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Front Cover Photograph:

Vacuum Skin Packed Meat Products.
Skin packing improves appearance as well as prolonging shelf life.

The CSIRO Food Research Quarterly will change in style and format from volume 49, number 1, 1989. The Quarterly will continue to publish papers from the CSIRO Division of Food Processing and other relevant CSIRO Divisions. Papers from other organisations carrying out food related research will also be considered and authors seeking further information are invited to contact the Editor. The Editorial Committee is particularly interested in concise, review type material which reaches a logical conclusion from studies available.

K.C. Richardson,
Editor.

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Destruction of *Escherichia coli* on cattle carcasses using hot water

By M.G. Smith

CSIRO Division of Food Processing, Meat Research Laboratory, P.O. Box 12, Cannon Hill, Queensland 4170, Australia

Introduction

Bacteria capable of causing food poisoning have been isolated from all the common animals and poultry used for human nutrition. In fact, it is believed that animals and poultry used for human food are the major reservoir of these organisms, constantly being carried to the human consumer on dressed carcasses and raw meat (Bowman, 1965; Hobbs; 1965; Bryan, 1980).

The organisms most commonly regarded as food-borne pathogens are the salmonellas and some countries such as Sweden have made heroic efforts to eradicate these bacteria from farm animals and human food. However, during the last decade, other organisms are also being implicated more often as food-borne pathogens capable of causing severe illness, such as *Yersinia enterocolytica, Aeromonas hydrophila, Campylobacter jejuni* or *coli, Listeria monocytogenes* and enteropathogenic *E. coli,* especially the strain 0157:H7 which causes haemorrhagic colitis or "bloody diarrhoea". Certainly these organisms have been isolated from meat in many countries and from the vicinity of abattoirs in Australia.

Recently, some alarm has been expressed, especially in the United States of America, regarding the presence of antibiotic-resistant *E. coli* and antibiotic-resistant salmonellas on meat (Bischoff 1987; Schell 1987). Because of the method by which cattle are raised in Australia, usually on open pastures or range land, these types of organisms are probably not of any great importance in this country at present, but they possibly could become more prevalent in future with the spread of intensive-rearing practices.

In spite of the strictest standards of hygiene, some potential food poisoning organisms are still transferred to the outside surfaces of carcasses during the slaughtering and dressing operations in abattoirs. The investigation described here was originally undertaken to determine whether a process could be developed that would destroy these organisms *in situ* on sides of beef.

At the beginning, we took the view that hot water, if effective, would be the safest treatment medium and no one would object to its use. This might not be so with irradiation, U.V. light, microwave heating, steam, a naked flame, hydrogen peroxide, or the application of various organic acids such as acetic or citric acids.

This paper describes the results of experiments conducted to determine the destruction of *E. coli* (and, by inference, other similar organisms) on beef using water at different temperatures, and the development and testing of a commercial process for treating sides of beef in abattoirs.

Experimental methods

Briskets, with the subcutaneous layer of connective tissue still present, were taken from cattle carcasses within two hours of slaughter of the animal and cut into squares *c.* 10 cm per side and about 2.5 cm thick. They were inoculated by swabbing a culture of *E. coli* (grown in Oxoid nutrient broth at 37°C for 24 h) liberally over the original outside surface and allowed to dry at room temperature (22°C) for 30 min. Tests showed this method resulted in at least 10^6 viable cells per cm^2 of surface tissue.

Tap water, adjusted to various temperatures, was poured over the inoculated pieces of meat for the required time intervals. The numbers of *E. coli* present were estimated before and after treatment. Ten tests were made at each time interval for each of the temperatures used.

A sharp, sterile cork borer (5 cm^2 cross-section) was used to mark out two areas on the meat. These were excised aseptically, placed in a sterile blender jar (Sunbeam Corp. Ltd.), 90 mL of sterile 0.1% peptone water added, and the meat tissue was blended at 20,000 rpm for 30 s. Then, either 0.1 mL of the blend, or of 10-fold dilutions of it, were spread over the surface of TYSG agar plates (Oxoid Tryptone Soya Agar to which 2 g glucose and 2 g Oxoid yeast extract were added per 1000 mL). The plates were incubated at 37°C for 24 h. The lowest count which could be obtained using this technique was 10^2 organisms/cm^2.

Dressed sides of beef from cattle slaughtered no more than about one hour beforehand were inoculated by swabbing a suspension of *E. coli* cells liberally over the freshly exposed surface tissues. A wad of cotton wool about 15 cm square was soaked repeatedly in the bacterial

suspension and rubbed over all the exposed meat tissue. It was found that three litres of such a suspension was enough to completely inoculate a side of beef in this way. The sides were then allowed to hang for another 15 min to let any excess moisture drain off before the initial samples were taken. This method of inoculation resulted in initial counts of at least 10^6 viable cells per cm^2 over the entire surface of each side of beef.

The sides were treated in a novel hot-water decontamination cabinet for either 10 or 20 seconds with water at different temperatures (Davey and Smith 1988). An average temperature (T_f) of the film of water flowing over the whole side was calculated (Davey 1988a). The temperature of the water in the reservoir was also recorded.

Surface tissue samples were taken and treated in the same manner as described below. Six sites on each side were sampled — the neck, thoracic cavity, rump, mid-back, brisket and shoulder — and 2 x 5 cm^2 areas of surface tissue were taken in duplicate both before and after treatment.

Results and discussion

The decreases in the $\log_{10}$ counts of the number of inoculated *E. coli* on pieces of beef treated with water at various temperatures and for different times are shown in Fig.1. These results are very similar to those obtained previously (Smith and Graham 1978). However, in the present experiments the surface area of a fresh beef carcass was used and a treatment time of 5 s was included.

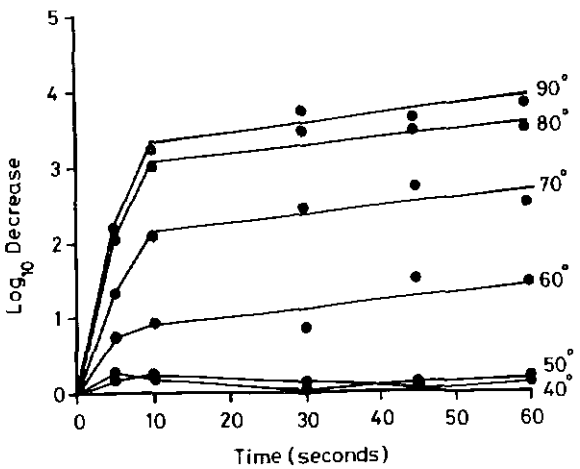

Fig. 1. $\log_{10}$ decrease in numbers of *E. coli* cells inoculated on pieces of beef after treatment with water adjusted to various temperatures for different times.

Very few organisms were removed with water at either 40° or 50°C even with 60 s treatment (no more than *c.* log 0.2). As the temperature was then progressively raised much larger decreases were obtained up to 80°C. Beyond this temperature, little further reduction occurred. It can also be seen from this graph that 5 s treatment is too short a time to obtain the full effect of the heat treatments applied. After 10 s, any further removal of the organisms present was only gradual at any of the lethal temperatures used. Therefore the shortest and most effective time of treatment would appear to be between 5 and 10 s and, if the lines of the graphs are extrapolated to intersect between these time intervals, this can be calculated to lie between 7 and 8 seconds.

These results can be rearranged to illustrate other points (Fig.2).

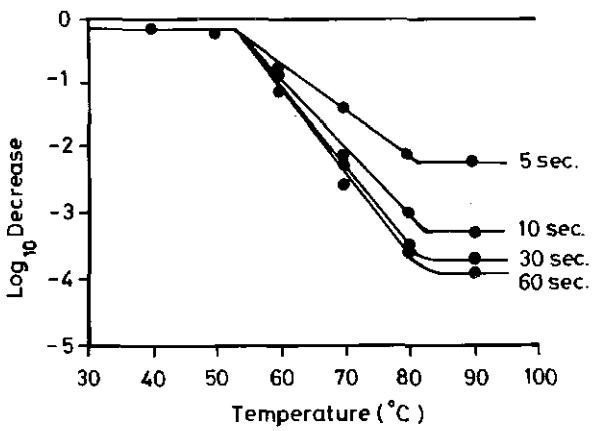

Fig. 2. $\log_{10}$ decrease in numbers of *E. coli* cells inoculated on pieces of beef after treatment with water adjusted to various temperatures for different times.

There was no lethal effect due to the action of the hot water on the bacteria present until the temperature reached at least 54°C. Below this any decrease can be regarded as due to a "wash-off" action of the film of water flowing over the surface of the meat tissue and was usually less than *c.* $\log_{10}$ 0.2. Again, increasing the temperature above 54°C led to greater removal of the organisms present up to about 82-83°C after which little or no further decrease was found. Also, the longer the time of treatment the larger the reductions obtained, but above about 10 s this rate of increase fell rapidly.

The pieces of meat had a bleached, cooked appearence immediately after treatment with water at temperatures of 70°C or above. The normal colour soon returned to the surface tissues after treatment at all temperatures up to and including 80°C. At 90°C, however, with an exposure time of as little as 10 s, this colour change was permanent.

The data obtained from the laboratory experiments were used to design and construct a novel hot-water decontamination cabinet (Davey 1988a). This was tested using whole sides of beef from freshly slaughtered animals (details given by Davey 1988b). Samples were taken before treatment and again within 30 min after treatment. The mean $\log_{10}$ decrease on six sites on each of two sides treated in this cabinet are shown in Fig.3.

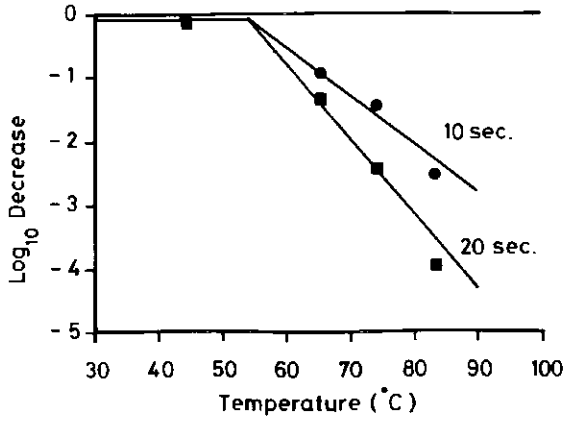

Fig. 3. $\log_{10}$ decrease in numbers of *E. coli* of cattle carcasses after treatment in a decontamination cabinet.

