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Identification of species in smallgoods products

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Introduction

Recent concern over possible substitution of one species for another in certain meat products has led to the development of improved techniques for species identification including enzyme-linked immunosorbent assay (Kang'ethe *et al.* 1982; Whittaker *et al.* 1982) and isoelectric focusing (IEF) (Kaiser *et al.* 1980; King and Kurth 1982; Slattery and Sinclair 1983). However, immunodiffusion (Ouchterlony method) is still the most commonly used procedure (Kurth and Shaw 1983). The simplicity of this technique favours its use for routine screening, especially when samples are being tested for only a few species. The use of IEF permits the identification of a wider range of species (Kaiser *et al.* 1980) and the detection of unsuspected contaminants and of low proportions of adulterants (King and Kurth 1982).

Commercially available antisera are of limited use for species identification of cooked meats. Although antisera to heat-stable antigens (Hayden 1981) and heat-denatured proteins (Karpas *et al.* 1970) have been prepared, they are unlikely to be adopted for routine testing (Kurth and Shaw 1983). Histidine dipeptides are reported to be stable to heat, and the marked variation in their ratio from one species to another is of value for species identification (Carnegie *et al.* 1982), but there is also considerable variation between muscles of the same animal (Tamaki *et al.* 1977). Heat-coagulation and denaturation of enzymes during cooking preclude species identification by enzyme-staining of IEF gels (King and Kurth 1982) when simple aqueous extraction is used. However, King (1984) showed that, by extracting samples with 6M guanidine hydrochloride, and dialysing the extracts against an appropriate buffer, the activity of adenylate kinase (AK) and creatine kinase (CK) may be at least partly recovered,

provided the meat was not heated above 120°C (AK) or 100°C (CK). By staining IEF gels for these two enzymes, the species of origin of cooked meat served at restaurants and fast-food outlets may be determined (King 1984). However, smallgoods present additional problems because their preparation involves heating in the presence of salts (e.g. nitrites) and/or other additives which may potentially alter the antigenic properties and/or the isoelectric points of proteins. Furthermore, many smallgoods contain meats from several species. Hybrid CK dimers then form during dialysis of guanidine hydrochloride extracts and complicate interpretation of isoelectric focusing patterns (King 1984).

In the present investigation both the immunodiffusion and the IEF methods were tested on a number of smallgoods products purchased from retail outlets.

Methods

Twenty smallgoods products (Table 1) were purchased locally. Each product was divided into two portions, one of which was examined by immunodiffusion and the other by IEF.

Immunodiffusion

A representative sample (20 g) was removed from each product. The sample, which included meat from the centre and the periphery, but not the skin, was placed in a plastic bag. Deionized water (20 ml) was added and the bag pounded for 120 s in a stomacher (Colworth 400). The suspension was then transferred to a 50 ml centrifuge tube which was centrifuged at $1000 \times g$ for 10 min. The supernatant was tested using the immunodiffusion test (Kurth and Shaw 1983). The volume of antigen and antiserum used was normally 20 μ l although when doubtful reactions were present or when there were discrepancies between IEF and

Table 1. Identification of species in smallgoods products

No.	Product	Ingredients ^A	Results							
			Pig		Beef		Sheep		Chicken	
			IEFB ^B	IDC ^C	IEF	ID	IEF	ID	IEF	ID
1	Luncheon	meat	+	+	+	+	—	+	—	—
2	Pork'n beef sausages	pork, beef	—	—	+	—	—	—	—	—
3	Kabanas	pork, beef	+	—	+	—	—	—	—	—
4	Kabana	beef, pork	—	—	+	—	—	—	—	—
5	Peperoni	beef, pork	+	+	+	+	—	—	—	—
6	Frankfurters	pork, meat	+	+	+	+	—	—	—	—
7	Salami	pork, beef	+	—	+	—	—	—	—	—
8	Pork brawn	pork	+	—	—	—	—	—	—	—
9	Chicken devon	chicken	—	—	—	—	—	—	+	+
10	Chicken roll	chicken	—	—	—	—	—	—	+	+
11	Polish salami	beef, pork	+	—	+	—	—	—	—	—
12	Luncheon	pork, meats	+	+	+	+	—	—	—	—
13	Ham and chicken	pork, meats	+	+	+	—	—	—	—	—
14	Liverwurst	pork	+	+	—	—	—	—	—	—
15	Strasbourg	meat	+	+	—	—	+	+	—	—
16	Windsor	beef, pork	+	—	+	—	—	—	—	—
17	Devon	meat, pork	+	+	—	—	+	+	+	+
18	Windsor	pork, meats	+	+	+	—	—	—	—	—
19	Devon	beef	+	—	+	—	—	—	—	—
20	Ham and chicken	meat, pork	—	+	+	+	—	—	+	+

^A Species listed on product label

^B Isoelectric focusing

^C Immunodiffusion

+ Species detected

— Species not detected

immunodiffusion results the tests were re-run using a double loading technique to give a final volume of 40 μ l in each well. All samples were tested against commercially available antisera (Wellcome Reagents Limited) to the following species; cow, sheep/goat, pig, hen, horse and kangaroo. The antisera were tested to ensure that they were sensitive at the 10% level i.e. that they could detect the minor component in a 10%/90% mixture of two different species. The plates were read, after overnight incubation, by an independent observer who was not aware of the origin of the samples.

Isoelectric focusing

● 1 g of each product was homogenized with 2 ml of deionized water and subjected to IEF as described by King and Kurth (1982). Gels were stained for phosphoglucumutase (PGM) and AK.

● 10 g of each product was ground under liquid nitrogen and freeze-dried. A portion of the freeze-dried powder was extracted with

6M guanidine hydrochloride and subjected to IEF in agarose gels containing 1% Triton X-100 and Pharmalyte (pH 5-8) ampholytes, as described by King (1984). Whereas 20 mg muscle powder were adequate, 50–100 mg smallgoods sample were required for extraction with 5 ml 6M guanidine hydrochloride in order to give sufficiently intense bands. Each gel was stained for either AK or AK + CK.

Results

Of the 20 products examined, 12 gave positive reactions with at least one antiserum. However, the remaining 8 exhibited no reaction with any of the antisera used, presumably because of loss of antigenicity during smallgoods production. As shown in Table 1, species detected by the immunodiffusion tests were consistent with label descriptions. However, species present at levels less than 10% of the total meat content may not have been detected by the immunodiffusion method. Hence no chicken



Fig. 1. Smallgoods typical of those sampled. The numerous varieties available, which differ widely in species composition, heat-treatment received, and content of fat, salt and spices present a challenge to the food analyst.

was detected in one product labelled as containing 1% chicken. All products gave negative reactions against both horse and kangaroo antisera.

When aqueous extracts were subjected to IEF and stained for PGM, no bands appeared, presumably due to denaturation of this enzyme during processing. Although AK bands appeared, they were distorted and not satisfactory for species identification. Consequently, only the guanidine hydrochloride extracts are considered further. These produced prominent bands from each smallgoods product when IEF gels were stained for AK. As shown in Fig. 2, several possible contaminating species exhibit AK isoelectric points distinct from beef and are thus readily detected in cooked meats. However, no AK bands corresponding to species other than beef, sheep, pork and chicken were evident in any of the smallgoods patterns. Beef, sheep and pork have similar AK patterns but may be

distinguished by staining for CK + AK (King 1984). Each smallgoods product exhibited CK bands but in some (e.g. pork brawn) the bands were faint. An example of the patterns obtained is shown in Fig. 3. When a mixture of species is present, interpretation is complicated by the hybrid CK dimers which form during dialysis (Fig. 4). However, comparison of the smallgoods CK patterns with standards containing one or more species yielded the results shown in Table 1.

Although guanidine hydrochloride extraction and dialysis resulted in partial recovery of AK and CK activity, no antigenic activity was evident.

Discussion

The antisera used in these tests have been shown to give positive reactions with samples that have been heated to 70 °C for 30 min but positive reactions do not usually occur with samples that have been heated to 80 °C for 30 min (Kurth and Shaw 1983). The

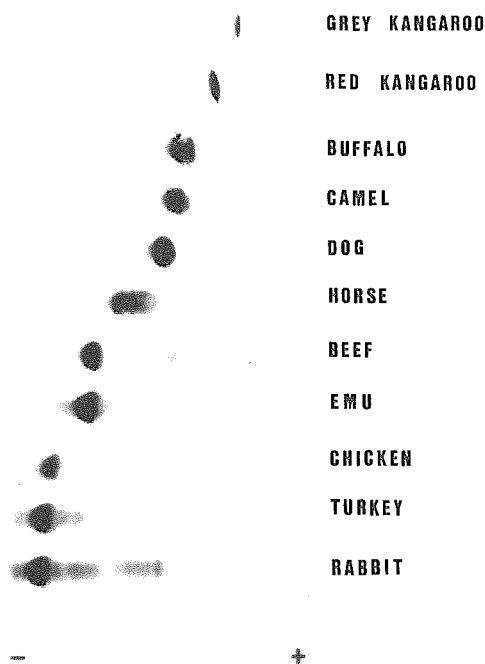


Fig. 2. IEF gel stained for AK.

temperatures used for cooking sausage products vary from about 70°C to 88°C depending on the type of sausage (Gerrard 1976) and thus it could be expected that the antisera would be of no value for species detection with some sausage products. Of the 20 samples tested, 8 failed to give a positive reaction with any of the antisera, presumably these 8 samples had been cooked to a temperature of about 80°C or higher.

