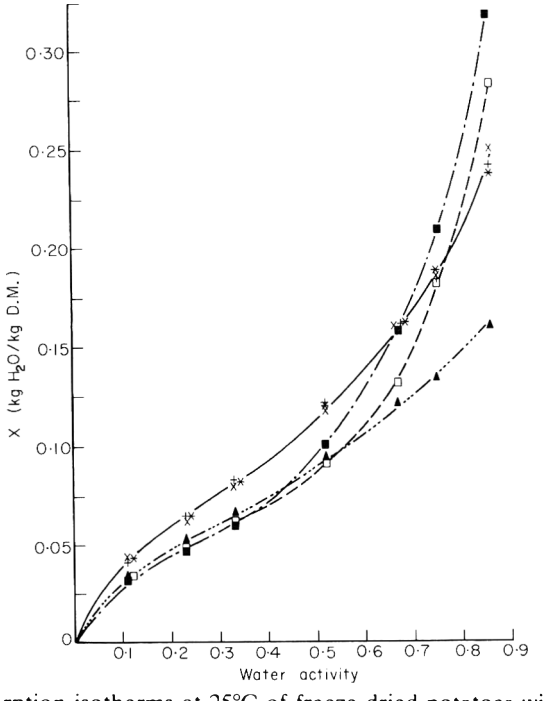

Figure 2. Adsorption isotherms at 25°C of freeze dried potatoes with and without added sucrose, glucose or α-lactose. *Norchip; +, Russet Burbank; ×, Norland; □, Norchip + sucrose; ■, Norchip + glucose; ▲, Norchip + lactose.

This is in agreement with the results of Strolle & Cording (1965) who reported that potato flakes made from eight potato cultivars, ranging in specific gravity from 1.066 to 1.094, had similar moisture sorption properties. Addition of sugars to potatoes caused equilibrium moisture content to decrease in the low and intermediate water activity ranges. With glucose and sucrose addition, the equilibrium moisture content increased significantly in the high relative humidity range. This increase is the result of the dissolution of the sugars in the water (Loncin, Bimbenet & Lenges, 1968; Audu, Loncin & Weisser, 1978).

The moisture sorption capacity of potatoes was lowered by the presence of lactose in the entire water activity range investigated. Lactose is known (Audu, Loncin & Weisser, 1978) to be more hygroscopic and less soluble than sucrose or glucose. Hence, its effect on the equilibrium moisture content of potatoes was less than the other two sugars at low water activity, and the opposite at high relative humidity.

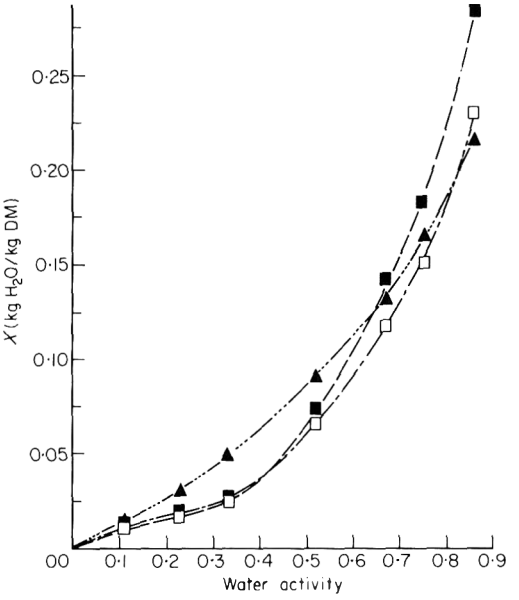

Figure 3. Adsorption isotherms at 25°C of vacuum dried potatoes with and without added glucose or sucrose. ▲, Norchip; □, Norchip + sucrose; ■, Norchip + glucose.

Figure 3 shows the equilibrium adsorption isotherms of vacuum oven dried Norchip potatoes containing 34.5% (dry basis) sucrose, 22.9% glucose, or no added sugar. As for the freeze dried samples, the effect of sugar addition was to lower the equilibrium moisture content at low water activity, and increase it at high values of water activity. The addition of sugar lowered the monolayer moisture content of the freeze dried samples from 6.34 kg H_2O/100 kg DM for the untreated potato to 5.05 for the lactose treated sample, to 4.90 for the glucose treated product and to 4.74 for the sucrose treated sample. The monolayer moisture content of the vacuum dried samples decreased from 4.20 kg H_2O/100 kg DM for the untreated product to 1.94 for the sucrose treated sample, to 1.68 for the glucose treated sample.

Equilibrium temperature has an effect on isotherms. An increase in temperature usually causes a lowering of the isotherm curve which increases the water activity at a constant moisture content, making the product more susceptible to microbiological spoilage (Rockland & Nishi, 1980). When the data for vacuum dried Russet Burbank potatoes at 10, 25 and 40°C (Fig. 4) were plotted in the conventional way, i.e., concentration as ordinates against activities as abscissae, the three curves coincided at lower water activities. The

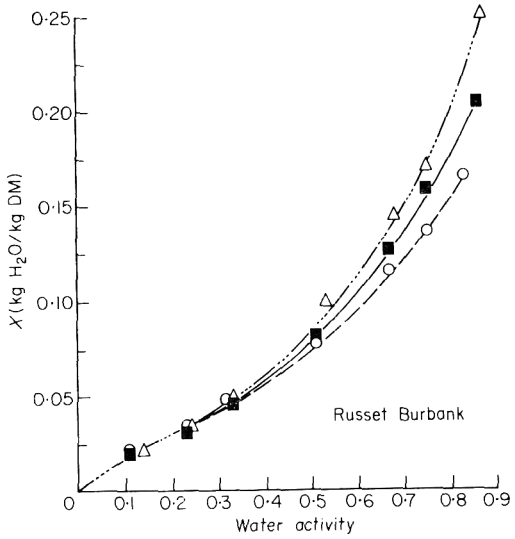

Figure 4. Adsorption isotherms of vacuum dried Russet Burbank potatoes at 10°C (■), 25°C (△) and 40°C (O).

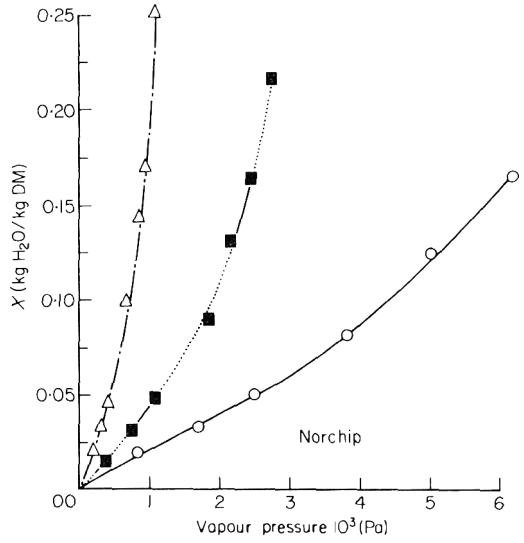

Figure 5. Adsorption isotherms of vacuum dried Norchip potatoes at 10°C (△), 25°C (■) and 40°C (O).

data for vacuum dried Norchip potatoes, plotted X (kg H_2O/kg DM) *vs* a_w, were similar to those for Russet Burbank. However, when these data were plotted using the vapour pressure instead of the corresponding activity (Fig. 5), the higher temperature isotherms were well below the lower temperatures, where they should be according to the classical physical adsorption theory. A zero or positive temperature dependence has been reported for freeze dried potatoes by Saravacos & Stinchfield (1965), for prunes by Bolin (1980) and for protein by Hermansson (1977). For protein, this behaviour is attributed to

structural changes of the protein matrix due to swelling. However, for potatoes, this behaviour is a result of their high fructose, sucrose and glucose content. Indeed, Audu, Loncin & Weisser (1978) clearly showed that a decrease in temperature tremendously increases the water activity of fructose and glucose.

Sorption data from several temperatures are often used (Mazza, 1980b) to compute the isosteric heat or sorption by application of the Clausius-Clapeyron equation. However, in order to do so, the amount of adsorbed water at each water activity must decrease with increasing temperature. Since this is not the case for potatoes, to evaluate the thermodynamics, calorimetric heat of sorption would have to be measured.

The adsorption-desorption isotherms at 25°C from freeze dried and fresh potato slices indicated a hysteresis loop at intermediate moisture levels (Fig. 6). The fact that there was no hysteresis above 0.65 a_w indicates that the food possessed a surface free of capillarity, and that condensation of water on this surface took place in association with solution effect.

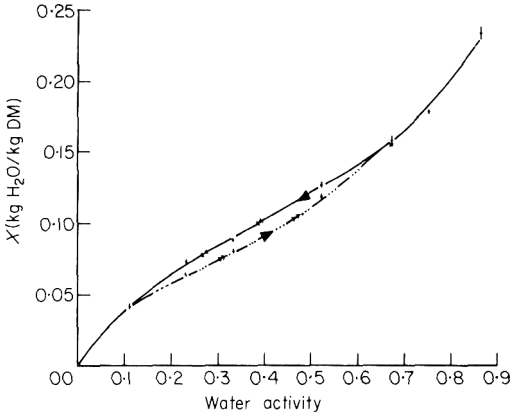

Figure 6. Adsorption-desorption isotherms of Norchip potatoes at 25°C.

Acknowledgments

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The status of fluoride added to bovine milk

I. Fluoride in cold milk

C. G. BEDDOWS

Summary

The use of bovine milk as a carrier for fluoride has been advocated as a prophylactic measure. The effect of the addition of sodium fluoride to cold milk, up to 1000 ppm, was examined by particle micro electrophoresis, centrifugation, and dialysis.

The fluoride had little effect on the electrokinetic potential, and no sedimentation calcium fluoride occurred. Even with centrifugation at 30 000 ***g***, the bulk of the fluoride remained in solution. Dialysis showed that the fluoride could easily be removed, and that the eluent contained fluoride in the ionic (free) form.

The addition of fluoride to calcium phosphate free (CPF, milk showed that the bulk of the fluoride remained with the protein, even after addition of excess calcium chloride. Thus the fluoride does not affect the stability of milk and the element forms a reversible ionic complex. The evidence suggests that it is held in solution by the smaller milk proteins.

Introduction

Milk has been advocated as a vehicle for supplying fluoride, to reduce the incidence of dental caries (Konikoff *et al.*, 1962; Ziegler, 1954). It has been proposed that in order to avoid unnecessary dilution of the milk a solution of milk containing 500 ppm should be prepared and diluted with milk to give a final concentration of 5 ppm; a 200 cm^3 drink would then supply 1 mg of fluoride (Borrow, 1971).

Such a concentration of fluoride in milk, theoretically, could present problems of stability as well as presenting the possibility of forming undesirable fluoride containing compounds. Therefore, it is essential to investigate the

Author's address: School of Health and Applied Sciences, Leeds Polytechnic, Leeds 1.

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status of the fluoride in the milk, and the effect that the ion may have at such a high concentration on the stability of milk, before application of the method is considered.

As milk contains on average 0.123% calcium (Nickerson, 1960) it might be anticipated that, although a lot of calcium is bound to phosphate and citrate, the addition of fluoride ions at 500 ppm could cause the formation of insoluble calcium fluoride as the solubility of CaF_2 is 16 ppm in aqueous solution (Ks. of $CaF_2 = 3.64 \times 10^{-11}$). Carroll *et al.* (1957) reported using fluioride as a sequestering agent in their electron microscopic study of the casein micelle and caused its breakdown into smaller sub units; the concentration of fluoride used, however, was not given.

The effect of fluoride at 1 and 4 ppm in milk was briefly examined by Ericsson (1958) who reported no deleterious effect on the stability of the milk.

Experimental

(a) The effect of fluoride on the electrophoretic mobility of the casein micelles was examined by adding sodium fluoride (A. R. grade BDH Ltd., Poole, Dorset) to skim milk, up to concentrations of 1000 ppm fluoride. It was compared with untreated milk, and with milk containing up to 1000 ppm chloride in a 'Particle Micro-electrophoresis Apparatus' (Mark II) (Rank Bros., Bottisham, Cambridge, U.K.). Sixty volts was applied and the rate of migration of milk particles was examined at room temperature. For each concentration, 30 readings were taken and their average recorded.

(b) The effect of fluoride on the pH was measured, up to 1000 ppm fluoride, using a EIL Vibron pH meter (EIL Ltd., Richmond, Surrey).

(c) The stability of fluoride containing milk was examined using the radio-isotope ^{18}F ($t_{0.5} = 102$ min) (MRC Cyclotron Unit, Hammersmith Hospital, London) as a marker. Samples were counted directly in glass vials on a Panax Scintillation Counter type D 657 (Panax Ltd., Redhill, Surrey). A general sampling procedure was adopted for further experiments. 10 cm^3 portions of milk were used in either centrifuge tubes, or test tubes. The distribution of fluoride in the tubes was determined by carefully removing the top 2 cm^3 by pipette (fraction 1), then the next 2 cm^3 (fraction 2), then the next 2 cm^3 (fraction 3) and finally 2 cm^3 from the solution from the bottom of the tube (fraction 4). The radioactivity of each fraction was measured.

A number of experiments were carried out to examine the stability.

(i) Portions of freshly pasteurized whole milk (Channel Island) (9.5 cm) was added to sodium fluoride solution (0.5 cm^3) containing ^{18}F, to give concentrations up to 1000 ppm F^-. After standing at 20°C for intervals up to 12 hr, the 2 cm^3 fractions were removed and analysed as described above.

In initial experiments, the fluoride solution was added to the top of the milk, with stirring, and then mixed thoroughly. With later experiments, the milk was added to the fluoride solution.

(ii) Portions (10 cm^3) were centrifuged (a) at 4000 *g* for 15 min, at ambient temperature, or (b) at 12 000 *g* for 30 min at 4°C or (c) at 30 000 *g* for 30 min at 20°C (MSE High Speed 18, MSE Ltd., Crawley, Surrey. In each case, 2 cm^3 portions were removed and the radioactivity determined as before. The protein content was determined on the supernatant liquor from (c) by the method of Lowry *et al.* (1950).

The effect of adding solid $CaCl_2$, up to 2000 ppm calcium, to the clear supernatant produced in (c) was determined by recentrifuging at 30 000 *g* for 30 min after addition, and then removing and counting the radioactivity of the top 2 cm^3 and the bottom 2 cm^3.

In a related experiment, freshly prepared radio-labelled CaF_2 was added to fresh milk to give a concentration of 10 mg in 10 cm^3, in a centrifuge tube. After shaking and allowing to stand for 10 min, the mixture was centrifuged at 4000 *g* for 15 min. The top and bottom 2 cm^3 were removed and analysed.

(d) Solutions of milk containing up to 1000 ppm, F^- (with radio labelled fluoride) were *dialysed* in a hollow fibre beaker dialyser (Bio-rad Laboratories, Bromley, Kent). Water was eluted through the fibres at a rate in excess of 1 l/hr and the milk sample (100 cm^3) was stirred, in the outer component.

The concentration of fluoride in the milk being dialysed, was followed by removing 2 cm^3 portions at intervals over a period of 5 hr, and measuring the radioactivity. A solution of sodium fluoride was dialysed under the same conditions.

In some experiments the dialysis eluent was collected and concentrated at 40°C under vacuum to 100 cm^3. The fluoride content was measured, by diluting with an equal volume of 0.1 M sodium chloride, and measuring the free fluoride ion concentration using the fluoride ion selective electrode (Orion Research Inc., Cambridge, Massachussetts, USA) by comparing with standards prepared by adding a known amount of sodium fluoride and 0.1 M sodium chloride to eluent obtained from the dialysis of a non-fluoride treated milk.

(e) The effect of fluoride and calcium on 'calcium-phosphate free' (CPF) milk was determined by preparing CPF milk according to the method of Pyne and McGann (1960a), by dialysing against milk initially (CPF1) and some by further dialysis against distilled water (CPF2). Fluoride containing ^{18}F, (0.5c) was added to 10cm^3 portions of CPF1 and CPF2 to give a concentration of 1000 ppm F, and then centrifuged at 30 000 *g* for 30 min to give a clear supernatant liquor. The radioactivities of the top and bottom 2 cm^3 samples were measured and compared with fresh milk treated similarly.

To this clear supernatant from CPF1 and CPF2, $CaCl_2$ solution was added to give 2000 ppm calcium. The solution was re-centrifuged at 3000 *g* for 30 min. The radioactivities of the top and bottom 2 cm^3 samples were measured.

Results and discussion

Below 100 ppm F^-, neither chloride or fluoride had much effect on the migration rates of milk micelles (Table 1). Even at higher concentration the change

Table 1. Effect of chloride and fluoride on the migration rates of milk micelles

Concentration (ppm)	Migration Rate	
	Chloride (mm/V/min)	Fluoride (mm/V/min)
0	390	390
1	388	395
10	393	396
100	430	452
1000	481	509

was relatively small. Thus the fluoride has very little effect on the zeta potential which plays a significant role in maintaining the integrity and nature of the casein micelles (Darling & Dickson 1979). The micelle size did not appear to be affected when examined, which would suggest that the concentrations are not as great as those used by Caroll *et al.* (1971).

The addition of 1000 ppm fluoride (as sodium fluoride) caused only a negligible change in the pH from 6.66 to 6.75.

Contrary to expectation, when sodium fluoride was added to milk to give a concentration of 1000 ppm F^-, no precipitation occurred, even after standing for 24 hr at 20°C.

When sodium fluoride was added to milk and mixed thoroughly, the fluoride tended to equilibrate in the top 'layer' in preference to the lower layers (Table 2). This effect was apparent in whole milk, raw milk, homogenized milk and in skim milk. The time taken for this uneven distribution of fluoride ion to occur was about 10 min. Temperature had no effect on the extent of the uneven distribution. When NaCl was used with a trace of ^{18}F, the same effect occurred at each concentration (Table 3).

Table 2. The concentration of fluoride (given as counts per sec of ^{18}F) in various fractions in a test tube, after adding sodium fluoride solution (0.5 cm^3) at given concentrations to the top of the milk (9.5 cm^3) mixing well and standing for 10 min

Sample	Initial concentration of NaF (ppm)					
	1000	500	250	100	50	25
Top layer	14,480	14,684	14,649	15,112	13,804	13,664
Second layer	14,464	14,480	14,702	14,526	13,808	13,440
Third layer	14,502	14,470	14,017	14,100	13,676	13,194
Bottom layer	14,596	14,160	11,054	13,012	13,202	12,502
Percentage difference*	+3%	−3%	−25%	−15%	−5%	−8%

*Represents the percentage difference between top and bottom.

Table 3. The percentage difference in the ^{18}F concentration between top and bottom layers after the addition of known concentration of sodium chloride (containing a trace of ^{18}F) (0.5 cm^3) to the top of the milk (9.5 cm^3) mixing well and standing for 10 min before determining the radioactivity using a scintillation counter

NaCl concentration (ppm)	Percentage difference (between top and bottom samples)
250	−27
500	− 5
1000	−15
2000	−11

However, when milk was added to the sodium fluoride solution, no difference in the F^- distribution occurred even on standing up to 12 hr at 1000 ppm, with each type of milk. It is difficult to explain the observation, unless it is likely that on standing, prior to the addition of NaF solution, some separation of protein or other components occurred. For example, it was possible that lipid micelles present (even though in very low concentration in the skim milk) may have separated out a little and they could contain stronger fluoride attracting groups.

Centrifugation at 4000 *g* produced no sediment in the milk. The radioactivity was evenly distributed throughout. When freshly formed radiolabelled calcium fluoride was added to milk at 100 ppm and centrifuged, nearly all of it (88%) precipitated out at 4000 *g*. This indicated that calcium fluoride itself was not being formed in the milk to which fluoride had been added.

At higher speeds (12000 *g*) a slight redistribution of the fluoride concentration occurred (Table 4). The top fraction was visibly clear. Very little diffusion of the fluoride occurred on standing. If CaF_2 had been formed, a precipitate would have resulted.

Table 4. The concentration of fluoride (given as counts per sec of ^{18}F) in various fractions before and after centrifuging at 12000 *g* or 30000 *g* for 30 min at 20°C with a skim milk containing initially 500 ppm F^- (from NaF)

	12000 *g*		30000 *g*	
Sample	Initial (cpm)	30 min diffusion (cpm)	Initial (cpm)	30 min diffusion (cpm)
Top layer	26109 (clear)	25535	19424 (clear)	17626 (clear)
Second layer	26201	25842	20406 (clear)	17117 (clear)
Third layer	26454	26012	20717 (clear)	18701 (clear)
Bottom layer	26611	26209	24714*	22899*

*Heavy precipitate present.

Centrifugation of milk at 30 000 *g* resulted in a clear supernatant in the top 8–9 cm^3. A layer of lipid was present on the surface. Although some uneven distribution of fluoride concentraton was present at least 85% of the fluoride remained in the visibly 'clear' solution. Only slight diffusion of the fluoride occurred on standing (Table 4). The solution would be expected to contain some 80 ppm free calcium ions. Analysis of the protein in the supernatant liquor showed that 2.63 mg/cm^3 was present. After addition of an excess of calcium ions (2000 ppm) only a further 10% of the fluoride was sedimented at 30 000 *g* which suggested that the fluoride was held in solution by the other components, such as the protein. This effect was observed with both whole and skim milks.

It is essential to know the nature of the bonding of the fluoride in the milk, because if any covalent fluoride compounds were to be formed, they would have to be investigated fully.

Dialysis using Visking tubing would be too slow for use with ^{18}F which has a short half-life (102 min). Consequently, a hollow fibre dialysis beaker was employed, with water being passed through the fibres. The time-course curve is given in Fig. 1. The rate of dialysis was slower compared with the dialysis from water but all the fluoride was removed. Thus the fluoride must have formed a weak ionic reversible complex in the milk. This would explain the slower uptake of fluoride into plasma, from fluoride treated milk, as reported by Ericsson (1958).

However, it was feasible that the fluoride might have formed dialysable low weight covalent complexe(s). Consequently, the aqueous dialysate was concentrated and the 'free fluoride' concentration was measured using a fluoride ion-selective electrode. All of the dialysed fluoride was found to be in the 'free' form, when compared with fluoride added to the dialysate from fluoride free milk. Thus the fluoride must be in a simple ionic equilibrium in the milk.

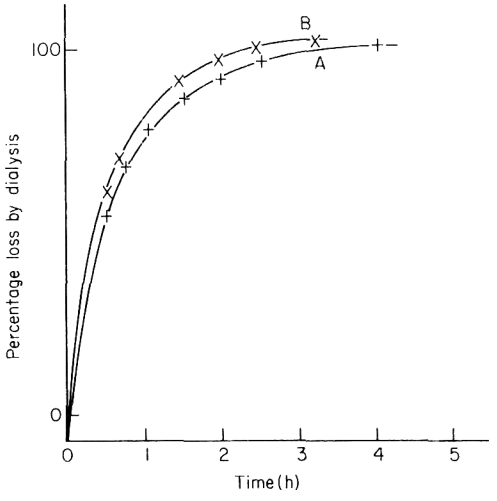

Figure 1. Dialysis of (A) raw milk containing 100 ppm F^- (B) water containing 100 ppm F^-, using the hollow fibre dialysis technique (^{18}F used as a marker).

In order to obtain some information as to what species the fluoride may have attached, calcium phosphate free milk was prepared (Pyne & McGann, 1960a) at 2°C. After dialysis against milk, the total calcium concentration of the dialysate (CPF1) was 12.7 ppm. The solution was not completely clear, unlike the solution obtained by Pyne & McGann (1960a), although it was viscous. Attempts to reduce the calcium concentration further were made by dialysing against water (CPF2). Within a short period, the calcium content was reduced to 6.9–7.3 ppm. However, it must be noted that citrate and other constituents important for the maintenance of micelle structure may have been removed (Pyne & McGann 1960ab) or disturbed (Downey and Murphy 1970). Fluoride was added to both CPF1 and CPF2 to give concentrations of 1000 ppm and both solutions were centrifuged at 30 000 *g*. When examined as before, the bottom fraction had approximately 15% more radioactivity than the top fraction, for both CPF1 and CPF2, these are similar to the results obtained with undialysed milk. The protein content of the supernatant from the fluoride treated – CPF2 was still quite significant (2.08 mg/cm^3). When calcium was added (2000 ppm) to these solutions which were then re-centrifuged at 30 000 *g*, the uneven distribution of fluoride concentration was greater; the bottom fraction contained 24% (CPF1) and 26% (CPF2) more fluoride than the top fraction. However, a lot of fluoride still remained in solution (80%). When calcium chloride was added directly to CPF1 and CPF2, the precipitate obtained on centrifugation was much greater than with fresh milk, as would be expected from the reports of Pyne & McGann (1960a). Thus it appears from the use of CPF milk that the fluoride could only form an ionic complex with the protein, as the calcium content was very small.

