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Transport of fresh horticultural produce under modified atmospheres

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Introduction

Successful storage of fresh produce is based on reducing the rate of ripening, or delaying the onset of ripening, and preventing decay and disorders, so that 'freshness' is maintained at a high level of acceptability to the consumer. Such storage is achieved through altering the environment of the produce immediately after harvest: by lowering the temperature, by application of chemicals, by changing the composition of the atmosphere, or by a combination of these treatments.

The three commonly used terms describing storage involving changes in the composition of the atmosphere, are controlled atmosphere (CA), modified atmosphere (MA) and low pressure or hypobaric storage (LPS). The distinction between CA and MA storage is that under CA storage, the concentrations of carbon dioxide and oxygen are regulated continuously by external equipment while under MA storage, although the atmosphere may be purged initially, the concentrations of carbon dioxide and oxygen are determined by the respiration of the produce and the permeability or gastightness of surrounding packaging material, storage room or vehicle. For some produce other gases may be added (e.g. carbon monoxide) or may be removed (e.g. ethylene). Under LPS, a reduced oxygen concentration is achieved by reducing the total pressure surrounding the produce.

Whenever it is desired to reduce the deteriorative changes during transport, first consideration should be given to pre-cooling the produce and to transporting in refrigerated vehicles. For most produce, particularly that which can be cooled to near freezing temperature without suffering chilling injury, this is sufficient to allow successful transportation to the market place. The desirability of modifying the atmosphere during transport, in addition to, or in place of, refrigeration, depends on the individual product, its economic value and the duration of the journey.

Recommended conditions for CA storage

Kader and Morris (1977) published a chart showing the limits of tolerance of some fruits and vegetables to elevated carbon dioxide and reduced oxygen (Fig. 1) at their recommended storage temperatures. This chart does not give recommendations, but specifies the 'levels of carbon dioxide above which, and the levels of oxygen below which, physiological damage would be expected'. However, these limits of tolerance are interdependent as well as temperature dependent. Other possible factors which may affect tolerance include maturity, cultivar and the presence of other gases (e.g. carbon monoxide and ethylene).

Various lists of recommended atmospheres have been published. Tables summarizing CA knowledge and recommendations for fruit and vegetables were compiled for the first National Controlled Atmosphere Research Conference (Hardenburg 1969) and a number of authors gave tables of recommendations at the second conference (Dewey 1977). The considerable changes in the recommended conditions for some produce between 1969 and 1977 indicates that this is a field where knowledge is still growing.

Smock (1979) and Isenberg (1979), in reviews on the CA storage of fruit and vegetables respectively, indicated that there was still no firm agreement between researchers on the effects of CA on the storage of some produce. The particular cultivar, the maturity when harvested, the growing area and conditions, were all mentioned as having an effect on CA storage.

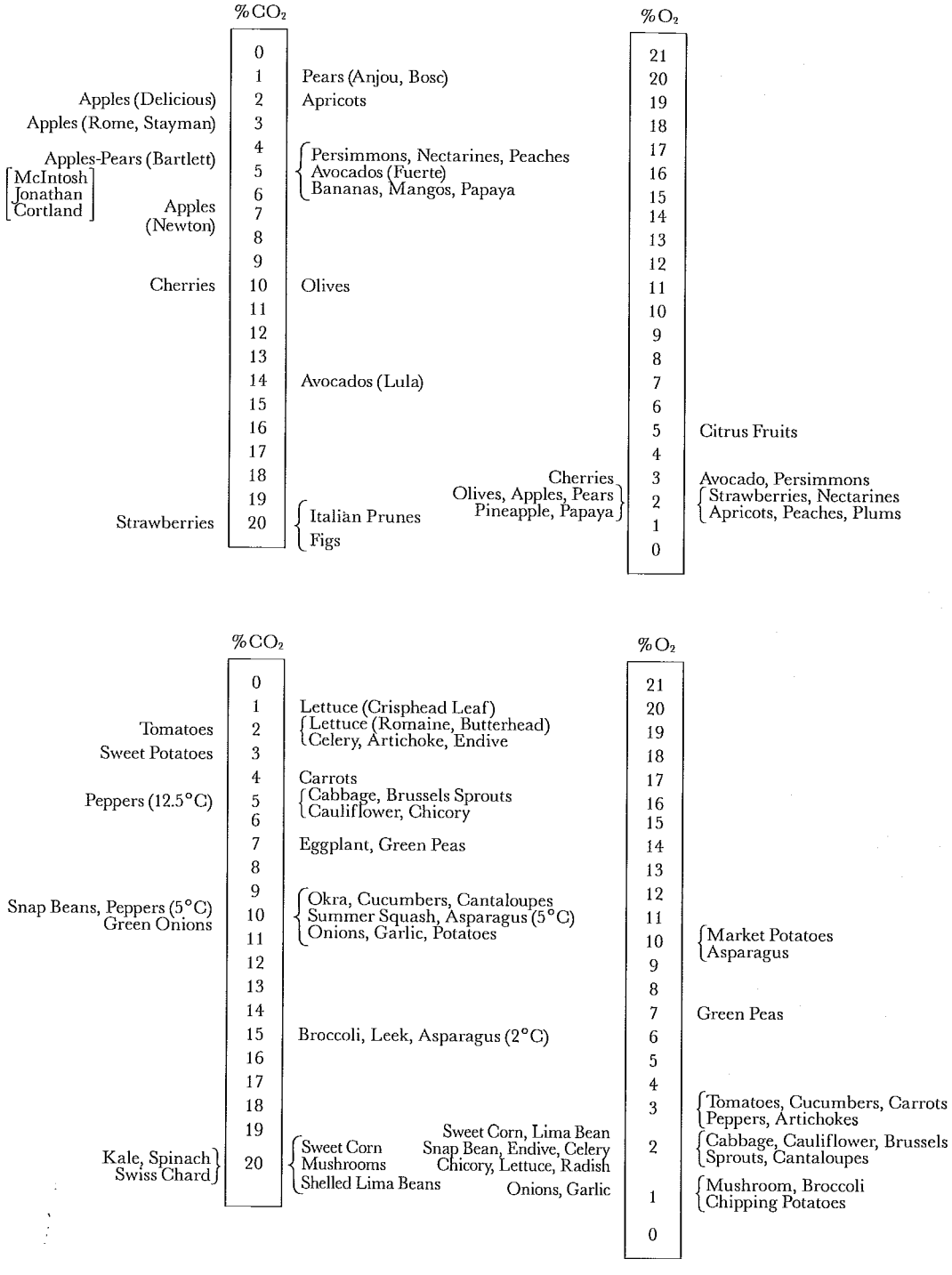

Fig. 1. Relative tolerance of fruits and vegetables to elevated carbon dioxide and reduced oxygen levels at recommended storage temperatures.

Recommended conditions for low pressure storage

In the 1960s Burg showed that for some produce, storage life was increased under reduced pressure and he patented the process. Burg (1975) gave a table showing the comparative life of produce under refrigerated (but not CA) and LPS conditions. He reported an increase of storage life of three to six times but did not give figures for the pressure used. Dilley (1977) reported good results for some produce at the following absolute pressures: apples 10 kPa, sweet cherries 7 kPa, mature green tomatoes 10 kPa, asparagus 3 to 5 kPa and mushrooms 2 kPa (1atm = 101 kPa). Spalding (1977) reported that LPS at 20 kPa was superior to CA for limes and mangos but inferior to CA for avocados.

Lougheed *et al.* (1978) reviewed the application of LPS to horticultural crops and found conflicting results had been reported. They were critical that, 'often researchers have failed to clearly indicate the quality attributes which were used to determine storage life and seem to have ignored the impact of LPS on flavour and nutritional quality of fruits and vegetables'. They concluded that LPS provides 'an elegant and unique system for creating a low oxygen atmosphere almost free of ethylene' and that it would be most useful for apples, leafy vegetables and ornamental plants. For produce susceptible to decay, some means of decay control would be necessary.

Methods of achieving atmosphere modification during transport

A modified atmosphere can be achieved during transport: either by enclosing produce in plastic film or by using gastight vehicles or vehicles fitted with gas control equipment.

Plastic film

A wide range of plastic films with different permeabilities to moisture and the gases used for atmosphere modification have become available since workers first suggested their use in the 1940s. Now plastic films of appropriate thickness and permeability can be chosen to maintain the desired atmosphere around the produce. Saguy and Mannheim (1973) tested plastics for use with strawberries, Marcellin (1974) discussed the principles involved in the use of plastic films and reported on the use of polyethylene and a silicone membrane with a number of fruits and vegetables, and Ben-Yehoshua *et al.* (1979) reported on the use of high density polyethylene with citrus.

Wolfe (1980) referred to the use of individual packages and pallet bags while Harvey (1969), Lugg (1977) and Macleod and Kasmire (1975) described the use of pallet bags or shrouds. Any absorbent material needed to remove undesirable gases (e.g. ethylene) was placed inside the bag or shroud which then was purged to obtain the required initial gas composition; the bag or shroud material must of course remain intact during transport to be effective. Individual packages within cartons would be protected sufficiently, but the exposed bag on a pallet would be prone to mechanical damage, particularly as the pallet is manoeuvred into position in the vehicle.

MA vehicles

'Transfresh' Corporation in the USA was the first major group to offer vehicles or containers for MA. The 'Transfresh' specification calls for extremely gastight vehicles, greater than 10 times the gastightness specified by ISO for refrigerated shipping containers (Sharp 1982). This high level of gastightness is achieved by careful sealing of connections between the refrigeration unit and the interior of the vehicle and by sealing the door opening with plastic film before the doors are closed. (Road vehicles in Australia are not built to a gastightness specification.)

Gas loss from vehicles/containers is a result of pressure differentials between the interior and exterior atmospheres. These are caused by (1) operation of the air circulation fans, (2) changes in barometric pressure associated with atmospheric changes or changes in altitude during transport (an altitude change of 1 m is equivalent to a pressure change of 10 Pa), (3) fluctuations in temperature due to cycling of the refrigeration unit, (4) wind action on the exterior walls, and (5) differences in the partial pressure of individual gases. 'Transfresh' initially fitted a 'breather bag' to compensate for cyclic pressure changes, but this is no longer general practice.

The two systems used by 'Transfresh' for applying atmosphere modifications in commercial shipments are 'Tectrol', for which 'Transfresh' hold the patent, and 'Oxytrol' for which 'Transfresh' is a non-exclusive licensee.

'Tectrol' is a passive system where a gas mixture specific for a particular product is added to a bag or gastight vehicle/container before shipment. The patent specifies 'an

artificial atmosphere composed of carbon monoxide substantially in excess of 5% and preferably above 10%, carbon dioxide, a significant amount of oxygen, and the remainder nitrogen'. For example, the gas mixture recommended for cauliflower is 10–20% carbon monoxide, 5% carbon dioxide, 5% oxygen and remainder nitrogen. No further additions of gas are applied as the system relies on the gastightness of the enclosure to retain the gas.

'Oxytrol' is an active system comprising an oxygen sensor placed within the vehicle/container, a controller and a liquid nitrogen tank. The controller can be set for 2, 3, 4 or 5% oxygen and the oxygen level will be maintained to within ± 1% by purging with nitrogen. The claim is made that low oxygen allows a mixture of products to be carried in the one vehicle at a compromise temperature – the products are said to 'sleep' in the low oxygen atmosphere.

