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The biosynthesis of flavour compounds in cheddar cheese

By A. J. Hillier and G. R. Jago

CSIRO Division of Food Research, Highett, Victoria.

Investigation of flavour deficiencies in fat-modified cheeses provided useful answers to general questions about cheese flavour.

Bovine milk has an approximate composition of water (870 g/l), lipids (40 g/l), proteins (30 g/l), carbohydrates (50 g/l), vitamins and minerals, distributed in three different physical states: solution, emulsion and colloidal suspension. The process of Cheddar cheese manufacture effectively removes a large percentage of the water and water-soluble compounds (lactose and non-casein proteins) from milk and concentrates the casein and lipid fractions to form a curd which is acidified and salted. The curd is then allowed to ripen or mature over several weeks or months in order to produce a digestible product with a distinct flavour, commonly described as Cheddar flavour.

The respective primary functions of bacteria (starter organisms) and rennet, which are added to the cheese milk early in the manufacturing process, are the conversion of milk lactose to lactic acid and the coagulation of the casein micelles. In addition, they provide a rich source of enzymes which, together with the enzymes normally present in milk, are chiefly responsible for the changes that occur in the curd during the ripening process. In particular, these enzymes act on the lactose, fat and protein of the curd, and on their degradation products, to produce Cheddar flavour. However, because lactose is water-soluble, its concentration in the curd is rapidly diminished by the expulsion and removal of whey during cheese manufacture. Therefore lactose does not appear to be an important substrate for the production of flavour in ripening cheese.

The protein and fat are retained in the curd, along with the starter organisms and a small fraction of the rennet, and undergo marked changes during the ripening process. The amino acids released from the milk proteins during the ripening process do not in themselves appear to have any marked impact on flavour. However, their numerous degradation products, which include acetaldehyde, fatty acids and sulphur compounds, may be all-important in the formation of Cheddar flavour. Likewise, individual fatty acids liberated from the milk fat could also influence the flavour of the cheese.

The chemical basis for the flavour of Cheddar cheese has been the subject of research in many countries, principally in the U.S.A. by Kristoffersen and Harper and their associates, and in England by a group at the National Institute of Research in Dairying. The subject has been extensively reviewed by Kristoffersen (1973), Evans and Manning (1973) and Evans and Mabbitt (1974).

While a multitude of products such as acids (particularly short-chain fatty acids, acetic and lactic acids), alcohols, aldehydes, ketones, esters, ammonia, amines, sulphides and mercaptans appear in cheese during ripening, Kristoffersen believes that the characteristic flavour of cheese is related to the concentrations and balance of relatively few key components. For example, in Cheddar cheese a relationship between flavour quality and the relative concentrations of free fatty acids and hydrogen sulphide was observed (Kristoffersen and Gould 1960). This implied that optimum flavour development in Cheddar cheese was dependent on the relative rates at which the fat and protein were degraded to produce the essential flavour components.

A similar relationship has been observed

in other cultured products such as cottage cheese, cultured cream, butter and buttermilk (Keen and Walker 1972), where an optimum ratio between diacetyl and acetaldehyde concentrations has been established. If this ratio is exceeded, a harsh or diacetyl flavour prevails, whereas below this ratio a 'yoghurt' or 'green' flavour is apparent.

On the other hand, Evans and Manning (1973) maintain that no one has yet advanced an integrated hypothesis to account for Cheddar flavour. They consider that the H_2S: fatty acid ratio proposed by Kristoffersen may correlate with flavour only because it indicates that reactions by which the flavour-important compounds are produced have proceeded to the required extent.

The role of fatty acids in the formation of Cheddar flavour remains inconclusive. It is known that cheese manufactured from skim milk does not develop Cheddar flavour. Furthermore, low concentrations of fatty acids in cheese are associated with flat, uninteresting and often mild flavours, while high concentrations are associated with flavour defects (Evans and Manning 1973). Little doubt remains, however, about the importance of sulphur-containing compounds in the development of Cheddar flavour.

Sulphur compounds

Extensive studies of the sulphur compounds present in the aroma or volatile fraction of Cheddar cheese have been made by the English workers (Manning and Robinson 1973; Manning 1974; Manning *et al.* 1976). They established the presence of H_2S, methanethiol, dimethylsulphide and diacetyl in the volatile fraction but found that the intensity of flavour in Cheddar cheese ranging in age from 2–12 months was related only to the concentration of methanethiol. When cheese was manufactured 'aseptically' from whole milk without starter organisms, or from skim milk with starter organisms, little or no flavour developed in the cheese and the presence of methanethiol could not be detected.

The absence of flavour in cheese made from skim milk could be due to the absence of degradation products and/or loss of essential volatile flavour compounds (e.g. methanethiol) which are fat-soluble and retained in whole milk cheese by the lipid present.

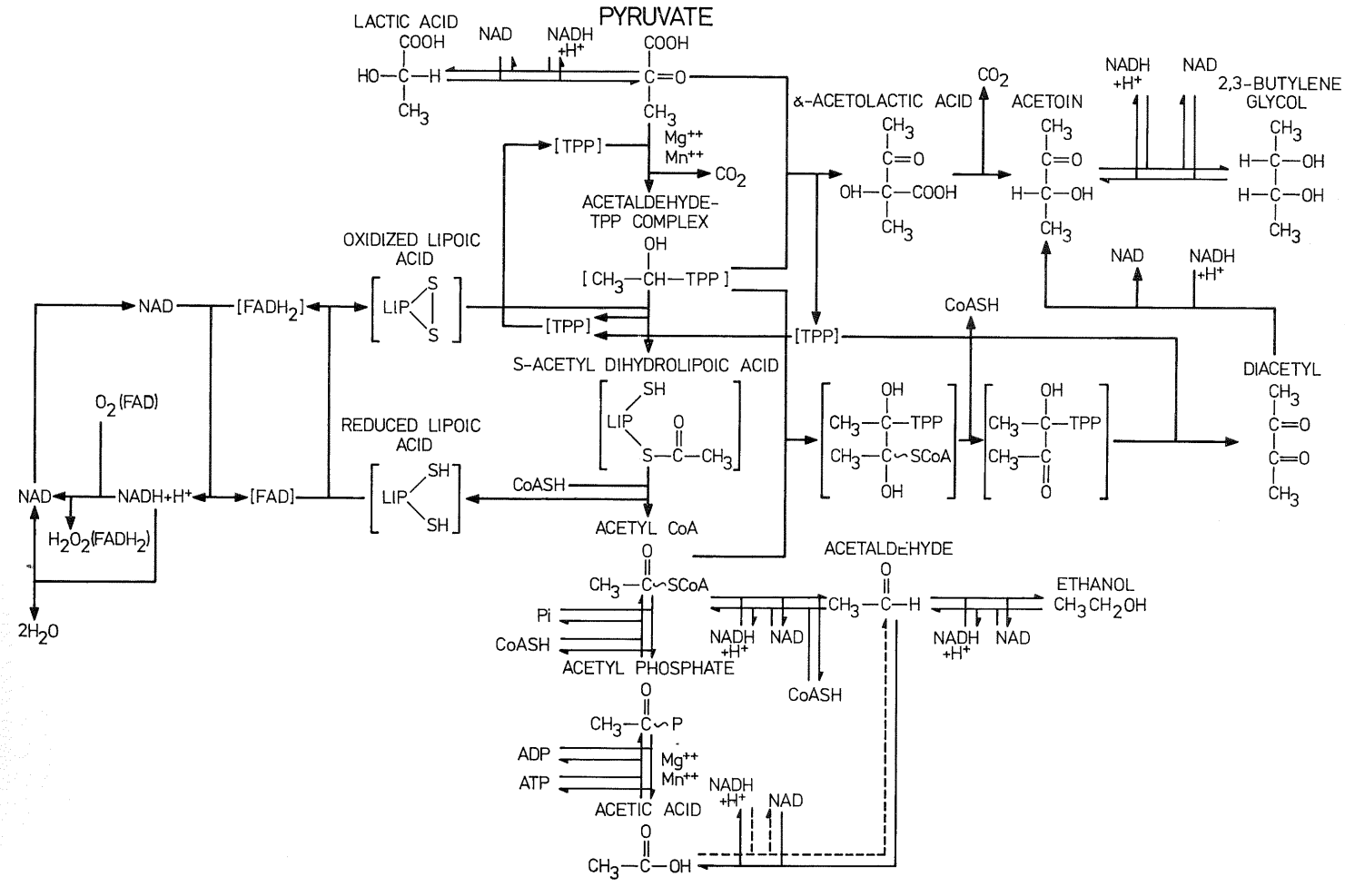
Strains of corynebacteria able to produce methanethiol from the amino acid methionine were recently isolated from Cheddar cheese by Sharpe *et al.* (1976). Corynebacteria have been shown to be present in raw milk and are not destroyed by the heat treatment milk receives before cheese manufacture. However, since cheese made without starter organisms does not contain methanethiol, it is likely that some symbiotic relationship between corynebacteria and the starter organisms is required to produce methanethiol in cheese.

The species of Group N streptococci used as starter organisms in the manufacture of Cheddar cheese are added in large numbers to the cheese milk. They then multiply and the end-products of their metabolism are lactic acid and flavour compounds such as acetaldehyde, ethanol, acetic acid and diacetyl. The metabolic pathways by which Group N streptococci produce these compounds from pyruvate are illustrated in Fig. 1. As shown in Fig. 2, Group N streptococci produce pyruvate as an intermediate in the metabolism of lactose, the amino acids alanine and serine, and in some strains, citrate.

Many other types of bacteria including pediococci, lactobacilli and corynebacteria can also be found in maturing Cheddar cheese, and the population of some of these microorganisms may reach that of the starter streptococci (Law *et al.* 1976). However, little is known about the ability of these organisms to produce flavour compounds.

Fig. 1. Pathways for the metabolism of pyruvate in Group N streptococci. Enzyme systems shown: Lactate dehydrogenase; pyruvate dehydrogenase; α-acetolactate synthetase; α-acetolactate decarboxylase; 2,3-butylene glycol dehydrogenase; diacetyl reductase; dihydrolipoyl transacetylase;* diacetyl synthetase;* dihydrolipoyl dehydrogenase;* reduced NAD oxidase; reduced NAD peroxidase; phosphotransacetylase; acetate kinase; aldehyde dehydrogenase (Co A dependent); alcohol dehydrogenase; aldehyde dehydrogenase (Co A independent).*

– – → Pathways yet to be established in Group N streptococci. * Enzymes yet to be established in Group N streptococci. Enzyme bound components are enclosed in square brackets.


