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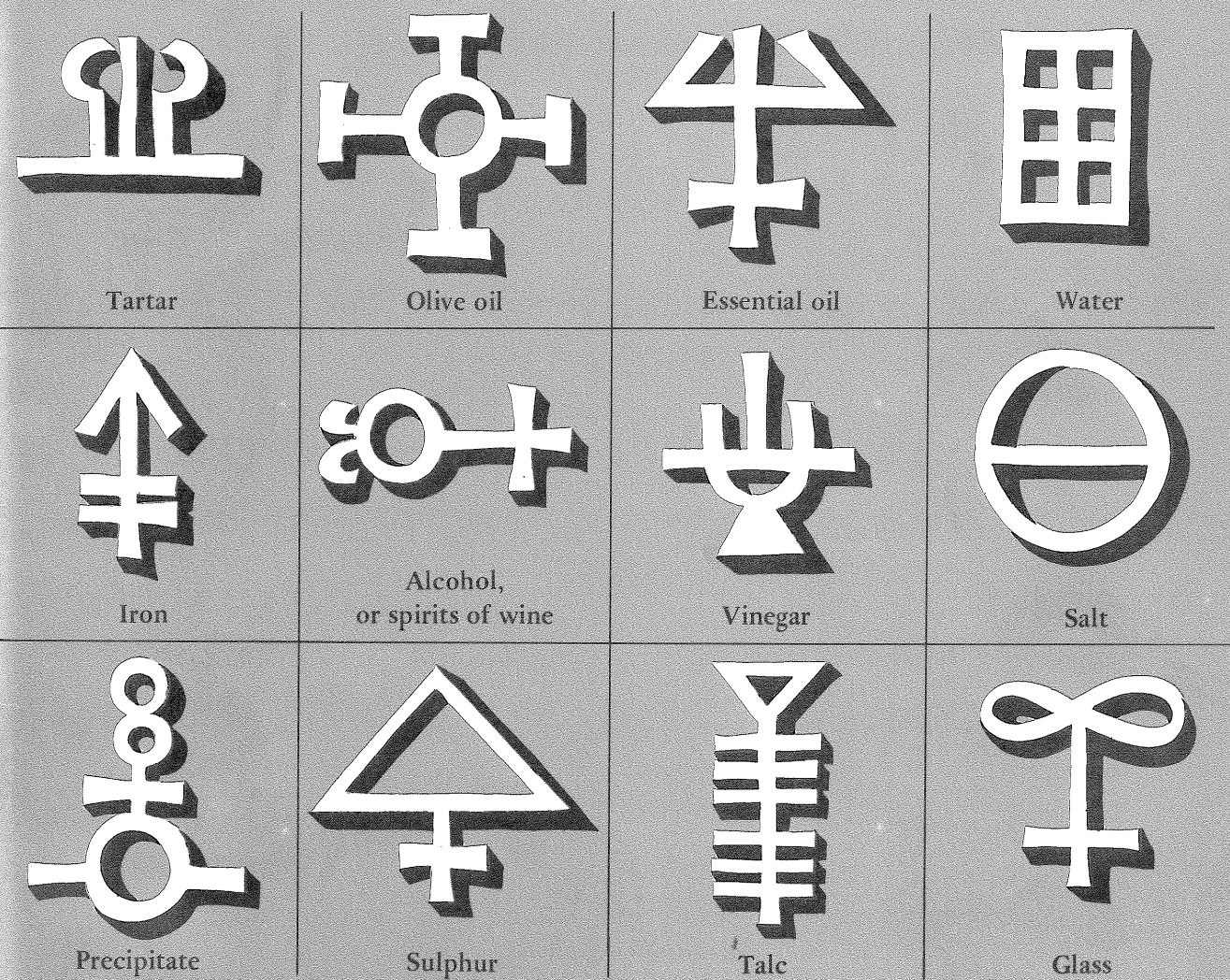

Dried meat products

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One frequently hears discussion of the possibility of making dried meat products which could be stored without refrigeration. In Australia their unavailability is often commented on, particularly in the wake of natural disasters such as cyclones, floods and earthquakes when fresh food is in short supply and cold storage facilities, and the electricity to run them, may be restricted.

During periods when there is a surplus of meat in Australia and when people in other parts of the world, especially in over-populated or underdeveloped regions, are starving in the aftermath of conflict or drought, the usefulness of such products seems obvious. Why, then, are dried meat products not mass produced in Australia?

History

The art of producing air-dried *raw* meat products has been developed over the ages since man first encountered the problem of preserving surplus meat. The main products that have evolved are biltong, charqui and pemmican. This article describes the methods used to prepare these products and comments on their applicability today. In modern times, particularly under the stimulus of global warfare, intensive efforts have been made to apply recently developed drying technology to produce a *cooked* dried meat product, but without great success. These efforts, too, are examined briefly.

Biltong

It may well be a practical proposition to make biltong in Australia, although this would probably be more expensive than is generally thought. However, it seems that even in countries where biltong is traditional, production for consumers outside primitive tribal communities is still mainly limited to the scale of what can be made at home or in the butcher's shop.

Instructions published by the South African Livestock and Meat Industries Control Board (Anon. 1974) make the point that the consumption of biltong in South Africa is not now confined to the ethnic originators and that a biltong cult has spread to all sections of the community. Thus, biltong is nowadays regarded as something of a delicacy. The connoisseur seeks tenderness in 'binnebiltong' or 'ouma se biltong' which is made only from fillet steak. Others claim that the best biltong is 'garing biltong' obtained from the eye muscle. Generally, the muscles of the hindquarter are used and 'a young animal must be chosen otherwise the biltong will be too tough'.

In the recommended method for making biltong, the selected muscles are dissected along their seams and cut into strips resembling tongues (hence biltong) 250–300 mm (8–12 in.) long and 50–100 mm (2–4 in.) diam. A comparatively lean well-fleshed 'buttock', i.e. hindquarter, will yield about 70% biltong, 12% trim pieces and 18% bone. During the drying process 60% of the mass of the meat is lost, so that a whole 6-kg cut of rump steak would yield only about 2·5 kg biltong.

Once the raw biltong has been dissected and prepared it is hung to dry suspended by strings or wire hooks. 'In dry weather or at a time of year when there are no flies, the strips may be hung in the sun on the first day. Thereafter, the biltong should be hung in the shade'. Mildew may form unless drying is rapid. If biltong is dried indoors an electric fan may be used to help keep flies away. The period of drying and the stage of dryness at which the biltong is considered suitable for further processing both appear to be flexible and a matter for the processor's own judgment.

The meat is salted either by immersion in brine or, more usually, by packing in dry salt. 'The longer the biltong is left to salt, the more salt is absorbed. Biltong that contains a lot of fat takes longer to absorb salt than lean biltong. The biltong also becomes more salty the longer it is left to dry out. As a result of these factors it is difficult

to determine the quantities exactly since personal taste also plays an important role'.

One or other of various spices such as aniseed, coriander, allspice or garlic may be mixed with pepper and added to the salt. Other optional ingredients are sugar, saltpetre to promote a red colour, and sodium bicarbonate to counteract mould. The final product when packed away for storage should be absolutely dry. 'Although the fat may become rancid, biltong will keep its qualities for many years if it is stored in a dry place'. It may also be vacuum-packed in plastic film and placed in frozen storage where it will keep indefinitely.

To judge from this account, it seems unlikely that biltong could be prepared under controlled conditions for less cost than it takes to prepare table meat. Also, because of the weight loss it would have to be sold for three times the price per unit of fresh meat. It is a matter for speculation what markets could be found for the product which, away from native communities, appears to be developing as a gourmet snack food flavoured in different ways.

If biltong were being prepared either for the Australian market or to a standard which would enable the product to be exported, it would be necessary to:

- Prepare the biltong from selected muscles of the hindquarters of young animals slaughtered in approved slaughter houses and subjected to a veterinary inspection in order to ensure that no pathogenic organisms were present. (It should be noted that drying and salting are the only preservative methods employed in subsequent processing. There is no heating and the product may be eaten without cooking.)
- Remove the muscles under standard boning-room conditions and cut them into the required strips *c.* 200–300 mm long and 50–100 mm diam.
- Salt and season the product to produce an acceptable flavour.
- Ensure that drying is done in a controlled environment under microbiologically clean conditions.
- Pack and distribute it either frozen or under conditions ensuring that it remains dry.

This is a long way from the popular conception of cutting a few strips of meat off a carcass, hanging it over a fence to dry in the sun, rubbing in some salt and hanging it up somewhere for later consumption.

Basically, the production of biltong is labour-intensive and the process is not amenable to automation particularly as critical steps require subjective judgments. The traditionally prepared product is probably successful largely because it is consumed within a small community and does not have to endure the rigours associated with wider distribution.

Charqui

Charqui, which is produced in South America, differs from biltong in that it is traditionally a very fat product. As recently as 1947, it was estimated that in Brazil alone as many as 600 000 cattle were converted into charqui each year. The method used is as follows. A fresh side of beef is cut into three pieces: the 'manta', which is the meat cut off the bone from the point of the thick rump down to the neck, and two 'postas' which are the shoulder and leg boned out in single pieces. These primal cuts of meat are then opened up with large-bladed knives, cut into strips similar to biltong and hung to cool at air temperature for about an hour. The strips are then immersed in brine for another hour, drained, dipped in coarse dry salt, stacked in heaps 1–1·5 m high, covered in salt and left overnight. The piles are turned each day for 4 days so that the strips from the top of one pile go to the bottom of a new pile and those from the bottom go to the top, and the piles are re-covered with salt. Drying begins on the fifth day. On the first day of drying the meat is hung over drying racks and exposed to the sun for no more than 1–2 h. Then it is removed from the racks and piled in stacks about 1 m high under a tarpaulin for 2–3 days to 'cure'. Drying and curing is repeated 5–7 times until the meat has lost 40% of its fresh weight. The best grade final product contains 20–35% fatty tissue.

It is not known whether charqui or the 'jerkey' made by the North American Indians was the original product. Jerkey was dried over fires and had a characteristic smoky flavour. Jerkey made in the traditional way may not be judged very palatable by modern standards, but commercially prepared versions are popular as snack foods in the United States (Fig. 1).

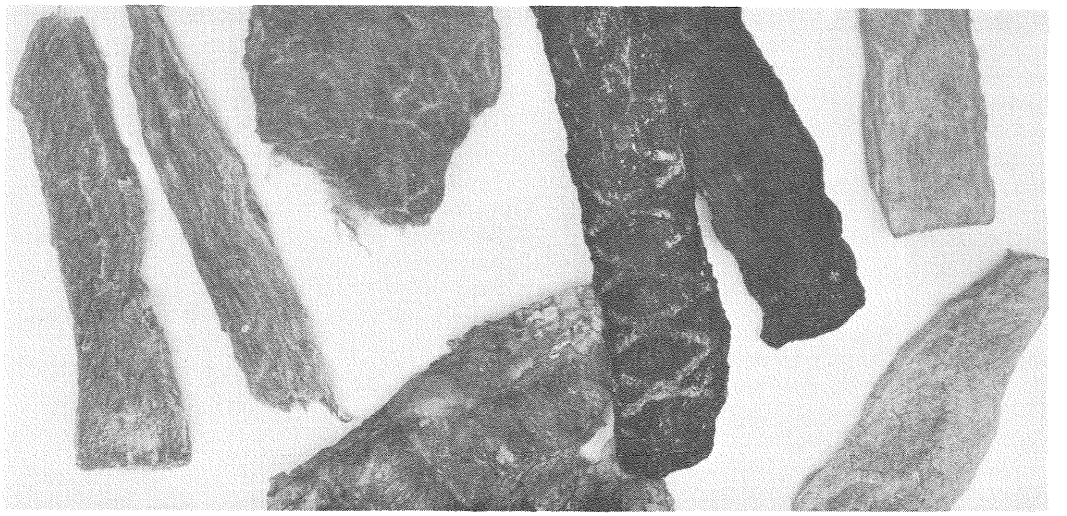

Fig. 1. Jerkey and beef steak prepared by the modern method of freeze-drying. The dark coloured sample is commercial jerkey from the United States. The other samples were made at the Food Research Laboratory, North Ryde.