Again, almost no decrease occurred using water at a temperature less than about 54°C. Below this, any removal can be regarded as due to a "wash-off" effect and is quite small (*c.* $\log_{10}0.2$). Above 54°C, the higher the temperature of water used, the greater was the degree of removal of bacteria. Also, when the temperature of the film of water (T_f) was 83.5°C there was more than a mean $\log_{10}^2$ reduction (99%) over the six sites tested on two sides after an exposure time of 10 s, and more than a mean $\log_{10}^3$ reduction (99.9%) if a 20 s exposure was used.

Although the sides looked bleached and cooked immediately after treatment, the normal colour returned almost completely within a further 15-20 min at room temperature when they were judged to be acceptable commercially.

Summary

Laboratory experiments using pieces of fresh beef inoculated with *Escherichia coli* showed that approximately 99.9% of these organisms could be destroyed *in situ* by applying water at a temperature of 80°C for 10 s. This was accomplished without permanently damaging the surface tissues of the meat to any disagreeable extent.

Using this information a hot-water decontamination cabinet was designed and constructed. Tests showed that with a film of water of 83.5°C and an exposure time of 10 s, a mean reduction of at least $\log_{10}2.57$ or 99.7% of inoculated *E. coli* could be obtained over a side of beef from a freshly slaughtered animal. An even larger mean reduction $\log_{10}3.98$ or more than 99.9% was obtained if the exposure time was extended to 20 s. Other micro-organisms, if present, such as the salmonellas which have the potential to cause food poisoning in humans should be destroyed *in situ* to the same extent. At neither exposure time (10 s or 20 s) was the appearance of the side of beef permanently impaired. After about 15 min at room temperature the surface tissues regained almost all their normal colour and the side was regarded as commercially acceptable.

Acknowledgement

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Packaging meat for greater consumer appeal

By P.M. Husband

CSIRO, Division of Food Processing, South Perth, Western Australia, 6151.

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Introduction

In 1880 the S.S. Strathleven sailed from Australia for the United Kingdom with the first export shipment of frozen lamb. To mark this occasion, Queen Victoria was presented with a frozen lamb carcass so she could experience for herself the quality of the colonial product. She was obviously impressed with what she ate, so much so in fact that we were still sending lamb to the United Kingdom in the same basic frozen form one hundred years later. Australia exports a lot of frozen meat; sheep meat in both carcass and boneless form, and beef, mostly as boxed boneless product. Twenty years ago, in 1969, another milestone was reached with the export from Australia to Japan of our first container of chilled, vacuum packed beef. Chilled meat exports per se were not new. As early as the 1930's Australia had sent chilled beef quarters to the United Kingdom in ships with holds which had a carbon dioxide atmosphere. However, the advent of the vacuum pack bag meant that boneless primal cuts could be packed in cartons and held for sufficient time to enable the meat to be shipped over long distances in a chilled condition.

Many problems arose during the development of the Japanese chilled meat trade. Some of these problems were of a technical nature and occurred because of a lack of understanding of the scientific basis of the new vacuum packing technology. A lot of valuable research has subsequently been done, especially in Australia. This, together with our now considerable experience with extended shelf life vacuum packing, has put the chilled meat trade on a sound technological footing.

We have a long way to go before we celebrate the centenary of vacuum packed chilled meat exports, and there is little chance that the Australian meat industry will leave it until then to embark on the next major initiative in meat storage and transportation. However, it is true to say that Australia has paid scant attention, up until now, to developments that have occurred since the introduction of vacuum packing. The last ten to twelve years has seen unprecedented development of flexible, semi-rigid and rigid plastic packaging materials. These have opened the way for a wide variety of packaging applications for the meat industry and these opportunities have led to much innovative product development in many countries.

Development of packaging film

The first people to recognise the merits of vacuum packaging were the French, who, at the Maginot Line in 1936, realised that the quality of frozen meat could be preserved by packaging it in evacuated rubber latex bags. The development in the 1960's of low permeability plastic film consisting of polyvinylidine chloride (PVDC) opened the way for vacuum packaging as we know it. Film properties were improved with the development of coating and extrusion technology for PVDC, and new films were introduced in the early 1970's using materials such as nylon and polyester as the oxygen barrier. Developments in lamination and co-extrusion technology have since given rise to a range of films that incorporate highly efficient barriers such as ethylene vinyl alcohol and aluminium foil as well as the barrier materials developed earlier. A range of other components that serve various purposes has also been incorporated into films following further recent sophistication of the extrusion and lamination processes. Films consisting of up to nine separate component layers can now be manufactured in a single production run. These extra components include materials such as ethylene vinyl acetate to improve sealing properties, ionomer resins for their extraordinary sealing and formability characteristics, polypropylene for its high temperature seal stability and retortability, and compounds such as polyester, polycarbonate and polyetherimide for hot fill applications, designed for reheating in either conventional or microwave ovens.

All this rapid development refocused attention on the questions of the interactions between plastic packaging materials and foods. There has been particular concern over the migration of chemicals, such as residual monomers and plastisizers from the package into the food. The renewed concern has

resulted in research being done around the world in an attempt to quantify the extent of chemical migration from various plastics. Our knowledge of the subject is now expanding rapidly.

Despite the existence now of a diverse range of polymers and composite packaging materials comprised of them, a lot of developmental research is still being done. The list of plastic materials mentioned above is far from exhaustive. Packaging film based on these materials now cover many applications, yet new products are being developed by the larger chemical corporations at such a rate that food packaging materials may undergo a major change again in five to ten years. One of the world's largest chemical corporations for instance spends over US$400 million, representing 40% of their research and development budget, on plastic packaging materials alone.

Much new meat product development work based on the application of modern packaging technology, has been done in various countries over recent years. Unfortunately, Australia has lagged behind in this area and has failed to capitalise on the advantages offered by modern packaging. It is therefore timely to review some of the new packaging options now available.

Chilled meat packaging

Whilst tenderness is an important criterion of meat quality, appearance, specifically colour, is also important, particularly to consumers when purchasing fresh meat. Most systems for the packaging of retail cuts of meat have therefore concentrated on the preservation of colour (bright red for beef). This has meant that vacuum packed meat, with its characteristic purple colour (caused by the presence of the meat pigment, myoglobin, in its non-oxygenated form), has never enjoyed any widespread public acceptance. This is unfortunate given the positive influence vacuum packaging has on shelf life.

Meat colour

As stated before, myoglobin is the pigment which imparts colour to meat and meat products. In its reduced form, the pigment is purple in colour. When exposed to oxygen, the pigment will oxygenate and form the compound oxymyoglobin, which is red and this produces the colour most consumers associate with fresh meat (Laurie, 1968). The intensity of the red colour, also an important quality criterion, is influenced by pH. Consumers favour meat with a bright red colour. Normal pH beef is bright red in colour but high pH meat is darker. The meat surface is more translucent and its reducing capacity is greater, and this diminishes the thickness of the oxygenated layer at the surface (MacDougall, 1982). A combination of these two factors make the darker purple colour more apparent.

When myoglobin is oxidised, the brown pigment metmyoglobin is formed. A layer of metmyoglobin is usually present just below the surface of fresh meat, between the myoglobin and oxymyoglobin layers. This is because the rate of oxidation of myoglobin to metmyoglobin is fastest at low oxygen concentrations. The rate of formation of metmyoglobin is also a function of pH, and it decreases as pH increases.

With the passage of time, fresh meat loses its bright red colour because the metmyoglobin layer migrates towards the surface, eventually causing fading, and then browning of the meat. This occurs less rapidly with high pH dark-cutting meat.

Modified atmosphere packing

Prepared fresh meat has for years been sold on polystyrene trays overwrapped with thin, oxygen permeable film. This prevents desiccation of the meat and yields a degree of hygiene control. However, the colour of the meat packed in this way is usually unacceptable before microbial spoilage is noted. Modified atmosphere packaging has provided a solution to this problem. An atmosphere which is highly enriched with oxygen ensures the existence of a deep layer of oxymyoglobin and prevents the colour problems caused by the formation of metmyoglobin. To take advantage of the enriched oxygen atmosphere, meat is packed in thermoformed, semi-rigid, unplasticised PVC trays. The trays are flushed with the gas mixture and then sealed with a top web consisting of an oxygen barrier (often PVDC) a sealant layer (often ethylene vinyl acetate) and other components. The gas mixtures usually contain between 70% and 80% oxygen. The balance of the atmosphere is carbon dioxide. This inhibits the growth of certain spoilage bacteria and does not have any effect on the colour of the meat (Taylor, *et al.* 1986). One of the problems associated with modified atmosphere packs is that they must contain a volume of gas two to three times that of the meat. This means the size of the pack is larger than conventional packs.

Another important fact to be noted is that the extension of shelf life provided by storage in modified atmosphere is greatly reduced if there is not good control of temperature. In parts of

Europe, where widespread use is being made of modified atmosphere packing, industry pays extremely thorough attention to temperature control. By comparison, there are problems in Australia with control of meat storage temperature, particularly in the merchandising sector. In any attempt to introduce modified atmosphere packaging this must be taken into consideration. A concept that utilises the same principle as the modified atmosphere pack, but which to some degree is less susceptible to premature spoilage problems, is the Master Pack. Meat cuts are individually packed in an oxygen permeable film, preferably skin tight, then bulk packed in an oxygen rich atmosphere contained within a low permeability outer bag. Apart from the space saving with this technique relative to the modified atmosphere pack, the individual packs are only in the oxygen rich atmosphere until they go out for retail display and sale. In this way the red colour is maintained for a prolonged period.

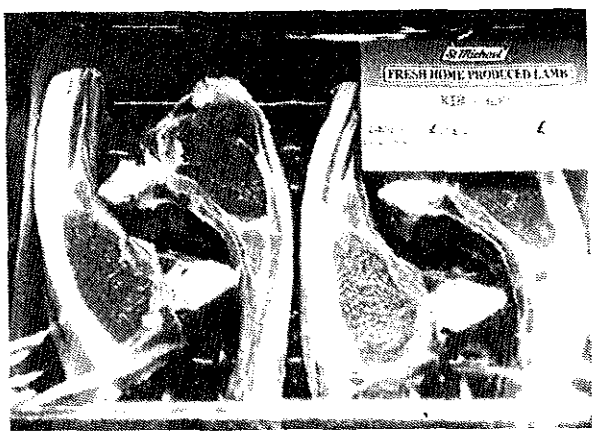

Fig. 1. Modified atmosphere pack of lamb chops — preservation of fresh meat colour.

