"STORAGE CHANGES IN PORK PIES"

by

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Abstract.

This research project was designed to investigate the chemical and physical changes in pork pies during storage. Lipid oxidation and moisture migration were found to be the parameters of most importance, with protein cross-linkaging and colour changes in the meat filling having less of a deteriorative effect on pie shelf life.

The extent of lipid oxidation and the development of rancidity (taste and odour) were found to be affected by cooking, the age of the meat and back fat in the meat filling and by their storage conditions (temperature and time). An increase in all or any of these parameters was found to increase the rate of rancidity development. On the other hand the rusk and the seasoning mix used in the meat filling of the pies, were found to reduce the rate of rancidity development. This antioxidant activity was investigated further. In the rusk, substances produced by the maillard browning reaction during the course of its manufacture were found to be antioxidative. However prolonged heating, although it produced a rusk with high antioxidant activity, had lowered its water binding capacity sufficiently to prevent its use in the meat products where water has to be bound to an inert filler.

The antioxidant component of the seasoning mix was found to be white pepper, with the ethanol soluble fraction exhibiting this activity.

Lipid analyses showed that rancidity developed parallel to the loss of the unsaturated fatty acids which had a chain length of 16 or above carbon atoms; and a concomitant rise in

the concentration of short chain fatty acids and aldehydes, especially those having five and six carbon atoms in the chain length. The level of hexanoic acid (C6:0) rose as rancidity developed, being development most marked in the pies with the fastest rate of rancidity development (i.e. those devoid of both the rusk and the seasoning mix).

Moisture migration, both from the atmosphere and from the jelly into the pastry (especially into the brown outer layer of pastry) resulted in an increase in moisture content, and a decrease in texture, of the pastry. Threshold levels for these two parameters were established above and below which respectively the pastry was deemed unacceptable, and these values were used subsequently in the investigation to assess the effect of various products on shelf life extension.

Simultaneous lowering of the relative humidity (r.h.) of the external atmosphere, and lowering of the jelly a_w, to levels at which to prevent moisture migration into the pastry resulted in a 35 day shelf life for the pies. However the use of glycerol to reduce the jelly a_w to 0.56 resulted in an unpleasant taste to the pie. Lowering the jelly a_w to 0.84 gave an extension (of at least 2 days) to the usual 8 days shelf life. Unfortunately attempts to achieve this a_w (0.84) by the use of binders and pH, were unsuccessful. Similarly the use of cetyl alcohol as a moisture barrier within the pork pie was not effective.

The formation of a gelatin-lactein cogel, showed excellent potential for reducing moisture migration in pork pies.

Acknowledgements.

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Chapter 1.

Introduction.

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Despite the fact that meat animals are an inefficient way of manufacturing protein, it is almost certain that the use of ruminants on marginal land will never be replaced totally by any alternative man-made process, capable of using such land to produce convenient nutritional and acceptable food.

As economic costs put the price of carcase meat beyond the budget of many consumers, the further development of nutritious meat products of high eating quality would clearly be appreciated by both the consumer and the meat industry. One meat product which is very popular in Great Britain is the pork pie. Its popularity is especially great during the summer months and at Christmas and New Year. There are several large manufacturers of pork pies in Britain today, along with many small producers (i.e, the village butcher) who have a limited supply and outlet. The major producers, however, supply pies to all parts of the country. This results in a very competitive market; and these producers are engaged in a continuous effort to try to gain the maximum share of the market. In order to achieve this, the manufacturer may change or improve his product. If the pie produced is already a quality product, then, one way it can be improved is to ensure that the pie does not deteriorate on storage as fast as those of competitors.

One of the main disadvantages of pork pies is the softening of the pastry case during storage, leading to a limited shelf life of about 8 days from manufacture. The other problem is off-taste and off-odour development in the pie, especially in the meat filling. This is a result of

lipid oxidation occurring, and limits the shelf life of the meat filling to between 8 and 15 days post manufacture.

If sufficient extension of the pork pie shelf life could be economically achieved, then there would be a considerable advantage obtained by both the consumer and the manufacturer. It was against this background that the present investigation was undertaken. This involved the study of the softening of the pastry of pork pies using various techniques, and criteria (threshold values) which were elaborated during the course of the study. This led to the use of various humectants, binders and pH values to control the available water (a_w) of the jelly and the relative humidity (r.h.) of the atmosphere with a view to delaying the attainment of the values for moisture content and texture above and below which respectively, the pastry became unacceptably soft.

The development of rancid odour and taste, due to lipid oxidation, was also studied, and the effects of various processes involved in the pie manufacture were among the many factors investigated.

This thesis describes the results obtained from an investigation of the problem of storage changes in pork pies, and some conclusions derived from these.

Chapter 2.

Literature Review.

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2.1. Pork pie manufacture.

The pork pie is considered a traditional food in the areas where it is manufactured, such as the Midlands and North of England (M.A.F.F., 1980). Hot water crust pastry was the earliest known form of pastry case. It dates back to the fourteenth century when it was used in a pie type product known as "pig pye" (Hartley, 1954).

Estimates in 1981 gave the consumption of meat pies to be 120,000 t annually (Butcher, 1981). The figure would be even higher if catering material, and frozen pies, were included. Meat pies can be considered to belong to one of two main categories:-

1. Hot eating pies, e.g. steak and kidney pies (representing 70% share of the market).
2. Cold eating pies (representing 30% of the market).

Cold eating pies experience strong seasonal and regional variations in demand.

Pork pies consist of hot water crust pastry (raised by hand or machine), with a meat filling of comminuted pork fat and lean, seasonings, filler, water and, if required, sodium nitrite to produce a cured pink colour. A typical recipe is as follows (Wilson, 1981):-

Compound	%
pork	56 - 59
pork fat	27 - 25
rusk	5.5
seasonings	2.3 - 3.6
nitrite	0.3
water	8 - 9

The fat and lean are minced together and then mixed or bowl-chopped with the other ingredients. This filling material is metered out into the formed pastry cases; held in hoops or tins. These are lidded, then glazed and baked.

The aim of baking is to produce a pie that is microbiologically safe and stable. This is achieved by developing temperatures of at least 80°C in the core of the pie during baking. The baking process causes changes in the pie. At 55°C, the meat fat fractions become molten. If the meat filling is of a coarse nature, the fat can migrate out onto the surface of the meat and out onto the baking trays via the pastry (Sperring and Webb, 1973). If tins are used instead of hoops to support the pies, this fat remains in the tin and virtually fries the pie (Wilson, 1981). When pastry hoops are used the fat re-enters the pie pastry during the initial cooling stage of manufacture. This results in a pastry texture gradient being formed, with the crispest pastry being at the base of the pastry wall, and the least crisp being at the lid.

As the temperature rises the proteins begin to denature. The molecules of gluten from the flour, and the proteins of the meat, start to lose their associated water and this water passes into the filling where it is absorbed by the filler present. Excess water is driven off along with excess fat as the temperature continues to rise. Water may "boil out" under the pastry lid and take with it soluble protein and fat. This causes undesirable

discolourations (Sperring and Webb, 1973): it can be overcome by creating a vent in the pastry lid to allow excess water to excape.

After initial cooling, to allow the pies to be removed from the tins or hoops, the pies are "jellied". The aim of "jellying" is to disperse a solution of gelatine throughout the pie, filling any gaps caused by the meat shrinking on cooking. The pies are injected with a gelatine solution at a temperature above 45°C (to reduce microbial contamination, Wilson, 1981).

The pies are best "jellied" at a core temperature of 65 - 70°C, when the pastry is sufficiently cool to minimize the absorption of the jelly solution. Usually a 6% gelatine solution (of 160-200 bloom strength) is used (Wilson, 1981). Blower (1981) found that as the pie core temperature was lowered from 80°C to 20°C, less jelly solution was absorbed by the pastry. Butcher (1981) reported that jellying the pies at a core temperature of 23°C significantly reduced the incidence of pastry softening. However this is not commercially feasible due to the possibility of microbial contamination of the jelly.

Pies are cooled to reduce their temperature to minimize microbial survival. Pork pie ingredients can alter the product in many ways:- The flour used in the pie crust contains 8-12% protein, and the actual protein concentration can have a variety of effects on the pastry. A high protein flour produces a paste (raw pastry) that is tough to handle and allows excess fat seepage, but

produces a crisp pastry upon baking. A low protein flour results in increased jelly absorption by the pastry (Wade and Meers, 1973).

For pie pastry a fat is required that will "set up" in the raw pastry yet still allow it to be pliable enough for blocking out or raising without splitting. This setting ability contributes to the firmness, as well as to the shortness and crispness of the pastry (Weedon, 1973).

Sperring and Webb (1973) claimed that lard pastry was crisper than vegetable oil pastry. Blistering of the pastry lid was encountered when using lard; but this was offset by the better sealing of the lid to the box. Lard will give a distinctive flavour to the pastry, which can be affected by the pigs' diet (Inglett, 1974).

Water is used to make a paste (dough) with the flour and fat. Excess water causes fat seepage and a paste which is soft and difficult to handle. Insufficient quantity of water produces a tough pastry which reduces jelly seepage into the pastry (Robb, 1973). The same author commented that the temperature of the water was important. The best paste and pastry was produced when the temperature of the water was at least 82°C. Lower temperatures resulted in more jelly absorption by the pastry, leading to a loss of texture (crispness).

The meat filling ingredients must obviously affect the pie quality, ideally the lean to fat ratio should be 2:1. If more lean is used, the filling is hard and dry, according to Wilson (1981). It would also be more expensive to

produce, whilst excess fat in the filling is discriminated against by the consumer.

Seasonings were added for their flavour characteristics, whilst the rusk is incorporated into the mix as a filler, it also 'soaks' up any water released during baking. If present in sufficient quantity (5%), it helps prevent "boil out" an undesirable occurance during manufacture (Sperring and Webb, 1973).

2.2. Deteriorative changes in pork pies occurring during storage.

Storage refers to the period of time elapsing from when the pies are wrapped to when they are consumed. During this time the pies will be kept in different environments e.g. in the transporter container, in the store/shop cold room, in the store/shop cold display and finally in the consumer's refrigerator. These changes of environments can accelerate the changes that are occurring in the pies, and these will be discussed in the following sections.

2.2.1. Colour changes in the meat filling.

Colour is the total impression as seen by the eye (Forrest et al., 1975) and cannot exist without an observer to perceive it (MacDougal, 1983). Any specific colour consists of three attributes: hue, chroma and value. Hue describes the wavelength of the light radiation in terms of the red, blue or green components. Chroma describes the intensity of the colour compared with white light, and

value is an indication of the overall brightness of the colour. Colour, although a physiological concept, can be measured in physical quantities if the hue and value can be defined relative to a standard observer as is done in the C.I.E. System of measurement.

Colour is due to pigments that absorb light at certain wavelengths and reflect light at other wavelengths. The predominant pigments in meat are haemoglobin, the blood protein pigment and myoglobin, the muscle pigment. Myoglobin contributes 80-90% pigment in a well bled carcass (Forrest et al., 1975). Also present (as minor pigments) are catalase and cytochrome enzymes.

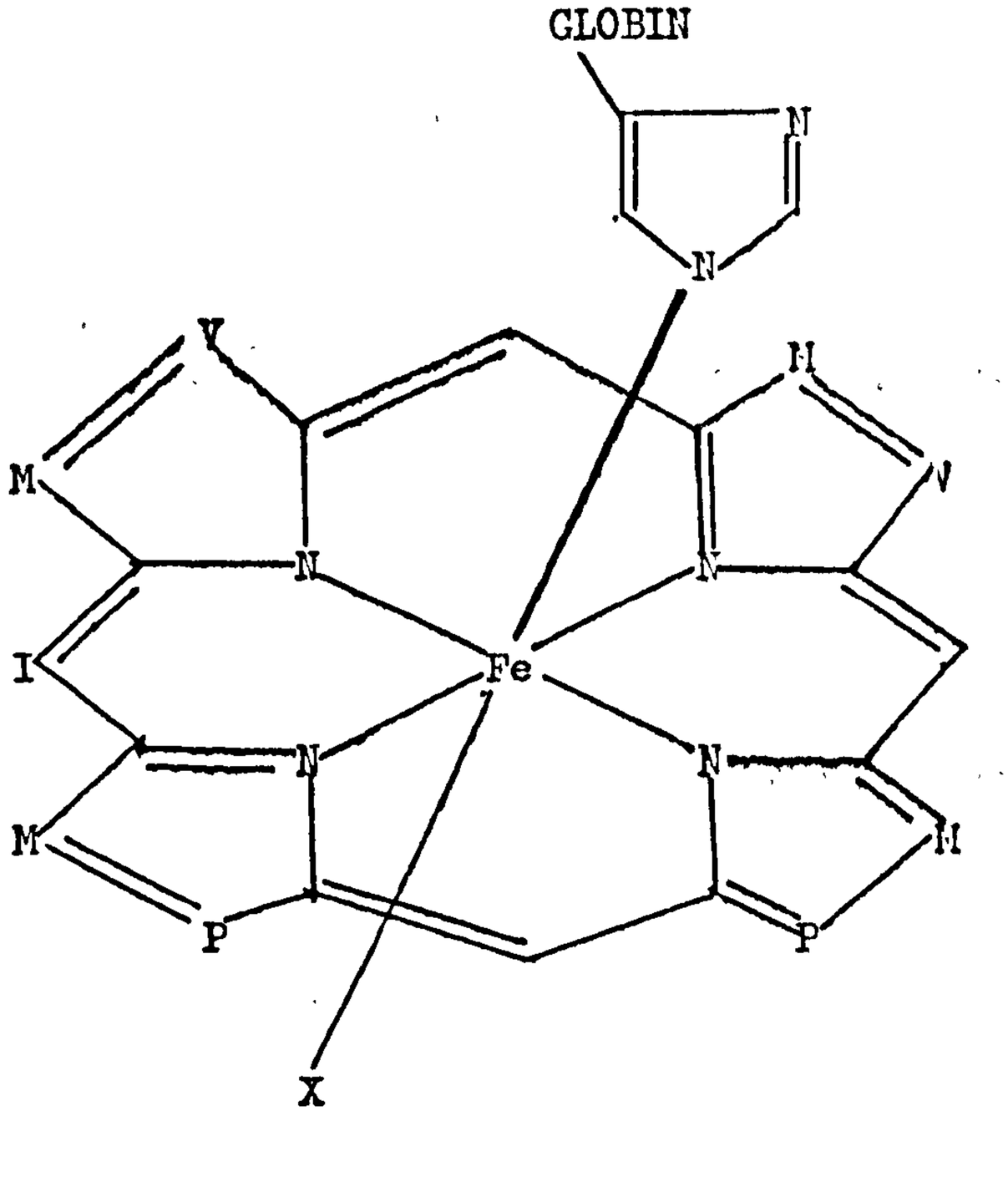
Myoglobin consists of a globular protein globin and a haematin ring. The iron in the haematin ring is co-ordinated with the four pyrrole nitrogens of porphyrin (Figure 2.1).

The colour of meat is partially dependant on the chemical state of the iron in the haematin ring. As the chemistry of myoglobin has been reviewed (Antonini and Brunori, 1971), with reference to meat colour (Giddings, 1977; Livingston and Brown, 1981) it will not be dealt with here in detail. However the main reactions and colour changes are illustrated in Figure 2.2.

When it is in the ferrous (Fe^{2+}) state the iron in the myoglobin can combine with such molecules as oxygen, nitric oxide and carbon monoxide. This ability is lost when the globin is denatured e.g. by heat. This leads to a tendency for the iron to oxidize to its ferric form (Fe^{3+}).

FIGURE 2.1

Schematic diagram of the haematin complex.



The globin and X molecules are not on the same plane as the haematin complex.

X is an atom or compound with the correct steric configuration to fit into the haematin pocket of the globin chain and coordinate to the iron atom.

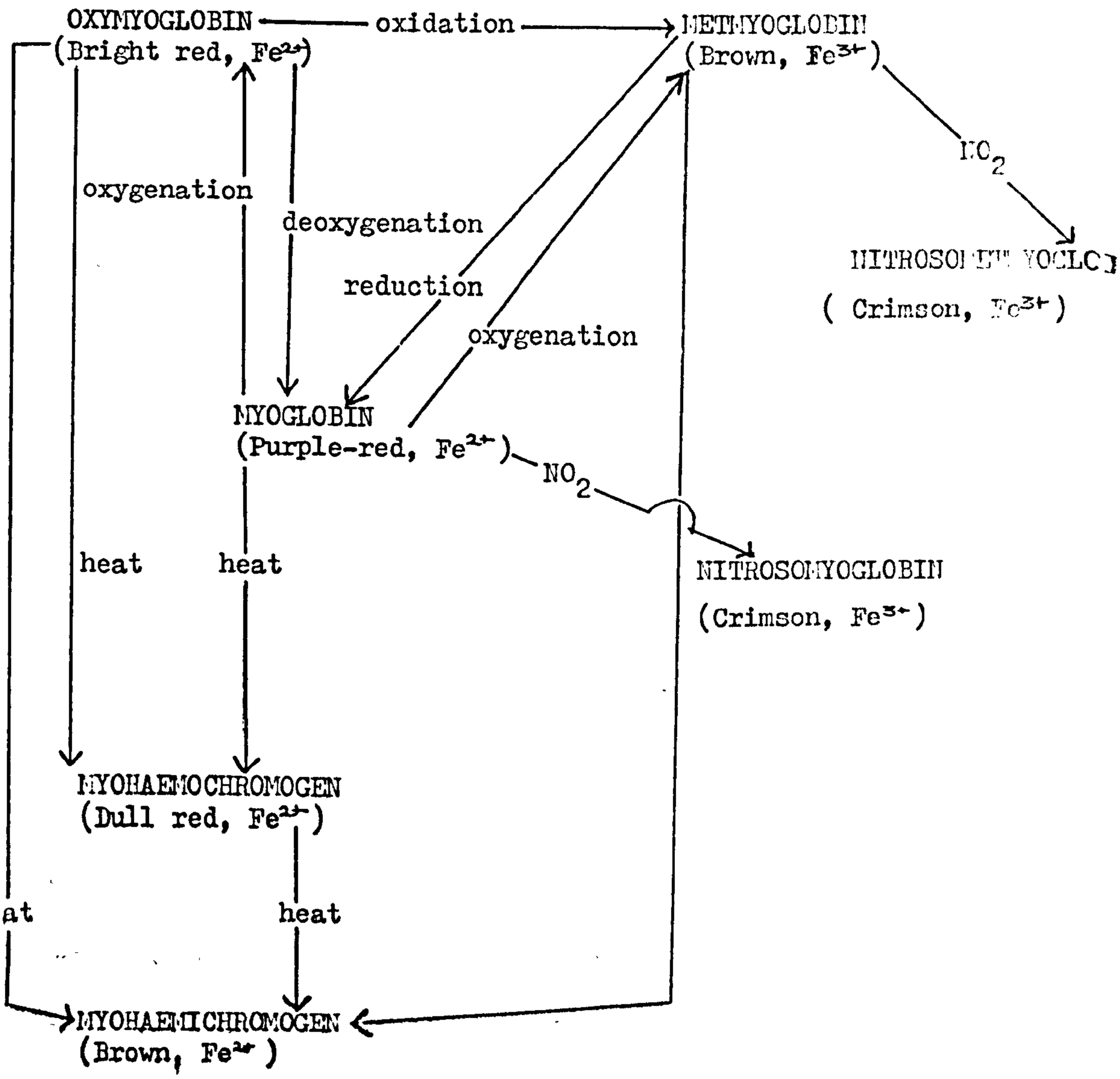
M = methyl radical

V = vinyl radical

P = propyl radical

FIGURE 2.2

Chemical state, reactions and colour changes of myoglobin as it applies to pork pie meat filling colour.



(Lemberg and Legge, 1949). If the ferrous iron(Fe^{2+}) in myoglobin and oxymyoglobin oxidizes to the ferric form the brown metmyoglobin is produced (Watts, 1954). These three pigments can be denatured by heat to form ferrous and ferric complexes of haematin and denatured proteins termed haemochromogen and haemichromogen respectively by Lemberg and Legge, (1949). The ferrous pigments (from oxymyoglobin and myoglobin) are red and are readily oxidized to the ferric form which is brown (MacDougall, 1983). Ledward (1974) postulated that the ferric pigments were haematin di-imidazole complexes. However Giddings (1977) doubted that two denatured protein - bound imidazoles could occupy the two haem axial co-ordination sites.

2.2.2. Changes in moisture and texture.

Unless consumed, most foodstuffs will eventually be spoilt by micro-organisms. This spoilage can be retarded by changing the environment. The available moisture (a_w), rather than the total moisture content is one of the controlling factors in microbial growth. Available moisture is a measure of the unbound free water that is available to support biological and chemical reactions (Insalata, 1972). A_W also determines the water (moisture) content of the food, its nature and extent of water binding (Loncin *et al.*, 1974), and thus contributes to the food's texture (Labuza, 1974). A_W is more closely related to the physical, chemical and biological properties of the food than is the total moisture content (Rockland and Nishi, 1980).

Exposing foodstuffs to environments having a different a_w will lead to the establishment of an equilibrium between the food and the new environment. Foods will lose or gain weight, therefore when stored at different relative humidities (rh). If weight losses are plotted against r.h. a moisture sorption isotherm can be obtained. The available moisture, a_w, may be defined as follows:-

$$a_w = \frac{P}{Po} = \frac{\% RH}{100}$$ (Labuza, 1971)

where:

P:- water vapour pressure exerted by the food material.

Po:- vapour pressure of pure water at temperature To.

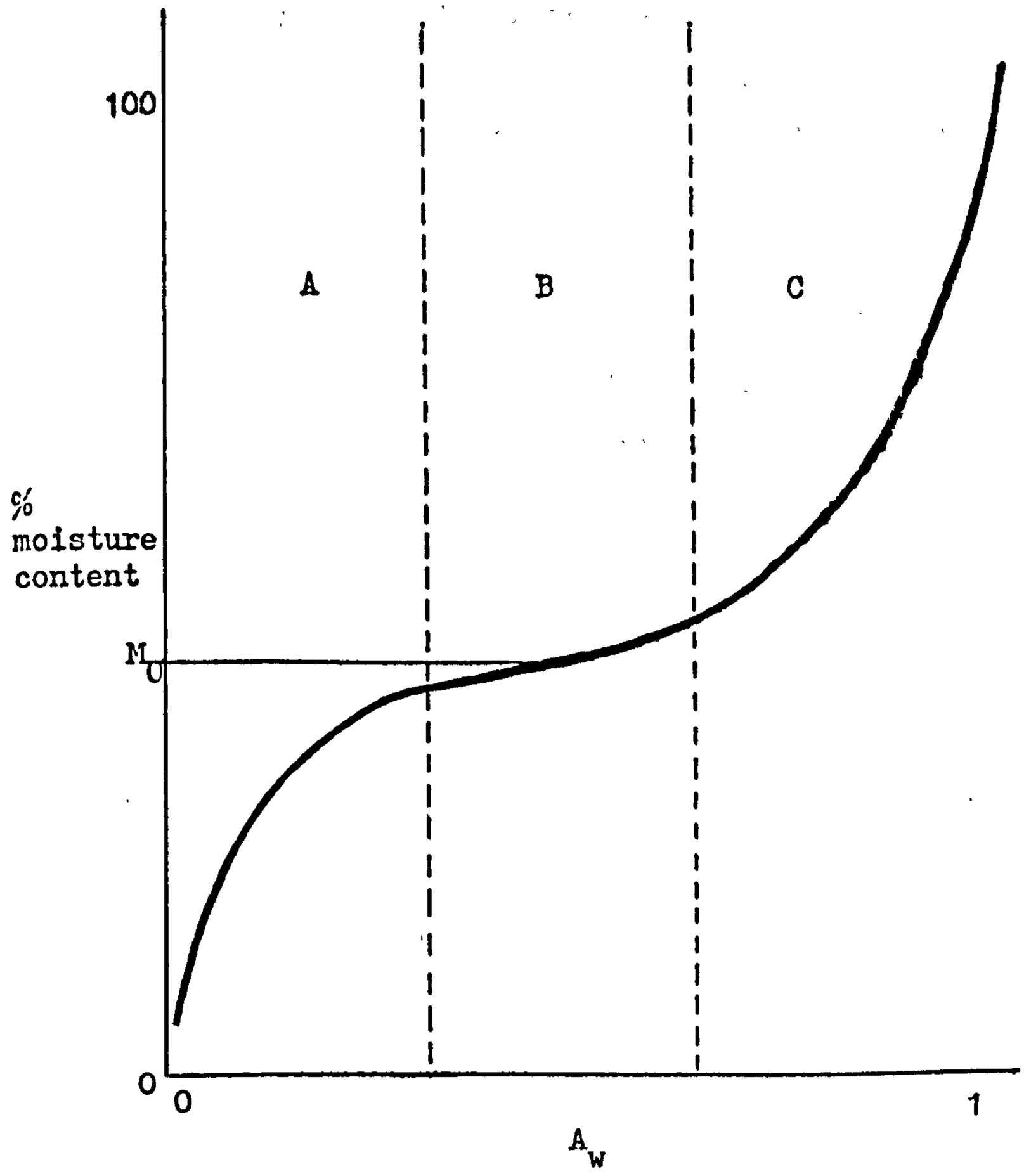
To:- equilibrium temperature of the system.

The adsorption isotherm (Figure 2.3), is prepared by placing completely dry material into atmospheres of increasing rh, and measuring the weight gain due to moisture adsorption.

Three regions can be distinguished in the sigmoidal shape of the isotherm, these are defined by their water content. In region A (of Figure 2.3) water is adsorbed as a monolayer film. According to Rockland (1969) the water molecules are believed to be bound to carbohydrate and to carbonyl groups. In this region the a_w is below 0.20 and the water is held tenaciously (Brockman, 1973). When all such sites are occupied, this state is known as monolayer completion (MO in Figure 2.3). It is near this point

FIGURE 2.3

Moisture adsorption isotherm for a food.



that the water content has a marked affect on a_w. At a_w's above the monolayer state (B in Figure 2.3) there is adsorption of additional layers of water over the initial layer. The additional water is bound to the latter by hydrogen bonds via hydroxyl and amide groups (Rockland, 1969). It is in this region (B),: a_w 0.2 to 0.6, that a more ordered structure appears (Brockman, 1973) and the water present shows a marked resistance to freezing.

In the third region (C) water condenses into the food material's pores and dissolution of the solute material occurs, thus causing a lowering of the vapour pressure of the food (Rockland, 1969).

When food comprises ingredients with different a_ws, stability problems arise, since moisture migrates from one component to another in an attempt to establish an equilibrium a_w. Bushill (1968) found that moisture migrated from the meat filling into the crust in a meat pie. This resulted in loss of pastry crispness, and eventual mould spoilage of the product. Butcher (1981) implicated moisture migration from the pie centre into the pie crust as the major cause of pork pie pastry softening. He also found that low temperatures lessened the pastry softening caused by such moisture migration; but they had a detrimental affect on appearance. Bushill (1968) claimed that moisture migration could be controlled by a knowledge of the water vapour pressure of the individual component parts of the product. The product formulation was adjusted, accordingly, to reduce or eliminate the different vapour pressures (a_ws).

The effect of a_w on texture is difficult to establish due to the general problems which are associated with attempts to quantify texture. Texture is the least defined, described and understood organoleptic property of food. It is not a single well defined attribute (Brennan, 1980) but, an important complex attribute of food quality (Brennan, 1980). Crispness is a term used to describe the textural characteristics of pastry, but even so, according to Vickers and Bourne (1976), the term is not an adequate description of the properties it purports to describe. Crispness differs from crunchiness and brittleness (Vickers, 1975). It is a universally liked characteristic (Szczesniak and Kahn, 1971).

Katz and Lubuza (1981) reported on the textural characteristics and a_w in snack foods, and concluded that as a_w increased, sensory acceptability decreased. Critical a_ws were found where these products were deemed unacceptable. This, they proposed, was due to gradual intermolecular bonding changes as the moisture content increased above the monolayer state, resulting in a decrease in cell wall stiffness and loss of crispness (Vickers and Bourne, 1976).

In pastry, hydrogen bonds and van der Waal forces form links between the carbohydrate macromolecular matrix in the monolayer phase. This causes strong macromolecular interactions, which form crystalline-like zones (Labuza, 1968). These zones probably give rise to "crispness" as a force is required to break them. When the water content is above the monolayer value there is a decrease in macromolecular interactions, since water breaks the bonds concerned.

Thus, there are less crystalline zones (Suggett, 1975). The water also acts to 'plasticize' the macromolecules, allowing them to 'slip' past each other. The effect is perceived as a loss in crispness by the consumer (Butcher, 1981).

2.2.3. Flavour changes.

Flavour is an important aspect of meat quality, and it would be difficult and unrealistic to separate it from odour, and taste as those contribute to the quality we know as flavour, Amerine et al., (1965). The characteristic odour and taste of cooked meat are produced from precursors in the fat and lean.

The flavour of cooked meat can be produced to some extent by heating a dialysate of lean meat. This contains glycoprotein and inosinic acid, which when reacted with glucose inosine and inorganic phosphate (all present in meat) give rise to meaty odours and flavours. According to Lea (1965), the maillard-type reaction, and other browning reactions, contribute to flavour. Volatile carbonyls, sulphur compounds, pyrazines, ammonia, acetone, diacetyl, propionic acid, butyric acid and formic acids all play significant parts in the flavour of cooked meats (Patterson, 1975).

There is no standard method to identify and quantify flavour. G.L.C. - M.S. (Gas Liquid Chromatography - Mass Spectroscopy) and integration by computer have allowed routine identification of complex odouriferous mixtures in

foods (Wong et al., 1975; Buttery et al., 1977). However this has led to some confusion as the compounds isolated have not always corresponded with subjective odour responses (Lawrie, 1979).

Consumer, or trained, taste panels can give reliable quantitative and qualitative information on the nature of odours and tastes in cooked meat (Ford and Park, 1981). However tenderness, and the nature of the illumination can interfere and can reduce the taste panel's sensitivity to flavour, causing disagreement over the more subtle odours and flavours.

Flavour can be affected by other factors:-

Thus females are more sensitive than males to Boar taint (Griffiths and Patterson, 1970), also Patterson and Stinson (1971) found a significant effect of breed on degree of boar taints between Landrace and Large White boars.

Use of high linoleic acid diets results in a faster rate of rancidity onset (Bremner et al., 1976) in meats derived from such animals, results that were verified by chemical analysis and taste panels,

Tuomy et al., (1969) found a positive correlation between oxygen uptake in meat products and undesirable odours and flavours.

In raw meat flavour changes occur with time of storage, even at -18°C - cooked meats stored at 4°C can undergo very rapid changes in flavour. The phenomenon was first reported by Tims and Watts, (1958), who terms it warmed over flavour (W.O.F.). It is due to the very rapid

onset of rancidity. Rancidity arises from the interaction of oxygen with unsaturated fatty acids present in neutral lipids and phospholipids, and produces off-flavours which make the food unacceptable on a consumer market level (Labuza, 1971).

WOF was assumed to be due to metmyoglobin-catalyzed lipid oxidation (Hirano and Olcott, 1971; Younathan and Watts, 1960) but it has been claimed since that non-haem iron is the major pro-oxidant in cooked meat (Love and Pearson, 1974). Igene et al., (1979b) found pork held at 4°C for 48 hrs was more susceptable to WOF than similar samples held at -18°C.

Wilson et al., (1976) observed a relationship between phospholipid levels and WOF development. Whilst Igene and Pearson (1979) presented evidence that phospholipids were the major contributors to WOF in cooked meat model systems, triglycerides enhanced WOF development only when combined with phospholipids.

WOF development can be retarded by use of 2% ethylenediamine tetraacetic acid (ETDA) which chelates the non-haem iron and significantly reduces lipid oxidation. Non-haem iron's origin is unknown (Igene et al., 1979b) but it is present as about 10% of the fresh meat total iron content.

Zipser et al., (1964) found lower rancidity scores (TBA values) in cooked cured pork than in uncured pork, although Liu and Watts, (1970); Sato and Hegarty, (1971); Bailey and Swain, (1973) reported that nitrite inhibited WOF development. Fooladi et al., (1978) determined the

role of nitrite in preventing WOF development, and found an inhibition of rancidity which was detectable both by taste panels and TBA analysis. They concluded that nitrite possibly acted by protecting the phospholipids, as the levels of the compounds related closely to TBA values.