In common with biltong, the production of charqui is a slow labour-intensive process of a type which would be extremely suspect in the eyes of hygiene-conscious communities and authorities.

Pemmican

Pemmican is the cold-environment equivalent of these products. The original pemmican was invented by American Indians, specifically the Cree. Borgstrom (1968) describes it as consisting of 'dried meat of buffalo, caribou, deer, and later beef, which was packed in melted fat into specially made rawhide bags. The meat was dried in the sun and pounded or shredded prior to being mixed with the melted fat. This preserving method is based on the air exclusion provided by the fat, which not only reduces oxidative changes but diminishes microbial growth. This is accomplished not only by suppressing the growth of aerobics, but because the combination of a fat medium and dry conditions deprives the micro-organisms of the water indispensable to metabolic functions.... Mostly the pemmican was flavored and partially preserved by the addition of dried, acid berries'.

The product supplied to polar travellers is basically the same but is made from beef. Sometimes dried fruits such as currants are added to increase its palatability. It is significant that this form of dried meat has been used in cold regions where the development of rancidity is relatively slow and where the higher calorific value of the fat affords a convenient way of providing the extra energy intake often required in cold climates. Pemmican would be unsuitable for use in most underdeveloped areas of the world as these lie in the warmer zones and this particular form of dried meat would have to be stored under refrigeration or canned.

Sun-dried meat

In many hot dry environments lean meat is dried, without curing, for local consumption. It will keep while in an arid environment but begins to putrefy on exposure to humidity. Such meat is prepared in inland parts of Australia and in native communities in other parts of the world with a similar climate. However, it is difficult to envisage how such a product could be utilized to relieve famine. In contrast with the slaughter of one or two animals for local consumption, any production on a scale large enough to make an impact on the numbers of surplus cattle or on the supply of food to overseas communities, would pose many expensive problems—and the end product is still a perishable commodity except under special storage conditions.

Air-dried cooked meats

When raw meat is air-dried the end product cannot be restored to a form where it

resembles fresh meat nor can it be used with any success in recipes in which fresh meat is normally used. The alternative is to dry cooked meat, and this approach yields an end product which more closely resembles meat in its familiar cooked state. Air-drying and freeze-drying have both been developed as commercial processes and applied to minces, cubes and slices of cooked meat.

Mince

Substantial quantities of air-dried pre-cooked mutton mince were produced in Australia during World War II (Fig. 2). There was a shortage of skilled boners at that time and hence the method of production included a rather severe pressure-cooking of the meat before drying in order to facilitate separation of the meat from the bones (Anon. 1962). The British Ministry of Food encouraged development of air-drying processes for meat in various parts of the world and by the end of 1944 the estimated total production capacities (tonnes dehydrated meat per year) of the plants were: Argentina, 20 000 t; Uruguay, 7000 t; Brazil, 5500 t; Australia, 4500 t; and New Zealand, 4500 t.

The bulk of Australian and New Zealand meat thus produced was consumed by the Armed Forces in the Middle East, while the best of the South American production was issued to the Armed Forces in Britain. This product was never issued to domestic consumers (Sharp 1953). Dried beef and pork products were also made in the U.S.A. and a raw nitrite-cured smoked product (which was said to taste 'kippery') was prepared in Germany.

During the post-war period the production and storage of air-dried mutton mince were studied exhaustively by the Division of Food Preservation.

Slices and chunks

Problems associated with the air-drying of beef mince and of mutton slices were examined by the Division at about that time, and it also investigated the shelf-life of dried beef chunks prepared by a New Zealand patented process.

That there is now no production of air-dried cooked meat, although there were 10 plants operating in Australia in the mid 1940s, is an indication that either these processes were uneconomic or the products were unsatisfactory in some respect. It is recorded (Anon. 1962) that most of the products had rather poor eating quality; off-flavours frequently developed and the storage life was relatively short. The beef chunks prepared by the New Zealand process were said to have a characteristic 'processed' or 'twice-cooked' flavour. Even after soaking overnight the reconstituted meat tasted slightly dry, slightly tough and 'woolly', and it was dark in colour. These defects increased with increase in storage time and temperature. Further development was apparently abandoned in favour of freeze-dried products.

Freeze-dried meats

In freeze-drying the water in the material being processed is frozen and then removed by sublimation under high vacuum and at low temperatures. Mince may be freeze-dried either from the raw or cooked state and the product is undoubtedly better than mince dehydrated by other methods. However, most reconstituted freeze-dried meat still suffers from the texture defect described as 'woolliness', i.e. after the flavour has dissipated the mouth-feel is of a fibrous material rather like cotton wool. The same defect is encountered also in air-dried meat and in some spun vegetable protein products.

Progress has been made in producing freeze-dried raw beef steaks and cooked meat slices and lamb, mutton and pork chops, but the products are generally expensive. In normal times there is little commercial demand for these products and much of the developmental work has been done in armed forces food research establishments in various parts of the world. However, capital equipment and operating costs are known to be high.

Freeze-dried meat shares with air-dried meat a requirement for special packaging. Both will absorb moisture and oxygen if exposed to the atmosphere, with consequent risk of mould and bacterial growth and the development of off-flavours. Minces may be compressed into blocks to reduce deterioration. The dried products must be packed either in tinplate containers or in impermeable foils and films from which the air has been evacuated or replaced by inert gases such as carbon dioxide or nitrogen.

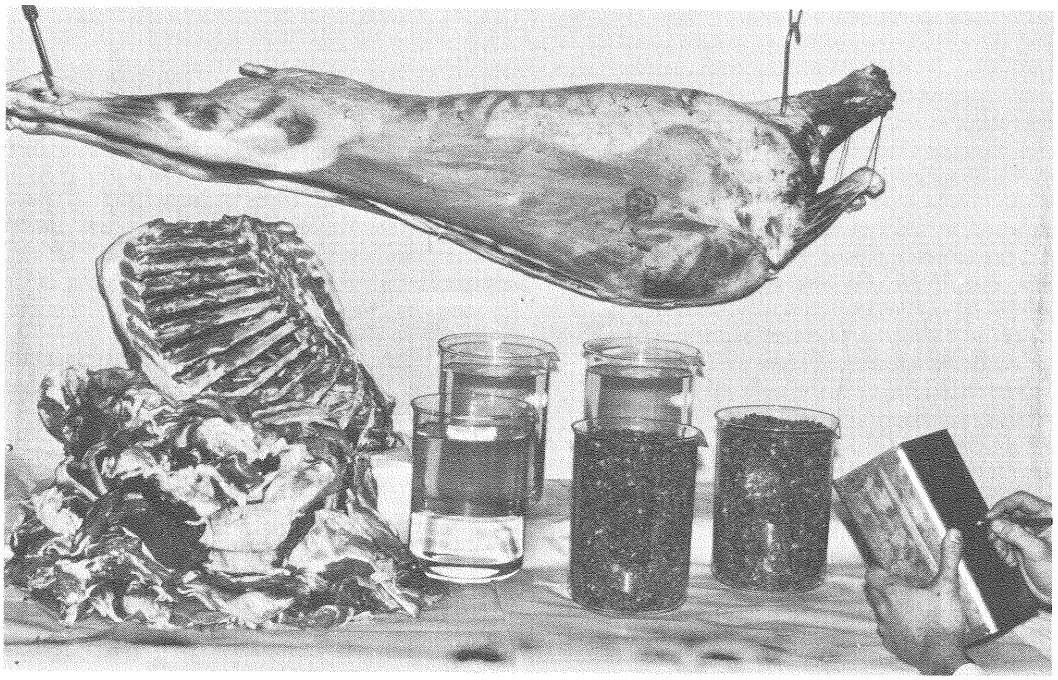

Fig. 2. Reduction of an 18-kg mutton carcass on dehydration, showing carcass, meat and bones, extracted water and dried meat, and the final pack in a 3-kg can.

Effect of drying on nutrients

Loss of water produced by drying results in increased concentration of nutrients in the remaining food mass. The proteins, fats and carbohydrates in dried foods are present in larger amounts per unit weight than in their fresh counterparts, and the nutrient value of most reconstituted or rehydrated foods is comparable to that of fresh items. Drying reduces the biological value of protein to a degree dependent on the method and temperature of drying. Prolonged exposure to high temperatures can render the protein less useful in the diet, whereas treatment at low temperatures may increase its digestibility.

The vitamin content of dried meat is usually less satisfactory than that of fresh meat. Most of its vitamin C is lost. Thiamine levels are reduced, with the greatest loss occurring at high drying temperatures; small losses of riboflavin and niacin also occur ('Encyclopaedia Britannica' 1973). Storage of freeze-dried beef fillet containing 5% water for 1 year at 20°C in air has been reported to cause a 40% loss of the essential amino acid, lysine (Dvořák and Vognarová 1965). Limited observations on cooked air-dried dehydrated pork packed in laminated lead foil (quoted by Sharp 1953) showed that there was an appreciable fall in the biological value of the protein after 56–112 days at temperatures of 21–43°C and that thiamine was lost rapidly at temperatures over 21°C.

Conclusion

The preparation of dried meat products is expensive, whether modern technology is used or the traditional methods, modified to suit the present, are revived. Traditional methods are extremely laborious, a proportion of the product is frequently spoilt, and the product is at best likely to be of limited appeal requiring the development of a special taste by the consumer. Moreover, the keeping properties of these products, unless stored under refrigeration, are likely to be poor. In any country where labour costs are high, the cost of commercial production would be prohibitive except to cater for a market demanding high-priced delicacies. Even the products obtained by sophisticated freeze-drying methods have a limited shelf life and while the eating qualities may be satisfactory the nutritional

value may deteriorate rapidly unless they are stored at cold or cool temperatures. In addition, the costs of production are high as a consequence of high capital equipment and operating costs.

A canned corned-beef type of product, which is acceptable to consumers in all parts of the world and which will meet the criteria imposed by all health authorities, would appear to offer better prospects for utilizing meat surpluses. Canned meats may be produced more economically than dried meats and the risk of deterioration when they are stored without refrigeration is less.

Nevertheless, to dismiss dried meat products as having no future would be to ignore the possibility of increasing the total consumption of meat in unconventional forms. As mentioned earlier, biltong is a widely consumed delicacy in Africa. In North America, jerkey is eaten in several forms—simply dry-cured, or else pickle-cured, hot pickle-cured or marinated. In Europe, a dried cured beef product is sold as 'Viande des Grisons', 'Binden Fleisch' or 'Bündner Fleisch'. This product, which has a low (6%) fat content, is used in weight-reducing diets. It is prepared from beef carefully trimmed to remove fat, aged for 1 week, rubbed in a special curing mixture and dry-cured in stacks for 3–4 weeks at 6–8°C (Frentz 1974).

Moreover, freeze-drying may have greater commercial application in the future, as technology improves. There is already a growing demand for imported freeze-dried mince, prepared in light-weight packs, among the increasing numbers of bush walkers and others who carry their own rations in pursuit of freedom away from civilization. It has been reported that an excellent raw material for frankfurter-type sausages can be prepared by freeze-drying fresh meat that has been salted and minced before the onset of rigor mortis, i.e. meat that has been removed from a carcass while it is still warm (Hamm and Potthast 1975). The CSIRO Meat Research Laboratory has made arrangements with West Germany for the evaluation, in the making of smallgoods, of frozen meat that has been 'hot-boned' before rigor sets in. A freeze-dried product that weighed less and that could be transported and stored with less attention to refrigeration might be considerably more attractive to processors if the cost of production was not excessive.