PYRUVATE
LACTIC ACID
NAD
NADH +H+
[TPP]
Mg++
Mn++
CO2
ACETALDEHYDE-TPP COMPLEX
α-ACETOLACTIC ACID
ACETOIN
2,3-BUTYLENE GLYCOL
OXIDIZED LIPOIC ACID
[FADH2]
[FAD]
O2 (FAD)
H2O2(FADH2)
2H2O
REDUCED LIPOIC ACID
S-ACETYL DIHYDROLIPOIC ACID
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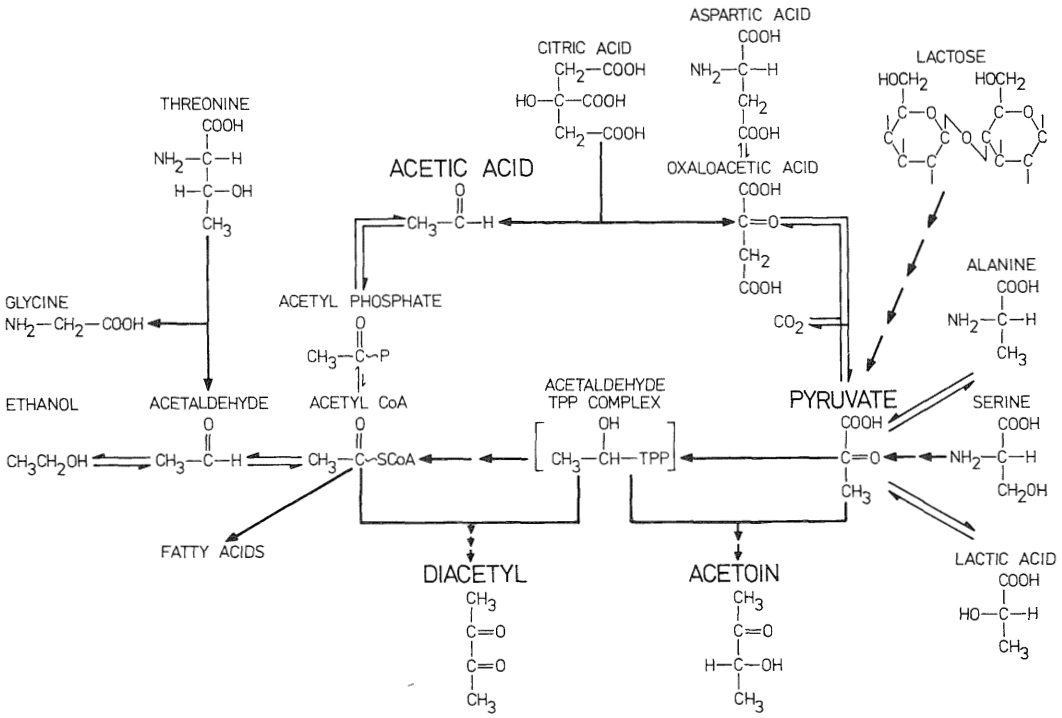

Fig. 2. Pathways for the formation of pyruvate and flavour compounds in cheese.

Since the flavour of Cheddar cheese involves several compounds as yet not clearly defined, the role of the Group N streptococci in the development of this flavour has not been fully elucidated. However, it is certain that Cheddar flavour does not develop and methanethiol is not detected in cheese from which the Group N streptococci have been excluded during manufacture.

If the development of full Cheddar flavour is dependent on a balance being maintained not only in the rate of formation but also in the ultimate concentration of each of the essential compounds involved, then any process which blocked the formation of any one of these compounds (e.g. methanethiol) would nullify the development of Cheddar flavour.

Therefore one way to test the essentiality of a flavour compound would be to suppress its formation in the cheese and then observe whether the flavour of the cheese is impaired.

Effects of unsaturated fatty acids

Acetic acid produced by bacteria is the dominant volatile fatty acid in Cheddar cheese (Evans and Mabbitt 1974) and has long been considered to form the basis of cheese aroma (Patton 1963). However, proof that it is essential to the development of Cheddar flavour has not been established.

It is known that unsaturated fatty acids, such as oleic and linoleic acids, are potent inhibitors of the pyruvate dehydrogenase system in Group N streptococci (Anders and Jago 1970). The inhibition of this enzyme system blocks the formation of diacetyl, acetic acid, acetaldehyde and ethanol from pyruvate (Figs 1 and 2). When Cheddar cheese was manufactured from cows' milk containing approximately 20% linoleic acid in the milk fat, no Cheddar flavour developed in the cheese. On analysis it was found that this cheese had very low acetic acid and ethanol contents as compared with cheese manufactured from conventional milk (Czulak *et al.* 1974).

If the inhibitory activity of the unsaturated fatty acids in cheese is specific for the pyruvate dehydrogenase system in the Group N streptococci, then acetic acid

and/or ethanol also become essential compounds in the development of Cheddar flavour. When a strain of *Lactobacillus bulgaricus* was added to the milk in combination with the normal starter organisms there was a marked increase in the levels of acetic acid and ethanol and a satisfactory flavour developed in the cheese.

L. bulgaricus appears able to bypass the block in the pyruvate dehydrogenase system by forming acetaldehyde from threonine (Fig. 2), while Group N streptococci grown at 37–38°C (the normal cooking temperature of Cheddar cheese) are unable to utilize this pathway (Lees and Jago 1976). The acetaldehyde produced from threonine by *L. bulgaricus* is converted to acetic acid and ethanol by the combined action of the aldehyde and alcohol dehydrogenases (Fig. 3) which are present in the Group N streptococci (Lees and Jago, unpublished data). In this reaction sequence the coenzyme nicotinamide adenine dinucleotide (NAD) is alternatively oxidized and reduced as acetaldehyde is converted to ethanol and acetic acid.

The advent of cheese containing high levels of polyunsaturated fatty acids as a result of feeding cows with protected lipid supplements has provided a new medium for studying the development of flavour in cheese, since the presence of unsaturated fatty acids blocks the formation of essential flavour compounds. Further investigation will identify how many of the essential compounds involved in the development of Cheddar flavour are inhibited by polyunsaturated acids. Suppression of the formation of specific compounds in the cheese during ripening should clarify the role of these compounds in Cheddar flavour.

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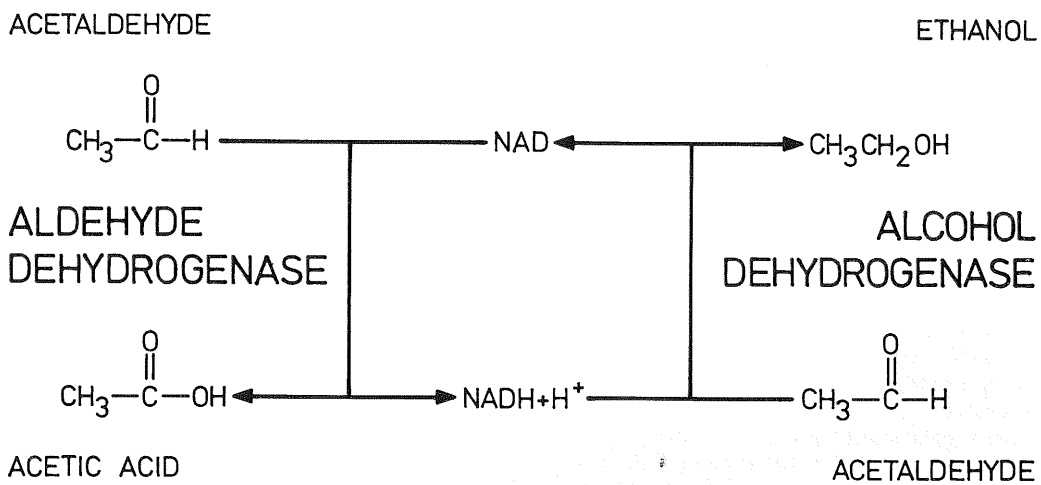

Fig. 3. The mechanism of formation of acetic acid and ethanol from acetaldehyde by Group N streptococci.

Recovery of functional meat proteins from abattoir by-products

By R. G. Hamilton

CSIRO Division of Food Research, Cannon Hill, Queensland.

The extraction of high-value edible proteins from meat by-products would result in a range of new and unconventional meat products. By-products that contain substantial amounts of meat tissue (e.g. trimmings and bones) are usually rendered down to yield tallow and meat meal because present methods of boning are too costly for recovering the meat. In 1976 100 000 t meat adhering to fat and bones were rendered into low-value products in Australia.

Commercial processes (Vickery 1968; Levin 1970; Paoli 1970) are available for the recovery of meat tissue from fat trimmings and bony residues normally processed by rendering, but the products are expensive, perishable and lack functional properties. One process, mechanical deboning, produces a paste of meat, bone particles, fat and connective tissue, but the paste is perishable and must be used immediately, or frozen. Another method, ultra-low temperature rendering results in a raw meat emulsion of reduced fat content, but it is similar in composition and perishability to the mechanically deboned product. Other methods involve the use of isopropyl alcohol, ethylene dichloride or acetone in solvent extraction processes to make meat protein concentrates for human consumption. In general, these concentrates have good nutritional value but lack functional properties such as solubility, water binding properties, and heat coagulability because the proteins are denatured during the process.

While these techniques may be adequate for processing marine and oil-seed by-products into functionless, almost tasteless protein pastes and flours for addition to foods to enhance the nutritional value, they have proved unsuccessful when applied to meat. Unlike fish proteins (Spinelli *et al.* 1975) or vegetable proteins (Melnychyn 1972) the colour and flavour of meat are appealing to the consumer. A new approach to meat protein recovery is needed that will result in a product which retains the valuable features, is stable (preferably dry) and cheap.

By-product raw materials

The raw materials (trimmings and bones) consist of fat, meat tissue, bone tissue and connective tissue (Duerr and Earle 1973). The proteins in bone tissue and connective tissue constitute a large proportion of the total protein present, but they are of secondary importance being nutritionally and functionally inferior. Meat tissue consists of muscle proteins and connective tissue proteins.

Muscle proteins may be classified into two groups—sarcoplasmic proteins and myofibrillar proteins. The sarcoplasmic proteins, about 40% of the total muscle proteins (Lawrie 1974), are soluble in salt solutions of low ionic strength and have relatively low molecular weights. They contribute to the colour and flavour of meat by reacting with carbohydrates present in the meat during cooking. The myofibrillar, or structural proteins, about 60% of the total muscle proteins (Lawrie 1974), have actin, myosin and tropomyosin as their main constituents and are responsible for the physical properties of meat, particularly gel formation, water binding and heat coagulability. The connective tissue proteins, collagen and elastin, are of little importance.

Investigations were made into selective solubilization of the muscle proteins to facilitate protein removal by filtration, centrifugation or pressing, leaving the residue to be rendered in the normal way to produce tallow and meal.

Solubilizing processes

There are several established laboratory procedures (Keller and Block 1960) for the solubilization of muscle proteins, but several of these procedures are unsuitable for commercial development because of high cost, reagent toxicity, reagent incompatibility with the final product, poor yield, or damage to the proteins.

Alkaline solubilization (Figs 1 and 2) warranted further investigation because only small quantities of simple, inexpensive reagents were required. This technique allowed simultaneous solubilization of sarcoplasmic and myofibrillar proteins at refrigerated temperatures in dilute sodium hydroxide solution.

The protein yield from this process depended on the pH of the solvent, the time and temperature of extraction, and the ratio of meat to solvent. The results obtained are summarized below.

Effect of pH on protein yield

The pH of the extraction mixture had a pronounced effect on the solubility and extractability of the meat proteins as illustrated in Fig. 3. Initial adjustment of the pH was found to be ineffective because the pH gradually dropped during extraction, probably as a result of diffusion of alkali through the meat tissue. Readjustment of pH was tedious and time consuming so this procedure was altered and a known volume of 30% sodium hydroxide was added to the mixture before extraction. Initial pH readings were disregarded and equilibrium pH was monitored.