2.3. Lipid oxidation.

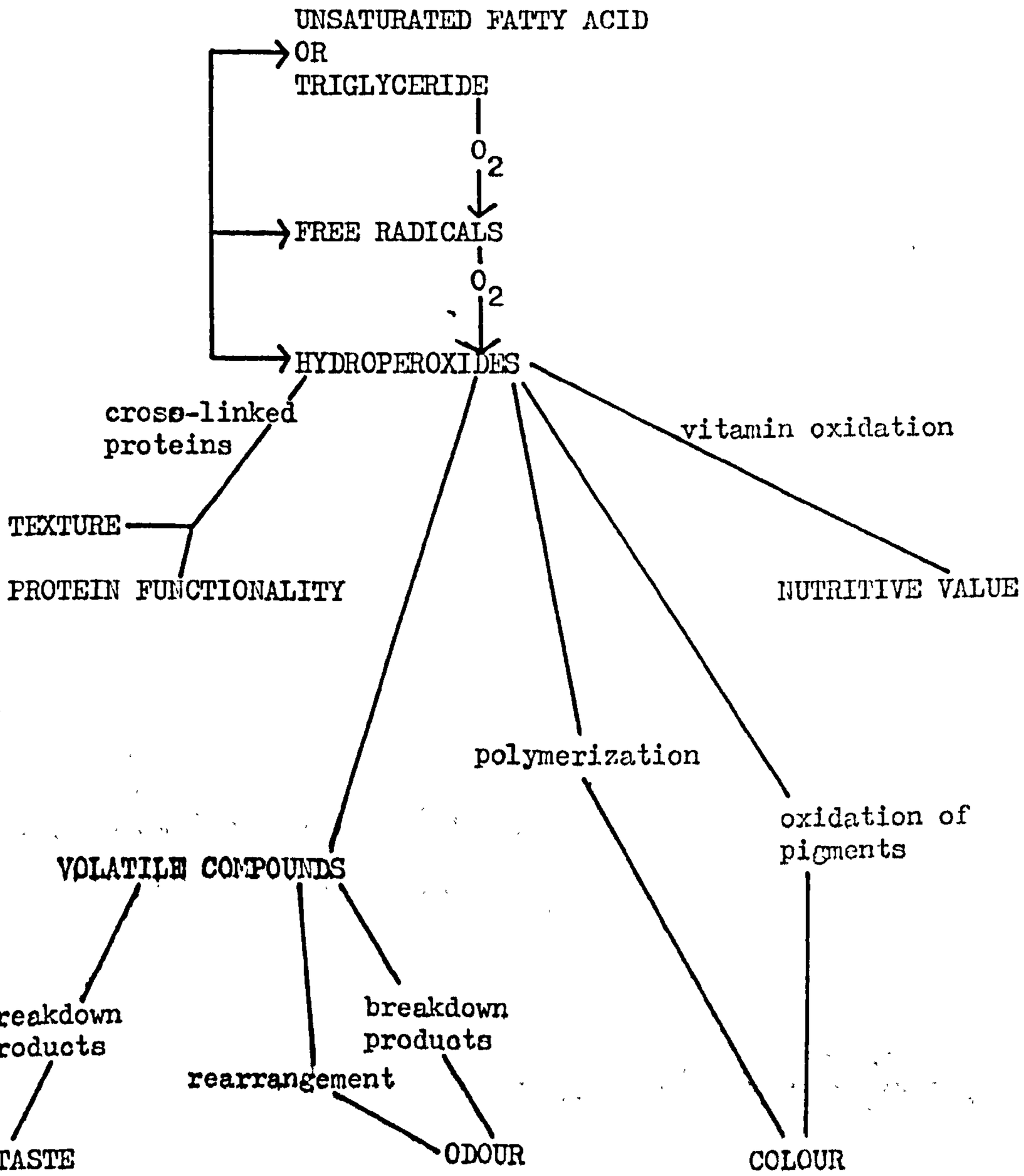
2.3.1. General.

The autoxidation of lipids is a natural chemical reaction between atmospheric oxygen and unsaturated organic compounds (Chan et al., 1982). It occurs in food systems containing unsaturated fatty acid moieties (especially those having one or more double bonds). The off odours and flavours caused by such lipid oxidation result in the rapid deterioration of foods, and thus to their rejection and to waste of their nutrients.

Lipid oxidation is one of the major causes of food deterioration, even in foods containing very little lipid (e.g. some vegetable products : Eriksson, 1982). It affects food quality in many ways:- odour, due to volatile compounds produced (Eriksson, 1979); taste by hydroxy acid production (Baur and Grosch, 1977); colour due to maillard type reactions (Porkorny 1981); texture due to protein cross-linkages (Shenouda and Piggott, 1977); and nutritive value due to loss of essential fatty acids and cholesterol oxidation. Figure 2.4 shows how lipid oxidation may affect food quality.

FIGURE 2.4

Outline of lipid oxidation



Oxidation is a free radical chain reaction which can be described in terms of initiation, propagation and termination (Bateman, 1954; Swern, 1961) and is shown schematically as follows:-

Initiation:-

$$RH + O_2 \longrightarrow R\cdot + \cdot OH \qquad (i)$$

Propagation:-

$$R\cdot + O_2 \longrightarrow ROO\cdot \qquad (ii)$$

$$ROO\cdot + RH \longrightarrow ROOH + R\cdot \qquad (iii)$$

Termination:-

$$R\cdot + R\cdot \longrightarrow RR \qquad (iv)$$

$$R\cdot + ROO\cdot \longrightarrow ROOR \qquad (v)$$

$$ROO\cdot + ROO\cdot \longrightarrow ROOR + O_2 \qquad (vi)$$

where:-

RH is an unsaturated fatty acid

R· + ROO· are free radicals

ROOH is a hydroperoxide (commonly called a peroxide).

Free radicals are compounds which have an unpaired electron. Once produced, they react readily with oxygen to form peroxides and hydroperoxides in food fats. These products are relatively unstable and break down to volatile compounds or produce further free radicals which continue to initiate further lipid oxidation. This breakdown

mechanism results in the production of a large number of volatile compounds and others of low molecular weight which are responsible for the rancid odours and tastes, and decreases the shelf life of the products (Labuza, 1971).

These compounds have a low olfactory perception Kochar and Meara, 1975; Kinsella, 1969) as only minute amounts cause off-flavours and odours. Thus, for example, 2 mg of 2-trans, 6-cis-nonadienal can spoil the flavour of one ton of pure fat. Lea and Swoboda (1958); Kinsella (1969); Forss (1972); Brown et al., (1973) and Parsons (1974) have published flavour threshold values for compound isolated from food fats. As only p.p.m. are necessary for detection (0.05 p.p.m. in milk for hexanal, Kinsella, 1969) these compounds must be masked by other food constituents since foods are not rejected at these low detection levels (Labuza, 1971).

The complex kinetics and reactions of lipid oxidation have been studied by Farmer et al., (1942); Bolland (1949); and Bateman (1954). It involves not merely the addition of oxygen to fatty acids to form epoxides (Ellis, 1926) and hydroperoxides, but a complex series of specific reactions which occur between methylene interrupted fatty acid chains and oxygen. Once initiated the reactions are self propagating (autocatalytical), and are stopped by a termination reaction.

Labuza (1971) states that the rate of autocatalization is not solely proportional to the number of double bonds present since small amounts of compounds may accelerate or retard the reaction. In pure materials there is a long

induction period, and the activation energy required is moderately high.

2.3.2. Initiation of the reaction.

Thermodynamically, direct attack by oxygen on an unsaturated fatty acid is unlikely, due to the high activation energy required (35-60 Kcal/mol: Privett and Blank, 1962). It has been suggested that peroxide formation is initiated by singlet oxygen (Rawls and Van Santen, 1970; 1971). Hydroperoxide formation requires a change in total electron spin, and as the substrate (fatty acid) and product are in the singlet state, and oxygen is normally in the triplet state, the conservation of electron spin would be violated, thus making the reaction improbable (Wigner, 1959).

Singlet oxygen ($^1O_2^*$) is produced by a photochemical reaction in the presence of a sensitizer. Sensitizers include chlorophyll, flavin, peroxidase enzymes, and haemoporphyrins (Krinsky, 1977; Korcycha-Dahl and Richardson, 1978).

The mode of $^1O_2^*$ production is as follows:

$$S + h \longrightarrow {}^1S^* \longrightarrow {}^3S^*$$

$${}^3S^* + {}^3O_2 \longrightarrow {}^1O_2^* + {}^1S$$

$${}^1O_2^* + RH \longrightarrow ROOH$$

$$ROOH \longrightarrow \text{Free radicals}$$

where:

^1S: Singlet state sensitizer

$^1S^*$: Excited singlet state sensitizer

$^3S^*$: Excited triplet state sensitizer

$^3O_2$: Normal triplet oxygen.

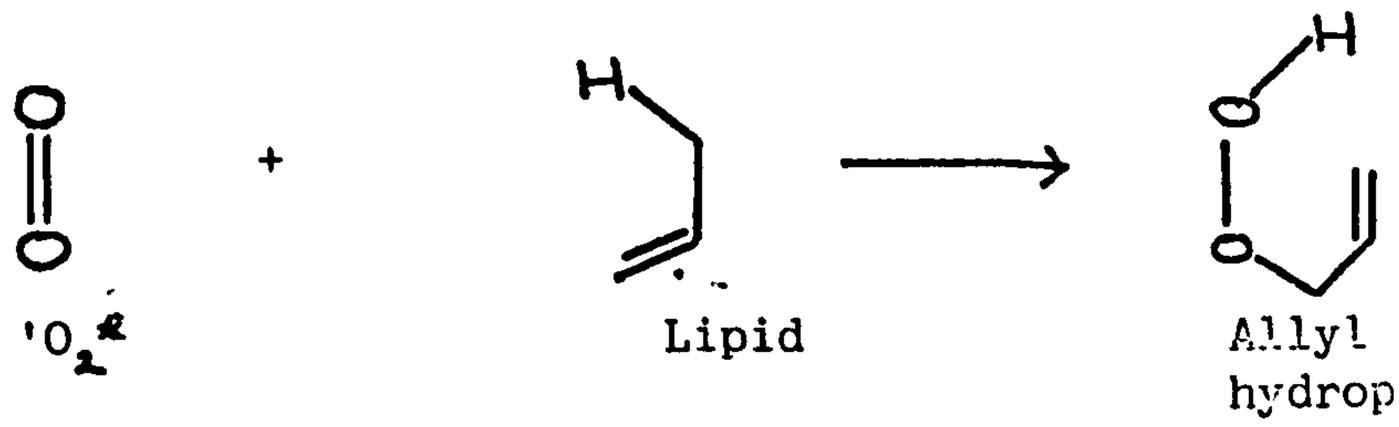
$^1O_2^*$: excited singlet oxygen

h : uv energy in photons

RH: fatty acid

ROOH: hydroperoxide.

Korcycha-Dahl and Richardson (1978) presented a review on the possible modes of production of $^1O_2^*$ and how it could react with food constituents. Singlet oxygen initiates autoxidation by an "ene" type reaction with lipids to produce allyl hydroperoxides as shown:-



$^1O_2^*$ Lipid Allyl hydroperoxide

The subsequent decomposition of these allyl hydroperoxides gives rise to free radicals which initiate new reactions in the propogation step of autoxidation. These allyl hydroperoxides can react with normal triplet oxygen to yield peroxyl radicals (Hanzlik 1976). Thus, once the reaction is initiated by singlet oxygen, it will quickly be autocatalytic in the presence of oxygen (triplet state). Other autoxidation mechanisms e.g. metal catalysed reactions can now be involved (Wallace et al., 1974).

The mode of peroxide generation in autoxidation is different from that produced by singlet oxygen (Clements et al., 1973). This is supported by work by Terao and Matsushita (1975; 1977) who found that methyl linoleate produced the 9-, 10-, 12- and 13-hydroperoxide isomers when reacted with single oxygen, but only produced 9- and 13-

isomers in autocatalitical oxidation.

In the presence of u.v. radiation Chahine and de Man (1971) found that the activation energy for the initiation of corn oil oxidation by singlet oxygen was only 4 Kcal/mol. Thus it is assumed that singlet oxygen initiates the first peroxide production. If only traces of singlet oxygen are present in food tissue and u.v. light is not present, then long induction periods occur before rancidity is detected Food stored exposed to the air and u.v. light in the presence of $^1O_2^*$ sensitizers, or where the metal content (free or bound) is high at the start of storage, result in a short product shelf life i.e. a short induction period.

2.3.3. Factors affecting lipid oxidation.

There are many chemical and environmental factors that can have pro- or antioxidant effects on lipid oxidation, some showing both activities under different conditions.

Temperature has a marked effect, especially on the propagation and peroxide decomposition steps (Lundberg, 1962). The rate of autoxidation does not show a linear relationship with increase in temperature. Waletzko and Labuza (1976) found that a shorter shelf life was predicted than actually occurred when the rate was extrapolated from studies carried out at high temperatures. Lea (1962) claimed that refrigeration would retard lipid oxidation, however, this has been shown not to necessarily be true by Igene et al., (1979a; 1979b; 1980; 1981). As at high temperatures and a deficiency of oxygen, the oxidation rate

is limited by the oxygen concentration, especially for linoleic acid (Bateman, 1954; Marcuse and Fredricksson, 1968). This is due to the reduced oxygen solubility at high temperatures. Hydroperoxyl radical production is retarded when oxygen is removed (Troller and Christian, 1978).

The degree of unsaturation of the lipids affects the oxidation rate, since it increases as the degree of unsaturation increases. Linoleic acid (18:2) oxidizes 10 times faster than oleic acid (18:1) and linolenic acid (18:3) 20-30 times faster than linoleic acid (Labuza, 1971). The volatile lipid oxidation products can accelerate the rate of oxidation, as El-Magoli et al., (1979) found when gases containing hexanal and decadienal were passed over safflower oil.

Lipids do not absorb u.v. light, but are oxidized by photosensitized oxidation products such as chlorophyll, porphyrins and haem pigments (Carlson et al., 1976).

As well as chemical activation, enzymes may also catalyse lipid oxidation and lipoxygenase is responsible for hydroperoxide production in inadequately processed foods. The biochemistry and kinetics of this reaction have been extensively reviewed elsewhere (Veldink et al., 1977; Gaillard and Chan, 1980; Nicolas and Drapon, 1981), and so will not be covered here.

Tocopherol is a well known antioxidant, but it can become prooxidant at high concentrations. Cillard et al., (1980a; 1980b) found that in an aqueous media the concentration ratio of α-tocopherol to linoleic acid was critical in

selecting its pro- or anti-oxidant properties. Tocopherol was found to be prooxidant above the molar ratio of 5×10^{-3} : 1 (α-tocopherol : linoleic acid).

Lipid oxidation may be catalysed by metals which have an oxidation-reduction potential between two valency states. These metals include cobalt, nickel, copper iron and manganese (Ingold, 1962). They influence the initiation reactions by acting as photosensitive intermediates, and by a series of electron transfer reactions (Haber and Willscatter, 1931) which cycles the metal between its two valency states. Free radicals being produced from the hydroperoxides formed.

(i) $ROOH + M^{2+} \longrightarrow RO\cdot + {}^{-}OH + M^{3+}$

(ii) $ROOH + M^{3+} \longrightarrow ROO\cdot + H^{+} + M^{2+}$

where

ROOH : hydroperoxide, $RO\cdot + ROO\cdot$: free radicals M^{3+} and M^{2+} metal in its valency states.

The kinetics and mechanisms are discussed in detail by Uri (1952); Ingold (1962; 1968); Bird (1971); Labuza (1971) and Waters (1971).

Haem compounds (myoglobin, haemoglobin, cytochrome C) catalyse the oxidation (Tappel, 1953a; 1955) and according to Labuza (1971) may introduce oxygen into the food or model system during processing of tissues. Haem compounds act by catalysing the decomposition of peroxides into free radicals (Tappel, 1962).

The role of phospholipids as antioxidants has been debated frequently (Brandt et al., 1973). Luckman et al., (1953); Ivanow et al., (1971); Bishov et al., (1960) described phospholipid antioxidant properties and quoted their stabilizing effects on oleqfin substances. Bishov et al., (1960) claimed that in a model system phospholipids, at certain concentrations, had a greater protective effect than BHT. Olcott and Van der Veen (1963) thought the action to be synergistic with other antioxidants rather than a true antioxidant effect.

Maillard reaction products have been reported to inhibit oxidation in food and model systems (Lingert and Waller, 1983; Kirigaya et al., 1969; Linnert and Eriksson, 1980; Eichner, 1980; Tomita, 1972; Lingnert, 1980; Lingnert and Lundgreen, 1980). However the results obtained are not consistent, and work is now in operation to elucidate the mechanisms involved (Eriksson, 1982).

Proteins, especially casein (Porkorny et al., 1961; Taylor and Richardson, 1980), serum albumen (Yukami, 1971) and soya protein (Porkorny et al., 1961; Pratt, 1972) - have been implicated in inhibiting lipid oxidation. Amino acids, acting as chelating agents, can retard oxidation; but they can act as pro-oxidants at high pHs (Marcuse, 1962). Proline has been reported to be synergistic with other antioxidants (Olcott and Kula, 1959) while polyphosphates in poultry are reported to decrease rancidity (Rao et al., 1976).

The antioxidant activity of herbs and spices was reported by Chipault (1957). Rosemary and sage were found to be good antioxidants. This is due to their content of camosolic acid (Brieskorn and Domling, 1969). Herrmann et al., (1981) reported that most herbs and spices showed antioxidant activity in lard kept in the dark; but, in the light, green spices and herbs were pro-oxidants. The antioxidant property may be due to phenolic compounds - hydroxycinnamic and hydroxybenzoic acids (Thumann and Herrmann,1980).

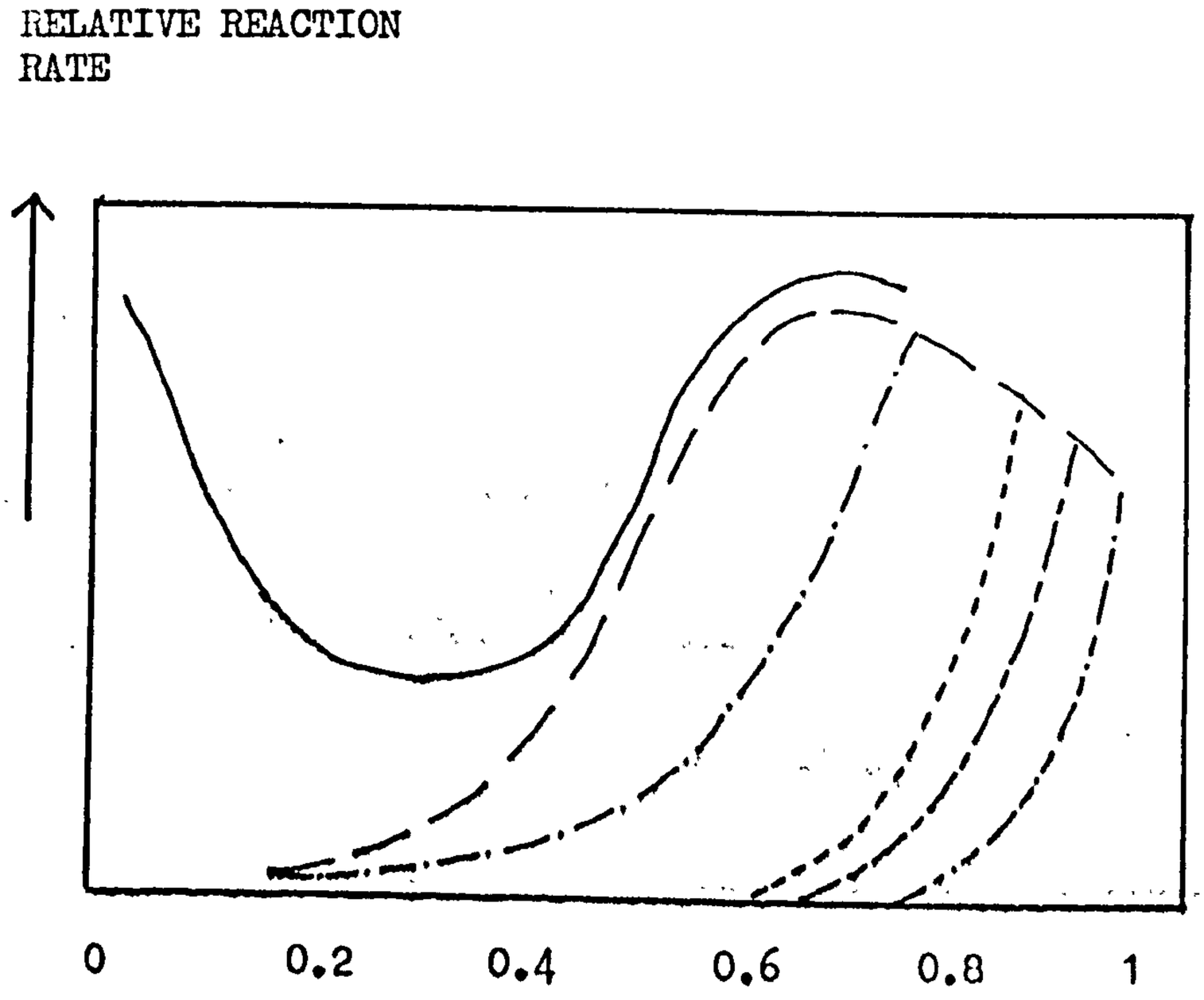
Drying and dehydration are used as methods of the preservation of foods but they can induce undesirable chemical changes, including the production of free radicals (Munday et al., (1962) and these can initiate or accelerate lipid oxidation (Matz et al., 1955; Martin, 1958).

Lipid oxidation is affected by the water content or a_w. (Figure 2.5). At low a_w 0 - 0.2, the food is susceptible to lipid oxidation (Salwin, 1962; Labuza, 1970; Labuza, 1971 ;). As the a_w rises from 0.2 to 0.55 oxidation is retarded due to water forming hydrogen bonds with the peroxides, so decreasing their activity. Water also dilutes or hydrates metal catalysts reducing their catalytical affect, and dilutes the reactant concentration (Karel, 1975). The effect of water appears to be in the early stages of oxidation (Quast and Karel, 1972).

Above an a_w of about 0.55, oxidation is enhanced due to mobility of catalysts and matrix swelling yielding new reaction sites (Heidlebaugh et al., 1971; Heidlebaugh, 1970).

FIGURE 2.5

Stability of food as a function of water activity.



Where

——— = Lipid Oxidation

— — = Non-Enzymic Browning

—·— = Enzyme Activity

- - - - - = Mould Growth

— — — = Yeast Growth

—·—·— = Bacteria Growth

(from Labuza, 1971).

2.3.4. Peroxide production and secondary products.

Hydroperoxides are the initial lipid oxidation products, and their production increases up to a maximum concentration. Then they undergo decomposition into secondary products (Karel, 1975). The reactions involved are:-

$$ROOH \longrightarrow RO^{\cdot} + {}^{\cdot}OH \qquad (i)$$

$$2ROOH \longrightarrow ROO^{\cdot} + RO^{\cdot} + H_2O \qquad (ii)$$

$$R^{\cdot} + O_2 \longrightarrow ROO^{\cdot}$$

$$ROO^{\cdot} + RH \longrightarrow R^{\cdot} + ROOH$$

where

RH : fatty acid

ROOH: hydroperoxide

R·
ROO·) peroxide radicals

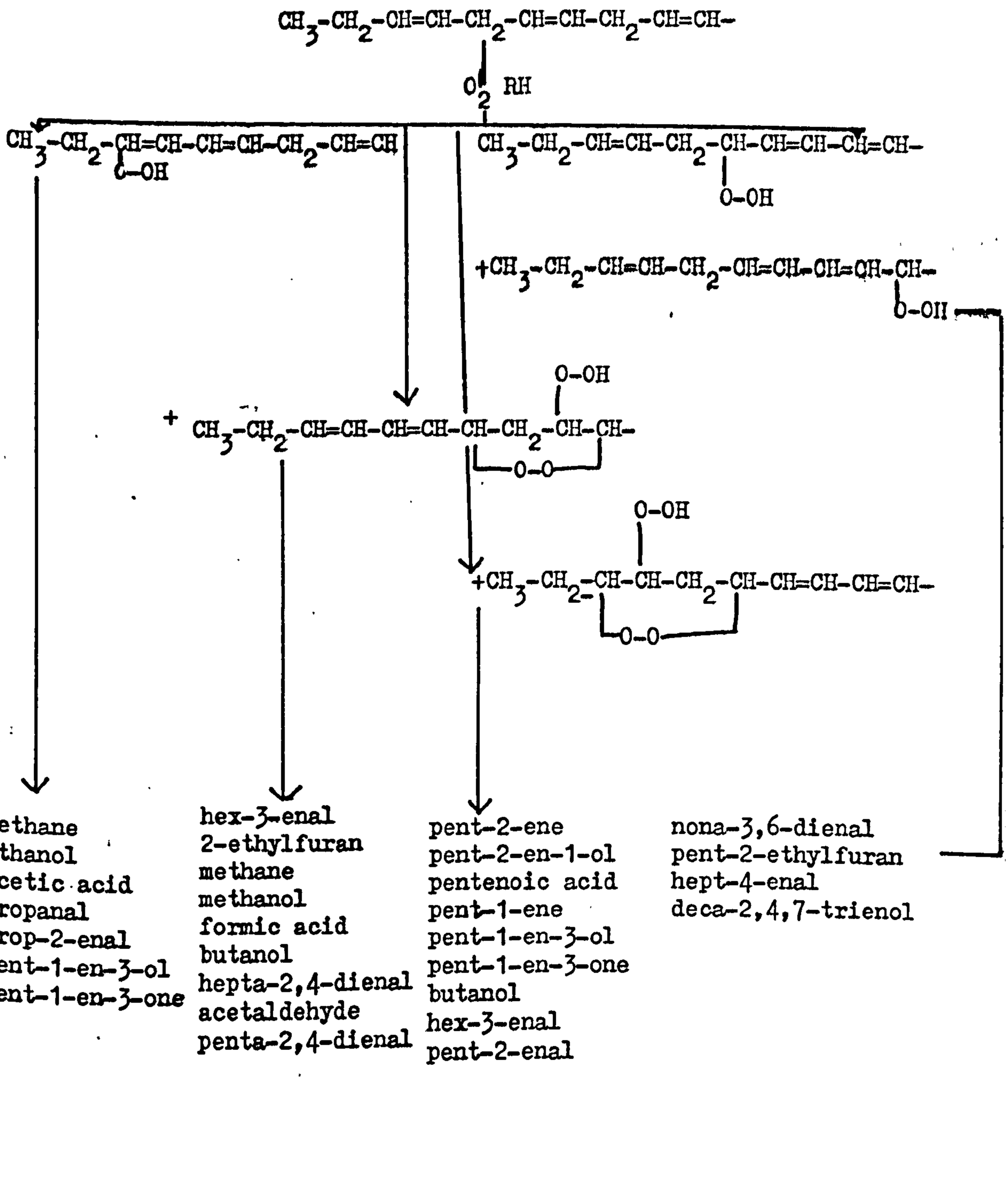
(Karel et al., 1975)

Initially the peroxide breakdown to free radicals follows reaction (i), and is a monomolecular decomposition. When the level of oxidized material rises above 0.5 on an α-molar basis the reaction follows equation (ii). This is bimolecular breakdown, the rate being directly proportional to the peroxide concentration (Labuza, 1971).

The peroxy radicals and hydroperoxides from oleic, linoleic and linolenic acids have been extensively studies. Figure 2.6 shows the pathways and products expected from linolenic acid.

FIGURE 2.6

Pathway of expected volatile products from Linoleic acid



(from Kochar and Meara, 1975).

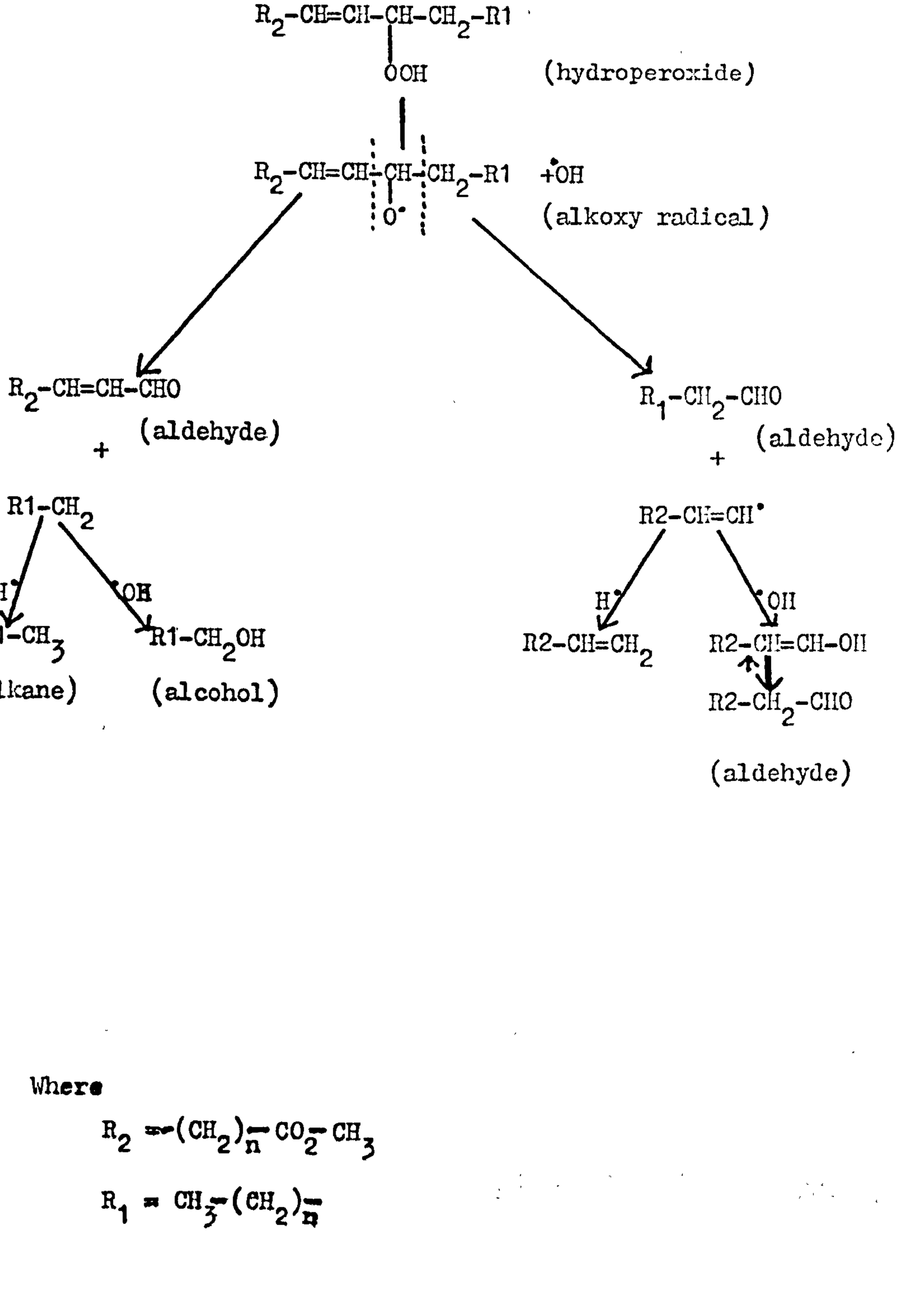
Hydroperoxide formation occurs at the propagation step of oxidation and involves the formation of a peroxy radical (ROO·) by oxygenation of an unsaturated lipid with one or more double bonds. Oxygenation can occur at either end of the radical to yield a hydroperoxide. In linoleic acid the 9- to 13-OOH hydroperoxides are formed, with two isomers of each being produced in equal proportions (Chan and Levett, 1977a). Linolenic acid yields eight hydroperoxides (9-, 12-, 13 and 15 and both isomers) and a mixture of diperoxides, which yield cleavage products unobtainable from the hydroperoxides (Chan et al., 1982).

Lipid hydroperoxide (peroxide) radicals are the initial product of oxidation, and they yield secondary products of which some are responsible for rancid odours and taste. The hydroperoxides are unstable and undergo homolytic cleavage to yield an alkoxy radical (Frankel, 1983) which decomposes by cleavage on either side of the carbon atom bearing the oxygen atom (Hamilton, 1983). These products can react with ·OH or ·H to form aldehydes, or tautomerize to form a saturated aldehyde (involves ·OH). The hydrocarbon free radical produced during the cleavage can react with ·OH to yield an alcohol, or can pick up an H· radical to form a hydrocarbon. Figure 2.7 shows the products yielded by cleavage of a hydroperoxide.

Thus peroxides breakdown or polymerise to form saturated and unsaturated compounds some of which are volatile compounds with low olfactory levels. Figure 2.8 shows the fatty acids from which specific aldehydes are produced.

FIGURE 2.7

Decomposition reactions of hydroperoxides to form volatile end products.



Where

$R_2 = -(CH_2)_n-CO_2-CH_3$

$R_1 = CH_3-(CH_2)_n-$

FIGURE 2.8

Origin of specific aldehydes obtained from various unsaturated fatty acid hydroperoxides.