At least three other companies now produce or plan containers designed specifically for modified atmospheres. 'Foodsources', a USA company incorporated in 1979, planned to have 1370 containers in operation by December 1982. Their 'Nitrol' system, essentially control of oxygen level by addition of nitrogen, is claimed to control oxygen to within ± ⅛% of the set point, to keep ethylene concentrations below 0.2 ppm, and to control humidity. Carbon dioxide and carbon monoxide levels can also be controlled if required. The refrigeration unit fitted to the containers is claimed to control cargo temperatures throughout the container to within ± 0.5 °C. Though a substantial improvement in produce quality is claimed by the Company, no independent assessments of the system have been reported, nor is there any indication of how much of the claimed improvement is due solely to the improved temperature control. A similar system, 'Freshbox', is being developed by a Dutch company, A & G Freshbox B.V. An Austrian company 3F – Kühl System G.m.b.H., offers a container which is claimed to control oxygen, nitrogen, carbon dioxide, relative humidity and ethylene; other gases also can be controlled if required. The temperature is controlled by a liquid nitrogen 'Polarstream' system.

The extra costs associated with the supply of gas and the control system on these containers mean that their use can be viable commercially only for produce that will command a high market price or where the only alternative is air transport.

Special containers, in effect vacuum chambers, are needed for LPS transport. Such containers, known as the Grumman Dormavac system, were built by Grumman Allied Industries of the USA. The containers' external dimensions (2.44 m × 2.44 m × 12.19 m) conformed to ISO standards but because of the additional equipment required to operate them, the payload and loadspace were reduced (19 t, 45 m^3 compared to 25 t, 54 m^3).

Only six prototype containers had been built by 1979 and although Grumman had plans to manufacture a production version in 1979–80 (Marmelstein 1979), and produce about 50 containers by 1981, the company has now abandoned the project. A new company, Dormavac Systems Corporation, Texas, USA, recently acquired all assets of the Dormavac system from Grumman Allied Industries. In March 1984, a Dormavac container cost $270 000 or could be leased for $4000 per month; the price of a conventional ISO refrigerated container was $23 000. It is unlikely that general horticultural produce could bear the additional transport costs.

Use of CA, MA and LPS in transit

There is little information available in the scientific literature on experimental trials or current commercial use of CA, MA or LPS in transportation of horticultural products. The greatest use by far is within, or for exports from, USA (Marcellin 1977).

Table 1 is taken from Kader (1980) and gives recommended CA conditions and the extent of commercial use in the USA for a number of fruits and vegetables. The only use mentioned for domestic transport in USA is for strawberries, where the use was said to be increasing, and for sweet cherries where there is some use with pallet covers or box liners. He claims that many of the listed products are shipped under MA for export marketing but no figures or references are given. Kader reported an increasing interest in the addition of carbon monoxide to CA storage. He concluded that 5-10% carbon monoxide in low oxygen atmospheres could be useful during transport and/or storage of some fruits to reduce the incidence and severity of decay.

In the UK in the 1950s, the transport of strawberries and raspberries in insulated rail containers, with atmospheres enriched with carbon dioxide derived from dry ice blocks, was investigated (Smith 1955–56). A beneficial effect, especially with raspberries was obtained,

with a concentration of 20–25% giving best results. Harvey (1969, 1977) reported similar use of carbon dioxide for air transport of strawberries from the USA. Pallets of strawberries were covered with a polyethylene cover and 3 kg of dry ice added. This gave an atmosphere of 20–35% carbon dioxide for the 17–18 h journey and a significant reduction in decay was achieved. In the second report, pallets of strawberries were covered with a polyethylene bag or a heat shrink polyethylene film and carbon dioxide was added as a gas or as dry ice. For journeys of 26–34 h from California to Tokyo and 44–50 h from

Table 1
Recommended CA conditions during transport and/or storage of fruits, their potential for benefit, and the extent of commercial use in USA (After Kader (1980)

Commodity	Temp. range (°C)	CA		Potential for benefit	Extent of commercial use
		$\%O_2$	$\%CO_2$		
Deciduous tree fruits					
Apple	0-5	2-3	1-2	Excellent	About 40% of US production is stored under CA
Apricot	0-5	2-3	2-3	Fair	No commercial use
Cherry, sweet	0-5	3-10	10-12	Good	Some commercial use (pallet covers or box liners)
Fig	0-5	5	15	Good	Limited commercial use
Grape	0-5	-	-	Slight or none	(No commercial use; incompatible with SO_2 fumigation)
Kiwifruit	0-5	2	5	Excellent	Limited commercial use
Nectarine	0-5	1-2	5	Good	Limited commercial use
Peach	0-5	1-2	5	Good	Limited commercial use
Pear	0-5	2-3	0-1	Excellent	Some commercial use
Persimmon	0-5	3-5	5-8	Fair	No commercial use
Plum and prune	0-5	1-2	0-5	Good	No commercial use
Strawberry	0-5	10	15-20	Excellent	Increasing use during transit
Subtropical and tropical fruits					
Avocado	5-13	2-5	3-10	Good	Limited commercial use
Banana	12-15	2-5	2-5	Excellent	Some commercial use
Grapefruit	10-15	3-10	5-10	Fair	No commercial use
Lemon	10-15	5	0-5	Good	Limited commercial use
Lime	10-15	5	0-10	Good	Limited commercial use
Olive	8-12	2-5	5-10	Fair	No commercial use
Orange	5-10	10	5	Fair	No commercial use
Mango	10-15	5	5	Fair	No commercial use
Papaya	10-15	5	10	Fair	No commercial use
Pineapple	10-15	5	10	Fair	No commercial use
Vegetable fruits					
Cantaloupe	5-10	3-5	10-15	Good	Limited commercial use
Honeydew melons	10-12	3-5	0	Fair	No commercial use
Peppers, Bell	8-12	3-5	0	Fair	Limited commercial use
Peppers, chilli	8-12	3-5	0	Fair	No commercial use; 10–15% CO_2 is beneficial at 5–8°C
Tomatoes					
mature-green	12-20	3-5	0	Good	Limited commercial use
partially ripe	8-12	3-5	0	Good	Limited commercial use

California to Hong Kong the addition of carbon dioxide in the form of dry ice gave the greatest reduction in incidence of decay as the dry ice had the additional effect of reducing the rise in temperature of the top layers.

White (1969) reported on the increasing number of piggyback trailers, highway trailers and shipping containers being fitted with the 'Oxytrol' system for low oxygen transport. He mentions the carriage of pineapple, papayas, lettuce and celery.

Lugg (1969) of Transfresh Corporation, reported on the number of 'Tectrol' systems being fitted to railcars, piggyback trailers, highway trailers, and shipping containers. Later, Lugg (1977) again reported on the 'Tectrol' system and also on the 'Oxytrol' system. He was not as enthusiastic as earlier, as the initial high volume use with lettuce had dropped off because of a change from rail to road transport. In 1969, lettuce was shipped by rail across the USA with a transit time of about seven days and MA had helped maintain quality. However, costs and delays on the rail system resulted in the industry transferring to road vehicles and MA was not economically justified with the shorter transit time. Lugg reported that the quantity of products being carried under the 'Transfresh' system was still increasing, with the break-up being about 40% meat and 25% lettuce, with the remainder being strawberries and mixed horticultural products. He also reported the move to sealed pallet-sized modules which meant that products requiring different atmospheres could be carried in the one vehicle. He also emphasized the need for proper temperature maintenance and commented that some people had used MA as a marketing tool rather than as a means of preserving freshness.

Woodruff (1977) of Transfresh Corporation, claimed that the carriage temperature of produce in practice was not ideal, e.g. produce that should be carried at 0°C was carried at 3.5 to 5.5°C to avoid freezing and produce that should be carried at 10 to 13°C was carried below 10°C to diminish problems of mould and decay. He concluded that research into MA was done under the wrong conditions for its usefulness in transportation to be assessed and that too much emphasis has been placed on low oxygen MA. He carried out research into the effects of the addition of carbon monoxide in the range 5 to 20% to MA and reported good results, particularly with produce where pathological disorders were the limiting factor, but he did not give any statistics to back his claims. He claimed good results with cauliflower, lettuce, grapes, citrus, peppers, melons, stone fruits, and some tropical fruits. He did not give any results for transport trials.

Macleod and Kasmire (1975) reported on a 'test shipment of vine-ripened tomatoes under MA'. Pallets were covered with sealed shrouds, the oxygen level was reduced to below 4% by an initial purging with nitrogen, and bags of lime to absorb carbon dioxide were placed under the shrouds. However, all the shrouds were torn during transit and at outturn oxygen levels were in the range 13 to 17%. In addition, the temperature of tomatoes under the shroud was 3 to 5°C warmer than that of tomatoes on a control pallet without a shroud.

Wolfe (1980) of Transfresh Corporation, referred to large plastic bags for pallets and claimed these were 'successful', particularly in handling strawberries. One advantage is the ease of handling mixed loads with the one vehicle. Institutional packs (4kg) of prepared vegetable products were being transported in MA. Wolfe noted the importance of good temperature control in achieving the full benefits of MA. His assessment of the benefits offered by carbon monoxide was more subdued than that of Woodruff. Wolfe claimed that 'carbon monoxide is not required in most instances but has been found to be beneficial in low concentrations (0–10%) in the prolongation of shelf life', for the produce referred to by Woodruff.

Hall (1971) reported on the use of polyethylene bag liners for pears exported under refrigeration from Australia. The naturally produced MA of reduced oxygen and increased carbon dioxide 'so improved the condition of the fruit overseas that almost all exported pears are now packed in packages lined with polyethylene film bags'. This procedure is still used.

An anonymous report (1978) referred to six experimental LPS containers which were filled with various foodstuffs and transported overseas. This experiment was carried out by the container manufacturers, Grumman, and the produce said to be satisfactorily carried were porkmeat, strawberries, papaya, avocado, carnations and limes.

Jamieson (1980), of Grumman Industries, reported highly favourable results for LPS of pork loins, strawberries, limes, papaya, lettuce

and Bell peppers. He referred to experimental, full container shipments of mangoes, limes, pork, pineapples, papayas, melons and lamb, but no details were given.

Possible use of atmosphere modification in Australia

Wade (1980) suggested that improvement of transport and distribution is the most pressing need in postharvest technology of Australian horticultural produce. Most produce is transported within Australia in non-refrigerated vehicles as refrigerated vehicles are not available or are too expensive or the journey is short. Where produce is carried in refrigerated vehicles, the journey is rarely sufficiently long or the value of produce high enough to warrant the additional costs of MA. However, for non-refrigerated transport, the use of MA to retard quality loss or ripening may be useful for some produce.

Scott *et al.* (1971) showed that commercial packs of bananas sealed in polyethylene bags, can be transported without refrigeration for 2500 to 4500 km with a duration of 8 to 18 days and remain in a hard green condition. The bananas generated their own MA within a wide range of low oxygen and high carbon dioxide concentrations. Best results were obtained when the bananas were treated with a fungicide to control rots and an ethylene absorbent was placed in the bag.