Above pH 9·5 a strong 'fishy' odour developed in the extract which disappeared

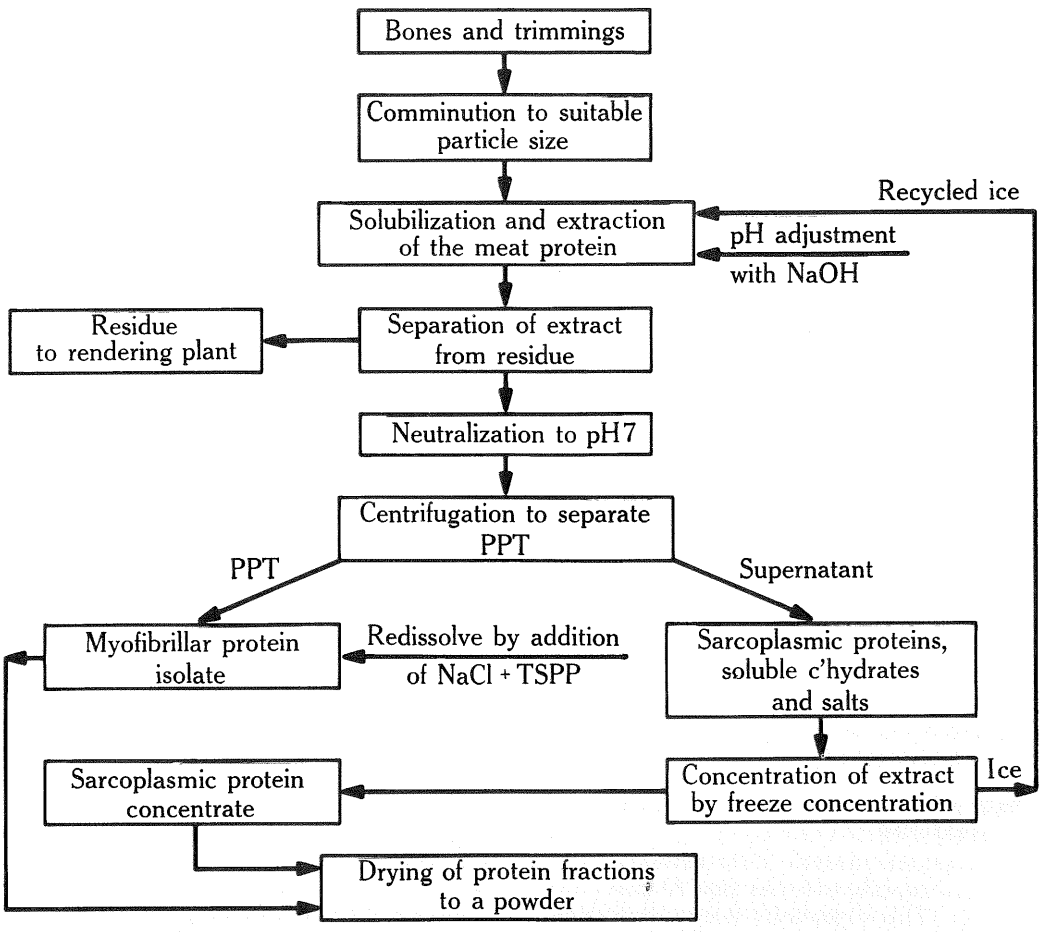

Fig. 1. Alkaline extraction of trimmings and bones.

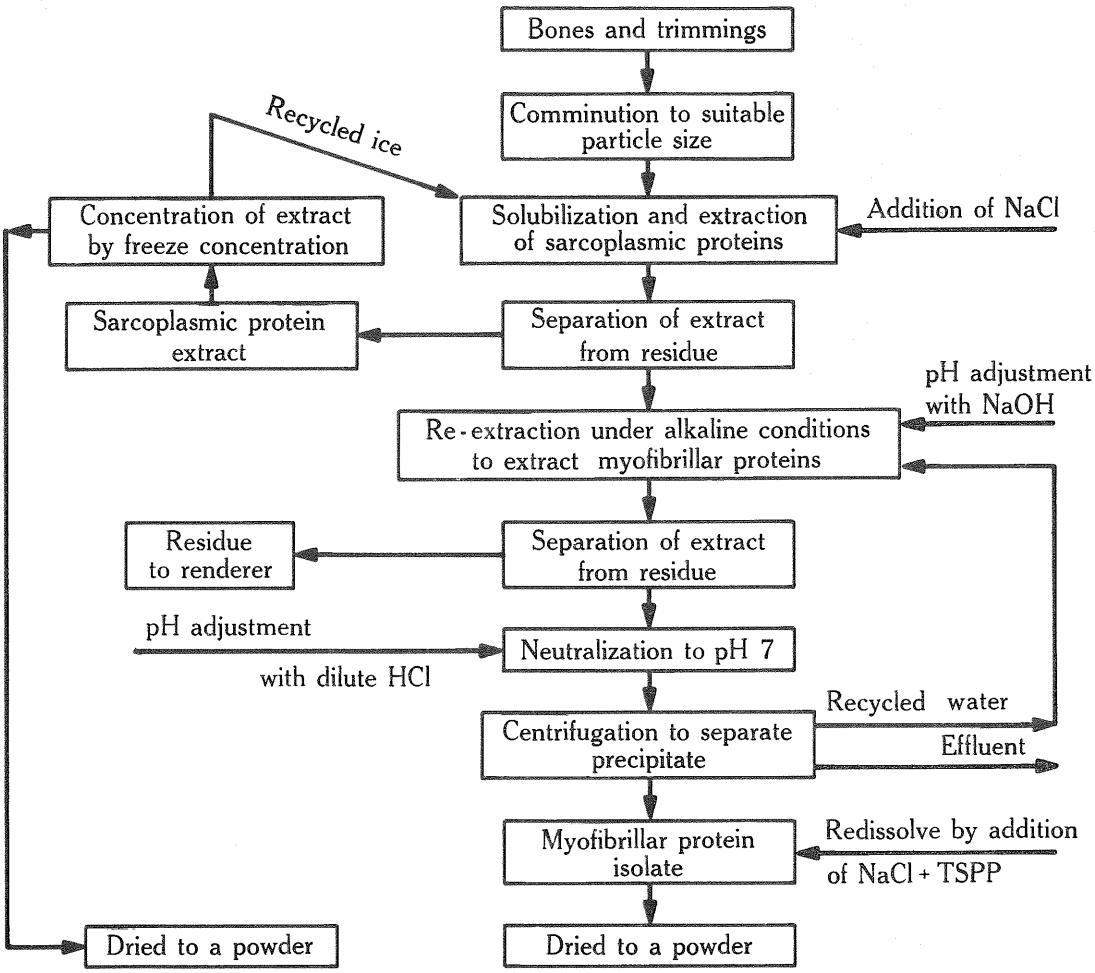

Fig. 2. Two-stage extraction of meat proteins in bones and trimmings.

after neutralization. The critical pH for extractability appeared to be pH 9. It was later found that the increased viscosity of the extract at this pH was affecting the extractability. Extraction of the meat proteins above pH 11 did little to increase the yield and required a large amount of alkali. Below pH 8·5 the yield not only decreased but the extract consisted mainly of sarcoplasmic protein (analysed by SDS gel electrophoresis).

Effect of extraction time on protein extractability

The protein content of the extract increased rapidly over the first 30 min (Fig. 4). This is partially due to elution of the soluble sarcoplasmic proteins and diffusion of the alkali through the meat tissue. After 30 min the rate of extraction decreased. The fat was separated more easily when the extraction time was increased to 40 min. It can be seen from Fig. 4 that whilst protein concentration increased with increasing time of extraction, the yield of extract was reduced, allowing flexibility in the extraction time between 30 and 120 min.

Extraction temperature

The temperature of extraction had little effect on the yield of protein. The choice of temperature is dependent on the type of separation equipment used, bacteriological considerations, and an appreciation of the undesirable secondary reactions between the

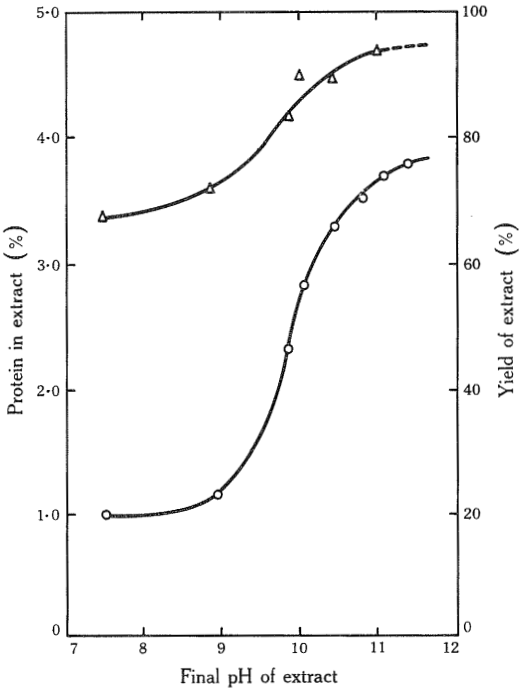

Fig. 3. Effect of pH on protein yield from trimmings (6 mm particle size) with a meat to solvent ratio of 1 : 5 at 10°C for 1 h. △, percentage yield of extract; ○, percentage protein in extract.

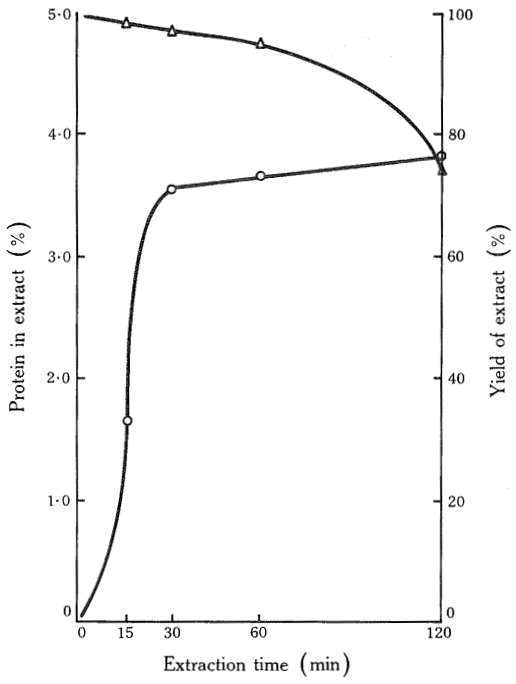

Fig. 4. Effect of extraction time on protein yield from trimmings (6 mm particle size) at pH 11 and 10°C with a meat to solvent ratio of 1 : 5. △, percentage yield of extract; ○, percentage protein in extract.

protein and alkali, such as protein hydrolysis and lysinvalanine* formation (Provansal *et al.* 1975). In the work reported here all extractions were carried out at 10°C.