Conclusions

The effect of adding sodium fluoride at concentrations up to 1000 ppm to bovine milk was examined. It was observed that:

(a) The fluoride did not have any real effect on the electrokinetic protential of the case in micelles.

(b) No significant change in the pH occurred with the addition of NaF.

(c) Even at 1000 ppm F^- (from NaF) no precipitate was formed upon standing for several hours.

(d) With centrifugation at 4000 *g*, (when freshly prepared calcium fluoride can be sedimented), no precipitation occurred with milk containing 1000 ppm F^-.

(e) With centrifugation at 30 000 *g*, a milk solution containing fluoride at an initial concentration of 500 ppm, gave a clear supernatant which still contained at least 85% of the fluoride. The addition of calcium fluoride solution to the clear supernatant and re-centrifuging, caused only a small decrease in the fluoride content.

(f) Dialysis of milk containing 100 ppm F^- initially, removed all the fluoride

from the milk, at a slower rate than from water; the dialysate was shown to contain the fluoride in the 'free' form.

(g) After addition of 1000 ppm F^- to calcium phosphate free (CPF) milk and centrifuging at 30 000 *g*, the fluoride content of the clear supernatant liquor decreased only by 15%. Thus the milk containing fluoride, even at 1000 ppm, was stable. The fluoride formed a reversible ionic complex, most likely with the protein present although the possibility of some of the fluoride entering the micelle which contains calcium phosphate, cannot be entirely ruled out. No evidence of any covalently bound fluoride containing complexes was found.

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The status of fluoride in bovine milk

II. The effect of various heat treatment processes

C. G. BEDDOWS AND C. BLAKE*

Summary

Raw, whole and skim milks containing fluoride up to 100 ppm fluoride were pasteurised by the HTST and low temperature holding (LTH), and UHT and 'in bottle' sterilization methods. The heat treated milks were stable on standing at room temperature while centrifugation at 4000 *g*, 12 000 *g* and 30 000 *g* showed that a fluoride containing precipitate was not formed.

Dialysis in conjunction with a fluoride ion-selective electrode showed that most of the fluoride was in an easily removable ionic complex. However a proportion of the fluoride, depending upon the heat treatment process used, was not removed by dialysis. A similar effect was observed with heat treated 0.25% casein suspension and with calcium phosphate free (CPF) milk. Examination of the non-dialysable fraction from milk, by addition of fresh fluoride and also by gel chromotography showed that the fluoride was held in a tightly bound but reversible ionic complex.

All of the fluoride could be recovered in an ionic form as shown using the ion-selective electrode on the milk after precipitation of the milk protein with 0.3 M citric acid.

Introduction

Fluoride has been widely reported as a prophylactic agent in the prevention of dental caries. Bovine milk has been used as a vehicle for the distribution of fluoride (Ziegler 1956, 1962, 1964; Konikoff, 1960; Konikoff *et al.*, 1962). One method advocated involves the preparation of milk, containing 500 ppm fluoride, which can then be diluted with milk to give a concentration of 5 ppm; this would give a nominal dilution of the milk (Borrow, 1971).

Authors' addresses: School of Health and Applied Sciences, Leeds Polytechnic, Leeds LS1 3HE, and *Department of Applied Biology and Food Science, Polytechnic of the South Bank, Borough Road, London SE1 0AA.

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Milk containing fluoride at 500 ppm, was shown to be quite stable; some evidence indicated that the majority of the fluoride became associated with the lower MW proteins and not with calcium (Beddows, 1982).

However if this method of providing fluoride containing milk were to be adopted pasteurization would be required in order to ensure microbiological safety. This paper reports the effect of various heat treatment processes on the status of the fluoride in milk. If the fluorine formed covalent compounds, then the milk would have to be tested for the presence of any toxic compounds.

To follow the fate of fluoride, ^{18}F ($t_{0.5}$ = 102 min) was used; it is a positron emitter and thus interacts with matter to produce γ-radiation, which can be counted on a scintillation counter.

The heat treatment processes investigated were the UHT method (Burton & Perkin, 1970), the HTST method (Franklin, 1965) the low temperature holding method (LTH) and the 'in bottle' sterilization procedure.

Materials and methods

(a) Heat treatment processes

Portions of raw bovine milk (whole or skim) containing 0, 5, 10, 20, 50 and 100 ppm fluoride (using NaF analar grade (BDH Ltd., Poole, Dorset)) and labelled with ^{18}F (MRC Cyclotron Unit, Hammersmith Hospital, London) usually at an initial activity of 0.5 mC 100 cm^{-3}, were subjected to one of the following heat treatment procedures. Plastic containers were used whenever possible, in all experiments.

(i) *Low temperature holding method (LTH).* Portions were maintained at 63°C for 30 min.

(ii) *HTST.* Portions of milk were heated to 71.6°C for 15 sec in a pilot scale HTST treatment apparatus (NIRD, Shinfield, Berks) (Franklin, 1965).

(iii) *UHT.* Portions were treated in a small scale UHT plant (NIRD Shinfield, Berks) (Burton & Perkin, 1970).

(iv) *'In-bottle' sterilization.* Portions were heated in glass bottles at 100°C for 30 min, as part of a normal heat treatment line (Express Dairies Ltd., Nine Elms, Vauxhall, London).

(b) *Examination of the Heat Treated Milks*

(i) *Stability of the solutions.* Portions of milk were held at room temperature for up to 24 hrs; other portions were centrifuged at 12 000 *g* and 30 000 *g* and samples were removed from the tubes as reported earlier (Beddows 1982). The ^{18}F activity was counted on a Panax Scintillation Counter, type D657 (Panax Ltd., Redhill Surrey).

(ii) *Dialysis.* Portions of each heat treated milk were dialysed against water, as described previously (Beddows 1982) using a hollow fibre beaker dialyser

(Bio-Rad Laboratories Ltd., Bromley, Kent). The percentage loss in radioactivity in the milk was plotted against time.

In certain experiments, the eluate was collected and evaporated to low volume (100 cm^3); the total fluoride content was determined by its radioactivity and the 'free' fluoride content was determined using an Orion fluoride ion-selective electrode model 94–07 with an Orion 90-20-00 double junction reference electrode. A standard was prepared by adding sodium fluoride to an eluent from the dialysis of fluoride-free milk treated in the same way.

In one experiment a portion of LTH – milk containing 10 ppm fluoride was dialysed. The milk was removed from the beaker and kept at 5°C overnight. Fresh fluoride (containing ^{18}F) was then added (to give 10 ppm and the milk was kept at 20°C for 5 hr. The milk was dialysed again and aliquots were removed and analysed as before.

In separate experiments, (a) a suspension of 0.25% casein adjusted to pH 6.8 and (b) a sample of calcium phosphate free (CPF) milk, prepared according to the method of Pyne & McGann (1960a,) were treated with sodium fluoride (containing ^{18}F) to give concentrations of 10 ppm fluoride and they were subjected to the LTH method. The resulting solutions were dialysed and analysed as above.

In another experiment, a sample of dialysed LTH – milk (1 cm^3) was added to a column of Sephadex G25 (Pharmacia Ltd., Uppsala, Sweden). The eluent was monitored at O.D.=280 nm and 3 cm^3 fractions were collected. The radioactivity in each tube was determined as before. Some of the eluent was evaporated to low volume at 38°C under vacuum and re-chromatographed. The eluent and collected fractions were analysed as before.

The dialysis was repeated with two portions of milk containing 10 ppm fluoride whose pH had been adjusted to 6.0 (with 2 M acetic acid) or pH 8.0 (with 2 M NaOH) respectively and had been treated by the LTH method.

(iii) *Precipitation.* Portions of heat treated milk were adjusted to pH 3.2 using 0.3 M citric acid and the solution was centrifuged at 5000 ***g***. The fluoride content of the precipitate and in the supernatant liquor were determined radiochemically and the free unionised fluoride was determined in the supernatant liquor using the Orion fluoride ion-selective electrode, with sodium fluoride in eluent from the dialysis of fluoride-free milk as standard.

Results and discussion

Examination of the fractions obtained after standing, from each heat-treated solution containing 100 ppm F^- showed that even after 24 hr no precipitation occurred. Centrifugation at 12 000 ***g*** (when freshly prepared solid CaF_2 separates out if added to milk) produced no uneven distribution of fluoride in the samples.

Centrifugation at 30 000 ***g*** for 30 min did not give a totally clear supernatant

Table 1. Effect of centrifugation at 30 000 g (for 30 min) on LTH-treated milks containing fluoride using ^{18}F as a marker (results given in cpm)

	Initial fluoride concentration (ppm)					
Sample	0*	5	10	20	50	100
(a) (Top)	8037	7739	7618	8243	8080	7369
(b)	8047	7866	7703	8173	8175	7365
(c)	8135	7889	7683	8281	8254	7390
(d) (Bottom)	8262	7967	7822	8420	8310	7552

*A trace of ^{18}F marker was added.

liquor, unlike un-heated milk (Beddows, 1982), although the upper portion (5 cm^3 to 10 cm^3) was clear. Some uneven distribution of the fluoride in the centrifuge tube did occur. Typical results after LTH treatment of whole milk are given in Table 1 and show that at least 90% of the fluoride remained in the supernatant liquor. Neither the fluoride concentration nor the type of milk nor heating process used, had any effect on the relative stability (even up to 100 ppm fluoride). This was a little surprising as it might be expected that with heating and cooling, milk undergoes some changes and the possibility of forming insoluble calcium fluoride might have been increased.

However dialysis using the hollow fibre beaker did show a difference for each heat treatment process used. This difference was present with whole and skim milks. A typical series is given in Fig. 1. The time and temperature of the heat treatment had an effect on the retention of some of the fluoride as the 'in-bottle' sterilization produced a greater effect than the LTH method. The UHT and HTST methods gave comparable results.

Two portions of milk containing 10 ppm fluoride were adjusted to pH 6.0 and pH 8.0 respectively and then subjected to the LTH method. After dialysis, 14 and 16% of the fluoride respectively still remained in solution.

When the whole procedure was repeated with a suspension of 0.25% casein or with a sample of calcium phosphate free (CPF) milk that had contained 10 ppm fluoride, the same effect was observed (Fig. 1, curves G and H respectively) which indicated that the fluoride must be interacting with protein.

In order to gain an insight as to the nature of the fluoride-protein interaction, a sample of the LTH-milk that initially contained 10 ppm fluoride was dialysed until 'equilibrium' was attained; 2.2 ppm fluoride remained.

The milk was allowed to stand overnight at 5°C in order to allow the ^{18}F to decay to a very low level. Fluoride with ^{18}F was then added to a concentration of 10 ppm and the milk was allowed to stand for 5 hr at 20°C. The milk was re-dialysed and again the amount of fluoride removed reached a maximum of 85% which was similar to that obtained with the initial dialysis. This suggested that the 'retained' fluoride was in equilibrium with the freshly added fluoride because if it were not, then all of the freshly added fluoride and ^{18}F would have

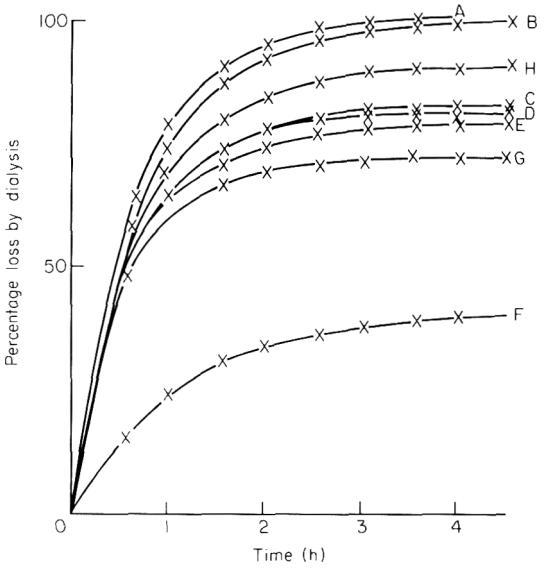

Figure 1. Dialysis of solutions containing initially, 10 ppm fluoride. (A), aqueous NaF solution; (B), raw unheated milk; (C) HTST pasteurized milk; (D), low temperature holding treated milk; (E), UHT pasteurized milk; (F), 'in bottle' sterilized milk; (G), LTH treated 0.25% casein solution; (H), LTH treated CPF milk.

been removed by the dialysis. Fluoride can be fully removed from pretreated cold milk (Beddows, 1982).

The dialysate of an LTH milk containing 50 ppm fluoride was evaporated at 38°C to 100 cm^3 and the free fluoride content was measured using the fluoride ion-specific electrode and compared with a standard prepared by adding NaF (with ^{18}F) to the eluate from the dialysis of a fluoride free milk. The total fluoride content was obtained by measuring the radioactivity. All the fluoride in the aqueous eluent was in the free unionised form and thus low molecular weight covalent fluoro-compounds could not have been formed.

To investigate the fluoride retention in heat treated milk further, a portion of dialysed LHT milk originally containing 10 ppm F, was passed onto a Sephadex G25 column and eluted with water. The fluoride content of the eluent was measured using a scintillation counter, and the protein concentration was monitored spectrophotometrically at 280 nm.

Several fractions (Fig. 2) were present. The first fraction (28–42 cm^3) appeared to contain only protein, the second fraction (43–66 cm^3) contained both fluoride and protein, the third fraction contained both fluoride and protein but the fluoride content was greater for the amount of protein present. However free fluoride appeared in fraction 4 (88–120 cm^3). As the fluoride is small in size it should be retained by the gel. Thus when sodium fluoride solution was chromatographed on the same column the fluoride appeared in the same fraction. Fractions 2 and 3 were quickly evaporated to low volume (~2 cm^3) at 40°C *in vacuo* and each was passed through the same column. The same effect,

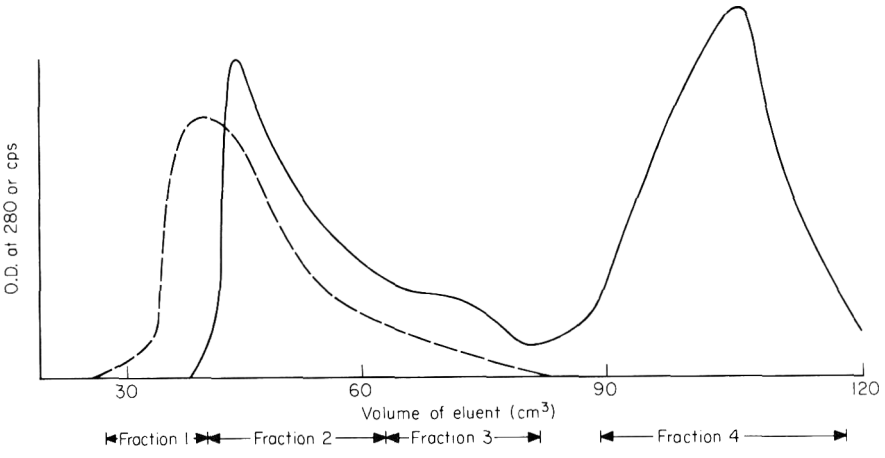

Figure 2. Elution of LTH treated milk dialysate (initially 10 ppm fluoride) using Sephadex G25 (30 cm × 1.2 cm int. diam) with water (- - - γ 280; ———, radioactivity (^{18}F)).

with the appearance of a large amount of 'free' fluoride (95–107 cm^3), occurred again when fraction 2 was separated. Fraction 3 contained only a small amount of protein (60–63 cm^3) and fluoride (100–105 cm^3). (The ^{18}F decays quickly so that the levels of radioactivity obtained were relatively low.)

Thus it appears that in the presence of the Sephadex gel the fluoride was in equilibrium with the protein; the gel disturbed this equilibrium by 'removing' the free fluoride as it passed through the column. However the equilibrium must be quite strongly biased towards the protein-fluoride complex, as a weaker equilibrium would easily separate all the fluoride because the Sephadex column has a large number of spaces available for the fluoride to enter on its passage through the column. Further separation of the protein-fluoride fraction was not possible owing to the decay of the ^{18}F and the low concentration of fluoride present could not be measured by other means. It was expected that all the fluoride could eventually be separated from the protein.

Thus the fluoride appeared to be in two equilibria with the protein, one which was easily disturbed by dialysis and a second which must be much more strongly bound.

In order to confirm that other fluoride compounds had not been formed, a portion of LHT milk containing 50 ppm F (and ^{18}F) was treated with 0.3 M citric acid to pH 3.2 to precipitate the protein. A large amount of precipitate was formed. This was centrifuged, washed with 0.01 M citric acid and the fluoride content of the precipitate was determined and also that of the supernatant. No fluoride was present in the precipitate (after redissolving in 0.1 M phosphate buffer, pH 6.5).

A portion (20 cm^3) of the supernatant liquor was adjusted to pH 5.0 (with 3.0 cm^3 1 M NaOH). The fluoride content of the solution was measured using the

fluoride ion-selective electrode. It was compared with a solution of fluoride added to the supernatant liquor from a fluoride free milk that had been treated similarly. All of the fluoride appeared as unbound fluoride and thus must be present in a reversible ionic form, even in the heat treated milks.

For confirmation the supernatant liquor after precipitation was dialysed against water, as before. All the fluoride was removed in 4 hr.

Conclusions

Various pasteurization procedures currently in use (UHT, HTST, low temperature holding (LTH) and 'in-bottle' sterilization) did not affect the stability of bovine milk to which fluoride had been added at concentrations up to 100 ppm fluoride; no sedimentation occurred on standing or even after centrifugation at 12 000 *g*. Centrifugation at 30 000 *g* gave a sediment but over 80% of the fluoride remained in supernatant liquor.

Dialysis of the heat treated milks, using a hollow fibre dialyser showed that most of the fluoride formed a simple reversible ionic complex probably with protein. However up to 60% of the fluoride, depending upon the heat treatment used, could not be dialysed easily. This effect was also observed with fluoride in LTH treated 'calcium phosphate free' (CPF) milk, and in a 0.25% casein suspension.

Examination of the non-dialysable fluoride fraction, using gel chromatography, showed that the fluoride was held in a reversible ionic complex but the point of equilibrium was strongly in favour of the protein-fluoride complex.

Precipitation of the protein of heat treated milks containing fluoride, by the addition of 0.3 M citric acid, released all of the fluoride into the supernatant liquor. Examination of this solution using a fluoride ion-selective electrode showed the fluoride to be in the free form. Thus all the fluoride was in reversible ionic complexes. No evidence of covalent compound formation was apparent.

The heat treatment of fluoride-containing milks does not affect their properties or produce any covalent compounds and so such products might be considered for use as a prophylactic measure.

Acknowledgments

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The use of soybean milk in soft-cheese making:

I. Effect of soybean milk on rennet coagulation property of milk

N. H. METWALLI, S. I. SHALABI, A. S. ZAHRAN
AND O. EL-DEMERDASH

Summary

The compositions of soybean milk and whole milk were compared. The two milks were similar in their pH, acidity and protein level, while they differed in the ash, carbohydrate and fat content. Pronounced differences were observed in their amino acid contents. Yield and amino acid content of soybean milk depend to a large extent on the method of extraction.

Addition of soybean milk to milk greatly inhibits the rennet coagulation of milk. Mixing soybean milk with raw milk in the ratio of 1:4 was found to be the most suitable proportion for cheese making, with the alteration of the cheese making process by increasing the amount of rennet, addition of calcium chloride and lowering the pH.

Introduction

Soybean is a native crop of eastern Asia where it has served as an important part of the diet for centuries. Hot water extraction of ground beans yields a milky mixture which resembles cow's milk in appearance and contains protein, carbohydrates and minerals (Woodruff, 1938: You Winston *et al.*, 1968).

Soybean milk has been given considerable attention as an economical nutritive beverage for overcoming protein malnutrition in developing countries. The nutritive value is about 80–90% that of cow's milk (Desikacher & Subrahenyan, 1946; Change & Murray, 1949). For this reason soybean milk can supplement as well as act as a substitute for cow's milk to a large extent. However the use of soybean milk has been limited by the bitter beany taste and undesirable flavour.

Authors' address: Food Technology and Dairy Department, Agriculture College, Minia University, Egypt.

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In this study we examined the possibility of mixing soybean milk with whole milk for soft cheese making. Unlike cow's milk, soybean milk cannot be coagulated by rennet. Yamanaka & Furukuwa (1972) postulated that the presence of soybean milk solids inhibits rennet action. The following series of experiments was designed to determine precisely the factors controlling the effect of soybean milk on rennet action. The compositions of the two milks were also compared.

Materials and methods

Preparation of soybean milk

Soybean milk was prepared from the Harrysoy variety of soybeans by the following two methods:

Method 1. The beans were soaked in water at 4°C for 24 hr, dehulled and then the milk extracted by warm tap water (40°C) in the ratio of 1:3 using a blender. It was finally filtered through cheese cloth to remove the insoluble residue. The yield was 6.5 l/kg of dried beans.

Method 2. The beans were first steam heated to 60°C, then milled to produce soyflour. Soymilk was obtained by mixing soyflour with water in the ratio of 1:8 blended and filtered through cheese cloth (Ferreia *et al.*, 1976). This yielded 5.5 l/kg of dried beans.

Milk supply

Cheese in Egypt is usually made from mixed cow's and buffalo's milk. Milk samples used were a 1:1 mixture of cow's and buffalo's milk obtained from the Faculty herd. This will be referred to as whole milk.

Chemical analysis

Acidity, total solids, total nitrogen and ash contents were determined according to Ling (1963). The fat content was measured by the Gerber method for milk and by Soxhlet for soybean milk. Amino acid analysis was performed by paper chromatography, n-butanol, acetic acid and water 4:1:1 being used as a solvent. Concentrations were measured colorimetrically at 530 nm.

pH Adjustment

The pH was adjusted by adding 1 N HCl to milk samples with constant stirring, left standing for 1 hr to equilibrate before the reading was taken.

Rennet clotting time

Rennet clotting time was determined visibly at 30°C in a shaker water bath, using 10 ml milk and 0.15% of calf rennet.

Curd firmness

A milk sample (500 ml) was clotted at 30°C in a 1-litre beaker by the addition of 0.15% calf rennet and left standing for 1 hr for complete clotting. A round aluminium plate (4 cm) with four pointed-end stands (0.2 × 10 cm) was placed on the surface of the curd. Weights were added to drive the stands into the curd and the results expressed numerically as follows: 30–35 g firm, 20–25 soft, 10–15 very soft, less than 10 g, weak curd.

Results and discussions

Results in Table 1 confirm earlier findings that soybean milk like whole milk contains protein, fat, carbohydrates and minerals. Acidity, pH value and protein level were the same in both milk and soybean milk either prepared by method 1 (soymilk 1) or by method 2 (soymilk 2). However, fat, carbohydrates and ash contents were higher in milk than in soybean milk, possibly due to the presence of buffalo's milk which contains higher percentage of fat and ash than cow's milk (Shalabi, 1969).

Table 1. Gross composition of whole milk and soybean milk (percentage)

Samples	pH Value	Acidity (%)	Total solids	Fat	Total protein	Carbohydrates*	Ash
Whole milk	6.48	0.17	13.87	5.58	3.69	4.93	0.83
Soymilk 1	6.43	0.16	8.99	2.28	3.55	2.5	0.65
Soymilk 2	6.43	0.16	8.64	2.21	3.15	2.4	0.63

*Carbohydrates were calculated by differences. Data are averages of 10 preparations of soybean milk and 10 milk samples.