Antisera to heat stable antigens (Hayden 1981) can be used for species identification with meat samples that have been autoclaved at 120°C for 30 min but cross reactions with sheep and beef do occur and thus problems in interpretation of the results may arise if these antisera are used for testing smallgoods products.

There was no evidence to suggest that any of the positive reactions obtained with the immunodiffusion tests were false positives. Tizard *et al.* (1982) obtained false positive reactions when using the capillary tube precipitin test (the ring test) for meat identification.

They claimed that the presence of sodium ascorbate, but not sodium erythrobate, could

lead to a false positive reaction. Neither ascorbic acid nor sodium ascorbate was listed as an ingredient in any of the 20 products tested.

In contrast to immunodiffusion, IEF showed the presence of one or more species in each product. However, some products contained more than two species, and the formation of CK dimers then impairs identification. For example, an artificial mixture of pork, beef and chicken (in equal proportion) was indistinguishable from a mixture of beef and chicken. Consequently, IEF revealed only beef and chicken in product No. 20 although all three species were detected by immunodiffusion (Table 1). Another discrepancy arose for product No. 1 where beef, pork and sheep were all detected

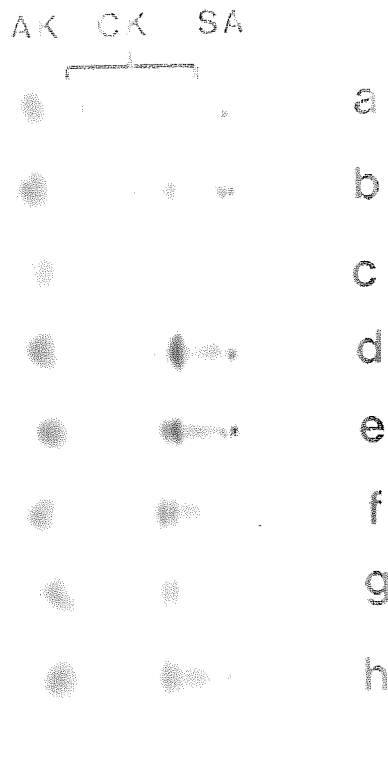


Fig. 3. IEF gel stained for AK and CK. Some stained material was present at the position of sample application (SA).

a sheep (80°C/30 min) b sheep:pork (1:1)
c Product No. 1 d pork (80°C/30 min)
e Product No. 3 f Product No. 6
g Product No. 7 h beef:pork (1:1)

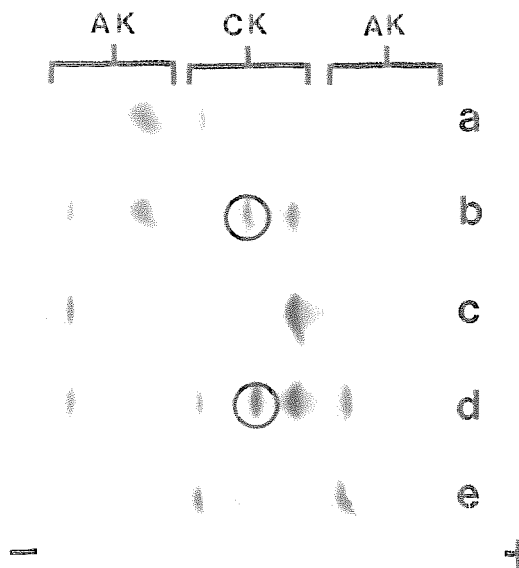


Fig. 4. IEF gel stained for AK + CK

a sheep b sheep chicken (1 1)
c chicken d red kangaroo chicken (1 1)
e red kangaroo

A prominent hybrid band in each of the two lanes of mixed species is encircled

by immunodiffusion but only beef and pork by IEF. Despite the formation of hybrid CK dimers, sheep and beef are distinguishable in artificial mixtures of pork, provided that they constitute at least 20% of the mixture. It is possible that all three species were present in product No. 1 but that the enzymic activity of sheep CK was too low to be detected.

Difficulties with other methods such as measurement of histidine/dipeptide ratios (Carnegie *et al.* 1982) would also be expected when several species are present. For example, while the measurement of the anserine/carnosine ratio can be used to detect chicken in cooked pork products the addition of sheep meat would obscure the detection of the chicken (Tinbergen and Slump 1976).

As shown in Table 1, for nine of the twenty products examined, there was complete agreement among label descriptions, IEF and immunodiffusion. For a further five products (Nos. 3, 7, 11, 16 and 18) IEF agreed with label descriptions, but loss of antigenicity during smallgoods processing prevented detection by immunodiffusion. In two products (Nos. 1 and 20), the three species detected by immunodiffusion agreed with

those on the label, but IEF detected only two species in each, owing to CK hybrid dimer formation.

For three products (Nos. 2, 4 and 13) there were more species listed on the label than could be detected by IEF and immunodiffusion. This may have been due to lack of sensitivity especially in product No. 13 where chicken was listed on the label as being present at only 1%. In the remaining product (No. 19) IEF detected a species (pork) not listed on the label. However, it was not possible to confirm this result by immunodiffusion as no positive reaction was obtained for this product.

Conclusion

The above results show that the species of origin of at least some of the meat present in any smallgoods product can be determined by the methods described. However, species present in small proportions (<10%) may not be detected. By using a combination of both immunodiffusion and IEF, the problems peculiar to each (*viz.* loss of antigenicity during smallgoods processing, and formation of CK hybrid dimers during dialysis prior to IEF) can, to a large extent, be overcome.

References

- Carnegie, P. R., Hec, K. P., and Bell, A. W. (1982). Ophidine and other histidine dipeptides in pig muscles and tinned hams. *J. Sci. Food Agric.* 33, 795-801.
- Gerrard, F. (1976). 'Sausage and smallgoods production' 6th Ed., p. 129 (Northwood Publications Limited : London).
- Hayden, A. R. (1981). Use of antisera to heat-stable antigens of adrenals for species identification in thoroughly cooked beef sausages. *J. Food Sci.* 46, 1810-3.
- Kaiser, K. P., Matheis, G., Kmita-Durmann, C., and Belitz, H. D. (1980). Identification of animal species in meat, fish and derived products by means of protein differentiation with electrophoretic methods. *Z. Lebensm. Unters. Forsch.* 170, 334-42.
- Kang'ethe, E. K., Jones, S. J., and Patterson, R. L. S. (1982). Identification of the species origin of fresh meat using an enzyme-linked immunosorbent assay procedure. *Meat Sci.* 7, 229-39.
- Karpas, A. B., Myers, W. L., and Segre, D. (1970). Serologic identification of species of origin of sausage meats. *J. Food Sci.* 35, 150-5.
- King, N. L. (1984). Species identification of cooked meats by enzyme staining of isoelectric focusing gels. *Meat Sci.* (in press).

- King, N. L., and Kurth, L. (1982). Analysis of raw beef samples for adulterant meat species by enzyme staining of isoelectric focusing gels. *J. Food Sci.* 47, 1608-12.
- Kurth, L., and Shaw, F. D. (1983). Identification of the species of origin of meat by electrophoretic and immunological methods. *Food Technol. Aust.* 35, 328-31.
- Slattery, W. J., and Sinclair, A. J. (1983). Differentiation of meat according to species by the electrophoretic separation of muscle lactate dehydrogenase and esterase isoenzymes and isoelectric focusing of soluble muscle proteins. *Aust. Vet. J.* 60, 47-51.
- Tamaki, N., Nakamura, M., Harada, M., Kimura, K., Kawano, H., and Hama, T. (1977). Anserine and carnosine contents in muscular tissue of rat and rabbit. *J. Nutr. Sci. Vitaminol.* 23, 319-29.
- Tinbergen, B. J., and Slump, P. (1976). The detection of chicken meat in meat products by means of the anserine/carnosine ratio. *Z. Lebensm. Unters. Forsch.* 161, 7-11.
- Tizard, I. R., Fish, N. A., and Caoili, F. (1982). False-positive reactions in the immunoprecipitation test for meat identification. *J. Food Prot.* 45, 353-5.
- Whittaker, R. G., Spencer, T. L., and Copland, J. W. (1982). Enzyme-linked immunosorbent assay for meat species testing. *Aust. Vet. J.* 59, 125.

Seafood research in New Zealand

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There is an exciting range of research in fish technology in New Zealand, with much of it being directed to foster quality in products for export. This recognition of the need to export to survive and the need to establish ways of maintaining quality to compete on world markets ensures a continued incentive for research.

The New Zealand offshore fishing industry has undergone a period of considerable expansion, particularly in joint fishing ventures with other fishing nations such as Russia, Japan and Korea. Information on these fisheries is readily available elsewhere (Armitage 1983) and a major portion of the June 1983 edition of Australian Fisheries has been devoted to the problems of the inshore fisheries where too many boats are chasing too few fish. This paper is not about the fisheries as such or about the processing industry although the author visited over 30 factories, but about recent research on seafood technology which is of particular interest. Where necessary, background information including the results of applications of research is included to help put that research into context. An obvious

observation is that the fishing industry is an export growth area for New Zealand and this has brought about a consciousness of the need for close liaison between industry and the research groups.