Effect of meat to solvent ratio on protein extractability

Meat to solvent ratios ranging from 1 : 1 to 1 : 10 were investigated and the results for protein extractability are presented in Fig. 5. It was decided to express the ratios in terms of meat rather than raw material or total protein because it is the meat that affects extractability. The trimmings and bones used in the experiments contained 20% meat, on average. Therefore the 1 : 1 meat to solvent ratio was equivalent to 5 : 1 raw material to solvent, and similarly 1 : 2 ≡ 5 : 2, 1 : 5 ≡ 1 : 1, 1 : 7·5 ≡ 1 : 1·5 and 1 : 10 ≡ 1 : 2. Extraction of the protein with a large quantity of liquid resulted in the best yield of extracted protein but at a very low concentration. Conversely, extraction with a small quantity of liquid resulted in a very low yield of viscous extract of high protein content. It was also found that the lower the meat content of the raw material the more difficult it was to separate.

Obviously neither extreme is desirable. The very dilute extract contained excessive water requiring large facilities and more costly processing and storage, while the more concentrated extract produced low yields. Fig. 5 shows the meat to solvent ratio that produces optimum extractability.

An extract that contained between 3–4% (85% of the extractable protein) protein was achieved, but this was too dilute for direct drying. Owing to the large amount of costly energy that was required to evaporate water from the extract on low temperature drying equipment, water content had to be lowered by less expensive means before the extract could be dried. All the techniques capable of evaporating water at sufficiently low temperatures so as not to damage the proteins have a high energy requirement. These techniques need expensive and specialized equipment, some of which is unsuited to handling the highly viscous concentrate.

* Lysinvalanine is known to be poisonous to rats.

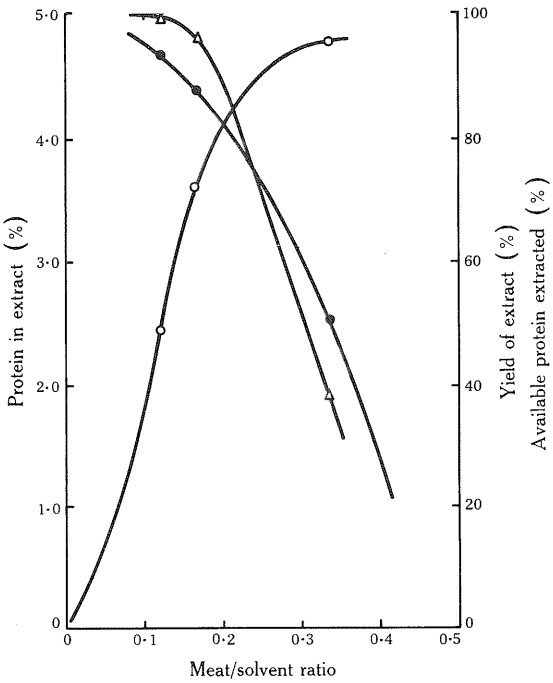

Fig. 5. Effect of meat to solvent ratio on protein yield from trimmings (6 mm particle size) at pH 11 for 1 h at 10°C. △, percentage yield of extract; ○, percentage protein in extract; ●, percentage of available protein extracted.

$$\frac{\text{meat}}{\text{meat} + \text{solvent}} = \text{ratio}$$

Concentration processes

One approach was to precipitate the proteins at their isoelectric points with food-grade acid. This produced excellent yields of concentrated protein, but the protein was denatured by the acid, stripping it of solubility, gel-forming capacity and red meat colour.

Ultrafiltration was also considered but was dismissed after discussions with specialists in this field, because the proteins would quickly clog the filtration membranes.

A technique that has been little used in the food-processing industry, freeze concentration, which relies on refrigeration rather than heating to remove water, was evaluated. This process, used at a temperature just below the freezing point of water, slowed down bacterial growth and offered saving in energy compared with evaporation. Crystals of pure ice are formed in the extract and removed by filtration. The ice can then be used for the chilling and extraction of incoming raw material, thus off-setting refrigeration costs. However, as the proteins were concentrated, the increased viscosity of the solution made separation of the ice difficult. In an attempt to overcome this problem, the viscosity of the extract was reduced by the addition of tetra sodium pyrophosphate (TSPP) (Bendall 1964; Yasui *et al.* 1964*a*) but this allowed only a marginal increase in concentration before the viscosity again increased. The viscosity increase was traced to the actomyosin in the myofibrillar fraction.

In other experiments, the myofibrillar proteins were first removed by fractional precipitation by neutralizing the chilled extract to pH 7 with very dilute food-grade HCl. A high speed agitator was used to prevent the formation of pockets of low pH which would denature the protein. The precipitated proteins were then recovered by centrifugation. Addition of NaCl and tetra sodium pyrophosphate (Yasui *et al.* 1964*b*) to the precipitate resolubilized the protein to a thixotropic gel, restoring the water binding and gel-forming capacities.

The sarcoplasmic proteins left in the supernatant were recovered as a 10% protein concentrate by freeze concentration, as no viscosity problems were encountered during the concentration of this fraction (Fig. 1).

The procedure outlined does require double handling, the water being first separated from the myofibrillar proteins and then later from the sarcoplasmic proteins. This may be avoided by first removing the sarcoplasmic proteins in a minimum quantity of salt (NaCl) solution of low ionic strength before alkaline extraction (Fig. 2). The supernatant from the precipitation of the myofibrillar protein would then contain negligible protein and could either be discarded or used to extract myofibrillar protein in subsequent batches. The sarcoplasmic protein extract obtained in this way would contain more protein and could be more efficiently concentrated by freeze concentration. A final decision on the system to be used would depend on an analysis of the economy of the two methods.

Evaluation of products

The concentrated proteins were evaluated qualitatively by heating to 90°C. The

sarcoplasmic protein coagulated to a light brown mass with no obvious weep, and had the flavour and aroma of cooked meat. The myofibrillar protein isolate set to an off-white coagulate which had a texture and cohesive strength similar to that of cooked egg white. Combined in their natural meat ratio, the concentrated sarcoplasmic and myofibrillar proteins were used to partially replace the meat in a typical 'Windsor' sausage formulation.

Two batches were prepared, one a control and the other having 50% of the meat replaced with the protein concentrate.*

The sausages were evaluated for weight loss on cooking, colour and gel strength (see table) and assessed by a taste panel consisting of CSIRO staff.

Evaluation of Windsor sausages for colour, weight loss on cooking and gel strength.

Sample	Sausage colour	Weight loss on cooking (%)	Gel strength (Instron Brake Test) (kg)
Control	Typical pale pink	0·6	1·21
50% meat replacement with meat protein conc.	Typical pale pink	1·6	0·98

The sausage with 50% of its meat replaced had comparable colour to the control. Water binding and gel strength were reduced slightly but these differences were of little significance as judged by the taste panel.

In a 'Duo Trio' test held over two morning sessions, 90 tasters were asked to pick the difference between the control and the protein mix sausages. Of the 58 people who could correctly tell the difference, 30 preferred the control and 22 preferred the sausage containing the 50% protein mix, indicating that statistically there were no significant differences.

The drying of these proteins at low temperatures is being studied and trials with an experimental fluidized bed drier show great promise.

Although development of these processes is still in the laboratory phase, protein concentrates and isolates prepared on a commercial scale should retain, to a significant extent, the desirable functional properties of raw meat, and should be acceptable to the food industry. Such functional concentrates and isolates could, undoubtedly, be applied in a wide range of processed meat products. Moreover, the composite protein powder, being fat free and low in cholesterol, recommends itself for use in special dietary foods.

The method clearly provides an opportunity to utilize meat by-products more profitably without interfering with the conventional production of tallow and meal.

* As the water content of the combined protein concentrate was 90% instead of the normal 80% for meat tissue, additional water was added to the control to compensate for this.

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Proteolytic enzymes in the dairy industry*

By R. Beeby

CSIRO Division of Food Research, Dairy Research Laboratory, Highett, Vic.

Milk is a secretion produced by female mammals to provide all the nutrients needed by their young in the early stages of their development. For centuries man has used the milk from animals that he has domesticated as a food source, because of its high nutritional value. However, milk was intended by nature for immediate consumption without any need for long-term storage. Therefore in order to exploit milk as a continuing supply of food, man has had to devise a technology for preserving its valuable food constituents. One effective means of doing this was to make cheese from the milk. The basic procedure for manufacturing cheese has remained essentially unchanged to the present day and cheesemaking is the major process in the dairy industry in which proteolytic enzymes are employed. Although cheese is made from the milk of several species, cows' milk is the most widely used, and the following discussion refers to cheese made from cows' milk.

The manufacture of cheese

Cheesemaking is essentially a de-watering process by which most of the protein and fat and appreciable quantities of the minerals in the milk are obtained in a concentrated form. The low moisture content and acidic nature of the cheese together with the relatively high level of salt, combine to hinder the growth of undesirable microorganisms, thus preserving a considerable proportion of the nutritional components of milk.

Enzymic coagulation of milk

Addition of a small amount of a suitable proteolytic enzyme to milk, at a temperature above 15°C, causes the milk to clot. The coagulum, which initially encloses the entire aqueous phase of the milk, spontaneously shrinks, especially if cut or disturbed, and expels most of the liquid present (syneresis). The result is a mixture of solid particles, the curd, consisting of fat enclosed in a network of protein; and a liquid, the whey, that contains the soluble components of the milk—the lactose, salts and soluble proteins. The curd particles are easily separated from the whey and they become the unripened cheese. How does the proteolytic enzyme bring about the coagulation of the milk? The answer lies in the particular properties of a protein fraction in the milk, casein, which is the protein forming the actual structure of the curd particles.

Casein micelles of milk

Cows' milk contains 3–4% protein of which approximately 80% is casein. Casein is not a single protein but a mixture of three main types α_s-, β- and κ- caseins, which respectively account for some 50%, 30%

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and 15% of the total casein. In order to maintain a low viscosity so that the milk can flow easily through the mammary gland, and at the same time to provide an effective vehicle for the transport of calcium and phosphorus to the developing young, nature has formed casein into roughly spherical complexes with the colloidal calcium phosphate of milk. Almost 60% of the calcium and phosphorus in milk is bound in these particles, each of which contains several thousand protein molecules. These complexes are known as casein micelles and are made up of subunits approximately 10 nm in diameter arranged in porous clusters 30–800 nm in diameter. Because their size is such that they scatter light of visible wavelengths, the casein micelles are mainly responsible for the turbid appearance of milk. One of the casein fractions, κ-casein, is responsible for stabilizing these casein micelles in the milk.

Enzymic cleavage of κ-casein

A protein is a polymer that is composed of approximately 20 different types of α-amino acids linked together by means of amide (peptide) bonds and arranged in a particular sequence which is characteristic for the protein. There is an amino group at one end of the protein chain, the N-terminus, and a carboxyl at the other, the C-terminus. Amino acids differ from each other with respect to the substituent side-chain groups they contain, and these vary from acidic to basic and from hydrophilic (water-loving) to hydrophobic (water-hating). The physical properties of a protein are determined by the number and type of these groups in the protein and the order in which they are arranged along the molecule. The protein chain of κ-casein consists of two quite different segments. One, the C-terminal third of the protein, is highly negatively charged and is very hydrophilic. The remaining two-thirds of the molecule carries a net positive charge and is hydrophobic in nature. The cleavage of a peptide bond between these two regions in the κ-casein splits off the hydrophilic portion of the protein and thereby markedly alters the character of the casein micelles. The net charge on the micelles is reduced, and positively charged sites are exposed which can react with negatively charged groups on other micelles. As a result, the altered casein micelles interact to form a three-dimensional network which becomes the milk coagulum. Further interaction brings the protein elements of the coagulum closer together and expels liquid from both within and between the casein micelles. The extent to which this occurs is affected by factors such as temperature and acidity which can be manipulated to control the moisture content of the curd.