References

Anon. (1962). Meat hydration. CSIRO Aust. Div. Food Preserv. Circ. 6-P.

Anon. (1974). Making biltong. *Meat Ind., Pretoria* **23**(2), 27–30.

Borgstrom, G. (1968). 'Principles of Food Science.' Vol. 1, p. 33. (Macmillan: New York.)

Dvořák, Z., and Vognarová, I. (1965). Available lysine in meat and meat products. *J. Sci. Food Agric.* **16**, 305–12.

'Encyclopaedia Britannica' (1973). Macropaedia Vol. 7, p. 491.

Frentz, J. C. (1974). Dried beef. *Euroviande-Courier des Abattoirs* **15**, 19–20 (in French).

Hamm, R., and Potthast, K. (1975). Freeze-dried meat for Frankfurter-type sausages. *Fleisch-wirtschaft* **55**, 87–9 (in German).

Sharp, J. G. (1953). Dehydrated meat. DSIR Gr. Brit. Food Invest. Bd Spec. Rep. No. 57.

Debittering citrus products with enzymes*

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In recent years, considerable attention has been paid to the use of enzymes to solve problems of bitterness in citrus products

Citrus fruits contain two types of bitter principles that are chemically distinct—flavonoids and limonoids—and these compounds affect the quality of citrus fruits and their products in distinctly different ways (see table p. 85). Consequently, there are differences in the problems they cause and in the methods that have been developed for their elimination.

Characteristics of flavonoid bitterness

The bitter citrus flavonoids are best represented by naringin, the main bitter principle of grapefruit. They are distributed throughout the fruit including the intact juice sacs, but occur in highest concentration (up to 2%) in the albedo (Fig. 1). They are sufficiently soluble in aqueous systems to produce a bitter taste immediately on consumption of the fruit or its freshly extracted juice, and bitterness will rapidly increase unless the pulp and rag are removed from the juice (Fig. 2). However, the immediate removal of all pulp particles (cloud) from the juice will not always give a non-bitter serum because the bitter principles exist in the intact juice sacs, and their concentration may exceed the bitterness threshold.

Flavonoid bitterness is generally mild, since even saturated solutions of naringin are not sickeningly bitter, and many people regard a moderate level of flavonoid bitterness as refreshing. Thus, Seville marmalade, fresh grapefruit and grapefruit juice are accepted items at the breakfast table. Grapefruit juice containing less than 0·03% naringin is, in fact, regarded as being of poor quality.

Moreover, flavonoid bitterness can be readily removed from the palate by normal salivary processes. It has been suggested that this process sharpens up the taste buds, leading to heightened perception of taste sensations; as a result, foods consumed immediately afterwards are accepted with lower amounts of sweetening agents. This sequence provides a rationale for the 'grapefruit slimming diet'.

Flavonoids, ubiquitous constituents of the plant kingdom, are present in quantity in all citrus fruits. Indeed, citrus fruits can be

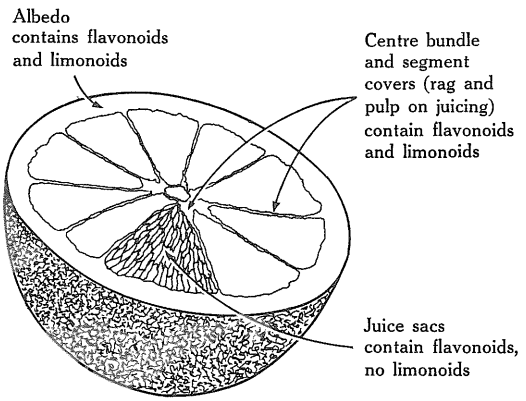


Fig. 1. Location of flavonoid and limonoid bitter principles in the various tissues of citrus fruits.

*This article is based on a talk given at the Ninth AIFST Convention in Adelaide, May 1975. It is a review of the subject covering literature mainly published since 1970; earlier studies have been reviewed by Kefford and Chandler (1970).

separated into two chemo-taxonomic groups according to which of two very similar disaccharides, neohesperidose (I) and rutinose (II), are dominant in their flavonoid components. The disaccharides differ only in that a rhamnose unit (I*i* or II*i*) is linked by its α-1-hydroxyl group to the 2-hydroxyl group of a glucose unit (I*ii*) in neohesperidose and to the 6-hydroxyl group of a glucose unit (II*ii*) in rutinose. The rutinoside group is represented by sweet oranges and lemons, and the neohesperidoside group by bitter (Seville) oranges and grapefruit, with hybrids showing some of the features of both parents (Nishiura *et al.* 1971).

For a short time, it seemed likely that the susceptibility of citrus fruits to flavonoid

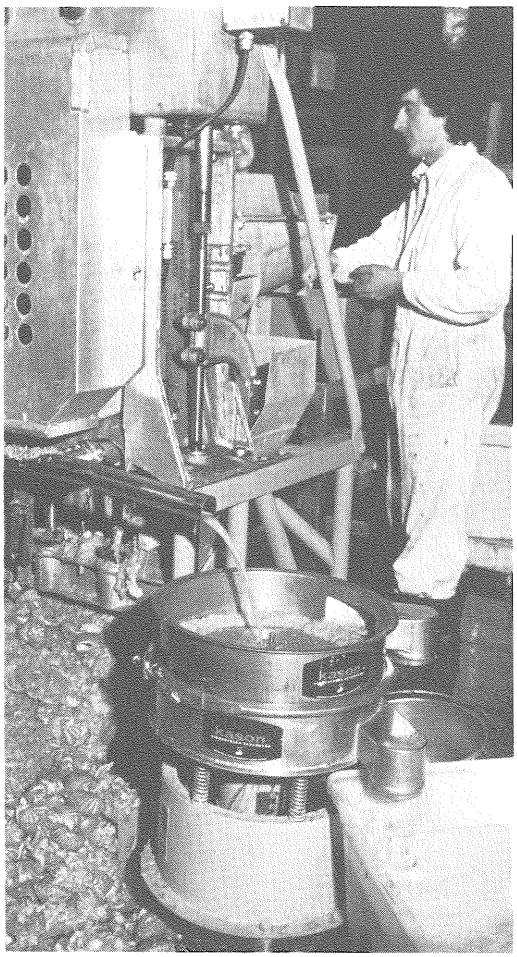
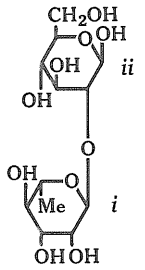
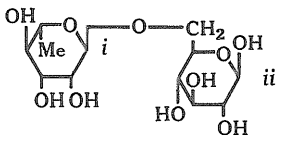


Fig. 2. Rapid removal of rag and pulp from freshly extracted juice may avoid flavonoid bitterness and will lessen limonoid bitterness.



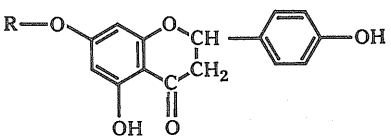
I Neohesperidose
i Rhamnose unit; Me = CH_3
ii Glucose unit



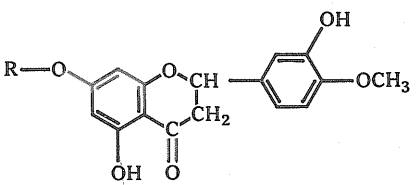
II Rutinose
i Rhamnose unit; Me = CH_3
ii Glucose unit

bitterness was determined simply by the glycosidic nature of their flavonoid components. Thus, although not all neohesperidosides are bitter, this disaccharide is a component of the two most bitter citrus flavonoids, naringin (III*a*) and poncirin. On the other hand, rutinose is the glycosidic component of a number of tasteless flavonoids including hesperidin (IV*a*) which occurs in very considerable quantities in a large number of citrus fruits.

However, the situation is not quite as clear-cut as this differentiation would suggest. On a molar basis, neohesperidin (IV*b*), the isomer of hesperidin corresponding to naringin, is only one-tenth as bitter as this



III*a* Naringin; R = Neohesperidose
III*b* Narirutin; R = Rutinose

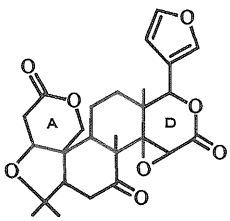


IV*a* Hesperidin; R = Rutinose
IV*b* Neohesperidin; R = Neohesperidose

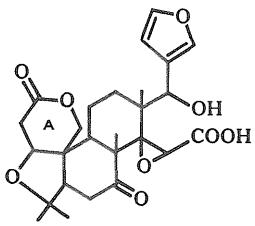
flavonoid and just as bitter as its glucosidic analogue. Moreover, recent work (Kamiya *et al.* 1975) contradicts earlier reports that narirutin (III*b*), the analogue of naringin containing rutinose instead of neohesperidose, is devoid of bitterness. Nevertheless, the relative bitterness of the various glycosidic types and their distribution among the citrus fruits results in bitterness being a technological problem only in those varieties capable of elaborating the specific disaccharide, neohesperidose.

Characteristics of limonoid bitterness

In contrast, limonoid bitterness involves, in effect, only the most common member of this unique group of compounds, limonin (V). Limonin, which is almost insoluble in water, occurs in the fruit in association with the derived hydroxyacid lactone (VI), which is non-bitter and comparatively water soluble. These compounds do not occur in the intact juice sacs in any significant quantity but are present in the albedo in total amount up to 0·06% by weight. Consequently, bitterness may be avoided if juice sacs only are squeezed or if the extracted juice is immediately separated as a clear serum (Fig. 3). An American patent taken out several years ago was based on this second observation.



V Limonin



VI Limonoic acid
A-ring lactone

During normal commercial extraction, albedo particles are necessarily incorporated into the juice, giving rise to bitterness, the level of which depends on the amount of albedo particles and their limonoid content. The bitterness is not detectable in the freshly extracted juice but appears after some hours at ambient temperatures, and more quickly if the juice is heated. This delay is caused by the time taken for limonin to pass into solution or for the hydroxyacid lactone (VI) to be converted to limonin; both processes are accelerated by heat.

Despite the fact that it is almost insoluble in water, limonin can give bitter solutions of sickening intensity, especially in the presence of sugar and pectin which increase its solubility. On a molar basis, it is more bitter than caffeine, naringin or the permitted beverage-bittering agent, sucrose octaacetate (Fig. 4). Limonin bitterness increases more steeply with increasing concentration than does naringin bitterness, and is particularly persistent on the palate, lingering for a considerable time despite frequent rinsings. Individuals vary in their ability to detect and respond to limonoid bitterness, and infants and children are particularly likely to react

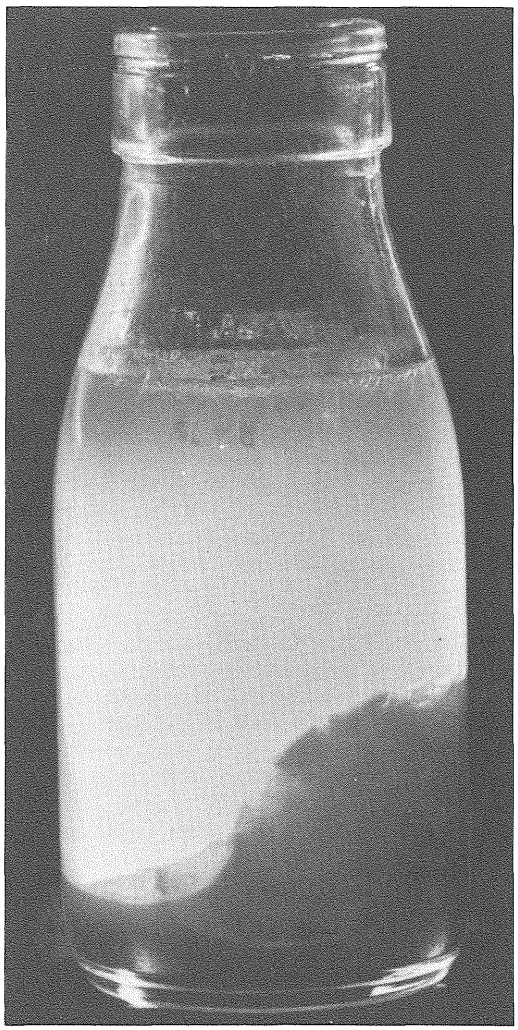


Fig. 3. Rapid centrifugation of freshly extracted juice to a clear serum may avoid flavonoid bitterness and will avoid limonoid bitterness.

against its presence in orange juice. Most adults detect limonin at concentrations of 9 ppm in orange juice and find concentrations of more than 12–15 ppm objectionably bitter.