Research centres

The main centres of seafood research in New Zealand are Nelson in the South Island (Massey University Fish Research Unit) and Auckland in the North Island (DSIR Fish Processing Research Unit) where the author spent seven months. Application of research results, extension, in-plant advice and construction of codes of practice is done by the New Zealand Fishing Industry Board in Wellington. Research into fish biology, population studies and catch statistics is handled by the Fisheries Research Division of

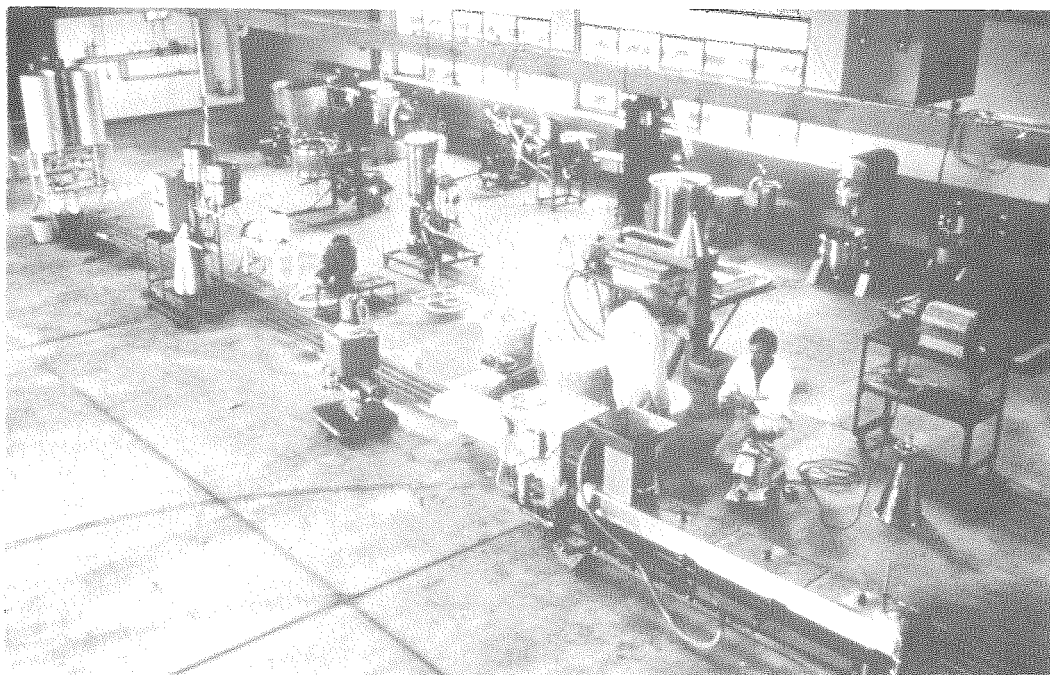


Fig. 1 The food process bay at DSIR, Mt Albert Research Centre.

DSIR, while the imposition of catch limits and policing of regulations is the responsibility of the Ministry of Agriculture and Fisheries. In addition, various Universities conduct basic research into the biology and zoology of species of commercial importance. Other DSIR divisions, such as Applied Biochemistry and Chemistry, also contribute to particular aspects of research where appropriate, e.g. nutritional and analytical work on wax esters from deep sea fishes.

The DSIR unit has been in operation about four years and is constituted within the Division of Horticulture and Processing located on campus at the Mt Albert Research Centre (MARC). This allows access to library and computing facilities, process equipment (Fig. 1) and the scientific equipment and expertise associated with a large research centre of over 300 persons. This population also provides sufficient numbers for sensory evaluation panels. Sensory evaluation plays an important part in the Unit's overall program of describing the changes that occur during storage of New Zealand's major commercial species, with the aim of giving

reasonable indications of their commercial shelf life. This work is reinforced with both microbiological and chemical assessment of fish in various forms — whole, gutted, filleted, vacuum packed and packed in modified atmospheres. Two taste panels are used, an inhouse panel of MARC staff known as the SERF panel (sensory evaluation research, fish) and an off-campus panel known as the SETOF panel (sensory evaluation team on fish). Establishment of this SETOF panel was a bold concept and it was recruited by advertising in the newspapers. Of the original hundred or so applicants, about twenty were found to be reliable in attendance and consistent in their scoring patterns. The ladies of the SETOF panel are all middle-aged, middle class women who are keen to attend regularly. They are not paid but are reimbursed for travel expenses. The idea is that this panel would be more representative of the New Zealand consumer than the MARC staff, even though the latter may have had more training at picking fine shades of difference between samples.

Quite independently a technique of profile evaluation has been derived at MARC which

is very similar to that used at the Tasmanian Food Research Unit (cf. Quarmby *et al.* 1983). This profile technique has been used in research programs supported by various companies to assess organoleptic changes in orange roughy, blue grenadier (*hoki* to New Zealanders), snapper and other processed seafood products. The DSIR can enter into contract research agreements or charge for their services.

Another interesting development is the formation of a panel of Japanese living in New Zealand to give information on Japanese consumer preference, not only on seafoods, but also on fruits, wines and dairy products. Japan is an important export market for New Zealand produce.

Offshore fisheries

Orange roughy

While the inshore fisheries suffer from the problem of a dwindling resource, the prospect for the off-shore fishery is much brighter. The major species in this fishery is the orange roughy (*Hoplostethus atlanticus*) (Fig. 2) which, although it can be caught all

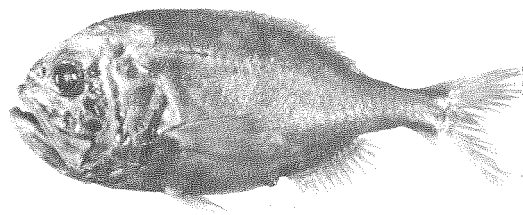


Fig. 2 An orange roughy

around both islands, is fished mainly on the Challenger plateau and along the 1000m contour, west of the South Island, and out to the East around the Chatham Islands. The bottom-dwelling (demersal) orange roughy has no swim bladder but uses extensive deposits of wax esters for buoyancy. Investigations by both the DSIR and Massey teams on the wax esters has shown that their composition gives them commercial potential as substitutes for whale oil and jojoba oil in applications such as high pressure lubricants (Buisson *et al.* 1982). The more lucrative and esoteric application in cosmetics is also being pursued.

Wax esters are well known as faecal lubricants, e.g. castor oil, and while the oil is mainly associated with the head and belly flap (up to 20% wax ester) it is also located under the skin (Grigor *et al.* 1983). This has necessitated development of deep skinning techniques to remove as much as practical, leaving a fillet with a relatively low oil content. However, consumption of a large meal of the fillets may still lead to the condition aptly described as intestinal hurry (Everage E. public comm.). For this reason toxicity trials using rats and pigs have been done on behalf of the New Zealand Fishing Industry Board. The hazards associated with eating orange roughy under normal conditions of consumption are considered to be very slight, indeed its effects may be advantageous to some. The fillets are white, of good texture, and have the decided advantage of not being strong in flavour — an attribute that consumers seem to prefer. While its shelf life is possibly not greater than that of other whitefish — snapper, for example — its bland character and good textural stability and appearance make it difficult to judge how long it has been stored until it is ammoniacal.

Work at DSIR has established that heading and gutting at sea then storage in ice offers no advantage over storage whole in ice. An increasing proportion of the catch is landed iced from N.Z. boats, while the joint venture boats (Fig. 3) and the two large boats operating out of Dunedin by the Fletcher Fishing group freeze the headed and gutted



Fig. 3 A Russian freezer trawler at Dunedin dwarfs the ice boat 'Otago Challenger'

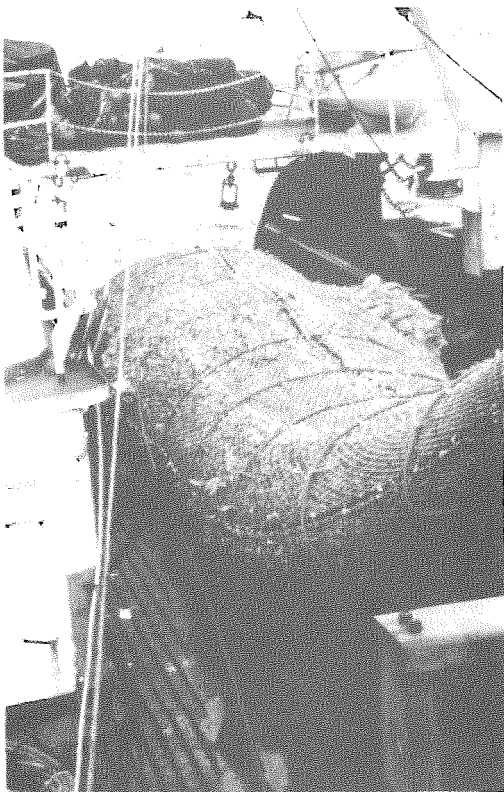


Fig. 4 A 60 tonne shot of orange roughy in the cod end

fish into blocks in vertical plate freezers. Large catches can be taken (Fig. 4) and hauls of 20 tonnes are common. Much more than this can cause considerable damage to the fish and large catches take a long time to stow. The oil is produced commercially in Dunedin using the low temperature wet rendering process developed by the Meat Industry Research Institute of New Zealand. In other plants the roughy offal is not kept separate from the general stream and hence there is a residual of wax esters in the fish meal produced. Care is required here since stock fed rations containing the fish meal can be subject to scouring, which results in loss of production.