Amino acid analysis (Table 2) show a remarkable difference between the two milks in their amino acid contents. Lysine, asparagine, tyrosine, methionine and leucine contents were in general less in soybean milk than in whole milk, whereas arginine, threonine, histidine, serine, cystine and cysteine were higher in the former than in the latter. These results are fully in line with the conclusion of Dutra De Oliveira & Scatena (1967) that soybean milk is a good source of all the essential amino acids except methionine. Accordingly mixing soybean milk with whole milk will enrich the nutritive value of both milks.

Table 2 also demonstrates that although soymilk of the same protein concentration can be obtained from both methods of extraction, there is a noticeable difference in their amino acid contents. Soybean milk prepared from soaked beans (method 1) contained higher percentages of serine, proline and methionine. Soybean milk from soy flour (method 2) had a higher percentage of lysine, arginine, threonine and valine. This suggests that the method of

Table 2. Amino acid contents of whole milk and soybean milk (g/100 g protein)*

Amino acids	Whole milk	Soymilk 1	Soymilk 2	Soymilk 1 heated†	Soymilk 2 heated
Cystine + cysteine	0.52	0.94	0.76	0.80	0.69
Lysine	8.39	4.06	5.20	3.04	4.62
Histidine	2.47	4.60	3.62	3.90	2.90
Arginine	3.30	6.79	8.29	6.79	7.72
Asparagine	7.20	3.70	6.33	3.34	5.49
Serine	6.04	13.33	10.13	13.30	10.13
Glutamic	21.11	20.20	19.60	19.59	18.40
Threonine	3.22	8.94	10.58	6.30	8.81
Alanine	3.14	2.23	3.67	2.23	3.67
Proline	9.04	6.72	3.92	5.45	2.46
Tyrosine	7.32	2.52	2.87	1.01	1.15
Methionine	2.42	1.66	0.95	1.25	0.96
Valine	5.39	3.58	6.31	2.93	4.09
Phenylalanine	4.02	2.30	2.63	2.29	2.62
Leucine – isoleucine	16.68	12.22	12.06	10.49	10.46

*Amino acid was calculated as a percentage of total protein content of milks.
†Heat treatment was by autoclaving at 120°C for 15 min.

preparing soybean milk may affect the composition of the extracted soybean protein.

Effect of soybean milk on rennet coagulation properties of milk

The data in Table 3 demonstrate that there is an increase in rennet clotting time when soymilk is added to milk prior to rennet action (0.15% rennet). This increase was accelerated by increasing the amount of soymilk from 5 to 20% (v/v), after which a sharp increase occurred. This increase has been attributed to the inhibitory effect of soybean milk solids (Yamanaka & Furukuwa, 1972). An

Table 3. Effect of added soybean milk on Rennet coagulation property of milk

	Rennet clotting time (min)		
Soymilk added (%)	Soymilk 1	Soymilk 2	Body of curd
0	28	28	Firm
5	36	35	Firm
10	45	43	Soft
15	52	52	Soft
20	64	60	Very soft
25	156	150	Weak curd
30	182	176	Jelly texture

alternative explanation is that the majority of soybean milk proteins are of the globulin type (Wolf, 1972) and contain free sulphydryl groups (Nash *et al.*, 1971). Therefore there is a possibility of an interaction between soybean milk protein and milk casein similar to that reported between the casein and whey proteins which inhibit rennet action (Shalabi & Wheelock, 1976; Wheelock & Kirk, 1974).

The firmness of the curd was greatly affected by the addition of soybean milk, prepared either by method 1 or method 2. The effect was more obvious as the amount of soybean milk was increased. Addition of soybean milk at concentrations of 25–30% resulted in the formation of a very weak curd. This demonstrates that apparently under similar conditions 20% of soymilk would be the maximum proportion of mixing with milk for cheese making.

When the amount of added soymilk was constant at 20%, increasing the amount of rennet was accompanied by a steady decrease in rennet clotting time whereas it had no detectable effect on curd firmness (Table 4). This effect was more obvious in whole milk than in soymilk/whole milk mixture.

The effect of addition of 0.15% rennet while varying the amount of calcium chloride added can be seen in Table 5. It can be seen that addition of calcium chloride as low as 0.01% greatly enhanced the coagulation of both milk and soybean milk/whole milk mixture. Higher concentrations produced the same effect but at a slower rate. However addition of calcium chloride even at a concentration of 0.1% did not increase the firmness of the curd.

The pH value has an important effect on the coagulability of both milk and the mixture with soymilk. Reducing the pH decreased the rennet clotting time

Table 4. Effect of varying rennet concentration on rennet coagulation property of milk and 4:1 mixture with soymilk

	Rennet clotting time (min)		
Rennet added (%)	Milk	Milk + soymilk 1	Milk + soymilk 2
0.05	58	117	100
0.10	38	100	78
0.15	27	62	58
0.20	20	49	39
0.25	18	36	35
0.30	17	34	32
0.35	16	32	30
0.40	10	30	28
0.45	8	20	19
0.50	5	18	17

Body of curd was firm with milk and very soft with soymilk and remained so with all rennet concentrations used.

Table 5. Effect of adding calcium chloride on rennet coagulation property of milk and 4:1 mixture with soymilk

Calcium chloride added (%)	Rennet clotting time (min)		
	Milk	Milk + soymilk 1	Milk + soymilk 2
0	28	64	60
0.01	20	25	22
0.02	18	22	20
0.04	15	17	16
0.06	13	15	14
0.08	10	12	11
0.10	8	9	8

Body of curd was firm with milk and very soft with soy milk and remained so with all calcium chloride concentrations used.

Table 6. Effect of pH on rennet coagulation property of milk and 4:1 mixture with soymilk

pH	Rennet clotting time (min)			Body of curd
	Milk*	Milk + soymilk 1	Milk + soymilk 2	Milk + soymilk
6.5	27	64	60	Very soft
6.0	8	25	22	Soft
5.5	4	8	6	Firm
5.0	2	2	2	Firm

*With milk there was a fast curd syneresis

(Table 6). It was also noted that reducing the pH to 5.5 or 5.0 before renneting greatly improved the curd firmness of soymilk and milk mixture whereas with milk alone it resulted in a fast curd syneresis.

In combination, it was observed that reducing the pH to 5.5 and addition of 0.02% calcium chloride before clotting improved both rennet clotting and curd firmness. In addition autoclaving soymilk at 120°C for 15 min before mixing with milk greatly improved curd firmness, possibly by causing denaturation of the soybean milk protein. This treatment is necessary to destroy trypsin inhibitors naturally present in soybean milk (Hackler *et al.*, 1965). However this treatment caused a slight loss of amino acids (Table 2).

Conclusion

The results of this investigation provide useful information on the use of soybean milk in soft cheese making. Soybean milk can be easily obtained by water extraction from previously soaked beans or soy flour. This yields soybean milk with same protein level as in milk but with different amino acid contents. The two milks were also quite different in their fat, carbohydrate and mineral contents. The results also demonstrated that the amino acid content of soybean milk was influenced by the method of preparation.

It was clearly observed that mixing soymilk with milk affected both rennet clotting time and the firmness of the curd, the effect being dependent on the amount of soybean milk added. It was found that addition of soybean milk at a level of 20% would be the maximum for adding to milk for cheese making. On the other hand, increasing the amount of rennet, lowering the pH and addition of calcium chloride reduced or eliminated the inhibitory effect of soybean milk when added to cow's milk in a 20% ratio. This effect was most pronounced when rennet was added at a level of 0.15%, the pH was lowered to 5.5 and 0.02% calcium chloride was added, provided that the soymilk had previously been heated at 120°C for 15 min.

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Changes in fatty acids of frog legs during frozen storage

A. SARVADEVA*‡ AND L. N. SRIKAR†

Summary

The changes in the fatty acids of frog leg meat during frozen storage were studied with column and gas-liquid chromatography. The major component of the lipids, the phospholipids constituted 90% of total lipids. 16:0, 18:0, 22:0 and 18:2 polyunsaturated fatty acid were the major fatty acids of the frog legs meat lipids. Short chain fatty acids were noticed to the extent of 10%. During frozen storage the proportion of phospholipids decreased, while that of neutral lipids increased. Since the total lipid content was constant, phospholipases were presumably responsible for this change. Alterations in the fatty acid composition of the neutral lipids during storage were consistent with such a hypothesis.

Introduction

Frog legs have attained a great importance because of their export in frozen condition from India, Cuba, China and other countries to France, Belgium, America and other countries. Frog legs being a protein-rich meat are susceptible to the changes with respect to the protein and lipid constituents as observed in other animal meats during frozen storage. Though numerous reports on the frozen storage changes in fish, meat and poultry are available, reports on the changes in frog legs during frozen storage are few. Pathak & Agrawal (1966) have determined the fatty acid composition of fats from various parts of the frog *Rana tigrina*. They have noticed a high percentage of C_{18} unsaturated fatty acids in all the tissues analysed. McMullin *et al.* (1968) have reported that the degree of unsaturation of the fatty acids was higher in bull frog, *Rana clamitus,* than in the terrestrial species like mouse and quail. The present investigation is an attempt to understand the changes in fatty acids of frog leg meat during the frozen storage.

Authors' addresses: *Syndicate Bank, West Coast Zone, Manipal-576119, India and †College of Fisheries, Mangalore-575002, India.

‡Address correspondence to L. N. Srikar, Associate Professor of Fishery Biochemistry, College of Fisheries, Mangalore-575002, India.

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Materials and methods

Frog legs of the species *Rana hexadactyla* were processed according to the procedure recommended by the Marine Products Export Development Authority and frozen in a contact plate freezer at −35°C. They were packed in cartons and preserved at −20°C for the periodical analyses.

Lipids from the frog leg meat were extracted according to the method of Bligh & Dyer (1959) and preserved at −10°C for further analyses. The neutral lipids and the phospholipids were fractionated by silicic acid column chromatography. While the neutral lipid fraction was quantified by weighing, the phospholipids were quantified by determining the phosphorus content according to Fiske & Subbarow (1925) and multiplying the phosphorus content by 25. The fatty acid composition of the lipids was determined by gas liquid chromatography. Methyl esters of fatty acids were prepared according to Kates (1964) and analysed with a CIC gas chromatograph with hydrogen flame ionisation detector. The analysis was carried out on 8′×1/8″ stainless steel column packed with 15% DEGS on Chromosorb W (80–100 mesh). Column temperature was 182°C and the detector temperature was 210°C. Nitrogen was the carrier gas. Retention times of a mixture of standard fatty acids was plotted on a semilog paper relative to C_{18} as 1.00 and from the graph obtained, the retention times of other fatty acids were calculated and identified on the chromatograms of samples. Quantitative analysis was made on the basis of the area percentage of each peak by triangulation.

Results and discussion

The changes in the neutral lipids and phospholipids during the frozen storage period of 150 days are shown in Table 1. The frog leg meat lipids were found to contain a high level of phospholipids (90%). During storage there was a gradual

Table 1. Changes in lipid fractions of frog leg meat during frozen storage

	Days of storage at −20°C						
	0	15	30	45	90	120	150
Total lipids (TL) (g/100 g of meat*)	0.59 ± 0.012	0.62 ± 0.006	0.61 ± 0.006	0.60 ± 0.017	0.60 ± 0.012	0.55 ± 0.006	0.61 ± 0.012
Neutral lipids (NL) (percentage of TL†)	8.6	10.6	10.8	11.6	14.2	14.5	15.5
Phospholipids (PL) (percentage of TL†)	90.2	89.7	89.3	87.5	86.2	86.9	85.2

*Mean of three determinations (± s.e. mean).
†Mean of two determinations.

decline in the phospholipids with a simultaneous increase in neutral lipids, the increase in free fatty acids being apparent. An obvious phospholipid breakdown resulting in the liberation of fatty acids was noticed. Phospholipases A and B which are present in animal tissues along with other phospholipases are known to liberate the free fatty acids from phospholipids. Thus, the phospholipid hydrolysis observed here, makes the activity of phospholipases obvious. Olley & Lovern (1960) in Cod, Bligh & Scott (1966) also in Cod, Davidkova & Khan (1967) in chicken muscle and Awad *et al.* (1969) in freshwater whitefish muscle have all noticed similar observations.

The total lipids fatty acid composition is given in Table 2 and the fatty acid composition of the neutral lipids of the 0 day and 150 days stored frog leg meat is presented in Table 3. Figures 1, 2 and 3 depict the gas-liquid chromatograms of methyl esters of fatty acids of initial total lipids, neutral lipids at 0 day and 150 days of storage respectively.

McMullin *et al.*(1968)have reported that the saturated fatty acid content is less than the unsaturated acids in bull frog. According to Pathak & Agrawal (1966)

Table 2. Fatty acid composition of total lipids of fresh frog leg meat*

Acids	Percentage
Saturaged acids	
10:0	1.9
12:0	2.8
13:0	2.4
14:0	3.6
15:0	3.8
16:0	21.1
18:0	7.6
20:0	1.0
22:0	9.9
Total	54.1
Mono unsaturated acids	
10:1	4.4
14:1	2.6
16:1	5.9
18:1	13.7
Total	26.6
Poly unsaturated acid	
18:2	9.1
Unidentified acids	
(8)	5.3
(8)	2.0
(10) or (11)	2.8
Total	10.1

*Mean of two determinations.

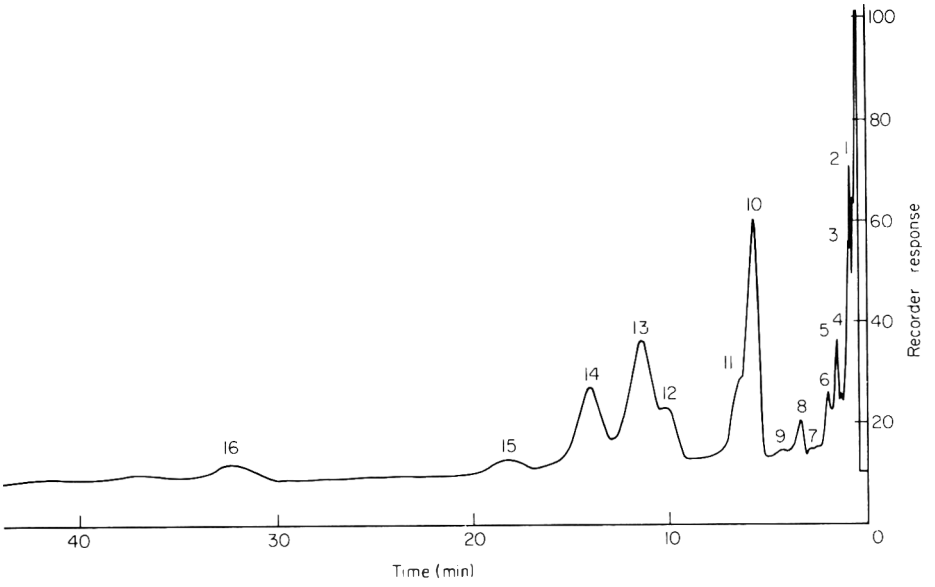

Figure 1. Gas-liquid chromatogram of methyl esters of fatty acids of total lipids of frog leg meat. Column: 8′ × 1/8″ stainless steel 15% DEGS on Chromosorb W (80–100 mesh). Temperature: 182°C.

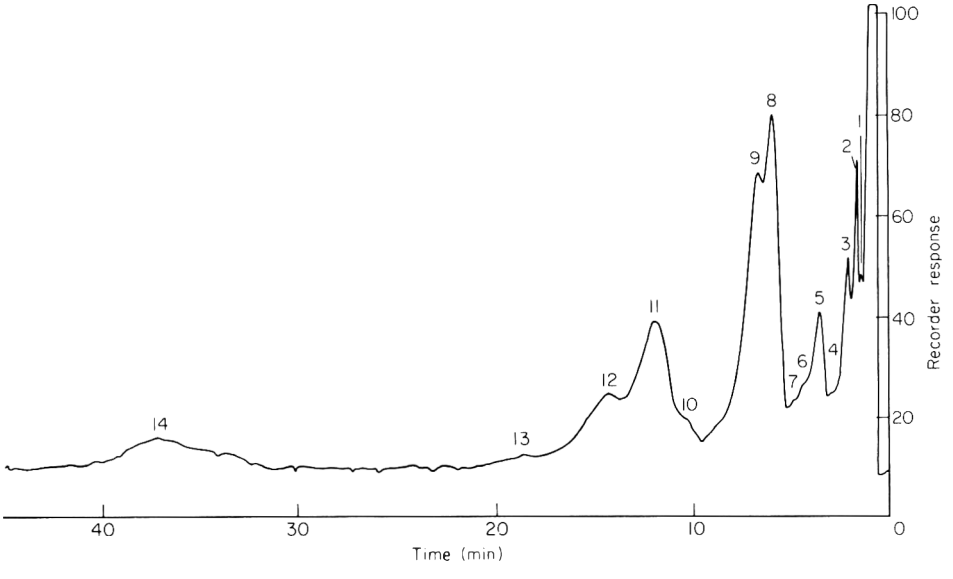

Figure 2. Gas-liquid chromatogram of methyl esters of neutral lipid fatty acids of fresh frog leg meat. Column: 8′ × 1/8″ stainless steel 15% DEGS on Chromosorb W (80–100 mesh). Temperature: 182°C.

the palmitic acid formed 25% of the total fatty acids of the Bull frog meat lipids. In the present study the saturated fatty acids were found in a higher quantity than the unsaturated fatty acids. The value obtained for palmitic acid (21.1%) is almost equal to that reported by McMullin *et al.* (1968) in bull frog. But Hilditch (1947) has reported a low value of 11%. 10:0, 12:0, 13:0, 15:0 and 22:0 fatty

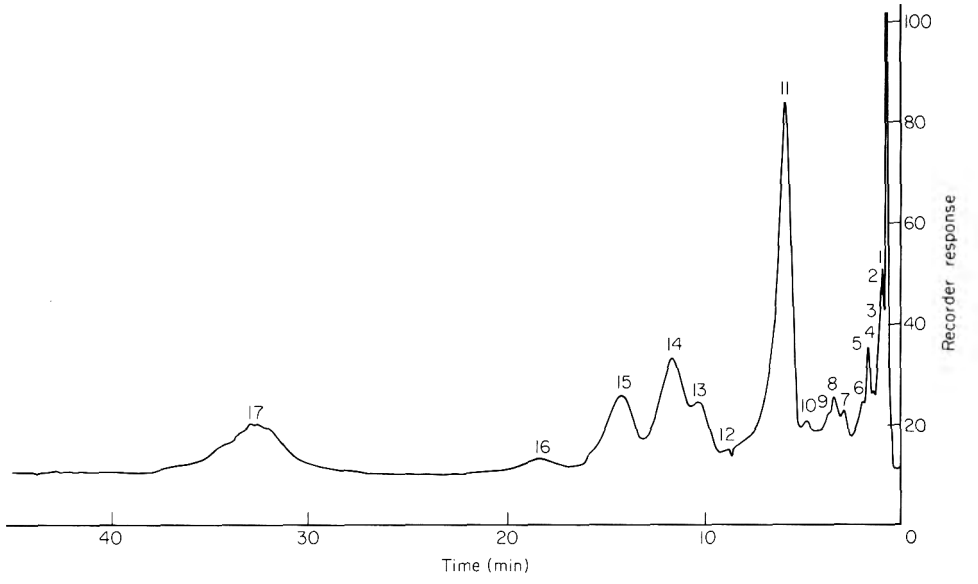

Figure 3. Gas-liquid chromatogram of methyl esters of neutral lipid fatty acids of frozen frog leg meat stored at −20°C for 150 days. Column: 8′×1/8″ stainless steel 15% DEGS on Chromosorb W (80–100 mesh). Temperature: 182°C.

acids were noticed in this investigation which have not been reported by other workers earlier. The stearic acid content (7.6%) agrees well with the values reported by Pathak & Agrawal (1966) and McMullin *et al.* (1968) which are 8.2 and 8.9 per cent respectively. Hilditch (1947) has reported the stearic acid content to be only 3.1% in frog fat which is a very low value when compared to the result obtained in the present study and those reported by earlier workers.

Among the unsaturated fatty acids, according to Hilditch (1947), frog fat has an oleic acid (18:1) content of about 52% which is higher than those reported by other workers and the value observed in the present study. While Pathak & Agrawal (1966) observed the C_{18} unsaturated fatty acid content to be 38%, McMullin *et al.* (1968) reported a value of only 18% for oleic acid. In the present study the value was found to be only 14%.

Some short chain fatty acids (suspected to be C_8 and C_{10}) were also noticed which has not been reported by other workers. These unidentified short chain fatty acids amounted to 7.3 per cent of total fatty acids.

The major fatty acids found were 16:0, 18:1. 18:0, 16:1 acids as also reported by McMullin *et al.* (1968). 22:0 acid was to the extent of 9.9% which has not been reported earlier.

These differences in the fatty acid composition may be due to species difference and also to the diet of the animals.

The neutral lipid fraction fatty acids (Table 3) showed slight variation from the total lipids fatty acid composition. Two short chain unidentified acids and 10:0 fatty acid were not observed in the neutral lipid fraction initially which indicates that these short chain fatty acids are associated with phospholipids.

Table 3. Fatty acid composition of neutral lipids of frog leg meat*

	0 Day (%)	After 150 days storage at −20°C (%)
Saturated acids		
10:0	—	2.2
12:0	5.6	3.4
13:0	2.0	0.8
14:0	6.0	2.9
15:0	4.7	—
16:0	16.5	16.3
18:0	2.9	7.8
20:0	1.4	2.6
22:0	6.4	3.1
Total	45.5	39.1
Mono unsaturated acids		
10:1	8.3	4.4
14:1	5.5	2.8
16:1	14.5	7.0
18:1	13.7	20.4
Total	42.0	34.6
Poly unsaturated acid		
18:2	8.9	13.3
Unidentified acids		
(8)	—	4.0
(8)	—	6.0
(10) and (11)	3.9	2.9
Total	3.9	12.9

*Mean of two determinations.

The unsaturated fatty acids were in a higher proportion to saturated fatty acids which is quite contrary to that of total lipids fatty acid composition.

The neutral lipids of 150 days frozen stored frog leg meat showed a slightly different fatty acid composition from the fatty acid composition of initial neutral lipids. The 18:0, 20:0, 18:1 and 18:2 fatty acids had increased considerably which means to say that these fatty acids were released by the hydrolysis of lipids. Since the hydrolysis of phospholipids was apparent (as already discussed earlier), these fatty acids may be derived from the phospholipid source. The appearance of short chain fatty acids after storage makes it clear that these short chain fatty acids are derived from phospholipid hydrolysis. According to Wierzchowski (1969), the presence of short chain fatty acids, such as butyric acid can be used as a spoilage index in fish. This may well apply here proving the

fact that short chain fatty acids released by lipid hydrolysis is an index of spoilage as suggested by him.

Conclusion

From this study it can be concluded that the changes in lipids during frozen storage of frog legs follow the same path as that of other animal meats under similar conditions, the phospholipids undergoing hydrolysis thus liberating the fatty acids and in particular the short chain fatty acids.

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Hydrolytic changes in the lipids of fish roe products during storage

J. K. KAITARANTA

Summary

Lipid hydrolysis and changes in the lipid components during cold and frozen storage of various rainbow trout and whitefish roe products were investigated over a 1-year storage period.

Rainbow trout roe lipids were composed of about 51% phospholipids and 46% triglycerides. The respective percentages in whitefish roe were 31 and 66. During the cold storage of salted roe products both of these lipids were hydrolyzed, the majority of the free fatty acids being produced from triglycerides. Lipid hydrolysis in frozen and pasteurized roe was very slow.

Lecithin as the major phospholipid comprised about 90% and 61% of the total phospholipid in rainbow trout and whitefish roe, respectively. The remainder was composed mainly of cephalin since all the other components amounted to less than 2%. Cephalin was found more susceptible than lecithin to being hydrolyzed although equal or higher amounts of free fatty acids were released in the roe products from lecithin due to its abundance.