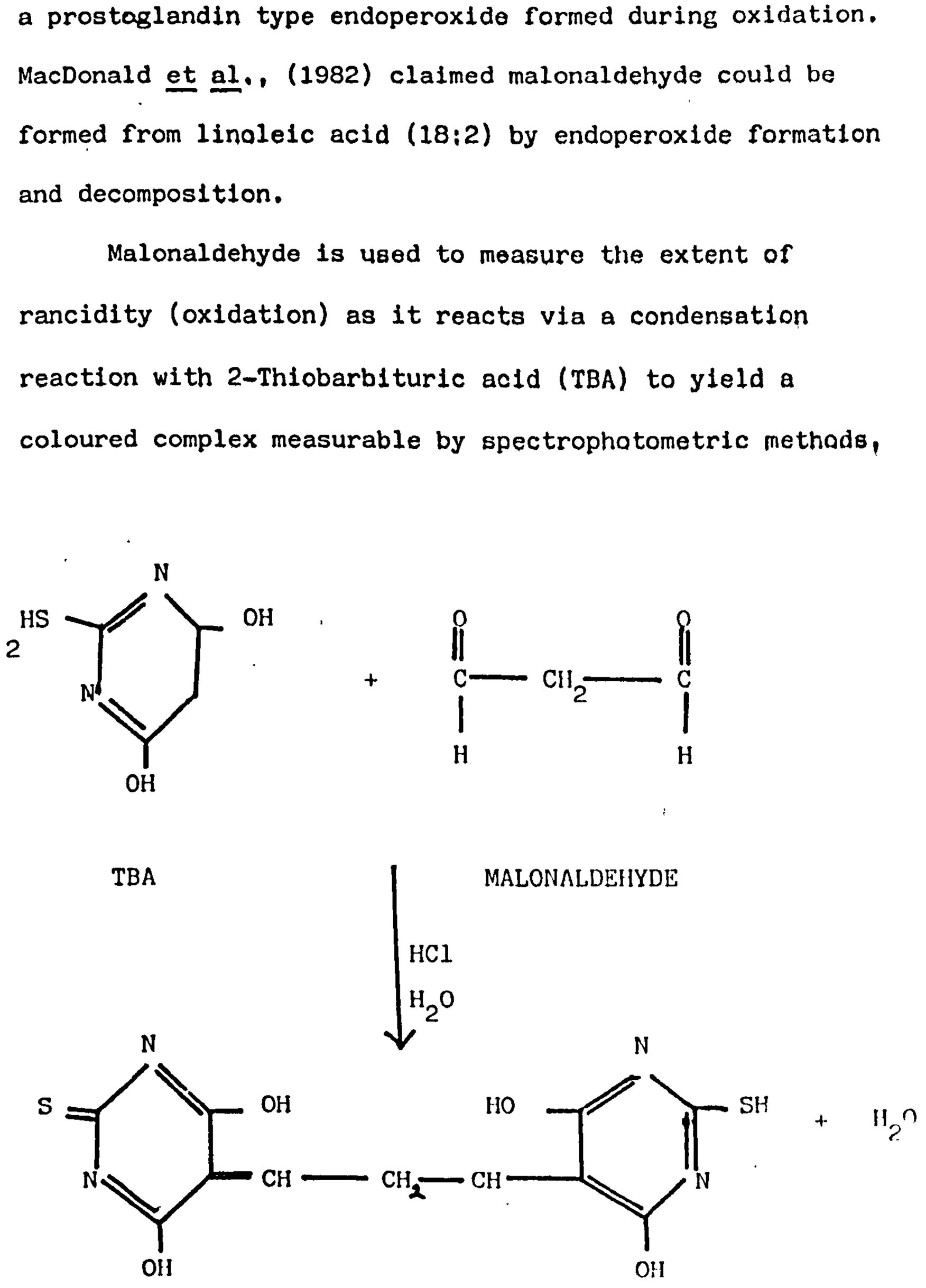
Fatty Acid	Hydroperoxide	Aldehyde formed
Oleic	C8	2-Undecanal
	C9	2-Decanal
	C10	n-Nonanal
	C11	n-Octanal
Linoleic	C9	2,4-Decadenal
	C11	2-Octanal
	C13	n-Hexanal
Linolenic	C9	2,4,7-Decatrienal
	C11	2,5-Octadienal
	C12	2,4-Heptadienal
	C13	3-Hexanal
	C14	2-Pentenal
	C16	Propanal
Arachidonic	C7	2,5,8-Tridecatrienal
	C10	2,5-Undecadienal
	C11	2,4-Decadienal
	C12	3-Nonenal
	C13	2-Octanal
	C15	Hexanal

Where

——— = aldehyde implicated in rancid odours and taste.

One breakdown product that has received much attention is malonaldehyde, which has been used extensively to follow the development of rancidity using the TBA test (Gray, 1978; Melton, 1983). Malonaldehyde is formed from β-γ aldehydes which originate from fatty acids with 3 double bonds (Pryor et al., 1976). This is achieved via a prostaglandin type endoperoxide formed during oxidation. MacDonald et al., (1982) claimed malonaldehyde could be formed from linoleic acid (18:2) by endoperoxide formation and decomposition.

Malonaldehyde is used to measure the extent of rancidity (oxidation) as it reacts via a condensation reaction with 2-Thiobarbituric acid (TBA) to yield a coloured complex measurable by spectrophotometric methods,



The reactions of lipid oxidation products and food constituents with malonaldehyde have been reported (Kwon, 1963; Kwon et al., 1965; Kwon and Watts, 1964). Malonaldehyde is present in its enolate form in meat tissue (Kwon and Watts, 1964) and is stabilized against further decomposition by metal chelates. It may be recovered by acid and heat as in the TBA test. When it is in its enolic form it can react with food compounds e.g. proteins rendering the proteins unavailable.

2.4. Introduction to the Project

Good manufacturing hygiene and refrigerated storage has led to the improvement of the microbiological quality of pork pies. It is now the chemical and physical changes previously described (Sections 2.2 and 2.3) that are causing quality deterioration, and so limiting the shelf life of pork pies.

If a one day old, and a one week old pork pie were given to a meat products trained taste panel, their comments of the changes between the two pies would be:-

i) Softening of the pastry

ii) Discolouration of the meat filling

iii) Flavour changes involving development of off odours and taste

Trained taste panelists can say what changes have occurred, but they cannot say what has caused these changes.

It is the determination of the relative importance of these changes (which are not easily quantified), the nature of the chemical and physical changes during storage, and their prevention, that is going to be investigated and discussed in the next four chapters.

Chapter 3.

Materials and Methods.

Chapter 3. Materials and Methods.

Throughout the experimental work all the raw pie ingredients (lean, fat and pastry) were supplied by the Queens Drive factory of Pork Farms plc. The rusk was supplied by Lucas food ingredients (Birstwith, Harrogate) and the seasoning ingredients (salt, white pepper, Apex starch and monosodium glutamate) by R.H.M. plc (Leeds).

3.1. Method of pie manufacture.

Pork shoulder, leg and belly meats were minced with pork back fat and then mixed with water, rusk and a seasoning mix containing salt, white pepper, apex starch and monosodium glutamate to form a standard recipe meat block. This was automatically weighed out (150g) and pressed into cases of hot water pastry in metal rings (10 cm. diameter). After lidding, the pies were sprayed with a continuous thin film of egg glaze supplied by Blyth Dairies Ltd. Baking at 460°C for 50 min was followed by initial air cooling for 15 min. The pies were deringed and injected with 45 ml of 4% gelatine solution and finally cooled to 4°C in an air blast chamber. The cooled pies were mechanically wrapped with cellophane and transported to Sutton Bonington where they were usually stored at 4°C and 80-90% r.h.

As far as possible the same production line and operators were always used. Except where stated standard recipes for the pastry, meat block and gelatine jelly were used.

3.2. Burger manufacture.

Shoulder cuts of pork were used. The longissimus dorsi were dissected out and any visible fat and connective tissue was removed and discarded. The muscles were minced using a Kenwood mincer attachment (5mm diameter) at a speed setting of 1.

The minced muscles were mixed with water, and (where appropriate) rusk and/or the seasoning mix were added. The proportions of lean: water: rusk: seasoning were in the amounts used in the factory standard recipe. The meat mix was formed by hand into burgers 100gm in weight before cooking. These burgers were 80-90 mm in diameter. They were cooked using a Tefal contact grill for 10 minutes, turning after 6 minutes, no red juice could be pressed from the burgers after this time. The burgers were left to cool at room temperature, and then stored in half litre plastic containers (6/box not in contact with each other). The boxes were lidded and stored at 4°C until required for analysis.

3.3. Analytical methods.

3.3.1. Sampling technique.

At least four pies were taken and each separated into the component parts:- Pastry, Meat filling and visible Jelly. All of each component was minced in a Kenwood mincer attachment (5 mm diameter), and mixed by hand to ensure randomization. Samples of each component were selected for subsequent analysis.

Initially all of the pastry case was sampled. Later, it was found that the brown layer was the area where most changes were occurring and thus only the brown layer of the side walls were sampled in subsequent studies. For sampling burgers - the entire burger was minced in a Kenwood mincer attachment (5 mm.diameter). A sample was selected at random for further analysis.

3.3.2. Protein solubility in sodium dodecyl sulphate (SDS) and β -mercaptoethanol.

The occurrence of cross-linking bonds involving proteins can be measured by the loss of protein solubility in sodium dodecyl sulphate plus β-mercaptoethanol. SDS readily breaks hydrogen and hydrophobic bonds, whilst β-mercaptoethanol cleaves disulphide bonds. Thus loss of protein solubility in this solvent indicates the formation of non-disulphide covalent bonds. The procedure involved standing for 30 mins at room temperature 0.5 g sample in 50 ml 3 : 1% SDS : β-mercaptoethanol, then heating in a boiling water bath for 30 min, followed by centrifugation at 17,000 g. for 30 mins, and filtering the supernatant through Whatman No. 1 filter paper. The nitrogen content of the filtrate and residue were determined by:- 10 ml. of filtrate and the residue wrapped in filter paper. Each separately were digested with 20 ml. conc. sulphuric acid (A.R. grade) and 2 Kjeltabs. After cooling the fluid was made up to 100 ml. volumetrically and allowed to cool again. This was connected to a Tecator distillation apparatus, 10 ml. 40% sodium hydroxide added, and steam distilled until 150 ml. were collected in 10 ml, 2% boric acid with methyl orange

indicator. This was titrated against 0.02N hydrochloric acid.

$$\% \text{ solubility} = \frac{\text{titre supernatant} \times 5}{\text{titre supernatant plus titre residue} \times 5} \times 100$$

3.3.3. pH measurement.

1g ± 0.1g of minced sample was homogenised with 10 ml. of distilled water and the pH recorded using a combined glass electrode and Pye Unicam pH meter. The readings were taken in triplicate.

3.3.4. Moisture content.

3.3.4.1. Vacuum oven method.

5 or 10g ± 0.1 mg samples were placed in preweighed aluminium dishes in a vacuum oven at 70°C for 4 hours (for jelly) or 12 hours (for meat and pastry). After cooling in a dessicator the samples were reweighed and the moisture content expressed as the percentage loss in weight,

3.3.4.2. By m-xylene distillation.

10 gm ± 0.1 mg minced sample was placed in a 250 ml. distilling flask, to which 100 ml. m-xylene or 2-octanol was added. The flask was connected to a condenser and a calibrated collecting vessel (0.2 ml. divisions). The sample was heated until the volume of water in the collecting vessel was constant (no change over at least

4 minutes). This volume was recorded and the % moisture content calculated as:-

$$\% \text{ moisture} = \frac{\text{volume of water} \times 100}{\text{weight of sample}}$$

Due to the odour and volatile nature of m-xylene, the vaccum oven method was the method used in the majority of the moisture content determinations.

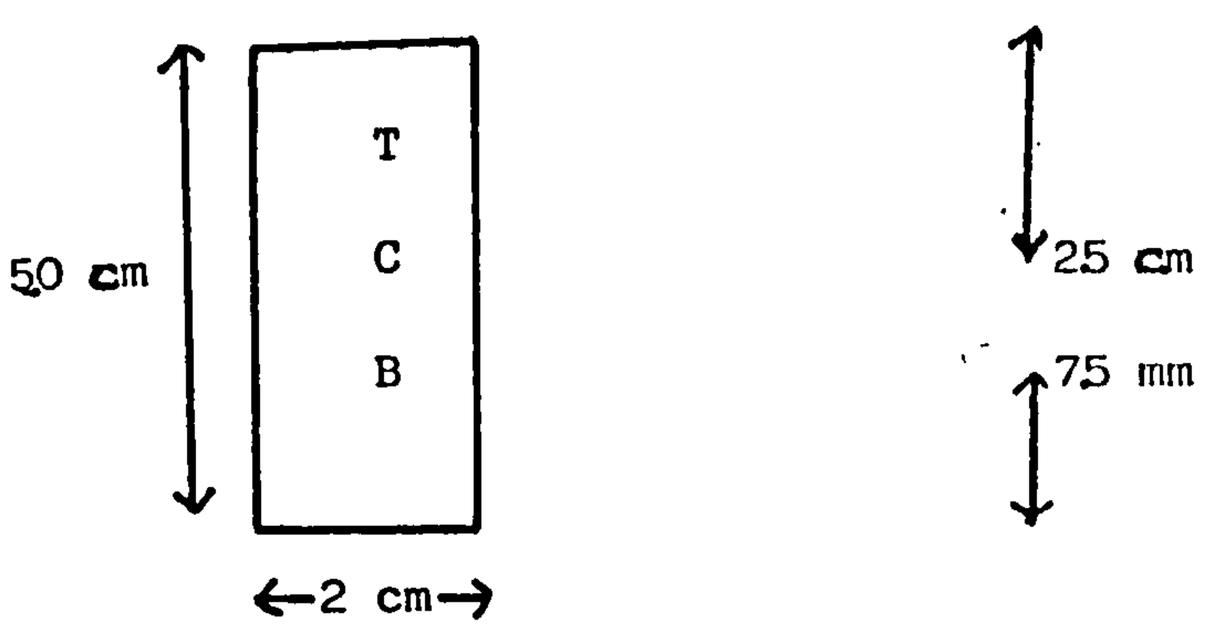
3.3.5. Texture measurements.

3.3.5.1. Stevens-LFRA texture analyser.

The Stevens-LFRA (Leatherhead Food Research Association) was employed to measure objectively the texture of the pastry and meat filling. It involves recording the test material's resistance to the passage of a probe (needle, cone, barrel) through it.

The meat filling was measured through the top surface at its centre point, and at various points approximately half way up the outside surface.

The pastry texture was measured after the inner white layer had been removed, leaving a brown pastry shell. The texture of which was recorded from the side walls:-



The texture was recorded by placing the pastry sample section, or meat filling on a polystyrene block placed on the texture analyser platform. A needle probe (1 mm dia.) travelling at 10 m/sec to a depth of 25 mm (for meat filling) or 9 mm (pastry) was used. The maximum load (g) to penetrate the sample was recorded.

3.3.5.2, Sensory evaluation,

A taste panel of ten people was used for assessment of pastry texture. Each panel member was asked to rate the pastry on a scale of 0 to 5 for 'crispness'. Two reference numbers were used: 0 was the value given to kneaded pastry dough and 5 was given to a 'fresh' Rich Tea biscuit (United Biscuits plc).

3.3.6. a_w measurement.

The minced samples were put in 3 cm diameter plastic pots and placed in the measuring chambers of a Rotronic Hygroskop DT device. This a_w meter had been calibrated using known standard solutions. Measurements of a_w were made at 25°C, one hour after the display value did not alter over a 20 min period (Rotronic handbook; Roberts and Elson, 1981).

3.3.7. Water-holding capacity (WHC).

The WHC of the proteins was determined using the method of Jauregui et al., (1981). It requires no special equipment; - a piece of Whatman No. 50 filter paper was

folded over an inverted 16 x 150 mm test tube and 3 pieces of Whatman No. 3 filter paper were folded over the first piece. The thimble thus formed was kept in shape using an elastic band and weighed before and after the addition of 1.5g $\pm$ 0.1g of sample. The thimble and sample were centrifuged at 2°C, 30,900g for 15 min. The sample cake was removed using forceps, and the thimble reweighed. All determinations were performed in triplicate. WHC was recorded at % expressible moisture (% weight loss from the original sample). The lower the % expressible moisture, the better the WHC.

To avoid weight loss and gain due to evaporation and condensation it was necessary to centrifuge samples immediately after weighing, and to reweigh immediately after centrifuging.

3.3.8. Colour.

The colour changes in the meat filling was determined by use of a reflectance spectrophotometer (Pye model 124 The range scanned was 370 to 700 nm. Zero % reflectance was a black disc and 100% reflectance was a MgO disc. The CIE co-ordinates were calculated using a 'CIE programme' on a Superbrain desk top computer.

3.3.9. Measurement of lipid oxidation.

3.3.9.1. Peroxide value (p.v.)

Hydroperoxides (Peroxides) are the primary products of lipid oxidation, so it could therefore be assumed that detection of the peroxide concentration in samples would be a measure of the extent of oxidation. The position is complicated, however, by the transitory nature of the peroxides, these being intermediate products in the formation of carbonyl and hydroxy compounds (Section 2.3.2.).

Peroxides in flesh foods may be measured in a variety of ways after the lipids have been extracted from them. In the present work an iodometric method (Pearson, 1970) was used after extraction of the lipid by the method of Bligh and Dyer, (1959).

The iodometric methods (Lea, 1931; Wheeler, 1932) depend upon the ability of peroxides to liberate iodine from potassium iodide in an acid medium (glacial acetic acid). The reaction may be summarised as:-

$$2I^- + O^- + 2H^+ \longrightarrow H_2O + I_2 + 2e^-$$

However, absorption of iodine by the unsaturated bonds of the lipid and liberation of iodine from potassium iodide by oxygen in the system to be titrated (oxygen error) are possible sources or error (Mehlenbacher, 1960).

The method of Pearson, (1970) was used, and the peroxide value was expressed as ml of N/100 sodium thiosulphate required to titrate the iodine liberated from potassium iodide by 5g of fat.

3.3.9.2. 2-Thiobarbituric acid value (TBA Value).

The 2-thiobarbituric acid test (TBA) is the one most widely used to detect oxidative deterioration of the lipids of fresh foods (Gray, 1978; Rhee, 1978a). Lipid oxidation is expressed as a TBA value which is defined as the concentration of malonaldehyde in p.p.m. i.e. as mg malonaldehyde/kg sample.

The TBA test can be performed on fresh foods (i) directly on the product, followed by extraction of the coloured complex (Sinnhuber and Yu, 1958; Yu and Sinnhuber, 1957), (ii) on an extract of the food (Witte et al., 1970; Vyneke, 1975) or (iii) on a portion of a steam distillate obtained from an acidified extract (Tarladgis et al., 1960).

It was the steam distillate method of Tarladgis et al., (1960) that was used to determine the TBA value in this study. All samples were analysed in triplicate.

3,3.9,3. Correlation between TBA value and subjective assessment of rancidity.

The TBA values were determined in the pie as a whole, in the pastry and in the meat filling. At the same time an eight member (untrained) taste panel recorded their comments on the pie, as a whole, and on the component parts.

3.3.10. Lipid extraction.

In order that further analysis of the lipids could be performed, they had to be extracted from the samples. For this purpose the method of Bligh and Dyer (1959) was used, since it is a cold extraction process, and thus limits the oxidation of lipids, which is possible when hot solvent extractions are performed (Mehlenbacher, 1960). Also the use of methanol in this extraction allows adequate etraction of lipid bound to protein and extracts phospholipids as well as neutral lipids.

10g $\pm$ 0.1g of sample was homogenized in 10 ml. chloroform and 20 ml. methanol for 2 min. A further 10 ml. of chloroform was added and again homogenized for 30 sec. 10 ml. of distilled water was then added, and the mixture homogenized for 30 sec. This procedure ensured on a volume basis a ratio of sample chloroform : methanol : water of 1 : 2 : 2 : 1. The homogenate was filtered through Whatman No. 1 filter paper in a Buchner funnel, along with 10 ml. of chloroform rinsings from the homogenizer flask. The filtrate was transferred to a separating funnel, where a biphasic system was established. The lower layer of chloroform contained the extracted lipids, and was dried over silica gel in a vacuum oven at 70°C, and finally rotary evaporated to dryness. Final traces of solvent were removed by a stream of nitrogen passing over the lipids. The samples were stored in the dark at -18°C in a nitrogen atmosphere.

3.3.11. Lipid separation.

Throughout this investigation two types of separation were performed. One involved separating the lipids into phospholipids and neutral lipids, the other separated the lipids into their lipid classes. The first involved column chromatography, the second, thin layer chromatography.

3.3.11.1. Column chromatography.

Silicic acid is a suitable medium for the separation of phospholipids from neutral lipids. As the phospholipids are trapped on the silica whilst the neutral lipids are eluted by $CHCl_3$, subsequent elution with CH_3OH releases them (Choudry and Arnold, 1960).

A 350 x 15 mm Id glass column was packed with activated Davidson Silica, using a slurry of 28 g silica in A.R. grade chloroform. The column was packed and used on the same day,: - The lipid extract, in chloroform was fed on to the column and eluted with 10 bed volumes (600 ml.) of chloroform (A.R. grade). The column was then eluted with 600 ml.of methanol (A.R. grade). Both solvents were removed by rotary evaporation.

3.3.11.2. Thin layer chromatography (T.L.C.)

The separation of the extracted lipids into their lipid classes was performed as described by Christie (1973).

3.3.12. Gas-Liquid Chromatography (G.L.C.)

3.3.12.1. Sample esterification.

After the TLC separation of the lipids, the method of Allen and Good (1971) was used to prepare the methyl esters of the lipids for subsequent gas liquid chromatography analysis:

The bands on the TLC plate were scraped into separate 25 ml glass stoppered "Quickfit" tubes containing 5 ml. of 5% H_2SO_4 in CH_3OH. These were heated to 68-70°C for 2 hrs., cooled and 5 ml of distilled water added. The solution was extracted three times with 2 ml. of petroleum ether 40-60°C B.P. (A.R. grade). The petroleum ether phase was washed with 5 ml. of a saturated solution of $NaHCO_3$ and evaporated to dryness.

The methyl esters were taken up in 1 ml of 2,2,4-trimethyl pentane (iso-octane).

3.3.12.2. Gas Liquid Chromatography (G.L.C.)

The separation of the lipid methyl esters and their subsequent identification was performed using GLC. Initially the analysis was performed using twin glass columns (213.5 cm x 4 mm I.D.) packed with GP-3%-SP 2310/2% SP 2300 on 100-120 mesh Chromosorb WAW, (Supelco Chemical Co., U.S.A.). However this procedure could not adequately separate C6 - C12 fatty acids, and was therefore discarded.

Two column packings and temperature programmes were used to separate C6 to C22 fatty acids:-

10% Diethylene glycol succinate (DEGS) on gas chrom Q 100 - 120 mesh (Phase Separations Ltd., Clwyd), using a temperature programme of 100 to 200°C at 8°C/min; and 6 min at 200°C. A Pye 104 gas chromatograph with a Flame ionization detector was used. Carrier gas (nitrogen) flow rate of 45 ml/min, hydrogen and air at 45 ml/min and 600 ml/min respectively. Injector and detector temperature were 200°C.

10% Silar 10CP on gas chrom Q 100 - 120 mesh (Phase Separations Ltd., Clwyd) in a Sigma 3B Chromatograph Perkin Elmer, Beaconsfield, Bucks) with a temperature programme of 3 min at 80°C then a 4°C/min rise to 185°C and 20 mins at 185°C. Nitrogen flow rate was 20 ml/min. Detector and injector temperatures were 250°C sample size was 5 µl.

The standards used were the methyl esters of hexanoic, heptanoic, octanoic, decanoic, dodecanoic, tetradecanoic, hexadecanoic, trans-3-hexadecanoic, octadecanoic, cis-9-octadecanoic, 9,12-octadecadienoic and cis-9-eicosenoic acids (Sigma Chemical Co. Ltd.).

The methyl esters were identified by their peak retention times as shown in the results (Section 5.5.2.).

Quantitative determination of the relative amounts present were performed using a Pye Unicam CDP1 computing integrator.

3.3.13. High pressure liquid chromatography (H.P.L.C.)

Aldehydes are one of the end products of lipid oxidation (Section 2.3.4.). As such they provide a means of detecting lipid oxidation at the stage where off odours and tastes occur. 2,4-Dinitrophenyl hydrazine (2,4-DNPH'S) derivatives of the aldehydes were prepared so that they could be detected by u.v. absorption following separation by HPLC.

3.3.13.1. Sample preparation.

The method of Reindl and Stan (1982a; 1982b) was used with modifications. Direct derivative formation in an acid aqueous solution used to be used to prepare 2,4-DNPH derivatives, but only 70% recovery of aldehyde as the derivative was collected (Selim, 1977). Another unsatisfactory feature of this methodology is that hydroperoxides will decompose to carbonyl compounds (Horikx, 1964). By use of a two phase mixture (see method) it is possible to obtain quantitative derivative formation from low aldehyde concentrations. The modified method of Reindl and Stan (1982) was devised to prevent hydroperoxide decomposition and yield quantitative amounts of 2, 4-DNPHS.

10g $\pm$ 0.1g of sample was homogenized with 50 ml of cold ethanol (A.R. grade) and immediately centrifuged at 0°C, 900g for 10 min.

The supernatant was vacuum distilled (20 - 30 torr) at 50°C with the condenser and collecting vessel being maintained at 0°C with crushed ice. The collecting vessel was connected by a short length of pressure tubing to a second vessel also held at 0°C. Both vessels contained 45 ml of 2,4-DNP solution (1g 2,4-DNP in 1 litre HCL-H_3PO_4 mixture (1:1) diluted to pH 1) (2,4-DNP supplied by Sigma Chemical Co.).

After the first distillation 50 ml of distilled water was added to the distillation flask, and a second distillation performed at 65°C. Upon termination, the 2,4-DNP solutions were combined in a 250 ml conical flask and kept stoppered at room temperature in the dark (wrapped in aluminium foil) for 1 hr.

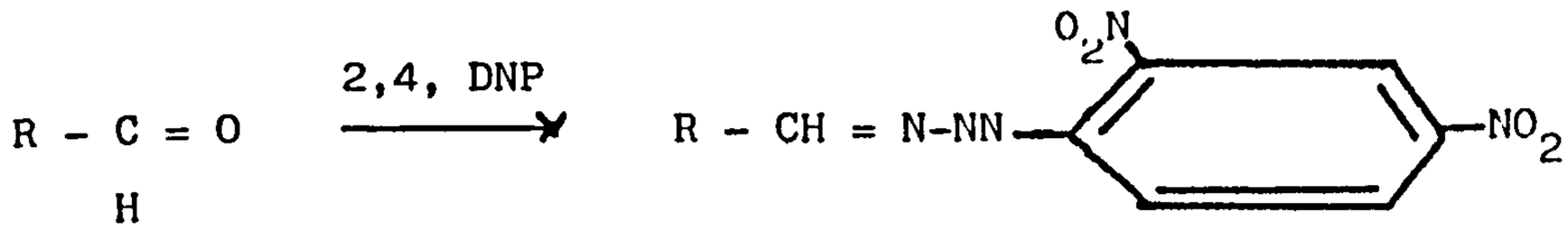
50 ml of n-hexane (HPLC grade) was added, and the moisture vigorously stirred with a magnetic stirrer for 2 hrs at room temperature. The n-hexane layer was separated and the acid removed by washing twice with 10 ml of distilled water. The hexane was removed by vacuum evaporation at 30°C, The 2,4-DNPH derivatives were taken up in 2 ml of methanol (HPLC grade).

3.3.13.2. Preparation of the standards.

Pentanol, hexanol, hex-2-enol and valericaldehyde were supplied by Sigma Chemical Co. Octanal, nonanal, nona-2, 4-dienal and Nona-2-enal were supplied by Aldrich Chemical Co. The derivatives were prepared according to Vogel (1978): To the clear solution obtained by warming

0.5 gm 2,4 DNP, in 1 ml of conc. HCl (A.R. grade) and 8-10 ml ethanol (A.R. grade) 0.25g aldehyde was added and the solution heated just to boiling. After cooling to room temperature the 2,4 DNPH derivative was filtered off and recrystalized twice from ethanol (A.R. grade).

The essential reaction is



(Knapp, 1979).

3.3.13.3. High pressure liquid chromatography (HPLC) analysis.

The separation of the hydrazones of saturated, mono and diunsaturated aldehydes was performed by reversed phase HPLC. The method was a modified version of Reindl and Stan (1982a). HPLC separation and analysis was carried out using Applied Chromatography Services (ACS) Pumps 750/03 model, controlled by an ACS gradient programmer (decilinear) 750/36 and S.C.O.V.T. model 750/51. Injection was facilitated by a Rheodyne valve 7120 with a 20µl loop. Detection was at 360 nm using a Shimadzu SPD-2A variable u.v. detector.

The 2,4-DNP derivatives were separated on a 49 x 250 mm ODS2 5µm column (Phase Separation Ltd., Clwyd), using an isocratic elution of 1.0 ml/min. The eluent was acetonitrite - water - tetrahydrofuran 75 : 24 : 1 (v/v/v) and the separation was performed at room temperature.

Peak identification was established by use of retention times and, as a double check, by use of an enrichment procedure. The retention times were established using pure standards as prepared in 3.3.13.2. To identify an unknown peak the column capacity ratio K' was calculated.

$$K' = \frac{tr - to}{to}$$

tr is the given peak retention time. to is the solvent peak retention time. A plot of the $\log_{10}$ K' against carbon no. yields a straight line for a homologous series.

3.3.14. Seasonings.

The seasoning mix and mix ingredients (60% salt, 19.1% Apex starch, 15.2% white pepper and 4.7% monosodium glutamate) were supplied by R.H.M. Foods Ltd. (Leeds).

3.3.14.1. Pepper extraction.

Pepper was divided into water soluble, ethanol soluble and insoluble residue fractions by the following procedure:-

25 gm of white pepper was homogenized with 100 ml of distilled water. After standing for 15 min it was rehomogenized and filtered through Whatman No. 1 filter paper. 20 ml of H_2O was used to rinse the flask and the washings were added to the homogenate and filtered. The filtrate was freeze dried.

The residue was dried in a vacuum oven at 70°C and then homogenized with 250 ml of ethanol (A.R. grade). The homogenate was filtered, and the ethanolic solution concentrated by rotary evaporation. The insoluble residue was dried in a vacuum oven at 70°C.

3.3.14.2. Thin layer chromatography of the ethanol soluble extract (oleoresin).

The oleoresin in ethanol (0.1g in 100 ml) was spotted on a silica gel G plate and eluted with 15% ethyl acetate in petroleum ether for 30 minutes (Ethyl acetate facilitates particle separation of the isomers of piperine).

The plate was examined under u.v. light and any spots marked. It was then sprayed with 2, 4-DNPH solution (saturated 2,4-DNPH in 2m HCl) and any spots recorded. After heating in an oven at 105°C for 30 mins the appearance and/or disappearance of all spots was recorded. Pure piperine (Sigma Chemical Co.) in ethanol (0.1 gm in 100 ml) was also spotted on the same plate,

3.3.15. Statistical analysis.

The Student t-test was used to establish if significant differences occurred between samples. It was performed using a Superbrain microcomputer (Multitex Ltd.) and a T-test programme where the significance levels, the confidence levels were 95% or P<>0.05 was used.

Linear regression by least square analysis was performed using a BBC microcomputor with a linear regression programme (giving Y intercept, slope, best line of fit and confidence level, and the regression coefficient r).

Chapter 4.

Preliminary investigations into the changes during storage of pork pies.

Chapter 4.

Preliminary investigations into the changes during storage of pork pies.

Initial investigations were necessary to ascertain what chemical and physical changes were taking place, and their relative rates so as to decide which were of importance in limiting the shelf life of these products.

4.1. Initial investigation into storage changes in pork pies.

The chemical and physical changes that could occur during storage of pork pies were investigated.

4.1.1. Experimental design.

11 oz pork pies were made as described in Section 3.1, and were wrapped in cellophane, and stored at 4°C, 80% r.h. until required. The following analyses were performed on alternate days for 3 weeks on samples from whole pies, meat filling, pastry and where appropriate the jelly (Section 3.3.1.) : - moisture content (Section 3.3.4.): pH (Section 3.3.3.): a_w (Section 3.3.6.): solubility in SDS β-mercaptoethanol (Section 3.3.2.): colour (Section 3.3.8.): TBA value (Section 3.3.9.2.) and peroxide value (Section 3.3.9.1.). Whole pies were used for texture assessment (Section 3.3.5.) and for following weight changes in individual pies. This was done by numbering the pies and recording initial weight and final weight when taken for analysis.

This experimental design was set up and performed as three separate trials. The quoted results represent the mean of the three trials.

4.1.2. Results and discussion.

4.1.2.1. Moisture content.

It was found that the total moisture content of the pies increased as storage proceeded, as did the pastry moisture content. The moisture content of the meat filling, however, remained constant (Figure 4.1), whilst that of the jelly decreased. Although the loss of moisture from the jelly was mirrored by the increase in moisture content of the pastry, it could not totally account for it. The jelly only provides ⅓ of the total moisture increase:- i.e. the 50 g of jelly in the pie loses 25.5g of moisture, while the 155g of pastry gains about 76g of moisture during the storage period (Figure 4.1). The remainder of the increase in the moisture content of the pastry must therefore have come from the atmosphere, as an equilibrium mechanism between the two environments.

The moisture content of the meat filling remained constant, a feature probably related to the water binding action of the meat proteins, and added salt and rusk.

4.1.2.2. pH.

Apart from the meat filling which remained constant the results for the pastry and whole pie were very variable and unpredictable (Figure 4.2).

FIGURE 4.1

Percentage Moisture Content Of Whole Pork Pies (◢—◢), and Of The Meat Filling (△—△), Pastry(○—○) and Jelly (■—■).