Vacuum packaging

A widely held view in the meat industry, both in Australia and overseas, is that vacuum packed meat in unsuitable for retail sale because the consumer will simply not accept the purple colour. Attempts have been made in the past in the United States and indeed in Australia to market vacuum packed steak, but given the opportunity to compare the colour of the two alternatives side by side on display, the consumer has always rejected the vacuum packed meat. This has been one of the industry's greatest dilemmas, because the purple colour is a good indicator of the integrity of the pack itself.

A bold move has been made recently by a large supermarket chain in the United States to shatter the myth that the industry cannot sell "purple" beef. This company examined the

Fig. 2. Modified atmosphere master packs of fresh (chilled) lamb.

merits of vacuum packing in conjunction with the merits of centralised packing and made the decision to adopt both. Centralised packing eliminates the problems associated with having prepack operations at every supermarket, it allows better standardisation of the degree of fat trim that is becoming so important these days, and it gives better control over product yield. Vacuum packing provides the shelf life necessary to distribute the product over long distances and still allows for a substantial extension of product shelf life after being purchased by the consumer.

The reason for the success of this marketing exercise, and the thing that sets it apart from earlier attempts by other supermarket chains, is the degree of commitment by the company. Two very important factors have been addressed. Firstly, the company demonstrated the advantage of the new pack in a short, concise video presentation that was mounted in all stores. The video explains the colour, the fact that the pack can be held chilled for ten days and, if the consumer wishes to freeze the meat, that it is the best possible method of packaging meat for this purpose. Secondly, they completely replaced the old overwrapped tray packs with vacuum packs, thus removing any means of colour comparison. The result of these efforts was that there was no negative reaction in terms of sales.

Temperature control is very important, as it is for most meat products, if optimum shelf life is to be achieved with retail vacuum packs. However, it is widely acknowledged to be less important than it is for modified atmosphere packs or conventional packs.

A recent development that appears to offer a compromise between the vacuum pack and non-vacuum pack is a film that has provision for the oxygen barrier layer to be stripped away

from the pack, leaving a fully sealed, oxygen permeable pack. This would offer the storage life advantages of a vacuum pack — up to the point of sale — and the aesthetic advantages of presenting a red product for retail sale. It may be that a package of this type could bridge the interim gap in the transition towards overall acceptance of vacuum packaged meat.

Fig. 3. Vacuum packed, retail ready steak. Maximum shelf life from a chilled retail pack.

Another recent development that offers advantages for presentation as well as packaging design variety is skin packaging. As the name implies this process allows the packaging film to conform exactly to surface profile of the product. This gives rise to many opportunities for enhanced product presentation, as well as further improving the integrity of the pack itself.

Skin packs can be prepared with oxygen barrier film or film that is permeable to oxygen. Skin packaging has application for both fresh meat and manufactured meat products. It will be further discussed under packaging of meat products.

Packaging of meat products

Packaging film developments leading to reduced oxygen permeabilities have been important for fresh meat, but not to the same extent as for manufactured products. A film with an oxygen permeability of around 25 cc/m²/24 hr is adequate for vacuum packaging fresh meat. There is not a lot to be gained by using lower permeabilities, within the range of conventional vacuum packaging films (Husband 1983). However it is widely acknowledged by packaging manufacturers and meat processors in Europe that manufactured meat products do benefit from films with lower permeabilities. An enormous variety of packaging concepts have therefore received widespread acceptance in this sector of the industry. Add to this the multitude of features that can now be incorporated into various packs and the options are numerous. Examples include sliced ham and sausage products vacuum packed in semi-rigid top and bottom webs, sliced product in gas flushed flexible film packs, and sausage in semi-rigid gas flushed packs with a closable lid.

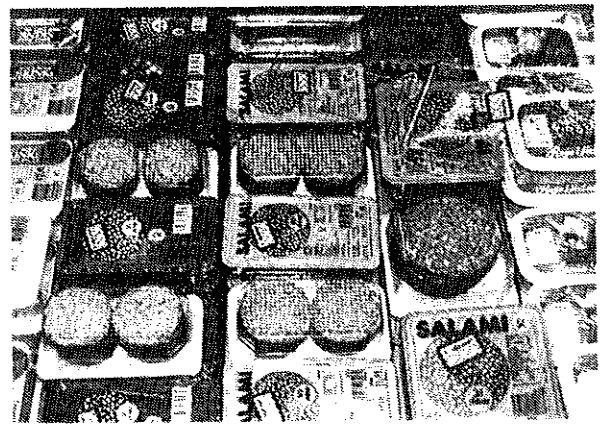

Fig. 4. Modified atmosphere, semi-rigid meat product packs. Attractive package as well as shelf life extension.

As mentioned above, skin packaging is a further development designed to enhance presentation and has application for fresh meat as well as manufactured meat products packaging. In a skin pack, the product becomes the die for the thermoform packaging operation. A semi-rigid bottom web may or may not be thermoformed. The top web is heated in the evacuating chamber until it is near its melting point, at which it drapes over the product and forms around all its contours. Upon sealing and cooling, it retains its new shape, ensuring intimate contact with the product irrespective of surface irregularities.

Skin packing is receiving wide acceptance, particularly where it is desired to show off a product's shape and appearance or to properly display an attractive shingle pack of several

Fig. 5. Vacuum skin packed meat products. Skin packing improves appearance as well as prolonging shelf life.

sausage types. It is an excellent illustration of how modern packaging can itself be a form of value added processing.

Heat resistant packaging materials

One of the more important features to be incorporated into packaging films in recent years is the ability to withstand heat, both in the processing and the reheating of the food products they contain. The reheat-in-bag concept was made possible by the development of nylon-based barrier films. Components of meals or indeed complete meals, can be hot filled into these materials, ensuring that few vegetative micro organisms are present. This results in a prolonged shelf life.

The concept has made it possible to hot fill pumpable products using form-fill equipment at very high speeds. Conventional form-fill equipment will not give the product an aseptic status, but it does guarantee a shelf life well in excess of that achievable by more traditional means. Films of this type can also withstand product reheating. The concept has potential for the catering industry as well as in the home.

Heat resistant flexible films have been further developed to the stage where they can withstand much higher temperature. There are films in existence that can withstand heat processing at 112°C for 20 minutes. This has enabled the production, for instance, of otherwise highly perishable sausage products that can be stored without spoilage for three months at ambient temperature.

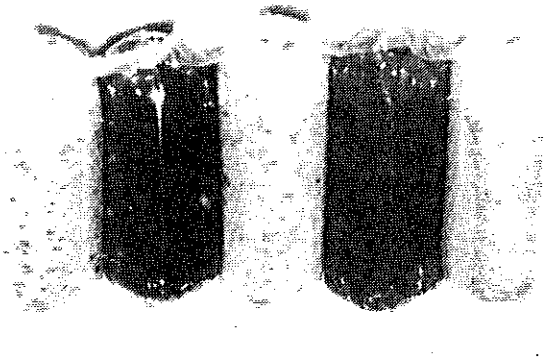

Fig. 6. Shelf stable vacuum packed sausage, heat processed for twenty minutes at 112°C (in the package).

Some semi-rigid and rigid packaging materials are now available that can withstand heat processing up to that used in full retorting (canning). These materials have enabled the development of a range of shelf stable products, including complete meals and parts thereof, that are vastly superior to their frozen, foil packed counterparts of yesteryear.

Prepared meals

Prepared pre-cooked meals have been on the fringes of a big market for many years but have been restrained by a lack of complete convenience. The early frozen dinners in foil trays were of mediocre quality at best. Lack of a good oxygen barrier led to flavour deterioration and they could only be heated in a convection oven. Rigid polypropylene trays and properly sealed, peelable plastic film tops have changed the appearance and acceptability of prepared meals. They can now be sold either chilled or frozen, oxygen barriers can be incorporated if necessary, and, due to the all plastic nature of the package, the product can be re-heated in a microwave oven.

The retort pouch, which never did achieve wide public acceptance, except in Japan, is being superseded by a range of rigid and semi-rigid plastic trays that are highly heat resistant. Compartmentalised trays made of materials such as polycarbonate can be hot filled and heat processed to a point where the meals are shelf stable at ambient temperatures for periods in excess of three months. High density polypropylene-based trays are fully retortable, extremely stable, attractive, and easy to use. These trays are comprised of multi-layered materials that contain oxygen barrier components such as ethylene vinyl alcohol. Because the permeability of ethylene vinyl alcohol deteriorates markedly under the influence of moisture, the tray is structured to allow for this. A moisture barrier is placed between the oxygen barrier and the internal surface. The outer layers are designed to allow the oxygen barrier to dry out following its contact with moisture during the process. No permanent change in permability is caused.

An additional convenience feature has been incorporated into these products. As well as being heatable in a microwave oven, some

Fig. 7. Fully retorted, shelf stable "ready meal". Plastic tray suitable for microwave oven reheating.

types of tray can also be heated in a conventional convection oven up to temperatures of 260°C.

Shelf stable meat product

Shelf stable meat products have been receiving a lot of attention in terms of their potential to expand the value-added possibilities for meat. These items are akin to snack foods, and indeed should compete with them in the marketplace. However, being based on an important commodity such as meat, they represent more than a mere snack. Most can be eaten as fun foods, but they should be considered as high value meat products.

Their inclusion in this discussion is relevant because they rely for their presentation, their convenience and, to a degree, their shelf stability, on the use of a very high standard of packaging. Storage of these products is intended to be at ambient temperature, and shelf lives of between two and twelve months are expected, depending on the type of product. Thus only the very best oxygen barrier materials can be used, and it is not uncommon to see foil laminates, special metallised films and clear films with multiple barrier layers being used for these applications.

Fig. 8. Shelf stable semi-dry fermented sausage. Vacuum packed to prolong shelf life.

Hence the correct choice of packaging film is one of a number of measures that need to be taken to ensure shelf stability particularly in the absence of a non-sterilising heat treatment. For instance, the water activity or moisture content of dried meat can be slightly higher when it is stored in a carbon dioxide atmosphere (using a pack of low permeability) than it would have to be otherwise. This allows for a slightly moister product, which is likely to be more acceptable to the consumer.