There is an increasing interest in Australia in tropical and sub-tropical produce. Most of this produce is highly perishable and is grown in areas remote from the large domestic markets. MA transport at ambient temperatures may allow more of this produce to be marketed as a supplement to the more traditional produce.

Chaplin and Hawson (1981) showed that ripening of avocados can be retarded by sealing them in polyethylene bags. Again MA with a wide range of composition of low oxygen and high carbon dioxide was generated naturally. The retardation of ripening, as compared to avocados without polyethylene bags, was sufficient to reduce substantially the wastage for the 5 day road journey at ambient temperature from Eastern to Western Australia.

Scott *et al.* (1981) showed that the natural colour of litchis can be retained and water loss and fungal wastage reduced when they are treated with a fungicide, packaged in polyvinyl chloride wrap and held for nine days. Again, there was a considerable effective range in the composition of the MA attained. The longer shelf life at 20°-30°C should enable the fruit to reach more markets by surface transport.

Marcellin and Ulrich (1983) have reviewed the work on the improvement of storage life of some produce achieved by exposure to high carbon dioxide or low oxygen atmospheres or to various thermal treatments before storage and by intermittent CA or thermal changes during storage. Some of these techniques may prove useful for the transportation of some produce. For example, Wills *et al.* (1979) have shown that respiration rates of some vegetables at ambient temperatures are reduced after short exposure to high carbon dioxide or low oxygen. If it can be shown also that rate of loss of quality is reduced, then this could prove to be a very useful pre-transport treatment.

Australia (with New Zealand) is more remote from most export markets than are other countries. While available MA techniques may give that extra benefit to allow short-lived produce to be shipped across the Atlantic (2-3 weeks), the shipping times from Australia (4-6 weeks) exclude such produce at present, and produce such as asparagus, stone fruits, berries, mangoes and cut flowers are exported now by air. Air transport will always have some use as it has the advantage that produce can be transported quickly to meet sudden shortages in the market. However, in the longer term it seems unlikely that the present favourable air freight rates will continue and the availability of larger quantities of produce, e.g. as a result of the large scale planting of mangoes, will require the development of MA techniques to allow sea transport if significant exports are to occur.

Conclusions

There are conflicting reports on the usefulness of MA in addition to refrigeration during transport. Much of the work has been done by those who have a commercial interest in the process. Woodruff (1977) of Transfresh Corporation, the largest group involved in MA transport has said, 'if produce was carried at ideal temperatures there probably would not be anyone in the MA business for transit of produce'. He maintains that because produce is not carried at ideal temperatures, MA has a role. Wolfe (1980), also of Transfresh, has said, 'one hidden benefit is that the attention given to the commodity being transported can frequently result in better handling throughout

frequently result in better handling throughout the distribution chain, augmenting the returns available from use of MA'.

It seems likely, from the evidence available, that if more care was taken immediately after harvest, particularly in prompt cooling of produce, and more effort put into proper temperature maintenance during refrigerated transport, then there would be little additional benefit to be obtained from MA for most produce in transit. However, to attain transport times of several weeks for highly perishable produce, MA will be necessary.

It does not seem likely that the use of highly specialized refrigerated shipping containers to provide MA conditions would be economically viable. The demand for MA transport is seasonal and it would be difficult to obtain produce requiring MA for the return trip. The development of a simpler, one-trip modification to conventional refrigerated containers would appear to be a more viable solution.

In Australia, the benefit of MA would appear to be in non-refrigerated transport of tropical produce. In this case the most favourable form of MA appears to be one of dynamic equilibrium, generated by the produce and packaging material.

References

Anon. (1978). A hypobaric transport and storage process for perishable foodstuffs. *Alimentaria, E. S.*, **11**, 82-3.

Ben-Yehoshua, S., Kobiler, I., and Shapiro, B. (1979). Some physiological effects of delaying deterioration of citrus fruits by individual seal packaging in high density polyethylene film. *J. Am. Soc. Hortic. Sci.* **104**, 868-72.

Burg, S. P. (1975). Hypobaric storage and transportation of fresh fruits and vegetables. *In* 'Postharvest Biology and Handling of Fruits and Vegetables', eds. N. F. Haard and D. K. Salunkhe, pp. 172-88. (AVI Publishing Co. Inc.: Westport, Conn.).

Chaplin, G. R., and Hawson, M. G. (1981). Extending the postharvest life of unrefrigerated avocado fruit by storage in polyethylene bags. *Sci. Hortic.* **14**, 219-26.

Dewey, D. H. ed. (1977). 'Controlled Atmospheres for the Storage and Transport of Horticultural Crops'. (Dep. Hortic. Michigan State Univ.: East Lansing, Michigan).

Dilley, D. R. (1977). The hypobaric concept for controlled atmosphere storage. *In* 'Controlled Atmospheres for the Storage and Transport of Horticultural Crops', ed. D. H. Dewey, pp 29-37. (Dep. Hortic. Michigan State Univ.: East Lansing, Michigan.).

Hall, E. G. (1971). Handling pears for export. *CSIRO Food Res. Q.* **31**, 70-5.

Hardenburg, R. E. (1969). Summary tables of controlled atmosphere recommendations. *In* 'Controlled Atmospheres for the Storage and Transport of Horticultural Crops', eds D. H. Dewey, R. C. Herner, and D. R. Dilley, pp. 122-31. (Dep. Hortic. Michigan State Univ.: East Lansing, Michigan.).

Harvey, J. M. (1969). Modified atmospheres in air transport. *In* 'Controlled Atmospheres for the Storage and Transport of Horticultural Crops', eds D. H. Dewey, R. C. Herner, and D. R. Dilley, pp. 50-3. (Dep. Hortic. Michigan State Univ.: East Lansing, Michigan.).

Harvey, J. M. (1977). In-transit atmosphere modification – effects on quality of fruits and vegetables. *In* 'Controlled Atmospheres for the Storage and Transport of Horticultural Crops' ed. D. H. Dewey, pp. 71-8. (Dep. Hortic. Michigan State Univ.: East Lansing, Michigan.).

Isenberg, F. M. R. (1979). Controlled atmosphere storage of vegetables. *Hortic. Rev.* **1**, 337-94.

Jamieson, W. (1980). Use of hypobaric conditions for refrigerated storage of meats, fruits and vegetables. *Food Technol.* **34**, 64-71.

Kader, A. A. (1980). Prevention of ripening in fruits by use of controlled atmospheres. *Food Technol.* **34**, 51-4.

Kader, A. A., and Morris, L. L. (1977). Relative tolerance of fruits and vegetables to elevated CO_2 and reduced O_2 levels. *In* 'Controlled Atmospheres for the Storage and Transport of Horticultural Crops', ed. D. H. Dewey, pp. 260-5. (Dep. Hortic. Michigan State Univ.: East Lansing, Michigan.).

Lougheed, E. C., Murr, D. P., and Berard, L. (1978). Low pressure storage for horticultural crops. *Hortscience*, **3**, 21-7.

Lugg, J. R. (1969). The Transfresh system of atmospheres for produce transportation. *In* 'Controlled Atmospheres for the Storage and Transport of Horticultural Crops', eds D. H. Dewey, R. C. Herner and D. R. Dilley, pp. 58-9. (Dep. Hortic. Michigan State Univ.: East Lansing, Michigan.).

Lugg, J. R. (1977). The Transfresh system for transportation of perishables. *In* 'Controlled Atmospheres for the Storage and Transport of Horticultural Crops', ed. D. H. Dewey, pp. 79-81. (Dep. Hortic. Michigan State Univ.: East Lansing, Michigan.).

MacLeod, R. F., and Kasmire, R. F. (1976). *In* 'Fresh Market Tomato Research Vegetable Crop Series 176, March 1976', pp. 101-3, (Univ. California: Davis, California.).

Marcellin, P. (1974). Conservation des fruits et légumes en atmosphere contrôlée, à l'aide de membranes de polymeres. *Rev. Gen. Froid.* **64**, 217-36.

Marcellin, P. (1977). Le transport des fruits et légumes en atmosphere contrôlée. *Rev. Gen. Froid.* **67**, 15-21.

Marcellin, P., and Ulrich, R. (1983). Comportement des fruits et légumes en conditions modulées et programmées. *Int. J. Refrig.* **6**, 329-36.

Marmelstein, N. H. (1979). Hypobaric transport and

storage of fresh meats and produce earns 1979 IFT Food Technology Industrial Achievement Award. *Food Technol.* **33**, 32-40.

Saguy, I., and Mannheim, C. H. (1973). Prolonging shelf-life of strawberries by packaging in selected plastic films. *Bull. Int. Inst. Refrig. Annexe* 1973-3, 149-58.

Scott, K. J., Blake, J. R., Strachan, G., Tugwell, B. L., and McGlasson, W. B. (1971). Transport of bananas at ambient temperatures using polyethylene bags. *Trop. Agric.* (Trinidad) **48**, 245-54.

Scott, K. J., Brown, B. I., Chaplin, G. R., Willcox, M. E., and Bain, J. M. (1981). The control of rotting and browning of litchi fruit by hot benomyl and plastic films. *Sci. Hortic.* **16**, 253-62.

Sharp, A. K. (1982). Measurement of gas-tightness with an automatic pressure decay timer. *Bull. Int. Inst. Refrig. Annexe* 1982–1, 361-7

Smith, W. H. (1955-6). The application of precooling and carbon dioxide treatment to the marketing of strawberries and raspberries. *Sci. Hortic.* **12**, 147-54.

Smock, R. M. (1979). Controlled atmosphere storage of fruits. *Hortic. Rev.* **1**, 301-36.

Spalding, D. J. (1977). Low pressure (hypobaric) storage of avocados, limes, and mangoes. *In* 'Controlled Atmospheres for the Storage and Transport of Horticultural Crops', ed. D. H. Dewey, pp. 156-64. (Dep. Hortic. Michigan State Univ.: East Lansing, Michigan.).

Wade, N. L. (1980). Atmosphere composition as an aid to refrigeration. *CSIRO Food Res. Q.* **40**, 84-90.

White, T. G. (1969). The Oxytrol system – function, commercial use and commodity research. *In* 'Controlled Atmospheres for the Storage and Transport of Horticultural Crops', eds D. H. Dewey, R. C. Herner and D. R. Dilley, pp. 55-7. (Dep. Hortic. Michigan State Univ.: East Lansing, Michigan.).

Wills, R. B. H., Wimalasiri, P., and Scott, K. J. (1979). Short pre-storage exposures to high carbon dioxide or low oxygen atmospheres for the storage of some vegetables. *Hortscience* **14**, 528-30.

Wolfe, S. K. (1980). Use of CO and CO_2 enriched atmospheres for meats, fish and produce. *Food Technol.* **34**, 55-8, 63.

Woodruff, R. E. (1977). Use of carbon monoxide in modified atmospheres for fruits and vegetables in transit. *In* 'Controlled Atmospheres for the Storage and Transport of Horticultural Crops', ed. D. H. Dewey, pp. 52-4. (Dep. Hortic. Michigan State Univ.: East Lansing, Michigan.).