The occurrence of limonin, like the occurrence of naringin, is under strict genetic control, but the control is different in effect. Limonoids are entirely restricted to plants of the related Rutaceae and Meliaceae families, and limonin occurs only in plants of the Rutaceae family. However, limonin apparently occurs in all fruits of this family, including citrus fruits, although in greatly varying concentrations. Limonoids are regarded as intermediates in one of the pathways in the metabolism of triterpenoid compounds in the developing and ripening fruit, and another similar pathway leads to the plant sterols; the triterpenoid cycloartenol (VII) and the sterol β-sitosterol (VIII) are citrus constituents. Of the citrus limonoids, only limonin and its precursor nomilin (IX) are bitter, and nomilin is considerably less

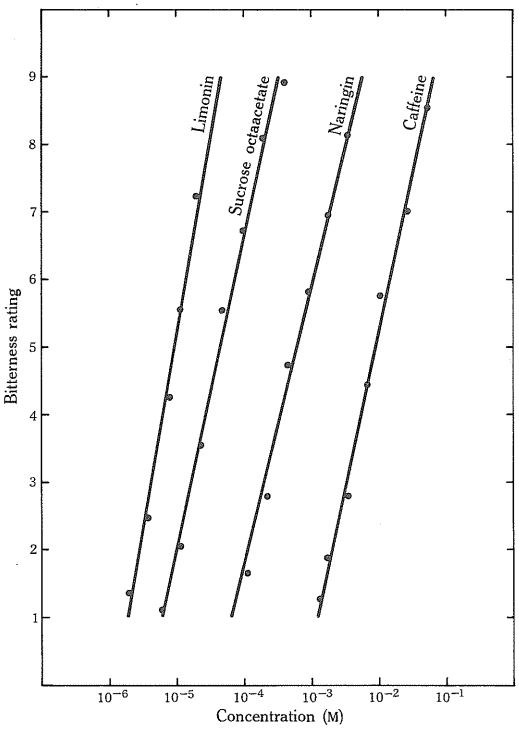
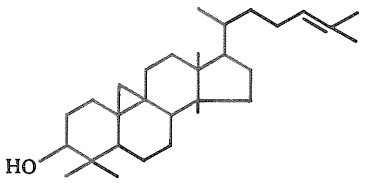
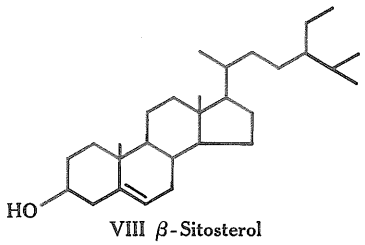


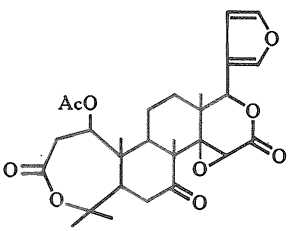
Fig. 4. Relationship between bitterness rating and concentration of aqueous solutions for several bitter principles. Bitterness scale: 0, no bitterness; 2, very slight; 4, slight; 6, moderate; 8, strong; 10, extreme.



VII Cycloartenol



VIII β-Sitosterol



IX Nomilin; Ac = $COCH_3$

bitter than limonin. Thus limonin bitterness in citrus products depends on the amount of triterpenoid undergoing the particular biodegradation in which limonin is an intermediate, and on the stage reached in this pathway at the time the fruit is processed.

Within the citrus family, therefore, fruits differ not so much in the type of limonoid they contain as in the amount of limonin present, whereas with their flavonoid components they differ primarily in the type of glycoside that predominates. Thus limonin bitterness is a possibility in products from all citrus varieties, although the problem is important only when there is a high concentration of limonin in the fruit. One of the varieties where high concentrations of limonin can accumulate is the grapefruit, and it is now recognized that the intensification in the bitterness of grapefruit products which occurs in processing is almost entirely due to limonin and not, as was earlier believed, to naringin.

Removal of flavonoid bitterness

It now seems clear that the contribution of limonin to the bitterness of grapefruit juice has been one of the factors militating against

the effectiveness of the enzymic methods that were developed for debittering citrus juices by removing their bitter flavonoid constituents. These methods, first reported in Japan in 1955, have since been the subject of many patents which claim to produce and utilize an enzyme capable of hydrolysing the neohesperidose moiety on which the bitterness of citrus flavonoids largely depends. The three most recent such patents are by Ito and Takiguchi (1970) and Fukumoto and Okada (1972, 1973). The enzyme, commercially known as 'naringinase', has been isolated from a wide range of moulds grown on media containing naringin or rhamnose, and in tests of 132 strains from 39 genera, *Aspergillus niger* and *Coniella diplodiella* have been reported to yield the most active preparations, hydrolysing naringin within a few hours at room temperature and at the natural pH of most citrus juices (3·0–3·8).

Other patent applications using naringinase have been for the preparation of sweetening agents such as hesperitin dihydrochalcone glucoside (Horowitz and Gentili 1968; Teranishi and Sato 1972); for a horticultural spray which is applied to the calyx and which, it is claimed, lowers the naringin and acid contents of Japanese oranges (Tanabe Seiyaku Co. Ltd 1971); and for the production of non-bitter sweet lemon wines (Sikkoman Syoyu Co. Ltd 1972) and citrus purées (Cruse and Lime 1973).

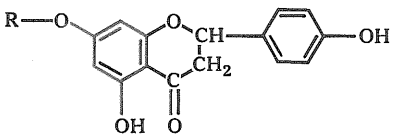
The properties of naringinase

The first commercial preparations of naringinase were so heavily contaminated with pectinase that pectin hydrolysis, which disrupted the complex hydrocolloid system that holds both limonin and naringin in solution, was probably more important in debittering than naringin hydrolysis. Since pectinase activity resulted in undesirable loss of cloud, attempts were made to purify the crude enzyme. It was found to contain a large number of enzymes, two of which were involved in the stepwise hydrolysis of the flavonoid bitter principles: a rhamnosidase which split off rhamnose from the disaccharide, and a glucosidase which split off glucose from the resultant glucoside. In the case of naringin, the two products were prunin (X*a*) and naringenin (X*b*), and only the first stage was necessary for effective debittering since the neohesperidose structure of naringin had been destroyed with the formation of the glucoside, prunin, which is less than one-third as bitter as naringin on a molar basis.

Eventually the two enzymes were isolated in crystalline form and, as a result, some of the ambiguities associated with naringinase activity were resolved. Conflicting reports on inhibition by natural fruit sugars were shown to be the result of rhamnose inhibition of rhamnosidase activity and glucose inhibition of glucosidase activity. More recently, workers in South Korea (Ki *et al.* 1973) used molecular sieving, gel electrophoresis and ion exchange chromatography in a three-step procedure for isolating a highly purified homogeneous enzyme which hydrolysed naringin to prunin and rhamnose only.

Separation of the two enzymes is, of course, not essential for commercial application, but for most debittering treatments it is necessary to remove pectinase to avoid loss of cloud. Japanese workers achieved this with some success by two commercially practicable methods: one made use of the different solubilities of the glycosidases and the pectinases in alcohol, and the other depended on the differential inactivation of pectinase at pH 8·0 and 37°C.

Differential heat inactivation was also the basis for an ingenious process for debittering citrus juices and segments by adding the crude enzyme to the contents of the can before processing: after heating for 10 min at 80°C, pectinase was inactivated but naringinase activity was retained, giving non-bitter juice within 2 hours and non-bitter segments within 2 weeks. The process also prevented the unsightly crystallization of naringin, and hesperidin crystallization in canned mandarins could be similarly avoided by the use of hesperidinase which splits off a rhamnose from the other flavonoid rhamnoglucosides, the rutinosides. However, the application of this 'in-can debittering' process to grapefruit juice and segments would lead to uncharacteristic products devoid of bitterness, unless bitter principles other than flavonoid neohesperidosides were



X*a* **Prunin; R = Glucose**
X*b* **Naringenin; R = Hydrogen**

present in sufficient quantity or unless a second, more severe heat treatment was given to inactivate the naringinase at an appropriate time.

Immobilized naringinases

The problem of pectinase contamination has led to attempts to compensate for the added costs of naringinase purification by immobilizing the enzyme onto solid supports, so allowing it to be re-used. Goldstein *et al.* (1971) in Israel were the first to report the preparation of water-insoluble derivatives of naringinase. They immobilized a commercial preparation on resins made by co-polymerization of maleic anhydride and various substituted ethylenes with hexamethylenediamine as the coupling agent.

The most active and the most stable preparations of immobilized naringinase were obtained with methyl vinyl ether or ethylene as the co-polymer, but even so the stability was poor and all activity was lost after storage for 1 month at 4°C or for 1 week at ambient temperature. The immobilized enzymes showed maximum activity at pH 5–6 and only about 80% of this activity at the natural pH of citrus juices. Furthermore, about 90% of the activity was lost in citrus juices at their natural pH when held for the 1 h required for debittering, although naringinase itself is not inactivated under these conditions. Moreover, about 55–85% of initial naringinase activity was lost in the immobilization process, and the size of the immobilized enzyme particles necessitated the clarification of the juice before it could be treated.

With all these difficulties, debittering processes that rely on immobilized naringinases seem to be neither practical nor economic, but Goldstein *et al.* optimistically foresee a use for them in batch or column processes for debittering clarified juices.

The same workers reported no success in immobilizing naringinase on the diazotized resin from condensation of dialdehyde starch and methylene dianiline (S-MDA resin). However, the first of two patents (Krasnobaev 1973) granted to Givaudan on the immobilization of naringinase claimed debittering of grapefruit juice after treatment with the enzyme immobilized on the same S-MDA resin, but no chemical analyses are available to support the organoleptic assessments. The second patent (Krasnobaev 1974), in which naringinase is immobilized on silica gel or porous glass pretreated with 4,4′-diaminodiphenylmethane and then diazotized is not yet available for detailed study. From what we know about the debittering of citrus juices by adsorption (Chandler and Johnson 1974), the S-MDA carrier resin itself probably has the ability to remove both naringin and limonin from grapefruit juice and so debitter it. Thus the mechanism for debittering in the Givaudan process remains in doubt, especially since Goldstein *et al.* failed to demonstrate enzyme activity in naringinase immobilized on S-MDA resin and tested for naringin hydrolysis—not merely for removal of bitterness.