The potential of shelf stable meat items based on dried meat, and the range of existing European meat products, is considerable. Sophisticated packaging films can help make this potential a reality in Australia.

Fig. 9. A range of shelf stable "meat snack" products that rely on high quality packaging for their shelf reliability.

Conclusion

Advances in packaging technology have revolutionised, and will continue to revolutionise, the food industry in general and the meat industry in particular. The consumer is becoming more discerning in his or her choice of food products and there is a trend away from traditional food items and eating practices.

Of the many factors that determine the success or failure of a product in today's market, important ones, such as attractive appearance, convenience and hygienic presentation, are catered for to a very large extent by appropriate packaging.

Packaging has become an integral part of the food production process, and is no longer simply an adjunct to it. To quote a large Japanese food manufacturer ". . . the package is now the face of the product." The meat industry must work to maintain its place in view of the changes taking place in eating habits. Therefore, the packaging options outlined above need to be seriously considered, and adopted as a necessary part of improving the image of meat, and expanding the basis of manufacturing meat products.

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Responses of fungi to modified atmospheres

By A.D. Hocking

Food Research Laboratory, CSIRO Division of Food Processing, P.O. Box 52, North Ryde, NSW 2113, Australia.

The use of controlled atmospheres (CA) for insect control in the bulk storage of grains can have the added benefit of controlling mould growth and mycotoxin production. Mould deterioration accounts for significant losses in stored grains, particularly in tropical countries where the temperature and relative humidity are high. The problem is exacerbated where grains are inadequately dried before entering storage.

Atmospheres high in CO_2 are more effective in controlling fungal growth than those which exclude O_2 by replacement with nitrogen. Although most fungi require some oxygen for growth, many spoilage species are efficient scavengers and are capable of near normal growth in O_2 concentrations of $<1\%$. Atmosphere containing about 20% CO_2 generally inhibit mould growth, but $>80\%$ CO_2 may be required to prevent fungal deterioration of high moisture commodities. Some *Fusarium, Aspergillus* and *Mucor* species are particularly tolerant of high levels of CO_2.

Mycotoxin production is more sensitive than fungal growth to low O_2 and high CO_2. Concentrations of CO_2 between 20 and 60% have been demonstrated to prevent or significantly reduce mycotoxin production by some *Fusarium, Aspergillus* and *Penicillium* species. Reduction of O_2 content is less effective in preventing mycotoxin formation.

Introduction

Medium to long term storage of grains in tropical regions presents many problems, as grain is frequently stored at a higher moisture content than is desirable, and invasion pressure from insects is often high. Both these factors will also encourage mould growth, and postharvest losses in stored grains due to insects and other pests, and fungal spoilage are considerable.

The introduction of controlled atmosphere storage of commodities for insect control also offers considerable scope for control of fungal deterioration. However, many storage fungi are capable of growth in low partial pressures of oxygen, and reduction of available oxygen is often not sufficient to prevent moulding, particularly of high moisture grains. Elevated levels of CO_2 are more inhibitory to mould growth, but other factors, such as temperature and moisture content, will affect the degree of inhibition exerted by controlled atmospheres.

Effect of controlled atmospheres on mycoflora of stored commodities

Investigations into the effects of reduced O_2 and increased CO_2 on moulds in stored commodities date back at least to the early 1950s, when hermetic storage of grains was proposed as a new technology (Vayssiere, 1948). The earliest studies were undertaken with maize (Bottomley *et al*, 1950) and wheat (Peterson *et al*, 1956) and dealt mainly with spoilage by storage fungi. Studies undertaken after the mid 1960s were more concerned with the proliferation of mycotoxingenic fungi, and the effects of controlled atmospheres on mycotoxin production.

Maize. Storage of high moisture content maize presents a significant problem in many parts of the world, including the USA. Bottomley *et al* (1950) investigated the effects of reduced oxygen on corn stored at relative humidities between 75 and 100%, and temperatures from 25 to 45°C, but their storage period was only 12 days. They found that mould growth was significantly reduced but not prevented by storage in an atmosphere of 0.1% O_2 and 21% CO_2. Different moulds predominated depending on the storage conditions. At 80% relative humidity, *Penicillium* species were dominant at 25°C, *Aspergillus flavus* at 30°C, and *Eurotium* species at 35°C (Table 1). Mould growth was less at 40 and 45°C, but *Mucor* was predominant at 45°C, especially when the oxygen concentration was 5% or less. In corn at 90% ERH or higher, *Candida* species proliferated in the 0.1% O_2 and 21% CO_2 atmosphere at 25°C, but not at higher temperatures.

Wilson *et al* (1975) investigated the effects of modified atmospheres on the survival of the toxigenic moulds *A. flavus* and *Fusarium moniliforme* in freshly harvested high moisture corn (moisture content 29.4%) and corn rewetted to 19.6% moisture. The corn was inoculated with *A. flavus*, and exposed to atmospheres of air, N_2 (99.7%, balance O_2),

TABLE 1

Predominant mycoflora in corn stored for 12 days at 80% ERH in 20% CO_2 and 0.1% O_2.

Temperature (°C)	Species (%)
25	*Penicillium* (55); *A. flavus* (45)
30	*A. flavus* (90)
35	*Eurotium* (50); *Penicillium* (50)
40	*Eurotium* (70); *A. flavus* (25)
45	*Mucor* (25); *Penicillium* (50); *A. flavus* (15)

(Data of Bottomley *et al*, 1950)

CO_2 (61.7%) and low O_2 (8.7%), and a CA mixture of 13.5% CO_2, 0.5% O_2 and 84.8% N_2. In the freshly harvested corn, *A. flavus* levels increased in the air control to 90% kernel infection after 2 weeks, but with the other treatments kernel infection rate was only 5-18% after 4 weeks (Fig. 1). *F. moniliforme* was recovered from 21% of the kernels initially, but in subsamples exposed to modified atmospheres for four weeks, then held for 1 week in air, was present in 100% of kernels from all three treatments. The CO_2 + low O_2 sample developed an unpleasant odour, and was visibly overgrown with an unidentified yeast. In the rewetted corn, *A. flavus* did not decrease in any of the treatments, and increased in the N_2 and CA treatments. The

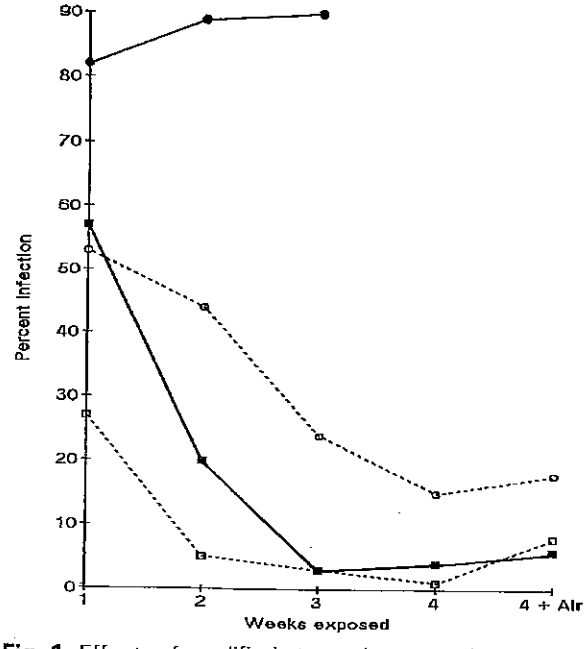


Fig. 1. Effects of modified atmospheres on *Aspergillus flavus* infection of inoculated high-moisture corn. Data of Wilson *et al*, 1975.
(●) Air (0.03% CO_2, 21% O_2, 78% N_2);
(o) CO_2 + low O_2 (61.7% CO_2, 8.7% O_2, 29.6% N_2);
(■) N_2 (99.7% N_2, 0.3% O_2);
(□) CA (13.5% CO_2, 0.5% O_2, 84.8% N_2)

incidence of *F. moniliforme* increased from 67% to near 90% in all treatments. The incidence of other fungi (*Penicillium, Eurotium,* other *Aspergillus* species, *Rhizopus* and *Mucor)* was low, and did not increase during modified atmosphere storage.

In a longer term experiment, Wilson *et al* (1977) used corn with a moisture content of 18.8% for a storage trial in an atmosphere of 14-15% CO_2 and 0.5-1.0% O_2. Corn stored for 35 and 109 days in this atmosphere was tested for aflatoxins and the presence of *A. flavus* and *F. moniliforme.* No aflatoxin was detected after 35 or 109 days, whereas a control sample stored in air contained 472 ug/kg total aflatoxins. A significant proportion of the kernels contained *A. flavus* (30-47%) and *F. moniliforme* (35-47%), after both storage periods, and 27% of kernels contained a *Penicillium* species after 109 days storage. The corn was not assayed for *Fusarium* or *Penicillum* toxins.

Controlled atmosphere storage of high moisture corn in atmospheres containing <1% O_2 could be used for temporary holding before drying, or, at lower temperatures, for longer-term storage, the main advantages being residue-free insect control and retardation of fungal growth. However, because most fungi are not killed by low O_2 atmospheres, the safe storage period for high moisture corn is limited and the corn will deteriorate rapidly upon exposure to the normal atmosphere.

Peanuts. Much attention has been paid to control of aflatoxin production in stored peanuts by use of controlled atmospheres, and this aspect will be addressed in this paper. However, relatively little has been published on the mycoflora changes that occur in peanuts stored under controlled atmospheres over long periods.

The effects of CO_2 on growth and sporulation of *A. flavus* on high moisture peanuts 20% O_2 were reported by Landers *et al* (1967) and Sanders *et al* (1968). Growth and sporulation were reduced with each 20% increase in CO_2 from 20% to 80%, with no growth occurring in 100% CO_2. Growth was much reduced in atmospheres of < 5% O_2, and almost completely inhibited at < 1% O_2. Concentrations of CO_2 in excess of 20% were required before there was any inhibition of growth of *A. flavus* in high moisture peanuts. However, Jackson and Press (1967) reported that incidence of *A. flavus* at 27°C on shelled peanuts of 5.0% moisture content (m.c.) or unshelled peanuts at 7.5% m.c. was not reduced by storage in atmospheres containing 3% O_2 or 82% CO_2 in air compared with air storage over 12 months.