Since coagulation of milk and subsequent syneresis of the coagulum is initiated by the cleavage of one peptide bond in the κ-casein, any protease, which is capable of splitting this bond at the natural pH of milk, might possibly be used for making cheese. However, degradation of the proteins at this stage of the process must be limited to avoid destruction of the interaction sites required for effective coagulation and syneresis. A proteolytic enzyme exhibits its maximum catalytic activity over a narrow range of pH values, and within this range it may be expected to catalyse the hydrolysis of all the available peptide bonds that its particular specificity allows. Thus, while any enzyme which shows maximum proteolytic activity at the pH of milk will certainly cause rapid splitting of the κ-casein, the proteolysis will not necessarily be confined to this protein, nor to only one bond in it. The ideal enzyme for clotting milk is therefore one which will catalyse only the specific and limited hydrolysis of the κ-casein. But how can we achieve a rapid action and such a high degree of specificity?

Fortunately milk is designed by nature to coagulate in just the way outlined, in order that the casein may be retained in the calf's stomach long enough to permit digestion. The arrangement of certain amino acids in the κ-casein is such that the particular peptide bond which links the two different segments of the protein is extremely susceptible to enzymic cleavage, while the calf is provided with an appropriate protease, chymosin or rennin, which catalyses the hydrolysis of this bond very rapidly at the pH of milk, but which possesses very little general proteolytic activity at this pH. Because the bond in κ-casein is so labile, other proteases which hydrolyse proteins only very slowly at the pH of milk are nevertheless able to cleave κ-casein rapidly under these conditions, and a variety of extracts of plant and animal

origin have been employed as milk coagulants for the manufacture of cheese. However, chymosin in the form of rennet, an extract of calves' stomachs, has been the preferred milk-clotting agent for cheese-making for a long time, no doubt because it performs very efficiently what is its natural function in the calf. In fact, the clotting of milk by chymosin is a very good example of the efficiency of action of enzymes. One gram of the pure enzyme is sufficient to coagulate some 22 700 l of milk in approximately 30 min at 30°C. In so doing that one gram of enzyme catalyses the splitting of approximately 100 000 g κ-casein in the milk.

Recently, expanding cheese production, rising costs and decreased availability of calf vells for the preparation of rennet extract, have stimulated the search for alternative milk-clotting proteases. Chicken pepsin, swine pepsin, a number of proteases of fungal origin, and mixtures of swine pepsin and calf rennet have been used successfully in place of calf rennet in the manufacture of a wide range of cheeses. The fermentation-derived rennet substitutes will probably play an increasingly important role in the manufacture of cheese in the future because they can be produced on a large scale by means of modern fermentation technology.

Proteolysis during maturation

So far I have described the part played by proteolytic enzymes in the coagulation and syneresis stages of the cheesemaking process. Many changes must take place, however, before the unripened curd becomes the product we enjoy as cheese, and proteolytic enzymes play an important role in this transformation. The proteins, which are major structural components of the curd, are slowly broken down so that the curd loses its rubbery character and acquires the desired texture of the final cheese. This transformation is due in part to the small quantity of milk-clotting enzyme that is retained in the curd. Although the protease used to coagulate the milk is chosen so that it does not cause extensive general proteolysis during coagulation, and despite the fact that the ratio of enzyme to protein is reduced 20–30-fold by syneresis, the prolonged period of maturation affords ample opportunity for the residual protease to modify the proteins in the curd. Also the acid produced by the starter organisms from lactose lowers the pH of the curd below that of the milk and this stimulates activity in those coagulating enzymes that are acid proteases. Not all the proteolysis occurring during cheese ripening is due to the residual milk-clotting enzyme. Other proteolytic enzymes stem from the ripening agents used for the maturation of the particular type of cheese.

Proteases that are contained in starter bacteria trapped within the curd are released when the organisms die and are then free to act on the structural proteins in the curd. In some varieties of cheese, additional ripening agents, such as moulds, are employed and their proteases also contribute to the modification of the curd. The proteolytic specificity of the enzymes present in the curd, together with the pH, the salt concentration, the water content and the temperature of storage, affects the extent to which the proteins are broken down and this in turn determines the physical characteristics of the cheese. As maturation is a continuous process, conditions must be controlled to ensure that the breakdown of proteins does not proceed to the point where the structure is destroyed.

It is not only the texture of the cheese that changes during maturation, for it is during this period that the characteristic flavour of the cheese develops. Although proteases do not contribute significantly to the development of flavour in ripened cheese—other enzymes such as lipases produced by the ripening agents are responsible for this—they can cause the cheese to develop a bitter taste. Bitterness in cheese is due mostly to the presence of protein fragments, peptides of a certain size and amino acid composition. If, during the breakdown of the proteins as the cheese matures, peptides of this type accumulate, the cheese will be bitter even though the body and texture and other aspects of flavour are satisfactory. Bitterness will not develop if the conditions of maturation are such that the proteases present are unable to break the particular peptide bonds which must be cleaved if bitter peptides are to form, or if the ripening agents produce sufficient peptidases to break them down as they are formed.

Cultured milks

Another dairy product in which proteolytic enzymes occur is cultured milk. Like cheese, cultured milks have been in existence for centuries and are considered by many to contribute to the good health and longevity of those who consume them. Although some differences in flavour and texture exist between the cultured milks indigenous to different countries, they are all basically similar, probably because they originated in similar fashion. Before the advent of mechanical separators, cream was obtained by holding milk for lengthy periods to allow the fat globules to rise. During this time adventitious lactic acid-producing bacteria in the milk would proliferate and the milk would become sour. The vessels used to hold the milk were generally earthenware or wooden and their porous surfaces retained sufficient bacteria after use to provide a substantial inoculum the next time they were used. The acid produced by the bacteria does two things. It prevents the growth of putrefying and other harmful bacteria and it reduces the charge on the casein micelles causing them to interact and form a weak gel. Thus although sour, the product is still wholesome as a food and has a much thicker consistency than milk. Today this type of product, of which yoghurt is the main example, is manufactured under carefully controlled conditions with strains of bacteria specially selected to produce the desired physical and flavour characteristics. Although the degradation of the proteins in yoghurt by the proteolytic enzymes produced by the bacteria is not very extensive, it is thought to influence the texture and consistency of the product. Because the rennin-sensitive peptide bond in the κ-casein is largely unaffected by these proteases, the coagulation and subsequent syneresis that would be initiated by its cleavage do not occur and the product remains uniform in texture for a considerable period. The peptides that are formed as a result of the limited proteolysis are rapidly broken down to amino acids by peptidases. As a result bitterness is not a common defect in this type of product.

Other applications

Control of oxidized flavour

An interesting effect of certain proteolytic enzymes on milk is that the treated milk becomes resistant to the development of the oxidative flavour defects that are induced by copper. It appears that proteolysis exposes sites in the casein fraction which effectively complex the metal ions and prevent them from catalysing oxidation of the fat. The enzyme trypsin has been found to be suitable for this purpose, but whether or not the specificity of the enzyme is important is not known. Clearly, proteases that cleave the rennin-sensitive bond in κ-casein would not be suitable, even though they might expose sites capable of binding copper, since they would also initiate coagulation.

Immobilized proteases

Newly developed techniques allow enzymes to be attached to the surfaces of solid particles or entrapped within porous insoluble structures and still retain substantial activity. Such enzymes may be used in continuous reactors where a high concentration of enzyme relative to substrate ensures rapid reaction, while the extent of reaction is controlled by the flow through the reactor. Immobilized proteases might provide the means for the continuous treatment of milk to reduce susceptibility to oxidative defects as already outlined, or they might form the basis of a continuous system for initiating coagulation in the manufacture of cheese.

Heat-stable bacterial proteases

Milk is cooled rapidly after it leaves the cow and is maintained at a low temperature during transportation and storage before processing in order to minimize the growth of microorganisms. While acid-producing bacteria multiply only slowly under these conditions, microorganisms that are adapted to growing at low temperatures and that are normally inhibited by the growth of the acid-producers, are able to proliferate in the milk. Some organisms of this type produce highly active proteases which are also very stable to high temperatures. The result is that, although subsequent processing destroys the organisms, their proteolytic enzymes survive and may cause defects such as coagulation in liquid milk products during storage.

Our knowledge concerning the ways in which proteolytic enzymes contribute to

both the desirable properties and the defects in traditional milk products has increased considerably, although it is still incomplete. The more fully we understand the mechanisms involved in these reactions the more we can hope to improve products, develop new processing techniques and control defects.

The collection and processing of edible blood

By A. Graham

CSIRO Division of Food Reseach, Cannon Hill, Qld.

The processing of edible and non-edible materials recovered as by-products of abattoir operations is an essential part of the Australian meat industry, and the value of the recovered materials contributes significantly to the industry's economic viability. For the year ending June 1976, by-products had an estimated value of $341 million. Abattoir by-products provide the raw materials necessary for the continued operation of secondary industries producing gelatine, leather, pharmaceuticals, and pet foods. However, the materials of interest to secondary processors are only a small proportion of the total by-products of the meat industry. The greater proportion of by-products is processed at the abattoirs to produce meatmeal, tallow, and blood meal. The meat and blood meals find a ready market as animal feedstuffs, but at a low price because they are sold in competition with cereal products.

The meat industry could increase its earnings from by-products by upgrading as much of the by-product output as possible from non-edible to edible products. The extent of the increase depends, of course, on the cost of the additional processing in relation to the augmented value of the final product.

Blood by-products

One of the most promising materials for profitable upgrading is blood. Dried blood has an extremely high level of crude protein (95%) and an excellent profile of amino acids essential for human nutritional needs (as shown in the table). There are many areas of the world where speciality products made from blood are already accepted human foods, e.g. blood sausages in Europe and black puddings in parts of the United Kingdom. Such products, however, could utilize only a small proportion of the total quantity of recoverable blood, which amounts to more than 80 000 t from cattle alone in Australian abattoirs each year.

Objections to the use of blood as a food are mainly on aesthetic grounds. For instance, cooked blood is almost black in

Essential amino acid composition of freeze-dried whole blood, spray dried plasma and resh beef as percentage of protein

	Freeze dried whole blood[A]	Spray dried plasma[B]	Beef[C]
Lysine	9·2	9·2	8·4
Histidine	5·6	3·5	2·9
Tryptophan	1·4	1·9	1·1
Threonine	5·2	6·3	4·0
Valine	9·1	7·0	5·7
Methionine	1·3	1·0	2·3
Isoleucine	0·9	2·9	5·1
Leucine	12·4	10·1	8·4
Phenylalanine	7·0	5·6	4·0

Sources: [A]Wilson 1974; [B]Dill 1976; [C]Schweigert and Payne 1956.

colour. Such objections may be overcome in two ways:

- the clear plasma fraction of the blood may be separated from the red haemoglobin fraction by centrifugation;
- the intense colouring of whole blood may be removed by acid precipitation and solvent extraction.