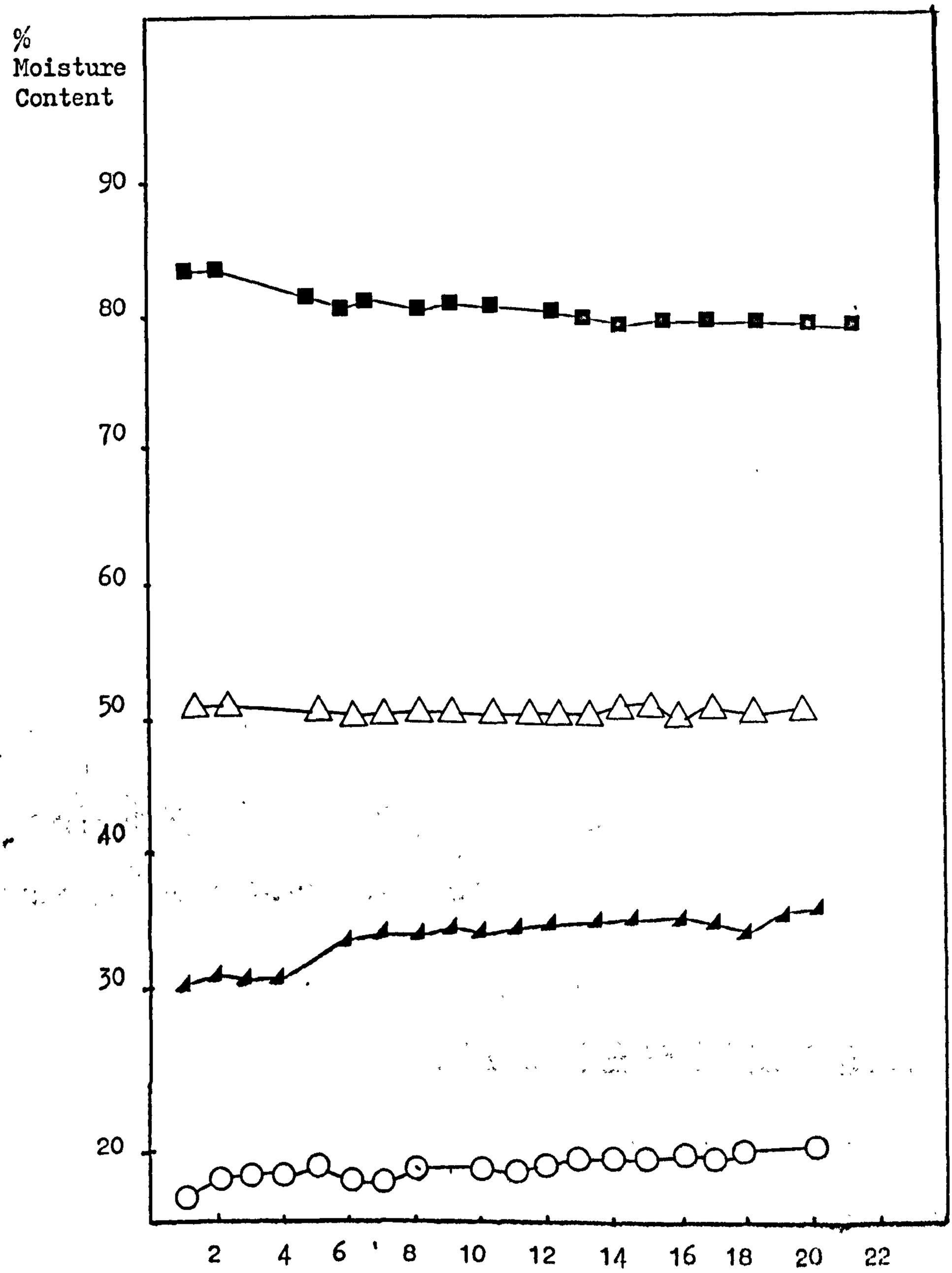
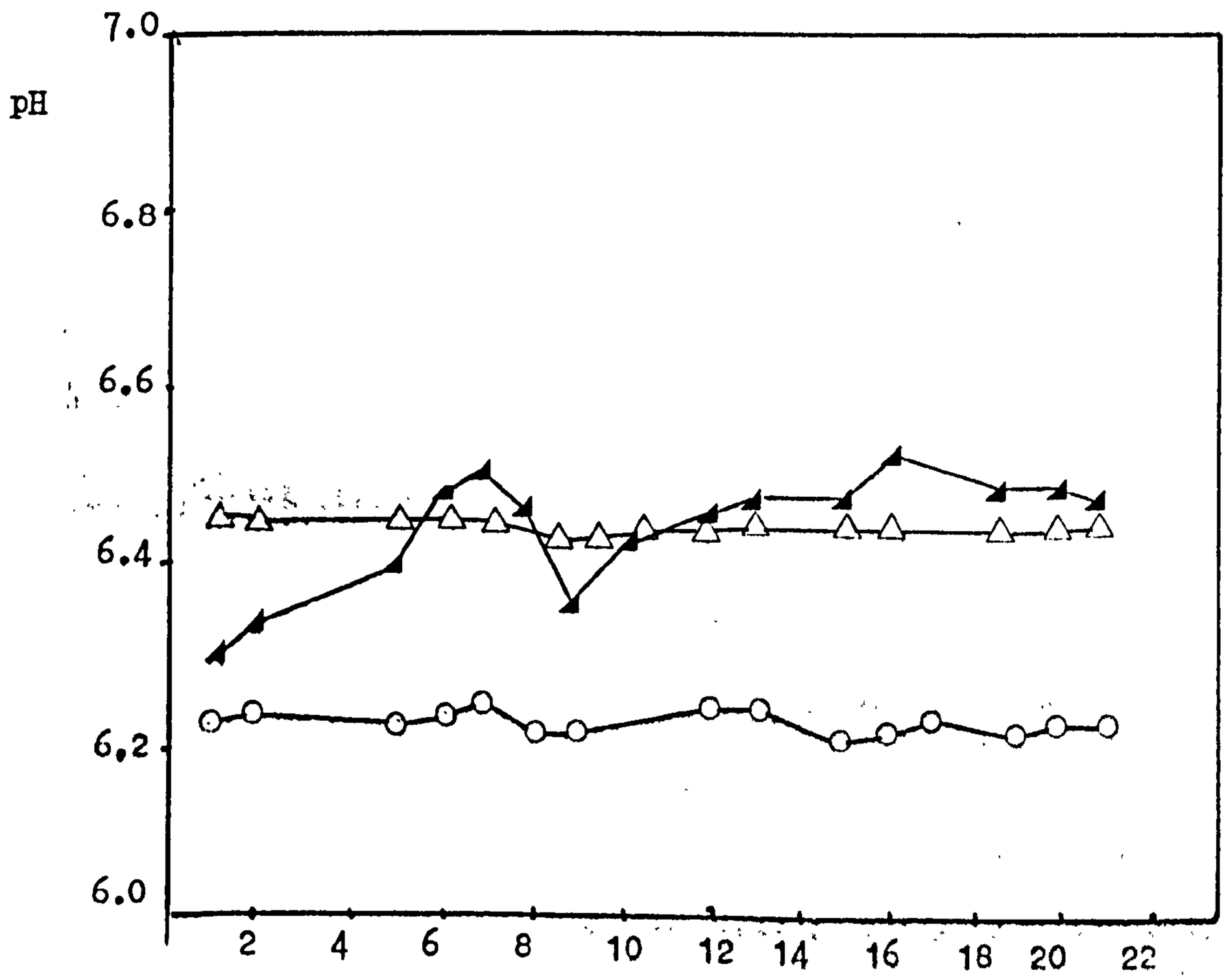


FIGURE 4.2

Changes in pH of the whole pork pie (◢—◢), meat filling (△–△) and pastry (○–○) stored at 4°C and 80% r.h.



4.1.2.3. A_w.

The changes in the a_w of the pie components followed the same trend as the moisture content, with the pastry a_w increasing, that of the jelly declining, and that of the meat filling showing little change (Figure 4.3). Of interest is that the pastry a_w starts below that which most moulds are inhibited. However as the a_w rises the potential for mould growth increases and such growth was observed.

4.1.2.4. Pie weight.

The changes in the wrapped pie were recorded against time. The pies increased rapidly in weight initially, then gradually levelled off as storage continued. If an inert object the same size and shape as a pork pie was wrapped in the cellophane wrapping material, only the wrap increased in weight. This was because the atmosphere and the inert object were in equilibrium with each other.
If there was no filling in the wrapped pastry case, then moisture would have to pass from the atmosphere into the pastry to create an equilibrium, thus increasing its weight.

The wrap increases in weight with storage due to condensation, which is due to moisture migrating into and from the pie depending on the storage conditions.

4.1.2.5. Texture.

Textural changes in the pastry and meat filling were recorded as the force in grams to penetrate the sample.

FIGURE 4.3

Change in a_w of the meat filling (▲—▲), pastry (○—○) and jelly (□—□) of pork pies stored at 4°C and 80% r.h.

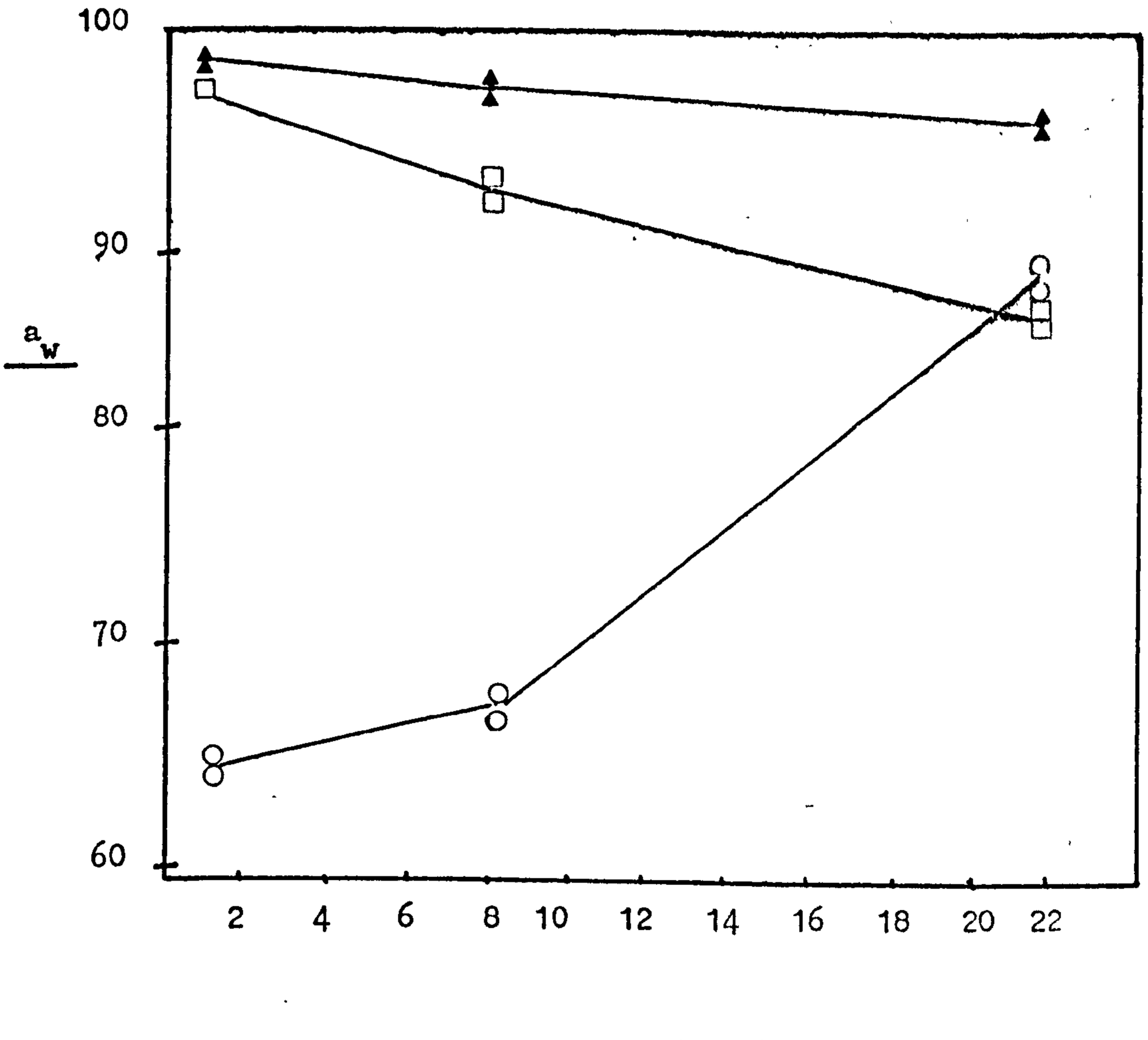
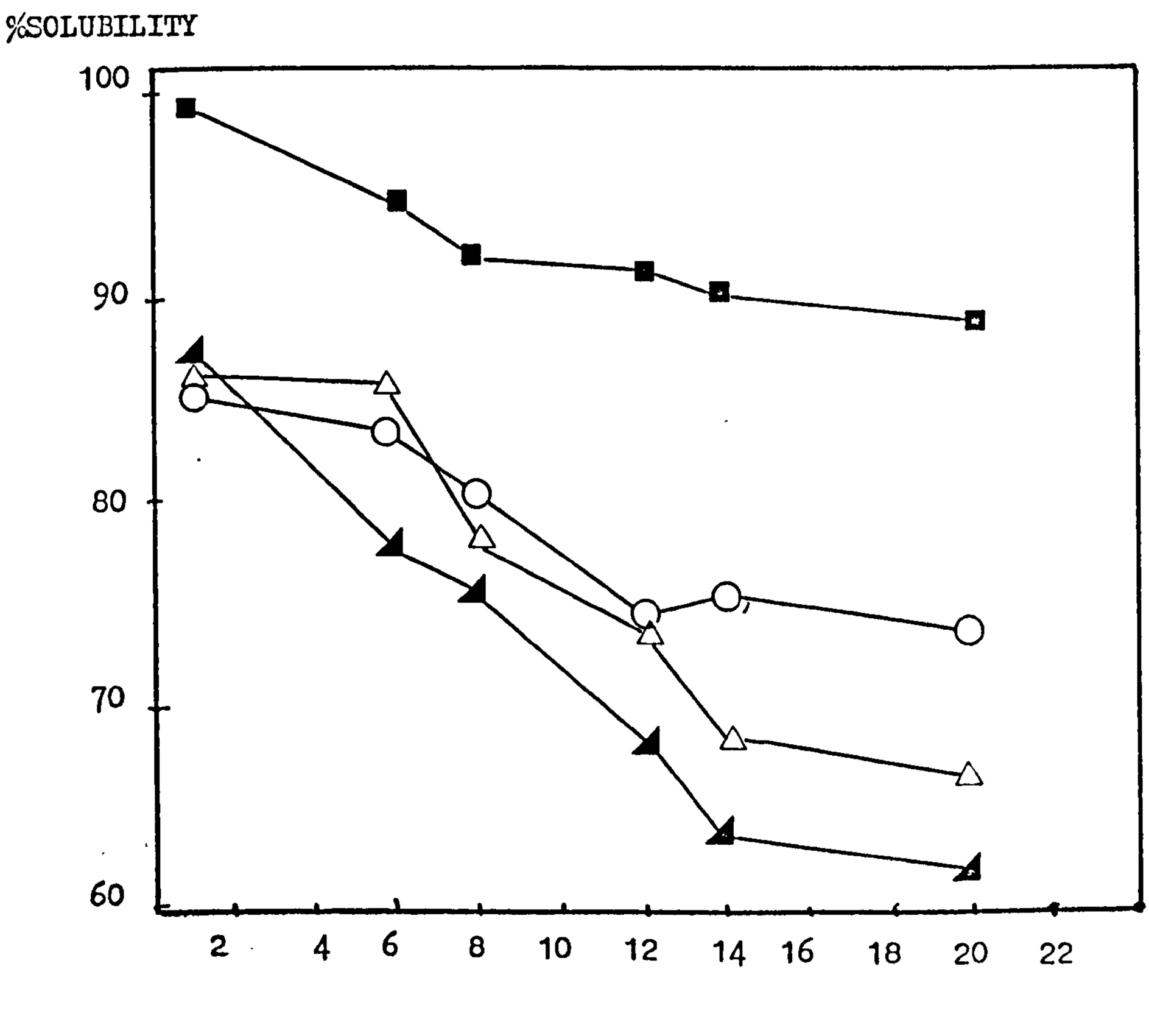


FIGURE 4.4

Change in protein solubility in SDS/β-Mercaptoethanol of the whole pie (◢–◢), of the meat filling (△–△), pastry (○–○), and jelly (■-■) of pies stored at 4°C and 80% r.h.



As time proceeded the meat filling required more force (more resistance shown to the needle probe). This may be due to the formation of cross-linkages between proteins or protein lipid products (see Section 4.1.2.6.). Also the water binding action of the rusk could result in a firmer meat filling texture. The pastry side walls showed a loss in texture (crispness), which was also detected subjectively by touch and taste. The loss in texture is presumably due to an increase in water content (Labuza, 1968) which reduces the number of starch crystalline areas (Labuza, 1968; Suggett, 1975) so there is less resistance to the probe's travel through the sample (See Section 2.2.2.).

4.1.2.6. Nitrogen solubility in SDS β-mercaptoethanol.

The soluble nitrogen or the solubility of the protein in 1% SDS β-mercaptoethanol is given in Figure 4.4. All the pie and pie component samples exhibited a decrease in solubility as storage proceeded. This indicated that covalent bonds were being formed, probably between proteins or lipid oxidation products (Andrews et al., 1965; Buttkus, 1970; Karel, 1980; Karel et al., 1975).

4.1.2.7. Colour.

When the C.I.E. co-ordinates X and Y were calculated very little difference was noted ($P > 0.05$) whatever the age of the sample (Table 4.1).

Table 4.1.

C.I.E. co-ordinates (X and Y) for the meat filling of pies stored at 4°C, 80% rh.

Storage time (Days)	C.I.E. Co-ordinates X	Y
2	.489	.406
	.489	.407
	.486	.406
6	.493	.408
	.495	.404
	.495	.404
	.498	.404
	.487	.408
13	.489	.406
	.484	.408
	.488	.408
	.492	.405
16	.496	.404
	.491	.406
	.492	.406
	.482	.409
20	.484	.412
	.479	.412
	.493	.413
	.490	.407

Yet, visibly the colour of the meat could be seen to change dramatically. There was a progressive development of a grey colour indicating destruction of the cooked meat pigments, moving from the outer surface of the filling towards the core. This discolouration was noticable after 7 days storage and very distinctive at 14 days.

Thus the method used to follow the meat filling colour changes was not sensitive enough to detect them. The human eye was a better detector, but unfortunately could not record the changes for comparisons at a later date.

4.1.2.8. Lipid oxidation.

The measurement of lipid oxidation was performed by the peroxide value test and the TBA test. The peroxide value (meat only) rose and peaked and then declined as the storage trial continued. This pattern of peroxide value rising and declining was expected as peroxides are the primary product of lipid oxidation (Gray, 1978). There is a build up in the peroxide level, and then a decline (Labuza, 1971) as the peroxides become involved in further reactions that eventually yield aldehydes, ketones and alcohols. The drawback to the use of peroxide value in the study of pork pies, is that if a single sample is taken and a low peroxide value obtained, it cannot be ascertained if oxidation has just started or if it is well advanced.

This does not apply to the TBA test, as values obtained for the pie, the pastry and the meat filling all increased with storage. This increase was different for each of the pie components and whole pie. Taste panel comments on the meat flavour were recorded along with the TBA values obtained. This revealed that when the TBA value was 5 or above, rancid flavours could be detectable by trained tasters both in the laboratory and factory.

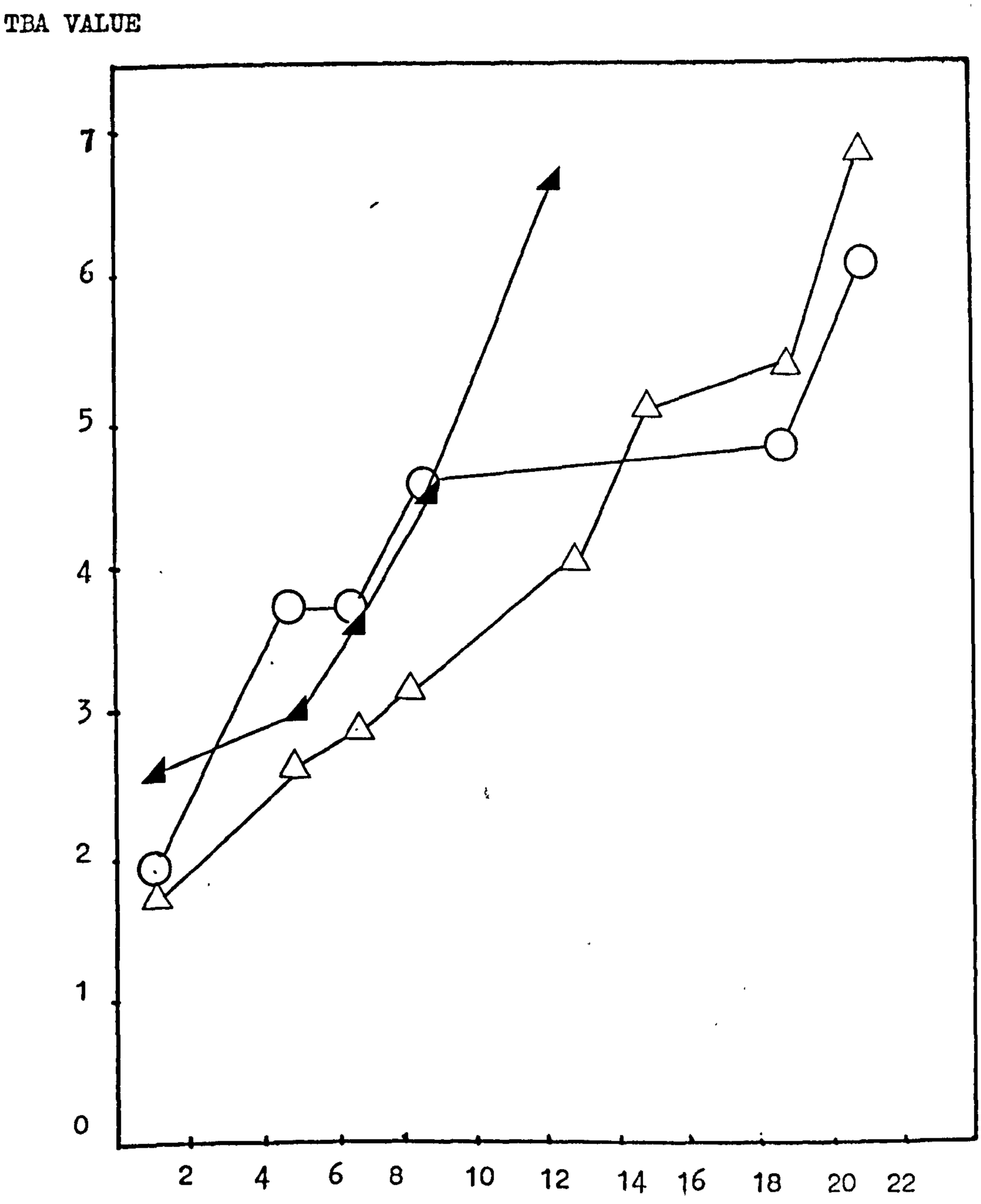
So this TBA value of 5 was used as the threshold of detectable rancidity in these products using the TBA value of 5 as the limit the whole pie shelf life was 10 days (Figure 4.5).

The TBA test involves measuring spectrophotometrically the colour complex formed from TBA reagent and malonaldehyde, According to Tarladgis et al., (1960) acid and heat are necessary for maximum colour development. During the course of this work it was found that the temperature of the water bath played an important role in the colour development. If temperatures below 100°C were employed, a yellow colour developed, this colour becoming more dominant the lower the temperature. The yellow colour had a maximum peak at 450 nm. All aldehydes produce a yellow colour with an absorption peak at 450 nm only on reaction with TBA reagent. 2,4-alka-dienals and 2-alkenals the compounds of interest produce the red 532 nm pigment, (Marcuse and Johansson (1973) Patton, (1973). So a temperature of 100°C must be used for the TBA test. Measurement at 450 nm and at 532 would also include glyceraldehyde (Patton, 1960) and hydroxymethylfural (Keeney and Bassette, 1959) which are not related to rancidity development.

Use of impure glacial acetic acid was found to reduce and impair the red colour development, in some cases preventing any colour development.

FIGURE 4.5

Changes in TBA values for the whole pork pie (◭-◭), and for the meat filling (△-△), and pastry (○-○) of pies stored at 4°C and 80% r.h.



4.2. Initial investigations into storage changes in pork pies stored at 4°C, 80% rh.

From the first set of trials the areas meriting further investigations were moisture migration and lipid oxidation (TBA values).

4.2.1. Experimental design.

The pork pies were made as described in Section 3.1., stored wrapped in cellophane at 4°C, 80% rh.. The moisture content (Section 3.3.4.) and TBA values (Section 3.3.9.2.) were recorded daily for the whole pie, meat filling and pastry. Lipid samples were taken (Section 3.3.10) from the whole pie, pastry and meat filling, converted into methyl esters (Section 3.3.12.) at day 1 of storage, and at the end of storage, when they were analysed by gas liquid chromatography (G.L.C. Section 3.3.12.2.).

4.2.2. Results and discussion.

The moisture content of the pies and the pie components (Figure 4.6) showed the same trends as previously recorded (4.1.2.1.). The moisture increase of the pastry being mirrored by the loss of moisture from the jelly. Again the total gain by the pastry could not be explained only by the loss of moisture from the jelly. The atmosphere around the pie also contributed to the increase in the pastry moisture content.

The meat filling moisture content remained unaffected throughout the storage period.

FIGURE 4.6

Changes in the percentage moisture content of the whole pie (◢—◢), and of the meat filling (△—△), pastry (○—○), and jelly (■—■) of pies stored at 4°C and 80% r.h.

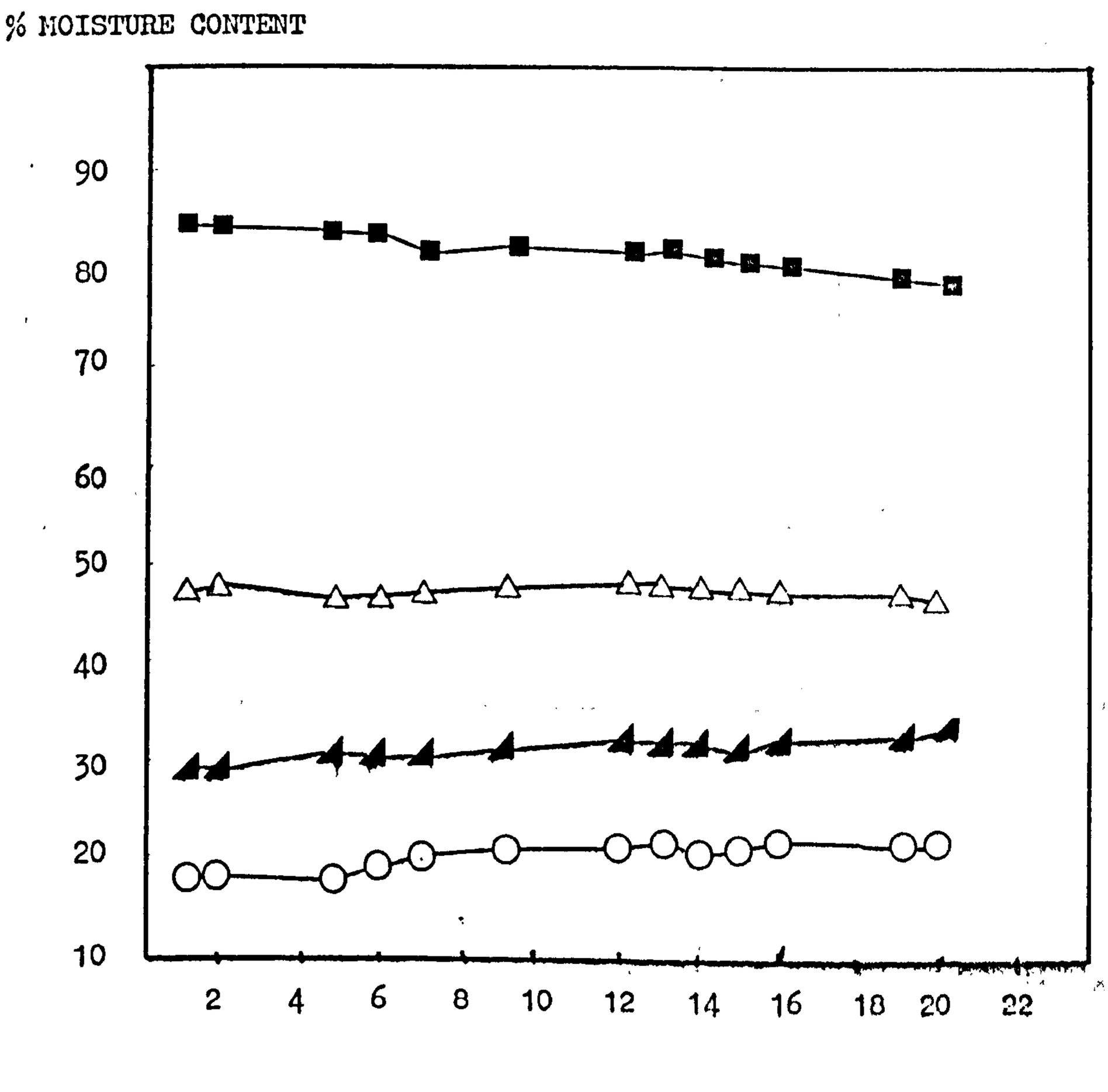
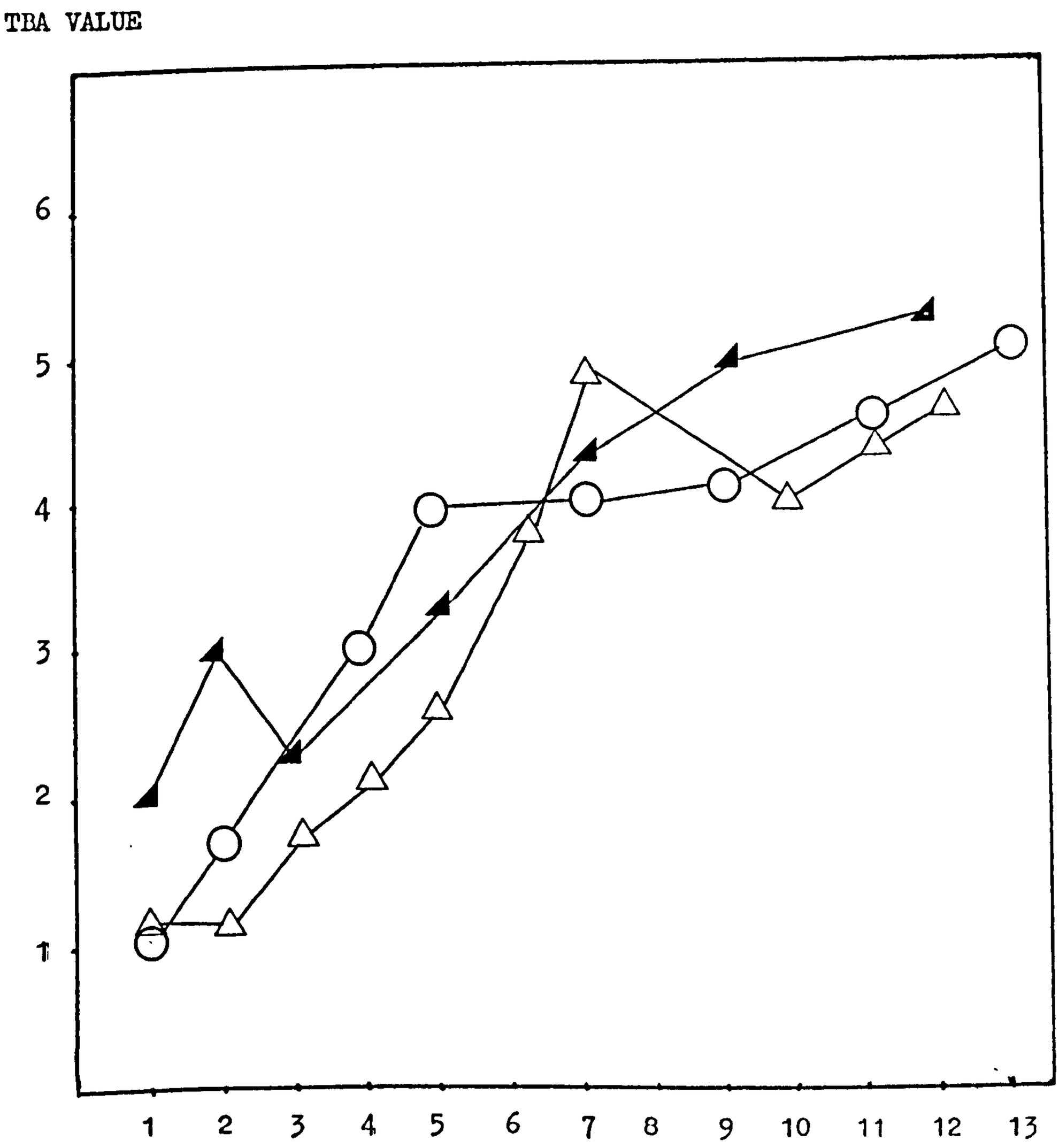


FIGURE 4.7

Changes in TBA values for the whole pie (◢–◢) and of the meat filling (△–△) and pastry (○–○) of pies stored at 4°C and 80% r.h.



The progress of rancidity was followed by the TBA method. The same pattern of increasing values was observed, but the values were not the same as before (Figure 4.7). This is presumably because the meat filling was made from different batches of lean and fat to the previous experiments and thus the lipids may be at different stages of oxidation. The age of the meat should affect the pastry and the whole pie values as during baking fat leaches out from the pie onto the baking tray. Upon cooling this molten fat is reabsorbed back into the pastry. It is by this method that some of the pork fat becomes incorporated into the pastry and thus can influence its keeping qualities.

Table 4.2 shows typical analysis of the changes in the fatty acids during storage. The system used did not adequately separate the low molecular weight fatty acids from the solvent shoulder. However by using computor integration and correcting for a steep base line, results were obtained. The results showed that oleic acid was the predominant fatty acid in fresh pork pies. Palmitic and oleic acids were also major fatty acids present. Upon storage an increase in the short chain low molecular weight fatty acids occurred, especially a six-carbon compounds (hexanoic acid?). The long chain unsaturated fatty acids were observed to decrease in concentration with storage. Thus indicating that they (palmitoleic 16:1, linoleic 18:2, linolenic 18:3) were involved in lipid oxidation.