News from the Division

Death – Arthur Kuskis

On 21 June 1984, FRL lost one of its best known and most popular personalities with the sudden death of Arthur Kuskis. A refugee from Latvia, Arthur came to Australia after the Second World War, having served a term in his professional capacity as dentist in a displaced persons' camp operated by the French occupation forces in Germany. On arrival in Australia, Arthur found himself unable to practise his profession and spent several years working in various jobs well below his capabilities until finding employment in 1955 as Technical Assistant with the then Division of Food Preservation and Transport at Homebush. For the next 17 years he assisted with investigations into tin plate corrosion and flexible film packaging, working with Eric Davis and Phil Moy whose retirement notices appear in the September issue of FRQ. Then, in 1972, he was given the responsibility for the day-to-day management of the taste test laboratory at FRL, initially under Miss Betty Christie and for the last 10 years under Dr Bob McBride. Here Arthur came into his element and his naturally gregarious disposition found full expression in the happy and efficient running of this essential FRL facility. Rarely, if ever, failing to obtain the ready cooperation of the staff members who served as guinea pigs or model consumers on his taste panels, Arthur was as successful as any sergeant-major in marshalling his troops for service, though relying on courtesy and rewards rather than on military discipline: his troops will never forget his courteous request/command as each left their taste booth: 'Same time again tomorrow, please'. Arthur's funeral service was attended by possibly a record number of his colleagues, many of whom were able to pay their last respects to him at the subsequent social gathering in a manner of which Arthur would have heartily approved.

BVC

Improving the quality of sunflower oil

By D. G. Bishop

CSIRO Division of Food Research, North Ryde, N.S.W., 2113

The production of sunflower oil has risen dramatically, on a worldwide basis, over the past decade. The oil is highly regarded as a food constituent, having a high content of the polyunsaturated fatty acid linoleic acid, but in addition has also been extensively tested as a possible substitute for petroleum-based diesel fuels.

Although Australian production of sunflower oil has increased in line with the rest of the world, and sunflower is now the largest oilseed crop produced in this country, problems of oil quality have been of continual concern to the food industry. The major factor is a variability in the content of linoleic acid in the oil, with the content falling below the level of 62% which is required by processors to meet the criteria of a polyunsaturated oil. Consequently, it is often necessary to add a more expensive oil, such as safflower oil, to meet the criteria demanded.

Although the oil composition of sunflower seeds is under genetic control, there is substantial evidence that environmental conditions can influence the linoleic acid content of the oil. It is over thirty years since

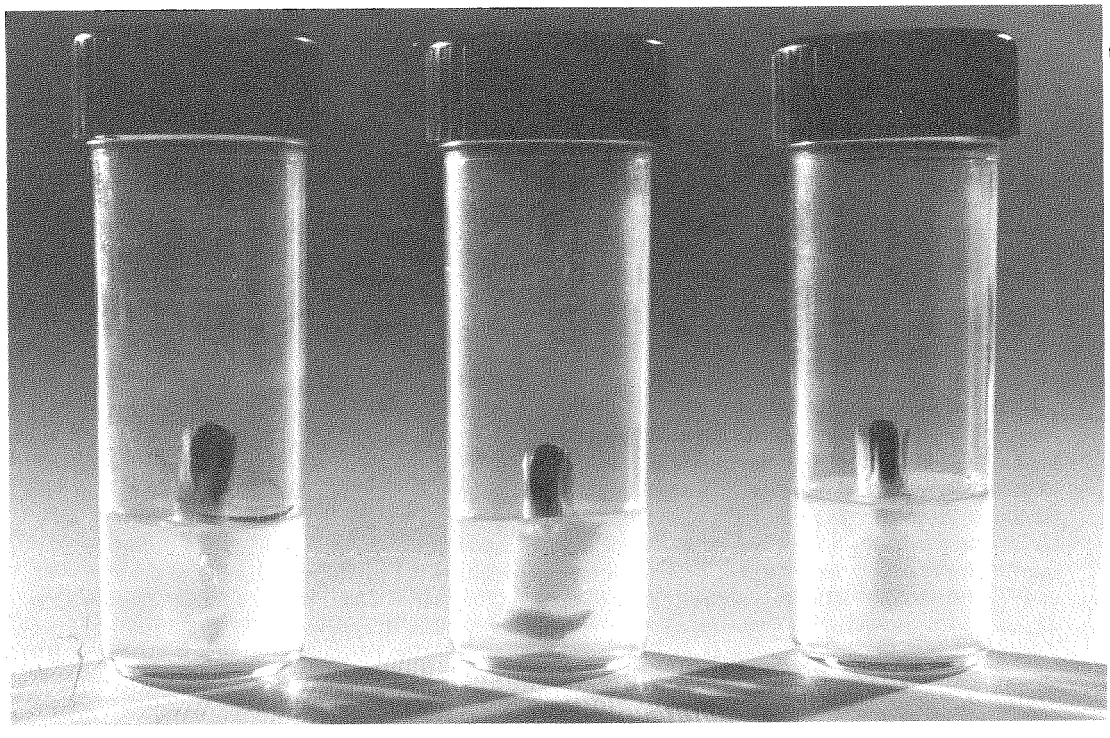

Fig. 1. Culturing system for isolated sunflower embryos.

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Bridge *et al.* (1951) showed that the linoleic acid content of the same strain of sunflower varied according to the latitude in Australia at which it was grown (Table 1). More recently it has been demonstrated (Harris *et al.* 1978) that the most important environmental factor influencing the fatty acid composition of the oil is temperature, with lower minimum (i.e. night) temperatures during the period of seed development leading to higher contents of linoleic acid in the oil. It is variability in temperature during seed development which is largely responsible for the fluctuations in the quality of the oil produced by Australian growers. A possible approach to overcome this problem would be the isolation and identification of temperature-stable genotypes.

TABLE 1
Effect of latitude on the fatty acid composition of sunflower oil

Latitude	Fatty acid composition (%)		
	Palmitic + Stearic	Oleic	Linoleic
N. Territory (lat. 14.5°S)	14	51	36
Queensland (lat. 27.5°S)	15	30	56
N.S.W. (lat. 32°S)	15	27	58
Victoria (lat. 36°S)	16	19	65

Data from Bridge *et al.* (1951).

During the past three years, a project funded by the Oilseeds Research Committee has been in progress both in the Department of Agronomy, University of New England and in the Plant Physiology Group of the Division of Food Research. The major aims of this collaborative effort have been to develop an *in vitro* technique for culturing sunflower embryos, so that temperature-stable material might be identified, and to study the control reactions in the biochemical pathways of linoleic acid synthesis in the seed.

The work at the University of New England has led to the development of a system for culturing isolated embryos under sterile conditions (Silver *et al.* 1982). Individual seeds, each with a core of spongy inflorescence tissue are removed from sunflower heads about fourteen days after pollination and cultured in sterile medium containing sucrose as a carbon source (Fig. 1). The inclusion of the spongy tissue, which contains vascular connections, serves to provide a larger surface area for the uptake of water and nutrients from the culture medium. Isolated seeds can then be allowed to develop further during the period of maximum oil synthesis at a range of temperatures and the oil content of each seed determined. Embryos cultured by this procedure grow at a rate of 70–80% of that of embryos allowed to complete their development in the sunflower head, and have a similar oil content and composition.

The effect of incubation temperature on the fatty acid composition of groups of cultured sunflower embryos is shown in Table 2 (Silver *et al.* 1982). While the content of the two saturated fatty acids, palmitic and stearic, remains relatively constant, a decrease in incubation temperature produces a marked increase in the content of linoleic acid and a concomitant decrease in the content of oleic

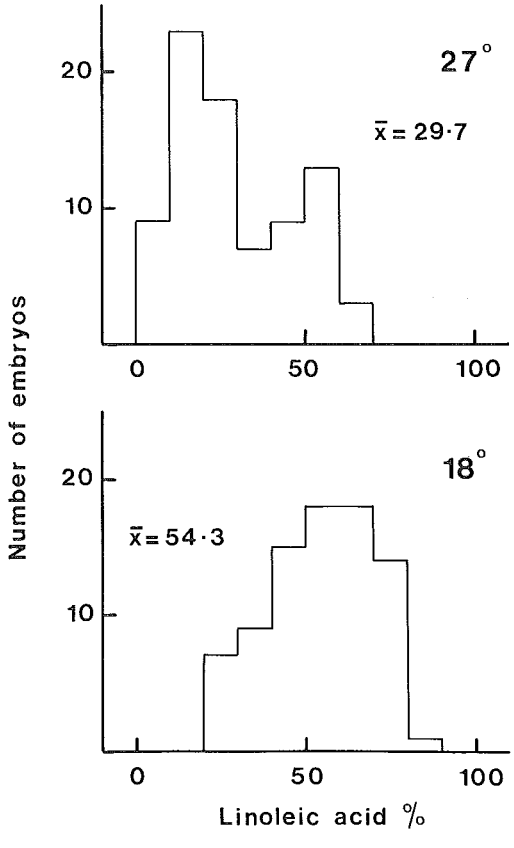

Fig. 2. Frequency distribution of linoleic acid content in individual sunflower embryos cultured at 27°C and 18°C. n = 82 in each case.

acid. However, it was also found that within each group there is a wide variation in the linoleic acid content of individual seeds, (Fig. 2) and that, although the frequency distribution shifts to higher linoleic acid content at lower incubation temperatures, individual seeds can be identified with a high linoleic acid content when incubated at 27°C. This property should be able to be used to select individual seeds whose synthesis of linoleic acid is relatively temperature stable, and then to propagate them.

Biochemical studies in the Plant Physiology Group have demonstrated that the biosynthesis of linoleic acid in subcellular fractions of sunflower seeds involves the participation of membrane lipids as substrate carriers (Rochester and Bishop 1982). Oleic acid, the precursor of linoleic acid, only undergoes desaturation if it is esterified in a phosphoglyceride, preferably phosphatidylcholine. Kinetics of these reactions show that the incorporation of exogenous oleic acid into phosphatidylcholine is very rapid (Fig. 3), occurring in less than ten minutes under the experimental conditions used, while the synthesis of linoleic acid continues for at least one hour. However, measurements of the effect of temperature on these two reactions has not so far indicated that either of them is likely to be the temperature-sensitive step controlling the level of linoleic acid in sunflower oil. At this stage, it appears likely that the temperature-sensitive reaction is one of the final steps in the synthesis of triacylglycerol, involving the

TABLE 2
Effect of temperature on the fatty acid composition of cultured sunflower embryos

Incubation temperature °C	Fatty acid composition (%)		
	Palmitic + Stearic	Oleic	Linoleic
15	7	14	79
18	11	19	71
21	9	29	62
24	10	38	52
27	11	52	37

Data from Silver *et al.* (1982).