Introduction

The keeping quality of fish roe products is highly dependent on the undesirable reactions in their lipids (Kaitaranta, Vuorela & Linko, 1979). The development of lipid hydrolysis which is a major reaction can be diminished by the appropriate processing methods and storage conditions. Moreover, the other ingredients, such as salt and preservatives may have an influence on the formation of free fatty acids (FFA) in roe products (Kaitaranta *et al.*, 1979). A relationship between the rate of lipid hydrolysis and the salt concentration was recently reported also for frozen sardine (Nambudiry, 1980) whereas the earlier studies have suggested that lipolysis in fish and fish products proceeds independently of the salt content (Lovern, 1962).

Author's address: Technical Research Centre of Finland, Food Research Laboratory, SF-02150 Espoo 15, Finland.

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Lipid hydrolysis is influenced by the enzymes of fish tissues themselves and is not noticeably affected by microbial enzymes and is not carried out by non-enzymic processes (Olley & Lovern, 1960). In some fish species and tissues, phospholipids (PL) alone have been found responsible for the formation of FFA (Lovern & Olley, 1962; Olley, Pirie & Watson, 1962; Bligh & Scott, 1966; Nair, Gopakumar & Nair, 1976), whereas in other studies triglycerides (TG) have been reported to break down into FFA (Addison, Ackman & Hingley, 1969; Awad, Powrie & Fennema, 1969; Bosund & Ganrot, 1969; Wood & Hintz, 1971; Braddock & Dugan, 1972; Nair *et al.*, 1978). Differences are also found in the susceptibilities of various phospholipid components to lipid hydrolysis (Bligh & Scott, 1966; Bosund & Ganrot, 1969; Wood & Hintz, 1971; Braddock & Dugan, 1972).

The aim of this study was to investigate lipid hydrolysis in fish roe products during cold and frozen storage. The hydrolysis rate was measured using the acid value while quantitative thin-layer chromatographic (TLC-FID) and high-performance liquid chromatographic (HPLC) techniques were applied to the studies on the changes in neutral lipids and phospholipids, respectively.

Materials and methods

Rainbow trout roe products

Mature roe of rainbow trout (*Salmo gairdneri*) was obtained from a commercial fish farm as a by-product during the slaughter of the fish. The roe bags were opened and loose eggs were thoroughly washed with tap water to remove ovarian fluid, blood and membrane particles. After draining for about 15 min on a wire net, the roe was used for processing. One part of the roe was immediately packed into glass jars with 130 g of roe in each. These tightly capped jars were then frozen in an air blast freezer and stored at −20°C (Product T1). The remaining part of the roe was cured with iodine-free sodium chloride to give a salt content of 5% (by weight). The roe was then left overnight at 2°C for the equilibration of the salt. The cured roe was then packed into glass jars, half of the jars being frozen and stored as above (Product T2) while the remaining jars were stored as such at 2°C (Product T3).

Whitefish roe products

Mature roe of whitefish (*Coregonus albula*) was obtained from a commercial fish catch during the spawning season. The roe bags were removed and the eggs washed as those of rainbow trout. One part of the roe was packed into loosely capped glass jars with 100 g of roe in each and then stored overnight at 2°C. The remaining part of the roe was cured in the manner described above except that 14% (by weight) of salt was used (Product W3). The following day all the jars were hermetically sealed. The cured roe was stored at 2°C. One part of the non-cured roe was frozen in an air blast freezer and stored at −20°C (Product

W1) while the other part was pasteurized for 40 min at 80°C before storage at 2°C (Product W2).

Assessment of lipid hydrolysis

Lipid hydrolysis in the roe products during storage was followed regularly by successive determinations of the acid value (AV). One jar of each product was opened at a time and the lipids were extracted with a mixture of chloroform and methanol following the method of Bligh & Dyer (1959). The extract was concentrated and dried under vacuum in a rotary evaporator for weight determination. The acid value of the total lipid was measured according to the AOCS Method Te 1a–64. The remaining part of the lipid was dissolved in $CHCl_3$ and stored under a nitrogen atmosphere at −20°C for further analysis.

TLC-FID

Aliquots of the lipids extracted from the roe products after 0, 30 and 52 weeks of storage were subjected to the lipid class determinations by the quantitative TLC-FID method (Kaitaranta, 1980; Kaitaranta & Ackman, 1981; Kaitaranta & Nicolaides, 1981). Samples composed of 1–10 μg of the total lipid were spotted on silica coated quartz rods (Chromarod-S, Iatron Laboratories Inc., Japan). The TLC separation was performed by the use of a two-step development applying the mixtures of petroleum ether : benzene : formic acid (92 : 17 : 1, by vol) and petroleum ether : diethyl ether : formic acid (97 : 4 : 1, by vol). After development the rods were scanned on an Iatroscan TH-10 analyzer (Iatron Laboratories Inc., Japan). The combination of the instruments was as described by Sipos & Ackman (1978).

Quantitative lipid mixtures resembling the anticipated compositions of the samples were spotted on Chromarods and developed simultaneously with the sample rods. Based on the compositions of these mixtures and the peak areas measured for the individual lipid components, appropriate conversion factors were separately calculated for each series of analyses and applied to the sample quantitations.

HPLC-Automated phosphorus analysis

The phospholipid determination of the total lipid samples extracted after 0, 30 and 52 weeks of storage was performed using an HPLC separation of the phospholipid components followed by the automatic phosphorus analysis of the eluate.

A Milton Roy Instrument mini-pump (LDC Division, Milton Roy Co., Riviera Beach, FL, USA) was used to provide a solvent flow of acetonitrile : methanol : water (80 : 15 : 6.5 or 50 : 45 : 6.5, by vol) for a stepwise elution system on a μPorasil column (3.9×300 mm, Waters Assoc. Inc., Milford, MA, USA). An automatic phosphorus analyzer (Alsab Scientific Products Inc., Los

Angeles, CA, USA) was used to monitor and quantitate the phospholipid components in the eluate as described in detail by Kaitaranta & Bessman (1981).

Results

The development of lipid hydrolysis in the roe products as indicated by the acid value is shown in Fig. 1. In the salted, cold stored products (T3, W3), the AV increased reaching the level of about 25 mg KOH/g of oil after one year of storage. As compared to the initial level of 3 mg KOH/g, the acid values of 6.3 and 7.3 mg KOH/g at the end of the storage time for the pasteurized and frozen whitefish roes, respectively, showed a very slow hydrolysis of lipids. In the frozen trout roe products (T1, T2) the rate of lipid hydrolysis was negligible.

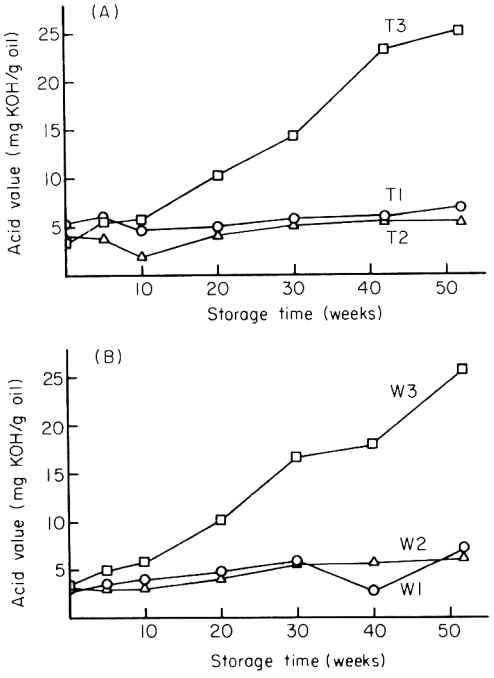

Figure 1. Changes in the acid value (AV) of various rainbow trout (A) and whitefish (B) roe products during storage. Trout roe products: T1 = frozen roe; T2 = salted (5%) and frozen roe; T3 = salted (5%) and cold stored roe. Whitefish roe products: W1 = frozen roe; W2 = pasteurized and cold stored roe; W3 = salted (14%) and cold stored roe. For the details of processing and storage see 'Materials and methods'.

Triglycerides and phospholipids occurred in almost equal amounts in the trout roe lipids and composed more than 95% of the total lipid (Table 1). Free fatty acids which were not detected in the Iatroscan analyses of the fresh roe lipids occurred in small amounts in all the trout roe products after 30 weeks of

Table 1. Lipid class compositions of fresh rainbow trout and whitefish roe, and changes in the component lipids during storage of various roe products

Roe product* + storage time	Lipids (percentage wet wt)	Percentage composition†							TG/PL ratio
		UNK‡	PL	CHOL	TG	FFA	WE+SE	HC	
Trout roe, unprocessed	8.8	—	51.0	2.4	46.4	—	0.2	0.1	0.9
T1, 30 weeks	8.4	—	51.3	2.1	46.2	0.1	0.3	0.1	0.9
52 weeks	8.5	—	52.0	1.9	45.8	0.2	traces	0.1	0.9
T2, 30 weeks	7.3	—	49.0	1.9	48.6	traces	0.2	0.2	1.0
52 weeks	8.6	—	50.1	2.3	47.4	0.1	0.2	traces	0.9
T3, 30 weeks	7.8	0.3	54.3	2.1	42.1	0.9	0.2	0.1	0.8
52 weeks	7.1	0.4	52.6	2.2	42.6	1.6	0.3	0.4	0.8
Whitefish roe, unprocessed	8.7	—	31.3	1.3	65.6	—	1.5	0.3	2.1
W1, 30 weeks	8.9	—	31.4	1.3	64.4	0.2	2.7	traces	2.1
52 weeks	8.8	0.1	34.0	1.5	61.6	0.2	2.4	0.2	1.8
W2, 30 weeks	8.9	—	31.0	1.4	66.2	—	1.4	0.1	2.1
52 weeks	8.8	—	32.5	1.6	63.8	—	2.1	traces	2.0
W3, 30 weeks	7.8	0.6	34.8	1.2	61.0	0.8	1.6	traces	1.8
52 weeks	7.3	2.1	31.3	1.6	59.5	2.8	2.4	0.2	1.9

*Roe products T1–T3 and W1–W3 as in Fig. 1.
†Abbreviations of lipid components as in Fig. 2.
‡UNK = unknown.

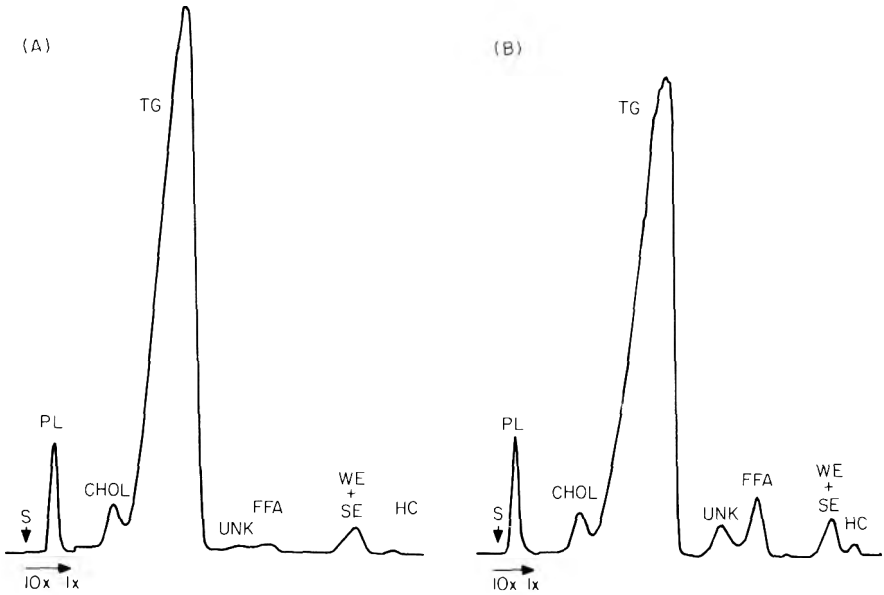

Figure 2. Iatroscan chromatograms of the lipids extracted from frozen stored (A) and cold stored (B) whitefish roe after one year of storage. Analytical methods are presented in the text. Note attenuation change from 10× to 1× after phospholipid position. S = start of scan; PL = phospholipid; CHOL = cholesterol; TG = triglyceride; FFA = free fatty acid; SE = sterol ester; WE = wax ester; HC = hydrocarbon; UNK = unknown.

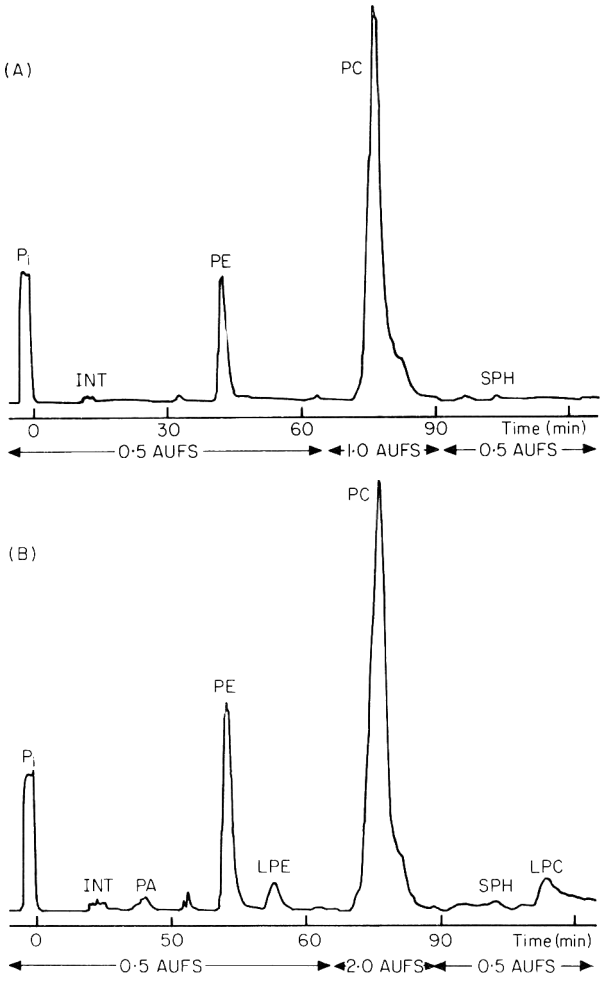

Figure 3. Separation of the phospholipids from fresh rainbow trout roe (A) and of those from salted (5%) roe after 1 year of storage (B) using a combined HPLC–automatic phosphorus analyzer. Analytical methods are presented in the text. Note absorption full scale (AUFS) changes during the analyses. PC = lecithin; LPC = lysolecithin; PE = cephalin; LPE = lysocephalin; SPH = sphingomyelin; PA = phosphatidic acid; P_i = external phosphate standard, 20 nmol; INT = neutral lipid interference.

storage while higher proportions (1.6%) were measured after 52 weeks in the cold stored roe (T3).

The proportion of triglycerides exceeded more than twice that of phospholipids in whitefish roe (Table 1) and as in the trout roe these two lipid classes dominated in the total lipid. In the pasteurized roe (W2) no FFA was detected even at the end of the storage while after 30 weeks of cold storage small amounts of FFA were found in the salted product (W3) and higher proportions (2.8%) after one year. An unidentified component appeared in the Iatroscan chromatograms of the lipids of the salted roe products (T3, W3) after 30 weeks of cold storage (Table 1). The same component was also detected after one year of storage in the frozen whitefish roe product (W1). This component which is more

Table 2. Phospholipid composition of fresh rainbow trout and whitefish roe, and changes in the component lipids during storage of various roe products

Roe product* + storage time	Percentage composition†						PC/PE ratio
	PA	PE	LPE	PC	SPH	LPC	
Trout roe, unprocessed	—	9.4	—	89.8	0.8	—	9.6
T1, 30 weeks	—	8.7	traces	90.4	1.0	traces	10.4
52 weeks	traces	8.8	0.2	89.9	1.1	traces	10.2
T2, 30 weeks	—	9.4	traces	89.2	1.4	traces	9.5
52 weeks	—	8.2	0.3	90.2	1.3	traces	11.0
T3, 30 weeks	0.7	7.8	0.5	89.3	1.0	0.8	11.5
52 weeks	0.7	6.9	1.0	86.4	1.6	3.4	12.5
Whitefish roe, unprocessed	—	36.6	1.1	61.4	0.5	0.4	1.7
W1, 30 weeks	—	35.0	0.7	63.0	1.4	traces	1.8
52 weeks	—	37.9	1.4	60.7	traces	traces	1.6
W2, 30 weeks	0.3	34.9	0.8	61.7	1.1	1.2	1.8
52 weeks	traces	33.9	0.5	64.9	0.7	traces	1.9
W3, 30 weeks	1.9	26.6	5.2	57.0	2.2	7.2	2.1
52 weeks	0.5	22.1	4.2	49.6	3.7	19.9	2.2

*Roe products T1–T3 and W1–W3 as in Fig. 1.
†Abbreviations of lipid components as in Fig. 3.

polar than free fatty acids in its chemical nature probably represents a group of oxidized fatty acids.

High-performance liquid chromatograms of phospholipids from fresh trout roe and from the product T3 after one year of cold storage are compared in Fig. 3. Lecithin and cephalin composed about 99% of the total phospholipid in fresh trout roe whereas their lyso forms together with phosphatidic acid (PA) formed about 5% of phospholipids in the salted product stored for one year at 2°C (Table 2). As a result of lipid hydrolysis small amounts of lysophospholipids were present in all the trout roe products after 30 weeks of storage but during the prolonged experiment their amount increased significantly only in the cold stored product (T3). Lysophospholipids occurred already in fresh whitefish roe (Table 2). After 30 weeks of storage, LPE amounted to 5.2% of the phospholipid in the salted, cold stored roe (W3) but no further increase was measured. The LPC content increased throughout the investigation period, reaching almost the 20% level at the end of the study. Phosphatidic acid, which also results from lipid hydrolysis, was not found to accumulate to any significant degree even in the products where the largest amount of lysophospholipids was found (Fig. 3, Table 2).

Discussion

Changes in the total lipids during storage

Lipids comprise approximately 9% of the wet weight in both rainbow trout and whitefish roe (Table 1). Their amount in the frozen or pasteurized roe products at different times of storage did not show any marked variation. This was not unexpected since only minor changes were measured for the respective acid values (Fig. 1). The lipid recoveries after 30 weeks of storage for the salted, cold stored roes (T3, W3) did not differ much from those for unprocessed roe whereas distinctly lower values were measured after the storage of one year (Table 1). Lipid losses up to 30% have been recorded by Hardy, McGill & Gunstone (1979) for frozen cod stored for 200 days at −10°C. Those losses were attributed to a selective hydrolysis of phospholipids resulting in free fatty acids and phosphatidic acid which were not quantitatively recovered by the extraction method. The chloroform-methanol extraction has also been criticized for a poor recovery of lysophospholipids (Bjerve, Daae & Bremer, 1974). As the lipid recoveries decreased also in the present study on roe products some components arising from lipid hydrolysis were apparently lost during the lipid extraction but the losses could not be accounted for any specific group of lipids.

Development of lipid hydrolysis

Previous studies on the keeping quality of various whitefish roe products (Kaitaranta *et al.*, 1979) demonstrated a decreased rate of lipid hydrolysis in heavily salted (12%, by weight) roe when compared to the respective product with a light salt content (5%, by weight). In a more detailed study on the effect of salt on lipolysis in frozen sardine Nambudiry (1980) observed that the increases from 2 to 8% in the salt content resulted in a gradually decreasing rate of lipid hydrolysis. In the present study differences in the development of acid value were not found between the salted, cold stored trout and whitefish roe products (T3, W3) in spite of the varying salt contents which were 5 and 14%, respectively (Fig. 1). This result suggests that the lipolytic enzyme systems in these roes have different sensitivities to the inhibitory effect of salt.

Freezing was an effective method to retard lipid hydrolysis as did pasteurization when combined with cold storage (Fig. 1). The 5% salt addition in trout roe (T2) before freezing has been found to improve the organoleptic quality of the product (Kaitaranta *et al.*, unpublished results) but it did not seem to affect lipid hydrolysis. In the pasteurized whitefish roe, lipid hydrolysis proceeded in a similar way to that in frozen roe (Fig. 1). Thus pasteurization effectively inactivates the lipolytic enzymes in roe though, on the other hand, it may cause discoloration as discussed earlier (Kaitaranta *et al.*, 1979).

The acid values which were measured for the salted, cold stored products T3 and W3 at the end of the storage period correspond to about 13% when converted to express the amount of free fatty acids as a percentage of the total

lipid in the product. Based on the lipid contents and compositions of these products before and after the storage (Tables 1 and 2) the decreases and increases in the individual lipid groups can be calculated. Assuming that these changes originate from lipid hydrolysis only, the released FFA amounts can be estimated using the conversion factors 0.956, 0.708 and 0.756 for triglyceride, lecithin and cephalin, respectively (Kinsella *et al.*, 1975). Such calculations predict the FFA values of 14.5 and 9.7% to the trout and whitefish roe products, respectively. These values are in a reasonable agreement with those based on the acid values. On the contrary, the Iatroscan analysis repeatedly gave lower FFA values than those obtained from the AV determinations. The acid values of the fresh roe products ranged from 3 to 5 mg KOH/g (Fig. 1) corresponding to the FFA values of 1.5–2.7% but no FFA was detected in the Iatroscan analysis. Further, the Iatroscan studies revealed only 1.6 and 2.8% FFA in the lipids of the products T3 and W3, respectively, after the storage period (Table 1). These percentages differ remarkably from those based on the AV determinations. Probably part of the free fatty acids formed during storage have oxidized and formed compounds which can influence the non-specific AV determination but which are not detected as fatty acids in the Iatroscan analysis. This explanation, however, does not cover the whole discrepancy since oxidation could not have occurred in the fresh products. Correlation between the titrimetric acid value determination and the thin-layer chromatographic Iatroscan analysis remain to be studied later.

Neutral lipids and the FFA production

The major lipids in the roe of trout and whitefish are triglycerides and phospholipids (Table 1) as shown earlier (Kaitaranta, 1980; Kaitaranta & Ackman, 1981). Considering the lipid pattern, these two lipid classes are the only components to release FFA in any significant amounts during lipid hydrolysis. In the cold stored trout roe (T3) the triglyceride content, as calculated from the data in Table 1 and taking into account also the 5% salt content in the product, decreased from 4.1 g/100 g of roe to 3.5 g/100 g within 30 weeks of storage and further to 3.2 g/100 g by the end of the experiment. This total change corresponds to a more than 20% decrease in the TG content. Accordingly it can be calculated that the concomitant change in phospholipids was only about 10%. Thus triglycerides are largely responsible for the FFA production in the cold stored trout roe (T3). The same result is obtained if the changes in the TG/PL ratio are compared (Table 1). This ratio in the trout roe product, T3, decreased from 0.9 to 0.8 during the storage showing a higher rate of hydrolysis in triglycerides as compared with phospholipids. Using the above mentioned conversion factors (Kinsella *et al.*, 1975) for the estimation of the FFA release corresponding to the losses in the TG and PL contents, respectively, it can be concluded that about two thirds of the FFA originate from TG and one third from PL.

Similar calculations concerning the salted, cold stored whitefish roe product

(W3) revealed a decrease of about 11% and 2% in the TG and PL contents, respectively, during the storage of one year. Concomitantly the TG/PL ratio decreased from 2.1 to 1.9. Both of these calculations show again an important role for triglycerides in the hydrolysis of roe lipids. About 60% of the total FFA release in this whitefish roe product when calculated as above was accounted for TG and the remaining 40% for PL. According to Awad *et al.* (1969) about 40% of the free fatty acids released in the muscle of another whitefish (*Coregonus clupeiformis*) during frozen storage was probably derived from triglycerides. The higher proportion of the FFA originating from triglycerides in the trout and whitefish roe products as compared to that in the whitefish muscle may be attributed to the differing amounts and compositions of lipids in these tissues. Moreover, the salt addition to the roe products may have had a diverse effect on the activity of lipases and phospholipases which are responsible for the hydrolytic reactions in triglycerides and phospholipids, respectively.