Table 4.2.

Changes in fatty acid content (%) in the

triglyceride fraction of lipids from whole pork pies.

Number of carbon atoms	Storage time (Days) at 4°C 80% rh.	
	1	21
6:0	5.4	23.0
7:0	8.4	10.4
8:0	3.0	6.9
9:0	1.1	5.6
10:0	0.1	2.9
11:0	-	3.1
12:0	-	2:0
14:0	1.3	2.4
14:1	0.1	0.3
16:0	20.7	19.8
16:1	4.1	0.76
18:0	13.3	12.6
18:1	30.2	7.6
18:2	8.1	2.3
18:3	1.6	0.9
20:1	0.5	-
20:4	1.2	-

4.3. Discussion of initial results.

From these initial trials certain areas of work appeared to merit further investigation. The two main areas of investigation appear to be moisture migration within and from outside the pie, and lipid oxidation.

Moisture migration had been shown to occur and to affect the pastry texture. Thus knowledge of the a_w of each component of the pie, and attempts to formulate systems to reduce the differences between the phases thus minimizing moisture migration need to be investigated.

For the oxidation of lipids, the production of short chain fatty acids was shown to occur, from the longer chain unsaturated fatty acids. However the meat filling ingredients appeared to influence the rate of rancidity development so the effect of different age meat and fat on pie quality needed to be studied. The G.L.C. system used in this study needs modification, and a new system to be developed to follow the changes in fatty acid profiles in the lipids. Thus to elucidate their possible role in rancidity development.

Chapter 5.

Lipid Oxidation.

Chapter 5. Lipid Oxidation.

5.1. Effect of age of backfat and shoulder meat on rancidity development in pork pies.

In Chapter 4 it was found that pies produced from shoulder meat 4 days after slaughter had a shorter useful life than those pies produced from 2 day old meat, as determined both by taste panel and TBA values. This finding clearly merited further investigation since shoulder meat is the major component of the meat-filling of the pies. Moreover, since delays of up to 7 days could conceivably occur between death of the animal and the use of the meat it was decided to compare the effect of "new" (1 day post mortem) and "old" (7 day post mortem) shoulder meat on rancidity development in pork pies. Because there was evidence that the age of the back fat used in the meat-filling was also a factor in the pie deterioration, the effect of the incorporation of "new" (1 day after delivery, 1-2 days post mortem) and "old" (7 days after delivery, 7-8 days post mortem) had on rancidity was also examined in the experiment.

5.1.1. Experimental design.

Accordingly, four batches of pies were made from a single pastry mix (Section 3.1), but having different meat and fat fillings as follows:-

i) "old" shoulder meat with "old" back fat.

ii) "old" shoulder meat with "new" back fat.

iii) "new" shoulder meat with "old" back fat.

iv) "new" shoulder meat with "new" back fat.

The "old" shoulder meat was 7 days post slaughter, and held at 2°C. "New" shoulder was 1 day post slaughter, and held at 2°C: "old" back fat was 7-8 days post mortem, and "new" back fat was 1 day post mortem, both being kept at 2°C until required. The leg and belly meat in all four meat mixes was 1 day post mortem.

After baking and jellying the pies were cooled to 4°C, wrapped, and stored at 4°C, 80% rh., until required, and TBA values were determined each day.

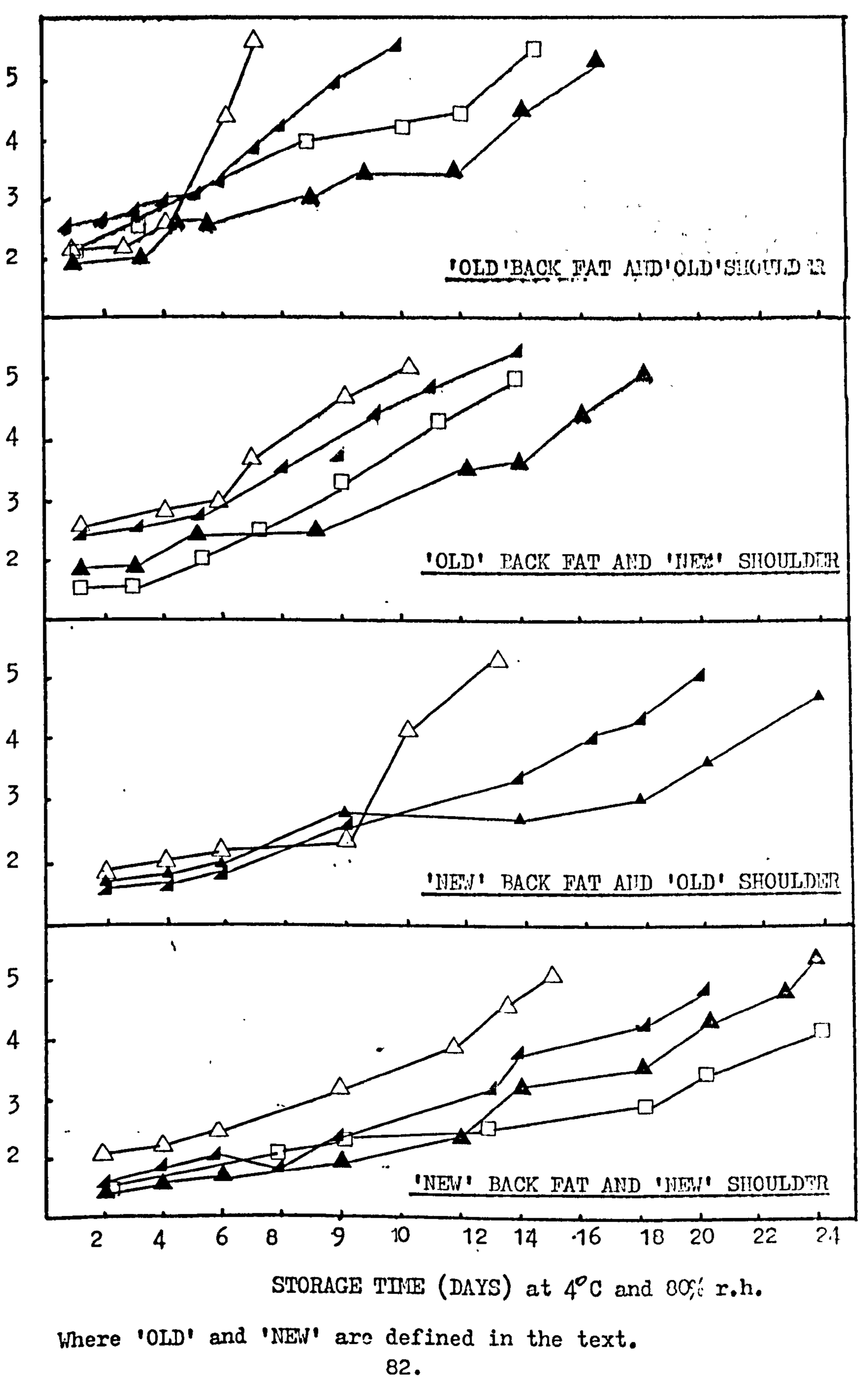
5.1.2. Results and discussion.

The increase in TBA values, as a function of storage, is shown in Figure 5.1. A TBA value of 5 was taken as the value above which the pies were organoleptically unacceptable (as found in Chapter 4). Using this criterion the shelf life (in days) for the pie components of the four batches of pies is shown in Table 5.1.

It may be seen that when back fat and shoulder meat are used, after keeping for 7 days after slaughter ("old"), the shelf life of the pie is considerably shorter than when back fat and shoulder meat, kept for 1 day post slaughter ("new"), are used. Use of "new" shoulder meat with "old" backfat extends the pie shelf life in terms of TBA values by 2 days, while use of "new" back fat with "old" or "new" shoulder meat extends the pie shelf life by 10 days when compared to meat pies containing both "old" back fat and "old" shoulder meat.

FIGURE 5.1

Change in TBA values for the whole pie (▲—▲), and for the meat filling (△—△), pastry (▲—▲), and meat filling core (□—□) of pies with the meat filling made using various combinations of 'old' and 'new' back fat and shoulder meat.



Where 'OLD' and 'NEW' are defined in the text.

Table 5.1.

Shelf life (days storage at 4°C, 80% rh)

of pork pies and pie components as determined by

attaining a TBA value of 5.

Component	Combination of Back fat/Shoulder meat in meat filling.			
	i) old/ old	ii) old/ new	iii) new/ old	iv) new/ new
Whole pie	9-10	12	20	20
Meat filling	8	10	12	15
Top surface of the filling	14-15	13	-	-
Meat core	12	16	24	26
Pastry	17	18	24	24-25

Combinations of back fat and shoulder are as defined in

the text.

" - ": not determined.

Minimum of six pies used in each daily TBA determinations.

The shelf life for the meat filling was shorter than that for the whole pie. "Old" back fat and "old" shoulder meat in the meat filling had a shelf life of 8 days; and when the back fat and shoulder meat were "new", the shelf life was 15 days. From the values in Table 5.1 it can be seen that when the shoulder meat is "new", use of "old" back fat in the meat filling of pork pies shortens its shelf life. Not surprisingly the best combination for maximum shelf life is "new" back fat with "new" shoulder meat. Use of "old" shoulder meat with "new" back fat is the second alternative. At all times the use of "old" back fat should be avoided, as it significantly ($P < 0.05$) reduces the shelf life of pies and their meat fillings (significance levels are as described in Section 3.15). With regard to the pastry, the shortest shelf life was obtained when "old" back fat and "old" shoulder meat were used together in the filling. "New" back fat, with "new" or "old" shoulder meat were used together in the filling. This combination produced the longest shelf life.

The pie filling affects the pastry shelf life (TBA values) because of the seepage of fats during baking. When the temperature is high enough to melt the fat, it becomes free to migrate to the surface of the filling and, via the pastry, out onto the baking tray (Wilson, 1981). The latter is reabsorbed by the pastry during the initial cooling phase of processing. Thus fat, from the meat filling, ends up in the pastry. Therefore "old" shoulder meat and "old" back fat with their higher levels of oxidized fats, will hasten the onset of rancidity in the pastry, and consequently shorten the pies' shelf life.

The incorporation of pork fat in meat products is known to shorten their shelf life (Benedict et al., 1975) in comparison with that of products containing beef fat. The probable cause of this is the high linoleic acid (C18:2) content of the former. Bremner et al., (1976) found that pork back fat with a high linoleic acid level developed peroxide values 2-3 times faster than that with normal linoleic acid levels; and rancid odours and tastes were produced earlier according to Bernardini et al., (1982).

Linoleic acid in pork muscle normally constitutes 8.0 - 10.7% of the total fatty acids. Hornstein et al., (1961) quoted a level of 7.9% in pork muscle, and Pearson et al,, (1977); Koch et al., (1968a) quote 10.5% in pork back fat. The level of linoleic acid in the pork pies studied in the present investigation was found to be 8.0% (see Section 4.2.).

It is possible to obtain back fat with high linoleic acid levels if the diet of pigs includes highly unsaturated fats such as those found in sunflower oil or seeds (Marchello et al., 1983) or maize. Since some pig feed formulations contain maize the problem of linoleic acid levels in the lean and fat could cause problems to the meat processing industry.

To reiterate, the age of the back fat and shoulder meat used in the pie filling affects the shelf life of the pastry, the pie filling and whole pie in terms of rancidity development (determined by TBA value). The age of the back fat had a greater influence on shelf life than the shoulder meat (Table 5.1) as there was a significant ($P < 0.05$) reduction (significance levels are as described in Section 3.3.15.), in

shelf life when "old" back fat was used compared to when "new" back fat was used. The age of the shoulder meat only influenced rancidity when used with "old" back fat ("old" shoulder shortening the shelf life).

Therefore, when at all possible "new" back fat should be used, preferably with "new" shoulder meat. If "old" back fat has to be used, for whatever reasons, "new" shoulder meat should be used with it. At all times "old" shoulder meat with "old" back fat should be avoided if good organoleptic pies are required.

Since the meat filling was evidently the factor which predominantly influenced storage life of the pastry and of the whole pie, the subsequent investigations concentrated on examining its behaviour.

5.2. Effect of storage conditions on rancidity development in pork back fat.

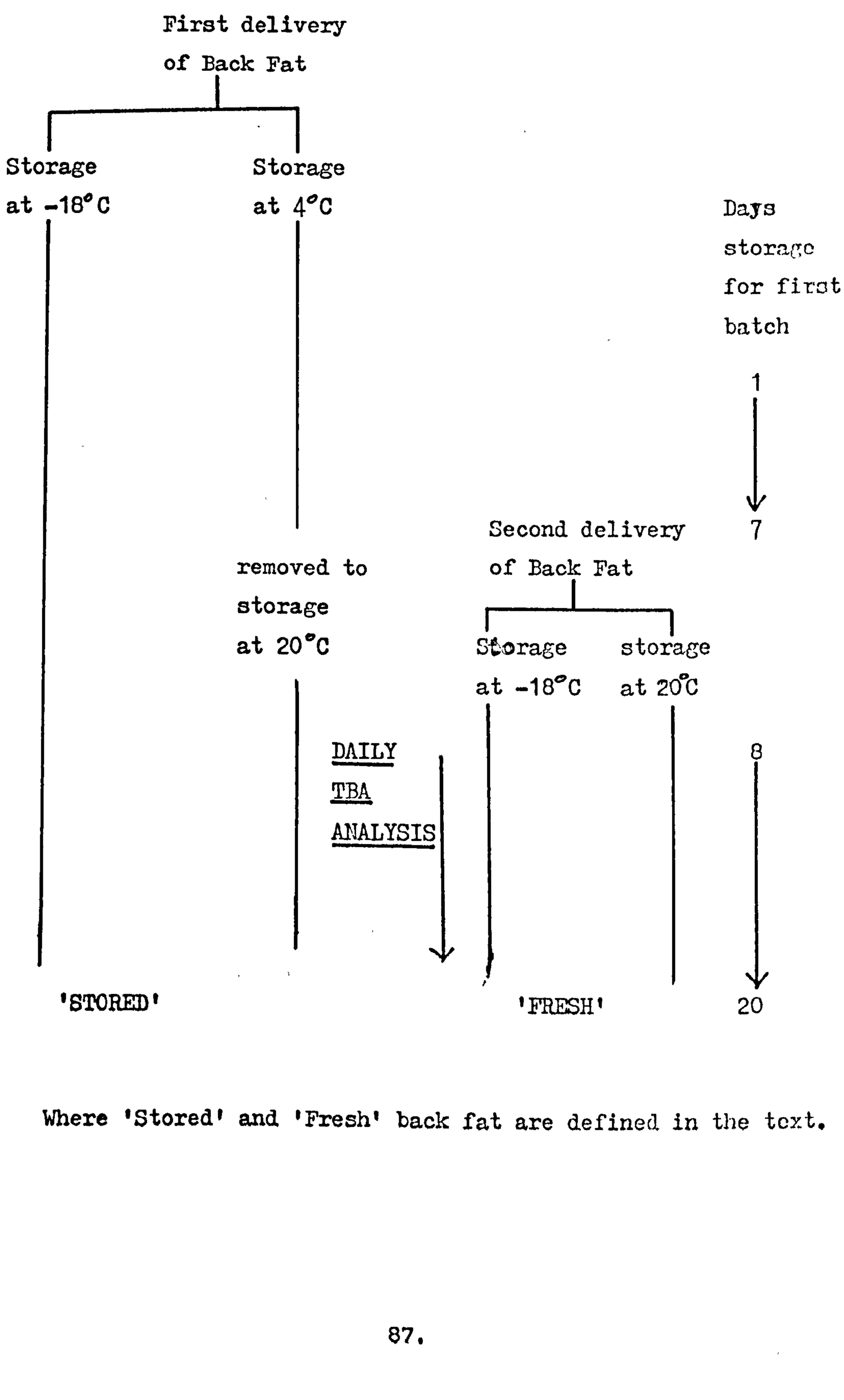
As the age of the back fat appeared to be more critical than that of the shoulder meat in influencing the shelf life of the pies (Section 5.1), further investigations of the back fat were deemed necessary. Since there is always the possibility of substandard meat management and/or of refrigeration failure in the processing factory, the effects of frozen and unrefrigerated storage was investigated.

5.2.1. Experimental design.

The design of the investigation to study the effect of frozen and non-refrigerated storage on rancidity development (TBA values) in back fat is illustrated in Figure 5.2.

FIGURE 5.2

Schematic diagram of the investigation into the effect of storage at -18°C and 20°C of 'stored' and 'fresh' back fat on rancidity development, as measured by the TBA test.



Where 'Stored' and 'Fresh' back fat are defined in the text.

Two batches of back fat were obtained from a supplier. The first batch was divided into two portions, and frozen at -18°C the other kept at 4°C for 7 days. Both portions were designated as "stored".

The second batch, designed as "fresh", was now obtained (7 days after the first batch) divided also into two portions, one being frozen at -18°C, the other kept at 4°C, for 2 hrs. The non frozen "stored" and "fresh" samples were then held at 20°C (room temperature). Thus there were four samples of back fat for TBA analysis:-

i) "stored" back fat kept at -18°C throughout.

ii) "stored" back fat kept at 20°C (after 7 days at 4°C).

iii) "fresh" back fat kept at -18°C throughout.

iv) "fresh" back fat kept at 20°C.

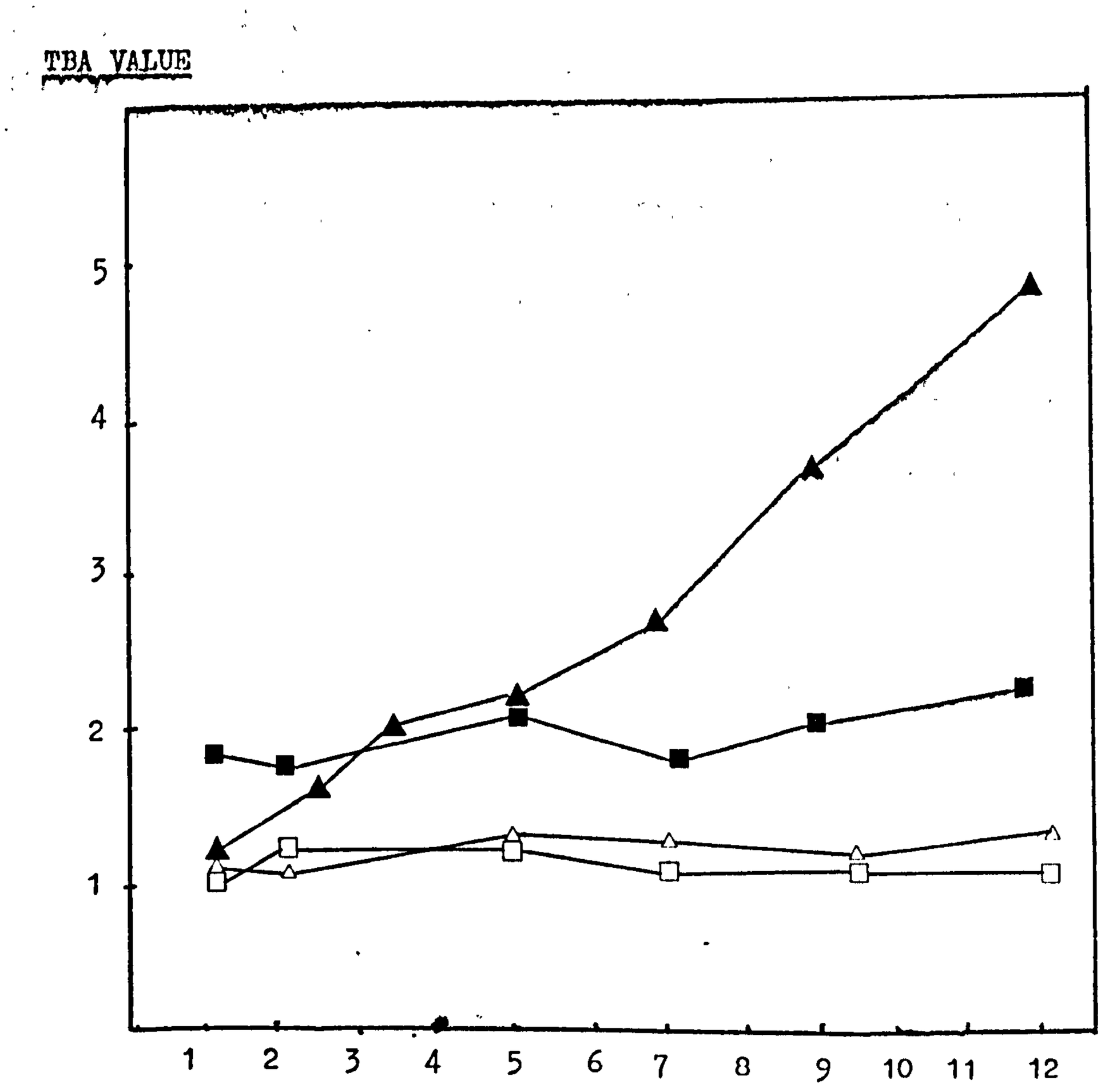
For each back fat sample (i to iv) TBA analysis was performed each day in triplicate (i.e. 12 analyses per day).

5.2.2. Results and discussion.

The TBA values obtained from this trial are shown in Figure 5.3. The "fresh" back fat samples, whether stored at -18°C or at 20°C, showed only a very slight increase in TBA values with time. In contrast the "stored" back fat kept at 20°C exhibited a rise in TBA values, as did that kept at -18°C (Frozen). In the "stored" back fat kept at 20°C the rate of rancidity development was significantly ($P < 0.05$) faster than that of the "stored" back fat kept at -18°C. Thus storage at 20°C markedly enhanced the development of rancidity in comparison with samples from the same delivery batch kept at -18C.

FIGURE 5.3

Effect of keeping 'stored' back fat at -18°C (■—■) and at 20°C (▲—▲), and 'fresh' back fat at -18°C (□—□) and at 20°C (△—△) on rancidity development, as measured by the TBA test.



Where the terms 'stored' and 'fresh' back fat are defined in the text.

The "stored" back fat kept at 20°C also showed signs of tissue breakdown, probably caused by enzymic action and it produced unpleasant odours after 9 days of storage at 20°C.

From Figure 5.3, it can be seen that the "stored" back fat kept at -18°C had higher TBA values than either of the "fresh" back fat samples, indicating a higher level of oxidized lipids.

Thus unrefrigerated storage, (or refrigeration failure) can lead to high TBA values in back fat and thus to rancid pork pies. If back fat is to be used in processing it should be kept frozen until 24 hrs prior to requirement, and then kept at 4°C. Once incorporated into the filling mix, it should be kept at 4°C, and utilized in production as soon as possible otherwise the pie shelf life (in terms of TBA values) will be reduced. Alternatively, the back fat should be obtained after slaughter for utilization in the following 24 hours.

5.3. Effect of cooking on rancidity development in the meat filling of pork pies, and the role of rusk and seasoning mix.

Cooking is claimed to enhance rancidity development (Younathan and Watts, 1959; Keller and Kinsella, 1973). It may also cause a rapid onset of rancidity (detectable, within 24 hours of cooking, on storing at 4°C) which has been termed "warmed over flavour" (WOF) by Igene *et al*., (1979a; 1979b; 1980; 1981).

The role of cooking on rancidity was thus investigated both in pork pies and pork burgers. The latter were included in the study because they contained longissimus dorsi muscle obtained from the shoulder region of the pig carcass. This muscle was used so as to provide a homogeneous source of muscle. Thus the development of rancidity in these products could thus only be affected by the test variables, which in this investigation, were cooking, and rusk and seasoning mix presence.

5.3.1. Experimental design.

To investigate the influence of cooking and rusk and seasoning mix on rancidity development, pies were made from a single pastry mix (Section 3.1), but with the meat filling containing varying amounts of rusk and seasoning mix, as follows:-

i) with 5.5% rusk, 0% seasoning mix

ii) with 0% rusk, 3.75% seasoning mix

iii) without rusk or seasoning mix

iv) with 5.5% rusk and 3.75% seasoning mix

which are the proportions of rusk and seasoning mix used by the manufacturer. The shoulder meat and back fat used were 2 days post slaughter.

Half of each set of pies was then baked, jellied and cooled. The other half was left uncooked. Thus there were 8 pie batches overall, and these were stored at 4°C, 80% r.h. The TBA analysis was performed daily on each of the different meat fillings.

In addition four batches of burgers were made (Section 3.2) from rusk and seasoning mix was added or omitted (as for the meat filling) in the same proportion as used in the meat filling recipe in the factory. Half of each burger batch was cooked (Section 3.2), the other half kept raw. The 8 batches of burgers were stored separately in plastic containers at 4°C. TBA analysis was performed daily in triplicate.

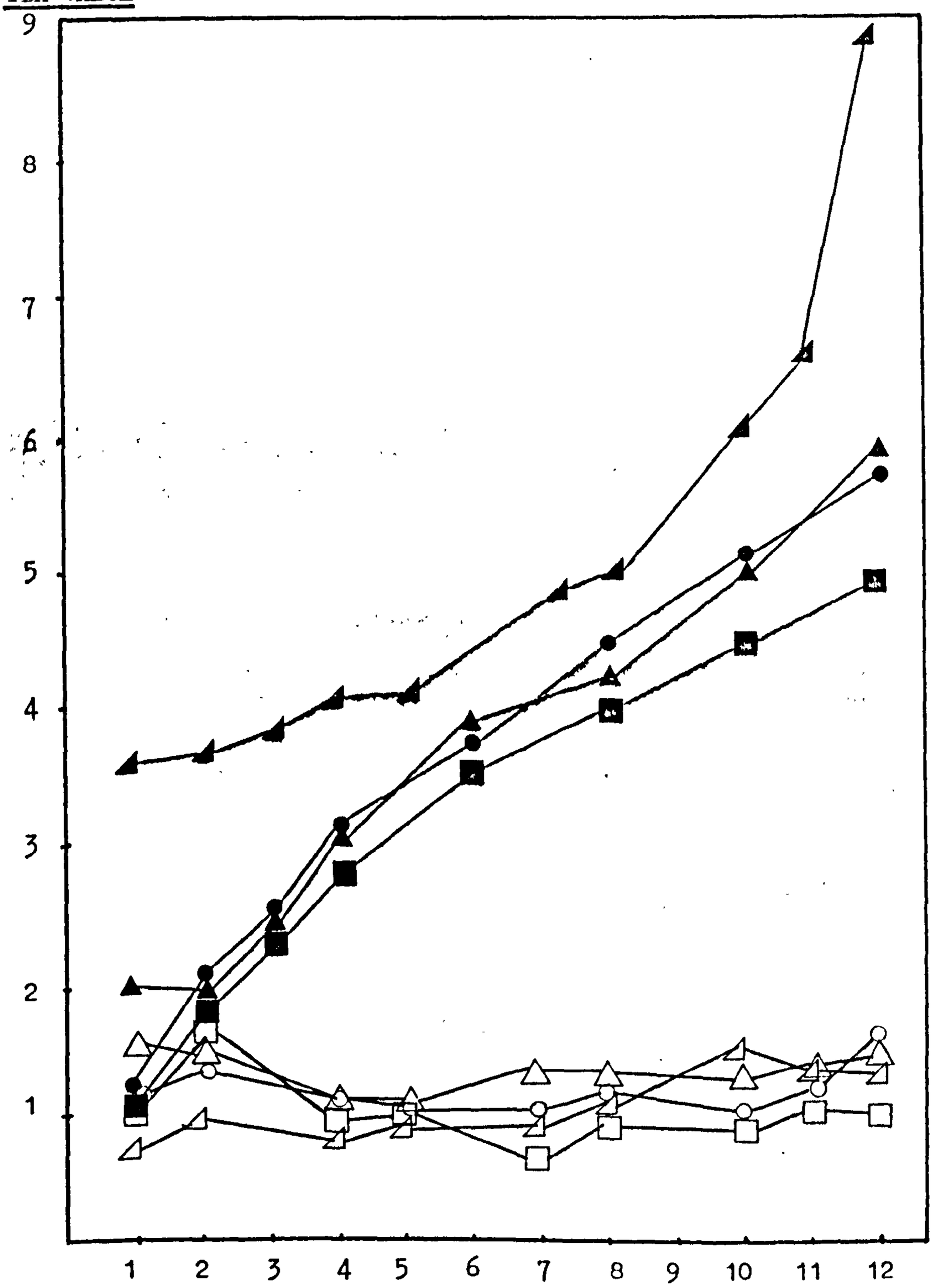
5.3.2. Results and discussion.

The TBA values obtained from the cooked and uncooked pies are shown in Figure 5.4. It is seen that the uncooked pie meat filling did not attain a TBA value of 5 throughout the 16 days storage at 4°C. However the meat filling had spoilt, as it had developed unpleasant odours and psycrophilic bacterial growth was observed. There was no significant different ($P > 0.05$) between any of the TBA values obtained from the uncooked meat fillings.

In all the cooked pies, meat filling rancidity developed but at different rates. The meat filling containing neither rusk or seasoning mix reached a TBA value of 5 by day 5/6 of storage i.e. it became organoleptically unacceptable. The meat fillings without rusk, and those without seasoning, attained this value after 9 days storage, whereas the control filling, containing both rusk and seasoning only did so after 15 days storage. The rate of rancidity development (TBA values) for the filling with no rusk or seasoning was significantly faster ($P < 0.05$) than those for the control filling and for those with no rusk or no seasoning, (by Student t-test, Section 5.3.15.). There was no significant

FIGURE 5.4

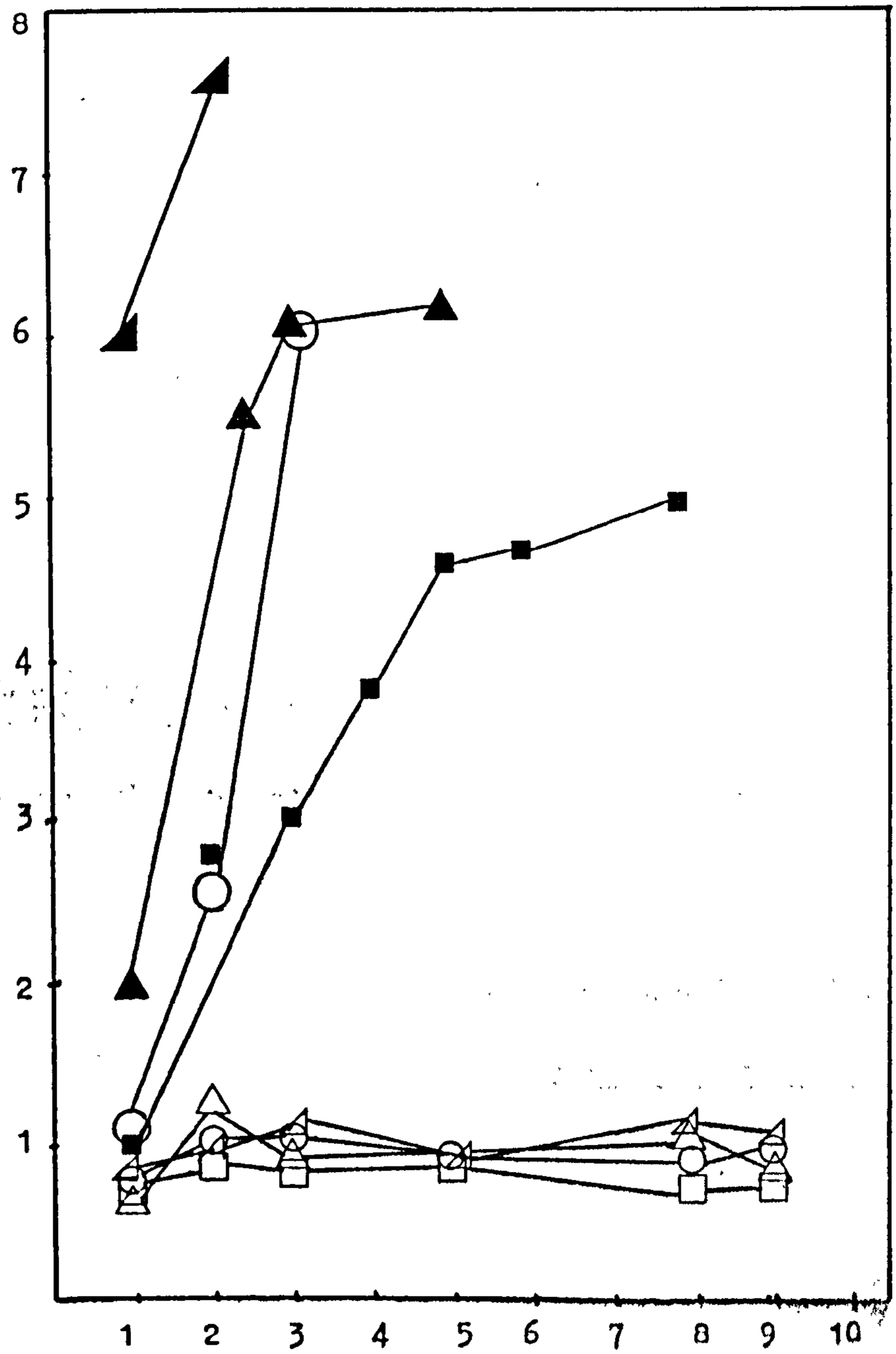
Effect of cooking on rancidity development in the meat filling of cooked (filled symbols) and uncooked (open symbols) pork pies stored at 4°C and 80% r.h.