Tuna

New Zealand fishermen have recently started to exploit tuna, in particular the southern bluefin which is frozen and sent to Japan for consumption as raw fish (*sashimi*). Handling and bleeding techniques on board

have been investigated by the Massey research team (Wilson 1982). The Massey fish research unit at Nelson has been established for a number of years and it does much of the practical research for the Fishing Industry Board. Recently it has investigated the process of thawing the large fish blocks from joint venture vessels for further processing. The system of controlled thawing using warm water at 16° – 20° C in specially designed tanks that have been recommended (Boyd and Wilson undated) is in use at several plants including the large Sealord plant at Nelson which thaws about 70 tonnes per day. They have also shown that suitably stable fish blocks can be made from the southern blue whiting (*Micromesistius australis*).

Inshore fisheries

Snapper

There is a lucrative inshore fishery for line-caught snapper (*Chrysophrys auratus*) killed by the *iki jime* process and sent chilled by air freight to Japan. The *iki jime* process involves spiking the live fish through the brain immediately it is brought on board (Fig. 5). The fish is then plunged into slush ice (ice and sea water) for one to three hours according to size until the centre temperature is near 0° C (Harvie 1982). They are then packed side by side belly down in ice for delivery to the factory where they may be rechilled if necessary in slush ice. Each fish is weighed, graded for appearance and packed in insulated boxes along with a sealed plastic

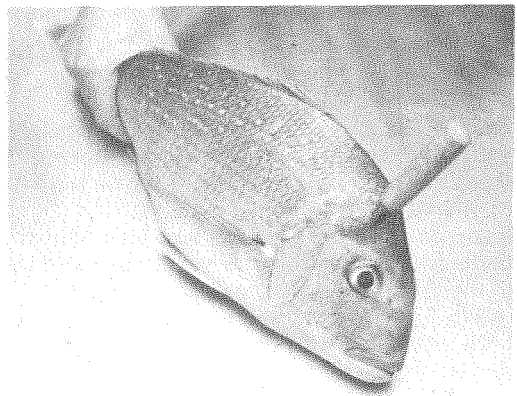


Fig. 5 The *iki jime* process of spiking snapper in the brain behind the eye.

bag of ice ready for airfreight to the Tsukiji market in Tokyo. Each fish must be carefully handled so as not to dislodge any scales or damage the eyes otherwise a top price will not result. The *iki jime* process is required by the Japanese who use it extensively themselves even on farmed snapper brought live to the market. Sometimes a thin wire is also pushed down the spinal cord to destroy the nervous system. This too is said to appreciably improve shelf life (Anon. 1982). The spike severs the *medulla oblongata*, does extensive damage to the *corpus cerebelli* and disrupts the midbrain. The result is that the fish retain for a longer time their natural bright colouring, flesh texture and appearance and flavour. The reasons for this are not known but some excellent work has been done at Nelson. Troll-caught Australian salmon (Kahawai in N.Z. — *Arripis trutta* esper) were spiked and placed in slush ice within 35 s of them striking the hook. Comparable unspiked fish were also taken. The spiked fish take longer to go into rigor mortis, 273 min. compared to 200 min. for the unspiked fish; their pH falls only slightly and the ATP increases from about 3–4 $\mu\text{mole/g}$ to 8–9 $\mu\text{mole/g}$ about 200 min. after spiking just when the ATP level of the unspiked fish falls steeply to a negligible amount and the fish enter rigor (Boyd *et al.* 1984). These results are intriguing and run counter to expectations; they do indicate that some change in post mortem biochemistry is occurring and this change may be responsible for the improved high quality shelf life characteristic of spiked fish. It is likely that this technique will become more widespread and be applied to other line-caught species of value.

Dogfish

Following a decline in catches in the Canterbury Bight, fishermen have turned their attention to the spiky dogfish which is readily caught in this area, but which has an unpleasant livery, faecal off-taste. Experiments by the Nelson unit have shown the taste to be independent of the age and sex of the shark and of the season or area of catch. The flavour responsible seems to be both water- and solvent-soluble and so far has eluded identification.

Shellfish

The main mussel growing areas of the

Marlborough Sounds are close to Nelson and development of process techniques and a specialized packaging and presentation technique have resulted in exports of a very sophisticated mussel package. Domestic consumption of pickled mussels in 500 g plastic pottles is also increasing. Like Australia, New Zealand has had problems with pollution of oysters and shellfish, and DSIR has investigated the performance of depuration plants and the factors that cause pollution in some areas.

Jack mackerel

New Zealand has a considerable resource of two species of jack mackerel which up to the present have been used mainly for fish meal (Fig. 6). Work at DSIR has shown the good keeping qualities of the most abundant species (*Trachurus novaezelandiae*) and its potential for the *sashimi* trade. The jack mackerel exhibit only slow post-mortem

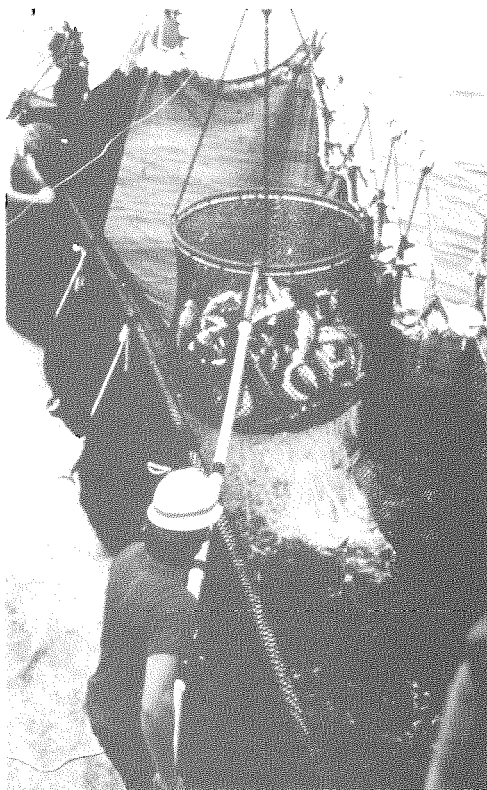


Fig. 6 Jack mackerel are brailled on board out of the purse seine.

breakdown in nucleotides and after seven days the K value (an index of breakdown) was only 20%, a value within the levels required for *sashimi*-grade fish. This agrees with the results of sensory evaluation trials, since by this stage no spoilage characteristics were detected by the panel.

Microbiologically, the fish remain sound for over two weeks on ice with an aerobic plate count in the flesh of below 10^5 cfu/g 7 days after catch. Smoked jack mackerel products have also been developed for the export trade and DSIR are advising on commercial production.

Further research

There are many obvious topics for research such as further investigation of the *iki jime* process and its application to other species, also development of live transport of lobster and shellfish. In particular, the industry needs a thorough investigation into all handling procedures from the moment the fish lands on the deck until it is through the process line. Improvement in this area would result in increased yields, a better product and greater profitability of the industry. It is to be hoped that both government and industry support will further the good research already being done.

References

- Anon. (1982). Fish marketing in Japan. N.Z. Fish. Ind. Board. Bull. Wellington. No. 68 (Dec.) pp 14-5.
- Armitage, R. O. (1983). Developments in the New Zealand fishing industry. *Aust. Fish.* 42(2), 7-10.
- Boyd, N. S., and Wilson, N. D. C. Thawing of fish blocks. Massey University Food Technology Research Centre, Fish Research Unit, Fish Quality Note No. 2.
- Boyd, N. S., Wilson, N. D., Jerrett, A. P., and Hall, B. I. (1984). Effects of brain destruction on post-harvest muscle metabolism in the fish kahawai (*Arripis trutta*). *J. Food Sci.* (in press).
- Buisson, D. H., Body, D. R., Dougherty, G. J., Eyres, L., and Vleig, P. (1982). Oil from deep water fish species as a substitute for sperm whale and jojoba oils. *J. Amer. Oil Chem. Soc.* 59, 390-5.
- Grigor, M. H., Thomas, C. R., Jones, P. D., and Buisson, D. H. (1983). Occurrence of wax esters in the tissues of the orange roughy (*Hoplostethus atlanticus*). *Lipids*. 18, 585-8.
- Harvie, R. (1982). A code of practice for air freight chilled fish. New Zealand Fishing Industry Board, Wellington.
- Quarmby, A. R., Bremner, H. A., and Thrower, S. J. (1983). On-board handling of gemfish Part II: Sensory profiles. *Aust. Fish.* 41(11), 42-5.
- Wilson, N. D. C. (1982). Some aspects of the handling and processing of tuna in New Zealand's South Island west coast fishery: A preliminary investigation. Massey University Food Technology Research Centre. Report No. 5.