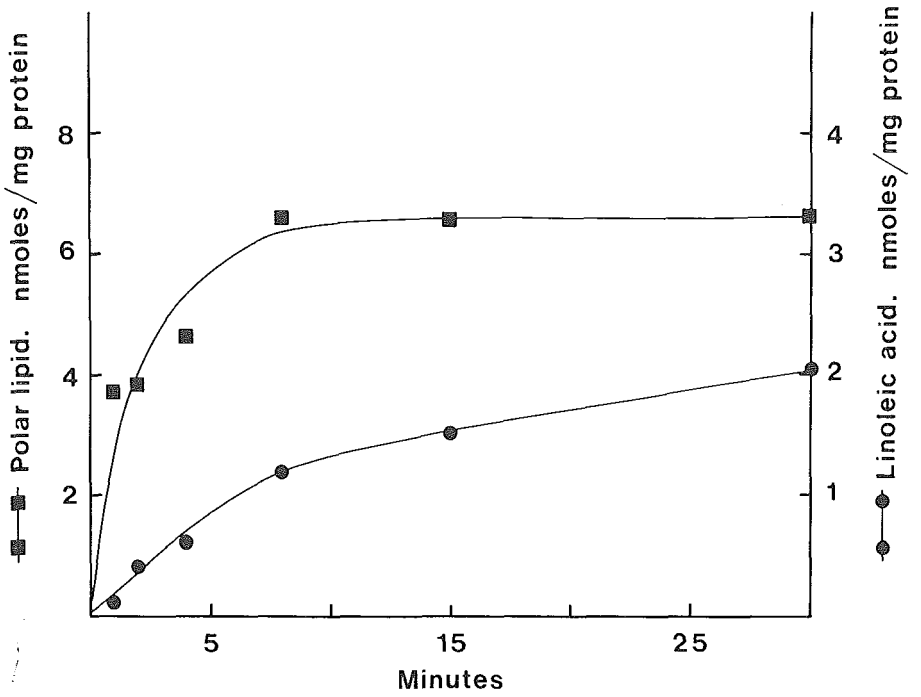

Fig. 3. Incorporation of radioactivity from ^{14}C-oleoyl-CoA into polar lipids and linoleic acid by a microsomal extract of sunflower seeds. Data from Rochester and Bishop (1982).

transfer of a molecule of linoleic acid from phosphatidylcholine to triacylglycerol.

Both these lines of investigation are continuing and it is hoped that a better understanding of the biochemical pathways of linoleic acid synthesis in sunflower seeds will assist the more empirical selection techniques, to enable the breeding of sunflower strains which are ideally suited to Australian growing conditions.

References

Bridge, R. E., Crossley, A., and Hilditch, T. P. (1951). The influence of environment upon the composition of sunflower seed oils. III. Oils from sunflower seeds grown in different regions of Australia. *J. Sci. Food Agric.* 2, 472-6.

Harris, H. C., McWilliam, J. R., and Mason, W. K. (1978). Influence of temperature on oil content and composition of sunflower seed. *Aust. J. Agric. Res.* 29, 1203-12.

Rochester, C. P., and Bishop, D. G. (1982). Biosynthesis of linoleic acid by cell-free extracts of sunflower seeds. *In* "Biochemistry and metabolism of plant lipids". Eds J. F. G. M. Wintermans and P. J. C. Kuiper, pp.57-60. (Elsevier: Amsterdam.)

Silver, J. G., McWilliam, J. R., and Harris, H. C. (1982). A technique for selecting sunflower genotypes with temperature-stable linoleic acid synthesis. Proc. Tenth. Int. Sunflower Conf. Surfers Paradise, pp. 59-63.

General reading

Beard, B. H. (1981). The Sunflower Crop. *Sci. Amer.* 244(5), 124-31.

Ralph, W. (1982). *Rural Research.* No. 115, pp. 4-9.

Seyman, J. (1981). *Ecos.* No. 27, pp. 3-10.

News from the Division

New research project on fatty acids

Dr K. A. Ferguson, Director of the CSIRO Institute of Animal and Food Sciences, has initiated an inter-divisional program on 'The occurrence, source, and role of long chain fatty acids in food and nutrition' with particular reference to those of marine sources.
Dr A. R. Johnson (FRL) has been asked to facilitate the coordination of the program.
As part of this program an inter-divisional workshop was held at FRL in March 1984. This was attended by Dr Ferguson and representatives of the CSIRO Divisions of Human Nutrition, Animal Production, Fisheries Research, and Food Research. Also invited were Dr A. Sinclair, Victorian Department of Agriculture, Dr K. O'Dea, Heidelberg Repatriation Hospital, Melbourne, Dr. P. J. Nestel and Ms S. Wong, The Baker Institute, Melbourne, and Dr R. Gibson and Dr I. Craig from Flinders University Medical School, Adelaide.

Work overseas

Dr L. Fisher spent ten weeks from mid-January to the end of March 1984 visiting thirty laboratories in France, U.K., U.S.A. and Canada. He also presented a paper 'Osmotic control of fusion' at the Annual Meeting of the Biophysical Society in San Antonio, Texas.

Among the laboratories visited were the Food Research Laboratories of the Agricultural Research Council (Norwich, U.K.) and the Unilever Research Laboratories (Port Sunlight, U.K.), where applications of current work in CSIRO to food emulsions were discussed. Collaborative experimental projects with the Institut Laue Langevin (Paris) and the Pathology Department, University of British Columbia (Vancouver) were also carried out, and seventeen invited seminars presented during the visit.

The Australian Nutrition Foundation

The Australian Nutrition Foundation recently moved its headquarters from Adelaide to Sydney, with the election of Miss J. F. Rogers, Director of Dietetics, Royal Prince Alfred Hospital, Missenden Road, Camperdown, N.S.W., 2050 as Chairman of Council, and the appointment of Mr G. Fisher (FRL) as National Secretary. The Registered Office is now C/o CSIRO Division of Food Research, P.O. Box 52, North Ryde, N.S.W., 2113.

Microwave drying of meat and meat products for rapid estimation of water and fat contents

By I. J Eustace[A] and P. N. Jones[B]

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Introduction

Rapid, reliable estimates of the water content of meat and meat products are of considerable value to meat processors for at least two reasons. Firstly, knowledge of water content of boneless meat, trimmings and mechanically recovered meat (i.e. the raw materials for manufactured meat products) permits better control of composition of the processed meats. The final moisture content of dried meat byproducts such as meat meal and blood meal can also be better controlled. Secondly, the close relationships between water content and fat and protein contents of boneless beef, pork and mutton (Karmas *et al.* 1961; Thornton *et al.* 1981) permit the estimation of fat and lean meat contents once the water content is known.

Conventional methods of estimating the water content of meat and meat products based on drying samples in air ovens maintained above 100°C are too slow for routine monitoring of cartoned meat packed to specification. Casey and Crosland (1982) measured the moisture content of beef mince by drying it for 30 to 40 minutes under an infra-red heating lamp. They found the results were highly correlated with fat content results.

Domestic-type microwave ovens have been used successfully to rapidly determine (in 2 to 10 minutes) the water content of meat (Lee and Latham 1976), butter (Marschke *et al.* 1979) and cheese (Shanley and Jameson 1981).

There is now an extensive range of domestic microwave ovens available in Australia which cost a few hundred dollars each. In the study described below a domestic microwave oven was evaluated for the estimation of the water and fat contents of meat and meat products. A major aim of the work was to dry the test samples in as short a time as possible to satisfy the need for a rapid method for monitoring the composition of cartoned boneless meat.

Development of microwave drying procedure

In our investigations it quickly became obvious that reliable drying of meat samples in a microwave oven was not possible unless certain precautions were taken.

It was found necessary to preheat the oven before any test samples were dried in order to prevent water from the meat condensing on cold sample containers and covers, and on interior surfaces of the oven. Ovens fitted with resistive elements are convenient for preheating. In ovens not fitted with radiant or convection heating facilities, preheating was accomplished by using a dry energy-absorbing load which was prepared by mixing 15 g of fine silicon carbide with 150 g of salt. A glass beaker containing the silicon carbide-salt mixture was placed in the oven which was operated for eight minutes. The silicon carbide-salt mix was left in the oven permanently as an energy-absorbing load to minimize the risk of microwave radiation being reflected back to the magnetron with resultant damage to the microwave oven.

The drying times necessary were selected by experimentation using comparative water content determinations by a reference method of drying in an air oven. Heating times were varied by 15-second intervals until estimates of water content obtained with the microwave oven compared favourably with values obtained by the reference method. It was found that 30 to 50 g of meat could be dried in 5 minutes in a National Model NE 8080 oven. A minimum of 3.5 minutes drying was required for triplicate 10 g samples. Excessive periods of heating resulted in some spattering and charring of samples and melting of the polypropylene containers used. It was not possible to satisfactorily dry single 10 g samples because of excessive charring and frequent holing of the containers. For loads greater than 50 g,

increasing the sample load required extension of the drying time. The required drying time for three 50 g samples (150 g total load) in the NE 8080 model oven was determined to be 9 minutes.

Pettinati (1975) and Hayward and Kropf (1980) achieved rapid drying and also combatted a spattering problem caused by rapid heating in microwave ovens by dispersing the meat samples in a mixture of salt and ferrous oxide. Ferrous oxide and silicon carbide are two of a number of compounds which strongly absorb microwave energy and heat up, thus acting as heat-generating agents. In some preliminary trials we used a pre-dried mixture of salt and silicon carbide in the weight ratio 10 to 1. Twenty grams of this mixture was added to each 10 g sample of meat. Drying of the sample was very rapid (3 minutes or less). However, we found that unless the silicon carbide-salt mixture was mixed thoroughly with the meat, 'hot spots' occurred which caused charring. The thorough mixing was time-consuming and negated any advantage of faster drying.

The metal dishes normally used for determinations of water content of meat are unsuitable for use in microwave ovens. It was established that samples could be dried in glass beakers or between layers of filter paper. However, polypropylene cups proved to be very convenient. It was found that if a sample was weighed into a container and simply left at the bottom, it heated so rapidly that spattering frequently occurred. Also, when the disposable polypropylene containers were used, melting and holing of the container sometimes occurred with loss of product. The spattering and holing problems were almost completely overcome by pressing the meat around the lower half of the wall of the container with a spatula or spoon. The use of loose-fitting covers of filter paper prevented the loss of product if any spattering did occur.

Once the basic procedure for microwave drying was established, analyses reported below were performed.

Evaluation of method

Materials and apparatus

Fresh meat. – For trial 1, quantities of minced lean and fat beef were mixed to give seven samples with fat contents ranging from 4% to 50%. Each of these samples was finely chopped in a silent cutter, then thoroughly mixed and placed in a polyethylene bag. Samples were stored at 0°C until required for testing.

For trial 2, 28 beef samples (approximately 400–500 g) were obtained from an export boning room. They were taken from samples obtained from cartons of boneless beef during the company's routine quality control procedures. The samples were composites, each consisting of core samples taken from 10 cartons of the boneless product. Each sample was minced three times through a 6.3 mm plate, and mixed after each pass.

In two further trials, 20 samples of mutton and 20 samples of pork were obtained from export boning establishments in the manner described above for trial 2. Each sample was minced three times through a 5 mm plate, and mixed after each pass.

Processed meats. – Samples of formulated sausage meat, frankfurter meat, corned meat loaf and canned corned beef were obtained from Brisbane processors. The samples were derived from samples routinely taken by company staff for quality control.

Meat meal, blood meal. – Twenty samples of meat meal and 16 samples of blood meal were obtained from Brisbane manufacturers of these products. The samples were taken from material collected by company quality assurance staff for routine analysis.

Sample containers. – Polypropylene cups (Code TCH-1, Vinyl Clad, Brisbane) were used as sample containers. Loose-fitting covers, made by fluting circles of filter paper (70 mm diameter), were used to prevent loss of sample due to spattering. The cups and filter papers were dried by heating them in the microwave oven for approximately one minute and then were stored in a desiccator until required.