TABLE 2

Fungal colonization of peanut kernels stored at 7% m.c. in 50-60% CO_2 in an outside bin at ambient temperatures for 12 months.

Species	Percent kernel invasion	
	Top of bin	Bottom of bin
A. flavus	95	18
A. candidus	5	1
A. niger	9	20
A. ochraceus	4	1
Eurotium	87	21
Penicillium	3	4
Rhizopus	12	38
Candida	100	11

(Data of Wilson *et al*, 1985.

Wilson *et al* (1985) used pilot scale experiments to determine if long term storage of peanuts was practical in modified atmospheres with minimal deterioration due to mould spoilage, aflatoxin contamination and insect infestation, without use of refrigeration or pesticides. Two large bins of peanuts (1996 kg and 6451 kg) were stored in an atmosphere of approximately 60% CO_2 (balance air), at a moisture content of 6-7% for one year.

The smaller (metal) bin experienced moisture migration due to condensation of water on or near the surface at night, the moisture content of the peanuts at the top rose to 11.1% and they were visibly mouldy after 16 weeks. After this time, the atmosphere was recirculated, and moisture contents rapidly equilibrated throughout the bin. The most common species at the top of the bin were *A. flavus, Eurotium* species, and an unidentified white yeast, possibly a *Candida* species (Table 2). Other *Aspergillus* species (*A. candidus, A. ochraceus* and *A. niger)* were also recorded on 18% of kernels, while *Rhizopus* and *Penicillium* were less frequently isolated. The same species of fungi were isolated from kernels at the bottom of the bin, but in much lower numbers (Table 2). Despite the high incidence of *A. flavus,* no aflatoxins were detected.

In the second trial with the larger (fibre-glass) bin of peanuts, the atmosphere of 55-60% CO_2 was recirculated, and there was no moisture migration. The only major change observed in the mycoflora was a decrease in superficial *Penicillium* contamination from 64 to 16%. Aflatoxins were not detected during the 54 week trial.

Wheat. The mycoflora of wheat differs from that of maize and oilseeds. Wheat is usually drier when harvested, and in general *A. flavus*

TABLE 3

Effect of oxygen concentration on mould population and distribution in wheat stored for 16 days at 18% moisture and 30 °C.

Oxygen %	Moulds/g	Species
0.2	7.0×10^3	*Eurotium*
2.3	1.9×10^5	*Penicillium*
	2.9×10^5	*Eurotium*
4.3	1.0×10^5	*Penicillium*
	8.0×10^5	*Eurotium*
	1.0×10^5	*A. flavus*
8.0	1.0×10^5	*Penicillium*
	5.6×10^5	*Eurotium*
20.6	6.8×10^5	*Penicillium*
	3.9×10^5	*Eurotium*
	5.6×10^4	*A. flavus*

(Data of Peterson *et al*, 1956)

and *F. moniliforme* cause fewer problems in this commodity.

Petersen *et al* (1956) stored wheat of 18% m.c. at 30°C for 16 days under atmospheres with varying concentrations of oxygen and carbon dioxide. In 4.3% O_2, the mycoflora was dominated by *Eurotium* species (80%), with *Penicillium* species and *A. flavus* also present (10% each). When O_2 was reduced to 2.3%, only *Eurotium* (67%) and *Penicillium* (33%) were present (Table 3). In 0.2% O_2, only *Eurotium* species were detected, and their numbers were much reduced (Table 3). With gas mixtures containing 21% O_2 and varying concentrations of CO_2, there was no significant change in numbers of fungi present in up to 18.6% CO_2. However, growth was almost completely inhibited by 50% and 79% CO_2 (Fig. 2). *Eurotium* species were the most tolerant of elevated levels of CO_2.

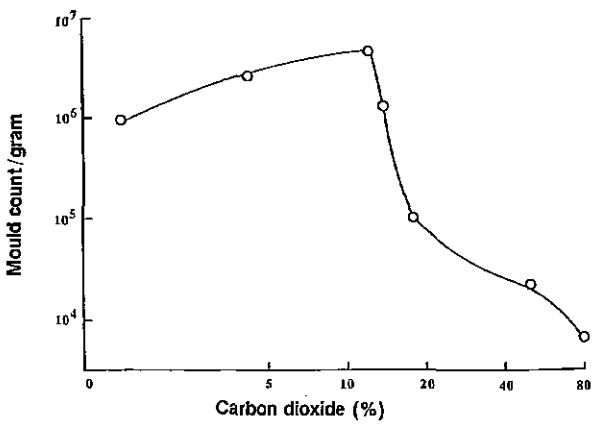


Fig. 2. Effect of CO_2 tension on mould count in wheat incubated 20 days at 30° and 18% moisture. All gas mixtures contained 21% O_2. Data of Peterson *et al*, 1956.

Shejbal and Di Maggio (1976) and Di Maggio *et al* (1976) stored wheat of 18% m.c. in pure nitrogen, and found that mould growth was inhibited, and fungi gradually decreased with time. After 30 weeks, there was an increase in *Aspergillus candidus*. After 54 weeks, the total mould count was 6 x 10^4/gram, a quite acceptable level for wheat. Under 0.2% O_2, mould growth at 18-26°C on wheat of 17.4% m.c. was substantially inhibited in comparison with the air control. However, with both treatments, *A. candidus* eventually proliferated, reaching counts of 6 x 10^5/gram after 3 and about 20 weeks respectively.

Rice. In a study on naturally contaminated rice, Richard-Molard *et al* (1986) investigated the effects of oxygen deficiency on microflora of grain rewetted to 0.87 and 0.94 a_W and stored for 2-4 months. They found that in the samples where the moisture content low enough to prevent bacterial growth (0.87 a_W), most storage fungi, including *Penicillium* and *Aspergillus* were inhibited by atmospheres of less than 1% O_2. However, yeasts (*Candida* spp.) and the yeast-like fungus *Aureobasidium pullulans* were able to develop, even with less than 0.5% O_2, and the higher the a_W, the more rapid the growth. In the complete absence of O_2 (under 100% CO_2 or N_2), there was no fungal growth. At a_W values higher than 0.90, lactic acid bacteria proliferated, and were not inhibited by any of the atmospheres studied.

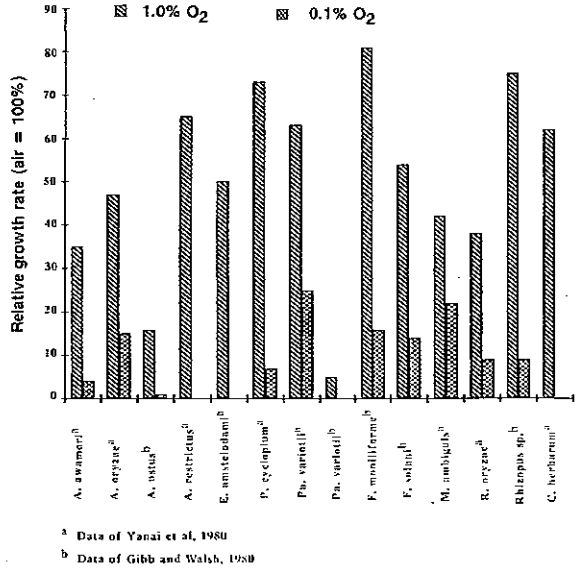
Effect of gas mixtures on growth of fungi

The two factors that need to be considered in preventing fungal growth in controlled atmospheres are (1) the minimum amount of oxygen required for fungal growth and (2) the inhibitory effects of high levels of CO_2. Atmospheres high in nitrogen are only effective because of their low O_2 content, as nitrogen itself has no inhibitory effects.

Oxygen requirements

Many fungi are able to grow in the presence of very small amounts of oxygen (Miller and Golding, 1949; Follstad, 1966; Wells and Uota, 1970; Walsh, 1972; Yanai *et al,* 1980; Gibb and Walsh, 1980; Magan and Lacy, 1984). Anaerobic growth has also been reported for several fungi, for example, *Fusarium oxysporum* (Gunner and Alexander, 1964) and some species of Mucorales that are used as starter cultures for food fermentations in Asia (Hesseltine *et al,* 1985). Tabak and Cook (1968) reported "good to very good" growth of a range of species under 100% nitrogen. The strongest growth was exhibited by *Geotrichum candidum,* a yeast-like fungus, *Mucor heimalis, Fusarium oxysporum* and *F. solani.* However, "good" growth was observed in *Aspergillus niger, A. fumigatus, Penicillium aurantiogriseum* and *P. brevicompactum,* and the black yeast-like fungus *Aureobasidium pullulans.* Such anaerobic growth can only take place if a number of growth factors (vitamins, oxygen donors in the form of higher oxidation states of certain elements) are supplied.

What is perhaps more relevant to CA storage of commodities, is the ability of many common field and storage fungi to grow in atmospheres containing < 1% O_2 (Fig. 3). Of the field fungi present on grains at harvest e.g. *Fusarium* species, *Alternaria,* other dematiaceous hyphomycetes, *Rhizopus,* yeasts etc., some grow very well in low levels of oxygen. *Fusarium moniliforme, F. oxysporum, F. culmorum* and *F. solani* all grow strongly in atmospheres containing 1.0% to 0.1% O_2 or even less (Gunner and Alexander, 1964; Tabak and Cook, 1968; Walsh, 1972; Gibb and Walsh, 1980; Magan and Lacey, 1984), provided that other growth conditions such as temperature and water activity are favourable. Some *Rhizopus* and *Mucor* species can also grow at low oxygen tensions (Wells and Uota, 1970; Gibb and Walsh, 1980; Yanai *et al* 1980) or even anaerobically (Hesseltine *et al,* 1985), and can proliferate in high moisture commodities stored under low oxygen atmospheres (Bottomley *et al,* 1950; Wilson *et al,* 1975).



[a] Data of Yanai et al, 1980
[b] Data of Gibb and Walsh, 1980

Fig. 3. Effects of reduced O_2 tensions on growth of some field and storage fungi. Data of Yanai *et al,* 1980 and Gibb and Walsh, 1980.

Other field fungi such as *Alternaria* and *Cladosporium herbarum* are more sensitive to reduced oxygen tensions (Magan and Lacey, 1984) and gradually die out during storage.