Where (■—■, □—□) are the cooked and uncooked control samples,
(▲—▲, △—△) are the samples containing no rusk,
(●—●, ○—○) are the samples containing no seasoning mix
(◢—◢, ◿—◿) are the samples with no rusk or seasoning mix.

FIGURE 5.5

Effect of cooking on rancidity development, as measured by the TBA test, in cooked (filled symbols) and uncooked (open symbols) pork burgers stored at 4°C and 80% r.h.



Where (■—■ , □—□) are the cooked and uncooked control samples,
(▲—▲ , △—△) are the samples containing no rusk,
(○—○ , ○—○) are the samples containing no seasoning mix,
(◢—◢ , ◿—◿) are the samples with no rusk or seasoning mix
reppectively

difference ($P > 0.05$) between the latter two fillings. Thus both the rusk and seasoning mix are in someway acting as antioxidants as their omission leads to faster rancidity development. This will be discussed in more detail later (Section 5.4).

In the burgers also, cooking accelerated rancidity development, the effect being even more pronounced than in the pies (Figure 5.5). This fast development of rancidity can be explained by the lack of pastry and gelatine jelly which can act as oxygen barriers in the pies. In pies the top surface of the meat filling has been shown to have the fastest rate of rancidity development (Section 5.1).

In the cooked burgers containing no rusk and seasoning, the rate of rancidity development was sufficiently marked to caused a warmed-over flavour. There was no significant difference ($P > 0.05$) between those burgers having no rusk, and those burgers having no seasoning mix, but in both of these the rate of rancidity development was significantly faster ($P < 0.05$) than in the control burgers. There was no difference ($P > 0.05$) between the rancidity (TBA) values between any of the four uncooked burgers (Figure 5.5).

Younathan and Watts (1959) reported that cooked cured meat developed off-odours and tastes, and TBA reactive material, at a faster rate than uncooked cured meat, when both were stored at 4°C. Keller and Kinsella (1973) obtained higher TBA values from cooked beef patties than from uncooked patties (burgers). These observations all seem to be confirmed

by the results obtained here: cooking accelerates rancidity in pork pies and burgers.

Several hypotheses have been suggested to explain how cooking accelerates rancidity (lipid oxidation). In uncooked meats, the undenatured haemoproteins, by stearic hindrance of the iron, preventing it calaysing lipid oxidation. Heating will denature the haemoproteins thus allowing the haem iron to catalyse oxidation (Eriksson 1975). This effect is greatest at pH 5.5 to 6.5 (Love, 1983) which is the pH of the meat filling (Section 4.1). Both states of haem and non haem iron (ferrous and ferric) may catalyse the reaction (Tappel, 1962; Greene and Price, 1975), the ferric iron being the more active (Kaschinitz and Hatefi, 1975).

Again cooking of pork causes a decrease in its linoleic acid (18:2) content (Gandermer, 1983), indicating its involvement in lipid oxidation (Corliss and Dugan, 1970). Cooking can also mobilize phospholipids bound to membranes, especially phosphatidyl ethanolamine (PE), (Igene et al., 1979b). This phospholipid is reported to be especially susceptible to oxidation (Igene et al., 1981), and so could play a key role in the lipid oxidation of cooked meats.

Whatever the causes, (probably a series of complex interactions), cooking of pork pies and pork burgers has been shown to accelerate rancidity (TBA values) development. This acceleration was usually not sufficiently marked to cause warmed-over flavour - except, possibly, in the case of the burgers without rusk or seasoning. To reiterate, therefore, the rusk and seasoning mix, both together and separately, act as antioxidants in the meat filling of pork pies, and in pork burgers.

5.4. Effect of the rusk and the seasoning mix on rancidity development in pork pie

Following the findings reported in Section 5.3, the role of the rusk and the seasoning mix as antioxidants was investigated further in this section, in order to try to establish the antioxidant components in both rusk and the seasoning mix.

5.4.1. Effect of quantity of rusk and the seasoning mix on the development

5.4.1.1. Experimental design.

The batches of pies were made from a single pastry mix (Section 3.1), but containing six meat filling mixes. All contained the same shoulder meat and back fat (2 days post slaughter), but with varying amounts of rusk and seasoning mix, which were varied as follows:-

i) 5.5% rusk and 3.75% seasoning mix, which are the 'normal' proportions used in the meat filling of the manufacturer (control).

ii) Without rusk or seasoning mix

iii) 5.5% rusk, with no seasoning mix

iv) 11% rusk, and no seasoning mix

v) 3.75% seasoning mix, and no rusk.

vi) 7.50% seasoning mix and no rusk.

All percentages are on a w/w basis with the meat and fat.

After baking, jellying and cooling, the wrapped pies were stored at 4°C, and 80% r.h. The meat filling of each pie batch was sampled as described in Section 3.3.1. and TBA analysis performed daily in triplicate.

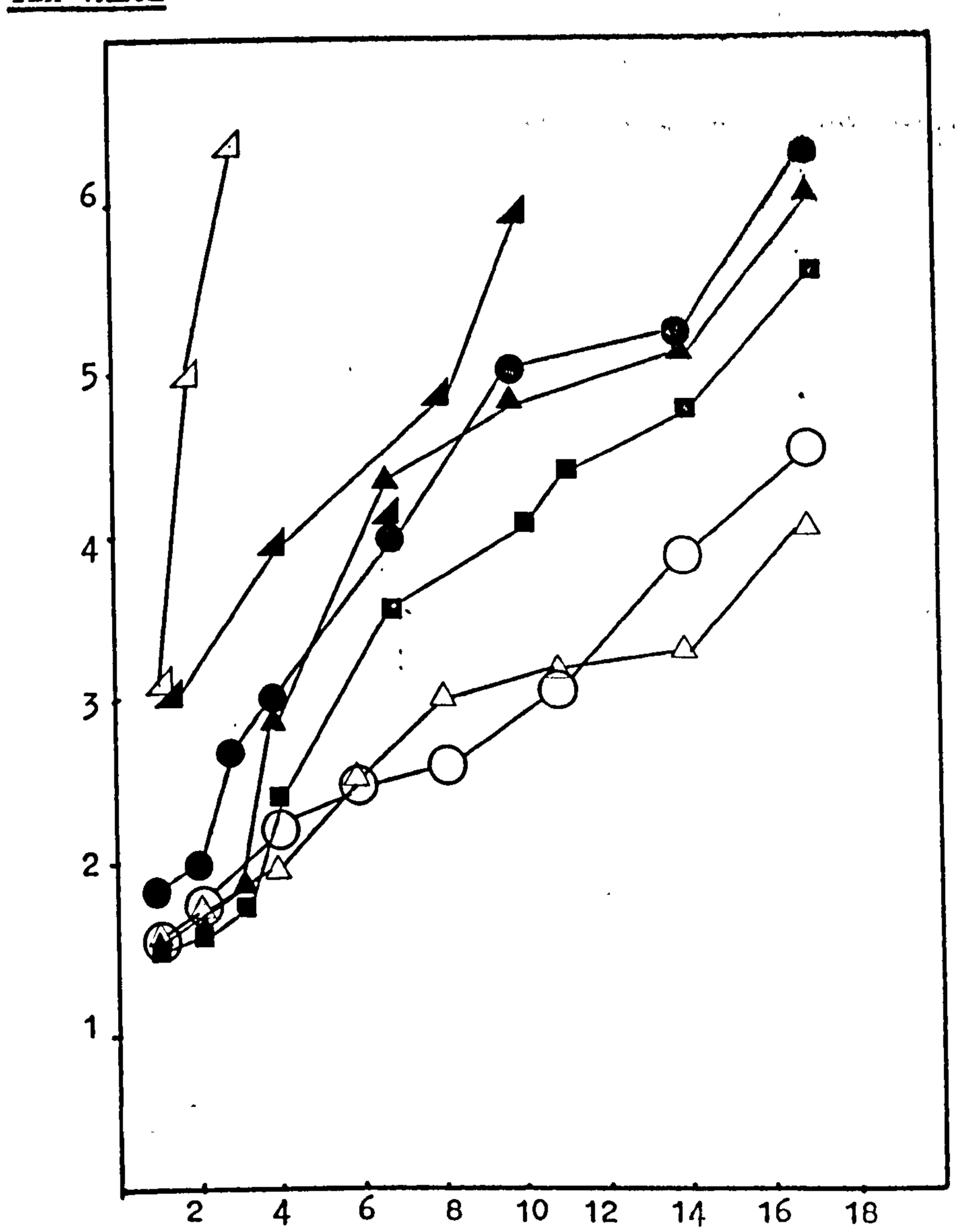
5.4.1.2. Results and Discussion.

Of considerable interest was the observation that the pies with no rusk or seasoning mix, when stored whilst still in the production hoops (normally removed during processing) exhibited a slower development of rancidity than those where the hoop had been removed (Figure 5.6). In the absence of rusk and seasoning mix the meat filling became rancid quicker than did pies with the rusk and/or seasoning mix present in the meat fillings. No significant difference was found between the pies with 5.5% rusk, and the pies with 3.75% seasoning mix in the meat fillings, nor was their any significance in rancidity development between pies with 11% rusk and pies with 7.50% seasoning in the meat fillings. Whereas, the use of 5.5% rusk, or of 3.75% seasoning mix in the meat filling was associated with TBA values higher than the control meat filling. Doubling the amount of rusk, or seasoning mix in the filling resulted in TBA values lower than those from the control filling, and they resulted in a longer time period before rancidity limited the pie shelf life, when compared to the shelf life of the control pie, or when rusk or seasoning are used at their usual levels (5.5% and 3.75% respectively).

The use of 7.5% seasoning mix, however gave a peppery taste to the filling; whilst incorporation of 11% rusk (twice the usual amount) appeared to toughen the meat filling (as

FIGURE 5.6

Effect of quantity of rusk and of seasoning mix on rancidity development, as measured by the TBA test in the meat filling of pork pies stored at 4°C and 80% r.h.



Where (■—■) contains 5.5% rusk and 3.75% seasoning mix, (●—●) contains 5.5% rusk, (○—○) contains 11% rusk, (▲—▲) contains 3.75% seasoning mix, (△—△) contains 7.5% seasoning mix, (◿—◿) contains no rusk or seasoning mix, (◢—◢) contains no rusk or seasoning mix stored in the metal rings.

determined by subjective assessment performed during the course of the study).

The probable explanation for the retardation in rancidity development caused by storing the pies in their production hoops, is that they reduce the amount of pie surface area exposed to the atmosphere, thus there is less oxygen available for involvement in lipid oxidation.

The antioxidant action of the seasoning mix is probably due to its pepper content as mono-sodium glutamate (MSG) and starch are not known to be either pro- or anti-oxidative. Salt is a known pro-oxidant (Chang and Watts, 1950; Zipser et al., 1964; Aberle et al., 1980; Gray 1978; Huffman et al., 1981; Neer and Mandigo, 1977; Schwartz and Mandigo, 1977; Keeton, 1983; Rhee et al., 1983,) whereas black pepper has been shown to be antioxidative in oil emulsions, but not in lard (Chipault, 1957). Palitzsch et al., (1969) found white pepper (and its extract) to lengthen the oxidation induction period in lard two-fold when measured either by peroxide values or TBA values.

The rusk's antioxidant properties could be due to the Maillard browning reaction which occurs during the baking of the rusk. Maillard pigments are formed via a complex of several interconnecting, consecutive chemical reactions. However an acceptable theory explaining all the reactions is not yet available (Dworschak, 1980). In the Maillard reaction amino acids or primary or secondary amines react with monosaccharides, to form aldosylamines, aldoses and ketosylamines. The aldosylamines are unstable, and undergo

further changes involving the formation of 'Amadori' products, and these rearrange to form ketoseamines. Amino acids also catalyse the reactions ensuring the formation of diketo and unsaturated diketo sugar derivatives. Amino acids link with these products irreversibly to form, eventually, melanoidines. Amino acids also react with hexosuloses from ketoseamines via a Strecker degradation process, yielding aldehydes as one end product.

Hannan (pers. comm.) cited the Strecker degradation products as having antioxidant properties. Other authors (Griffith and Johnson, 1957; Hwang and Kim, 1973; Morita et al., 1976) have reported antioxidant properties for carbonyl compounds and amino acids, but have not reported on the structural determinants of the antioxidant action.

Lea et al. (1975) ascertained that the greatest antioxidant activity occurred at the beginning of the Maillard browning reaction. Park and Kim (1979) also claimed that the melanoidin compounds which were eventually found were antioxidative. Huyghabaert et al., (1982) reported that there was a positive relationship between antioxidant activity and the concentration of Amadori products present. As these colourless Amadori products are produced early in the browning reaction, they, accord with the hypothesis (Lea et al., 1975) that the greatest antioxidant effect is at the beginning of the Maillard reaction.

The present results have shown that the rusk and seasoning mix have antioxidant properties and that use of twice the usual amount of rusk (11%) or (7.5%) seasoning mix

reduced rancidity development more effectively than at the combination of the usual amounts of rusk and seasoning mix, Pepper is probably the main antioxidant component in the seasoning mix and Amadori products produced by the Maillard browning reaction, most likely account for the antioxidant action of rusk.

5.4.2. The pro- and antioxidant properties of the ingredients of the seasoning mix.

In Sections 5.3 and 5.4.1 the seasoning mix was shown to have antioxidant properties (even better, when used in combination with rusk). Since one of the mix ingredients : salt, is a known pro-oxidant (Gray, 1978), whilst pepper can be antioxidant (Palizsch et al., 1969) or exhibit no action at all (Chipault, 1957) depending on the system it is employed in, further investigation was clearly desired.

The effects were studied in 'burgers' as described in Section 3.2 as they developed rancidity faster than pie fillings (cf 5.3). The use of burgers also reduced the variables involved since longissimus dorsi was the muscle used, and so the only variable was the seasoning mix ingredients. Therefore, any significant change in rancidity development must be due to the mix ingredients.

5.4.2.1. Experimental design.

The burgers were made (Section 3.2) up as six batches, which differed and were as follows:-

i) no addition of mix ingredients (no additive)

ii) with seasoning mix in normal proportion of 3.75% (control)

iii) with Apex starch in usual mix proportion of 0.69%

iv) with M.S.G. in usual mix proportion of 0.17%

v) with salt in usual mix proportion of 2.21%

vi) with white pepper in usual mix proportion of 0.55%

After cooking, the burgers were stored at 4°C, 80% r.h, and analysed daily using the TBA test.

5.4.2.2. Results and discussion.

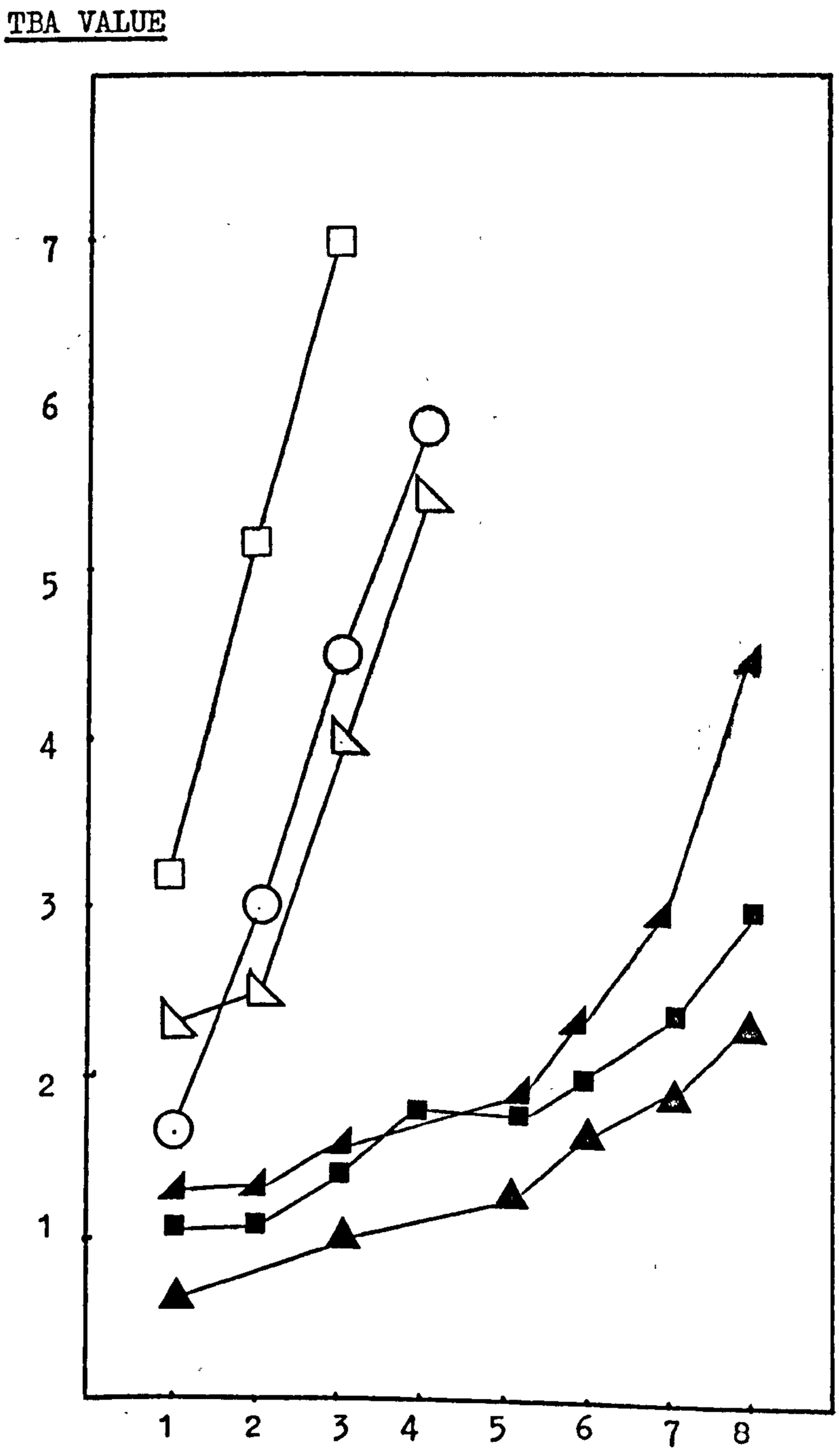
In terms of the TBA values which developed, the seasoning mix ingredients used in the burgers fell into two categories: those which had no effect, and those which slowed down rancidity development (Figure 5.7). In the former category were Apex Starch and M.S.G.; and there was no significant difference ($P > 0.05$) found in rancidity development from these burgers and those burgers with no seasoning mix ingredients.

The burgers containing salt showed a slightly higher rate of rancidity development that the control burgers. After six days storage in 'salt', burgers showed a marked acceleration of rancidity development (Figure 5.7).

In the burgers containing the white pepper rancidity developed at a slightly slower rate than in the controls, but this was not significant ($P > 0.05$). White pepper has been shown to lengthen the induction period of oxidation 2-fold in lard (Palitzsch et al., 1969). This was found not to occur in the burgers, probably due to catalysts present

FIGURE 5.7

Effect of seasoning mix (■—■), NO seasoning mix (□—□), and the seasoning mix ingredients: salt (◢—◢), white pepper (▲—▲), Apex starch (○—○) and mono-sodium glutamate (◺—◺) on rancidity development in pork burgers stored at 4°C and 80% r.h.



in the burgers e.g. iron; lard is a relatively pure system with few potential catalysts present.

5.4.3. Effect of the water soluble and ethanol soluble, and insoluble fractions of white pepper on rancidity values (TBA) in pork burgers.

The results of Section 5.4.2. had shown that white pepper was a major antioxidant component of the seasoning mix, but little work has been carried out to establish what component is responsible. An investigation of the effect of water soluble, ethanol soluble and insoluble fractions of white pepper was thus undertaken.

5.4.3.1. Experimental design.

The investigation was carried out using burgers for the reasons given in Sections 5.4.1. and 5.4.2.

The white pepper was separated into the three fractions as described in Section 3.3.14.1. Each fraction was incorporated in a burger mix in the same proportion as it occurred in the pepper, and at the same level of pepper usually used, which is 0.55% w/w with meat (lean and fat). (25g white pepper yields 1.97g ethanol soluble fraction, 7.8% ethanol soluble, 11.08% water soluble, 81.08% insoluble residue. In 600g meat mix 3.98g pepper is added, so 0.31g ethanol soluble fraction is used with 600g meat.)

Five batches of burgers were made containing:-

i) no white pepper fraction added (control)

ii) the water soluble fraction

iii) the ethanol-soluble fraction

iv) the insoluble residue

v) the unfractionated white pepper.

After cooking, the burgers were stored at 4°C, 80% rh. TBA analysis (in triplicate) was performed daily on each set of burgers.

Ethanol was used in the extraction process, even though on repeated extraction it can extract water soluble, non-flavour substances e.g. polysaccharides and gums (Purseglove et al., 1981). However repeated extractions were not carried out. Had repeated extractions been required, then dichloroethane would have been used, as it does not extract the water soluble substances (Purseglove et al., 1981).

5.4.3.2. Results and discussion.

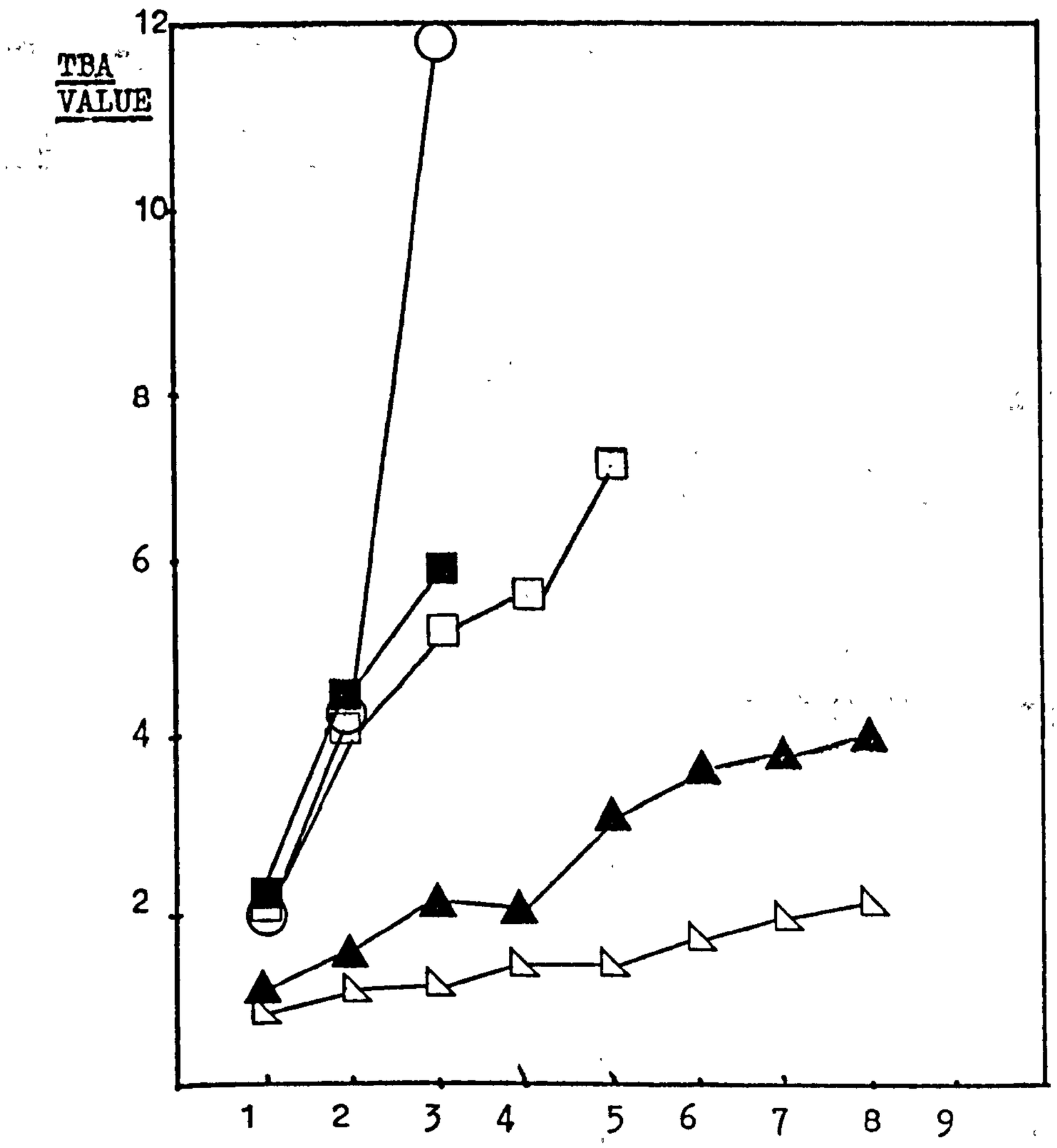
The TBA values obtained from the storage of the different burgers are shown in Figure 5.8. It is seen that the water soluble fraction and the insoluble residue had no effect in retarding rancidity development, (as measured by TBA values); there was no significant difference ($P > 0.05$) between the burgers with no pepper fractions (control) and those containing the insoluble residue.

The water soluble fraction may have exhibited a slight pro-oxidant activity, but this could not be proven or disproven conclusively. Watanabe and Ayano (1974) found this fraction only to be antioxidant when extracted from cloves.

The ethanol-soluble fraction (also known as the oleoresin) showed an antioxidant activity: - one as high as the unfractionated pepper. Thus it would appear that the antioxidant component of pepper lies in the oleoresin.

FIGURE 5.8

Effect of the presence of various fractions of white pepper on rancidity development in pork burgers stored at 4°C and 80% r.h.



Where (▲—▲) are the burgers containing whole white pepper, (■—■) are the burgers containing no additive - control, (◺—◺) are the burgers containing the ethanol soluble fraction, (○—○) are the burgers containing the water soluble fraction, and (□—□) are the burgers containing the insoluble fraction

Pepper oleoresin contains the odour, flavour and pungent principles of the pepper. Thus it is the piperine,volatile oil, monoterpene hydrocarbons or sequiterpene hydrocarbons that are responsible for the antioxidant property of pepper.

5.4.4. Thin layer chromatography (T.L.C.) of the pepper oleoresin.

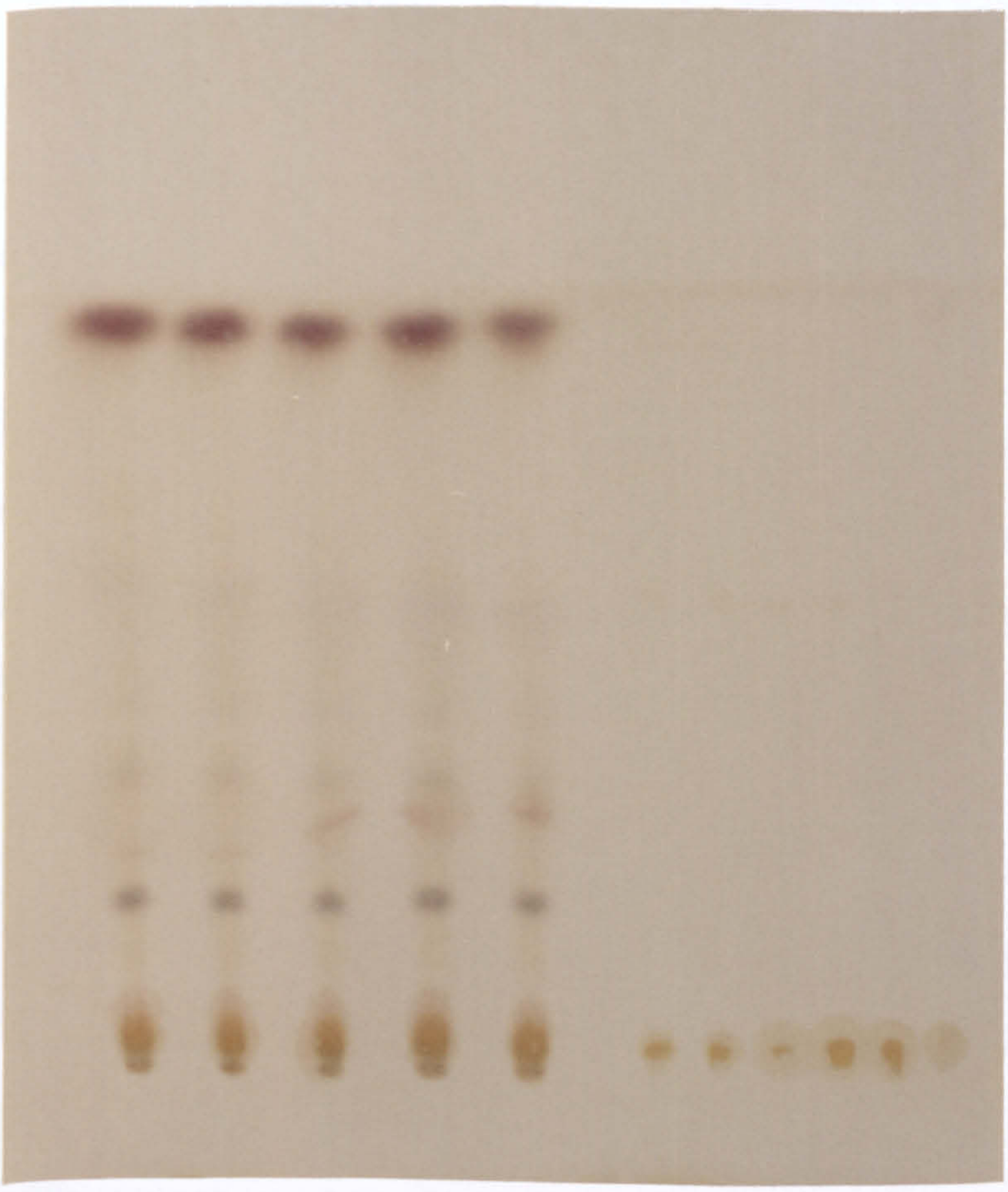
It has been shown that the ethanol-soluble fraction (the oleoresin) of white pepper is that which is responsible for its antioxidant property (Section 5.4.3). The oleoresin has been reported to be a complex mixture of many compounds (Wrolstand and Jennings, 1964; Richard and Jennings, 1971; Nigam and Manda, 1964; Ikeda et al., 1972; Govindarajan, 1977; Debrauwere and Verzele, 1975; Saltzer, 1977; Pyrseglove et al., 1981).

T.L.C. of the pepper oleoresin was carried out using a freshly extracted oleoresin sample (Section 3.3.14.1) and the T.L.C. method described in Section 3.3.14.2, which separates essential (volatile) oils from each other. A pure sample of piperine (Sigma Chemical Co.) which is the major single component of the oleoresin (Nambudiri et al., 1970) was compared with the oleoresin.

Figure 5.9 is a photograph of the separation of the components in the oleoresin and in pure piperine. It shows that the pure piperine sample (right hand side of the plate) remains on the base line. In the oleoresin sample, piperine can be identified therefore as the component remaining at the base line. An array of other compounds were present in the oleoresin, some of which were found only to be detectable

FIGURE 5.9

Photograph of the t.l.c plate of oleoresin and pure piperine, with 15% ethyl acetate in petroleum ether 40-60 b.p as the solvent.



when the plate was viewed under ultra violet light.

Since piperine is apparently the major single component in the oleoresin (35-56% according to Nambudiri et al., 1970; Pintauro, 1971) it may well be responsible for retarding rancidity development in pork pies and pork burgers.

5.4.5. A comparison of the antioxidant activity of piperine, pepper oleoresin and white pepper.

White pepper, and the oleoresin extracted from it, have been shown to have antioxidant properties (Sections 5.4.2. and 5.4.3). As piperine is the major single component of the oleoresin, (Section 5.4.4.) it may well be responsible for the antioxidant effect. In order to test this hypothesis a comparison was made of the antioxidant activity of piperine and of the oleoresin, pepper and seasoning mix.

5.4.5.1. Experimental design.

Two separate trials were carried out. In the first, four batches of burgers were prepared (Section 3.2), these contained either i) no additives (control), ii) 0.31g oleoresin, iii) 3.98g pepper or iv) the seasoning mix (in 600g of meat in each case).

In the second trial the burgers (6 batches contained either i) no additives (control), ii) 0.1g piperine, iii) 0.2g piperine, iv) 0.3g piperine, v) 0.31g freshly extracted oleoresin or iv) 3.98g pepper (in 600g of meat).

In both trials the burgers were cooked, and then stored at 4°C, 80% rh. TBA analysis was carried out daily, in triplicate, for each burger batch.

5.4.5.2. Results and discussion.

The results from the first trial are given in Figure 5.10, they represent the mean of three of determinations. In the burgers where there was no addition to the meat (control) the TBA values obtained initially were higher than the rest, and continued to be so throughout the duration of the trial. These control burgers attained a TBA value of 5 by the second day of storage, and thus were deemed rancid.

Different TBA values for the burgers containing the oleoresin, pepper and seasoning mix were obtained in each trial, probably due to differences in the meats used. Statistical analysis (Students t-test, Section 3.3.15) showed no significant difference ($P > 0.05$) between the burgers containing the oleoresin and pepper. The seasoning mix appeared slightly less effective in inhibiting rancidity, but the difference was not significant ($P > 0.05$). If the slightly less effective antioxidant properties of the mix is real, it is probably due to the pro-oxidant action of the salt present in the mix (Gray, 1978; Rhee et al., 1983; Keeton, 1983).