Phospholipids and lipid hydrolysis

A typical pattern of phospholipids in marine fish having phosphatidyl choline (lecithin) as the major component followed by phosphatidyl ethanolamine (cephalin) is also seen in roe (Table 2). About 90% of the total phospholipid in trout roe was composed of lecithin, the two other components, cephalin and sphingomyelin making up 9.4 and 0.8%, respectively. Excluding sphingomyelin this result is quite in agreement with that of Nakagawa & Tsuchiya (1976) who reported 84.5, 8.4 and 7.1% for lecithin, cephalin and sphingomyelin, respectively, in the phospholipids of unfertilized trout roe. By contrast, a different phospholipid pattern having only 71% lecithin and more than 22% cephalin has been found in studies on trout muscle (Gray & MacFarlane, 1961). In the phospholipids of fresh whitefish roe lecithin and cephalin were present in percentages of 61 and 37, respectively, and were accompanied by small proportions ($< 2\%$) of their lyso forms (Table 2). High amounts of lysophospholipids are not usually allowed to accumulate in fish tissues because of their faculty to disrupt cells (Yurkowski & Brockerhoff, 1965).

During the course of storage the lyso forms of lecithin and cephalin were found in all roe products, this suggesting that both of these lipids were hydrolyzed. Noticeable amounts of lysolipids appeared only in the cold stored products (T3, W3), which also showed the most extensive lipid hydrolysis (Fig. 1). Accumulation of lysolipids may be due to the effect of salt on the hydrolytic enzyme systems. The process of lipid hydrolysis in fish and fish products involves normally two steps which are supposed to be catalyzed by two separate enzymes (Yurkowski & Brockerhoff, 1965). Thus the added salt may inactivate the enzyme responsible for releasing the second fatty acid from the phospholipid backbone and results in an accumulation of lysolipids. In frozen fish, however, this accumulation is not usually found (Lovern & Olley, 1962). Moreover, the existence of two separate enzymes for the completion of hydrolysis of a phospholipid molecule has recently been questioned (Hardy, 1980).

Based on the data in Table 2 and the lipid contents given in Table 1 it can be calculated that approximately 35–40% of PE was hydrolyzed in the cold stored rainbow trout and whitefish roe products (T3, W3) during the storage of one year while the respective losses of PC were 15–20%. This higher hydrolysis rate of PE was also reflected to the PC/PE ratios (Table 2) which during the storage time increased from 9.6 to 12.5 and from 1.7 to 2.2 in the trout and whitefish roe products, respectively. The calculations based on the decreases in these two lipids during the storage show that despite the fast PE hydrolysis more fatty acids originate from PC in the trout roe product and roughly equal amounts in the whitefish roe product. In their studies on frozen cod muscle Bligh & Scott (1966) also observed a higher hydrolysis rate for cephalin as compared to lecithin and the same was later confirmed by Wood & Hintz (1971) for cold stored rockfish. On the contrary, the lecithin hydrolysis is faster than that of cephalin in frozen Baltic herring and coho salmon (Bosund & Ganrot, 1969; Braddock & Dugan, 1972).

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The influence of cooking technique on dietary fibre of boiled potato

D. E. JOHNSTON*† AND W. T. OLIVER*

Summary

Samples of twenty-five different cultivars of potatoes were prepared and examined after cooking by boiling and by pressure cooking, peeled and 'in the jacket' in a factorial design to study the effect of cooking on dietary fibre fractions of potatoes. Raw samples were prepared as control.

The samples were analysed for acid detergent fibre (ADF) and lignin by published procedures. Filtration problems were encountered with the published procedure for neutral detergent fibre (NDF) giving rise to lack of reproducibility. Reproducibility was restored by introducing a short incubation of the sample with α-amylase enzyme (*Bacillus subtilis*) prior to the normal detergent digestion.

The lignin content of all samples was $< 0.25\%$ on a dry matter basis. Both ADF and NDF increased on cooking but boiled samples did not differ from pressure cooked samples. Cooking 'in the jacket' gave higher ADF values than peeled samples but the effect was not observed for NDF values.

Cultivar differences were significant for both ADF and NDF values but as the samples were all from a single season's crop in a single field a definitive ranking is not possible.

Introduction

There is currently a great deal of interest in the component of the human diet known as dietary fibre. This interest was stimulated mainly by the observations of Burkitt (1973) and Trowell (1960) on the relative incidence of certain diseases in population groups having high and low intakes of dietary fibre. Dietary fibre is not a precise term and many workers' opinions vary, especially on which minor

Authors' addresses: *Department of Agriculture for Northern Ireland, Agricultural and Food Chemistry Research Division, Newforge Lane, Belfast BT9 5PX, and †The Queen's University of Belfast, Department of Agricultural and Food Chemistry, Newforge Lane, Belfast BT9 5PX, Northern Ireland.

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components should be included or excluded under the definition (Van Soest & Robertson, 1976; Southgate, 1976). However there is broad agreement that the insoluble polymeric materials of plant cell walls such as cellulose, hemicellulose, pectin and lignin constitute the major part of dietary fibre.

The national average intake of dietary fibre has been estimated as 20.4 g per head per day based on the 1974 national food survey (Southgate, Bingham & Robertson, 1978). Figures published for the Irish diet are slightly higher at 25.9 g per head per day (Gibney & Upton, 1978). Vegetables make a greater contribution to this intake than cereals and cereal products and by virtue of being consumed widely on a regular basis potato provides a greater contribution than any other vegetable. The actual consumption of potatoes declined from 1.8 kg per head per week in 1952 to just over 0.9 kg per head per week in 1976 but had recovered to 1.2 kg per head per week by 1978 (Hollingsworth, 1978; Anon, 1980).

Textural changes which take place on cooking potatoes are linked to alterations in the cell walls (Reeve, 1977). Hughes, Faulks & Grant (1975) have studied factors influencing pectin loss in the potato but there is little information on the behaviour of other components of potato dietary fibre on cooking. The choice of steam or water as cooking medium has been reported to influence the amount of cell wall damage (Reeve & Notter, 1959) as also has the presence of cut surfaces (Hoff, 1972) – these observations being highly relevant to the domestic cooking of potatoes.

The cultivars of potato produced and consumed in the British Isles display regional variations due to local soil conditions, climate, consumer preferences and other economic pressures (Anon., 1978, 1979). The variations in cooked texture between cultivars are well known (Anon., 1977).

The current study was undertaken to determine the relative influence of cultivar and cooking method on dietary fibre and hence the potential variation in dietary fibre intake.

The difficulties of establishing an agreed definition of fibre have given rise to a multiplicity of analytical procedures. The older term 'crude fibre' is now generally agreed to be inadequate but no single analysis has been found which conveys an adequate picture of the complex situation. The methods available for the analysis of dietary fibre have been reviewed by Selvendran, Ring & Du Pont (1979) and by Southgate, Hudson & Englyst (1978). In particular the procedures involving digestion in acid detergent or neutral detergent solutions developed by Van Soest (1963) and by Van Soest & Wine (1976) are less complicated than some of the multi step schemes which have been proposed and were therefore chosen for this study.

Materials and methods

Potatoes of twenty-five different cultivars were obtained from the stocks grown by the Plant Pathology Division DANI in the 1977 growing season at the

Agriculture and Food Science Centre, Newforge Lane, Belfast. Compound fertilizer (7:21:14) at a rate of 0.63 tonnes per ha was placed in the drills at planting. After harvesting potatoes were stored for about 60 days at a temperature of 12°C prior to cooking.

Samples of the twenty-five different cultivars of potato were cooked by boiling and pressure cooking at 103.4 kPa (15 lb per square inch) peeled and 'in the jacket' in a factorial design, raw samples served as controls. Two samples of each cultivar were taken for each treatment and each of these was analysed in duplicate by the methods described.

Cooking procedures

Boiling

Four tubers (combined weight about 650 g) were added to 1.5 l distilled water at room temperature in a saucepan brought to the boil in 5–10 min and then boiled for a further 35 min.

Pressure cooking

Four tubers (combined weight about 650 g) were placed in a pressure cooker (Prestige Hi-dome, the Prestige Group, Prestige House, 14–18 Holborn, London EC 1N 2QL) containing 400 ml distilled water at room temperature, brought to the boil in 5 min and steamed at 103.4 kPa for 20 min then removed from the heat and depressurized. The cooking conditions were chosen to give normal acceptable product.

Sample preparation

Those tubers which were cooked 'in the jacket' were first peeled and then all cooked tubers were allowed to cool for 1 hour. Individual tubers were then mashed into pre-weighed aluminium dishes, weighed and freeze dried. Raw samples were peeled, diced as quickly as possible into 1 cm^3 dice and blended to a smooth paste in a coffee mill (Moulinex Ltd., Station Approach, Coulsdon, Surrey CR3 2UD) weighed into aluminium dishes, quickly frozen in liquid nitrogen and freeze dried. The freeze dried samples were stored at 4°C prior to analysis.

Dry matter determination

Samples of freeze dried powder were oven dried at 100°C.

Acid detergent fibre (ADF)

Samples of freeze dried powder were analysed according to the method of Van Soest (1963).

Neutral detergent fibre (NDF)

Originally the Van Soest methods for dietary fibre were evolved for use with animal feeding stuffs but with materials high in starch the NDF method can have difficulties at the filtration step due to incomplete solubilization of the starch. Such difficulties were experienced in the early stages of this work giving rise to a serious lack of reproducibility. The standard procedure (Van Soest & Wine, 1967) was therefore modified by introducing a short incubation of the samples with bacterial α-amylase (*Bacillus subtilis*) EC 3.2.1.1. from Sigma, London (product number A6380). Freeze dried samples (1 g) were incubated in a waterbath of 40°C for 30 min with α-amylase (770 units assayed at 40°C (Robyt & Whelan, 1968)) in 10 ml of 0.2 M phosphate buffer pH 7.0 containing also 10^{-4} M calcium chloride. These conditions gave a negative iodine test for the presence of starch. The normal NDF procedure was then carried out. This modification gave reproducible results.

Lignin determination

The lignin determination was carried out by digesting the ADF residue in 72% sulphuric acid (Van Soest, 1963).

Results

The performance of each cultivar on cooking is presented in Table 1, which shows the percentage dry matter, ADF on a dry matter basis and NDF on a dry matter basis for the respective raw and cooked samples. The cooked values are the means of all four cooking treatments. The apparent increase in dry matter on cooking (significant at the 0.1% level) was partly due to the evaporative loss of water while the cooked potatoes were cooling. The range of dry matter contents of the raw potatoes was from 16.6% to 22.3%, a fairly typical range considering the early and main crop cultivars chosen but some of the main crop cultivars had lower dry matters than might have been expected from their listed characteristics.

The results for ADF on a dry matter basis ranged from 1.8–3.3% in the raw material and from 2.2–3.8% in the cooked samples. The majority of cultivars had higher ADF values when cooked but eight of the twenty-five had non-significantly higher values in the raw samples. The NDF values on a dry matter basis for the raw potatoes ranged from 1.8–4.9% and for the cooked material from 3.6–5.5%. The tendency for cooked samples to have higher NDF values was clearly seen and only three cultivars had non-significantly higher NDF values in the raw samples. No clear pattern emerged linking either ADF or NDF values to other listed characteristics of the cultivars.

The variations in ADF and NDF due to different cultivars were both significant at the 0.1% level. Nevertheless Table 1 should not be regarded as a

Table 1. Effect of cooking on dry matter, ADF and NDF of potato cultivars; means of all cooking techniques

	Percentage dry matter		Percentage ADF (DMB)		Percentage NDF (DMB)	
Cultivar	Raw	Cooked	Raw	Cooked	Raw	Cooked
Pentland Crown	18.5	18.7	2.9	3.8	3.5	5.4
Pentland Dell	20.2	21.6	2.3	3.2	4.9	4.9
Pentland Hawk	21.3	21.5	2.9	3.1	4.1	4.4
Pentland Squire	21.2	22.2	2.2	2.6	3.3	5.5
Arran Banner	19.0	20.7	1.8	2.4	3.0	4.7
Arran Consul	21.3	22.9	2.0	2.3	3.1	3.8
Arran Victory	22.0	22.6	2.2	2.9	3.7	3.8
Maris Page	20.9	22.7	2.3	2.7	3.9	4.0
Maris Peer	20.3	22.6	2.0	2.5	2.6	4.7
Maris Piper	21.6	22.4	2.0	2.7	1.8	3.8
Kerrs Pink	20.7	23.7	2.9	2.8	4.2	3.9
King Edward	17.4	19.2	3.3	3.2	4.3	4.8
Majestic	19.0	22.1	3.1	2.9	4.8	4.6
Record	22.3	23.0	3.0	2.8	4.3	5.2
Home Guard	20.2	23.4	2.3	2.2	3.3	3.7
Ballydoon	16.6	20.9	2.0	3.0	3.9	4.4
Desirée	17.4	19.9	2.8	2.9	4.2	4.4
Wilja	21.0	21.6	2.7	2.5	3.4	3.6
Stormont Enterprise	18.9	23.7	2.7	2.5	3.5	4.4
Ulster Concord	20.2	25.5	2.6	3.0	3.0	4.1
Ulster Prince	18.3	18.9	2.0	2.5	3.4	4.3
Ulster Sceptre	17.6	18.8	2.4	2.6	3.1	3.7
Ulster Torch	19.7	21.3	2.4	3.2	3.2	5.1
Up-to-date	19.1	21.0	2.7	2.6	4.0	3.9
Dunbar Standard	20.8	21.3	2.3	2.8	3.1	3.9

definitive ranking of cultivars on an ADF or NDF basis as the potatoes all came from one field in one growing season.

The effects of the various cooking methods on ADF and NDF, averaged over all cultivars are presented in Table 2. There was an increase in ADF on cooking which was significant at the 0.1% level and cooking 'in the jacket' gave a greater gain than cooking peeled with a 1% level of significance. Boiling gave results not significantly different from pressure cooking. An increase in NDF also took place on cooking which was significant at the 0.1% level but there were no significant differences between either pressure cooking and boiling or between cooking 'in the jacket' and peeled.

All the results of analysis for lignin were $< 0.25\%$ on a dry matter basis, the lower limit of detection with the sample size used.

Table 2. Effect of cooking technique on potato ADF and NDF; means (DMB) all cultivars

	Percentage ADF			Percentage NDF		
	Raw	Boiled	Pressure-cooked	Raw	Boiled	Pressure-cooked
'In the jacket'	2.47	2.82	2.91	3.58	4.37	4.47
Peeled	2.47	2.65	2.77	3.58	4.36	4.27
s.e. of a difference		0.08			0.14	

Discussion

The wide range of analytical methods described in the literature and the diverse chemical and physical properties of the components of dietary fibre inevitably lead to difficulties in fairly comparing different bodies of work and as a consequence interpretation of results is often more tenuous than precise.

The Van Soest detergent digestion procedures are widely used, particularly by agricultural scientists, for the determination of fibre. Originally the methods were applied to animal feeding stuffs but as interest in fibre in human nutrition grew the range of samples broadened and it became more obvious that the application of the NDF method to high starch food often caused difficulty. Various modifications have been proposed to overcome the problem. Potentially α-amylase should free the fibre from contaminating starch but opinions vary on when to introduce the enzyme and some sources of enzyme have been criticized. Baker, Norris & Li (1979) applied α-amylase treatment after detergent digestion and reported that their α-amylase from *Bacillus subtilis* had contaminating carbohydrase activity which led to unacceptable losses of NDF material. Hog pancreatic α-amylase was recommended as being free from such contamination. This experience of α-amylase from *Bacillus subtilis* was not shared by other workers (Marlett & Lee, 1980; McQueen & Nicholson, 1979). Marlett and Lee compared four modifications of the basic Van Soest procedure and found that incubation for 1 hr with α-amylase from *Bacillus subtilis* before digestion gave values which were generally not different from those obtained by post-digestion treatment of the residue with hog pancreatic α-amylase for 18 hr. The conditions evolved independently for the present study are similar to 'Modification A' reported by Marlett & Lee (1980). It was found in the present study that extending the incubation time from 30 min to 18 hr produced no significant lowering of NDF values. The coefficient of variation was 2.6%. It was presumed therefore that if contaminating carbohydrases were present they would not give rise to serious losses of NDF material under the chosen conditions.

Reports in the literature of the influence of cooking on the dry matter content of vegetables are variable but Toma *et al.* (1978) reported increases in dry

matter on boiling potatoes peeled or 'in the jacket' which were significant for two of the four cultivars studied. Although such increases were also observed in the present study dry matter measurements of cooked potatoes with and without a period of cooling demonstrated that part of the increase was due to evaporative loss of moisture during the cooling period.

Cereal products (Van Soest, 1965) and vegetables (Mathee & Appledorf, 1978) have been previously reported to have increased ADF and NDF values after cooking. Where Maillard type reactions are involved, as in toasted cereal products, the increase was reported to be due to lignin like products, insoluble in 72% sulphuric acid. The lack of measurable increase in lignin on cooking observed in this study would suggest that the apparent increases in ADF and NDF are the result of a different interaction of food components to give rise to products insoluble under the conditions of the digestion. Although cooking 'in the jacket' gives rise to higher ADF values than cooking peeled a corresponding effect is not observed for NDF values. It may be speculated that this difference arises due to substances present in ADF but not in NDF. The ADF fraction is generally considered to consist of cellulose and lignin while the NDF fraction consists of hemicellulose, cellulose and lignin. Strictly speaking however other compounds are also present. The conditions during NDF hydrolysis ensure that all pectin is rendered soluble but the low pH used during ADF hydrolysis allows part of the pectin to survive while all the hemicellulose is rendered soluble (Van Soest & Robertson, 1976). This pectin may be a cause of the difference. On cooking, pectin is lost to the cooking water (Hughes *et al.*, 1975), it is possible therefore that the intact skin of a potato cooked 'in the jacket' retards the outward movement of pectin or other materials and thereby gives rise to the increased ADF value. Alternatively, calcium ions are known to stabilize pectin and reduce its loss from the cooked tissue (Hughes *et al.*, 1975) and significant losses of minerals (Toma *et al.*, 1978; True *et al.*, 1979) including calcium are reported to occur from boiled peeled potatoes but not those boiled 'in the jacket'. The advantage to be gained by cooking potatoes 'in the jacket' would be slight, about a 6% increase on average in ADF intake on a dry matter basis.

Boiling and pressure cooking differ in the temperature and duration of cooking and in heat transfer medium. Considering the differences individually might lead to differences being expected between the results from the two methods.

Longer cooking times are reported to give rise to increased values of ADF and NDF in vegetables (Mathee & Appledorf, 1978) and less cell wall damage was observed in potatoes which had been steamed rather than boiled (Reeve & Notter, 1959). However no significant differences between methods were observed for either ADF or NDF values, which would suggest that choosing conditions so that both methods gave similar normal cooked product, as judged subjectively, results in similar changes taking place in the ADF and NDF material.

The variations in both ADF and NDF which arise as a result of cultivar variation are potentially more interesting. Cell size and cell wall thickness can

vary widely with cultivar (Reeve, Timm & Weaver, 1973). The incidence of dietary fibre linked diseases is known to vary from region to region (Eastwood, 1974) so it is possible that local availability or preferences for a particular cultivar could have relevance to the fibre intake. An approximately 1½ fold variation in NDF and ADF exists between the extremes of the cultivars in this study so that careful choice of cultivar could contribute to increased fibre intake without actually increasing the weight of potato consumed.

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Effect of pre-freezing on the stability of carotenoids in unblanched air-dried carrots

S. S. ARYA, V. NATESAN, K. S. PREMAVALLI AND
P. K. VIJAYARAGHAVAN

Summary

Freezing before conventional hot-air drying considerably improved the stability of carotenoids and lipids in dehydrated carrots. Freezing, however, did not influence the peroxidase activity, glycine, alanine, glutamic acid, aspartic acid, arginine and serine exhibited pro-oxidant effect while threonine and valine did not have a significant effect on β-carotene stability. Histidine, leucine, isoleucine, tryptophan, ascorbic acid and quercetin exhibited antioxidant effect on β-carotene degradation.

Introduction

The effect of various processing parameters on the stability of carotenoids in dehydrated carrots was reported previously (Arya *et al.*, 1979). It was observed that the rate of carotenoid destruction was significantly higher in blanched freeze-dried carrots than in unblanched freeze-dried carrots. However, in the conventional hot air drying process blanching is known to significantly enhance the stability of carotenoids during storage (Feinberg *et al.*, 1964). Since in the freeze drying process, the carrots are first frozen and subsequently moisture is removed by sublimation, the effect of freezing before conventional hot-air drying on the stability of carotenoids during storage was investigated.

Materials and methods

Dehydrated carrots

Good quality carrots (*Daucus carrota*) purchased from a local market were trimmed, hand peeled and washed. The washed carrots were sliced (3–4 mm thick), mixed thoroughly and divided into three equal lots; one lot was dried as

Authors' address: Defence Food Research Laboratory, Mysore-570011, India.
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such without any treatment. The second lot was blanched in boiling water for 6 min and subsequently dried while the third lot was frozen overnight in a blast freezer at − 10°C, thawed and subsequently dried. Sliced carrots (3 kg) were also dipped in 3% salt solution containing 0.2% sodium metabisulphite for 20 min, frozen and dried. All drying operations were carried out in a Kilburn hot-air drier at 70°C to a final moisture level of 4–5%.

Storage

The dehydrated carrots (50 g) were packed in paper-aluminium foil-polyethylene laminate pouches and stored at room temperature (16-35°C). Initially and periodically samples were analyzed for total carotenoids, TBA value, peroxide value and moisture content according to procedures described previously (Arya *et al.*, 1979). Peroxidase activity was measured according to the method of Maehly and Chance (1954) and expressed as mg equivalents of horse radish peroxidase per g of dry carrot.

Isolated systems

Weighed quantities of glycine, leucine, histidine, valine, arginine, tryptophan and isoleucine (each 0,007 moles) and ascorbic acid (200 mg) and quercetin dihydrate (100 mg) were suspended in alcohol (20 ml), quantitatively transferred to microcrystalline cellulose (50 g) and thoroughly ground in a pestle and mortar for uniform mixing. The powder was transferred to a RB flask and β-carotene (85 mg) dissolved in 50 ml benzene was added to the flask and contents swirled for uniform mixing. The solvent was evaporated under vacuum using a rotary vacuum evaporator. The impregnated cellulose was stored in petri dishes over saturated salt solutions in desiccators.

Results and discussion

Changes in total carotenoids, TBA value and peroxide value of unblanched, blanched and pre-frozen hot-air dried carrots during storage are shown in Table 1. It may be observed that relatively carotenoids are more stable in blanched than in unblanched air-dried carrots. Also, freezing before conventional hot-air drying considerably enhanced the stability of carotenoids and lipids in dehydrated carrots. It is interesting to observe that in pre-frozen dehydrated carrots about 90% of initial carotenoids were retained compared to 19.5% in unblanched and 40.1% in blanched dehydrated carrots after 3 months of ambient storage. Also the rate of lipid peroxidation as measured by changes in TBA and peroxide values was considerably lower in pre-frozen dehydrated carrots than in blanched and unblanched dehydrated carrots. Pre-frozen dehydrated carrots, however, browned at a faster rate and accordingly the

Table 1. Changes in total carotenoids, TBA value and peroxide value during processing and storage of air-dehydrated carrots.

Storage period (months)	Unblanched			Blanched			Frozen unblanched		
	Carotenoids (μg/g)	TBA value*	Peroxide value†	Carotenoids (μg/g)	TBA value*	Peroxide value†	Carotenoids (μg/g)	TBA value*	Peroxide value†
0	697.3	0.65	10.8	1056	0.57	10.0	817.3	0.38	8.1
1	491.5 (70.5)	0.87	12.8	753.2 (71.3)	0.69	11.8	774.0 (94.7)	0.56	8.1
3	135.5 (19.5)	1.20	13.3	423.0 (40.1)	0.83	10.6	739.8 (90.5)	0.64	6.4
6	101.5 (14.6)	1.38	—	254.3 (24.1)	1.05	—	670.0 (82.6)	0.71	—
8	60.5 (8.7)	1.33	—	217.3 (20.6)	1.00	—	652.7 (79.9)	0.68	—

Values in parenthesis indicate percentage retention of carotenoids.
*mg of malonaldehyde per kg sample.
†milliequivalents of O_2 per kg sample.