Storage fungi such as *Penicillium* and *Aspergillus* species are generally more sensitive to low levels of O_2 than the more tolerant field fungi. With the exception of *P. roquefortii,* the growth rates of most *Penicillium* species are reduced by more than 50% in atmospheres of 1% O_2 or less (Yanai *et al* 1980; Magan and Lacey, 1984). Of the Aspergilli, *A. candidus* is the most tolerant of reduced O_2 conditions (Magan and Lacey, 1984) and thus can proliferate in CA stored wheat (Shebjal and Di Maggio, 1976; Di Maggio *et al,* 1976). Some *Eurotium* species are also reasonably tolerant of low O_2 levels (Petersen *et al,* 1956; Yanai *et al,* 1980).

In our laboratory, studies on a number of spoilage fungi isolated from low O_2 environments have shown that most are inhibited only slightly when grown in nitrogen atmospheres, with 0–1.0% O_2 (Fig. 4). Isolates of *Penicillium corylophilum* and *P. glabrum* from vacuum packed jams were able to grow at 66–90% of their control rate (air) when sealed in barrier film with an atmosphere of nitrogen. *Fusarium equiseti* and *F. oxysporum* which caused fermentative spoilage of UHT fruit juices grew at 88–97% of their normal rate. A *Cladosporium* species isolated from the inside of a UHT pack of apple juice was little affected by lack of oxygen, growing at 95–100% when sealed in an atmosphere of nitrogen. *Mucor plumbeus* and *Absidia corymbifera* also grew strongly in nitrogen. The xerophilic fungus *Eurotium repens* grew at 60–90% of the control rate, depending on the growth medium, and the extreme xerophile *Xeromyces bisporus* grew at the same rate in air and in nitrogen (Fig. 4).

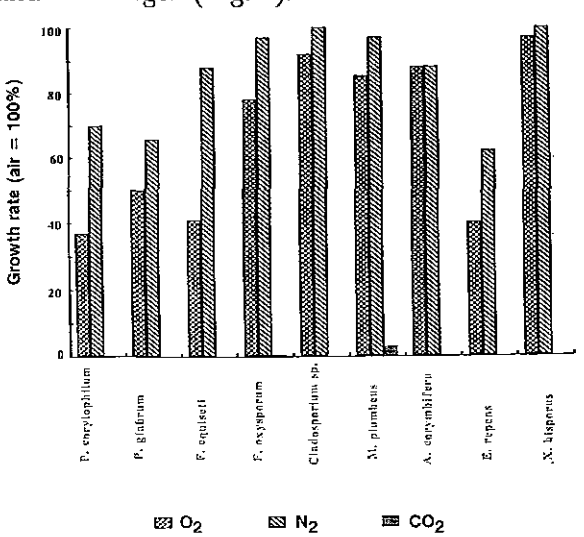


Fig. 4. Growth rates (relative to air) of nine fungi in O_2, N_2 and CO_2. Fungi were grown in pure culture of Petrislides (Millipore Corp.) sealed inside barrier film containing the appropriate atmosphere, and incubated at 25°C. Growth was measured by radial growth rate of colonies.

Effects of increased carbon dioxide levels

Levels of CO_2 from 4% to 20% can be stimulatory to growth of many fungi in atmospheres containing low levels of O_2 (Wells and Uota, 1970; Gibb and Walsh, 1980), conditions that may well arise during sealed storage of commodities. However, elevated CO_2 concentrations are generally much more effective in controlling fungal growth than oxygen depletion. Thus, atmospheres rich in CO_2 are more likely to prevent mould deterioration of CA stored high moisture commodities than atmospheres of nitrogen with traces of O_2. Typical insecticidal atmospheres used for grain storage are 1% O_2 in nitrogen and 60% CO_2 40% air (Banks, 1981), and while both may be equally effective in controlling insect populations in stored grain, the CO_2-enriched atmosphere would be more effective in controlling fungal growth in high-moisture commodities.

Atmospheres containing >50% CO_2 will substantially inhibit growth of most spoilage fungi (Peterson *et al,* 1956; Wells and Uota, 1970) but there is little information in the literature on their actual CO_2 tolerances. Stotzky and Goos (1965) recorded slight growth of *Rhizopus stolonifer, Mucor hiemalis* and a *Trichoderma* species in 100% CO_2. The same three species grew well in an atmosphere of 50% CO_2, 45% N_2, 5% O_2. *Fusarium oxysporum* grew in 95% CO_2, 5% N_2 but not in 95% CO_2 5% O_2. *Paecilomyces lilacinus* did not grow in either of these atmospheres, but grew reasonably well in 50% CO_2, 45% N_2, 5% O_2.

Wells and Uota (1970) showed that growth of *Alternaria alternata, Botrytis cinerea, Rhizopus stolonifer* and *Cladosporium herbarum* in atmospheres of 10, 20, 30 and 45% CO_2 plus 21% O_2 decreased linearly with increasing CO_2 concentrations and was inhibited about 50% in an atmosphere of 20% CO_2 (Fig. 5). Growth of a *Fusarium* species, cited as *F. roseum* was stimulated at 10% CO_2, and inhibited 50% at 45% CO_2.

Magan and Lacey (1984) reported that >15% CO_2 was required to halve the linear growth rate of most of the 14 species of field and storage fungi tested at 0.98–0.90 a_W and 23°C. The species most sensitive to elevated CO_2 concentrations were *Penicillium brevicompactum, Aspergillus fumigatus, A. nidulans* and *A. versicolor* (Table 4). However, no upper limits of CO_2 tolerance were determined, as the maximum concentration of CO_2 tested was 15%.

Nine species were tested in our laboratory for their ability to grow in an atmosphere of 97–99% CO_2 with trace amounts of O_2 and N_2. Only *Fusarium oxysporum* and *Mucor plumbeus*

TABLE 4

Concentrations of CO_2 required to halve the linear growth rate of field and storage fungi at 23 °C.

	Water activity		
	0.98	0.95	0.90
Field fungi			
A. alternata	>15.0	>15.0	>15.0
C. cladosporioides	>15.0	>15.0	>15.0
C. herbarum	13.0	>15.0	>15.0
E. nigrum	>15.0	>15.0	>15.0
F. culmorum	*14.0*	*13.5*	>15.0
Storage fungi			
P. brevicompactum	11.5	8.5	15.0
P. aurantiogriseum	4.5*	4.0*	>15.0
P. hordei	>15.0	8.5	9.5
P. piceum	>15.0	>15.0	14.5
P. roquefortii	>15.0	>15.0	4.5
A. candidus	>15.0	>15.0	>15.0
A. fumigatus	>15.0	5.2	12.5
A. nidulans	>15.0	6.5	13.5
A. versicolor	12.0	>15.0	14.5
E. repens	>15.0	>15.0	>15.0

* Stimulation of growth occurred at higher CO_2 concentrations

(Data of Magan and Lacey, 1984)

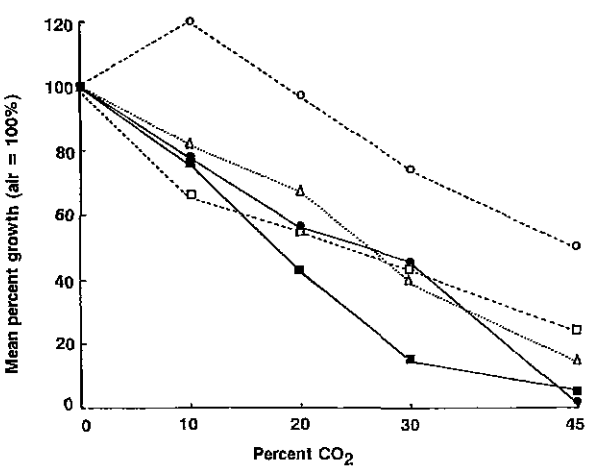


Fig. 5. Growth of five fungi in 21% O_2 with different levels of CO_2, cultured on liquid media at 19 °C. Growth was measured by dry weight of mycelia. Data of Wells and Uota, 1970.
(●) *Fusarium "roseum"*; (△) *Rhizopus stolonifer*
(□) *Alternaria alternata*; (●) *Botrytis cinerea*
(■) *Cladosporium herbarum*.

grew, and their growth rates were only 0.5–4% of those in air (Fig. 4).

The effects of CO_2 concentrations on fungi growing in stored commodities, rather than in pure culture, seem to vary. Landers *et al* (1967) and Sanders *et al* (1968) reported that growth of *A. flavus* on high moisture peanuts was inhibited by 80% CO_2/20% O_2, but Jackson and Press (1967) found no reduction in *A. flavus* on peanuts stored at 5% m.c. (approximately 0.7 a_w) in 82% CO_2 in air for 12 months. This perhaps indicates that although 80% CO_2 will inhibit growth of *A. flavus*, conidia of this species are not killed by exposure to high levels of CO_2 at low a_w. Peterson *et al* (1956) reported that *Eurotium* species survived and grew in wheat stored in 50% CO_2/21% O_2 and 79% CO_2/21% O_2. However, there is little evidence that *Eurotium* species are particularly tolerant of high concentrations of CO_2 in pure culture. Magan and Lacey (1984) found that >15% CO_2 was required to halve the linear growth rate of *E. repens*, but this species will not germinate or grow in an atmosphere of 85% CO_2 12% N_2 3% O_2 (Hocking, unpublished).

The exact mechanisms of CO_2 inhibition of microbial growth are unknown. It is obvious that it is not simply an oxygen displacement effect. Most studies have been carried out on bacteria, and little is known of the effects on fungi. Research on mechanisms of inhibition of bacterial growth have been summarised by Daniels *et al* (1985) as follows:
(a) the exclusion of oxygen by replacement with CO_2 may contribute slightly to the overall effect; (b) the ease with which CO_2 penetrates cells may facilitate its chemical effects on the internal metabolism; (c) carbon dioxide is able to produce a rapid acidification of the internal pH of cells with possible ramifications relating to metabolic processes; and (d) carbon dioxide appears to exert an effect on certain enzyme systems, though these effects differ for different species and with differing growth conditions.