Comparisons between the oleoresin, piperine and white pepper are given in Figure 5.11. As can be seen there was very little difference between the TBA values obtained for the burgers containing 0.1g or 0.2g of piperine; and those for the oleoresin were not significant between these samples ($P > 0.05$). This may be because 7.9% white pepper (7.8% in this study, Section 5.4.3) is ethanol-extractable

FIGURE 5.10

Comparison of the effect of whole white pepper (▲—▲) oleoresin (◺—◺), seasoning mix (■—■) and no additive -(control) (□—□) on rancidity development in pork burgers stored at 4°C and 80% r.h.

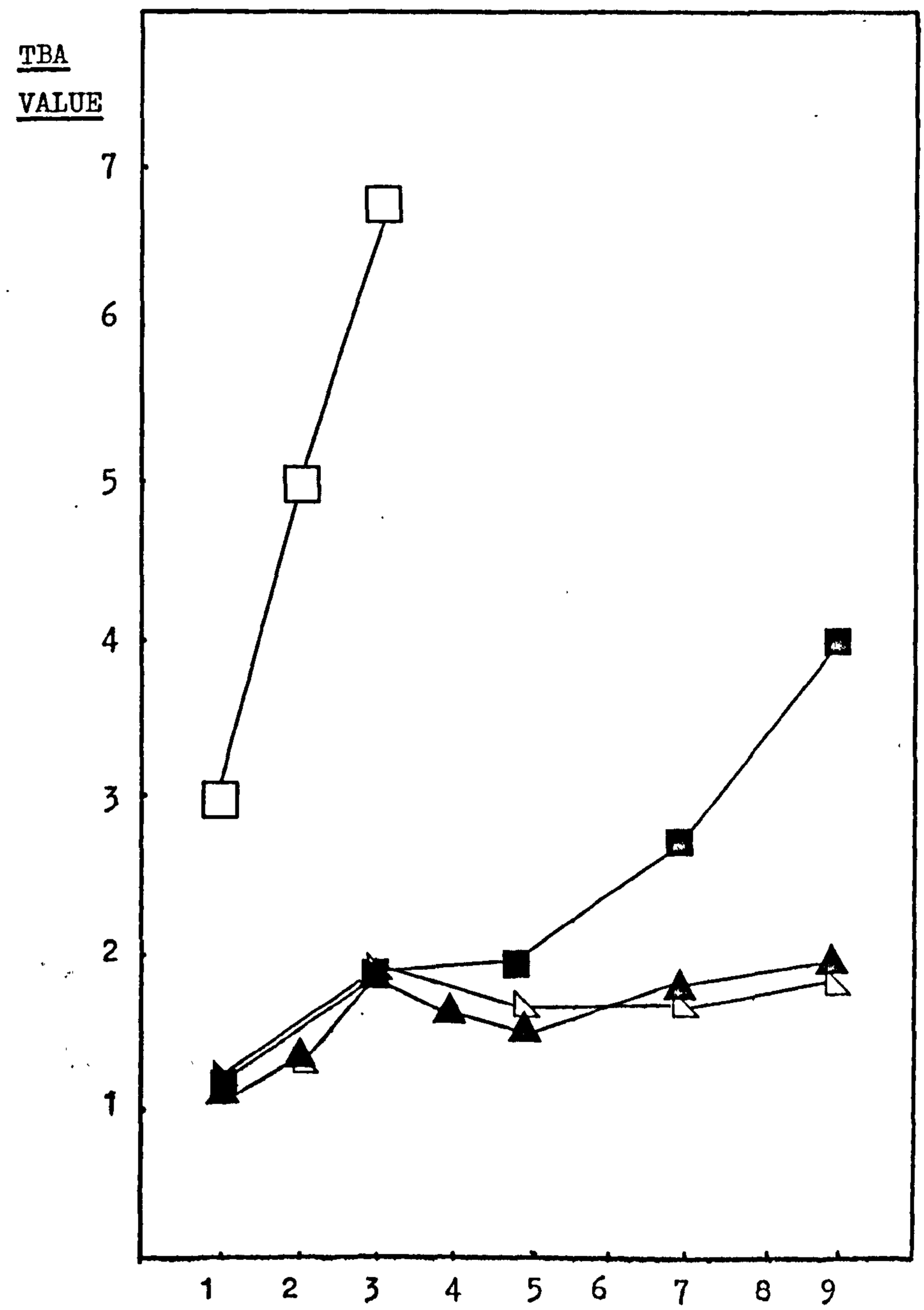
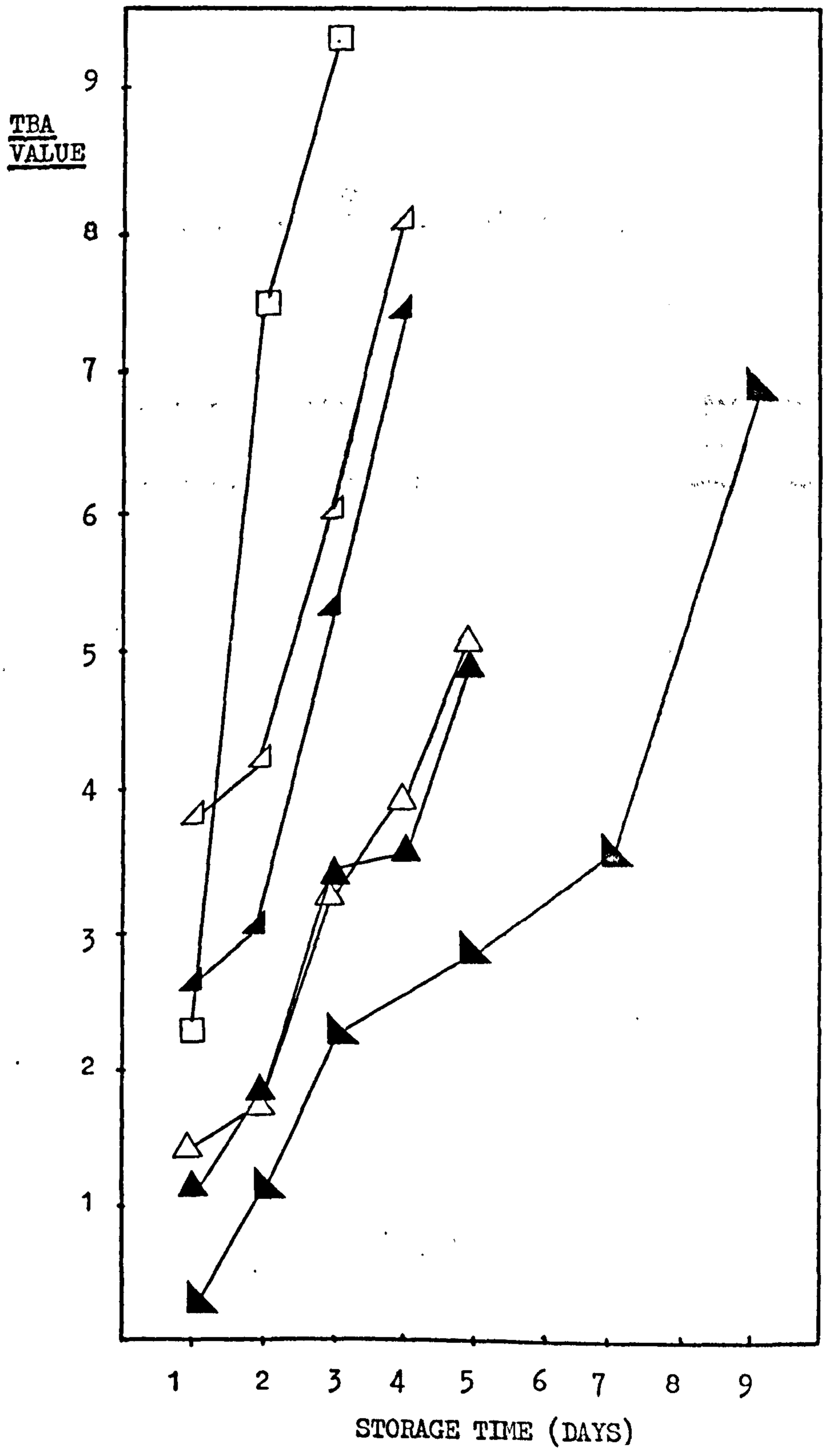


FIGURE 5.11

Comparison of the effect of whole white pepper (▲—▲), 0.31g oleoresin (△—△), 0.1g piperine (◿—◿), 0.2g piperine (◢—◢), 0.3g piperine(◣—◣)no additive(□—□) on rancidity development in pork burgers stored at 4°C and 80% r.h.



(Dwarakanath et al., 1959). Of this extractable material between 35-56% (Pintauro, 1971) and 41-61% (Nambudiri et al., 1970) is piperine. The actual values vary for different cultivars (Salzer, 1977; Govindarajan, 1977) and with the maturity of the pepper (Jansz et al., 1984). In the making of the test burgers 3.98g of white pepper was mixed with 600g of minced meat. Of this pepper, 0.31g is ethanol-soluble (Section 5.4.3), of which 0.11 to 0.20g would be piperine, assuming the figures of Pintauro (1971) and Nambudiri et al., (1970) are correct. If piperine is the active principle in the pepper then, (as found) the oleoresin should have similar antioxidant properties to 0.1 - 0.2g of piperine.

0.3g of piperine had significantly better antioxidant properties than all the other systems, suggesting that elevated levels of this compound will markedly extend the shelf life of meat products.

Figure 5.11 also shows that pepper is a more effective antioxidant than the oleoresin or 0.1, 0.2g of piperine. This suggests that there are other antioxidants in pepper. These could be the volatile (essential) oils, as it is known that the monoterpene hydrocarbons in the volatile oils are susceptible to changes during storage and cooking (Thiessen and Scheide, 1970; Purseglove et al., 1981). It is possible that these compounds also exhibit antioxidant properties.

Thus, piperine, and unknown compound(s), are the active principles in pepper, piperine playing the major role. This effect of piperine can also be enhanced by its utilization at elevated levels. However organoleptic and financial considerations may limit its use in extending meat products' shelf life.

5,4.6, Antioxidant development during the manufacture of rusk.

The rusk used in the meat filling of pork pies has been shown to have antioxidant properties (Sections 5.3, 5.4.1) and this is dependant on the concentration of rusk. As already indicated the compounds responsible for this activityin rusk are probably products of the Maillard browning reaction :- melanodins (Park and Kim, 1979); Amadori products (Huyghabaert et al., 1982). Heating increases the development of these compounds (Baltes, 1982). An examination was therefore carried out to elucidate this possibility.

5.4.6.1. Experimental design.

Burgers were used as the study medium, the different rusks used were the only variables.

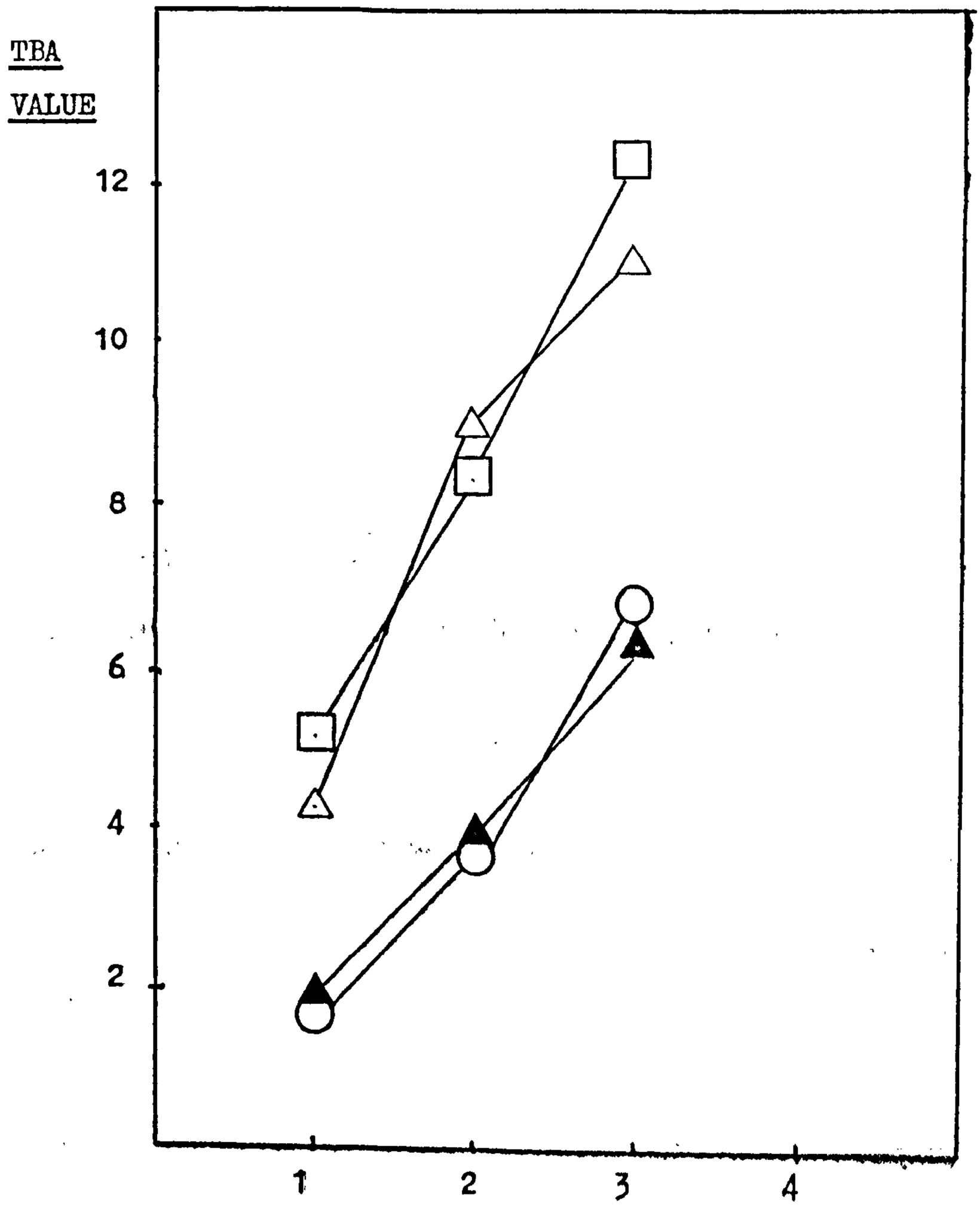
Four batches of burgers were made. These contained either i) no rusk (control), ii) 5.5% of rusk A, iii) 5.5% of rusk B, or iv) 5.5% of rusk C. Rusk A was produced after baking the flour, salt and dough for 15 minutes at 450°F (230°C). Rusks B and C were products taken at two points during the subsequent drying process. Once cooked the 4 sets of burgers were stored at 4°C, 80% rh., and sampled daily, the TBA analysis being performed in triplicate.

5.4.6.2. Results and discussion.

Figure 5.12 shows the TBA values obtained during the storage trial. There was no significant difference ($P > 0.05$) in TBA values between the burgers with no rusk (control)

FIGURE 5.12

Effect of 5.5% rusk sampled from various points during its manufacture, on rancidity development in pork burgers stored at 4°C and 80% r.h



The burgers contained no rusk (□—□), or 5.5% of rusk A (△—△), rusk B (▲—▲) or rusk C (○—○), where A,B,C are the treatment described in the text.

and those containing rusk A. However both rusk B and C exhibited antioxidant properties, (although there was no significant difference ($P > 0.05$) between them).

It is surprising that rusk A did not show any antioxidant properties, since it would have been expected that, following baking, some browning reaction products would form. According to Baltes (1982) preliminary thermal stress can yield intermediate melanoidin products, which have antioxidant properties (Park and Kim, 1979). Yet these products do not appear to have been produced in rusk A, although it had been exposed to temperatures of 230°C. for 15 min. Sato et al., (1973); Siu and Draper (1979); Porter (1980) found that low temperature cooking was inadequate to produce these 'browning' antioxidants Since the baking temperature in the manufacture of rusks is high (230°C), it must be presumed that the relatively short time (15 mins) at this temperature prevented the formation of melanoidin and Amadori products.

By the drying state antioxidant compounds have been formed, as shown by the reduction in TBA values with rusk B and C. Thus heating does develop the antioxidant compounds in rusk. However the actual effect of heat and time in the drying stage of manufacture could not be assessed due to reticence on behalf of the manufacturers.

5.4.7. Effect of heat on the antioxidant activity in rusk.

The antioxidant component in rusk is developed by heat, as in the drying stage in its manufacture (Section 5.4.6) but

as the exact nature in the drying process is not known, further investigation of the effect of thermal treatment on the development of antioxidants was made.

5.4.7.1. Experimental design.

Burgers were used in this investigation. They were made up to contain one of the following:-

i) 5.5% of rusk C.

ii) 5.5% of rusk R

iii) 5.5% of rusk A

iv) 5.5% of rusk A heated for 30 mins at 230°C (rusk A_{30})

v) 5.5% of rusk A heated for 60 mins at 230°C (rusk A_{60})

Rusk C was sampled during the drying process of manufacture (as described in Section 5.4.6). Rusk R was the final rusk product as used in the manufacture of pork pies. Rusk A was sampled after the initial baking process of rusk manufacture as described in Section 5.4.6.

After cooking the burgers were stored at 4°C, 80% rh, and sampled daily, TBA analysis being performed in triplicate.

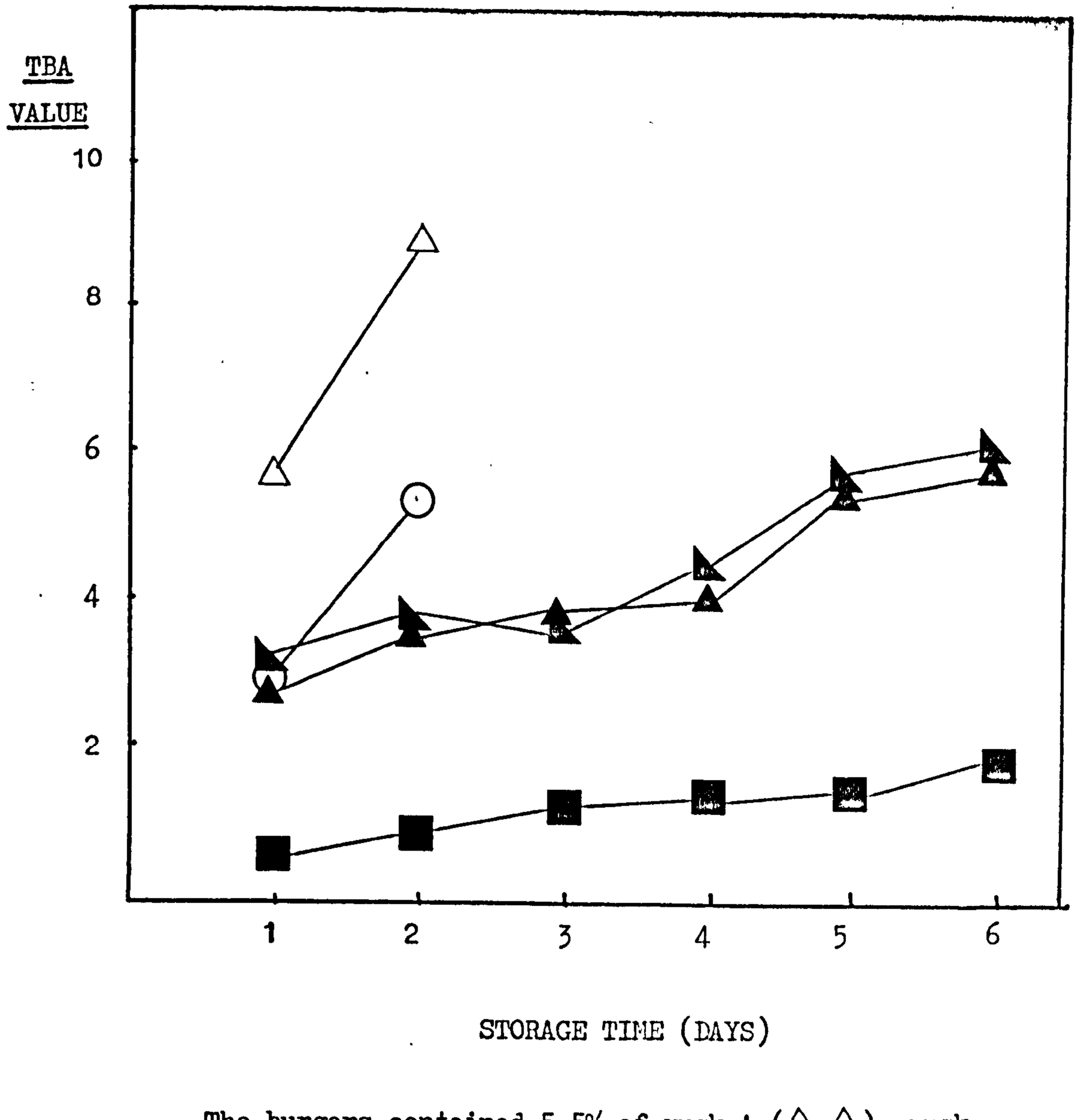
5.4.7.2. Results and discussion.

Comparing the TBA values obtained for burgers containing either rusks A, C or R (Figure 5.13) it can be seen that the final rusk product (rusk R) had a greater effect in reducing rancidity in burgers, than the baked product (rusk A) or the partially dried material (rusk C). Rusk A which had little heat treatment showed (as before) little or no antioxidant activity.

When rusk A was heated to 230°C for 30 mins (rusk A_{30}) it showed the same activity as rusk R, suggesting that this

FIGURE 5.13

The antioxidant activity of 5.5% rusk as affected by different heating times (measured by the TBA test) in pork burgers stored at 4°C and 80% r.h.



The burgers contained 5.5% of rusk A (△—△), rusk A_{30}(▲—▲), rusk A_{60}(■—■), rusk C (○—○) or rusk R (◣—◣), where A, A_{30}, A_{60}, C and R are treatments defined in the text.

level of heat treatment is similar to that employed in rusk manufacture. Further heating of rusk A (60 minutes at 230 °C, rusk A_{60}) yielded far greater antioxidant activity. The initial TBA values of the burgers incorporating this material (A_{60}) was significantly less than those of all the other samples tested and on subsequent storage for several days at 4°C the TBA values of these burgers containing rusk A_{60} remained low.

From these observations it would seem that further heat treatment of the final rusk product could yield a product with greater antioxidant potential. As rusk A_{30} is equivalent in antioxidant activity to rusk R, rusk A_{60} should be greater in antioxidant activity. However heating rusk A for 80 mins at 230°C resulted in carbonization of the rusk. Thus the product was black in appearance and had virtually no water holding ability. Since this latter property is the main purpose of adding rusk to comminuted meat products, development of this rusk would be useless for incorporation in meat products.

Thus, although heating develops antioxidant compounds in rusk, this development is limited by carbonization of the product, rendering it unusable in meat products.

5.5. Chemistry of rancidity development in pies.

In the hope of elucidating the rancidity changes more clearly, this area was studied in more depth, as some of the factors affecting rancidity development have been recorded in Sections 5.1 to 5.4.

Initially the changes in the fatty acid profiles of the lipid classes were monitored using Gas Liquid Chromatography (G.L.C.). Thereafter the role of lipids (as phospholipids and neutral lipids) in rancidity development was studied in order to see which played any significant roles in rancidity development.

Finally as aldehydes are one of the end products of lipid oxidation their production in pie meat filling was recorded using H.P.L.C. The relationship(s) between aldehyde production (one or total) and rancidity (as measured by the TBA test, and taste panels) was also investigated.

5.5.1. Changes in fatty acid profiles during refrigerated storage.

In the preliminary study (Section 4.2) the long chain fatty acid content was found to decrease whilst the short chain fatty acid content increased. Unfortunately the G.L.C. system used was unable to completely separate the short chain fatty acids from the solvent shoulder, unless computor integration was used. The following investigation involved the development of a G.L.C. system capable of good separation of all the fatty acids in the range C6 to C22 (which the original system was unable to do so completely).

The devised system was then to be utilized in the establishment of which fatty acids decreased, and which increased during storage. Rusk and the seasoning mix are known to retard rancidity development (Section 5.3, 5.4.1, 5.4.6), and so the G.L.C. system was employed to see if these compounds affected the fatty acid profiles of the meat filling during storage.

5.1.1. Experimental design.

Four batches of pies were manufactured from one pastry batch and one meat mix in which the shoulder meat and back fat were used 2 days post slaughter. The batches differed only in respect of the rusk and seasoning contents, they contained respectively:- i) 5.5% rusk and 3,75% seasoning mix, ii) 5.5% rusk only, iii) 3.75% seasoning mix only, iv) no rusk or seasoning mix. The four pie batches were baked, jellied, cooled and stored at 4°C and 80% rh.

On days 1, 14 and 21 of storage pies from each batch were sampled. The filling from at least 6 pies from each set was minced using a Kenwood mincer attachment (5mm dia.).

The lipids were extracted (Section 3.3.10) and separated into the lipid classes i.e. phospholipids, monoglycerides, diglycerides, free fatty acids and triglycerides by thin layer chromatography (T.L.C.) (Section 3.3.11.2). The separated lipids were converted into their corresponding methyl esters (Section 3.3.12.1) which were stored at -18°C in a nitrogen atmosphere, until analysed using G.L.C. as detailed in Section 3.3.12.2.

The 10% silar 10CP column packing material was used as the main column system. 10% DEGS was used as a second packing material, to give positive peak identification.

5.5.1.2. Results and discussion.

A chromatogram of a mixture of pure fatty acid methyl esters (separated on 10% silar 10CP on gas chrom Q 100-120 mesh column) is shown in Figure 5.14. In order to identify each peak, further chromatograms were produced using the

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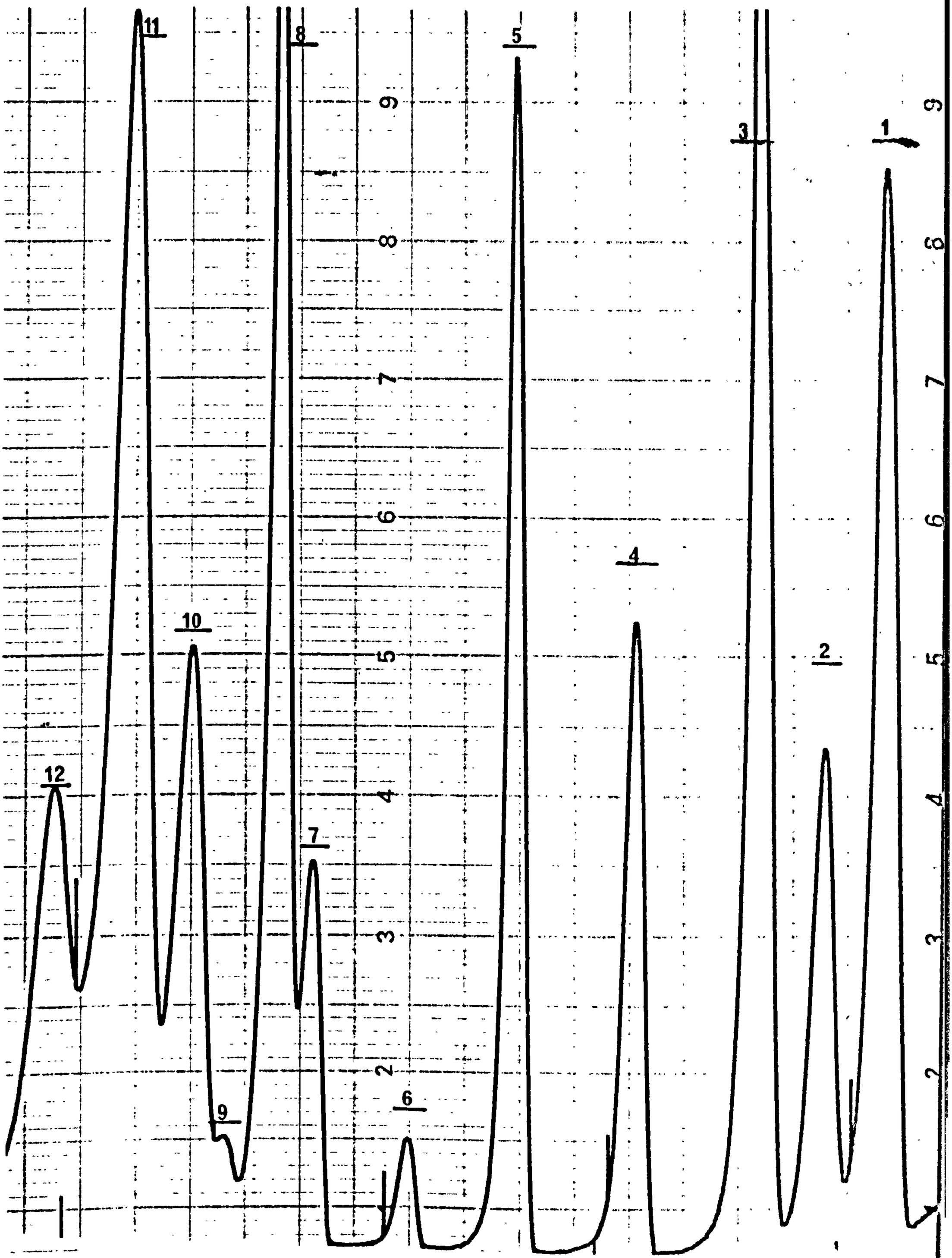
FIGURE 5.14

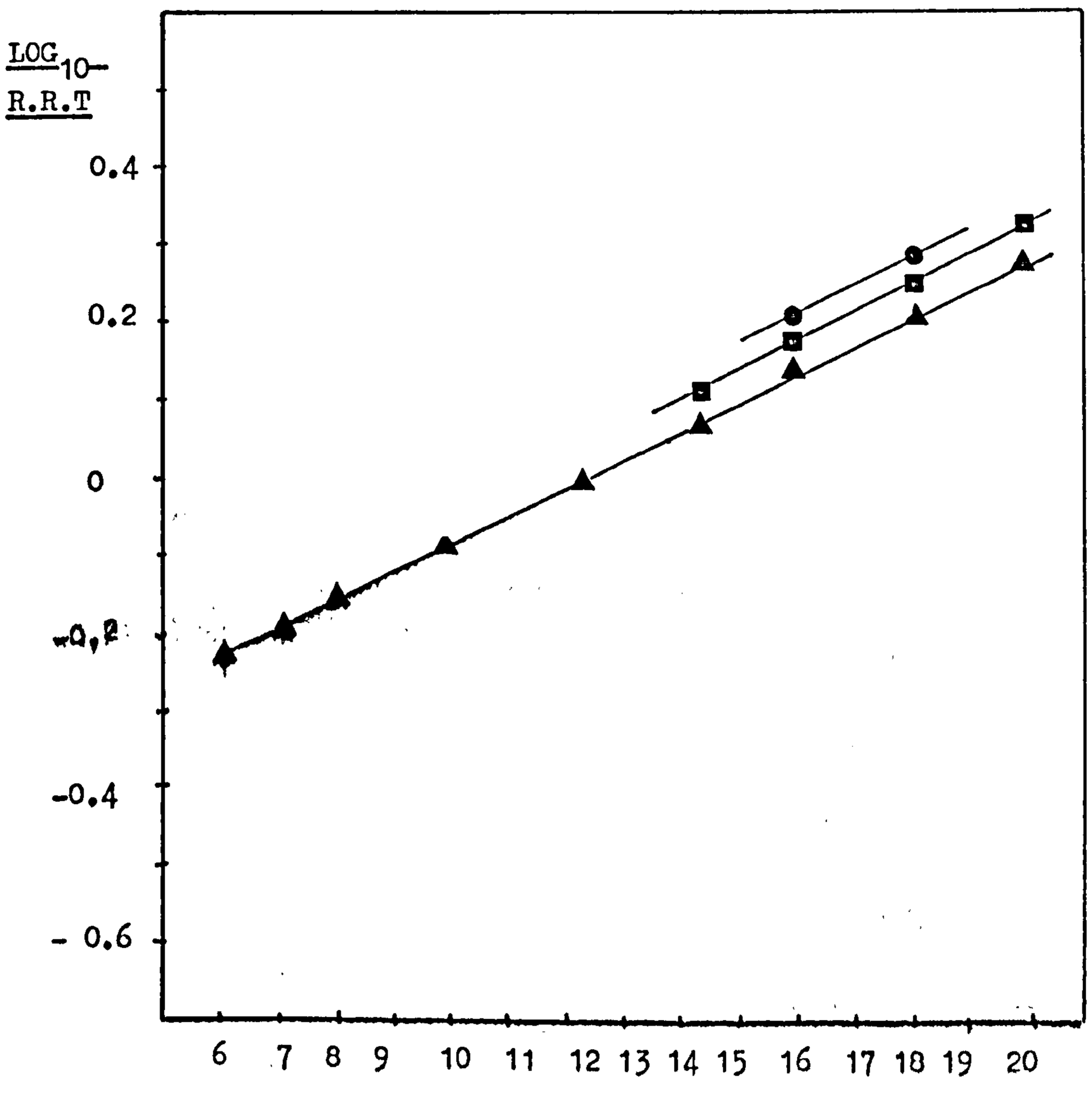
Chromatogram of Fatty Acid Methyl Ester mix separated on 10% Silar 10CP on Gas Chrom Q (100-120 mesh), using a temperature programme of3 mins. at 80°C rising to 185°C at 4°C/min. with a 20 mins. period at 185°C. Injector and detector temperatures at 250°C. Nitrogen flow rate of 20ml./min.



Where the idendity of the numbered peaks are given in the text.

FIGURE 5.15

Plot of Log_{10} Relative retention times against Fatty Acid Methyl Ester Carbon Number. Where (▲—▲) is the line for Saturated Fatty Acid Methyl Esters, (■—■) is the line for Unsaturated Fatty Acid Methyl Esters (mono unsaturated), and (●—●) for the poly-unsaturated Fatty Acid Methyl Esters.