Similar comments apply to an Italian patent (Dinelli and Morisi 1972) which describes the preparation of an immobilized naringinase by incorporating the enzyme into cellulose acetate threads obtained by emulsifying cellulose acetate dope with the enzyme solution before spinning. Again, the carrier itself may play some part in the debittering action of this preparation, although in this patent organoleptic assessments are at least partially backed by chemical analyses.

Present status

These observations indicate the difficulties of assessing the efficiency of the naringinase debittering processes so far developed. The many uncertainties in the results obtained account in part for the fact that, after a burst of activity between 1958 and 1965, the citrus industry in the U.S.A., which is the world's biggest producer of grapefruit juice, has shown little interest in naringinase, either in industry or research. Instead, American processors minimize problems of bitterness in grapefruit products by carefully selecting the fruit, by controlling the pressures used in juice extraction, and by blending juices from a variety of sources.

Any further attempts to develop debittering procedures based on naringinase should be carried out with adequate analytical control and with recognition of the following five important facts.

- The commonly used analytical method for assessing naringinase activity (e.g. Inoue and Okada 1969) does not distinguish between naringin and its

considerably less bitter hydrolysis product, prunin.

- The present uncertainty concerning the contribution of hesperidosides such as narirutin to citrus bitterness further complicates the assessment of the efficiency of naringinase for debittering citrus products.
- Pectinase contaminants in an enzyme preparation, even in small amounts, will themselves effectively reduce limonin and naringin bitterness in citrus juices.
- Carriers used for the immobilization of naringinase may themselves be active adsorbents of limonin and naringin.
- Even if naringin and other bitter flavonoids are removed by enzymic hydrolysis, there is still the possibility that the product will be objectionably bitter because of its limonin content.

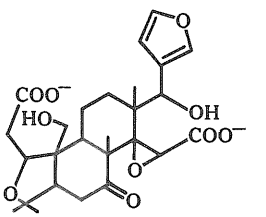
Bitterness in citrus fruits and their products

Flavonoid	Limonoid
Fruits vary in flavonoid type	Fruits vary in limonoid content
Bitter principles in tissue and juice sacs	Bitter principles in tissue, not in juice sacs
Bitter principles comparatively water-soluble	Bitter principles almost insoluble in water
Bitterness immediately apparent	Bitterness delayed
Bitterness in juice not avoided by instantaneous and complete pulp removal	Bitterness in juice avoided by instantaneous and complete pulp removal
Bitterness generally mild	Bitterness generally objectionable
Bitterness readily removed from palate	Bitterness persistent on palate

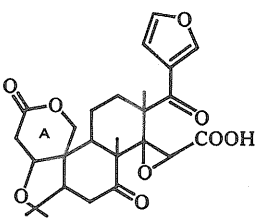
Removal of limonoid bitterness

As mentioned above, limonin (V) and, to a lesser extent, its precursor nomilin (IX) are entirely responsible for the bitterness in a number of citrus products, notably navel orange juice. As early as 1950, Americans were investigating enzymic processes for eliminating limonoid bitterness. Pectin-destroying enzymes from fruits or fungi were found to debitter orange juice after several hours' treatment at 4–10°C, but the process was associated with the development of off-flavour and loss of cloud. As in the treatment of grapefruit juice with crude naringinase, the debittering apparently involved the breakdown of colloidal systems stabilized by pectin, without chemical alteration of the limonin molecule.

It seems likely that pectinase activity was also responsible for the slightly favourable results obtained in Japan about 10 years ago in studies which used preparations from *Aspergillus* and *Penicillium* spp. to debitter Natsudaidai juice. More recently, Misawa *et al.* (1972) took out a Japanese patent on 'limonin decomposing enzymes' from *Aspergillus niger*, but from the details available these would appear to be crude preparations subject to heavy contamination with fungal pectinases.



XI Limonoate ion



XII 17-Dehydrolimonoic acid A-ring lactone

Limonin-degrading enzymes from bacteria

Recently, more attention has been directed to the study of enzymes of bacterial origin able to degrade limonin in the form of the derived hydroxyacid lactone (VI). These enzymes were isolated from soil bacteria adapted to grow on the limonoate ion (XI) from the dihydroxyacid form of limonin as the sole carbon source. The first enzyme (Hasegawa *et al.* 1972*a*,*b*, 1973), was isolated from *Arthrobacter globiformis* and characterized as a limonoate oxidoreductase, which requires nicotinamide adenine dinucleotide (NAD) as a cofactor. Although it has a pH optimum of 9·5, well above the pH of orange juice, a U.S. patent application has been

made for its use to debitter orange juice (Hasegawa and Brewster 1972). The second enzyme, a limonoate oxidoreductase, requiring either NAD or its phosphate as cofactor, was isolated from *Pseudomonas* sp. 321-18 (Hasegawa *et al.* 1974*b*); it operates over a wide pH range with optimum activity at pH 8–8·5, but retains some activity at pH 3·5–4·5. These enzymes, besides being different proteins and having different cofactor requirements, differ markedly in their stability and activity under various pH and temperature conditions.

Addition of these enzymes to fresh navel juice and reconstituted navel orange concentrate, both adjusted with alkali to the appropriate pH values, resulted in the formation of 17-dehydrolimonoic A-ring lactone (XII) from the limonoic A-ring lactone (VI) present in the juice. The 17-dehydrolimonoic A-ring lactone is non-bitter and is not converted to a bitter compound on re-acidification of the juice, so that enzyme-treated juices with their lower limonin contents were less bitter than untreated juices. With the *Arthrobacter* enzyme, substantial conversion of the limonoic A-ring lactone to 17-dehydrolimonoic A-ring lactone occurred with only that amount of cofactor naturally present in the juice, but addition of NAD (0·06 μmole/ml) allowed more efficient use of the enzyme and gave the same limonin conversion (50–60%) at pH 4·3 as was obtained at pH 5·5 without NAD. No detailed results have yet been published for the *Pseudomonas* enzyme except that at pH 4·0 it retains about 10% of its maximum activity (shown at pH 8·0–8·5), thus requiring higher concentrations or longer holding times for effective use. The claim is made that by attacking the hydroxyacid lactone (VI) present in the freshly extracted juice the limonin content in the final product would be reduced to well below the bitterness threshold.

Several problems arise when commercial usage of these microbial enzymes is considered. Unless their low activity at the natural pH of citrus juices (3·0–3·8) can be satisfactorily overcome by increasing either the time of treatment, the enzyme concentration or the amount of available cofactor, the juice would have to be brought to a pH at which the enzyme has sufficient activity to effect the amount of degradation necessary for debittering. The pH would have to be lowered again before consumption Such pH adjustments would alter the ionic composition and flavour balance of the juice; they would not only be unacceptable from a practical viewpoint but would probably be considered contrary to good manufacturing practice.

Moreover, the high cost of enzyme production and isolation must be considered especially since a medium containing limonin as the sole carbon source is required, and pure limonin is not readily available. For the process to be economic, the enzyme would probably need to be immobilized to allow its re-use, but immobilization would also be costly and the immobilized enzyme, like immobilized naringinase, may have a higher pH optimum and a lower stability. In view of these considerations, the commercial production and utilization of microbial enzymes to debitter orange juice is not yet in sight.

Limonin-degrading enzymes in citrus albedo

Earlier evidence for the presence of limonin-degrading enzymes in the orange albedo (Chandler 1971) has been confirmed by the recognition of such an enzyme in albedo tissue cultures (Hasegawa *et al.* 1974*a*). In the earlier work, albedo enzymes were used without isolation to lower the limonin content of bitter navel juice from 16·8 to 7·0 ppm. After addition of alkali to bring the pH of the juice (3·2) into the range at which the enzyme is most active (5·6), albedo homogenates that contained the limonin-degrading enzyme and were naturally at pH 5·6 were added and the blend was held overnight (16 h) at 0°C; readjustment of the pH gave a non-bitter product of acceptable flavour.

The problem associated with adjustment of pH arises once again in treatments with albedo diffusate. However, at pH 4·0 the albedo enzyme retains about 50% of its maximum activity and the possibility of its activation by cofactors has yet to be investigated. Moreover, the use of a fruit-derived enzyme poses fewer problems in terms of food regulations than does a bacterially derived one, and the presence of pectin in the homogenate ensures that the treated juice retains its cloud-holding ability during the treatment. On the other hand, albedo of high limonin-degrading activity is encountered only over a narrow period

during maturation of the fruit, and the isolation of extracts of high activity presents considerable difficulty.

In vivo *enzymic processes*

The limonin-degrading enzymes in citrus albedo are responsible for the decrease in limonin contents in the maturing orange. About 25 years ago, there were studies in the U.S.A. and Israel on ethylene treatments to promote fruit maturation and so reduce bitterness in the processed juice but the results were erratic, probably because of lack of adequate controls. This approach has recently been taken up again in California (Maier *et al.* 1971, 1973). After a short ethylene treatment of 3 h at 20 ppm concentration and subsequent storage for 5 days under ambient conditions, navel oranges gave juice with a limonin content of 13·2 ppm. This value may be compared with that of juice from fruit stored without ethylene treatment (19·4 ppm) and that of juice from the fresh fruit (24·3 ppm). As yet the mechanism for regulation or control of the limonoid metabolism in the fruit remains to be determined: ethylene may regulate the synthesis of enzymes involved in limonoid metabolism, or ethylene may influence the activities of these enzymes, or both.

The major disadvantage with ethylene treatment is the development of off-flavours in oranges exposed to conditions even slightly in excess of those recommended. Also, because the treatment reduces the limonin content by no more than 35–45% of the amount present in juice from untreated fruit, juices from early season fruit will still contain limonin in concentrations above the bitterness threshold. Since the treatment cannot be repeated, the ethylene debittering process will only be effective with fruit known to contain moderate levels of limonin, and thus the use of the limonin-degrading enzyme *in vivo* provides only a partial and as yet imperfect solution to the bitterness problem. Nevertheless, greater knowledge of the factors controlling the activity of limonin-degrading enzyme systems in the orange during both growth and storage has fundamental and practical implications, and work on this subject will be continued in the Food Research Laboratory.

Present status

While progress is being made in current studies on enzymic methods for reducing limonoid bitterness in citrus products, a number of problems inhibit their immediate commercial application.

- The enzymes of microbial origin would be expensive and have very limited activity at the natural pH of citrus juices.
- The enzymes present in citrus albedo, although retaining more of their activity under conditions of commercial applicability, require the availability of albedo of high activity since their isolation and concentration are difficult.
- Ethylene treatments to promote the activity of the albedo enzyme *in vivo* are subject to the general problems of ethylene treatments and can only be applied with a certainty of success to fruit of moderate limonin content.

References

Chandler, B. V. (1971). A limonin-degrading enzyme in citrus albedo. *J. Sci. Food Agric.* **22,** 634–7.

Chandler, B. V., and Johnson, R. L. (1974). Debittering of orange juice. Aust. Prov. Pat. Applic. PB6670.

Cruse, R. R., and Lime, B. J. (1973). Citrus purée. U.S. Pat. 3,749,585; *Chem. Abstr.* **79,** 90675g.

Dinelli, D., and Morisi, F. (1972). Enzymic removal of bitter taste from fruit juice. Ital. Pat. Applic. 20645; *Chem. Abstr.* **78,** 109526n.

Fukumoto, J., and Okada, S. (1972). Fermentative production of naringinase. Jap. Pat. 72,05,714; *Chem. Abstr.* **76,** 139078u.

Fukumoto, J., and Okada, S. (1973). Naringinase production by fermentation. Jap. Pat. 73,06,554; *Chem. Abstr.* **80,** 94271r.

Goldstein, L., Lifshitz, A., and Sokolovsky, M. (1971). Water-insoluble derivatives of naringinase. *Int. J. Biochem.* **2,** 448–56.