Table 2. Effect of pre-freezing and bisulphite treatment on the stability of carotenoids in unblanched air-dried carrots.

Storage period (months)	Frozen-unblanched			Salt and bisulphite dipped frozen unblanched		
	Carotenoids (μg/g)	TBA value*	NEB†	Carotenoids (μg/g)	TBA value*	NEB†
0	717.4	0.28	0.42	770.8	0.19	0.19
2	602.7 (84.0)	0.48	0.50	688.7 (89.3)	0.48	0.25
4	542.1 (75.6)	0.33	0.56	634.0 (82.3)	0.23	0.43
6	491.7 (68.6)	0.47	0.58	573.0 (74.0)	0.28	0.44

Values in parenthesis indicate percentage retention of carotenoids.
*mg of malonaldehyde per kg substance.
†non-enzymic browning reported as optical density 420 nm.

effects of sodium metabisulphite and salt treatment were investigated. The results are shown in Table 2. Metabisulphite treatment significantly reduced the formation of browning compounds both during dehydration and subsequent storage. Salt and metabisulphite treatment also improved the colour, texture and reconstitution characteristics of pre-frozen dehydrated carrots. The product retained excellent flavour and was devoid of hay-like odours, normally associated with carotenoid degradation in stored dehydrated carrots.

The beneficial effect of blanching on the stability of carotenoids and lipids during storage is generally believed to be due to the inactivation of peroxidase and lipoxidase activities which otherwise catalyse their destruction during dehydration and storage. Freezing of carrots, before conventional hot air drying, however, did not significantly influence the peroxidase activity which varied between 75.6 and 107.6×10^{-5} for fresh carrots and between 76.7 and 109.4×10^{-5} for frozen carrots. Peroxidase activity in unblanched dehydrated and pre-frozen dehydrated carrots was also not significantly different and ranged between 1.8 and 8.0×10^{-5}. There was no detectable lipoxidase activity in carrots. Previously, Pinsky, Grossman & Tropp (1971) did not find any lipoxidase activity in carrots but Rhee & Watts (1966) reported very low lipoxidase activity in carrots. Obviously, enhanced stability of carotenoids and lipids in pre-frozen dehydrated carrots compared to unblanched dehydrated carrots is not due to changes in peroxidase or lipoxidase activities. Pinsky, Grossman & Tropp (1971) have reported the presence of water-soluble antioxygenic compounds in carrots. It is most likely that these substances exist in physically bound form in intact carrot resulting in very limited access to the actual site of oxidation. Freezing seems to disrupt this binding, thereby increasing the mobility of these naturally occurring antioxidants which results in their enhanced antioxygenic action. Earlier, Weier (1944) had suggested the liberation of water soluble antioxidants in carrots as a result of blanching. However, direct evidence for the same has not been obtained.

Blanching is known to result in significant loss of water soluble substances. It has been reported that about 10–30% of total solids are lost due to leaching during steam and hot water blanching (Baloch, Buckle & Edwards, 1977; Gooding, Tucker & MacDougall, 1960); some of these substances may have antioxygenic effect. The antioxygenic effect of bioflavonoids and other polyphenolic compounds present in carrots had been reported previously but the role of amino acids and ascorbic acid, which also get leached out during blanching, on carotenoid stability is not known. In the present study the effect of these was studied in isolated systems at three levels of water activities and the results are given in Table 3. For the sake of brevity only the means of the ratios of β-carotene lost with and without additives are presented. All values less than 1.00 ± 0.05 indicate significant antioxygenic effect while more than 1.00 ± 0.05 indicate a pro-oxidant action.

It can be seen that glycine, alanine, arginine, glutanine, glutamic acid and aspartic acid accelerated the rate of β-carotene degradation while valine and threonine had practically no effect on β-carotene stability. Histidine, leucine, isoleucine and tryptophan, however, significantly, stabilized β-carotene in isolated systems. Free amino acids composition of carrots has been reported by Otsuka & Take (1969); glutamic acid, serine, threonine and alanine were present in highest amount. As is evident none of these amino acids have significant antioxygenic effect on β-carotene degradation. Ascorbic acid also exhibited only a slight stabilizing action but quercetin exhibited a strong protective effect. In freeze dried emulsions containing safflower oil or linoleic acid,

Table 3. Effect of amino acids, ascorbic acid and quercetine on the degradation of β-carotene

Additive	Relative degradation*		
	0.00	0.43	0.73 a_w
Glycine	1.18	1.29	1.23
Alanine	1.50	1.47	1.50
Valine	1.00	1.03	1.50
Leucine	0.76	0.78	0.74
Isoleucine	0.87	0.80	0.79
Arginine	1.15	1.12	1.10
Histidine	0.72	0.71	0.68
Serine	1.23	1.20	1.24
Glutamic acid	1.14	1.16	1.13
Glutamine	1.20	1.17	1.12
Threonine	1.05	1.03	1.03
Aspartic acid	1.15	1.18	1.17
Ascorbic acid	0.87	0.88	0.87
Quercetine dihydrate	0.62	0.64	0.65

*Mean of ratios of β-carotene lost with additive to that without additive after 7, 10 and 13 days of storage at room temperature.

amino acids, except cysteine have been reported to exhibit a slight antioxidant effect (Farag *et al.*, 1978; Riisom, Sims & Fioniti, 1980) but in aqueous emulsions, amino acids had both pro- and anti-oxidant effects (Marcuse, 1961; Farag *et al.*, 1978; Riisom, Sims & Fionti, 1980).

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Effect of collagen content and heat treatment on protein digestibility and biological value of meat products

A. LASER-REUTERSWÄRD,*§ N.-G. ASP,† I. BJÖRCK‡ AND H. RUDÉRUS*

Summary

The digestibility and biological value of meat mixed with various amounts of collagen were measured after heat treatments.

The M. biceps femoris muscle was used as the source of meat and pigskin as the source of collagen. The latter contained 78.9% collagen and digestibility both heated and raw was 95%.

The NPU of mixtures of meat and pigskin showed a relation with collagen content defined as $y = 82.8 - 0.6\,x$.

Supplementation of pigskin at a pigskin-meat mixture containing 50% collagen with methionine did not increase NPU.

The amount of collagen in untreated meat products is 15–30% and will be of negligible nutritional significance in a whole diet.

Introduction

The nutritive value of protein from muscle is known to be very high. The muscle proteins also suffer little or no damage when processed alone and denaturation does not affect protein quality (Bender, 1978). The proteins of the connective tissue, on the other hand, are generally considered to have little or no nutritive value. Furthermore collagen fibres are more resistant than most other protein fibres to attack by proteolytic enzymes (FDA, 1978) and meat collagen is considered to be only partly digestible. The low digestibility of collagen as well as of many unprocessed proteins, is considered to be due to naturally occurring crosslinks (Cheftel, 1977, 1979). With this background the Swedish National Food Administration has proposed legislation to limit the collagen content in meat products (Jansson, 1978).

Authors' addresses: *Swedish Meat Research Institute, Kävlinge, Sweden, and †Department of Food Chemistry, Chemical Center, University of Lund, and ‡Department of Industrial Nutrition, Chemical Center, University of Lund, Lund, Sweden.

§To whom correspondence should be addressed.

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Different animal tissues and cuts of meat differ in content of connective tissue, depending on anatomic localization and physiological functions (Vognarova, Dvorak & Böhm, 1968). The proteins of the connective tissue are mainly collagen and elastin, but the amounts of elastin are very small both in muscle (Vognarova *et al.*, 1968) and in pigskin (Johns, 1977). Cuts with relatively high contents are usually in comminuted products such as sausages. A content of about 25% collagen, calculated on the crude protein is common in Sweden in comminuted products (Jansson, 1978). More than half of this collagen comes from pigskin and back fat with or without pigskin. The nutritive value of collagen in meat products is therefore of considerable interest.

The biological value (BV) of gelatin, which is solubilized collagen, is known to be very low (Bender, Miller & Tunnah, 1953). This is due to complete absence of tryptophan and low content of cystine (Eastoe, 1967). The total amount of essential amino acids is less than half of that in muscle proteins. The nutritive value of products rich in collagen has been investigated by some authors with rat assays. Studies on raw mixed meat products have shown decreasing NPU-values when the amount of collagen is increased (Dvorak, 1972; Bender & Zia, 1976). The PER-value decreases linearly with increasing collagen content in a mixture of raw beef round and partially defatted chopped beef (YuBang Lee *et al.*, 1978). The highest amount of collagen used in these studies was 50% of the total protein. In nitrogen balance experiments with adult humans however, Kofranyi & Jekat (1969) showed that 50% of muscle protein could be replaced with gelatin without lowering the nutritive value.

Thus data on digestibility and overall nutritional value of collagenous products are partly contradictory and difficult to evaluate. The data on NPU values so far reported are derived from carcass analysis, where BV and digestibility cannot be distinguished. The purpose of the present investigation was (a) to measure the protein digestibility of collagen of various particle sizes and after different heat treatments and (b) to study the protein quality of a highly collagenous product alone, mixed with various amounts of meat, and supplemented with amino acids.

Materials and methods

Mixtures of beef and pigskin were prepared in order to obtain samples with controlled contents of collagen and calculated on the basis of nitrogen. Heating parameters were chosen to be as close as possible to the conditions for sausage production and reheating respectively. The design of the experiments is shown in Table 1.

Preparation of meat samples

Muscles biceps femoris from beef and pigskin were obtained directly after the cutting process from a commercial slaughterhouse. The beef sample was carefully trimmed of excess fat and connective tissue and the pigskin was freed from

Table 1. The nutritional values of mixtures of beef and pigskin heated under different conditions

Sample	Material	Mixture* (%)	Heating† conditions (°C)	TD‡	BV‡	NPU‡
1:1	Reference§			100.3 (0.9)	93.4 (1.4)	93.7 (1.9)
1:2	Beef/pigskin	100/0	raw	99.8 (0.4)	81.3 (4.2)	81.1 (3.9)
1:3	"	50/50	raw	99.2 (1.1)	66.3 (8.4)	65.8 (8.3)
1:4	"	100/0	74	100.3 (0.6)	78.1 (4.7)	78.4 (5.1)
1:5	"	50/50	74	99.3 (1.5)	58.8 (3.8)	58.4 (4.6)
1:6	"	100/0	95	99.0 (1.9)	83.2 (2.2)	82.3 (2.6)
1:7	"	50/50	95	99.2 (1.5)	64.9 (7.8)	64.3 (7.3)
2:1	Reference§			99.8 (0.8)	92.2 (2.2)	92.0 (2.5)
2:2	Beef/pigskin	100/0	74	99.4 (0.9)	78.4 (5.9)	77.9 (5.3)
2:3	"	80/20	74	100.4 (0.9)	68.5 (3.1)	68.8 (2.5)
2:4	"	60/40	74	100.6 (1.8)	64.1 (3.9)	64.5 (3.6)
2:5	"	40/60	74	99.3 (0.8)	56.1 (3.0)	55.7 (2.8)
2:6	"	20/80	74	97.6 (1.6)	44.6 (5.9)	43.5 (5.0)§§
2:7	"	0/100	74	95.3 (3.2)	32.9 (7.0)	31.2 (5.6)
3:1	Reference§			100.4 (0.7)	93.1 (1.9)	93.5 (1.9)
3:2	Pigskin + 0.40% Methionine		74	99.4 (0.7)	39.3 (7.0)	39.1 (7.3)**
3:3	Pigskin + 0.40% Methionine 0.15% Tryptophan		74	98.1 (1.3)	42.8 (1.8)	42.0 (2.1)
3:4	Beef/pigskin + 0.40% Methionine	40/60	74	98.5 (1.5)	56.2 (2.9)	55.3 (2.7)
4:1	Reference§			101.5 (1.4)	90.8 (1.5)	92.1 (0.9)
4:2	Pigskin ∅ < 0.8 mm		74	95.2 (2.2)	24.8 (8.3)	23.7 (8.2)
4:3	Pigskin ∅ 0.8–2 mm		74	97.1 (0.8)	22.7 (3.9)	22.1 (3.7)

* Mixtures are based on nitrogen contents
† 30 min.
‡ ± standard deviation in parenthesis
§ Reference casein + 0.22% Methionine
§§ Calculated on four rats
** Calculated on three rats

meaty parts. The samples were finely cut in a bowl chopper, deep frozen and stored for 2 months. After thawing, portions of beef and pigskin were separately packed into plastic bags of saran lacquered polyester in 1-cm thick layers, heated for 30 min in a waterbath of 74° ± 1°C and 95° ± 1°C, and then immediately chilled in ice-water. Samples were separately freeze-dried and then cut into a fine powder. The pigskin in experiment 4 was used directly after cutting, the particle diameter, measured with a sieve, being between 0.8 and 2 mm. In experiments 1–3 samples of pigskin were ground together with maize starch through a hammer mill with a filter of ∅ 0.8 mm before being mixed into the diet for rat assay.

Chemical analyses

Chemical analyses was performed on raw samples before freeze-drying. Nitrogen (N) was determined by the method of Kjeldahl. Hydroxyproline (HPRO) was determined by the method of Stegeman (1958) modified by Weber (1973). The calculation of the content of collagen was made according to Prändl, Haas & Polke (1967):

$$\text{Collagen} = \text{HPRO} \times 7.1; \; N_{\text{Collagen}} = \text{Collagen}/5.55,$$

$$\% \text{ Collagen} = \frac{\text{Collagen}}{6.25 \times (N_{\text{total}} - N_{\text{collagen}}) + \text{Collagen}} \times 100$$

Amino acid analysis

The amino acid composition was determined by duplicate analysis of beef and pigskin heated to 74°C. Acid hydrolysis was employed and the equipment used was a Beckman 120 B amino acid analyzer. Cystine and methionine were determined after performic acid oxidation. Tryptophan was determined after alkaline hydrolysis according to Vangala and Menden (1970) and analysed according to Miller (1967).

Protein quality

The nutritive value of protein was evaluated with N-balance studies on growing rats. Male Spraque Dawly rats from Anticimex, Sweden, were placed individually in metabolic cages according to Shiller (1960) and Eggum (1973). After four days of acclimatization, urine and faeces were collected during a five day balance period with 5 rats in each group. The temperature was kept at 25°C and the relative humidity at 50–60%. The diet was composed according to Eggum (1973) of 15 g N/kg dry matter and with modified contents of vitamins and minerals according to Forsum, Hambraeus & Siddiqi (1973). Maize starch was used as source of carbohydrate. ANRC Reference Protein High Nitrogen Casein (Sheffield Chem. USA) was used as reference protein. Amino acid supplementation was carried out with L-Methionine (Biochemische Zwecke, Merck) and L-Tryptophan (chromatographically homogenous, BDH). Amino acid supplementations are expressed as % of total diet prepared to contain 1.5% nitrogen, corresponding to 9.4% crude protein. Supplementation design is shown in Table 1.

The N contents of the feed, urine and faeces were analyzed by the method of Kjeldahl. Values for metabolic and endogenous N used in the calculations of TD and BV were obtained from experiments with 0.6% N from fat extracted, lyophilized whole egg as source of protein.

Results and discussion

Chemical composition

The beef contained 3.39 gN and 0.15 g HPRO per 100 g. The corresponding figures for pigskin were 4.91 and 3.10 respectively. So the collagen content in beef and pigskin were 5.1 and 78.9 per cent of the total protein respectively.

Digestibility

Data from experiment 1, Table 1, indicate that there are no significant differences in digestibility between raw and heated samples. The digestibilities of mixtures of equal parts of beef and pigskin, which correspond to 42% collagen, were more than 99% and comparable to the values for beef with only 5.1% collagen content. The lowest value of digestibility, 95%, was obtained for pigskin alone (2:7, 4:2, Table 1). In experiments 1–3 the pigskin was milled into particles with a diameter of less than 0.8 mm. Experiment 4 (4:2, 4:3 Table 1) showed that the digestibility was more than 95% even with particles of about 2 mm diam., as in most comminuted products.

Our findings are in agreement with earlier publications, all indicating that collagenous products are well digested. Mitchell & Carman (1926) considered the protein digestibility to be the same in cheap and expensive cuts of meat. Happich *et al.* (1975) showed that partially defatted chopped beef with about 50% collagen, heated to a maximum of 49°C, had a digestibility of 88%. Both raw and cooked pigs ears with high amounts of collagen were found to be 95% digestible (Vaughn, Wallace & Forster 1979). Brüggeman *et al.* (1964) refer to *in vitro* studies on raw and cooked pigskin where the digestibilities were 97.2 and 97.8% respectively. To our knowledge no investigation has been published that clearly shows low digestibility of collagen *in vivo*.

Vognarova *et al.* (1968), suggested that the content of solubilized collagen in heated muscle could be an indication of digestibility. Heating of the samples in this investigation has probably partly denatured the collagen. Johns & Courts (1977) refer to extraction studies of pigskin which gave a gelatin yield of only 4.5% after 2 hrs at 80°C of neutral pH. Hill (1966) found that in young as well as old animals less than 20% of the collagen in muscle was solubilized after one hours heating at 77°C. The heating used during sausage production and mild reheating evidently does not solubilize collagen to any larger extent. Furthermore in our study the digestibility was very high even in the raw product.

Apparently the opinion that collagen has a low digestibility is based not only on data concerning the low solubility of collagen after heating, but also on the fact that some proteolytic enzymes are ineffective in degrading collagen *in vitro*. Thus Neumann & Tytell (1950) did show that trypsin, chymotrypsin and papain were practically ineffective on collagen from different sources. However, from several early investigations, reviewed by Brüggeman *et al.* (1964), it seems that

collagen might be broken down in the stomach before passing into the intestine. These investigations show that pepsin and stomach acid do hydrolyze native collagen.

Biological value and NPU

Since the digestibility of collagen in pigskin is so high, NPU values mainly reflect the BV. Thus the differences of NPU are mainly due to variations in the amino acid composition of different collagenous products. Results of BV and NPU are shown in Table 1. Data from experiment 2 (Fig. 1) show a direct relationship between NPU and content of collagen, with the regression equation defined as $y = 82.8 - 0.6x$ and a correlation coefficient of 0.99. For pigskin, with 78.9% collagen, a NPU value of 36 can be deduced from the equation. Linear extrapolation to 100% collagen could then be related to a NPU of 23. This is in accordance with the concept that BV approaches 20 when the tryptophan content of a protein, limited by this amino acid, is decreasing towards zero (Bender, 1973) as in collagen. Present results are also in accordance with those of Bender & Zia (1976) who showed a NPU value of 69 for shin with 23.6% collagen and a NPU value of 82 for fillet with 2.5% collagen. NPU values of 69 and 81 respectively can be deduced from the equation in Fig. 1 for these contents of collagen. Corresponding NPU values deduced from the study by Dvorak (1972) are 65 and 83 respectively.

Amino acid supplementation

Supplementation of pigskin with methionine (3:2 Table 1) and methionine plus tryptophan (3:3 Table 1) did not significantly increase the NPU-value (compared to 2:7, Table 1). The reason for this is probably that the isoleucine

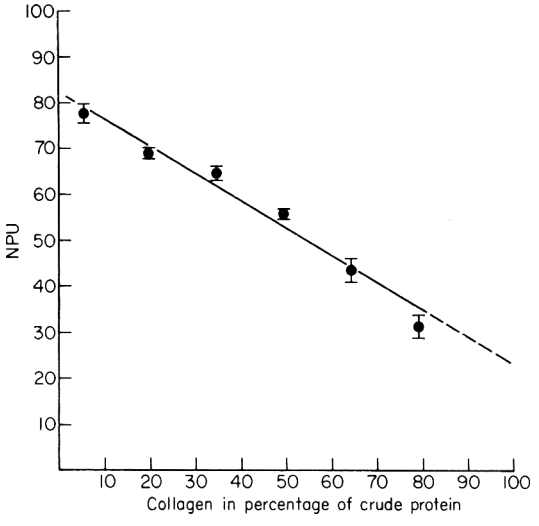

Figure 1. Relationship between NPU-value and collagen content in mixtures of M. biceps femoris from beef and pigskin (mean values and s.e. mean).

content of pigskin is also low, which immediately makes this amino acid limiting after the supplementation. The chemical score values from Table 2 indicate a value of about 30 for both tryptophan, methionine plus cystine and isoleucine. Supplementing a 50% collagen mixture with methionine (3:4, Table 1) did not increase the NPU. Table 2 shows that chemical scores for isoleucine, valine, methionine plus cystine, leucine, threonine and tryptophan of such a mixture are all between 57–66%. Thus it is obvious that supplementation must be performed with several amino acids in addition to methionine and tryptophan to increase the quality of a mixture of 50% collagen to the same level as muscle protein. At levels of about 25% of collagen it seems however enough to supplement just one amino acid. Thus Bender and Zia (1976) supplemented shin with methionine, increasing the NPU-value to a level above that of fillet.

Table 2. Essential amino acids and chemical scores for beef and pigskin

	Beef (g/16gN)	Pigskin (g/16gN)	Pigskin (Chemical score)*	Beef/pigskin 40/60 (Chemical score)†
Isoleucine	3.7	1.3	33	57
Leucine	6.6	2.9	41	63
Lysine	7.1	3.2	58	87
Methionine + cystine	3.6	1.2	34	62
Phenylalanine + tyrosine	6.0	2.9	48	69
Threonine	3.9	1.8	45	66
Tryptophan	1.2	0.3	30	66
Valine	4.0	2.1	42	57

*Calculated from figures from column 2 and data from FAO/WHO (1973).
†Calculated from figures from column 1 and 2 and data from FAO/WHO (1973).

The discussions above concerning supplementation are only relevant for growing children. In the study of Kofranyi & Jekat (1969) 50% of muscle protein could be replaced with gelatin without lowering the nutritive value for adult humans. The obvious explanation for this is that the need for essential amino acids is much lower for adults than for growing children (FAO/WHO, 1973). Comparison of essential amino acid patterns (Table 2) with estimated needs for adults (FAO/WAO, 1973) indicate that methionine plus cystine is limiting in the mixture containing 50% collagen, and that the content of these amino acids is only slightly below the need.

Conclusions

Collagen from pigskin has been shown to have a digestibility of about 95% in rat assay, when the particle size of collagen is about 2 mm as in comminuted meat

products. This was true both for raw and heated samples, and probably applies also to other collagenous materials used in food.

BV and thus NPU decreased linearly with increasing collagen content. The regression equation between NPU and % of collagen of the crude protein is defined as $y = 82.8 - 0.6x$. Supplementing a 50% collagen mixture with methionine did not increase the NPU value. In order to obtain an increase, supplementation must be performed with several amino acids.The acceptable NPU level in mixed meat products is a matter for discussion. For weaning foods PAG (1971) recommends a NPU value of not less than 60 and preferably above 65. This corresponds to a collagen content of 38% and 30% respectively in the present study. PER value of 2.5 proposed for interim legislation by USDA 1976 corresponds to 28.5% collagen in the investigation of YuBang Lee *et al.* (1978). From these data it is evident that even if 25–40% of the protein in a meat product is collagen the protein nutritional value of that product would be adequate as the sole source of protein even for weaning children.

In a mixed diet the practical levels of collagen in industrial meat products (15–30%) will be of negligible nutritional significance.

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Isohalic sorption isotherms

I. Determination for dried salted cod (*Gadus morrhua*)

P. E. DOE,*§ RAHILA HASHMI,† R. G. POULTER† AND JUNE OLLEY‡

Summary

The sorption behaviour of dried salted cod *(Gadus morrhua)* can be predicted from the physical properties of sodium chloride solutions and the sorption characteristics of fish muscle.