Effects of gas mixtures on mycotoxin production

Aflatoxins. A number of studies have investigated the effects of various atmospheres and other environmental conditions on aflatoxin production, both in stored commodities and in pure culture. Landers *et al* (1967), investigating aflatoxin production in stored peanuts, reported that aflatoxin production decreased with increasing concentrations of CO_2 from 0.03% (air) to 100%, and that in general, reducing the O_2 concentration also reduced aflatoxin production, particularly from 5% to 1% O_2 (Fig. 6). The inhibitory effect of CO_2 was greater at 15 °C than at 30 °C. At 15 °C, aflatoxin production in 20% CO_2 5% O_2 75% N_2 was less than 1% of that in air, and was barely detectable in an atmosphere of 40% CO_2 5% O_2 55% N_2. Sanders *et al* (1968) reported similar results in storage experiments with peanuts at reduced a_w and temperature. They found that aflatoxin levels decreased as

a_w decreased from 0.99 to 0.86. At a constant temperature, an increase in CO_2 concentration caused a decrease in aflatoxin formation, and lowering the temperature also decreased the amount of toxin formed.

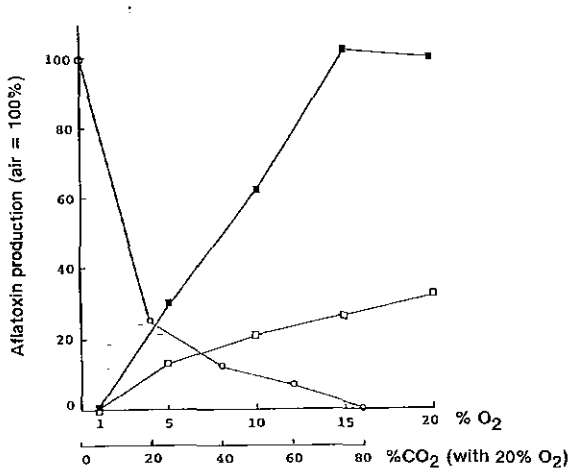


Fig. 6. Influence of various concentrations of O_2 and CO_2 on aflatoxin production in peanuts with kernel moisture content of 27-30% held at 30°C for 2 weeks. Data of Landers *et al*, 1967.
(●) CO_2 with 20% O_2; (■) O_2 with no CO_2; (□) O_2 with 20% CO_2

Epstein *et al* (1970) studied the effects of controlled atmosphere (10% CO_2, 1.8% O_2, 88.2% N_2) on aflatoxin production in liquid medium and in inoculated corn at room temperature (which varied from 25 to 35°C) and at temperatures from 29°C to 1°C. At room temperature, *A. flavus* grew well and produced toxin in both air and CA. At 15°C, aflatoxin production, but not growth, was inhibited in CA. Aflatoxin was not produced at 12°C, and there was little growth at this temperature in air and none in CA. The minimum temperature for aflatoxin production varies with strains, but is generally 10–12°C (Northolt *et al*, 1977).

Wilson and Jay (1975) found that corn inoculated with *A. flavus* and stored at 27°C for four weeks in three different modified atmospheres accumulated less than 20 μg/kg total aflatoxin compared with up to >1021 μg/kg for the air control. Remoistened corn was more susceptible to aflatoxin production than freshly harvested high moisture corn. Aflatoxin production in moistened (18.5% m.c.) wheat incubated at 32°C for up to 21 days was minimal (<1 μg/kg) in an atmosphere of N_2 compared with 123 μg/kg in air (Fabbri *et al*, 1980). Clevstrom *et al* (1983) also found that small quantities of aflatoxins were produced when *A. flavus* was cultured under an atmosphere of nitrogen, and that production increased approximately 15-fold with the addition of B vitamins and a supply of traces of air. Carbon dioxide enrichment hindered aflatoxin formation on a defined medium even in the presence of B vitamins, but small quantities (5 to 15 μg/litre) were formed when formic acid was added.

Carbon monoxide can also suppress growth of *A. flavus* and aflatoxin formation. Buchanan *et al* (1985) reported that after growth of *A. flavus* for 32 days in cooked rice medium or raw pistachio nuts in an atmosphere containing 2% O_2 and 10% CO, aflatoxin production was <2% of the production in an atmosphere containing 2% O_2 or air without CO.

Other Aspergillus toxins. Ochratoxin is the only other *Aspergillus* toxin that has been studied under modified atmospheres. Paster *et al* (1983) grew *A. ochraceus* on solid synthetic medium at 16°C±1°C for 14 days in atmospheres containing various concentrations of O_2 and CO_2 (Fig. 7). In atmospheres of 1% and 5% O_2 without CO_2, ochratoxin production was similar to the air control. Increasing the O_2 level up to 40% reduced ochratoxin production by 75%, whereas at 60% O_2, ochratoxin production was enhanced. In atmospheres of 10% and 20% CO_2, ochratoxin production decreased when O_2 concentrations were below 20%, and was enhanced when they were 40% or 60%. Ochratoxin production was completely inhibited by 30% or more CO_2, regardless of the oxygen concentration. Colony growth was partially inhibited at 60% CO_2, and there was no growth in 80% CO_2.

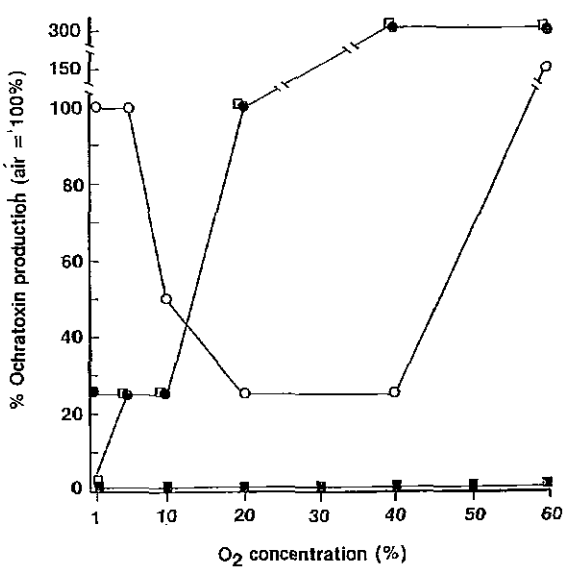


Fig. 7. Ochratoxin production by *Aspergillus ochraceus* grown under modified atmospheres on solid synthetic medium at 16°C ± 1°C for 14 days. Data of Paster *et al*, 1983.
(○) 0% CO_2; (●) 10% CO_2; (■) 30% CO_2.

Penicillium toxins. The effects of modified atmospheres on growth and toxin production by *Penicillium* species has not been thoroughly investigated, and there are few reports in the literature. However, in general, it can be assumed that elevated levels of CO_2 will inhibit toxin production to some degree. The effects of limiting O_2 supplies is less predictable.

The effect of modified atmospheres on patulin production by *Penicillium patulum* (now *P. griseofulvum)* has been investigated by Paster and Lisker (1985) (Fig. 8). Cultures grown for 7 days in 1% or 5% O_2 but no CO_2 produced less toxin than the control (1 and 14 mg/40 mL compared with 45 mg/40 mL for the control). In 10% O_2 without CO_2 patulin production and mycelial dry weight were similar to the controls. Increasing the O_2 content to 60% or 70% decreased patulin production to 20 and 1.3 mg/40 mL respectively. Toxin production was also inhibited when CO_2 concentration was raised to 20% or more in the presence of 20% O_2. Spores incubated in 100% CO_2 or N_2 did not germinate, but grew normally and produced patulin in amounts comparable to the controls when subsequently exposed to air.

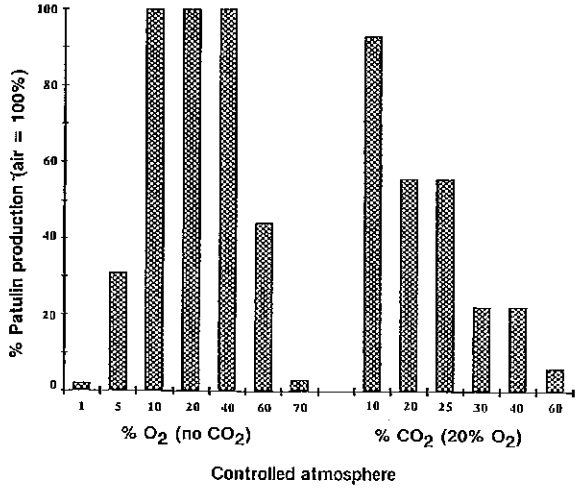


Fig. 8. Effects of controlled atmospheres on patulin production by *Penicillium aurantiogriseum* grown for 7 days in Czapek agar at 26°C. Data of Paster and Lisker, 1985.

Penicillic acid production by *Penicillium martensii* (now *P. aurantiogriseum)* was studied in mould inoculated corn over a temperature range of 5° to 20°C in air and in atmospheres containing 20%, 40% or 60% CO_2, with 20% O_2 (Lillehoj *et al,* 1972). Penicillic acid production decreased with increasing CO_2 concentration. Toxin production was greatest in air at 5°C, but was completely blocked at this temperature by 20% CO_2, and by 40% CO_2 at 10°C over a four week incubation period.

Fusarium toxins. As with *Penicillium* species, little work has been done on the effects of modified atmospheres on toxin production by *Fusarium* species, although it is known that many Fusaria are tolerant of low O_2 tensions and high CO_2 concentrations.

The effects of MA on production of T-2 toxin by *F. sporotrichioides* has been investigated both in synthetic media (Paster *et al,* 1986) and in remoistened irradiated corn (Paster and Menasherov, 1988). In the synthetic medium, T-2 production after 7 days at 27°C in an atmosphere of 50% CO_2/20% O_2 was reduced to about 20% of the air control (Fig. 9). At 60% and 80% CO_2 with 20% O_2, there was a significant reduction in fungal growth. Toxin production in 80% CO_2 was only 1.1 µg/45 mL. When the same strain of *F. sporotrichioides* was grown for 14 days at 26°C ±1°C on irradiated corn remoistened to 22% m.c., the production of T-2 toxin was totally inhibited under 60% CO_2/20% O_2, and only trace amounts were detected when the gas combination was 40% CO_2/5% O_2 (Fig. 10). Fungal growth was not inhibited by any of the gas mixtures examined, and the growth rate was identical to that for grains kept under air.