Hasegawa, S., and Brewster, L. C. (1972). Limonoate dehydrogenase and debittering of citrus juice therewith. U.S. Pat. Applic. 250,764.

Hasegawa, S., Bennett, R. D., and Maier, V. P. (1972*a*). Metabolism of limonin and limonoate by micro-organisms: the conversion to nonbitter compounds. *J. Agric. Food Chem.* **20,** 435–7.

Hasegawa, S., Bennett, R. D., Maier, V. P., and King, A. D. (1972*b*). Limonoate dehydrogenase from *Arthrobacter globiformis*. *J. Agric. Food Chem.* **20,** 1031–4.

Hasegawa, S., Brewster, L. C., and Maier, V. P. (1973). Use of limonoate dehydrogenase of

Arthrobacter globiformis for the prevention or removal of limonin bitterness in citrus products. *J. Food Sci.* **38,** 1153–5.

Hasegawa, S., Maier, V. P., and Bennett, R. D. (1974*a*). Detection of limonoate dehydrogenase activity in albedo tissues of *Citrus sinensis*. *Phytochemistry* **13,** 103–5.

Hasegawa, S., Maier, V. P., and King, A. D. (1974*b*). Isolation of new limonoate dehydrogenase from *Pseudomonas*. *J. Agric. Food Chem.* **22,** 523–6.

Horowitz, R. M., and Gentili, B. (1968). Enzyme preparation of hesperitin dehydrochalcone glucoside. U.S. Pat. 3,583,894; *Chem. Abstr.* **75,** 47652c.

Inoue, M., and Okada, S. (1969). Assays for naringinase, hesperidinase, anthocyanase, and pectinase. *Kagaku To Kogyo* (*Osaka*) **43,** 642–9; *Chem. Abstr.* **73,** 373f.

Ito, T., and Takiguchi, Y. (1970). Naringinase. Jap. Pat. 70,14,875; *Chem. Abstr.* **73,** 65046w.

Kamiya, S., Konishi, F., and Esaki, S. (1975). The synthesis of narirutin. *Agric. Biol. Chem.* **39,** 733–4.

Kefford, J. F., and Chandler, B. V. (1970). 'The Chemical Constituents of Citrus Fruits.' Adv. Food Res. Suppl. 2. (Academic Press: N.Y.)

Ki, W. K., Kim, J. K., and Kim, M. C. (1973). Naringinase of mold. 2. Purification of Aspergillus naringinase. *Han'guk Sikp'um Kwahakhoe Chi* **5,** 78–83; *Chem. Abstr.* **79,** 113966u.

Krasnobaev, V. (1973). Insoluble naringinase preparation to deprive grapefruit juice of bitterness. Germ. Pat. 2,247,022; *Chem. Abstr.* **79,** 17231e.

Krasnobaev, V. (1974). Immobilized water-insoluble enzymes. Germ. Pat. 2,333,834; *Chem. Abstr.* **80,** 117717g.

Maier, V. P., Brewster, L. C., and Hsu, A. C. (1971). Development of methods for producing non-bitter Navel orange juice. *Citrograph* **56,** 351, 373–5.

Maier, V. P., Brewster, L. C., and Hsu, A. C. (1973). Ethylene-accelerated limonoid metabolism in citrus fruits: A process for reducing juice bitterness. *J. Agric. Food Chem.* **21,** 490–5.

Misawa, Y., Matsuhara, M., and Doi, N. (1972). Limonoid decomposing enzymes. Jap. Pat. 72,46,916; *Chem. Abstr.* **79,** 124725r.

Nishiura, M., Kamiya, S., and Esaki, S. (1971). Flavonoids in citrus and related genera. III. Flavonoid pattern and citrus taxonomy. *Agric. Biol. Chem.* **35,** 1691–706.

Sikkoman Syoyu Co. Ltd (1972). (Sweet lemon wine.) Jap. Pat. 72,26,713.

Tanabe Seiyaku Co. Ltd (1971). (Orange quality improvement.) Jap. Pat. 71,10,023.

Teranishi, H., and Sato, T. (1972). Production of hesperitin dihydrochalcone glucoside. Jap. Pat. 72,46,347; *Chem. Abstr.* **80,** 48339w.

On the measurement of smell

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Our evaluation of the flavour of foods is derived mainly from the qualities of smell, taste and texture. It is the smell or odour, however, that is commonly considered to make the most important contribution to flavour

An odour can be described in terms of its quality, intensity, stimulating efficiency (i.e. the lowest concentration at which it creates an olfactory sensation) and olfactory fatiguing properties. As yet it is not possible to explain why an odour exhibits any of these characteristics. This is primarily because it is not known

- what physical features a molecule must possess to stimulate the olfactory receptor cells,
- what the peripheral reception process is, or
- how the olfactory system processes incoming information.

With such an alarming paucity of data, it is little wonder that workers do not agree about the most suitable methods for measuring the characteristics of an odour. Significant improvement in the methods of measurement will only be achieved when we have greater knowledge of the reception and

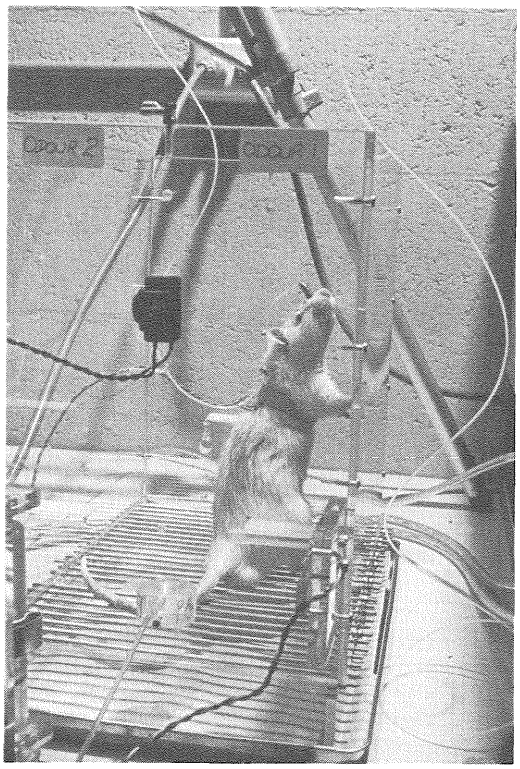


Fig. 1. A rat with a cannula implanted in the nasal cavity, sniffing for an odour cue; it has been trained to perform a specific task on receipt of the cue.

perception processes. Olfactory research with humans, unfortunately, is usually restricted to psychophysical studies. A better understanding of the mechanisms underlying human olfactory processes can only be achieved indirectly, by comparative studies with other vertebrates from which it is possible to obtain both psychophysical and physiological data. There is, however, a lack of quantitative psychophysical methods for studying olfaction with vertebrates and this has restricted the correlation of psychophysical and physiological data from the rat, rabbit, pigeon and frog, for which considerable anatomical and physiological data are available. For several years now, workers in this laboratory have been attempting to develop quantitative psychophysical methods for studying olfaction in vertebrates, particularly in the rat.

Rats were chosen because anatomical and physiological data existed and because the limited psychophysical data available suggested that rats and humans had a similar sensitivity to odours (Moulton 1960; Moulton and Eayrs 1960). In addition, rats were readily obtainable and can be trained to perform a variety of tasks that could prove useful in developing non-descriptive sensory analytical methods for use with humans. However, the absence of a suitable technique for delivering odours to unrestrained animals restricted psychophysical studies on olfaction. Hence a technique was developed by Laing *et al.* (1972, 1974) for delivering quantitative volumes of odorants to the nasal cavity of the unrestrained rat (Fig. 1). When used in conjunction with an air-dilution olfactometer of the type described by Laing *et al.* (1974), it provided precise control of the amount and duration of the odorant. This technique has been used successfully in studies of discrimination of odour quality (Laing *et al.* 1974), olfactory sensitivity (Laing 1975) and olfactory adaptation in the rat (Laing and Mackay-Sim 1975).

The results of these studies will be referred to in this article, where the aim is to discuss some of the problems in measuring the characteristics of odours, and to suggest ways of improving the current methods of measurement.

Odour thresholds

Factors influencing measurement of olfactory sensitivity

There is wide variation in the threshold values for olfactory sensitivity reported from different laboratories for both humans and rats (Moulton 1960; Davis 1973; Stahl 1973). This is not surprising in view of the complexity of factors that can influence threshold measurements. These include age, sex and motivation of the subjects, training, test method and response criterion, as well as purity of the odorants. However, the effect of these factors on the variability of the results can be significantly reduced.

It has become apparent, from the results obtained in this laboratory and others, that there are at least three factors that have been neglected in most studies on olfactory sensitivity. These are

- the influence of olfactory adaptation,
- the determination of the concentration of odorant vapour available to the nose, and
- the sampling or sniffing procedure.

Olfactory adaptation

Differences between the adaptation properties of odorants are often overlooked. As a result, long exposure or sampling times may be used and inadequate intervals may be allowed between the presentation of samples. This has occurred where workers have attempted to accumulate the highest number of responses from test subjects in the shortest possible time. In other words, test conditions have been chosen without regard to the odorant or to the capacity of the olfactory system to adapt.

The following examples have been chosen to illustrate the importance of selecting the correct test procedure for each odorant. Griffiths (1974) observed that olfactory adaptation persisted in humans for 6–12 min following a single sniff of moderate concentrations of 2,3,4,6-tetrachloroanisole, a compound which contributes to a musty taint in chickens. This fatigue or adaptation was experienced not only during successive presentations of this compound, but also when it was followed by other chloro-anisoles; an evaluation procedure had to be used which minimized the effects of adaptation. Interestingly, detection of the chloro-anisoles was unaffected by prior exposure to vanillin. In addition, Köster (1971) has established that recovery times from olfactory adaptation vary for many different odorants, and he commonly reported recovery times of 90–120 s following 1–2 sniffs of odorants presented at low concentrations. Köster also reported that even after recovery began the rate might slow or be reversed, particularly in the region of 75–100 s. Examples of these reversals are shown in Fig. 2. The reason for the phenomenon is not known.

Other examples come from psychophysical and physiological studies with animals. Thus Laing and Mackay-Sim (1975) showed in psychophysical studies with rats that the detection of propanol can be facilitated or hindered by preceding samples of propanol or heptanol. The effects were obtained with short presentation times (3 s) and intervals (3 s) between presentations and were dependent on the concentration of the odorants. Low concentrations of adapting odours facilitated detection of low concentrations of test odours, whilst high concentrations of adapting odours usually resulted in masking the test stimulus.

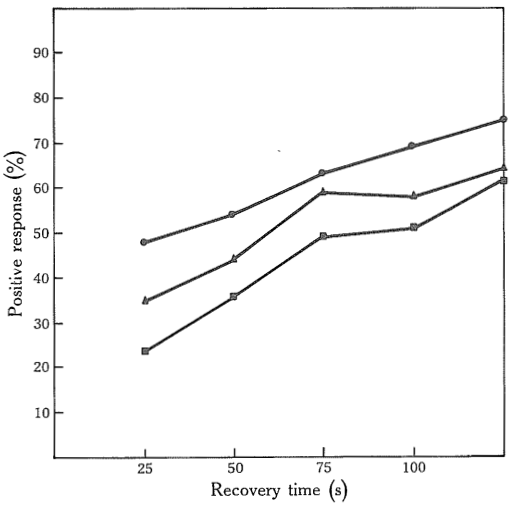


Fig. 2. Recovery of sensitivity in humans after olfactory adaptation to the three xylene isomers (from Köster 1971). ● p-xylene; ▲ o-xylene; ■ m-xylene.