Shelf-stable intermediate moisture meat products*

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Meat products have long been available which have prolonged shelf-life at ambient temperatures because of their reduced moisture content, and sometimes their increased acidity. Traditional products incorporating these characteristics include many salami types, plus a wide variety of other fermented and dried products of European origin.

In recent years there has been increasing manufacture in Europe and the USA of products derived from these traditional foods but which in their presentation as 'intermediate moisture' (IM) food products owe much to modern developments. These modern products represent one means of selling meat in convenient forms as snack foods. Much of the associated advertising deliberately projects these products as ready-to-eat snack foods, thereby clearly distinguishing them from fresh meat.

Corresponding developments in Australia have been very limited, and the type of marketing activities necessary to encourage success has not as yet been evident in this country. Even overseas, the range of products is quite limited. However, the possibilities for product development and market success appear to be substantial within Australia. Exports may also be a possibility.

Introduction

In 1982 a study was made in overseas countries of reduced moisture, shelf-stable meat and meat-containing products, including types of products available, their formulation, processing and packaging. Marketing data and the possibility of developing newer derivatives of these foods were also investigated.

Meat and meat products have long been preserved by such techniques as salting, drying and smoking in various combinations. Examples include biltong, charqui, and many salamis. Sometimes the product would need subsequent soaking and cooking to render it more palatable. More recently, successful variations of formulations,

seasonings and processing have led to increasingly diverse products which are shelf-stable at ambient temperatures.

During the 1960s, a product generally described as 'dried beef sausage stick' appeared on the USA and Canadian retail markets, as a packaged, shelf-stable snack item. This was widely imitated and eventually a large number of different brands were available. As a further diversification, 'jerky' also became available in individual or multi-piece packs and was strongly promoted.

These were effectively the first distinctly meat-based snack foods to become widely available. The dried beef sausage stick derived fairly directly from the continental smoked, cured and partly dried sausage known as pepperoni.

There has been very little similar activity in Australia. A puffed pork rind product has been available for several years. A few items based on beef with reduced water activity are available but do not appear to enjoy

*Suggested formulations, processing methods and product data are available on request.

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substantial marketing support or sales. They include smoked beef sausage products of about finger-size which are packaged as individual items, and are intended for sale as snack items. However, their water activities are too high for them to be considered shelf-stable, and they are immediately disadvantaged.

The following report includes comments about product types and qualities, various processing details, and observations on the possibilities in this country for meat-derived snack foods.

Intermediate moisture foods and water activity

IM foods are those in which a reduced moisture content is critically important to their shelf stability. Generally, moisture contents of these foods are in the range 20–50%. However, there must be dissolved in this moisture sufficient solutes to decrease the food's water activity below the point where microbial growth can be supported. Some common examples of IM foods are honey, confectionery with high sugar content, jams, fruit cakes, dried fruits, biltong, and various dried or smoked salami and other sausage products.

Water activity (a_w) can be defined in various ways, but it is effectively a measure of the availability of water in a system. It is a_w ,

not the total water content in a food which determines whether it is shelf stable, or whether it requires other procedures for its preservation. Consequently, two foods can have similar moisture contents but sufficiently different a_w such that one is shelf stable, and the other is not.

Water activity is usually expressed as a decimal value between zero and unity, and may be defined as the ratio of the vapour pressure of the water in the food (P_{food}) to that of pure water ($P_{\text{H}_2\text{O}}$) at the same temperature.

$$\text{Thus, } a_w = \frac{P_{\text{food}}}{P_{\text{H}_2\text{O}}}$$

Consequently, the a_w of a food can be determined very easily, and with sufficient accuracy for quality control and production control purposes using a suitably calibrated sample hygrometer. A sample hygrometer has a sample chamber which can be hermetically sealed to the instrument. This permits the instrument to measure relative humidity as a function of the sample. More elaborate systems are also available for more detailed work.

Many traditional European sausage products are shelf stable due to either a suitable pH- a_w combination at the end of



Fig. 1. Two well-presented shelf-stable European sausage snack sticks.



Fig. 2. Typical dried beef sausage stick single-serve packs found in the USA.

processing, or to post-processing evaporation which causes reduction in a_w . Many salamis fall into one or other of these categories. Two of the many traditional low-moisture-content meat products widely available in Europe are *landjaeger* and *bundnerfleisch*. Both of these are shaped into rectangular blocks with the use of casings and formers. *Landjaeger* is purchased as individual units, whilst *bundnerfleisch*, being much larger in cross-section is usually bought by the slice or the piece. *Landjaeger* serves largely as a snack, and can be viewed as an early development of meat into a convenient snack food item.

Dried beef sausage sticks, also known in Europe as mini-salamis, are a recent innovation. These are fairly widely available in Europe, and are a deliberate attempt to present meat as an attractive snack item.

Of the several brands of beef sticks available in Europe, two very well presented products are *Bi-Fi** — the mini salami, and *Saletti*† — the baby salami (Fig. 1). Both are available as individually packaged items, and are presented in very high quality, colourfully printed pouches which, being opaque, give good protection from light as well as from oxygen.

*Registered trade name of Scharffit Ansbach, W. Germany

†Registered trade name of Karl Könecke, Fleischwaren Fabrik, G.m.b.H. & Co, W. Germany

In the USA, there is considerable effort on marketing dried beef sausage sticks and beef jerky as snack items. Emphasis is achieved through the design of advertising materials, the location of the products in retail display areas, and product names themselves. Labelling laws require that ingredients be listed on the package in descending order of content. However, with dried beef sausage sticks, product names often do not refer directly to the meat origin of the product, or else combine that origin with some emphasis on the snack food aspects of the product (Fig. 2).

None of the products seen in the USA use the high quality (and expensive) packaging materials used for the two European products mentioned above. All USA manufacturers use flexible packaging materials which are basically transparent and are to varying degrees covered with colour printing.

The use of chemical antioxidants was indicated only on products derived from pork. However, all products were vacuum packaged, possibly with the use of gas-flushing.

Many of the sausage stick and jerky products are available in a range of pack sizes, from single-serve packs to approximately 44-stick bulk packs, and packaging includes clear glass jars fitted with screw caps, aluminium-lined cardboard canisters, and flexible vacuum packages of

varied size. One manufacturer markets a 2.4 m 'coil' of dried beef sausage stick as a 'family pack' to be used as required. This idea seems to have more potential than is realized by the manufacturer, for packaging costs are reduced per unit weight of product, and the opportunity to establish the product permanently in the home is clearly evident.

Commercial processes

Sausage sticks

The meat mix is prepared by comminuting the selected meats and fat to the required texture while adding the dry ingredients. If glucono- δ -lactone (GDL) is used as an acidulant, it is included with the other dry ingredients. If a starter culture is used, it is first made up according to the supplier's instructions, and then added during comminution.

If the meats used are at or slightly below 0°C at the start of comminution, smearing is reduced, and cutting and particle size are easier to control. Grinders or bowl choppers used for comminution and filling machines need to be in very good condition to ensure that the lean and fat are cut cleanly and that smearing and squashing are avoided. After cutting, the meat mix should either be immediately filled out or kept refrigerated until required for filling out.

Natural, cellulose, or regenerated collagen casings, which are all suitable for smoking and drying are used. The two European products mentioned earlier (*Saletti* and *Bi-Fi*) were filled into cellulose casings which had a release agent coated onto their inner surfaces, and then linked into approximately 140 mm long sausage units for subsequent smoking and drying. They were then separated at each linking point and, still inside their cellulose casings, packaged into colourful unit packs for distribution. The advantages of this system include avoidance of post-processing handling of the products' surfaces, the lower costs of cellulose casings, and avoidance of 'off-cuts'.

All of the products seen in the USA had regenerated collagen casings, which, like cellulose casings, are parallel-sided and available in shirred lengths of 25-30 m, and so are suited to mechanical handling. The diameters of casings vary from 12 to 17 mm.

By far the most common method of filling out these products in the USA comprises:

- filling entire lengths of the regenerated collagen casings
- winding these onto rectangular metal frames
- suspending these frames on trolleys
- conveying the trolleys to the smokehouse.

Thus, at each point of contact between the casings and the frames, i.e. about every 75 cm, there are two flattened sections of casings containing some of the meat mix. After processing, the straight sections of sausage in between are cut into uniform lengths for packaging, but the flared ends become 'off-cuts' and are usually recycled after fine-cutting in a bowl chopper. Although these 'off-cuts' are not lost, recycling them clearly adds to production costs.

The sausage sticks are then packaged, usually as unit items, in heat-sealable, thermo-formed packages. These appear to be vacuum packages, although there could also be an intermediate step of gas-flushing with a carbon dioxide-nitrogen mixture. The upper web is usually printed in the colours red, yellow and orange, with black or white printing being very common. The lower web is usually of clear film and therefore offers little protection against incident light.

The meat bar concept

Another form of the dried sausage stick is the 'Flat Snack' concept, promoted by the Devro Co., which produces regenerated collagen casings. The product is a hybrid of the dried beef sausage stick and the chopped and formed jerky products. It is essentially the formulation used for the dried beef sausage stick, but it is filled into oversized collagen casings, which are then passed between suitable rollers so that the product emerges as a flat strip of preselected thickness. This is then wrapped onto frames as for sausage sticks, or hung in lengths on sticks for drying and smoking.