Microwave ovens. – 1. National Model NE 8080 (Matsushita, Osaka, Japan). The oven had a turntable which rotated continuously during microwave heating. It was fitted with a magnetron with a variable 60 to 600 watt microwave output. It could be used as a convection oven as well as a microwave oven. The resistance heating element had a power output of 1300 watts. This oven was used for drying the meat meal samples, processed meats and the beef samples in trial 1.

2. National Model NE 6880. This oven was identical to Model NE 8080 except that it did not have a resistance heating element. This oven was used for drying samples of beef (trial 2), mutton, pork and blood meal samples.

Procedure

Fat determination. — 1. Reference method. Meat samples were dried at 102°C then analysed for fat by exhaustive extraction (approx. 48 hours) with diethyl ether in a large volume Soxhlet apparatus. Analyses were done in triplicate.

2. Babcock method. The fat content of each sample was determined in triplicate using the sulphuric acid digestion procedure (method 2) used by Muhl and Eustace (1977).

Moisture determination — 1. Reference method. Meat or meal (approx. 10 g) was weighed accurately into a weighed moisture dish and dried for 16 to 18 h at 102°C in a convection air oven. Triplicate subsamples from each composite sample were analysed.

2. Microwave method. Each test meat sample (approx. 10 g) was placed into a pre-weighed cup and pressed into a ring around the lower wall with a spatula. A pre-weighed cover was placed on the cup and the total weight was measured.

Three 10 g samples (triplicates of each meat sample) were dried simultaneously for 4 minutes (trial 1) or 5 minutes (later trials) in a pre-heated microwave oven.

For samples of processed meats, meat meal and blood meal, triplicate 10 g samples were heated for 4 minutes.

TABLE 1

Analyses of water content of fresh meat, processed meats and meals by microwave drying and by drying by a reference method

Product	No. of Samples	Sample size (g)	Mean water content (%)		
			Microwave method	Reference method	Comparison of methods[A]
Beef[B]	7	10	58.7	58.6	n.s.
Beef[C]	28	10	64.7	65.5	***
Mutton	20	10	61.5	62.6	***
Pork	20	10	52.5	53.6	***
Processed meats	8	10	61.3	61.3	n.s.
Meat meal	20	10	7.4	7.8	***
Blood meal	16	10	16.9	17.2	n.s.

[A] Least significant difference (l.s.d.) test
[B] Trial 1
[C] Trial 2
*** Significant at 0.1% level

Results and discussion

Determination of water content with microwave oven

The water contents of the seven beef samples prepared by mixing various proportions of lean and fat tissue (trial 1) ranged from 40.3 to 73.0%. Mean contents determined on triplicate 10 g samples by the microwave drying method and by the reference method are given in Table 1. The difference between the means was not statistically significant when the least significant difference test (L.S.D.) was applied ($P > 0.05$).

For the 28 samples of boneless beef analysed in trial 2, and for the mutton and pork, there were significant differences between the mean water contents determined by microwave drying and by the reference method (Table 1). The mean water contents determined by microwave drying were 0.8, 1.1 and 1.1 per cent less than the means determined by the reference method for beef, mutton and pork respectively. The analyses on these products were performed in a different microwave oven from the one used to dry the beef in trial 1, and the results suggest that five minutes drying in this particular oven was slightly too short to dry the samples completely.

For eight processed meat samples the mean difference between the water content values from the microwave method and the reference method was not statistically significant ($P > 0.05$).

Thus, the water contents of beef, mutton, pork and processed meats as determined by the microwave drying method are suitable for normal quality control purposes.

The water contents of 20 samples of meat meal ranged from 4.0% to 14.0%. The mean water content determined by the microwave method was less than that determined by the reference method (Table 1). The difference between the means was highly significant ($P < 0.001$). Notwithstanding this difference, the determinations by the two methods were highly correlated ($r = 0.994$). The linear regression equation relating reference results (Y) to microwave results (x) was $Y = 0.41 + 1.00x$. Consequently, microwave method determinations can be conveniently corrected by adding 0.4% to the result.

The water contents for 16 samples of blood

TABLE 2
Relationships between fat content determined by a reference ether extraction method and by three other methods of determining fat or water content

Independent variable	Meat species	Regression coefficients		Variance accounted for (%)
		a	b	
Babcock method of determining fat content	Beef	−0.12	0.97	92.6
	Mutton	0.46	0.92	97.3
	Pork	−0.18	0.96	97.5
	Overall	−0.13	0.96	96.9
Reference method of determining water content	Beef	97.21	−1.23	97.8
	Mutton	97.25	−1.22	95.1
	Pork	98.72	−1.24	99.2
	Overall	98.17	−1.24	98.8
Microwave method of determining water content	Beef	94.56	−1.21	96.5
	Mutton	97.47	−1.25	93.8
	Pork	98.94	−1.27	98.9
	Overall	98.29	−1.26	98.2

meal ranged from 6.3% to 27.6%. The mean difference between the microwave method results and those determined by the reference method was not significant ($P > 0.05$).

Relationship between fat content and water content

Once the domestic microwave oven used had proved satisfactory for drying meat samples, data were obtained on the water content and fat content of samples of boneless beef, mutton and pork, from commercial boning rooms.

Linear regression equations describing the relationships between the fat content of boneless beef, mutton and pork, as determined by a reference (ether extraction) method and three other fat or water content measurements are given in Table 2. For each of these three measures separate equations are given for the three meat species tested. Statistical analysis revealed no differences between species. Consequently, overall equations covering beef, mutton and pork are also presented. Plots of the reference fat content against Babcock fat content, reference water content and microwave water content are presented in Figs 1, 2 and 3 respectively.

Reference water content data were obtained by drying meat samples overnight at 102°C. Variation in the water content data for beef accounted for 97.8% of the variation in the fat

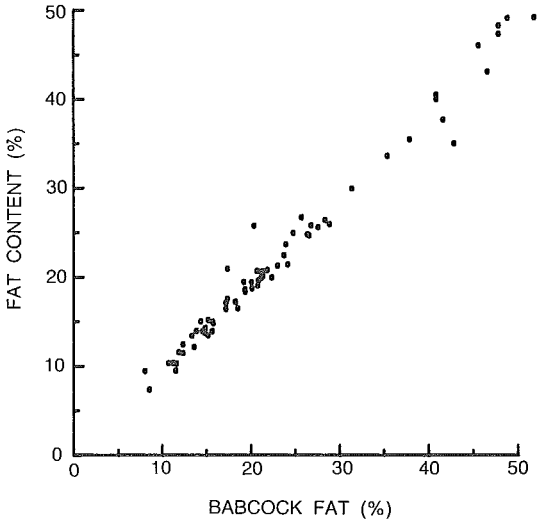

Fig. 1. Plot of fat content (reference) against fat content (Babcock) for boneless beef, mutton and pork (n = 68).

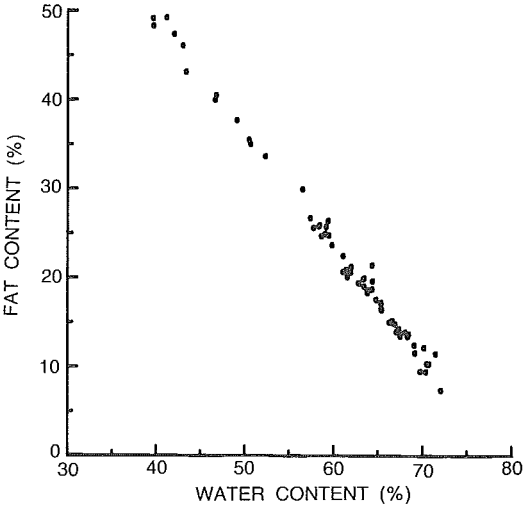

Fig. 2. Plot of fat content against water content (reference method) for boneless beef, mutton and pork (n = 68).

content results determined by the reference method, which agrees very closely with that reported by Thornton *et al.* (1981) for data on cartoned boneless beef typical of the production of commercial boning rooms.

The correlation coefficient for the overall equation relating reference fat content to microwave water content was not statistically different ($P > 0.05$) from that for the overall

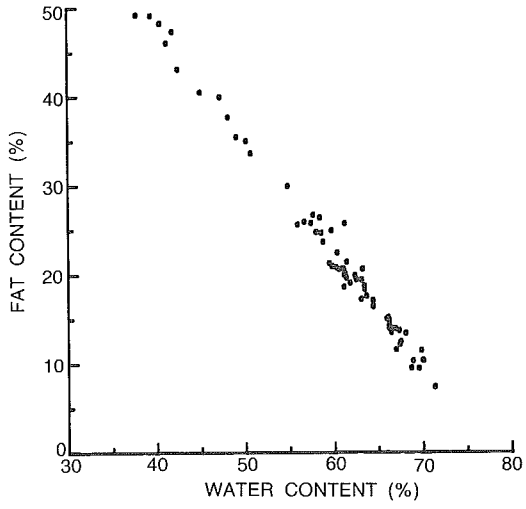

Fig. 3. Plot of fat content against water content determined by microwave drying of boneless beef, mutton and pork (n = 68).

equation in which reference water content is the independent variable. (Hotelling's test, in Williams 1959). The relationship between reference method fat determinations and microwave method water determinations is quite adequate for estimation of fat content of boneless beef, mutton and pork.

For routine quality control purposes it is convenient to prepare tables which present fat content values for specified water contents. This can be done, for example, using the overall equation for microwave water content given in Table 2.

It is normal for specifications to be written in terms of chemical lean content rather than fat content. The equations in Table 2 may be transformed by the use of the equation:

chemical lean (%) = 100 - fat content (%)

Measures of the inherent variation of the test methods are presented in Table 3, in which the variances of triplicate determinations on composite samples of beef, mutton and pork are given for each of the four test procedures.

The variance values for triplicate water content determinations are generally less than the values for fat content determinations. Consequently, it follows that the precision or repeatability of water contents determined by microwave drying compares more than favourably with that of reference (i.e. ether extraction) fat results.

For each of the four analytical methods the variance for the set of triplicate determinations for each of the samples was calculated. There was no evidence of an influence of fat content on the variance over the range of fat contents studied.

General discussion

Microwave energy distribution and intensity

A problem with uneven energy distribution in some microwave ovens used for moisture analysis has been recognized. Haywood and Kropf (1980) found 'hot spots' in the oven they used. The moisture content estimates for milk and meat samples placed at these 'hot spots' were higher than the estimates on samples placed at other locations. Lee and Latham (1976) and Pettinati (1975) recognized the significance of possible positional effects and recommended placing samples at the centre of the oven.

Many domestic microwave ovens now have features to minimize the positional effect. These include turntables to rotate the food continually, stirrer fans to improve the microwave distribution within the oven, and multiple magnetrons. Nevertheless, it is the authors' experience that energy distribution can still be uneven. Samples will heat very rapidly in 'hot spots' with occurrence of charring and spattering, whereas samples in areas of low energy intensity will take much longer to dry. It is important to check the energy distribution within the particular oven to be used. Details of how to rapidly check the energy distribution and determine suitable

TABLE 3
Variance of triplicate determinations from composite samples of beef, mutton and pork

	Beef (n = 28)	Mutton (n = 20)	Pork (n = 20)
Reference method for fat content (ether extraction)	0.458	0.305	1.039
Reference method for water content (air oven)	0.146	0.248	0.450
Microwave method for water content	0.264	0.268	0.407
Babcock method for fat content	0.369	0.468	0.428

positions within the oven for reliable drying of samples are given by Bill *et al.* (1983).