Plasma is an almost colourless liquid which dries to a fine-grained white powder. It has excellent nutritional properties, and a protein efficiency ratio of 2·14:1, which is very near that of casein (Dill 1976). Dried plasma may be used to supplement the cereal protein in bread, and results in improved bread quality as measured by loaf volume and crumb texture. Because of its high solubility and good water binding capacity, plasma is also a useful ingredient in emulsified meat products (Dill 1976). The plasma fraction, however, contains only part of the 20% solids in whole blood. Plasma makes up 60% of the whole blood volume and has a solids content of 8%. On the other hand the haemoglobin fraction, which is 40% of the whole blood, has a solids content of 40%. As the solids of both fractions are almost entirely protein, the recovery of plasma alone for edible purposes upgrades only a part of the total available protein.

The intense colouring of blood is due to the oxygen-transporting protein (haemoglobin) of the red blood cells. A process developed by the Division of Chemical Engineering, CSIRO (Wilson 1974), removes the haem pigment from either whole blood or the haemoglobin fraction by acid precipitation and solvent extraction. When the recovered proteins are air dried, the powder obtained is almost white in colour and bland in flavour.

Processing of blood

The use of blood in speciality foods in Europe has encouraged the development of processes for the collection and treatment of blood for edible purposes. The quantity of whole blood required for speciality products is, however, small and it is fairly standard practice to recover the plasma from the excess blood. In many instances the liquid plasma is incorporated into smallgoods immediately after recovery. When it is necessary to transport or store the liquid plasma it is usually frozen. The development of a fluidized bed drier suitable for use in abattoirs and capable of drying both the plasma and haemoglobin fractions has resulted in a great deal of new development work in blood collection and processing. In the drier, manufactured by Ecal–Nateko AB, Sweden, plastic spheres are used to support a thin film of the material to be dried. The coated spheres, or material carrying bodies (MCB) are passed through a warm air drying zone then through a higher turbulence zone where the dried film is removed. The MCB are then recirculated to the feed application zone for re-coating (Fig. 1).

In some countries, particularly the U.S.S.R., the processing of blood is much more extensive and products such as blood serum, fibrinogen, haemoglobin and enzymes are recovered for use in the pharmaceutical industry (Palmin 1975).

Blood clots rapidly after it is removed from an animal. Clotting is a complex process in which the soluble fibrinogen in the plasma is converted to threads of insoluble fibrin. The threads form a network of fibrous strands throughout the blood which retains the red and white blood cells in a jelly-like mass. Clotting can be prevented by removal of the fibrin, which can be done by rapidly cooling the blood to below 2°C, or by adding chemical substances such as sodium citrate, ammonium oxalate or sodium pyrophosphate which act as anti-coagulants. Sodium citrate is the most common additive used for the prevention of clotting, and in some countries it is the only substance accepted as an anti-coagulant when the blood is to be used in food products.

Two main requirements must be satisfied before blood can be considered to be suitable for processing as an edible product:

- only blood from animals passed as fit for human consumption can be processed;
- the blood must be collected in a way that minimizes contamination.

Collection of blood

There are two alternative methods for the hygienic collection of blood, the open collection technique and the closed technique. In the former, which is the traditional method for edible blood collection, the animal is stuck in the normal way and the blood is collected by placing

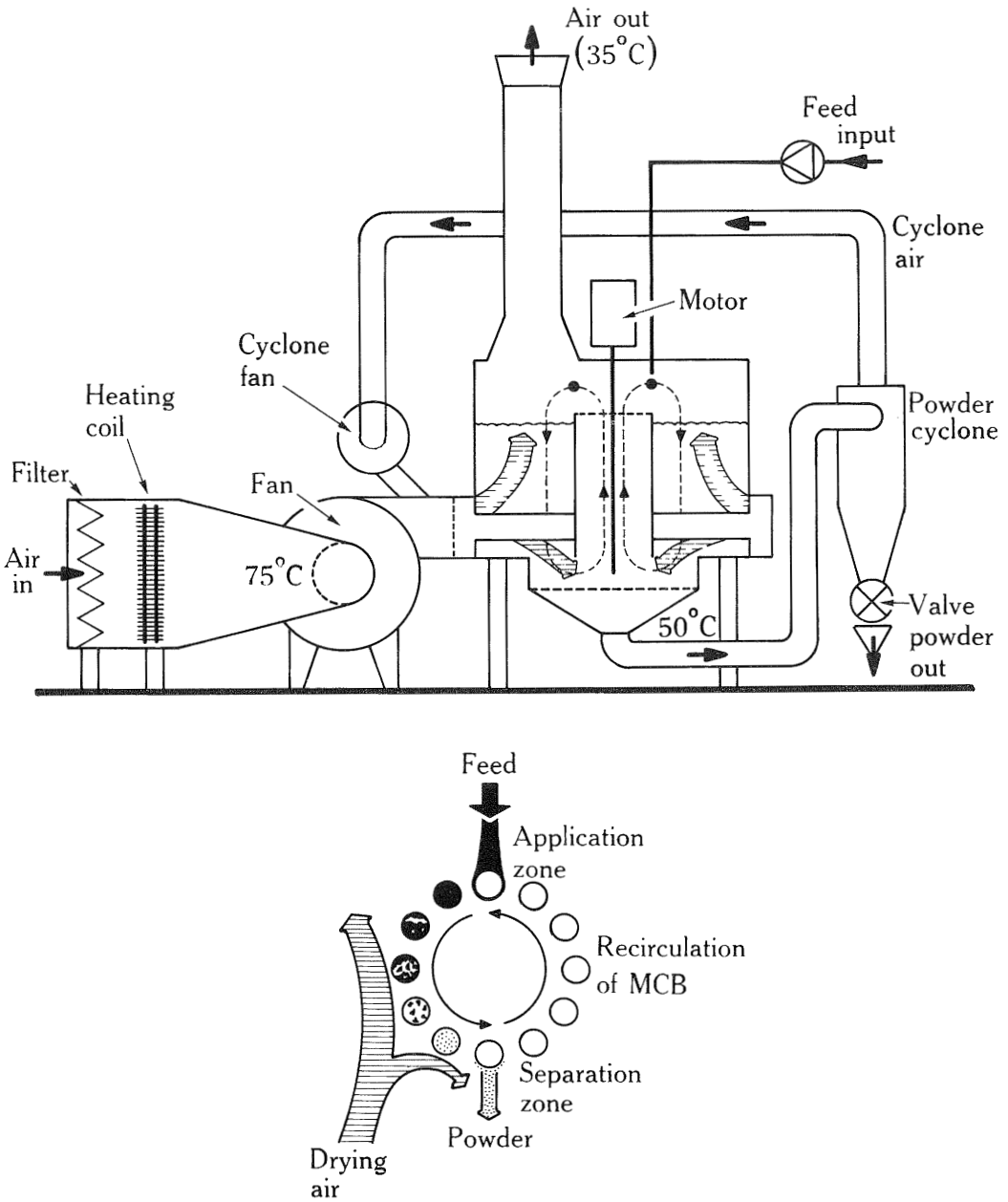

Fig. 1. Ecal-Nateko food drier

specially shaped vessels directly against the stick wound. Variations in the technique are generally in the extent to which the hide around the stick area is cleared and the shape of the collecting vessel (Fig. 2). Low levels of microbial contamination of the collected blood can be achieved by this method (Graham and McPhail 1974) provided care is taken not to contact the hide when placing the collecting vessel against the exposed tissue of the stick wound.

The closed technique of blood collection is the more recent development. It differs

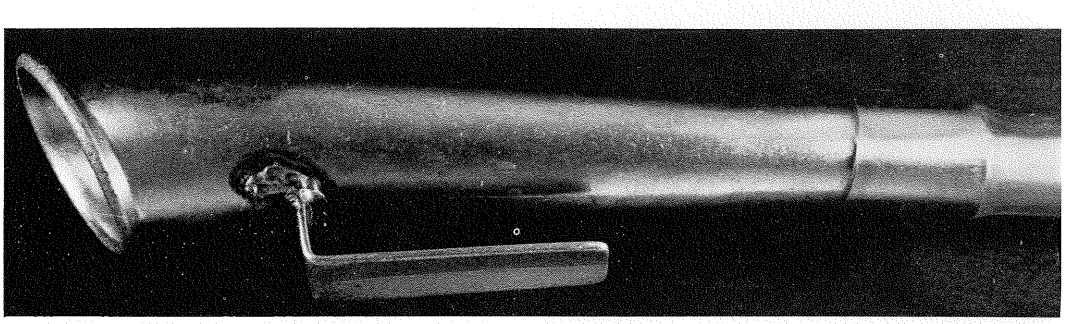

Fig. 2. Collecting cone and tube.

from the open method in that sticking is done with a knife with a hollow handle through which the blood is conveyed to tubes leading to storage vessels (Figs. 3 and 4). Where this technique is applied to pigs, it is possible to collect blood with a total bacterial count of around 100 organisms/ml (Heinz 1969), but this extremely low level is achieved only after effective sanitation of the entire collection equipment and special treatment, by singeing and scraping, of the carcass surface at the sticking site. If the sanitizing procedures are omitted, total bacterial counts may exceed 10^5/ml.

In the closed bleeding of cattle the preparation of the sticking site is simplified by the removal of a small section of hide which exposes an area of tissue. If a clean knife is then used contamination of the area is minimal, and bacterial counts as low as 10 organisms/ml are possible (Graham and McPhail 1974).

Variations in closed methods of blood collection are generally in the equipment used rather than in the basic procedures. All systems use a cutting blade to pierce the muscular tissues, and sever the major blood vessels close to the heart, e.g. vena cava and aorta. It is also common to use a hollow metal tube to support the cutting blade and connect with the flexible tubing conveying the blood to batch storage tanks. The hollow tube also acts as a convenient

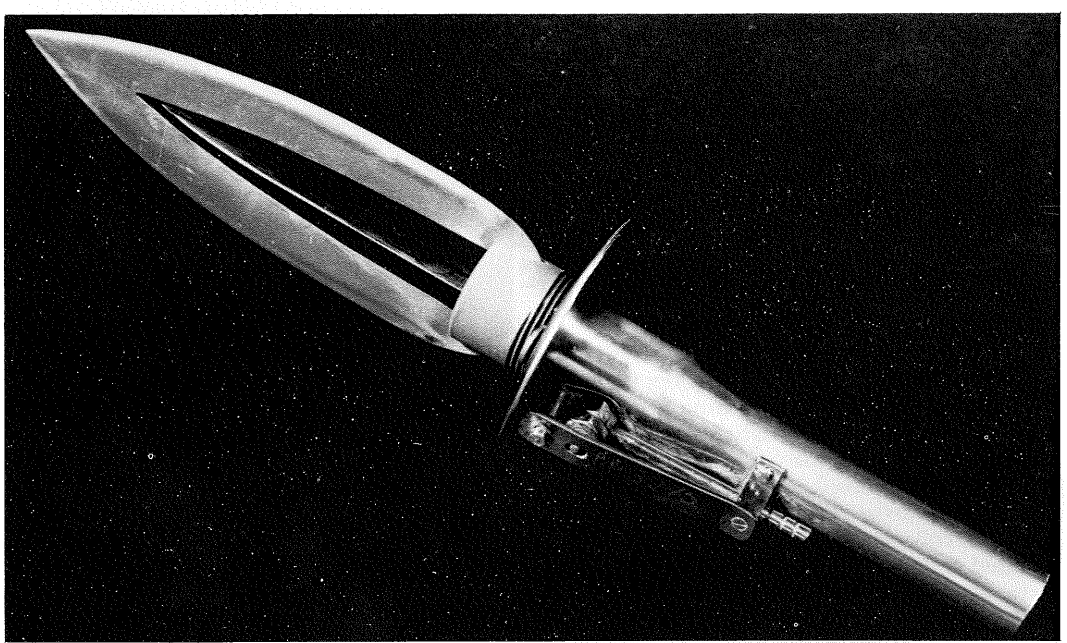

Fig. 3. Hollow handled sticking knife.