Advantages include the pleasantly plastic chewiness of the sausage sticks, the possibility of automated operation, and much greater dehydration rates than for dried sausage sticks. The main disadvantage appears to be the cost of the regenerated collagen casing.

The 'Flat Snack' has a high fat content, because basically the formulation for the dried beef sausage stick is used, and in this respect it contrasts with beef jerky, which has

a very low fat content. Whilst the increased fat content improves the product's chewiness, it can also result in a greasy or oily appearance. Attention to the formulation and preparation of the meat emulsion can probably eliminate this.

Jerky

Jerky is produced directly from solid muscle by two different methods. In the first, beef rounds, after removal of all visible fat, connective tissue and tendons, are pumped with a curing solution and then intensively massaged. The massaging softens the meat, allowing it to be filled into casings to produce a log of uniform cross-section. After cooking to an internal temperature of about 65 °C, the logs of meat are cooled and sliced into discs about 1.5-2 mm thick, which are then spread onto mesh trays, smoked and dried. Consequently, the discs of product are largely, although not totally, sliced at right angles to the muscle fibres. The product has a very attractive, translucent red colour, although this probably darkens with age and exposure to light. It is rather tough, and would benefit very much from mechanical tenderization of the muscles (beef rounds) before pumping. This product is vacuum packaged in clear, heat-shrinkable plastic pouches, which probably give reasonable

protection from oxygen, but not from light. However, they do display the product's attractive appearance to advantage.

Few processing details are available for the second product, but its appearance indicates much about its manufacture. Primal cuts, after trimming as above, are sliced into strips about 2-3 mm thick and 20-25 mm wide, in the direction of the muscle fibres. These are soaked in a curing solution, rinsed with water to remove excess solution, and the pieces are then spread in a single layer on mesh trays. After loading the trays onto trolleys, the raw product is placed in a smokehouse for smoking and drying. The somewhat translucent product is reddish-brown on completion of processing, and it is vacuum-packaged in single and various multiserve packs.

Jerky is also widely manufactured as a chopped and formed item. This product differs considerably from the traditional intact-muscle jerky, but does offer certain manufacturing advantages. These include control of composition, and very efficient use of the available meats. Collagenous material which would be a trimming loss in the production of intact-muscle jerky can be incorporated in the chopped and formed version.

Once the meats have been formed into thin

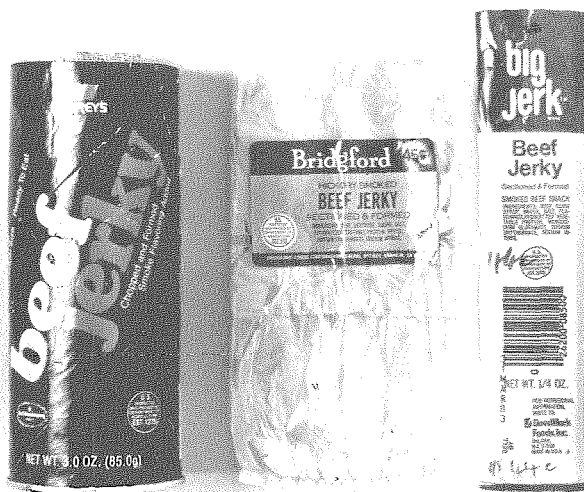


Fig. 3. Examples of single- and bulk-serve packs of jerky in the USA. The centre pack is used for the disc form of intact-muscle jerky mentioned in this article.

strips, processing is virtually identical to that for intact-muscle jerky. However, processing details differ before that stage, and in some instances meat restructuring technology is used.

The selected meats are comminuted by an appropriate method, which may be mincing, bowl chopping or 'flaking' with a Comitrol* or similar machine. Desired additives, such as salt, phosphates, sugars and flavourings are added during the comminution step, and the mix is then formed into rectangular blocks or 'logs'. These are tempered to approximately -2° to -5°C , and are then sliced into strips of the required dimensions. Pressing to shape between tempering and slicing is incorporated in some processes. The strips are loaded onto trays and then processed as above.

Important factors in manufacture

pH

The pH reduction in dried beef sausage sticks serves three purposes — dehydration is facilitated by bringing the pH close to the iso-electric point of meat proteins, a desirable acidic, or 'tangy', background flavour is introduced, and there is a substantial inhibitory effect on further microbiological activity in the product.

Most jerky products rely for their shelf stability on an a_w lower than that necessary for dried beef sausage sticks, as few jerky products are subjected to pH reduction during manufacture. It is generally believed that the ultimate pH of sausage sticks should be in the range 4.7–4.9, and GDL or lactic acid starter cultures are used as alternative acidulants. The principal advantage of starter cultures appears to be that they provide a microflora which competes with that already present and will improve the microbial stability of the product. GDL does not provide this particular benefit, but does permit fairly accurate control of the final pH of the product, as well as saving processing time.

a_w

Water activity assumes critical importance in shelf-stable but non-sterile foods. It must be low enough to ensure that along with pH

considerations, microbial activity is suspended indefinitely. If the a_w is reduced below the necessary level, unnecessary dehydration has occurred and therefore money has been lost. If it is too high, there is a very real risk of product spoilage, or worse, a potential public health risk.

Close control of formulation and processing should result in low a_w products exhibiting little batch-to-batch variation, suggesting that moisture content could be used as a means of determining the end-point of drying. However, direct a_w measurement is a much more appropriate quality control tool, and will overcome any non-uniformities in ingredients or blending. Sample hygrometers calibrated to read a_w directly are available at reasonable cost and are adequate for in-plant quality control purposes.

Different manufacturers suggested 'safe' a_w varying between 0.82 and 0.88. Lastner and Rödel (1976) state that if either the a_w is less than 0.85, or the pH is below 5.0, protection against staphylococcal enterotoxin production is obtained, and also advocate the use of vacuum packaging materials impermeable to oxygen. Since dehydration causes weight loss, and consequently economic loss, it is important to know the precise a_w required and also to ensure that the a_w of the whole batch does not exceed it.

Anti-mycotic agents

Several acceptable fungistatic additives, including sorbic acid, glycerol, parabens and propylene glycol assist in improving the shelf-stability of IM foods. Although specific approval for intended applications in Australia will be necessary, a precedent exists in that potassium sorbate/sorbic acid is already permitted in sliced processed cheese. A revision of the relevant statute has been gazetted, and will read as follows:

'Sliced processed cheese may contain sorbic acid in an amount not exceeding 0.2 parts per centum.'

Smoking

Smoking has long been an important process in the production of manufactured meat products. All dried beef sausage sticks and jerky examined were smoke-treated. Some contained artificial smoke additives, but most were smoked naturally.

Different woods give different smoke tastes to the finished product. Sometimes these

*Registered trade-mark for a range of equipment manufactured by Urschel Laboratories Inc., of Valparaiso, Indiana, USA.

differences are subtle, but at times they are quite marked. The intensity and duration of smoking is significant in determining the strength and quality of the final smoke taste. Woods commonly used in the USA include hickory (which is highly rated, although there seems to be no data to objectively confirm its claimed superiority), maple, oak, beech, and oregon. As in Australia, juniper berries are sometimes added to the smoke generator.

Although most operators have evolved their own schedules, the end results are much the same, the most obvious product differences being due to the seasonings and the amount of smoking applied. The type of wood used to produce the smoke generally has relatively a minor effect.

Drying

Drying is necessary to reduce the a_w to the desired level. Of the various methods available the most convenient is usually via smokehouse processing, as most jerky and sausage stick products are smoked naturally as well as being partially dried. Consequently, smoking and drying can be carried out independently, or in part concurrently in a smokehouse.

The drying necessary to achieve the required a_w increases production costs, because of weight losses involved. The leaner the product to be dried, the greater the amount of drying necessary. Conversely, less drying is necessary to achieve a given a_w as the amount of fat and non-meat ingredients such as cereals or hydrolyzed corn starch in the product is increased.

Product development possibilities

Research on IM foods is not evident at a significant level at either universities or government laboratories overseas. Instead, there are very limited programs of product development at the laboratories of some of the large supply houses and manufacturers of low a_w products. Given the continuing success of the two basic products (i.e. dried beef sausage sticks and jerky) as meat-derived snack foods, there are many avenues which could be developed.

Information gained in the USA regarding production of low a_w products provides a background in considering product development possibilities. The following points are relevant:

- the basic meat mix for dried beef sausage sticks and similar items is formed into sausage shapes because of convenience and familiarity to both manufacturers and consumers.
- equipment and technology exists, or is very readily adaptable, to permit production of a wide variety of other shapes.
- 'bite-size' pieces, products with or without casings, products with varying shapes and contours, can all be made either using existing equipment and technology, or after simple adaptation. One manufacturer of dried beef sausage stick uses the off-cuts in 'nibbling packs', which appear to be a successful and resourceful way of avoiding recycling of product. This suggests that a market for 'bite-size' pieces, as a deliberate product concept, may be readily developed.
- the virtually universal use of pepperoni-derived seasonings in the USA for dried beef sausage sticks is just one type of flavouring possibility. Other obvious possibilities include various roast flavours, and blends of meats, cheeses and other ingredients to take advantage of the popularity of Italian foods such as pizza.
- although the proportion of fat present has a major effect on the plasticity of the product, corn syrups can be useful both as humectants and for some further, or alternative contribution towards plasticity control.
- products intended for consumption immediately the package is opened (e.g. single-serve packs of jerky or sausage sticks) can be safely packaged at slightly higher a_w than bulk packs likely to be opened and closed repeatedly over a period of days or weeks. The extent of the attendant saving in weight-loss seems worthy of investigation from a commercial viewpoint.
- marketing of beef stick products in the USA has generally tended to emphasize the snack food aspect rather than relate the products explicitly to sausage. However, a broader range of low a_w meat-derived products would be easy to develop and should appeal to a wider market. Associating meat with the snack-food concept is apparently not difficult, but does require a thorough marketing approach.