Similar effects were obtained with humans by Engen and Bosack (1969) and Corbit and Engen (1971) with the same odorants or with related alcohols. In physiological studies with frogs, Ottoson (1956) showed that the electrical activity produced in the olfactory receptor region in response to odours, decreased significantly during successive presentations of an odour, indicating decreased sensitivity of the receptors.

These examples indicate that specification of presentation times and intersample intervals is an important and integral part of threshold measures. It is therefore suggested that for most odorants subjects should not be permitted more than one or two sniffs per trial, i.e. approximately 5–6 s to sniff the odorant. In addition, intersample intervals should be determined for each odorant with a small panel before commencing any major study. These suggestions apply equally to testing with humans or rats.

Measurement of concentration

Most thresholds have been determined either by means of a dynamic system for delivering the odorants to a subject, i.e. an air-dilution olfactometer, or by means of a static system such as a flask; many have been determined by both methods. It is often assumed that thresholds obtained with these two methods are similar but often this is not the case.

Among the most commonly used substances for diluting odorants are air, water, ethanol and paraffin oil. All these vary widely in their physicochemical properties. Hence, with the exception of measurements made with an olfactometer, the quantity of odorant vapour available to the nose when these other systems are used is seldom known. In a limited number of cases thresholds have been expressed in terms of the concentration of odorant in the diluent. This can be highly misleading as was shown by Lea and Swoboda (1958), who demonstrated that the threshold of decanal in water and paraffin oil was 0·007 and 7 ppm respectively. It is therefore obvious that attention must be paid to the choice of diluent when determining olfactory sensitivity by this method.

Different problems of measurement are encountered with air-dilution olfactometers. Until recently almost all concentrations were calculated on the basis of dilutions of saturated odorant vapour by known air-flows. However, this method has deficiencies; it does not account for back pressures or venturi effects which may occur when large and small flows meet, for inadequate mixing of odorant and diluting air streams or for adsorption of the odorants on the walls of the delivery tubing.

However, the concentration may be determined accurately with the aid of a flame ionization detector (FID) of the type used in gas chromatographs. This has been achieved for many odorants by connecting the outlet of the olfactometer (the sniffing point) to a FID. With a knowledge of the saturation vapour pressure of the odorant, the concentration can then be calculated from the data obtained by comparing the response of the detector to a known volume of the diluted vapour with that obtained from a known volume of saturated vapour of the particular odorant. Modifications of this general method have been described by Moulton *et al.* (1970) and Kauer (1974).

When an olfactometer is not available, it is possible to estimate accurately the concentration of odorant vapour in a flask by means of a modified version of the technique of headspace analysis, in which nitrogen or air is passed slowly over the surface of the diluted or threshold solution to a FID. A similar procedure is then followed with a saturated solution of the odorant. From the responses of the FID the concentration may be calculated in the same manner as above.

The FID can also be used to determine the composition of vapours emitted from a solution containing a mixture of odorants. This usually differs markedly from the composition of odorants in solution. In this instance, a known volume of odorous vapour is collected in a gas-sampling valve or porous polymer adsorbent before it is injected into a gas chromatograph for analysis.

Sampling or sniffing procedure

As mentioned earlier, quantitative delivery of odorants to unrestrained rats was achieved by directing the odours into the nasal cavity, which is adjacent to the receptor region. However, with most procedures and instruments designed to test the sensitivity of humans to odours, it has not been possible to prevent subjects inspiring laboratory air when the odour has been sniffed, and so an unknown dilution of the odorant has occurred during sniffing. This has been overlooked in most studies. In addition, the magnitude of the dilution varies from subject to subject (Laing, unpublished data) and differs for most olfactometers in use. As yet there has been no attempt to standardize the sniffing procedure or the instruments used. Flow rates from olfactometers, for example, vary from 0·04 to 100 l/min through outlets or sniffing ports with a cross-sectional area of 1–200 cm^2. Where flasks have been used, they have varied in size from 50 to 500 ml, their neck diameters have differed markedly and they have contained varying quantities of solution.

Because of this unsatisfactory situation, an investigation aimed at determining sniff volumes and rates under different conditions has begun in this laboratory and a device, based on the principle of the hot-wire anemometer, has been constructed for measuring these parameters. It is hoped that the study will also establish a sniffing procedure in which air other than that used to deliver the odorant is eliminated. Then it would be possible to make recommendations about the design of olfactometers from a knowledge of human physiological requirements.

Odour quality

Studies of odour quality have traditionally been in two distinct areas: one has attempted

to determine which physicochemical properties of a molecule are important for specifying quality, whilst the other has attempted to measure quality.

Physicochemical properties

It is not yet known which physicochemical properties specify quality. This is in contrast to the visual and auditory senses where colour or tonal quality are related to the frequency of light or of vibrating air.

In an effort to establish a relationship for olfaction, many attempts have been made to correlate descriptions of odours with molecular features such as shape, size, functionality (Amoore 1970) and spectral characteristics (Demerdache and Wright 1967) but no satisfactory correlations have been obtained. In contrast, Laffort (1969) reported good correlations between human olfactory thresholds and several factors concerned with intermolecular interaction forces. Disappointingly low correlations, however, were obtained between these factors and odour quality.

Other studies have aimed at correlating descriptions of odours or physicochemical properties with the electrical responses of olfactory cells that were either in the peripheral receptor area or in the forebrain (olfactory bulb). In many of these studies attempts have been made to use the results to classify odours into distinct quality categories, but as yet no generally acceptable classification has been compiled.

In view of the problems involved in determining the physicochemical basis of odour quality, it is little wonder that the various methods that have been used to measure quality have been empirical rather than quantitative.

Measurement of odour quality

Two types of psychological scaling methods, profile rating scales and proximity analysis, are commonly used to assess quality.

The procedure for obtaining a profile is based on the premise that odour quality can be represented by a number of descriptive terms, with each odorant assigned an intensity value for every descriptor. This method is illustrated in Fig. 3. Proximity analysis, however, requires only that the observer rate or rank the degree of difference between pairs of odorants or odorant descriptors. From these data estimates of

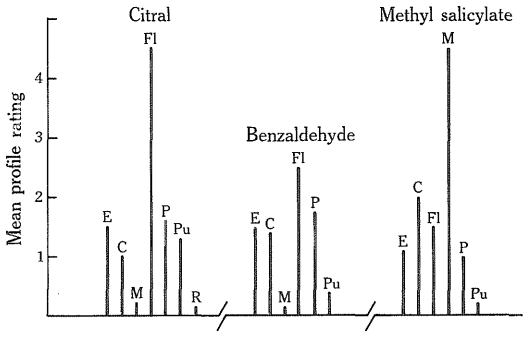


Fig. 3. Profiles obtained for three odorants from seven descriptive terms (from Moskowitz 1974). Order of descriptive terms: E, ethereal; C, camphoraceous; M, musky; Fl, floral; P, pepperminty; Pu, pungent; R, repulsive.

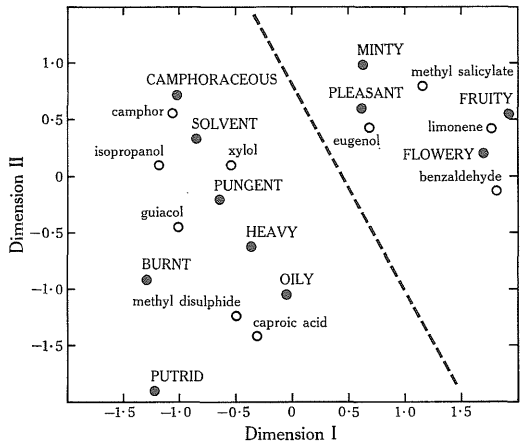


Fig. 4. Two-dimensional odour spaces obtained from proximity analyses. The data for the analyses represent mean dissimilarity estimates either between pairs of odorants or between pairs of odour descriptors (from Moskowitz and Gerbers 1974).

distances between odorants or descriptors are evolved and these distances are processed mathematically to yield a geometric configuration. Thus in Fig. 4 a point in space corresponds to an odour or descriptor and distances between points correspond to the dissimilarity between pairs of odorants or descriptors.

A major problem with both methods is that of semantics. The profile procedure requires an observer to relate a test odorant to several usually pre-assigned descriptors. The validity of the profile therefore depends on the proper selection of descriptive terms. In proximity analysis the problem is to select precisely the semantic description which

best applies to the dimensions or elements that are evolved mathematically. In both types of measurements the major quality or dimension often relates to pleasantness. Thus in Fig. 4, the odorants or descriptors to the left of the dashed line are considered to be unpleasant whilst those to the right are usually classified as pleasant. Other elements contributing to differences between odours are more difficult to name even though they may account for a large part of the odour's qualities (Moskowitz and Gerbers 1974). It is obvious then that our inadequate range of descriptive terms is a major obstacle to quantitative measurement of the quality of odours.

Compounding the problem of semantics is the failure of any psychophysical procedure to extract from subjects all the qualities or notes that a single odorant may have. This, perhaps, is partly because many workers neglect to determine whether the quality of the odour changes with the concentration of the odorant. It seems important that the method chosen to measure quality should ensure that the qualities discerned by each subject are related to individual threshold levels, rather than to concentration alone. There is little point, for example, in analysing the responses of two subjects at a single concentration if this concentration is five times above the threshold for one subject and fifty times above for the other. If this factor is not taken into account, disagreement between subjects may be substantial.

A new approach to these problems has been described by Laing *et al.* (1974). By means of the odour delivery system described earlier, rats were trained in a two-choice discrimination procedure to distinguish between the odours of benzaldehyde and benzonitrile (both 'almond-like'), benzaldehyde and isobutyl-n-butyrate (fruity), and benzonitrile and isobutyl-n-butyrate. The results indicated that the rats found that benzonitrile and benzaldehyde had similar odour qualities, and that these in turn were very different from those of isobutyl-n-butyrate. In addition, it appeared that the rats used several characteristics or notes of odours to distinguish between the odorants. In view of these observations, the methods employed with the rats may provide an objective approach to investigating the number of qualities which characterize an odour. As mentioned earlier, in profile ratings humans describe an odorant with the aid of several descriptors, but as yet no satisfactory psychophysical method has been developed to extract this data quantitatively from them.

However, although animal studies may improve our knowledge of odour characteristics, there are areas of olfaction, particularly in flavour work, which require the continued use of semantics. In these instances the thesaurus of 200 or so descriptive terms for odours being compiled by the ASTM Subcommittee E-18.04 on Sensory Evaluation may serve in the interim period until the relationship between physicochemical properties of odorants and odour quality is known. Only then might it be possible to define odour quality as readily as colour.

Odour intensity

It has long been recognized that man has difficulty in distinguishing between different concentrations or intensities of an odorant. This limited ability can perhaps be accounted for in terms of physiological evidence. The only major change observed in the electrical activity of the olfactory receptor cells or nerve fibres to the brain, in response to a small change in concentration, is an increase or decrease in the amplitude of the nerve pulses; their frequency remains unchanged. Hence the change in informational input to the brain with small changes in concentration is often not sufficient for it to register the difference. This suggests that it may be more important for the brain to detect changes in the type of odours in the vicinity of the body than changes (providing they are fairly small) in the concentration of a single odorant. Thus for many odorants, humans can detect a difference only when a 30–60% change in concentration occurs.

Why different odorants are perceived with different intensities at similar concentrations is not known, but a useful indicator of the relative intensities of different odorants is found in the power function, $S = KC^n$, where S is the intensity rating of the subject, C is the concentration of the stimulus, and K and n are constants which refer to the intercept and slope of the line obtained when the average values of S and C are plotted in double logarithmic coordinates. This relationship appears to fit over much of the

stimulus–response range except at the extremities.