Introduction

Salting and drying are widely used for preserving fish, particularly in South East Asia (Shamsuddoha, 1964; Waterman, 1976). Salting is achieved by immersion in sodium chloride brine or by incorporation of dry salt. Drying is mainly by direct exposure to sunlight on mats placed on the ground or on racks; there is an increasing interest in the use of improved drying techniques (Doe *et al.*, 1977; Doe, 1979). It is well established (Scott, 1957; Chirife & Iglesias, 1978) that the microbial stability of salted dried fish depends on its water activity, a_w, which is a measure of the water available to sustain bacterial or fungal action. The relationship of a_w to moisture content is usually presented in the form of a sorption isotherm; in the case of salted dried fish the salt content must also be considered. A sorption isotherm for heavily salted cod has been determined (Lupin, 1978) and a linear relationship between a_w in moist salted fish products and the molality of sodium chloride has been shown (Lupin, 1981). However the detail of how the sorption isotherms vary with salt content (isohalic sorption isotherms) has not, until now, been available. Differences in salting and drying methods, and fish species introduce too many variables to handle by multiple regression. This paper puts forward a theory which is based on a combination of

Authors' addresses: *Department of Mechanical Engineering, University of Tasmania, Hobart, Tasmania 7005, Australia; †Tropical Products Institute, London and ‡CSIRO, Tasmanian Food Research Unit, Hobart, Tasmania, Australia.

§To whom correspondence should be sent

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the sorption behaviour of fish muscle and known physical properties of sodium chloride solutions and supported by measurements on dried salted cod fish. It can be extended to give useful predictions of storage times for other dried fish in tropical climates (Poulter *et al.*, 1982).

Materials and methods

Fish species

Isohalic sorption isotherms were determined for fillets of cod, *Gadus morrha*, a temperate marine low-fat fish.

Salting methods

Two methods of salting were used. Firstly, fish were immersed at 25°C in a stirred saturated sodium chloride solution for periods of 1.5, 3 and 6 hr or longer. Secondly, dry salt (rock salt) was rubbed into coarsely minced fish in a manner similar to traditional practice in SE Asia.

Water activity measurements

The salted and unsalted samples were equilibrated, in partially evacuated vacuum desiccators, on perforated nylon discs over saturated solutions of various inorganic salts which had water activities in the range 0.07 to 0.9 at a temperature of 25±2°C. Samples reached constant mass within 2–3 weeks. In the determination of the sorption isotherm of unsalted cod, fillets were freeze-dried before equilibration. Salted samples in the range of a_w=0.75 or greater were equilibrated by desorption. Salted samples with a_ws less than 0.75 were equilibrated either by desorption or by adsorption after freeze drying.

Moisture content

Moisture contents were determined by drying 2–5 g samples in a convection oven for 24 hr at 105°C.

Salt content

Sodium chloride was measured using the ammonium thiocyanate and silver nitrate method with ammonium ferrous sulphate as indicator (AOAC, 1965). For salt contents of fish in excess of 45% the following method was used: a 2-g sample was macerated with distilled water and made up to 250 ml. Then a 25 ml aliquot was titrated against 0.1 N silver nitrate using potassium chromate as indicator. Salt content was calculated from 1 ml 0.1 N $AgNO_3$=0.0058 g NaCl.

Calculation of isohalic sorption isotherms

Ross (1975), in a paper on the estimation of a_w in intermediate moisture foods, justified taking the product of the water activities of the individual solutes as the a_w of the mixture. He extended the principle to include insolubles such as starches and proteins. Thus, this protein, or fish muscle, and water can be taken into account along with common salt so as to give the a_w of salted fish from the expression

$$a_w = a_{wn}\, a_{wo} \tag{1}$$

where a_{wn} is the water activity of the sodium chloride solution and a_{wo} is the water activity of the fish muscle.

The water activities of sodium chloride solutions were taken from published data (Robinson & Stokes, 1959) giving a_w as a function of molality. Molality, m, of the sodium chloride can be expressed as

$$m = \frac{M_s}{58.5}\,\frac{1000}{M_w}\,, \tag{2}$$

where M_s is the mass of sodium chloride dissolved in a mass M_w of water. This equation can be rewritten as

$$m = 17.09\,\frac{M_s}{M_b}\,\frac{M_b}{M_w}\,, \tag{3}$$

so that the molality may be calculated from the salt and moisture contents of the fish in terms of the salt-free, fat-free dry mass M_b.

Values of a_{wo} at different moisture contents are found by applying equation (1) to the measured sorption isotherm for unsalted cod and the measured salt content of the unsalted cod ($M_s/M_b = 0.05$) and equation (3) to give molality hence a_{wn}.

Results

Measured sorption data of salted and unsalted cod

Table 1 gives the measured sorption isotherm for unsalted cod as a list of moisture contents of freeze-dried cod equilibrated to a_ws ranging from 0.07 to 0.90. Table 2 shows the sorption data obtained after brining cod for different periods to give different values of M_s/M_b in the range of a_ws where the effect of salt is dominant. Table 3 shows the results for fish salted by extended brining or dry salting and held at a_ws in the lower range.

Table 4 shows the calculation of the sorption isotherm of cod muscle using data from a smooth curve through the measurements of Table 1; in Fig. 1 this isotherm is plotted together with the isotherms for sodium chloride solutions.

Table 1. Water adsorption isotherm for freeze-dried unsalted cod at 25°C

Water activity	0.07	0.11	0.22	0.33	0.44	0.54	0.57	0.68	0.71	0.75	0.80	0.85	0.90
Moisture content (dry basis)	0.05	0.05	0.09	0.09	0.12	0.14	0.16	0.20	0.21	0.25	0.27	0.32	0.44
	0.04*	0.05	0.08	0.09	0.12	0.14	0.17	0.19	0.22	0.25	0.27	0.32	0.45
							0.17	0.21					
								0.20					

*Replicate determinations

Table 2. Sorption data at 25°C in the a_w range where the effect of salt is dominant; cod brined for different times

	Brining times					
	1.5 hr		3 hr		6 hr	
Water activity	M_w/M_b	M_s/M_b	M_w/M_b	M_s/M_b	M_w/M_b	M_s/M_b
0.75	2.63	0.91	1.91	0.67	1.49	0.60
	2.55	0.88	2.69	0.99	2.76	1.00
			2.90	1.03		
0.80	1.55	0.45	1.75	0.57	2.16	0.69
	2.86	0.87	3.04	0.91	3.15	0.93
	2.99	0.86	2.83	0.87		
0.81	3.30	0.93	3.14	0.99	3.25	0.96
0.84	3.49	0.86	3.17	0.84		
0.85	3.38	0.80	3.33	0.82	3.31	0.84

M_w/M_b = Moisture content dry basis.
M_s/M_b = Salt content dry basis.

Table 5 shows the calculation of the isohalic sorption isotherms for salted cod. The values of M_w/M_b are for integral values of molality corresponding to the particular salt contents calculated according to equation (3). The values of a_{wn} corresponding to these values of molality were taken from Robinson & Stokes (1959). The isohalic sorption isotherms are plotted in Fig. 2; in effect it is the product of the two sorption isotherms of Fig. 1. The isohalic $M_s/M_b = 0.05$ is the experimentally determined adsorption isotherm for unsalted cod given in Table 1. Also shown in Fig. 2 are measured sorption data (Tables 1 and 2); these data are plotted as numbers representing the measured salt contents of dried salted cod at positions on the figure corresponding to the measured moisture contents and a_ws.

Table 3. Sorption data for cod salted by different methods at 25°C

Method	Water activity	Moisture content dry basis (M_w/M_b)	Salt content dry basis (M_s/M_b)
Brined for more than 6 hr	0.07	0.05	1.0
	0.33	0.11	1.1
Dry salted	0.33	0.10	1.6
	0.44	0.12	1.3
	0.57	0.21	1.2
		0.23	1.2
	0.68	0.33	1.1
		0.30	1.1
		0.31	1.1
Salted, freeze-dried	0.07	0.03	1.2
		0.04	1.2
		0.04	1.2
	0.11	0.06	1.3
		0.05	1.3
	0.22	0.08	1.4
	0.33	0.09	1.4
		0.10	1.4
	0.44	0.12	1.4
		0.12	1.4
	0.57	0.16	1.4
		0.16	1.4
	0.68	0.22	1.3
		0.21	1.3
	0.71	0.36	1.3
		0.37	1.3

Table 4. Determination of a_{wo} from the sorption isotherm of unsalted freeze-dried cod with salt content $M_s/M_b=0.05$

Moisture content	M_w/M_b	0.04	0.05	0.08	0.10	0.12	0.14	0.17	0.21	0.28	0.34	0.43	0.85	1.71	4.2*
Water activity	a_w	0.07	0.11	0.22	0.33	0.43	0.53	0.62	0.71	0.82	0.87	0.90	0.97	0.99	0.99
Water activity due to salt content	a_{wn}	0.75	0.75	0.75	0.75	0.75	0.75	0.80	0.85	0.89	0.91	0.93	0.97	0.99	0.99
Water activity of salt-free cod	a_{wo}	0.09	0.15	0.29	0.44	0.59	0.71	0.78	0.84	0.92	0.96	0.97	1.0	1.0	1.0

*Fresh unsalted cod (J.D. Mellor, pers. comm.)

Table 5. Calculation of the isohalic sorption isotherms for cod

Salt content	M_s/M_b=0.1				M_s/M_b=0.2				M_s/M_b=0.4				M_s/M_b=0.6				M_s/M_b=1.0			
	M_w/M_b	a_{wo}	a_{wn}	a_w	M_w/M_b	a_{wo}	a_{wn}	a_w	M_w/M_b	a_{wo}	a_{wn}	a_w	M_w/M_b	a_{wo}	a_{wn}	a_w	M_w/M_b	a_{wo}	a_{wn}	a_w
	.04	.09	.75	.07	.04	.09	.75	.07	.04	.09	.75	.07	.04	.09	.75	.07	.04	.09	.75	.07
	.05	.15	.75	.11	.05	.15	.75	.11	.08	.29	.75	.22	.08	.29	.75	.22	.08	.29	.75	.22
	.08	.29	.75	.22	.08	.29	.75	.22	.12	.59	.75	.44	.12	.59	.75	.44	.12	.59	.75	.44
	.10	.44	.75	.33	.10	.44	.75	.33	.17	.78	.75	.59	.17	.78	.75	.59	.17	.78	.75	.59
	.12	.59	.75	.44	.12	.59	.75	.44	.28	.92	.75	.69	.28	.92	.75	.69	.28	.92	.75	.69
	.14	.71	.75	.53	.17	.78	.75	.58	.43	.97	.75	.74	.43	.97	.75	.74	.43	.97	.75	.74
	.17	.78	.75	.58	.28	.92	.75	.69	.57	1.0	.75	.75	.57	1.0	.75	.75	.68	1.0	.75	.75
	.21	.84	.75	.63	.43	.97	.75	.73	.68	1.0	.75	.75	.68	1.0	.75	.75	.85	1.0	.75	.75
	.28	.92	.76	.70	.57	1.0	.76	.76	.85	1.0	.75	.75	.85	1.0	.75	.75	1.1	1.0	.75	.75
	.34	.96	.81	.78	.68	1.0	.81	.81	1.1	1.0	.76	.76	1.1	1.0	.75	.75	1.7	1.0	.75	.75
	.43	.97	.85	.83	.85	1.0	.85	.85	1.4	1.0	.81	.81	1.7	1.0	.76	.76	2.0	1.0	.75	.75
	.57	1.0	.89	.89	1.1	1.0	.89	.89	1.7	1.0	.85	.85	2.0	1.0	.81	.81	2.8	1.0	.76	.76
	.85	1.0	.93	.93	1.7	1.0	.93	.93	2.3	1.0	.89	.89	2.6	1.0	.85	.85	3.4	1.0	.81	.81
	1.7	1.0	.97	.97	3.4	1.0	.97	.97	3.4	1.0	.93	.93	3.4	1.0	.89	.89	4.3	1.0	.85	.85

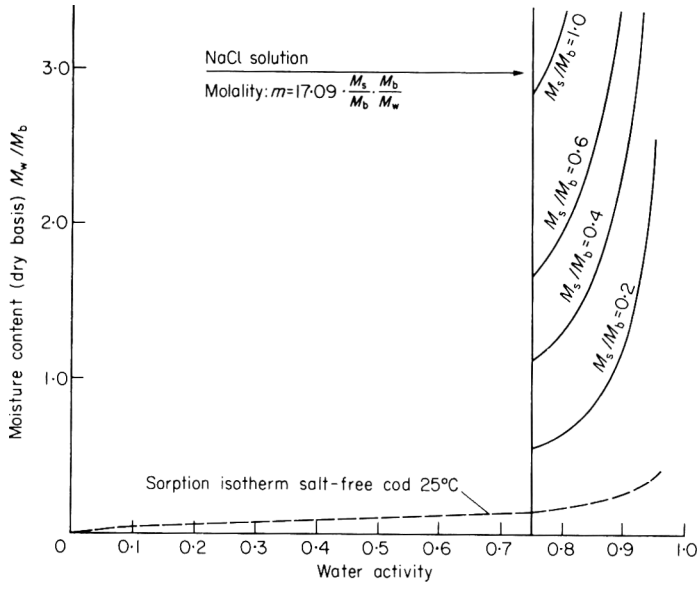

Figure 1. Calculated sorption isotherm for sodium chloride solutions of different salt content together with the sorption isotherm of salt free cod a_{wo} calculated from the measured sorption isotherm for unbrined cod.

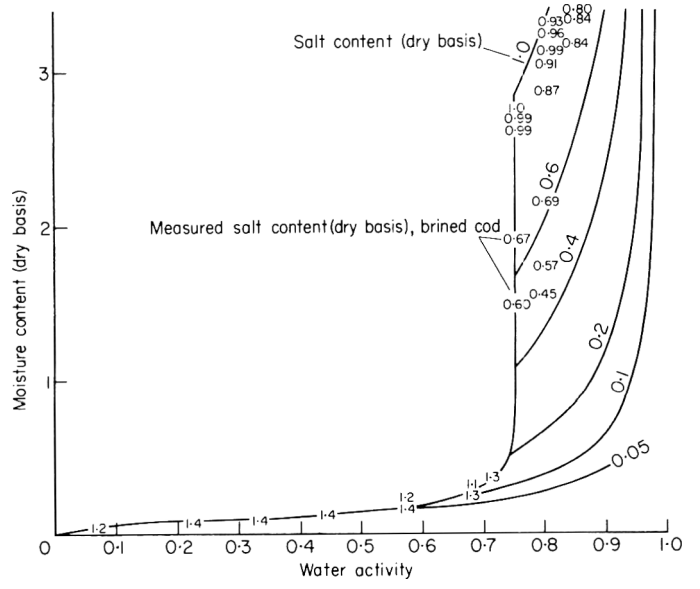

Figure 2. Isohalic sorption isotherms for salted cod at 25°C showing measured sorption data as plots of the measured salt content. The decimal points of the plotted salt contents are at the positions corresponding to the measured moisture contents and water activities.

Discussion

The data and calculations in this paper represent a first attempt to derive isohalic sorption isotherms for salted fish. However, several other factors need to be considered. The salt content of unsalted fish varies with several factors including the size and nutrition of the fish (Love, 1970). The measured sorption data were obtained from fillets of cod finely sliced to allow equilibration in a reasonable time; the application of these data to whole fish has not been studied. In the experimental investigation both adsorption and desorption methods were used although, in some foods, they lead to different sorption characteristics (Sloan & Labuza, 1975). Jason (1958) presents a desorption isotherm for cod which is at most 0.022 kg/kg higher in moisture content than the adsorption isotherm presented in Table 1. Cooper (1938) presents adsorption and desorption isotherms for heavily salted fish which differ by a similar amount.

If a_{wo} values, determined as described above, are to be used to calculate isohalic sorption isotherms for sun-dried fish (Poulter *et al.*, 1982) the effect of drying temperature on a_{wo} must be considered.

Changes in cod flesh occur when it is heated to 31.5°C. At this temperature some of the tissue water becomes less strongly bound to protein and appears as free fluid (Anon., 1968). At about 43°C the fish becomes somewhat more translucent followed by an increase in opacity due to the precipitation of thermally denatured sarcoplasmic proteins which begins at about 45°C (Anon, 1968; Aitken & Connell, 1979).

Thus the sorption isotherms of dried fish are probably influenced by the temperature of drying. Iglesias & Chirife (1976) have shown that the sorption isotherms at 30°C of pre-cooked dried beef vary with drying temperature. The same effect may be inferred for fish. There are no published isotherms on uncooked fish at temperatures above 30°C though, for cod, cooking may be assumed to start at 31.5°C. Isotherms for cooked fish dried at 50–70°C have been published by Shewan (1953), Tarr (1945), and Cutting, Reay & Shewan (1956). The results show a higher a_w for a given moisture content than would apply with fish dried below 31.5°C and hence an increased susceptibility to mould growth at the same moisture content. Another effect is that of evaporative cooling (Jason, 1958; Lewicki *et al.*, 1978) which can lower the temperature of the fish below that of the surrounding air in the early stages of drying. On the other hand, direct exposure to sunlight can result in the fish attaining a higher temperature than ambient.

Salting has the effect of removing water from the fish and then slowing down the subsequent drying rate (Jason & Peters, 1973). Thus the a_{wo} values presented in Table 4 are not strictly applicable to fish heated above 31.5°C in the presence of salt; further experimental work is necessary to determine whether these a_{wo} values are acceptable for calculating isohalic sorption isotherms for fish dried at other temperatures.

Conclusions

A method has been developed to establish sorption isotherms of fish with different salt contents. Fish are considered as a ternary system composed of salt, water and fish muscle. The water activity of the salt-water component is accurately known, but problems exist in characterizing the fish solids-water component of the system. The isohalic sorption isotherms derived in this paper use experimental data for unsalted freeze-dried cod with a_w adjusted by adsorption of moisture. The validity of their application to heated, salted cod with water activity adjusted by desorption and to other fish species has not been established. There is, however, evidence that experimentally determined a_ws for salted cod dried at temperatures less than 30°C agree well with the theoretically determined values.

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Technical note: Observations on the glutamic-oxaloacetic transaminases of turkey breast muscle during chill storage

J. M. JONES AND CHRISTINA C. ROONEY

Introduction

The skeletal muscles of commercially important mammalian and avian species contain two isoenzymes of glutamic-oxaloacetic transaminase (GOT; aspartate amino transferase; E.C.2.6.1.1), one (GOT_m) is localized in the mitochondrion, the other (GOT_s) in the sarcoplasm (Kormendy *et al.*, 1971; Guillot *et al.*, 1974; Vizzani, 1978). GOT_s is responsible for almost all the GOT activity of press juice of nonfrozen muscle, while the press juice from frozen-and-thawed meat also contains GOT_m activity resulting from mitochondrial damage occurring during the freeze-thaw cycle.

Hamm & Kormendy (1969) developed a routine electropheretic method for differentiating between fresh (i.e. chilled) and thawed meat based on the presence or absence of GOT_m in the press juice. The method was called into doubt, however, in the case of pork where both GOT_s and GOT_m were found in the press juice of non-frozen meat (Vanderkerckhove *et al.*, 1972). It was supposed that this was due to stress susceptibility of the pigs leading to mitochondrial damage. Since turkeys are also susceptible to stress, which is characterized post-mortem by an acceleration in the rate of glycolysis and by a rapid decline in muscle pH (van Hoof, 1979), it was decided to reassess the applicability to turkey meat of the Hamm and Kormendy method. In the first instance, the effect of chill storage on the GOT of turkey breast muscle was investigated.

Materials and methods

Materials

Intact deep breast muscles (*M. supracoracoideus*) were obtained from the carcasses of 24-week-old turkey stags processed locally. Single muscles were packaged using the wrapping materials described by Barnes *et al.* (1979) i.e. low

Authors' address: ARC Food Research Institute, Colney Lane, Norwich, NR4 7UA.

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density, oxygen permeable polyethylene film and oxygen impermeable 'Barrier Bags' (Cryovac Division, W.R. Grace & Co. Ltd). The latter were used to produce vacuum packs. Wrapped portions were held overnight at 1°C before starting the tests (day 0) and were stored at 1 ± 0.5°C throughout the experimental period.

Two experiments were performed using oxygen permeable packs and one using vacuum packs.

Measurement of muscle pH values

This was carried out at room temperature as described by Jones (1972).

Preparation of muscle press juice

The method was essentially that described by Rehbein (1979). 40 g of muscle were chopped into small pieces and centrifuged for 1 hr at 40 000 × ***g***. The clear supernatant was decanted off and held at 4°C until required for analysis – usually within 6 hr of preparation.

Separation and detection of isoenzymes

Press juice was diluted with an equal volume of 0.1 M phosphate buffer, pH 7.6 and 1 μl of the resulting solution applied to the middle of a strip of cellulose acetate (Celagram II, 2.5 × 18 cm. Shandon Southern). Electrophoresis was carried out for 18 h at approximately 1 mA/cm of the strip width.

After electrophoresis the wet strip was lightly sprayed with a solution prepared by dissolving the contents of 1 phial of 'GOT–UV System' (Boehringer Mannheim GmbH) and 2 mg of reduced nicotinamide adenine dinucleotide (NADH) in 4.75 ml of 0.6 M phosphate buffer, pH 7.6 containing 0.002% (w/v) pyridoxal phosphate. The strip was then examined under an ultra-violet (UV) lamp at 365 mm. Against a fluorescent background the isoenzymes appeared as dark bands moving towards the anode, GOT_s having the higher mobility.

Results and discussion

The GOT isoenzymes were identified in a preliminary experiment by comparing the electrophoretogram of the juice prepared from chilled muscle on day 0 with that of juice prepared from the same muscle after freezing and thawing.

In our initial experiment using breast muscle stored in permeable packs, only one UV absorbing zone (GOT_s) was observed in the electrophoretograms of juice from muscles held up to 3 days at 1°C while at day 10 a second enzyme band (GOT_m) was present in all extracts examined. A second experiment showed that

in some instances both enzymes were present in extracts prepared from muscles held at 1°C for as little as 4d (Table 1). It was not until the 10th day of storage, however, that the two isoenzymes were found consistently in all extracts. Thereafter all electrophoretograms of juice from permeable packaged muscles contained GOT_s and GOT_m. The variability between individual muscles in the

Table 1. GOT isoenzymes in the press juice of muscles held in oxygen permeable packs

	Expt 1		Expt 2	
Day	Muscle No.	Isoenzyme	Muscle No.	Isoenzyme
0	1	GOT_s	1	GOT_s
	2	GOT_s		
	3	GOT_s		
3	4	GOT_s	2	GOT_s
	5	GOT_s	3	GOT_s
	6	GOT_s		
4			4	GOT_s, GOT_m
			5	GOT_s, GOT_m
			6	GOT_s
6			10	GOT_s
			11	GOT_s
			12	GOT_s
7			13	GOT_s, GOT_m
10	7	GOT_s, GOT_m	14	GOT_s, GOT_m
	8	GOT_s, GOT_m	15	GOT_s, GOT_m
	9	GOT_s, GOT_m		

Table 2. Effect of prolonged storage and packaging on the occurrence of GOT enzymes in muscle press juice

	Oxygen permeable packs		Vacuum packs	
Day	Muscle pH	Isoenzymes	Muscle pH	Isoenzymes
12	6.17	GOT_s, GOT_m	6.04	GOT_s
	6.31	GOT_s, GOT_m	6.14	GOT_s
	6.25	GOT_s, GOT_m	6.11	GOT_s
14	6.23	GOT_s, GOT_m	6.27	GOT_s
	6.14	GOT_s, GOT_m	6.28	GOT_s
	6.18	GOT_s, GOT_m	6.13	GOT_s
21			6.34	GOT_s
			6.16	GOT_s
			6.15	GOT_s
24			6.14	GOT_s
			6.06	GOT_s
			6.29	GOT_s

early stages of storage at 1°C may be a reflection of the variation in mitochondrial damage caused by differing rates of decline in muscle pH post-slaughter.

In contrast to the situation using oxygen permeable material for packaging, GOT_m was not released into the sarcoplasm of breast muscle held in vacuum packs for a period up to 24 days (Table 2). It was not immediately clear why GOT_m should be released from the mitochondria of meat stored in air but not from those of meat stored in vacuum. However, since the microbial flora present on the meat are different in the two types of packages (Mead *et al.*, 1981) it was possible that mitochondrial damage and hence release of GOT_m, in meat stored in air resulted from the action of enzymes of aerobic bacteria.