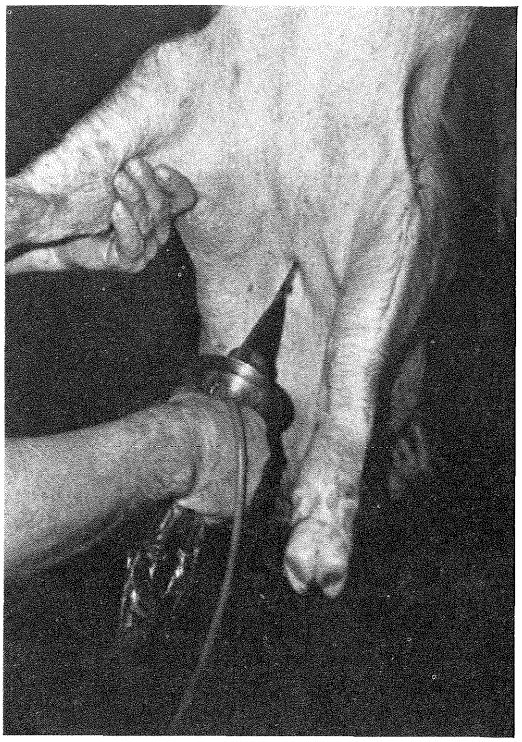

Fig. 4. Sticking knife in use with disposable tubing and anti-coagulant supply line. (See inside front cover for full colour representation.)

handle to the cutting blade. Anti-coagulant may also be introduced through a valve mounted on the metal tube.

Some systems in which the blood flows under the forces of heart action and gravity permit the use of light, flexible tubing, such as synthetic sausage casings, which may be discarded when cleaning is necessary. Other systems operate with the collection knife and tubing connected to a vacuum system. Originally it was thought that the reduced pressure in the system facilitated more rapid bleeding of the animal, but it has been shown (Popov and Vućković 1963) that the bleeding time is reduced, and then only slightly, only when the pressure is very low ($63-81 \times 10^3$ $N\,m^{-2}$ below atmospheric). The low pressures do assist removal of the blood from the collection system after it is released from the animal's circulatory system. Another advantage of using low pressures is that it is possible to leave the knife in the bleeding position unsupported. Many other minor differences are seen in entire systems, such as front leg restrainers, and means of holding the knife in the bleeding position.

Public health aspects

Health regulations require that blood must be segregated until the carcass has been subjected to veterinary examination for fitness for human consumption. In abattoirs processing small numbers of animals per day, blood from each animal is held in a small container. As the numbers slaughtered increase, however, the problem of handling a large number of containers becomes a major one. Identification, storage and final disposal of the blood to edible or non-edible processing, and the cleaning and sanitation of equipment make serious demands on both space and labour. By increasing the volume of blood held in each container to that collected in a prescribed period of time, e.g. one hour, or by collecting blood from a selected number of carcasses, e.g. 60, it is possible to reduce the labour requirements considerably. The saving in

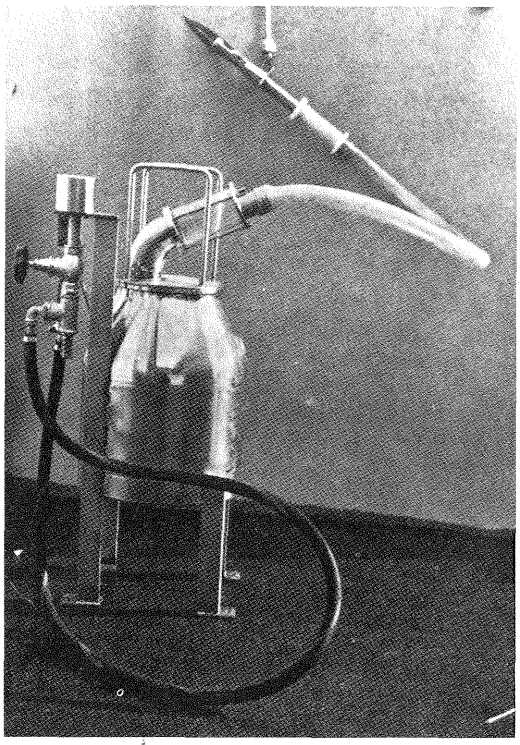

Fig. 5. Single knife unit capable of collecting the blood from up to 100 pigs per hour.

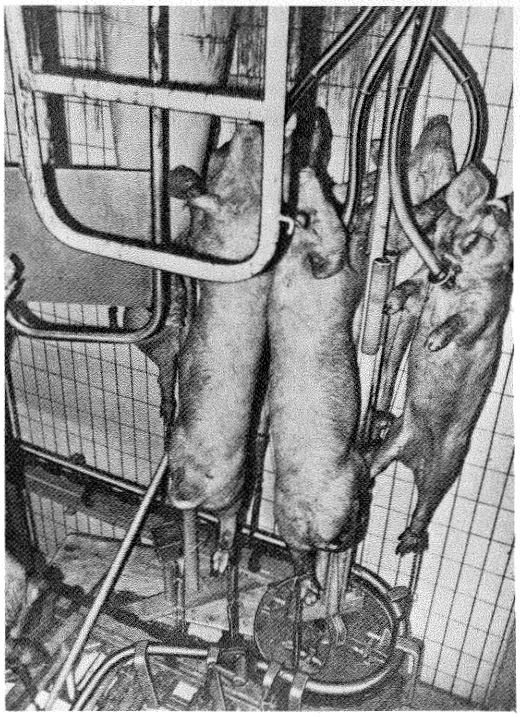

Fig. 6. Extraction carousel for pigs.

labour must, however, be set against the possibility of rejecting increased volumes of blood, since rejection of a single animal in a batch as unfit for human consumption means that the entire batch of blood must be rejected. Batch collecting appears to be the most common method used in Europe.

Commercial systems available in Europe for the collection and processing of blood range from a single knife unit (Fig. 5) capable of handling up to 100 pigs per hour, to a multi-knife unit arranged around a carousel device with a capacity of 300 pigs per hour (Figs. 6 and 7).

Under Australian conditions it is possible to use either open or closed blood collection systems (Graham and McPhail 1974). As yet, however, only a few Australian abattoirs are collecting and processing blood as an edible product, and processing is limited to the production of liquid plasma for immediate inclusion in emulsified meat products. There is an increasing awareness of the potential value of blood fractions, but future developments are very much dependent upon viable markets for the products in Australia and overseas.

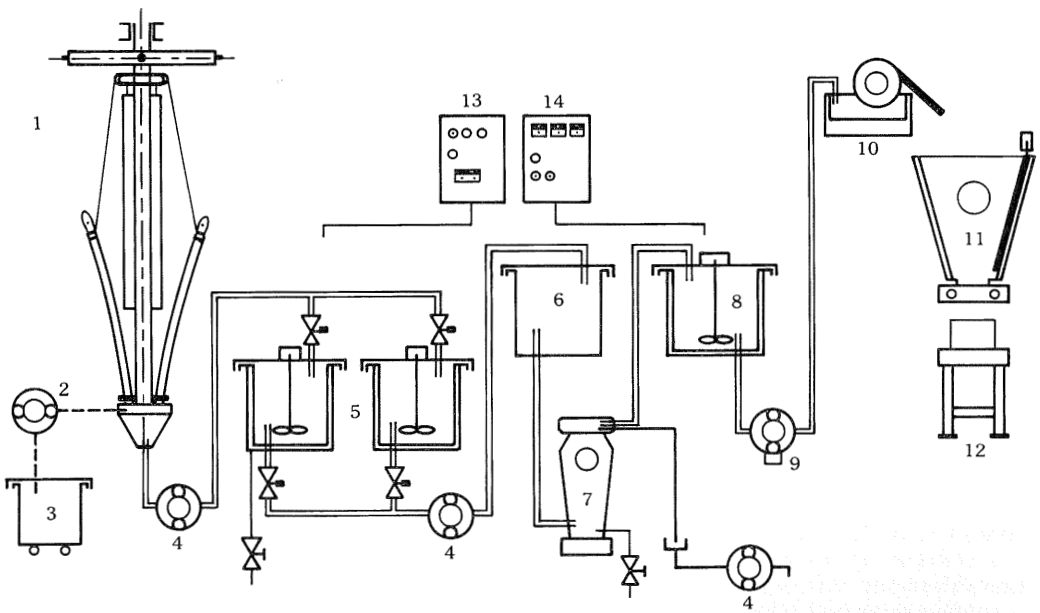

Fig. 7. Diagrammatic layout of blood collection and processing system including plasma freezer.
1. Extraction carousel with five extractor knives. 2. Fibrisol mixing pump. 3. Fibrisol tank. 4. Blood pumps. 5. Automatic separation system with stirrer and water cooling. 6. Storage tank. 7. Separator. 8. Refrigerating tank with stirrer. 9. Regulatable plasma pump. 10. Automatic ice machine. 11. Silo with loosening device and discharge belts. 12. Shaking table for packing purposes. 13. Blood extraction control system. 14. Plasma production control system.

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Genetic regulation of tomato ripening*

By E. C. Tigchelaar[A] and W. B. McGlasson

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

Fruit ripening involves a series of coordinated changes in chemical and physical characteristics which, in tomatoes particularly, transform relatively inedible tissue to a high quality delectable product. Ripening is commonly followed closely by senescence and ultimate deterioration—in many crops the entire process from initiation of ripening to the onset of deterioration may take only a few days.

Handling and shipment of tomatoes for fresh use presents particularly difficult problems in quality control, especially when long distances or prolonged periods of holding are required. Conventional low temperature storage has serious limitations as the fruit cannot be stored for long below 10°C (50°F) because of resultant 'chilling injury'. As a consequence, tomatoes are commonly harvested when immature and allowed to ripen naturally during transit, or are induced to ripen with ethylene gas before shipment. The resulting consignments often represent a heterogeneous collection of fruit at different stages of maturity, and the flavour is generally inferior to that of fruit ripened 'on the vine'.

Criticism of deficiencies in fruit quality resulting from existing handling methods has stimulated a search for new technologies.