The most common method for measuring S is by scaling procedures such as magnitude estimation. However, the slope n is the most interesting parameter, since its value reflects the magnitude of the change in the subject's response with changes in the concentration of the odorant. The value of n for several odorants is shown in the table.

Intensity slopes for some odorants (from Berglund *et al.* 1971)

Odorant	n
n-Amyl acetate	0·13
n-Butyric acid	0·22
Acetone	0·54
n-Heptanol	0·71

Similar differences for n have been obtained for a number of odorants in physiological studies, when the amplitude of the integrated electrical activity, which is generated in the olfactory receptor area in response to an odour, has been plotted against different concentrations of the stimulating odorants.

Unfortunately the value of n for a particular odorant as reported by different workers has varied widely. It is possible that the variability is primarily a consequence of the estimation and sniffing procedure and the method of presentation of the odorant.

However, in view of the seemingly practical nature of the stimulus–response equation, the ASTM Subcommittee E-18.04 has recommended that the equation be adopted in a standardized procedure for measuring odour intensities (Moskowitz *et al.* 1974). n-Butanol has been proposed as a reference odorant so that results from several laboratories may be compared. Briefly, it has been proposed that the odour intensity of n-butanol at a concentration of 250 ppm by volume in air be designated arbitrarily as 10 odour intensity units. Subjects are to assign numbers to other intensities of the same or different odours which are proportional to odour magnitude, i.e. intensities are scaled by the method of magnitude estimation.

A similar standardized procedure has been proposed by Svensson and Lindvall (1974), but since these workers are more concerned with odorous air pollutants, hydrogen sulphide is proposed as the reference odorant.

As yet such standard methods are suitable for use only with air-dilution olfactometers and not with any other method of odour presentation. Before a standardized method can be achieved for diluents other than air, it will be necessary to standardize the method of presentation of the odorants and of assessing the concentration. How successful these standardized methods will be is as yet unknown. Although several workers have used the butanol reference scale and obtained similar values for n, the limitations of the method have not yet been determined (Cain 1969; Laffort and Dravnieks 1973).

References

Amoore, J. E. (1970). 'Molecular Basis of Odor.' (Thomas: Springfield, Illinois.)

Berglund, B., Berglund, U., Ekman, G., and Engen, T. (1971). Individual psychophysical functions for 28 odorants. *Percept. Psychophys.* **9,** 379–84.

Cain, W. S. (1969). Odor intensity: Differences in the exponent of the psychophysical function. *Percept. Psychophys.* **6,** 349–54.

Corbit, T. E., and Engen, T. (1971). Facilitation of olfactory detection. *Percept. Psychophys.* **10,** 433–6.

Davis, R. G. (1973). Olfactory psychophysical parameters in man, rat, dog and pigeon. *J. Comp. Physiol. Psychol.* **85,** 221–32.

Demerdache, A., and Wright, R. H. (1967). Low frequency molecular vibration in relation to odor. *In* 'Olfaction and Taste', Proc. 2nd Int. Symp. pp. 125–65. (Pergamon Press: Oxford.)

Engen, T., and Bosack, T. N. (1969). Facilitation in olfactory detection. *J. Comp. Physiol. Psychol.* **68,** 320–6.

Griffiths, N. M. (1974). Sensory properties of the chloroanisoles. *Chem. Senses Flavor,* **1,** 187–95.

Kauer, J. S. (1974). Response patterns of amphibian olfactory bulb neurones to odour stimulation. *J. Physiol.* **243,** 695–715.

Köster, E. P. (1971). Adaptation and cross-adaptation in olfaction. Ph.D. thesis, Univ. of Utrecht.

Laffort, P. (1969). A linear relationship between olfactory effectiveness and identified molecular characteristics extended to fifty pure substances. *In* 'Olfaction and Taste', Proc. 3rd Int. Symp. pp. 150–7. (Rockefeller Univ. Press: New York.)

Laffort, P., and Dravnieks, A. (1973). An approach to a physico-chemical model of olfactory stimulation in vertebrates by single compounds. *J. Theor. Biol.* **38,** 335–45.

Laing, D. G. (1975). A comparative study of the olfactory sensitivity of humans and rats. *Chem. Senses Flavor* **1,** 257–69.

Laing, D. G., Cairncross, K. D., King, M. G., and Murray, K. E. (1972). The use of behavioural methods in studying the physiology of olfaction. *Proc. Aust. Physiol. Pharmacol. Soc.* **3,** 203.

Laing, D. G., and Mackay-Sim, A. (1975). Olfactory adaptation in the rat. *In* 'Olfaction and Taste', Proc. 5th Int. Symp. (Eds D. A. Denton and J. P. Coghlan) pp. 291–5. (Academic Press: London.)

Laing, D. G., Murray, K. E., King, M. G., and Cairncross, K. D. (1974). A study of olfactory discrimination in the rat with the aid of a new odor delivery technique. *Chem. Senses Flavor* **1,** 197–212.

Lea, C. H., and Swoboda, P. A. T. (1958). The flavour of aliphatic aldehydes. *Chem. Ind. (Lond.)*, 1289–90.

Moskowitz, H. R. (1974). Combination rules for judgements of odor quality difference. *J. Agric. Food Chem.* **22,** 740–3.

Moskowitz, H. R., Dravnieks, A., Cain, W. S., and Turk, A. (1974). Standardised procedure for expressing odor intensity. *Chem. Senses Flavor* **1,** 235–7.

Moskowitz, H. R., and Gerbers, C. L. (1974). Dimensional salience of odors. *Ann. N.Y. Acad. Sci.* **237,** 1–16.

Moulton, D. G. (1960). Studies in olfactory acuity. III. Relative detectability of *n*-aliphatic acetates by the rat. *Q. J. Exp. Psychol.* **12,** 203–13.

Moulton, D. G., Celebi, G., and Find, R. P. (1970). Olfaction in mammals—two aspects: proliferation of cells in the olfactory epithelium and sensitivity to odours. *In* 'Taste and Smell in Vertebrates', pp. 227–50. (Churchill: London.)

Moulton, D. G., and Eayrs, J. T. (1960). Studies in olfactory acuity. II. Relative detectability of *n*-aliphatic alcohols by the rat. *Q. J. Exp. Psychol.* **12,** 99–109.

Ottoson, D. (1956). Analysis of the electrical activity of the olfactory epithelium. *Acta Physiol. Scand.* **35,** Suppl. 122.

Stahl, W. H. (Ed.) (1973). 'Compilation of odor and taste threshold values data.' (ASTM: Philadelphia, Pa.)

Svensson, L. T., and Lindvall, T. (1974). On the consistency of intramodal intensity matching in olfaction. *Percept. Psychophys.* **16,** 264–70.

News from the Division

Appointments

Mr G. M. Ross, B.Sc., has joined FRL's Biochemistry Section where a team is carrying out biochemical, chemical and parasitological studies of tropical prawns, with the aim of discovering the cause of 'white spot' in prawns. The work is supported by the Fishing Industry Research Trust Account.

The position at MRL vacated by Mr P. L. Thomas (*CSIRO Fd Res. Q.*, 1975, **35,** 70) has been filled by Mr S. C. Williams, M.Sc.; his functions will include those of editor and consumer liaison officer. Mr Williams, who graduated in 1972, has taught business management, social psychology and communication at the University of Queensland and the Queensland Institute of Technology, while he gained his M.B.A.

Mr W. F. Spooncer, B.Sc., has been appointed Extension Officer attached to the Industry Section at MRL; he will be stationed at Hawkesbury Agricultural College, Richmond, N.S.W. Mr Spooncer has held several positions as a chemist with the Beecham Group Food Division in Britain and last year he was Technical Manager, Franchise Operations, for Cottees General Foods (Australia).

Visiting workers

Dr I. Adato of the Department of Tropical Horticulture, Agricultural Research Organization, The Volcani Centre, Israel, recently returned home after spending a year at FRL working with Dr W. B. McGlasson in research on non-ripening tomato mutants.

Professor Z. E. Sikorski has taken up a Senior Research Fellowship, tenable for one year, at TFRU; he will work on protein denaturation of frozen foods. Dr Sikorski is Chairman of the Department of Food Preservation and Technology at the Technological University, Gdansk, Poland.

Work overseas

Dr R. A. Buchanan's secondment to the Australian Department of Foreign Affairs has been extended. Dr Buchanan (DRL) is now located at Kuala Lumpur, as Australian Liaison Officer to the ASEAN subcommittee on protein.

Mr F. G. Kieseker (DRL) recently returned from a 4-week visit to Zambia and Tanzania, where he took part in a survey of the dairy manufacturing industry while on secondment to the Department of Overseas Trade. He paid particular attention to the manufacture of recombined UHT milk, flavoured milk, icecream and cheese.

Dr A. F. Egan (MRL) is spending 7 months from September 1975 at the Max-Planck-Institut für Immunbiologie, Freiburg, Federal Republic of Germany, where he is studying methods for isolation and estimation of the components of the outer membrane of the cell envelope in salmonellae. He will also visit research centres in Britain.

Dr A. R. Johnson (FRL) is spending 11 months as a guest worker in the Department of Food Science and Nutrition, Queen Elizabeth College, London. He will attend conferences in several European countries while he is overseas.

Mrs Judith Ruello has been released from duty during October and November to lecture on prawn technology, in Bangkok, at a FAO/DANIDA Regional Workshop on fish handling, plant sanitation, quality control and fish inspection.

Mr L. Herbert (MRL) recently spent 2 months in New Zealand at Massey University's Department of Bio-Technology as a guest lecturer.

Mr P. W. Board (FRL) was Guest Lecturer at the 11th Annual Conference of the New Zealand Institute of Food Science and Technology held at Massey University.

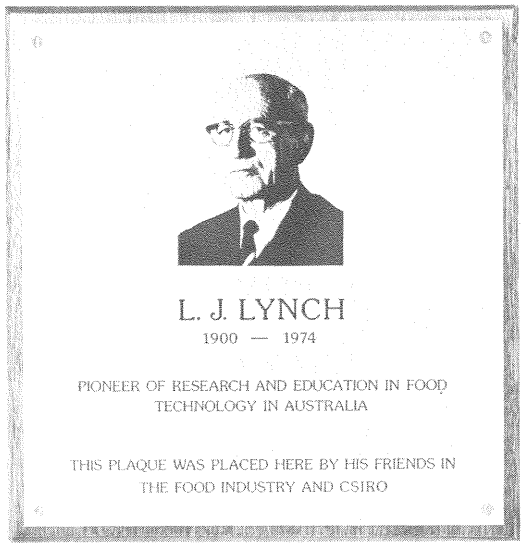
Conference

During October, Mr L. L. Muller and Mr S. C. Marshall of DRL attended a Whey Utilization Workshop at the University of Ohio, Columbus, Ohio, conducted under the auspices of the U.S.–Australia Science Agreement.

Award

Mr L. L. Muller has been made a Life Member of the Australian Society of Dairy Technology, in recognition of his outstanding contribution to the dairy manufacturing industry.

In memoriam



A memorial plaque to the late Lawrence J. Lynch has been placed in the Hicks Meeting Room at the Food Research Laboratory, North Ryde. L. J. Lynch, who died on 1 December 1974, was an officer of the Division of Food Preservation (as it then was) from 1935 to 1965, and at the time of his retirement was Senior Principal Research Scientist in charge of the Canned Foods Section (see *CSIRO Food Pres. Q.*, 1965, **25**, 55).

A similar plaque donated by the New South Wales Branch of the Australian Institute of Food Science and Technology has been placed in the School of Food Sciences, Hawkesbury Agricultural College, Richmond, N.S.W., in recognition of the contribution of L. J. Lynch as adviser, lecturer and examiner in the establishment of food technology courses at the College.

J. F. K.