- manufacturing low a_w products based on the more plastic texture of the dried beef sausage stick, but in a thin form, e.g. bars or analogues of the savoury biscuit concept, is favoured by at least the following points:
 - ▶ shorter processing time (more rapid drying)
 - ▶ the potential for closer control of ultimate a_w (with thinner products there should be more even drying)
 - ▶ the increased diversity of product shapes and sizes that can be manufactured
 - ▶ the elimination of off-cuts, which would permit better yields and avoidance of reprocessing
 - ▶ the emphasis is usually on beef as the major component, and the only meat ingredient in these products. However, some products include chicken or turkey meat, and 'Genoa salami sticks' (pork) are also available. Within Australia, combinations of beef and lean mutton appear worthy of investigation.

Conclusion

In various parts of the USA beef jerky is a traditional product. Both 'solid muscle' and the comminuted forms of jerky available are quite tough, and require a great deal of chewing (the more so with the 'solid muscle' version). The seasoning/spice mixes used, however, are frequently very attractive. Even so there is no obvious tradition of eating these

types of products in Australia, and it would undoubtedly be a long and expensive process to educate the community to accept them.

On the other hand, the much greater plasticity of the dried beef sausage stick makes its textural properties immediately appealing. There has been no well-coordinated attempt to market products of this type in Australia, or for that matter to consider them for export. Inescapably, with dehydration the price of the remaining meat escalates. Equally inescapable, however, is the marketing success story behind potato crisps — we all know they come from potatoes, but we buy them for their own particular characteristics — few of us would ever consider the price of fresh potatoes when buying a packet of crisps.

There appears to be a real opportunity for enterprising meat industry operators in Australia to develop a new market. It would require astute marketing activities which have not as yet been applied in this country to meat or meat products. Further, as these types of products are somewhat sophisticated, an intending manufacturer would be wise to ensure that he has an adequate understanding of the technology involved.

Reference

- Leistner, L., and Rödel, W. (1976). The stability of intermediate moisture foods with respect to microorganisms. In 'Intermediate Moisture Foods', Eds R. Davies, G. G. Birch and K. J. Parker, pp. 120-37 (Applied Science Publishers, London).

Packaging films: new techniques in permeability measurements

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Introduction

In a recent article (Holland *et al.* 1981), a spectrophotometric technique is described for measuring permeabilities of packaging films to oxygen. A detector film clamped between two pieces of test film in a simple cell absorbs oxygen from the air which has diffused through the test films. The absorbance change in the detector is a measure of the gas permeation. Other applications of this cell of interest to the food and packaging industries are described in this paper.

Theory

The permeability constant P for a film provides a convenient measure of the amount of gas or vapour transferred through the film.

By definition, $P = \frac{ql}{At\Delta p}$ where q is the quantity of gas or vapour diffusing through a film of area A and thickness l in time t , and Δp is the partial pressure difference of the gas or vapour across the film. For vapours, the simpler concept of vapour transmission rate $\frac{q}{At}$ is often preferred. This is especially the case for laminated films, where the overall thickness is not as relevant as the thickness of each web. In the cell described in the previous paper, the area A is defined by the pressure seal, l is measured by a film micrometer, q is monitored by absorbance change in the detector material, and Δp is given by the pressure p of gas or vapour surrounding the cell. The detector material must be capable of absorbing the diffusant more rapidly than it is passing through the test films, otherwise $\Delta p < p$.

A fairly simple analysis also shows that ideally $P = DS$, where D is the diffusion coefficient of the gas in the polymer and S is its solubility. Approximately, D measures

how fast the diffusant molecule moves through the interstices between the polymer molecules, whereas S represents the steady state concentration. D depends on the size of the molecule (the bigger the molecule, the slower the diffusion in general) and S depends on the similarity of the diffusant to the polymer (the more similar the higher the solubility). For water vapour and organic vapours, the solubility is more important than molecular size for most polymers, and the permeation rates are usually several orders of magnitude greater than those for oxygen or nitrogen.

The diffusion coefficient may be measured by the time lag required for the diffusant to penetrate the film in those cases where the film is sufficiently thick or the diffusion rate is low. In other cases, when no appreciable time lag occurs, only the steady state permeability will be measured. In some cases the kinetic and solubility parameters can be measured by weighing on a sensitive balance.

Spectrophotometric permeability measurement

In the normal gas chromatographic method a test film is sealed between two compartments of a cell, the permeant gas is passed into one compartment at a known pressure, and the amount diffusing into the second compartment filled with an inert gas is measured. This requires a gas supply, a film sealing system, and a gas chromatograph.

In the spectrophotometric method described in previous papers (Holland *et al.* 1980, 1981) the cell design is simple, and no special expertise is required for the procedure. The area of both the test film and the detector film need be no greater than that of the beam from the spectrophotometer light source. Sensitivity is high because all of the diffusant is absorbed by a detector film of

small volume. The sensitivity is also much greater than that obtained by weighing, as a microgram of permeant can be detected during determinations.

There are, however, several disadvantages to the method. Gas chromatographic detection is capable of dealing with almost all classes of gas or vapour, separately or simultaneously, whereas specific detectors have to be designed for each class of permeant in the spectrophotometric method. Normally, however, the food packager is only interested in one or two diffusants such as oxygen and water vapour, for which adequate detectors exist (Holland *et al.* 1981). Secondly, the detector film has to be more permeable than the test film, or measurement of very high permeation rates may not be possible. In practice it may be possible to increase the thickness of the test film, such as by the use of multiple layers of film. However, very low permeation rates are usually of greater interest in the packaging industry. Another fairly obvious disadvantage is the need for the test film to be at least translucent, so that light can reach the detector film. In principle, the permeability of reflective metallized films could be measured by reflection, employing a transparent and impermeable window, such as silica glass.

Design of detection system

The first detection system designed was used for oxygen permeability measurements (Holland *et al.* 1980). This consisted of a light activated singlet oxygen forming system, which was capable of determining rapidly and accurately very small oxygen permeation rates. A dye and a singlet oxygen acceptor were incorporated into ethyl cellulose film which is highly permeable to oxygen and most other diffusants.

The second system (Holland and Santangelo 1982) was used for measuring water vapour permeability. In this case extreme sensitivity was inappropriate, as water vapour permeabilities are orders of magnitude greater than their oxygen counterpart. A sensitive system would be saturated in seconds, rather than minutes or hours which are more suitable time scales for accurate measurement. Hence in this case a reagent with low molar absorbance and high concentration in the detector film was required. Again, the detector material had to

be incorporated into a suitable polymer with sufficient permeability to permit measurement on standard packaging materials. Regenerated cellulose films (30 μm thick) were soaked in a 2.6–2.8 M aqueous solution of cobalt chloride, air dried and stored in a desiccator as a bright blue film. The water vapour permeability of this detector film is low at low humidities, but under higher humidity conditions ($\geq 75\%$ R.H.), the permeability is high. The blue colour fades in proportion to the amount of water vapour present, and it can be monitored in the spectrophotometer at 690 nm. Fig. 1 shows the transmission of moisture through several packaging materials, measured by the absorbance change. In spite of the reduced sensitivity (low molar absorbance) of the detector, this method is much faster than the standard gravimetric method (Holland and Santangelo 1982).

Another example of detector design is a CO_2 sensitive film. Since CO_2 is only slightly more soluble in polymers than oxygen, a fairly high sensitivity is required in most cases. An obvious detector system (useful for

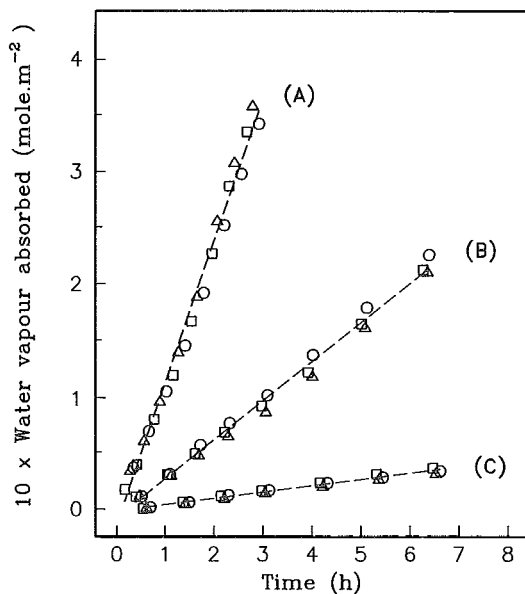


Fig. 1. Plots of water vapour absorbed at 25°C and 75% R.H. three determinations on each film.
(A) Nylon 6 (16 μm) (B) polyethylene terephthalate (13 μm)
(C) low density polyethylene (58 μm)
wavelength of absorbance : 690 nm
 $\epsilon (\text{CoCl}_2)$: 158

detecting other acids as well) is an alkaline indicator dissolved in a suitable polymer. Conversely, an indicator in the acid state could detect ammonia or amines. Alkaline phenolphthalein in ethyl cellulose appears to work as an acid detector system, but requires further development because of the low stability of the dye.