Implications for CA storage of commodities

Storage of commodities in controlled atmospheres containing high (>60%) levels of CO_2 to prevent insect infestation can also inhibit mould growth and mycotoxin

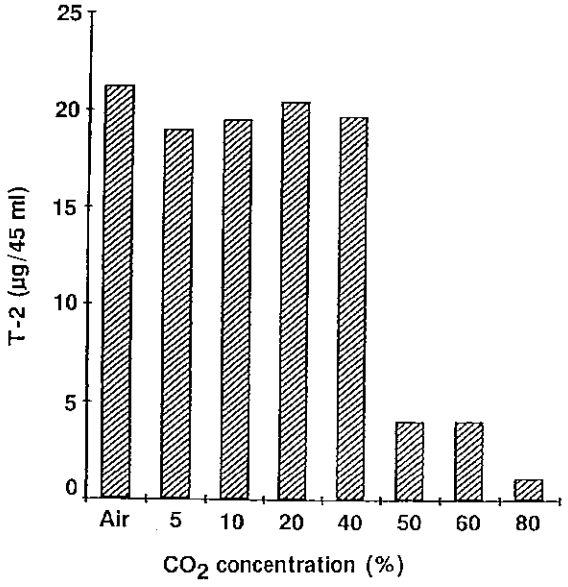


Fig. 9. T-2 toxin production by *Fusarium sporotrichioides* under controlled atmospheres containing various concentrations of CO_2 in 20% O_2. Cultures were grown on potato dextrose agar for 7 days at 27°C. Data of Paster *et al,* 1986.

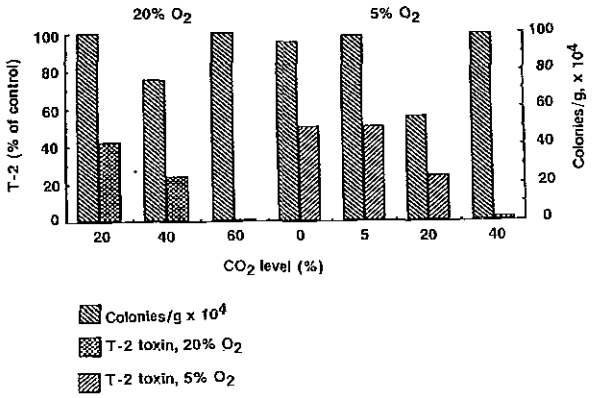


Fig. 10. Effects of various levels of CO_2 and O_2 on colony counts of *Fusarium sporotrichioides* and T-2 toxin production of corn stored under modified atmospheres at 26 °C ± 1 °C for 14 days. Data of Paster and Menasherov, 1988.

production, while atmospheres of nitrogen need to contain <1% O_2 to retard fungal growth. Mycotoxin production is more sensitive than fungal growth to CA conditions, but may still occur if other conditions (temperature and a_w) are favourable.

Fungal deterioration cannot be completely prevented in high moisture commodities (a_w between about 0].90 and 0.80) by CA storage, as some fungi, particularly some *Fusarium*, *Mucor* and *Aspergillus* species, are tolerant of levels of 60–80% CO_2. Yeasts and yeast-like fungi can also develop in CA stored high moisture commodities, causing rancidity and off odours. At very high moisture levels, above 0.90 a_w, lactic acid bacteria may develop, irrespective of the concentrations of CO_2 or O_2 used in the storage atmosphere.

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The biotin content of Australian convenience foods

By R.L. Hood and C.R. Beales

Introduction

The consumption of convenience foods is increasing in Australia, particularly with adolescents and low to middle income families. The Australian food processing industry is becoming geared to producing and marketing convenience foods. Innovative product promotion, high per capita disposable income, demand for leisure time, reasonable cost of convenience foods and more women in the work force all contribute towards this trend. The move away from traditional meals to convenience foods raises questions about the quantity and variety of vitamins taken in by some consumer groups. Biotin deficiency is not commonly encountered in humans, however, pregnant and lactating women, alcoholics, infants with inborn errors of metabolism, the elderly and athletes may be at risk of biotin deficiency, since these groups have been reported to have lowered urinary excretion of biotin and/or lowered circulatory concentrations of the vitamin.

Data available on the bioton content of foods is limited. The biotin contents of breakfast cereals (Hood 1984), infant foods (Hood 1987), selected fast foods (Hoppner and Lampi 1987) and a composite Canadian diet (Hoppner *et al* 1978) have been investigated. Wills, Greenfield and coauthors have reported on the mineral and vitamin content, excluding biotin, of many Australian foods. Although biotin contents of selected foods are listed in United Kingdom Food Composition Tables they serve only as a guide, since direct comparisons may not be possible. This report lists the amount of biotin in a range of convenience foods available in Australia.

Methods

Twenty-four convenience foods were purchased from super-markets and other retail outlets in the Sydney metropolitan area. Items were selected to incorporate a wide variety of manufacturers. On the same day as purchased duplicate samples were dried overnight in a vacuum oven to determine the water content and to facilitate grinding and sampling of the foods. The dried food was crushed and ground in a Wiley Mill fitted with a 1 mm mesh screen and stored frozen in sealed containers.

Duplicate ground samples (5 g) were hydrolysed in 50 mL of $2NH_2SO_4$ for one hour at 121°C in an autoclave. All hydrolysates were neutralised with 20% NaOH, filtered, and the volume of filtrate recorded before biotin analysis. The assay employs the principle of isotope dilution and is based on competition between a known quantity of radioactive biotin and an unknown quantity of non-radioactive biotin for the binding sites of avidin. After precipitation of the avidin-biotin complex, the amount of excess ^{14}C-biotin remaining in the supernatant is measured. The concentration of biotin in the unknown sample is then calculated from the dilution of radioactive biotin by non-radioactive biotin. All reagents of calibrating procedures used in the radiochemical assay have been described previously (Hood 1975, 1977), except that ^{3}H-biotin (Amersham (Australia) Pty Ltd) replaced ^{14}C-biotin to increase the sensitivity of the assay and eight scaled dilutions of avidin were used rather than six dilutions in order to improve precision.

Results and discussion

Convenience foods comprise the growth sector of the Australian food industry. The spectrum of convenience foods is broad, including fast-foods available at takeaway restaurants, snack foods and easy-to-prepare home meals. Ingredients are listed on the containers of convenience foods and they vary greatly due to the diversity of convenience foods which are available. Vitamins are not normally added to these foods. Published values of vitamin contents for a particular food can only represent an average value for that product and process, since variations in the natural ingredients (e.g. cereal grains), in processing conditions and in analytical methodology influence the available vitamin content of processed food. The average biotin content of 24 commercially available convenience foods and their water content are listed in Table 1.

Percent water varied greatly depending on food type. Prepared foods (e.g. hamburgers) and ready-to-serve foods sealed under sterile conditions (e.g. baked beans) have a high water content whereas snack foods (e.g. potato crisps) are low in water; which is in agreement with the data of Wills and Greenfield (1982). Biotin concentrations varied from 0.44μg/100g in

french fries to 6.92μ-g/100g in a savoury casserole base. A number of foods with high biotin concentrations were foods (tomato soup, casserole base and custard mix) that required addition of water before consumption.

Cereals are considered to be a good source of biotin and this is reflected in the high concentration of biotin in corn chips and popcorn and in museli bars, which have a high content of oats. Bran is the major biotin-containing component of cereal grains (Hood 1984).

Variation in the biological availability of biotin from different foods, particularly cereal grains, is considerable. Most biotin exists in a form which is bound to other food components; this biotin may not be released by gastric or intestinal digestion and hence may pass through the gastrointestinal tract and be excreted. A negligible portion of microbiologically assayed biotin in wheat is available to the chick (Frigg 1976) wereas all biotin in corn and 25 percent of biotin in oats is biologically available (Anderson *et al* 1978). Studies in biotin availability have not been carried out in human subjects. Biotin contents listed in Table 1 provide information on the total amount of biotin present in the food but provide no information on the amount of biotin which is nutritionally available.

A recommended daily allowance (RDA) for biotin has not been set due to uncertainities as the the extent of the contribution of biotin from intestinal synthesis by micro-organisms. As a compromise, the United States Food and Drug Administration has published an 'estimated safe and adequate daily dietary intake' for biotin of 100-200μg for adults and adolescents. Most convenience foods in the form and quantity eaten would contribute less than 5μg of biotin to the diet. It is unlikely that most Australians would achieve a daily intake of 100-200μg of biotin and in view of the lack of biotin deficiency symptoms in man, the estimated safe and adequate daily dietary intake has probably been set too high. Limited consumption of convenience foods by individuals eating a varied diet poses no problem of a biotin deficiency in man.

TABLE 1

Biotin content of selected convenience foods

Food	Water (%)	Biotin[a] (μg/100g)
French fries (McDonalds)	29.6	.44 ± .05
Prawn Crackers (Gold Medal Snacks)	3.5	.78 ± .09
Potato crisps (A.P.D. Snack Foods)	2.8	1.79 ± .07
Salada crackers (Arnotts)	3.5	1.92 ± .10
Twisties (A.P.D. Snack Foods)	2.3	2.44 ± .12
Big Mac (Hamburger) (McDonalds)	43.8	2.57 ± .11
Crunchy Snacks (Crackers) (Nabisco)	3.1	2.60 ± .09
Cheeseburger (McDonalds)	41.9	2.82 ± .19
Popcorn (Kandy Kist)	4.0	3.56 ± .02
Corn chips (Arnotts)	2.3	3.67 ± .14
Museli bar (Uncle Tobys)	10.4	4.69 ± .23
Meat pie (Frozen) (No Frills)	53.1	.80 ± .09
Beef, vegetables and rice (Frozen) (Findus)	74.1	.81 ± .05
Fish in seafood sauce (Frozen) (I and J)	79.9	1.33 ± .23
Baked beans (Edgell)	71.1	1.66 ± .24
Pizza (Frozen) (No brand)	53.7	2.33 ± .44
Three minute noodles (White Wings)	8.0	1.54 ± .10
Shell macaroni (Kraft)	12.3	4.06 ± .18
Chicken curry and rice (Dried) (Vesta)	12.2	2.24 ± .11
Chocolate cake mix (Dried) (White Wings)	4.9	1.78 ± .10
Prawns and chicken variety (Dried) (Huis Suimin)	10.4	2.51 ± .13
Quick custard mix (Foster Clark's)	15.3	4.63 ± .02
Tomato Soup (Dried) (Continental)	10.1	5.48 ± .55
Savoury casserole base (Continental)	8.7	6.92 ± .86

[a] Biotin concentration in food as purchased.

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