The charring of samples and melting of polypropylene sample containers which occasionally occurred was evidence that temperatures well in excess of 100 °C were sometimes reached when meat samples were being dried. Heating meat samples at too high temperatures (150 °C or greater) in conventional air ovens is known to produce charring and erroneously high results (Cohen 1971) and is therefore undesirable. The melting of polypropylene at about 135 °C serves as a useful indication that charring might have occurred. Corrective action, such as reduction of the power output of the microwave oven or reduction of the drying time, should be taken. Should adoption of a new combination of microwave energy and drying time be necessary it must be shown that the conditions chosen give complete drying.

The drying cycle

Shanley and Jameson (1981) recommended a drying cycle for cheese which included a final two-minute period of resistive heating after the microwave heating to evaporate condensate from the sample containers and the paper covers. One oven we used had the facility for convection cooking by the circulation of dry, heated air throughout the oven. However, we found that, provided the oven was preheated so that the container warmed quickly, condensation was not a problem. Therefore, we did not include a convection heating step when carrying out the determinations reported above. Such a step increases the total drying time required and also eliminates the possibility of using one of the many microwave ovens which do not have convectional heating facilities.

Elimination of sample cooling period

In our trials the dried samples were cooled in a desiccator before the final weighing. However, we found that the polypropylene container and sample cooled quickly and in practice the difference in weight of a sample weighed approximately 30 seconds after removal from the oven and again after 10 minutes cooling in a desiccator was small (0.05 g or less). For quality control this difference is small and is tolerable when immediate results are required.

When the samples are weighed hot a small correction can be applied, as convection currents may result in underestimation of the dried sample weight and overestimation of the water content. Comparison of water contents, determined on approximately 10 samples by weighing before and after cooling, will provide a satisfactory hot weighing correction.

Conclusions

The results of the comparative studies reported above show that the rapid method using microwave drying is useful for the estimation of the water content of cartoned boneless beef, mutton and pork, processed meats, meat meal and blood meal. The method would probably have other applications in quality control laboratories in Australian meatworks, including the drying of wastewater and sludge samples.

Drying of samples of minced boneless beef, mutton and pork in the microwave oven also proved to be a reliable, simple and quick method for estimating fat content and chemical lean content of unprocessed meat. Estimates of water content obtained by the drying of triplicate 10 g ground meat samples for five minutes are highly correlated with estimates of fat content determined by the reference ether extraction procedure. The close relationships mean that a suitable domestic microwave oven can be used to obtain estimates of fat content or chemical lean content that are suitable for quality control. The convenience of the method has the additional advantage that one overall linear equation relating fat content and microwave water content applies for beef, mutton and pork.

Domestic microwave ovens are inexpensive and are comparatively simple to use provided certain precautions are taken. The only other items of equipment necessary are a mincer and a suitable balance, equipment which is needed for most fat analysis procedures.

References

Bill, B. A., Eustace, I. J., Gibbons, R. A., and Jones, P. N. (1983). Rapid analyses for quality control: 1. Chemical lean in boneless beef, mutton and pork. 2. Water in meat, processed meats and byproducts. CSIRO Div. Food Res. Meat Res. Rept. 1/83.

Casey, J. A., and Crosland, A. R. (1982). Indirect measurement of the fat content of beef mince using the Infra-Tester. *J. Food Technol.* **17**, 567-72.

Cohen, E. H. (1971). Comparison of the official AOAC method with rapid methods for the analysis of moisture in meats. *J. Assoc. Off. Anal. Chem.* **54**, 1432-5.

Haywood, L. H., and Kropf, D. H. (1980). Sample position effects on moisture analyses by a microwave oven method. *J. Food Prot.* **43**, 656-7, 663.
Karmas, E., Thompson, J. E., and Wistreich, H. E. (1961). Relationship between pork leanness and moisture content. *Food Technol.* **15**, 8-10.
Lee, J. W. S., and Latham, S. D. (1976). Rapid moisture determination by a commercial-type microwave oven technique. *J. Food Sci.* **41**, 1487.
Marschke, R., Snow, A., and Tommerup, J. (1979). Rapid moisture testing of butter. *Aust. J. Dairy Technol.* **34**, 36-7.
Muhl, G. J., and Eustace, I. J. (1977). A comparison of some modified Babcock Methods. CSIRO Div. Food Res. Meat Res. Rept. 8/77.
Pettinati, J. D. (1975). Microwave oven method for rapid determination of moisture in meat. *J. Assoc. Off. Anal. Chem.* **58**, 1188-93.
Shanley, R. M., and Jameson, G. W. (1981). A study of the rapid determination of moisture in cheese by microwave heating. *Aust. J. Dairy Technol.* **36**, 107-9.
Thornton, R. F., Husband, P. M., and Larsen, T. W. (1981). The relationships between the fat, protein and water content of boneless meat. *Food Technol. Aust.* **33**, 468-73.
Williams, E. J. (1959). Comparison of two independent variables *In.* 'Regression Analysis' p. 73. (Wiley and Sons: New York.)

S-ovalbumin in eggs—a review

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The occurrence of S-ovalbumin in eggs was first reported 20 years ago from this laboratory. In this review, work carried out here and in other laboratories on the basic chemistry of the stability change in ovalbumin is discussed.

Occurrence of S-ovalbumin in stored eggs

Ovalbumin, the main protein in egg white, was first crystallized in 1890, and because of its ready availability and apparent purity has since served as a model in physicochemical research on proteins.

During studies of the heat denaturation of proteins, it was found that the ovalbumin behaved as a mixture of two proteins with different rates of denaturation (Smith and Back 1962). The less reactive fraction (S-ovalbumin*) was formed during storage of the egg, so that ovalbumins prepared from eggs from different sources, with different storage histories, showed differences in specific rotation, reduced viscosity, and solubility at the isoelectric point, after the same heat treatment.

It was estimated that the amount of S-ovalbumin varied from 5% in fresh eggs to 81% in eggs cold-stored for 6 months (Smith 1964). This increase in stability could also be observed with the purified protein *in vitro*. Over 95% of ovalbumin was converted to S-ovalbumin by holding a solution of ovalbumin at pH 10.0, 55°C for 20 h (Smith and Back 1965). Differential scanning calorimetry showed that the ovalbumin of stored eggs denatured as a mixture of three components, i.e. the formation of S-ovalbumin from ovalbumin proceeded through an intermediate (ovalbumin $\rightarrow S_1 \rightarrow S_2$) (Donovan *et al.* 1975, Donovan and Mapes 1976). This is illustrated in Fig. 1 (Smith and Nguyen unpublished work).

Rate of conversion in egg white and isolated ovalbumin

The process of conversion was studied by

*The name "S-ovalbumin" is discussed in an earlier review (Marshall and Neuberger 1972).

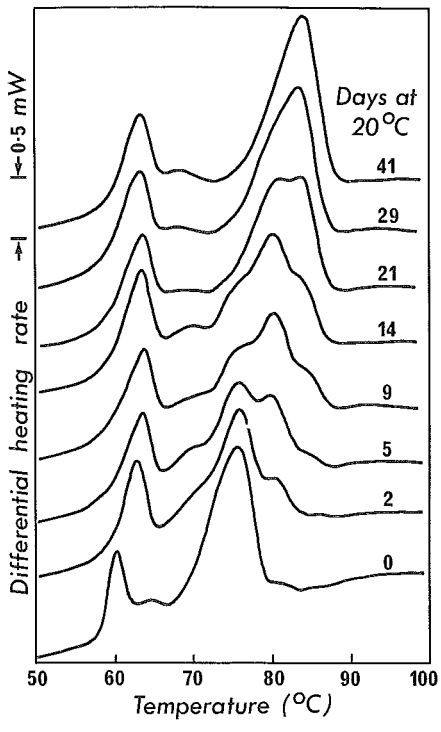

Fig. 1. Thermograms of egg whites held various times at 20°C. Heating rate 0.5 deg C/min.

measuring the amount of protein that was denatured by a standard heating procedure (Smith and Back 1965). The change in solubility at the isoelectric point was used to measure the degree of denaturation. Thus:

$$\% \text{ S-ovalbumin} = 100\left(\frac{A_n - A_o}{S - A_o}\right)$$

A_o, S, A_n were the percentage soluble protein in pure ovalbumin, S-ovalbumin and the mixture respectively.

The conversion to S-ovalbumin in shell eggs as well as in isolated ovalbumin is markedly affected by pH and temperature. Fig. 2 shows the rates of conversion of ovalbumin to S-ovalbumin on holding shell eggs at 20°C (Smith 1962). Table 1 (Smith 1969) shows the effects of both pH and temperature on the rate of conversion in ovalbumin solutions. The process was found to follow first order kinetics and was not reversible.

From the thermograms obtained from egg whites stored at 22° and 37°C, Donovan and Mapes (1976) determined the relative amounts of ovalbumin, S_1- and S_2-ovalbumin by computer fit, assuming Gaussian endotherms. On the assumption that the reactions were irreversible, and sequential first order, the rate constants were selected on a trial and error basis. The rates of transformation which were obtained by this method from stored shell eggs appear to be comparable to those of purified ovalbumin (Smith and Back 1965).

Differences in physical properties

S-ovalbumin is indistinguishable from ovalbumin in most physical aspects. Work done by Smith and Back in Australia as well as by Nakamura and his co-workers in Japan have not revealed the actual change in ovalbumin which confers the greater stability.

No significant difference was found when ovalbumin and S-ovalbumin were compared by measurements of sedimentation rates, molecular weights, electrophoretic and serological properties, sulphydryl and disulphide groups, u.v. absorption spectra, specific rotation, crystal form and solubility (Smith and Back 1965). However, from these results certain changes in structure could be discounted:

(i) dissociation into subunits and association to oligomers
(ii) gross change in shape
(iii) loss of a peptide fragment
(iv) change in ionizable side-chain groups

Kint and Tomimatsu (1979) reported that slight (3–4%) conformational changes, α-helix to antiparallel β-sheet, were involved in the conversion.

It was suggested that changes in surface charge occurred during the transformation of

TABLE 1
Rates of conversion of ovalbumin to S-ovalbumin *in vitro*. Times for 50% conversion, calculated from first-order rate constants

Temperature (°C)	Time (h)		
	pH 9.0	pH 9.5	pH 10.0
20	312[A]	216[A]	33
30	89[A]	47	12
40	27	11	3.2
50	8.8	2.6	1.0

[A] Extrapolated values.

ovalbumin to S-ovalbumin. Nakamura *et al.* (1980, 1981) observed broadening of the band in ion-exchange chromatography and isoelectric focusing and minor differences in the titration curves between ovalbumin and S-ovalbumin. They concluded that surface charge played an important role during the conversion. Kurisaki *et al.* (1982) disagreed, because the two proteins showed the same behaviour in electrophoresis and chromatography. Nakamura and Ishimaru (1981) found that S-ovalbumin was more compact and had a larger surface hydrophobicity than ovalbumin.