A final example of the use of polymer films as detectors is shown in Fig. 2. Various films were exposed to the saturated vapours of thymol or vanillin, two odorants of low volatility (< 1 mm Hg at 25°C).

It can be seen that ethyl cellulose and nylon 11 continue to absorb thymol and vanillin for days and can be considered good solvents. Polyethylene is soon saturated with thymol and does not absorb appreciable amounts of vanillin. Nevertheless, using ethyl cellulose as a detector the transmission rates of both thymol and vanillin in polyethylene can be monitored as they appear to be slower than in ethyl cellulose. Hence, absorption and transmission rates of microgram quantities of such materials can be measured if suitable molar absorbances ($> 10\,000$) occur at appropriate wavelengths; otherwise the sensitivity will be lower. Ethyl cellulose

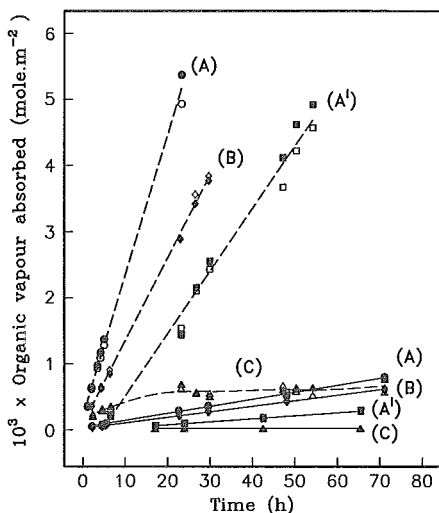


Fig. 2. Duplicate absorption plots of (A) ethyl cellulose ($15\mu\text{m}$) (A') ethyl cellulose detector ($15\mu\text{m}$) — transmission through low density polyethylene ($58\mu\text{m}$) (B) Nylon 11 ($81\mu\text{m}$) (C) low density polyethylene ($58\mu\text{m}$) Polymer films stored at 25°C in saturated vapours of Thymol wavelength of absorbance : 276 nm
 ϵ : 2188 ————— Vanillin wavelength of absorbance : 309 nm
 ϵ : $10\,471$

nearly always appears to be a good solvent, but whether the diffusion coefficient is fast enough for use as a detector has to be determined in each case.

In principle, then, flavour or off-flavour compounds can be collected, and their diffusion properties measured with great sensitivity by means of well chosen polymer detection systems. Separation of components might also be possible because of different solubilities and rates of permeation in different polymers. Further work is proceeding on these interesting systems.

Future work

Improvement of the performance of permeability measurements by the spectrophotometric method requires

- A more permeable detector
- A detector with a greater absorption capacity
- A detector support with lower absorbance over the entire visible and u.v. range of the spectrum (i.r. spectra may also be of interest)
- A detector support that is a more universal solvent than, say, ethyl cellulose so that all or most of the components of complex mixtures (odorants) may be absorbed and measured quantitatively.

To some extent such a system might not only be used for permeability work but also for separation and identification of odours by comparison of spectrophotometric profiles rather than by gas chromatographic or mass spectrometric analyses.

New detector materials which may improve all the above parameters are being investigated. Such materials need to have higher permeabilities than other permeable materials and to be good absorbants. These materials may lead to a more useful range of detectors than are presently available.

References

- Holland, R. V., Rooney, M. L., and Santangelo, R. A. (1980). Measuring oxygen permeabilities of polymer films by a new singlet oxygen technique. *Angew. Makromol. Chem.* 88, 209-21.
- Holland, R. V., Rooney, M. L., and Santangelo, R. A. (1981). New methods of measuring permeabilities of packaging films. *CSIRO Food Res. Q.* 41, 47-50.
- Holland, R. V., and Santangelo, R. A. (1982). Spectrophotometric determination of water vapour permeation through polymer films. *J. Appl. Polym. Sci.* 27, 1681-9.

News from the Division

Work overseas

Dr B. V. Chandler, Leader of FRL's Chemical Bases of Food Acceptance Group, undertook a month's consultancy in Hunan Province, China, late in 1983. The consultancy called for an Agricultural Economist (Mr Paul Nankivell) and a citrus juice expert to report on the feasibility of establishing a modern citrus processing plant in that province. Among the points to be specifically considered were:

(a) the potential local market (both within and beyond the province) as well as the export market for good quality citrus juices, especially orange juice;

(b) local laws and customs relating to juice purity, addition of preservatives and food pigments and other pure food matters;

(c) the suitability of the existing citrus demonstration farm at Lingling or alternative locations as a site for the juicing plant;

(d) type and cost of suitable juice plants if the project was deemed feasible.

The visit covered about 1500 kilometres within the Hunan Province and took Dr Chandler to the major citrus producing areas near Lingling, Shaoyan and Yuankiang where he inspected five citrus orchards and five citrus processing operations run under state farm, commune, production brigade or private ownership principles and representative of the citrus industry in the province. During his term in China, Dr Chandler spent many days in meetings and discussions with Hunan agricultural and food experts and gave (through interpreters) several talks on modern methods of citrus processing.

ACIAR

The Division has recently obtained approval, jointly with the NSW Department of Agriculture and the Queensland Department of Primary Industries, to carry out research projects in the South East Asian region, under the sponsorship of the Australian Centre for International Agricultural Research (ACIAR).

ACIAR was established as a statutory authority by an Act of the Australian Parliament in June 1982. Its purpose is to encourage and support research into the agricultural problems of developing countries in fields in which Australia has special competence. It does this by commissioning research by Australian institutions in partnership with research groups in developing countries. The projects are carried out both in Australia and in developing countries.

The four projects with which the Division is associated fall into the broad category of post-harvest technology. They are:

- a) Prediction and control of spoilage of fresh, cured and dried tropical fish in Indonesia
- b) Transport and storage of fresh fruit and vegetables in Papua New Guinea
- c) Postharvest physiology and technology of bananas in South-East Asia
- d) Physiological, chemical and storage characteristics of mango in the ASEAN region

We intend to report on these ACIAR projects in future issues of the Quarterly.

Bhutan aid project

The Division's involvement in Australian assistance to Bhutan in the area of postharvest storage and handling of fruit and vegetables, described in *CSIRO Food Res. Q.* Vol. 40, p. 43, and Vol. 41, pp. 58-89 and 87, has now entered another phase.

The UN's Food and Agriculture Organization has requested Australia to provide training for five more Bhutanese, in order to further the aims of the original project run by the Royal Government of Bhutan. The five, who arrived in Australia in November 1983, are: Singay Dukpa, Madan Kumar Chettri and Parouran Sharma who will receive training in refrigeration/mechanical engineering, Meghraj Gurung, a graduate in science wishing to specialize in analytical food science and Nagtong Dukpa who requires training in horticultural marketing.

After initial training to improve their English, the visitors will be based at FRL but undergo 'sandwich' courses at tertiary education establishments in the Sydney area.

Visiting workers

Dr Gregory Brewer from Southern Illinois University School of Medicine, Springfield, Illinois, USA, is a visiting scientist at the FRL. He is working with Drs L. R. Fisher and N. S. Parker on the effect of proteins on adhesion and fusion of lipid bilayers.

Following three years postdoctoral work at the University of Illinois, **Dr Max Keniry** spent three and a half months at FRL, on a collaborative project with Dr B. A. Cornell and using the n.m.r. facility, studying membrane systems.

Professor W. Breipohl, Department of Anatomy, University of Essen, West Germany and **Dr B. Rehn**, Department of Biology, University of Tübingen, West Germany, spent 4 and 12 weeks respectively at FRL late in 1983 as Visiting Scientists. With Dr D. G. Laing and Mr H. N. Panhuber they studied the structure and physiology of olfactory receptor cells following long-term exposure of the cells to odours or deodorized air.

Continuing international cooperation

In a recent issue of the Quarterly (Vol. 43, No 1, March 1983) we reported on a group visit to Australia by 16 students from France's Ecole Nationale Supérieure de Biologie Appliquée à la Nutrition et à l'Alimentation (ENS.BANA), located on the campus of the University of Dijon. The visit took place following a year's appointment of Dr Don Casimir (FRL) as Associate Professor at Dijon.

As a measure of the success of the group's stay in Australia, two of the students obtained permission to undertake their third year practical project as external students in the Division's Applied Food Science Group at FRL, using the facilities available in the pilot processing plant.

Under Dr Casimir's supervision, Phillipe Castan's project is concerned with the production of low alcohol wine and Benoit De Laval is working on the counter-current extraction of fish and poultry meat and residues.

After 6-7 months at North Ryde, the students will return to France to complete their studies.