Genetic mutations affecting tomato ripening

A potential solution to the problem has evolved from studies on the genetic regulation of tomato ripening. A number of tomato mutants which do not ripen normally have been extensively examined in order to determine how ripening is controlled in tomato fruit. One of these abnormal types, known as the non-ripening (*nor*) mutant, is particularly interesting because it shows a profound genetic influence on tomato shelf life.

The *nor* mutant was initially identified in an obscure variety known as Italian Winter, which was obtained from Dr E. A. Kerr of the Horticultural Research Institute of Ontario, who, in turn, had received it from an Italian immigrant to Canada. Apparently, the variety was grown in a local garden and when the fruit failed to ripen the plants were pulled and stored

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[A] Department of Horticulture, Purdue University, West Lafayette, Indiana, U.S.A.

during the long Canadian winter. Present seedstocks trace back to fruit which was brought to the Vineland Horticultural Research Station the following spring. The variety was later submitted to the Purdue University Agricultural Experiment Station at Lafayette, Indiana, U.S.A., where genetic and physiological studies were initiated. Investigations at Purdue, the University of Arkansas in the U.S., and the Division of Food Research, CSIRO,(Australia) have clearly demonstrated the potential practical value of the *nor* mutant, and cooperative studies are currently in progress to evaluate its commercial significance.

Several key events which occur during normal fruit ripening are effectively inhibited or retarded in the *nor* mutant:

- The onset of ripening, as signalled by a dramatic rise in fruit respiration and ethylene production, occurs six to seven weeks after flowering in ripening fruit but is not detected in mutant fruit (Fig. 1).
- Fruit softening and the associated activity of pectolytic enzymes responsible for degradation of cell wall pectic materials increase dramatically during normal ripening. Mutant fruit, in contrast, fail to produce detectable activity of the pectin hydrolysing enzyme polygalacturonase and consequently the fruit remain firm (Fig. 2) and exhibit a remarkably long shelf life.
- Colour development, which constitutes the first visual sign that ripening has begun, occurs one to two days after the onset of the respiratory rise in normal fruit. Mutant fruit begin to colour much later than normal and never achieve the intensity of colour found in full ripe fruit.

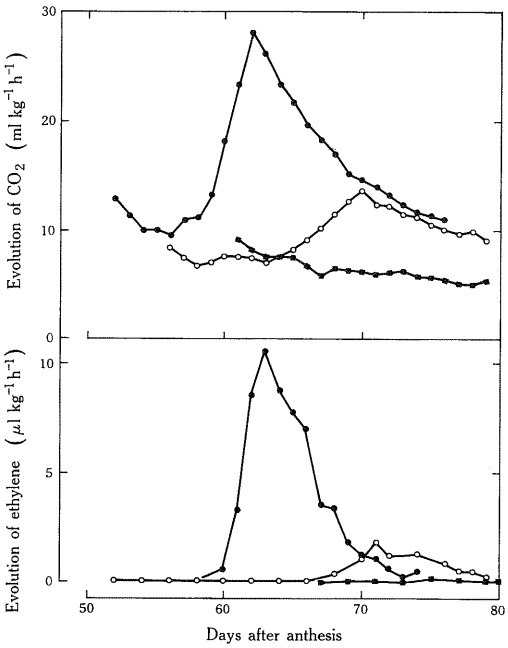

Fig. 1. Respiratory behaviour and ethylene production of normal, non-ripening (*nor*) and F_1 hybrid fruit during maturation. ●, normal; ○, F_1 hybrid; ■, non-ripening mutant.

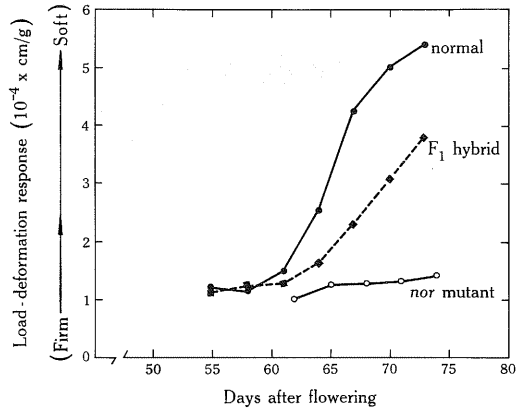

Fig. 2. Firmness changes in normal, non-ripening (*nor*) and F_1 hybrid fruit during maturation.

Utilization of non-ripening mutants

The practical value of non-ripening mutants has become apparent from comparative physiological studies of mutant and normal fruit. Mutant fruits appear to lack the ability to perform a vital step which initiates or regulates subsequent ripening changes. The ultimate objective of research with the mutants is to identify this vital step which 'triggers' fruit ripening, since such knowledge would improve our ability to store and handle fresh fruit.

Attempts to overcome the 'genetic block' by applications of growth regulators which are known to affect fruit ripening have been ineffective in inducing mutant fruit to ripen, and have shown conclusively that the primary genetic control of ripening does not occur directly through known plant growth hormones. The inability of mutant fruits to produce normal amounts of the ripening hormone, ethylene, appears to be a secondary effect of the genes involved and

Table 1. Storage life of normal and *nor* mutant hybrid fruits harvested 58 days from anthesis (red stage)

	Storage time (days) required for 50% of the fruits to be unacceptable	
Variety	15·5°C	20°C
C17 (normal)	24	15
F_1 (C17 × *nor*)	50	42

Table 2. Effect of the *nor* mutant on quality parameters in F_1 hybrid varieties

Variety	°Brix	pH	Titratable acidity	Viscosity	Colour a/b ratio
Normal	5·99	4·14	10·1	53	1·93
nor mutant	6·00	3·85	13·7	86	—
F_1 hybrids	6·05	4·18	9·6	77	2·03

the control of ripening occurs at some point prior to that of ethylene action.

A direct practical application of these abnormal ripening types of tomato has emerged from studies of F_1 hybrids developed by crossing normal ripening and non-ripening varieties. These F_1 hybrids ripen acceptably but more slowly than conventional varieties. The entire ripening process as measured by respiration rate, ethylene production, rate of colour development, softening and poly-galacturonase activity is modulated in F_1 hybrids so that there is a two- to three-fold increase in the shelf life of the hybrid tomatoes (Table 1). Despite this delayed ripening, the quality of F_1 hybrid varieties is equal to 'vine ripened fruit' of standard varieties (Table 2). This genetic attenuation in the ripening rate may allow fruit to be harvested at an optimal stage of maturity, thus improving quality and decreasing variation in maturity without undue spoilage. Cooperative pilot-scale studies are currently in progress in Australia and the U.S. to quantify the potential benefits of genetically modulating fruit ripening. These studies may provide solutions to the difficult task of providing the consumer with high quality fresh tomatoes all through the year.

News from the Division

Retirements

Mr L. H. Dickenson retired in November 1977 after an extended illness. Len joined CSIRO in 1947 and since then, has always been associated with dairy research, giving valued service as Administrative Officer at DRL.

Mr John Goldman recently retired from DRL after 25 years' service. During his last few years at DRL, John was associated with the Microbiology Section of the Cheese and Fermented Dairy Products Group.

Mr L. A. Hammond has resigned from his position at DRL to take up a position with the Australian Dairy Corporation. Apart from a brief period Les has been at DRL since 1952. During the last few years he has acted as a trouble-shooter for the cheese industry. Les will continue this role in his new position.

Obituary

The former Technical Secretary of the Division of Food Preservation, Mr R. B. Withers, died on 22 September 1977, aged 72 years. Mr Withers was for many years Chairman of the *Food Preservation Quarterly* Editorial Committee.

Grants

The Division, in a joint proposal with the N.S.W. Department of Agriculture, has received a three-year grant from the Rural Credits Development Fund of the Reserve Bank for work on improvement in quality of tomatoes. Dr W. B. McGlasson (FRL) will supervise the project.

A three-year extension of funds has been approved by the Australian Apple and Pear Corporation for continuation of work on processed products from apples and pears by Miss Helen Woods (FRL).

Visiting workers

Mr Ted Cunneen, of Asia Dairy Industries (a subsidiary of the Australian Dairy Corporation) is currently working with Mr Graham Kieseker at DRL on projects associated with recombined milk products plants in South-east Asia.

Dr A. D. King Jr, of the Microbiological Research Unit, U.S. Department of Agriculture Western Regional Laboratory, Berkeley, is working with Dr J. I. Pitt at FRL for about 10 months. His main interest is the action of mycotoxins in food spoilage.

Miss Mansoureh Fekri, UN/FAO Fellow from Iran, recently spent three months at FRL to add to her experience in the quality control aspects of fruit and vegetable processing.

Professor C. A. Ernstrom, head of the Department of Nutrition and Food Sciences, Utah State University, spent six months at DRL. He worked on the manufacture of hard and semi-hard cheeses from milk concentrated by ultrafiltration.

Work experience

FRL has actively participated in a work experience program organized by a number of high schools in the Sydney area. Under the program, selected students in years 10–11 are attached to Sections for a week or more and given the opportunity of doing some laboratory work as well as seeing at first hand a cross section of the research of FRL.

Awards

Mr Greig Zadow has been awarded the Australian Society of Dairy Technology's Silver Medal—the Loftus Hills Dairy Science Award for published work that has made a significant contribution to the knowledge of dairy science and technology.

Dr K. E. Murray, FRL, has been elected a Fellow of the Australian Academy of Technological Sciences.

Mr I. R. McDonald, Divisional Secretary, has won a CSIRO Jubilee Study Award to review management procedures in overseas research institutions. He will visit centres in Britain, Europe, and the U.S.A.

Conferences

The First Australian Food Microbiology Conference for the exchange of information on microbiological problems associated with food poisoning and spoilage in Australia, was held in Sydney on 18–19 August. The eminent food microbiologist, Professor D. A. A. Mossel from the University of Utrecht, was the keynote speaker. The conference was organized by the AIFST N.S.W. Branch Food Microbiology Group, with Dr W. G. Murrell, FRL, as coordinator. Six symposia were held on the following subjects: Indicator organisms; Microbiological food standards; Food-borne disease agents; Food spoilage; Microbiological hazards of international trade and travel. The conference attracted about 250 people.

A Post-harvest Research Workshop was held at Glenelg, S.A. from 13–16 September 1977, and was attended by some 30 participants. The Workshop, organized by the Fruit and Vegetable Post-harvest Research Subcommittee (Chairman: Dr D. Graham, Secretary: Mr G. Fisher, FRL) discussed three broad topics: Role and mode of action of mineral elements; Residues in post-harvest chemicals; Towards an integrated system of handling produce from farm to consumer. Proceedings will be printed by CSIRO and readers requiring a copy should contact the Technical Secretary.

Appointments

Mrs G. L. Veith, B.Sc., has joined the Tasmanian Food Research Unit (TFRU) for a year as an Experimental Officer, to work on the processing of rock lobsters.

Dr T. L. Lewis, Principal Research Scientist, formerly of the Division of Horticultural Research, has transferred to the Division of Food Research and is working with TFRU on the physiology of bitter pit in apples.

Dr J. Speirs has been appointed to FRL for three years to participate in a research program investigating the regulation of protein synthesis in mature cells; he will join the Plant Physiology Unit at Macquarie University.