Whatever the explanation, these results suggest that transaminase detection cannot be used to decide if turkey muscle has been frozen because the chilled muscle is likely to display GOT_m after as little as 4 days at 1°C and possibly even sooner at the higher temperature of storage commonly used.

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J. Fd Technol. (1982) **17**, 139–141

Technical note: The use of the rapid 'catalase' hygiene test to detect blood residues

J.J. MILLEDGE*

Summary

The use of a rapid 'catalase' test for establishing the effectiveness of cleaning in meat processing is examined and the detection limit for blood established.

Introduction

To ensure product safety and quality it is important that the cleaning of meat process plant is effective. Microbiological and visual methods have been widely used to routinely assess the effectiveness of cleaning, but despite the wide usage of these techniques they have a number of disadvantages. With microbiological methods there is generally a considerable delay between sampling and the result which can mean that the plant is used for several product runs before the results of the test are known. There may also be little correlation between the amount of soil on a surface and the microbiological count (Holland *et al.*, 1953; Abele, 1965; Kulkarni *et al.*, 1975). A microbiological count from a surface may be low, but the surface retains a considerable quantity of stale product which can provide an excellent growth medium for the residual flora. Visual appraisals although rapid have only a limited sensitivity which is dependent on the perception of the observer, lighting intensity and nature of the surface.

Belica (1975) has reported a 'catalase' test for hygiene control in slaughterhouses and meat processing plants, which is rapid, simple, cheap and little affected by the observer perception. The test is based on the reduction of hydrogen peroxide to water and oxygen by the catalase enzymes produced by many meat spoilage microorganisms and blood residues, but Belica (1975) gave no estimate of the detection limit of blood residues.

The object of this work was to establish the sensitivity of the rapid 'catalase' test for blood residues.

Author's address: Unilever Research, Colworth House, Sharnbrook, Bedfordshire MK44 1LO.

0022–1163/82/0200–0139 $02.00

Table 1. Detection of blood residues by the 'catalase' test.

	Concentration of horse blood										
	0	Undiluted	1/10	1/50	1/100	1/500	1/1000	1/5000	1/10000	1/50000	1/100000
Expt 1	−	+++	+++	+++	++	+	+	+	+	−	−
Expt 2	−	+++	+++	++	++	++	+	+	−	−	−
Expt 3	−	+++	+++	++	++	++	++	+	+	−	−

+++ Foaming.
++ Bubbles
+ Slow evolution of bubbles.
− No bubbles.

Method

The 'catalase' test (Belica, 1975) consists of placing a small drop of freshly diluted 3% hydrogen peroxide solution on to the test surface, the presence of catalase enzyme or blood being indicated by bubbles or foam.

On the central area (36 mm diam.) marked out by the inner expansion ring of clean unused tin can lids which had been degreased with trichloroethylene was placed 0.5 ml dilutions of horse blood in distilled water. The drop was spread evenly over the surface and dried for 2 h at 45°C. After cooling, the 'catalase' test was carried out on the blood soiled tin lids and on the clean surfaces of tin, Formica, PTFE, stainless steel and aluminium.

Results and discussion

The results in Table 1 show that the 'catalase' test is very sensitive and can detect blood residues at loading rates as low as 0.05 ml/m^2 which is 100 times lower than visually detectable. No 'false positives' were found on clean tin, Formica, PTFE, stainless steel and aluminium indicating that the 'catalase' test can be applied to the wide variety of surfaces found in the meat processing industry.

The 'catalase' test would appear to be a useful rapid hygiene test for the meat processing industry, but it is limited to surfaces which are visible and approximately horizontal. It is not proposed that the 'catalase' test should replace present microbiological hygiene tests, but be a supplement to them allowing a rapid appraisal of cleaning effectiveness between product runs.

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Book reviews

The Safety of Foods, 2nd ed. Ed. by H. D. Graham.
Westport, Connecticut: AVI, 1980. Pp. xvi + 774. ISBN 0–87055–337–2. $34.00.

The first edition of this book comprised the Proceedings of an International Symposium on 'The Importance and Safety of Foods' held in Puerto Rico in 1967. This second edition consists of a revision of those Proceedings, and does not derive directly from a similar, subsequent symposium. Thirty four contributors provide in 24 chapters a text which is intended to cover 'as completely as . . . possible . . . the major areas which bear on the safety of foods'. These chapters cover Food Spoilage and Food-borne Infection; Sources of Food Spoilage Micro-organisms; Microbial Problems in Food Safety with Particular Reference to *Clostridium botulinum*; Staphylococcal Food Poisoning; Salmonella Food Poisoning; Viruses in Foods; Mycotoxins in Foods and Feeds; Control of Food-borne Diseases; Food-borne Diseases of Animal Origin; Nitrosamines; Mercury in Foods; Trace Metal Problems with Industrial Waste Materials Applied to Vegetable Producing Soils; Polychlorinated Biphenyls and Polybrominated Biphenyls in Foods; Sources of Pesticide Residues; Antibiotics and Food Safety; Safety and Wholesomeness of Irradiated Foods; Toxins in Plants; Poisonous Marine Animals; The Need of Additives in Industry; The Proper Use of Food Additives; Regulating Additives from Food Contact Materials; Food Regulations in the Americas; Food Control Under the Canadian Food and Drugs Act; Safety of Food Service Delivery Systems.

It can be seen that the intention to cover 'the major areas which bear on the safety of foods' has been achieved.

There is a bibliography/list of references at the end of each chapter, but no overall author index. Although, as is customary in multi-author works, the comprehensiveness of the treatment and the extent of the literature coverage varies from chapter to chapter, most of the chapters provide extremely useful literature surveys. However, for the most part, the information incorporated ranges up to 1977 with relatively few references after this date.

In the reviewer's copy page numbers had been omitted from the chapter contents list, and the index is relatively short and not sufficiently comprehensive for a reference work of this size.

There are many useful figures, tables and graphs, but the book suffers from the customary and very tiresome AVI fault of poor reproduction of photographs. Typographical errors are also very frequent, and the typeface selected is not particularly easy to read.

Nevertheless the book will provide a very useful reference work for food scientists and technologists, perhaps especially for advanced undergraduate and postgraduate students.

W. F. Harrigan

Carbohydrate Sweeteners in Foods and Nutrition. Ed. by P. Koivistoinen and L. Hyvönen.
London: Academic Press, 1980. Pp xiv+289. ISBN 0–12–417050–1. £17.60.

This book is a record of papers presented at the 1978 Helsinki Symposium on carbohydrate sweeteners. Twenty papers provide an extensive coverage of present knowledge on carbohydrate sweeteners in foods from physiological, nutritive and chemical aspects with bias to the interests of the food scientist. A good balance has been achieved in the coverage of varying subjects by recognized authorities in specialized fields.

Paper 1 introduces the subject of added sweet carbohydrates in food and diet. It is followed by three papers on their effect on metabolism and disease, dental caries and nutritional policy surrounding added sweeteners.

Paper 5 reviews the present knowledge on sweetness theory. It discusses the stereochemical features of carbohydrates and their derivatives which contribute to their functional properties. The author also examines the temporal aspects of taste which until recently have been given little attention in the literature.

Papers 6, 7 and 8 discuss the psychophysics of sweetness perception. Comparative evaluation of sucrose and other carbohydrate sweeteners in food systems are the subjects discussed in the following two papers (9 and 10).

The effect of the environment on sweetness is the topic discussed in papers 11 and 12. Until recently, blends of sugars in food systems would normally consist of two components. Interest in multiple component blends demands a greater understanding of synergistic behaviour between component mixtures to help food formulation attain the desired characteristics.

Two papers on the processing of sugars in food by heat and fermentation follow this section (papers 13 and 14). Paper 13 deals with *Sugars in thermal processes.* It was presented by O. Theander and is worthy of special mention in its clarity of structure, directness and providing a thorough review of the subject.

Papers 15 and 16 are on the developments in enzymatic methods for improved glucose syrup production and the technological development and properties of their hydrogenated counterparts.

Papers 17 and 18 examine lactose syrups and lactitol for their potential application in consumables.

The concluding two papers discuss fructose and xylitol not only as sweeteners but as sweetening agents with desirable features such as low cariogenicity and low calorific values.

The book is clearly and well presented, bearing sub-titles for quick reference, with each paper including a summary and notes on future trends. Ideas and adequate illustrations and a uniform presentation combine to form a fine record of present knowledge in the field of carbohydrate sweeteners. Good value.

S. Z. Dziedzic

Food Microbiology – A Framework for the Future. By A. N. Sharpe. Springfield, Illinois: C. T. Thomas, 1980. Pp. 224. $24.75.

This I found to be a fascinating and very enjoyable book. It is not a conventional book on food microbiology and as its title implies it looks very much to the future in its concepts and content. Some traditional food microbiologists may well be offended by Dr Sharpe's (dare I say sharp) attack on many of the sacred cows of food microbiology but I am sure most will be stimulated by the author's attempt to propose alternative (and better) means of assuring the quality and safety of foods and the bringing of a breath of fresh air into the stuffy world of food microbiology.

The author has been concerned for many years with the development of methods for the microbiological analysis of foods and has been responsible for the innovation of a number of notable new techniques. This book arises from this research into methods and his identifying the severe difficulty of developing rapid, instrumented methods based on conventional microbiological techniques. In essence what Dr Sharpe has done in this book is to indicate what is wrong with traditional approaches and why the development of rapid methods based on these is not on; to specify the type of information relevant to quality and safety that the microbiologist (together with chemists, physicists and instrumentation experts) should be generating and how this information might be obtained. He rightly questions why microbiologists and other persons concerned with food control should continue to spend so much time and effort in developing methods that are indirectly or directly related to such non-specific tests as the 'The Total Viable Count' and then spending even more time trying to agree what level of these organisms constitute a quality defect or safety risk in a food, knowing in their hearts that any relationship to quality/safety is highly tenuous. The consumer is, or should be, not concerned with the microorganisms in the food *per se*, but with the products of their growth, as it is these, not the organisms that lead to unacceptable organoleptic changes or illness. He then logically reasons why not measure these products of metabolisms under controlled conditions and relate decisions as to acceptability or otherwise on such measurements. By so doing, tests can be related more specifically to quality and safety and by the use of existing chemical analytical procedure truly rapid instrumented tests can be developed.

The principal problem with Dr Sharpe's concept is the identification of the parameters that are concerned with quality and the establishment of the response of consumers to these parameters and by taste and other forms of panelling. It is also necessary to agree the threshold level of response/agreed tolerance to a particular parameter. This is particularly difficult in the case of infectious and/or toxigenic microorganisms and much of such data will have to be based on epidemiological evidence and decisions as to threshold levels agreed by consensus with the groups concerned.

This alternative approach to food microbiology has much to offer and as Dr Sharpe suggests, it would be more beneficial to food control if food microbiologists would spend less time attempting to count the microorganisms in food and more time establishing what products of their metabolism are concerned with quality defects in foods. He illustrates in the book how this may be achieved, but accepts that these are long term developments and that in the medium term methods based on counts must form the basis for control. The book is a must for all persons concerned with food control and in particular should be read by persons interested in developing methods, procedures and instruments for the microbiological analysis of foods.

A .C. Baird-Parker

Autoxidation in Food and Biological Systems. Ed. by M. G. Simic and M. Karel.
New York: Plenum Press, 1980. Pp. xii + 659. ISBN 0–306–40561–X. $65.

This book comprises sections on Mechanisms, Antioxidants, Biochemical Systems and Biological Systems. It is based mainly on contributions to an International Workshop held in 1979 at Natick, to which are added some further papers presented at an IFT Symposium on 'Food Lipids' at St Louis, also in 1979. Whilst most of the 33 chapters are thus in the nature of research reviews of very specific topics, others are of a broader and more general character, and could appeal to a less specialized readership.

'Mechanisms of Autoxidation' comprises 16 specialized chapters, mostly dealing with aspects of lipid oxidation, though proteins and cholesterol also receive attention. 'Antioxidants' (6 chapters) is a curious mixture of general and highly specialized material. 'Biochemical Systems' (6 chapters) and 'Biological Systems' (5 chapters) reflect the developing preoccupation of oxidizing lipid and sterol systems in relation to disease.

Many of the chapters will be likely only to be of value to specialist research workers. Perhaps half of them, however, could profitably be studied by a wider readership, including food scientists and technologists, toxicologists, pharmacists and medical practitioners. In most cases a reasonable economy of style and clear presentation is achieved, and good reference lists are given.

Food specialists may well be surprised at the wide variety of means that are disclosed for the preservation of food systems against oxidative deterioration. The second section of the book will be of particular interest to those whose information is beginning to be dated in this respect.

The book is printed in typewriter script, and therefore has a rather unfinished appearance. The text is marred by frequent mistakes. Editing and proof-reading seem to have been rather casual, but on the whole the book can be recommended to readers particularly concerned with food quality and keeping properties.

B. J. F. Hudson

Contemporary Developments in Nutrition. By Bonnie S. Worthington-Roberts.
St. Louis, Missouri: The C.V. Mosby Company, 1981. Pp. x + 603. ISBN 0–8016–5627–3. £12.75 (softback).

This attractive paperback book is composed of 20 chapters, each of which represents an up-to-date and comprehensive review of selected topics and reflects many popular nutrition issues. Each chapter is extensively illustrated and well-endowed with references. Very recent references relating to every topic are included in a separate section at the end of the book. The chapters are: 1. 'Carbohydrates in health and disease' – discussing the major dietary sources (starch, sucrose and lactose), together with a short section on the non-nutritive sweeteners (cyclamate, saccharin and aspartame). 2. 'Dietary fibre' – containing information on the components of fibre, fibre deficiency and disease in humans, and the adverse consequences of high fibre consumption. 3. 'Questions related to dietary fats' – a review of the basic facts about fats, recent trends in fat consumption, and a particularly useful section on the aetiology of atherosclerosis. 4. 'Proteins and amino acids' – covering protein nutrition, consumption and deficiency, and a summary of the use and mis-use of the so-called protein-sparing modified diet. 5. 'Vitamin E and the accessory lipid antioxidants' (by J. Bland) – including discussions about the forms of vitamin E and their biological activity, vitamin E as a free-radical trap, the free-radical theory of ageing, the interactions of vitamin E with selenium and the sulphur-containing amino acids to protect against free-radical perioxidation and pathology *in vivo*, and the association of these nutrients with some of the degenerative diseases. 6. 'The fat-soluble vitamins A and D' – summarizing the role of these two vitamins in human nutrition. 7. 'The water-soluble vitamins' – covering vitamin C and the B-complex. 8. 'Calcium and sodium' – paying particular attention to the relationship between sodium and hypertension. 9. 'Trace element nutrition' (by J. Bland) – a review of the physiological function of the trace elements in chronic and acute malnutrition, the influence of trace elements on development and as environmental toxicants, and diagnosis of

trace element deficiencies. 10. 'Iron: a case study in nutrient availability' (by E. R. Monsen) – covering the physiological need for iron, sources of dietary iron and its absorption. 11. 'The American food supply' (by C. S. Martinsen) – including short summaries of food legislation in the USA, and the use and technical effects of food additives. 12. 'Diet and cancer' – discussing the epidemiological evidence which relates to the possible role of nutrition in the aetiology of some forms of cancer. 13. 'Obesity: new knowledge and current treatments' (by L. K. Mahan) – a review chapter. 14. 'Nutrition, diet and oral health' (by O. F. Alvares) – discussing the growth and development of oral tissues, the effects of malnutrition on oral health, periodontal disease and dental caries. 15. 'Vegetarianism as a way of life' (by C. M. Trahms) – describing the various types of vegetarian diet, the proposed health benefits of a vegetarian regime, problems associated with inadequate vegetarian diets and the nutritional status of vegetarians. 16. 'Nutritional issues related to pregnancy' – reviewing weight gain and dietary requirements. 17. 'A new look at breast-feeding' – containing a useful section on the nutritional benefits of breast milk and the nutrition of lactating women. 18. 'Feeding babies in the 1980s' (by P. Pipes). 19. 'Suboptimal nutrition and behaviour in children – an excellent review chapter on malnutrition and learning, iron deficiency and behaviour in children, and nutrition and hyperactivity. 20. 'Diet and athletic performance' – covering dietary needs for athletes and nutritional preparations for strenuous physical activity.

The book, which is predominantly aimed at the North American market, is very well written, but many of the chapters seem to discuss only those topics which can be found in other excellent reviews of the subjects. This is particularly true of the chapters on dietary fibre, the water-soluble vitamins and calcium and sodium. Although the stated purpose of the book is to provide an up-dated summary of nutrition-related issues of concern to the general audience, it is rather difficult to define precisely to which audience it is directed. The book cannot be described as an introductory text, nor as a comprehensive reference book. Furthermore, the subject areas which may have popular appeal such as 'hypoglycaemia', 'dietary fibre', 'food additives', 'Zen macrobiotics', 'hyperkinesis', 'protein-sparing diets', 'physical activity', etc., are tucked away inside many of the chapters and are probably treated in such a way as to be too technical for a general audience. The book definitely requires a reasonable depth of knowledge for understanding and may be suitable for medical practitioners, social workers, nurses and home economists who are dealing with the general public and facing questions on these popular issues. In conclusion, the role of this book is difficult to define. Nevertheless, it is very readable and the copious illustrations set this book apart from other reviews of similar pertinent topics.

D. P. Richardson

Standards in Absorption Spectrometry. (Techniques in Visible and Ultraviolet Spectrometry, Volume 1.) Ed. by C. Burgess and A. Knowles. London: Chapman and Hall Ltd, 1981. Pp. 142. ISBN 0–412–22470–4. £9.50.

In 1977 the UV Spectrometry group set up a number of working parties to gather information on selected topics relevant to standardization of instrumentation. Originally the intention was to circulate this material to members of the group only it was realized this would be of interest also to scientists outside the group and hence the reports are to be published in a series of monographs. The first volume covers UV-visible Spectrophotometry with particular reference to those aspects of the subject 'which are vital to production of accurate and precise data'.

The first chapter covers briefly the basis of UV-visible spectrophotometry and includes some of the topics which are discussed more fully in subsequent chapters, e.g. stray light. The next two chapters cover respectively cell and instrument design considerations. The former is well written and contains much useful information on cell materials and related topics. The chapter on instrument design is very short (five pages) and includes only a brief discussion on those instrumental factors which influence cell design. A more pertinent title for this chapter could have been used or the material included in chapter 2. Chapters 4 and 5 are concerned with liquid and solid absorbance standards and provide an excellent coverage of the most commonly used methods. Chapter 4 also includes some revealing data from collaborative trials on the determination of the molar absorptivity of dichromate solutions. The wide range of results reported must cast doubt on the accuracy of many published data.

The often quoted, but poorly understood parameter 'stray-light' forms the subject matter for chapter 6. The importance of this parameter in terms of instrumental specification is well explained but some of the illustrative figures would have more impact if they contained a complete description of the experimental parameters employed. For example the effect of differing percentages stray-light on absorbance linearity is shown without quoting the actual percentages. Wavelength calibration receives attention in chapter 7 whilst the remainder of the book is concerned with matters such as cell handling, cell testing and recommended standardization procedures. Indeed, this last section is essentially a summary of the previous chapters and expresses concisely those procedures that should be in routine use in all laboratories using spectrophotometry.

The book overall is well written and provides an excellent 'users handbook' for UV-visible spectrophotometry. It is perhaps churlish to criticize the inconsistent use of the terms spectrometry and spectrophotometry but having used the latter at the outset of Chapter 1 this should have been used throughout, including in the book title. The practical nature of the book recommends itself to all prospective spectrophotometer users. In fact all new UV-visible spectrophotometers should be sold with a complimentary copy of this volume.

R. Macrae

Flavor Microbiology. By Pinhas Z. Margalith.
Springfield, Illinois: Charles C. Thomas, 1981. Pp. xiii+309. ISBN 0–398–04083–4. $31.50.

This book is an extensive and interesting survey of the diversity of flavoured compounds produced by microbes. It is presented as chapters according to commodities – dairy products, meat, vegetable products, bread, wine, beer and microbially produced flavours not associated with any particular food.

The author introduces each chapter with an apt quotation of greater or lesser age: the oldest biblical quotations underline the interest that man has taken, either voluntarily or by necessity, in the flavours produced by microbes in his stored food. Recently such activities have drawn wide interest and are covered by the umbrella of biotechnology. Sadly, the material presented just predates the most recent upsurge of interest in the 'natural' production of food flavours and control of the flavours produced in fermented products.

The book provides good source material for those seeking greater understanding of a particular process or contemplating making a transition from the art to the science of fermentation and for this purpose it is considerably stronger in some product areas than in others. Alhough these strengths and weaknesses reflect to a certain extent the different amounts of formally recorded information on each of the commodities, some areas where considerable knowledge is available are covered by the author in scant detail, as though he has no feeling for the subject matter – for example meat and the production of flavours. Information is presented as a series of statements, apparently painstakingly condensed from the many papers cited, and, because of the scale of this, the book represents an important compilation of facts setting out and explaining the range and significance of products associated with particular foods.

The author does not set out with any clarity the unifying principles of metabolism which govern flavour production; for example attention is not directed to groups of natural flavours spanning several products which appear to arise in a related manner or from the synthesis of a specific intermediate and are then subjected to a variety of interactions. The objectives of the book were not clear to me and this was most noticeable in the introductory chapters. For example the chapter on the sensation of flavour is very interesting but not very instructive for investigators of flavours: taste panelling, instrumental techniques and analysis of results receive only passing mention. Similarly the microbiology for the non-specialist contains interesting basic biology but is not an introduction to the production of flavours by microbes. It would be useful to point out in such a chapter the way limited numbers of microbes can safely be used for the production of flavours in food or whose products can be extracted as flavour additives.

The successful commercial exploitation of flavours produced by microbes is limited by all manner of economic, sociological and legal constraints. This being so the prospective flavourist must consider more than the biochemical powers of microbes when aiming for the promotion of food flavour through appropriate

control of microorganisms involved. Unfortunately, the book is limited to the more academic aspects of flavour production and does not tackle these broader issues.

In conclusion, the book is a thorough presentation of the factual data available, but it does not educate the reader in the unifying principles of flavour microbiology.

M. Brown

Errata

J. Fd Technol. (1980) **15**, 59–70
The prediction of water activity in acqueous solutions in connection with intermediate moisture foods IV. a_w Prediction in acqueous non-electrolyte solutions.

J. Chirife, C. Ferro Fontán and E.A. Benmergui

On p. 62 in Fig. 3 the a_w-concentration curve for mannitol has been drawn incorrectly; however, the a_w-lowering behaviour of mannitol can be calculated correctly by Equation (2) with K = 0.906 as indicated in Table 4.

J. Fd Technol. (1981) **16**, 21–30
The evaluation of water activity in acqueous solutions from freezing point depression.

C. Ferro Fontán and J. Chirife

Equation (6) on p. 24 should read
$-\ln a_w = 35.127.10^{+3}\ \theta_B - 1.1195.10^{-1}\ \theta_B^2$

Journal of Applied Bacteriology

The Society for Applied Bacteriology, which has just celebrated its 50th anniversary, launched the *Journal of Applied Bacteriology* in 1954. Since that time the *Journal* has grown in size, two volumes per year being published from 1975 onwards, in prestige and in the subject matter covered. It has established an international reputation with readers and authors; indeed each number of the *Journal* contains papers from worldwide sources. The Society's interest in the systematics and ecology of groups of microorganisms is reflected in the *Journal* and a major change in the organization of papers on systematic microbiology is detailed in a revised Instructions to Authors published early in 1982 as a supplement to Volume 52. The Instructions also give details of a novel form of publication, observation papers. These are intended to provide the *Journal's* readers with a succinct up-date of rapidly developing areas of microbiology. Such articles will be of particular interest to young scientists who seek conceptual enlightenment and senior persons in industry, Universities, etc., who need to keep abreast of developments in many areas of microbiology. Comprehensive reviews have become another important feature of the *Journal* and the publication of critical book reviews has attracted enthusiastic comment.

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

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

SI UNITS

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

NON SI UNITS

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

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

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.

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