Stability in different denaturing agents

Although physical differences between the native ovalbumin and S-ovalbumin are slight, they can be readily distinguished by their different resistance to denaturing agents such as heat, urea, guanidine hydrochloride, formamide and detergent.

Heat

At pH 3.0 and 55°C ovalbumin was 95% denatured after 90 min, S-ovalbumin was 12% denatured.

At pH 7.0 and 73.5°C ovalbumin was 88% denatured after 60 min, S-ovalbumin was 16% denatured (Smith and Back 1965).

The difference in heat stability between ovalbumin and S-ovalbumin has also been demonstrated by Nakamura *et al.* (1980) from measurements of specific rotation and change in solubility.

Urea and guanidine hydrochloride

In urea alone there was no significant difference between ovalbumin and S-ovalbumin at pH 3: they were both rapidly and fully unfolded. However, at pH 7, urea had no effect on S-ovalbumin, therefore mixtures of urea and guanidine hydrochloride were used. S-ovalbumin was more resistant to denaturation by mixed urea and guanidine hydrochloride (Smith and Back 1965).

Formamide-water mixtures

S-ovalbumin was more resistant to formamide than ovalbumin. The effect of different formamide concentrations on the reduced viscosity and specific rotation of ovalbumin and S-ovalbumin was studied. Although both proteins were fully unfolded in 70% formamide, subsequent aggregation was different (Smith and Back 1968a).

Alkylamines at pH 3.0

The stability of ovalbumin and S-ovalbumin to detergents was studied with alkylammonium cations at acid pH. Ultraviolet spectra were used to detect changes in internal structure, but no difference between ovalbumin and S-ovalbumin was observed (Smith and Back 1968a).

Chemical structures

Sulphydryl and disulphide groups

Because S-ovalbumin was found to aggregate less than ovalbumin after denaturation, it was suggested that there was a difference in covalent bond structure. One possibility was that a sulphydryl-disulphide interchange occurred during the transformation, moving the single disulphide cross-link to a position of greater influence on stability.

The peptide fragments obtained by enzymic digestion of each protein were fractionated and corresponding fractions were analysed and compared. Results showed that there was no difference between the peptides linked by the disulphide bond in ovalbumin and S-ovalbumin (Smith and Back 1968b).

That conversion of ovalbumin to S-ovalbumin did not involve a shift in the position of the disulphide bond was subsequently confirmed by Webster and Thompson (1980). They isolated two half-cystine and four cysteine peptides from ovalbumin, the intermediate, and S-ovalbumin which had been labelled with [2-^{14}C] iodoacetic acid and digested with thermolysin. The disulphide bond was found to be located between the third and fourth labelled residues from the N-terminus for all these ovalbumins.

Phosphate

High-resolution ^{31}P-n.m.r. was used to study the environment of the phosphoserine phosphate groups of ovalbumin and S-ovalbumin (Sleigh *et al.* 1983). There was a small difference in chemical shift but this did not indicate a significant difference around the phosphates in the two proteins.

Plakalbumin

Plakalbumin is derived from ovalbumin by mild proteolysis with subtilisin (Smith 1968). Under the same conditions as the ovalbumin-S-ovalbumin transformation, plakalbumin was found to convert to a heat-stable form, S-plakalbumin (Shitamori and Nakamura

1983). The properties of plakalbumin and S-plakalbumin suggested that the mechanism of S-plakalbumin formation is the same as that of S-ovalbumin formation from ovalbumin.

Applied aspects

Eggs are used in a wide range of products. Most of them are dependent on the heat coagulation properties of the egg proteins. The result of ageing on these functional properties has often been noted, e.g. Meehan, Sugihara and Kline (1962) reported that the baking properties of stored egg white could be improved by the addition of ovalbumin from fresh egg, and Lowe (1955) stated that old eggs give a lower viscosity when used in custard than fresh ones. Egelandsdal (1980) studied the heat induced gelling of ovalbumin by measuring rigidity and showed that increasing the S-ovalbumin fraction reduced the rigidity.

During work in this laboratory on the culinary properties of stored egg white (Smith and Nguyen 1983) eggs were stored in the shell and egg white was stored aseptically at 20° and 30°C for up to 7 weeks. Scanning calorimetry was used to confirm the expected increase in the proportion of S-ovalbumin (Fig. 2). The rigidity of samples cooked for 10 min at 90°C was measured with an Instron Universal testing machine. Cooked egg white from eggs stored in the shell showed a progressive *increase* in rigidity which was apparently caused by the increase in protein concentration brought about by evaporation of water. Egg white stored in closed containers showed no change in rigidity in the same test but if it was diluted before cooking there was a loss of gel strength during storage.

In other experiments, measurements of shear modulus on gels formed from mixtures of ovalbumin and S-ovalbumin showed that a 50% higher concentration of S-ovalbumin was required to give the same gel strength as ovalbumin. Thus, although in normal culinary usage the conversion has little effect when undiluted egg white is cooked, stored eggs are likely to be less effective than fresh for thickening sauces and custards and in some types of cakes.

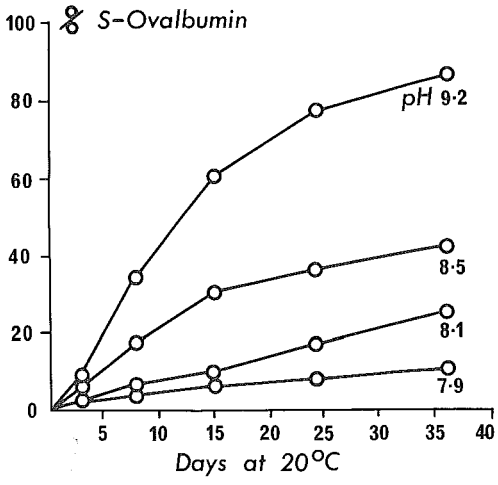

Fig. 2. Conversion of ovalbumin to S-ovalbumin in shell eggs stored at 20°C. Eggs were oiled at different times after laying to vary the pH of white.

Conclusion

Increase in heat stability on holding at alkaline pH at high temperature seems to be a specific phenomenon for ovalbumin. Ovalbumins prepared from duck and turkey eggs were converted to S-ovalbumin (Smith and Back 1970), while other proteins, such as lysozyme, ovotransferrin, β-lactoglobulin and bovine serum albumin, did not show the increase in heat stability after the same treatment (Nakamura *et al.* 1980).

Despite the work already done, the actual mechanism of the conversion is still obscure. Its biological significance is also unknown, although it may be calculated that about three-quarters of the ovalbumin in a fertile egg has been converted to S-ovalbumin after the first week of incubation. Thus, S-ovalbumin is a natural (and possibly preferred) state of ovalbumin in embryonic development.

References

Donovan, J. W., and Mapes, C. J. (1976). A differential scanning calorimetric study of conversion of ovalbumin to S-ovalbumin in eggs. *J. Sci. Food Agric.* **27**, 197-204.

Donovan, J. W., Mapes, C. J., Davis, J. G., and Garibaldi, J. A. (1975). A differential scanning calorimetric study of the stability of egg white to heat denaturation. *J. Sci. Food Agric.* **26**, 78-83.

Egelandsdal, B. (1980). Heat-induced gelling in solutions of ovalbumin. *J. Food Sci.* **45**, 570-3.

Kint, S., and Tomimatsu, Y. (1979). A Raman difference spectroscopic investigation of ovalbumin and S-ovalbumin. *Biopolymers* **18**, 1073-9.

Kurisaki, J., Murata, Y., Kaminogawa, S., and Yamauchi, K. (1982). Heterogeneity and properties of heat-stable ovalbumin from stored egg. *J. Agric. Food Chem.* **30**, 349-53.

Lowe, B. (1955). 'Experimental Cookery'. 4th Ed. (J. Wiley & Sons: New York.)

Marshall, R. D., and Neuberger, A. (1972). In 'Glycoproteins: their composition, structure and function' 2nd Ed. Part B. (Ed. A. Gottschalk) (Elsevier Publishing Company: Amsterdam).

Meehan, J. J., Sugihara, T. F., and Kline, L. (1962). Relationships between shell egg handling factors and egg product properties. *Poult. Sci.* **41**, 892-900.

Nakamura, R., Hirai, M., and Takemori, Y. (1980). Some differences noted between the properties of ovalbumin and S-ovalbumin in native state. *Agric. Biol. Chem.* **44**, 149-53.

Nakamura, R., and Ishimaru, M. (1981). Changes in the shape and surface hydrophobicity of ovalbumin during its transformation to S-ovalbumin. *Agric. Biol. Chem.* **45**, 2775-80.

Nakamura, R., Takemori, J., and Shitamori, S. (1981). Liberation of carboxyl groups on conversion of ovalbumin to S-ovalbumin. *Agric. Biol. Chem.* **45**, 1653-9.

Shitamori, S., and Nakamura, R. (1983). Plakalbumin converts to heat stable form under the same condition as an ovalbumin-S-ovalbumin transformation. *J. Agric. Food Chem.* **31**, 513-6.

Sleigh, R. W., Mackinlay, A. G., and Pope, J. M. (1983). NMR studies of the phosphoserine regions of bovine α_{S1}- and β-casein. *Biochim. Biophys. Acta* **742**, 175-83.

Smith, M. B. (1962). A naturally-occurring modification of ovalbumin. *Int. Congr. Food Sci. Tech. Proc.* 1st **1**, 81-7.

Smith, M. B. (1964). Studies on ovalbumin: I. Denaturation by heat and the heterogeneity of ovalbumin. *Aust. J. Biol. Sci.* **17**, 261-70.

Smith, M. B. (1968). The isolation of a large peptide from denatured plakalbumin. *Biochim. Biophys. Acta* **154**, 263-6.

Smith, M. B. (1969). Changes in the proteins of egg white during handling and processing. *Australas. Poult. Sci. Conv. Proc.* 149-57.

Smith, M. B., and Back, J. F. (1962). Modification of ovalbumin in stored eggs detected by heat denaturation. *Nature* **193**, 878-9.

Smith, M. B., and Back, J. F. (1965). Studies on ovalbumin: II. The formation and properties of S-ovalbumin, a more stable form of ovalbumin. *Aust. J. Biol. Sci.* **18**, 365-77.

Smith, M. B., and Back, J. F. (1968a). Studies on ovalbumin: III. Denaturation of ovalbumin and S-ovalbumin. *Aust. J. Biol. Sci.* **21**, 539-48.

Smith, M. B., and Back, J. F. (1968b). Studies on ovalbumin: IV. Trypsin digestion and the cystine peptides of ovalbumin and S-ovalbumin. *Aust. J. Biol. Sci.* **21**, 549-58.

Smith, M. B., and Back, J. F. (1970). Studies on ovalbumin: V. Amino acid composition and some properties of chicken, duck and turkey ovalbumins. *Aust. J. Biol. Sci.* **23**, 1221-7.

Smith, M. B., and Nguyen, Ly (1983). The gelling properties of heat-denatured proteins: ovalbumin and its natural heat-stable form, S-ovalbumin. *Proc. Aust. Biochem. Sci.* **15**, 22.

Webster, D. M., and Thompson, E. O. P. (1980). Position of the disulphide bond in ovalbumins of different heat stability. Elimination of thiol-disulphide interchange as a mechanism for their formation. *Aust. J. Biol. Sci.* **33**, 269-78.