'standard' mixture, and enriching it with one or other of the pure esters. Thus the peak corresponding to the methyl ester concerned was considerably enlarged. The retention time (time in seconds from the negative air peak at the start of the run to the centre of the component peak, also called 'retention volume') for each methyl ester was recorded. Thus peak identification was achieved. Unfortunately purified samples of all the fatty acids were not available but James (1960) showed that for any homologous series whether branched, saturated or unsaturated there is a constant retention factor which is produced by an increase in chain length of one $-CH_2-$ unit. Thus if the Log_{10} relative retention volumes or times (Log_{10}R.R.V.) are plotted against carbon number, the values for all the homologous fatty acids will fall on a straight line.

In this study dodecanoic acid methyl ester was taken as the standard, and the retention volumes relative to this component were calculated. A plot of the Log_{10} R.R.V. against fatty acid methyl ester carbon number (Figure 5.15) indicates that peaks 1, 2, 3, 4, 5, 6, 7, 9 and 12 on the chromatogram (Figure 5.14) belong to the straight chain saturated fatty acids series with carbon numbers of 6, 7, 8, 10, 12, 14, 16, 18 and 20 respectively.

If the Log_{10} R.R.V. values obtained from one column packing material are plotted against those from another (i.e. 10% silar 10CP against 10% DEGS (both on gas chrom Q 100-120 mesh) co-ordinates for fatty acids of similar structure (saturated, unsaturated or branched) will be linearly related (James, 1959). The Log_{10} R.R.V. values for both column packing materials are given in Table 5.2 and are plotted in Figure 5.16.

Table 5.2.

Log_{10} Relative retention volume values
(relative to octanoic and methyl ester)
for 10% DEGS and 10% Silar 10CP.
Used for Figure 5.15.

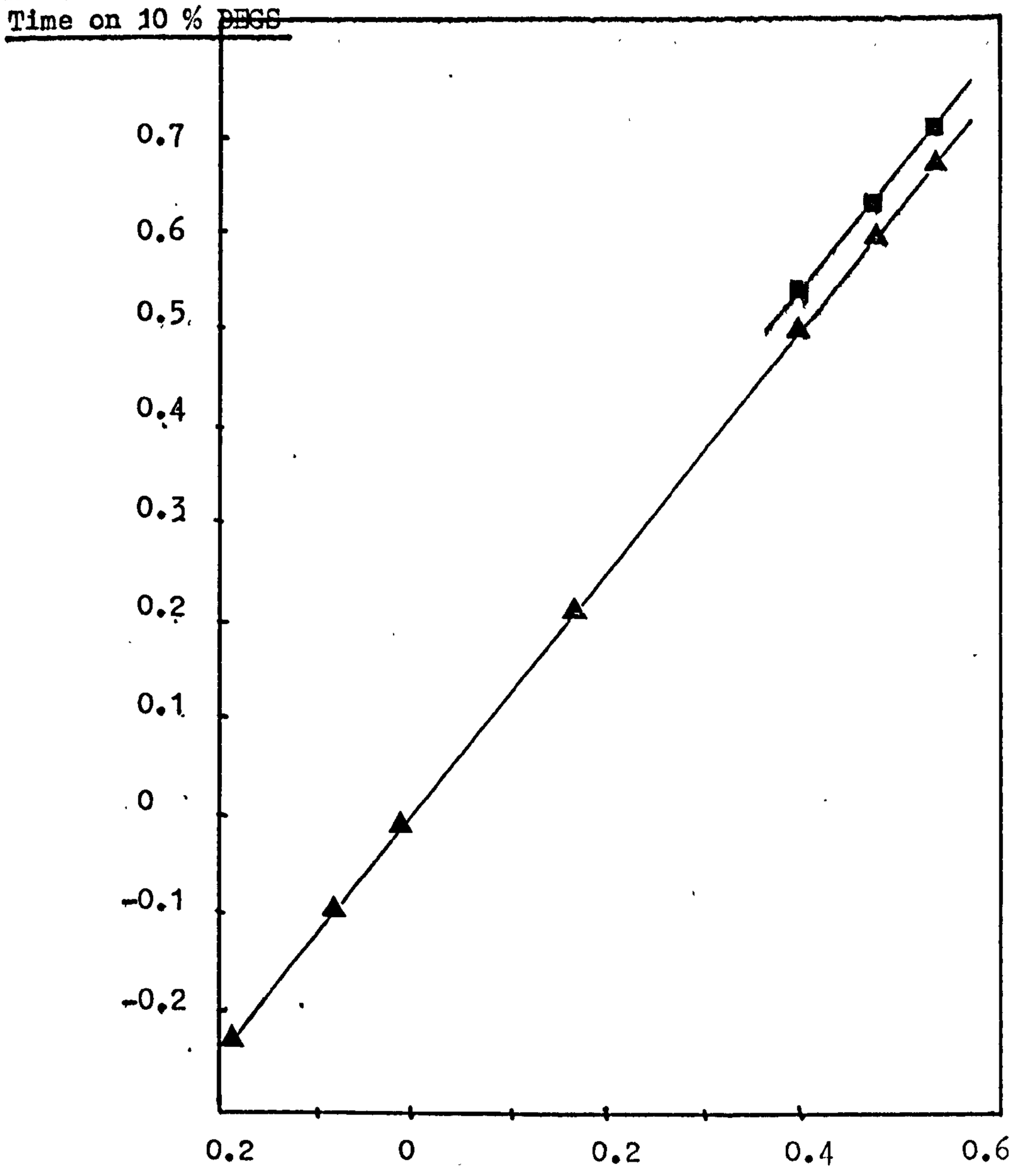
	10% DEGS x	10% SILAR 10CP *
carbon number	Log_{10} R.R.V.	Log_{10} R.R.V.
6	-0.230	-0.196
7	-0.101	-0.075
8	0	0
10:0	0.209	0.168
14:0	0.501	0.386
14:1	0.538	0.423
16:1	0.627	0.477
18:1	0.715	0.539
18:2	0.763	0.540
Temperature programme	100-200°C at 8°C/min and 6 min at 200°C	90 - 250°C at 8°C/min
Nitrogen flow rate	45 ml/min	Nitrogen flow rate 45 ml/min

x = Diethylene glycol succinate polyester on Gas Chrom Q 100 - 120 mesh.

* = 10% Silar 10CP on Gas Chrom Q 100-120 mesh.

FIGURE 5.16

Plot of Log_{10}Relative Retention Time(R.R.T) obtained for the Fatty Acid Methyl Esters mix on 10% DEGS against the Log_{10}R.R.T values from 10% Silar 10CP.



Where (▲—▲) are straight chain saturated fatty acid methyl esters, and (■—■) are monounsaturated fatty acid methyl asters.

The numbers on the lines refer the carbon number of the fatty acid methyl ester.

Thus, if an unidentified peak is found, if its Log_{10} R.R.V. value on both columns are known, its coordinate can be plotted on Figure 5.16, and its carbon number and degree of saturation established, Thus identifying the fatty acid. The Log_{10} values for 10% silar 10CP on gas chrom Q are different to those in Figure 5.15. This is because a different temperature programme and machine were employed and this was the data used to identify the peaks obtained from G.L.C. analysis of the different model systems in Section 5.5.2. In this Section (5.5.1.) the data in Figure 5.15 was used to identify the fatty acid peaks in each sample.

The results obtained from the G.L.C. analysis are presented in Tables 5.3 - 5.6, which show the fatty acid composition changes in the different meat filling batches:- i.e. with rusk and seasoning (control) (Table 5.3), with seasoning, but no rusk (Table 5.4), with rusk, but no seasoning, (Table 5.5), and with neither rusk or seasoning (Table 5.6).

In all cases the fillings showed a loss in percentage total unsaturated fatty acids, and a concomitant increase in percentage total saturated fatty acids. This rise is largely accounted for by the appearance of shorter chain length fatty acids of 6 to 10 carbon atoms in length. On storage hexanoic (C6:0) and heptanoic (C7:0) acids increase particularly and concomitently with the fall in the concentrations of oleic (C18:1) and linoleic (C18:2) acids.

In this context oleic acid concentration falls (cf Table 5.3) along with palmitoleic (C16:1), linoleic (C18:2), linolenic (C18:3) and arachidonic (C20:4) acids, as a

Table 5.3

Fatty acid composition changes in pork pie meat filling (control) containing rusk and seasoning.

Fatty acid	PHOSPHOLIPIDS Storage Time (Days)			MONOGLYCERIDES			DIGLYCERIDES			FREE FATTY ACIDS			TRIGLYCERIDES		
	1	14	21	1	14	21	1	14	21	1	14	21	1	14	21
6	2.9	9.75	29.89	0.93	0.14	13.93	-	22.70	33.85	-	29.90		-	7.5	26.5
7	7.9	-	10.19	-	-	1.91	-	22.80	18.00	-	13.20	-	8.01		15.97
8	5.5	0.73	1.81	-	0.14	-	-	1.58	2.30	-	1.19		-	0.54	5.17
9	0.09		0.53	0.39	-	-	-	-	0.25	-	-		-	-	0.50
10:0	1.89		0.59	0.99	0.41	0.08	-	-	-	-	-		-	0:07	0.23
12:0	4.04		0.65	0.2	0.16	0.31	-	-	-	-	0.62		-	0.27	0.56
14:0	0.64	0.62	0.68	0.38	1.22	1.87	1.72	0.52	0.85	-	1.29		2.01	1.70	1.03
14:1	0.29	0.16	0.40	1.45	1.11	-	-	-	-	-	-		-	0.07	-
16:0	4.29	9.42	9.01	12.60	7.82	9.86	7.40	4.64	6.56	16.30	14.40		11.41	15.75	9.74
16:1	12.81	-	8.40	12.09	7.99	8.43	2.10	1.09	-	-	-		-	4.40	1.90
18:0	13.95	11.08	9.87	9.87	9.74	9.78	17.80	12.00	5.88	16.89	11.02		15.64	14.80	10.46
18:1	31.68	45.42	26.79	59.9	58.87	41.63	50.10	34.03	19.5	61.10	43.28		44.77	30.75	22.49
18:2	10.2	5.02	5.05	5.54	3.42	2.01	11.80	8.15	0.60	-	-		12.49	9.54	4.77
18:3	-	-	-	-	-	-	1.70	-	-	-	-		-	-	-
20:0	-	-	-	-	-	-	-	-	-	-	-		-	1.24	
20:4	2.13	0.45	0.07	0.54	-	-	0.90	0.11	-	-	-				
Total saturated	38.4	34.95	62.83	24.3	29.48	37.3	26.92	54.53	70.44	33.19	61.80		29.09	48.57	70.16
Unsaturated	57.43	51.89	40.28	79.5	71.39	52.1	76.70	43.38	21.50	61.1	43.28		57.26	50.48	09.16

"-" not detected. Values expressed as % of total fatty acid content.

Table 5.4.

Fatty acid composition changes in pork pie meat filling containing seasoning but no rusk.

Fatty acid	PHOSPHOLIPID			MONOGLYCERIDE			DIGLYCERIDE			FREE FATTY ACID			TRIGLYCERIDE		
	Days storage														
	1	14	21	1	14	21	1	14	21	1	14	21	1	14	21
6	-	9.46	45.88	-	-	47.91	-	28.22	47.27	-	0.87	35.16	-	2.17	33.71
7	-	4.14	18.65	-	0.25	30.86	-	13.34	25.35	-	-	26.34	-	1.09	16.15
8	-	-	2.86	10.48	-	2.50	-	1.02	1.47	-	0.16	2.62	-	1.22	1.39
9	-	-	0.21	-	-	0.39	-	-	-	-	-	0.32	-	-	0.15
10:0	-	-	-	16.03	0.15	-	-	0.25	1.17	-	-	0.47	-	-	-
12:0	-	-	-	-	0.34	-	-	-	-	-	0.24	-	-	1.63	0.20
14:0	0.65	0.70	0.57	-	1.16	0.61	1.15	1.21	0.44	-	0.64	0.90	0.96	1.94	1.54
14:1	0.18	0.21	-	-	0.55	0.30	-	0.21	-	-	0.12	-	-	-	-
16:0	23.43	22.08	11.01	-	29.83	0.34	15.76	8.09	0.75	7.07	24.23	2.54	13.91	22.18	11.83
16:1	-	-	-	-	-	-	-	3.38	0.25	-	-	3.22	-	-	-
18:0	21.75	17.08	6.39	12.94	11.83	0.85	12.77	5.37	1.04	30.10	27.63	3.78	14.50	8.22	3.41
18:1	49.15	35.40	11.31	30.47	16.13	0.46	68.06	26.75	12.63	58.87	34.01	9.85	65.44	51.42	23.85
18:2	-	-	1.39	9.7	5.87	-	0.68	4.39	0.75	1.58	0.50	3.84	4.03	1.34	6.44
18:3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
20:0	0.86	0.36	-	-	-	-	-	-	-	-	-	-	-	-	-
20:4	0.76	0.59	-	-	-	-	-	-	-	-	-	-	-	-	-
22:4	2.25	0.23	-	-	-	-	-	-	-	-	-	-	-	-	-
Total Saturated	45.83	50.82	85.5	55.45	43.56	82.6	29.68	57.47	77.5	37.17	53.77	72.14	29.37	38.45	68.38
UnSaturated	52.35	36.64	12.7	40.17	22.00	11.6	68.74	34.6	13.6	60.45	34.75	26.91	69.47	52.76	30.29

"-" not detected.

Table 5.5.

Fatty acid composition of pork pie meat filling containing rusk but no seasoning mix.

Fatty acid	PHOSPHOLIPID Days storage.			DIGLYCERIDE			TRIGLYCERIDE		
	1	14	21	1	14	21	1	14	21
6	-	16.43	27.69	4.90	32.13	45.81	-	-	10.80
7	-	1.91	28.11	5.62	23.10	31.76	-	-	0.49
8	-	7.49	-	-	3.70	2.17	-	-	-
9	-	-	-	-	0.93	-	-	-	0.65
10:0	-	2.32	4.97	2.67	1.25	0.32	-	-	0.39
12:0	-	0.78	-	-	-	-	-	-	-
14:0	0.65	1.23	2.32	2.79	2.40	0.60	-	-	1.36
14:1	0.2	-	-	-	-	-	-	-	-
16:0	7.5	9.48	8.47	12.84	4.01	2.49	18.41	-	21.44
16:1	6.3	9.95	4.77	-	1.56	-	2.10	-	6.59
18:0	21.7	9.51	3.70	21.86	4.83	7.49	28.89	-	12.59
18:1	47.7	39.19	19.42	41.84	17.84	6.49	50.92	-	6.77
18:2	10.5	-	-	7.15	3.15	2.02	-	-	7.28
18:3	2.8	-	-	-	-	-	-	-	-
20:0	1.7	0.64	0.40	-	-	-	-	-	-
20:4	0.2	-	-	-	-	-	-	-	-
22:4	0.8	0.80	0.69	-	-	-	-	-	-
Total Saturated	31.55	49.78	75.68	50.68	72.35	90.66	47.4		78.56
Total Unsaturated	68.5	49.9	25.8	48.99	23.5	8.51	52.92		20.64

"-" not detected.

Table 5.6.

Fatty acid composition changes in pie meat filling containing no rusk or seasoning.

Fatty acid	PHOSPHOLIPID Days storage.			MONOGLYCERIDES			DIGLYCERIDE			FREE FATTY ACID			TRIGLYCERIDES		
	1	14	21	1	14	21	1	14	21	1	14	21	1	14	21
6	15.30	36.20	45.75				-	46.06	45.77	-	4.84	43.90	-	2.82	19.2
7	4.03	16.50	26.14				-	15.67	25.21	-	2.14	31.86	-	2.75	4.3
8	-	2.04	2.72				1.08	-	1.75	-	0.13	-	-	0.85	0.17
9	-	0.44	0.54				-	-	0.14	-	-	-	-	-	-
10:0	-	-	-				-	-	-	-	-	-	-	0.16	0.22
12:0	-	-	-				1.02	-	0.27	-	0.22	-	-	0.21	0.85
14:0	-	0.64	0.62				1.43	0.49	0.51	1.17	0.89	0.89	1.33	1.42	1.08
14:1	-	-	-				-	-	-	-	0.39	-	0.30	0.18	0.19
16:0	7.50	2.47	4.06				14.74	10.75		14.75	20.33	0.89	23.27	22.83	17.01
16:1	-	1.75	-				-	-	-	-	-	0.375	-	2.50	6.50
18:0	13.40	10.24	12.50				29.98	11.74	6.17	12.59	4.49	2.62	21.88	23.15	12.50
18:1	39.70	16.10	5.70				43.52	11.00	12.08	64.42	64.72	15.38	53.05	40.53	31.5
18:2	26.50	9.05	1.54				6.63	0.33	0.24	1.70	-	0.66	tr	6.11	5.5
18:3	-	-	-				-	-	-	1.34	-	-	-	0.65	0.34
Total Saturated	40.22	66.06	90.3				48.26	88.67	87.68	32.24	34.89	83.59	50.48	62.4	55.24
Unsaturated	66.00	29.69	7.24				50.15	11.33	12.32	67.76	65.11	16.41	53.35	48.2	43.69

DETECTED - SAMPLE LOST.

"-" not detected.

percentage of the total fatty acid content. A comparison of the tables (5.3, 5.4, 5.5 and 5.6) suggests that both the rusk and the seasoning mix protect the unsaturated fatty acids of all fractions, and especially those in the phospholipid fraction.

Pentanoic acid (5:0) arises from linolenic acid (Kochar and Meara 1975; Parsons, 1974), hexanoic andheptanoic acids from oleic acid (Parsons, 1974; Horikx, 1964) and hexanoic acid from linolenic acid (Stark and Forss, 1962; Urbach et al., 1972; Parsons, 1974), and these findings are borne out by the present results.

The only direct connection between rancidity and the fatty acid profiles in all four sets of meat fillings is possibly the level of hexanoic acid in the phospholipid fractions. Up to 14 days of storage, (which is the shelf life of the control pie as determined by TBA values and taste panels), the rate of the increase of hexanoic acid is matched by the decrease in acceptability (due to rancidity). The control meat filling had the slowest rate of hexanoic acid development in the phospholipid fraction (cf Table 5.3) and the slowest rancidity development (14 days, Sections 4.1, 5.3). While the filling containing neither rusk nor seasoning mix had the fastest rate of production of hexanoic acid (cf Table 5.6); and the shortest shelf life (5-6 days) (Section 5.3).

However this link is tentative, as no correlation between hexanoic acid development and TBA values was established. A stronger link could probably be found with the level of aldehyde production.

To summarize: the results revealed a loss of 18:1, 18:2 and 16:1 (the long chain unsaturated fatty acids) and a concomitant increase in 6:0 as storage proceeded. This confirmed the results recorded previously (Section 4.2) also a tentative link between hexanoic acid development in the phospholipid fraction and TBA values was observed.

5.5.2. The role of phospholipds, neutral lipids and total lipids in rancidity in a model system.

The study was designed so as to investigate the role the phospholipids, neutral lipids and total lipids played in rancidity development. The use of a model system was employed so as to reduce the number of variables that could influence rancidity. The ultimate aim was for a system where the only variable was the lipid under investigation.

5.5.2.1. Experimental design.

Pork pies were manufactured (Section 3.1) using a single pastry mix and employing meat filling with a known history (shoulder meat and back fat, 2 days post slaughter). After cooling the pastry and jelly were discarded and the meat filling minced using a Kenwood mincer attachment (5 mm dia.). From this a 50g sample ($\pm$ 0.1g) was selected, and the lipid extracted as described in Section 3.3.10.

The residue was vacuum dried at 40°C to remove any remaining solvent and stored in a nitrogen atmosphere at 4°C, until required.

The extracted lipid was separated by column chromatography (Section 3.3.11.1) into phospholipids and neutral lipids. The solvents were removed from both fractions by rotary evaporation at 20°C (so as to avoid deteriorative changes in the lipids).

In one system phospholipid was added back to a sample of the meat residue at a level of 0.87%. In a second system neutral lipids were added back at a level of 10.92%. These levels were equivalent to the percentages in which the phospholipids and neutral lipid were found to occur in the lipid samples from pork pies. They are also in agreement with the levels reported by Igene and Pearson (1979); Hornstein et al., (1961).

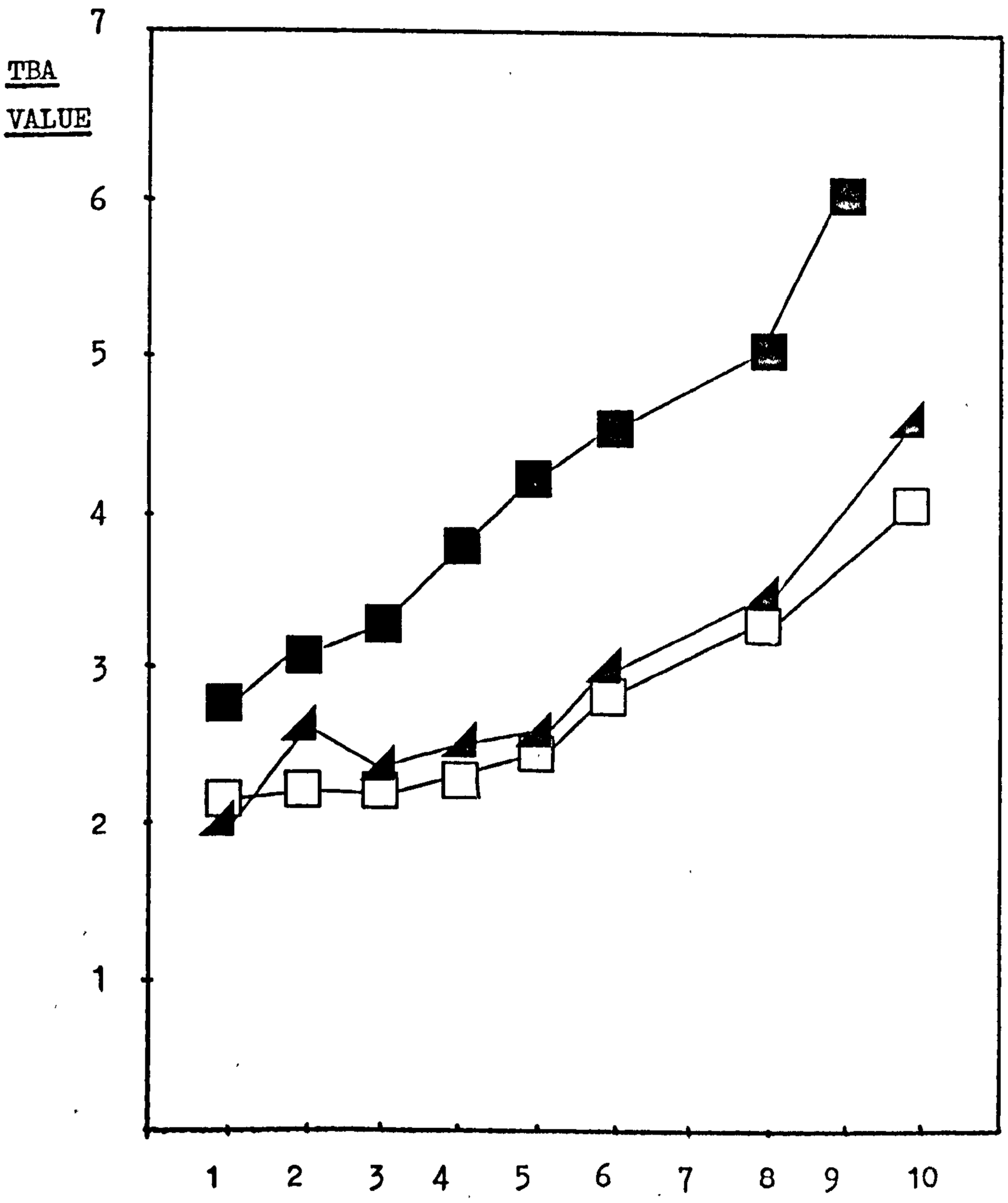
These two systems together, with an unextracted minced meat filling sample (control) were stored at 4°C and 80% rh; and analysed daily by the TBA test. Lipid samples of all three systems were taken at the beginning and at the end of 14 days storage, and analysed by G.L.C. as described in Sections 3.3.10 to 3.3.12. Peak identification was facilitated by the method described in Section 5.5.1. and by using the data presented in Table 5.2 and Figure 5.16.

5.5.2.2. Results and discussion.

The TBA values obtained for the two model-systems and the control are given in Figure 5.17 which shows the phospholipid system to have an elevated TBA value compared to the control, throughout storage. There was no significant difference found

FIGURE 5.17

Rancidity development in a model system containing Phospholipids only (■-■), or Neutral Lipids only (□-□), or both Phospholipids and Neutral Lipids (◢-◢), stored at 4°C.



($P > 0.05$) between the TBA values from the control system and the neutral lipid system.

The phospholipid system attained a TBA value of 5 (Section 4.1) after 7-8days of storage. The neutral lipid system took 12 days to attain a TBA value of 5: it was achieved in 11 days in the control system. These results confirm that the phospholipid fraction influences rancidity. Acosta et al., (1966) reported similar findings. Keskinal et al., (1964) Jacobson and Koehler, (1970); Wilson et al., (1976); Fooladi et al., (1978); Igene and Pearson (1979) all agree that phospholipids are responsible for rancidity development in meat and meat products. It has also been demonstrated that the TBA reactive material in pork is largely associated with the proteolipid fraction (Younathan and Watts, 1960).

However, in the meat filling there are both phospholipids and neutral lipids (as in the control system) yet there was little difference in the TBA values found for this system (the control) and one containing neutral lipids only. It is possible that, in the control system, the phospholipids are in some way protected from oxidation, and that during their extraction, this protection is lost.

Several authors (Watts, 1962; Love and Pearson, 1971; Igene et al., 1979b; 1980) have reported on the instability of polyunsaturated fatty acids (PUFAs) and their involvement in rancidity, especially the C18:1, C18:2, C20:4 and C22:4 fatty acids of the phospholipid fraction.

G.L.C. analysis was performed to evaluate the stability/ unstability of the fatty acids of each fraction. Changes in fatty acid composition of lipids provide an indirect measure of susceptibility to lipid oxidation (Keller and Kinsella, 1973).

Tables 5.7, 5.8 and 5.9 show the changes in the fatty acid composition of the phospholipids and neutral lipids of the three systems (where 5.7 is the changes in the phospholipid system, 5.8 the changes in the neutral lipid system and 5.9 the changes in the control). It can be seen that C6:0 and C7:0 fatty acids are present after only 1 day's storage in the phospholipid only system (Table 5.7). These fatty acids are absent at this time in the control system (cf Tables 5.7 and 5.9). Also the level of C18:2 is far less in the phospholipid only system suggesting that the protective agent(s) present in the control filling act by inhibiting the breakdown of C18:2 to C6:0 and C7:0 fatty acids.

In both phospholipid containing systems (Tables 5.7 and 5.9) there was an apparent decrease in C18:1 (oleic acid), C18:0 (stearic acid), C16:0 (palmitic acid) C18:2 (linoleic acid) and C16:1 (palmitoleic acid) with a concomitant rise in the percentage concetration of C6:0 (hexanoic acid)and C7:0 (heptanoic acid) during 14 days storage. The pattern of change in the fatty acid composition of the neutral lipids (Table 5.7) was similar to that observed in the phospholipids although the changes were less marked.

Table 5.7

Changes during storage in the fatty acid composition of the phospholipids of a defatted meat filling to which the phospholipids were added back.

Fatty acid	Day of Storage	
	1	14
6:0	13.2	45.5
7:0	10.5	12.5
8:0	-	17.7
9:0	1.5	0.9
10:0	-	5.4
12:0	-	-
14:0	-	6.5
14:1	0.6	0.1
16:0	20.2	6.9
16:1	10.6	-
18:0	6.5	2.8
18:1	32.8	7.8
18:2	4.7	3.1
Total saturated	51.9	88.2
Total unsaturated	48.7	10.1

"-" not detected

Calculated as % of total fatty acid content.

Table 5.8

Changes during storage in fatty acid composition of the neutral lipids of a defatted meat filling to which the neutral lipids were added back.

Fatty acid	Days of storage	
	1	14
6:0	13.9	19.5
7:0	13.1	9.3
8:0	2.6	-
9:0	1.7	-
10:0	8.1	14.1
12:0	-	-
14:0	1.6	3.2
14:1	1.5	0.3
16:0	0.4	16.8
16:1	2.3	1.8
18:0	1.0	6.1
18:1	49.0	28.2
18:2	10.4	5.8
Total saturated	40.4	64.0
Total unsaturated	60.2	36.1

"-" not detected

Calculated as % of total fatty acid content.

Table 5.9.

Changes with storage in the phospholipid and neutral lipid fatty acid composition in the control system.

Fatty acid	Phospholipid		Neutral lipids	
	Days of Storage			
	1	14	1	14
6:0	-	31.8	-	18.6
7:0	-	25.1	-	5.1
8:0	-	3.8	-	9.2
9:0	4.7	-	1.2	3.6
10:0	-	-	11.8	2.4
12:0	-	-	-	-
14:0	0.1	5.7	1.8	2.3
14:1	4.3	1.5	-	0.5
16:0	10.7	7.9	13.0	14.9
16:1	27.8	1.4	19.2	2.7
18:0	10.2	4.4	10.9	9.1
18:1	31.3	9.7	30.8	25.1
18:2	14.7	8.2	10.9	6.1
Total saturated	25.7	78.9	38.7	65.2
Total unsaturated	78.0	20.8	60.9	34.4

"-" not detected.

Calculated as % of total fatty acid content.

From these results, it would appear that the loss of 16:1, 18:1 and 18:2 fatty acids in both the phospholipids and neutral lipids and the increase of C6:0 fatty acid (and to some extent C7:0 and C8:0 fatty acids) contribute to the increase in TBA values. Gokalpet et al., (1983) found that C16:1 and C18:2 fatty acids in the neutral lipids of beef significantly contribute to sensory rancidity; while Igene et al., (1980) claimed that C18:1 and C18:2 in chicken phospholipids were the major contributors to rancidity.

In summary, phospholipids on their own accelerate rancidity development. When they are present with the neutral lipids (as is usual), however, do not rapidly accelerate rancidity and presumably must be protected in some manner. Whereas the breakdown of linoleic acid (C18:2) to hexanoic and heptanoic acids (C6:0, C7:0) is prevented.

The decrease in C18:0, C18:1, C18:2, C16:0 and C16:1 fatty acids and the increase in C6:0 and C7:0 fatty acids occurs quicker in the phospholipids than the neutral lipids which backs up the theory of El-Gharbawiand Dugan (1965) that lipid oxidation occurs first in the phospholipid fraction, and then in the neutral lipid fraction.

The results do indicate that C18:1, C18:2, C6:1, C6:0 and C7:0 fatty acids of the phospholipid and neutral lipid fractions are involved in rancidity, with the longer chain length fatty acids being broken down to the short chain fatty acids, which have unpleasant odours (personal experience!).

5.5.3. Aldehyde production and its relationship to sensory evaluations of rancidity in pork pies.

Aldehydes are one of the major end products of lipid oxidation and as such, are largely responsible for the many unpleasant odours and tastes associated with spoiling food-stuffs. The aldehydes have been used successfully to follow lipid oxidation in oils and foods (Buttery and Teranishi, 1963; Bengtsson et al., 1967, Dupuy, 1976; Rayner et al., 1978; Reineccius 1979; Warner et al., 1978). Some authors (Warner et al., 1978) claimed to have found a relationship between aldehyde levels and sensory rancidity in several foods; but not in meat products. The latter product was investigated.

5.5.3.1. Experimental design.

Four batches of pies were made, from a single pastry mix and one meat mix. Four batches contained respectively one of the following:-

i) Rusk and seasoning mix (control)

ii) no rusk

iii) no seasoning mix

iv) no rusk or seasoning mix

After baking and jellying the pies were stored at 4°C and 80% r.h.

After 1, 7, 14 and 21 days storage at least six pies from each batch were sampled. The meat filling was minced in a Kenwood mincer attachment (5mm dia.) and samples taken for TBA testing, and aldehyde determination. The latter

were derivatized to form 2, 4-Dinitrophenylhydrazones (Section 3.3.13.1.). H.P.L.C. analysis was performed on the samples (Section 3.3.13.3.).

5.5.3.2. Results and discussion.

A trace obtained from H.P.L.C. analysis of a typical sample is shown in Figure 5.18. Peak identification was carried out using the procedure described in Section 3.3.13.3. It can be seen that the unsaturated aldehydes are eluted before the saturated aldehydes of the same carbon number, which is in agreement with the findings of Reindl and Stan (1982).

The concentrations (ɑs to peak heights) of the aldehydes in the meat fillings are recorded in Tables 5.10 and 5.11. Each of the pie batches had a control which was analysed at the same time. It had been expected that, since the total aldehyde content should increase as lipid oxidation proceeded the absolute concentration of pentanal and hexanal would increase during storage. This was not observed however, (Tables 5.10, 5.11) the concentrations appearing to vary in a random fashion. There is no obvious explanation but it may be related to the volatile nature of these products which would cause their loss from the products during storage, random losses during extraction and derivatization, or reaction of the aldehydes with other compounds in the filling.

Obanu et al., (1976) showed that malonaldehyde may react with proteins and Yong and Karel (1978) showed that hexanal reacts

FIGURE 5.18

Typical Trace Obtained Using H.P.L.C analysis of the Aldehydes produced from meat filling of pork pies,stored at 4 C and 80% r.h Where the numbers on the peaks refer to the carbon number and saturation of the aldehydes.

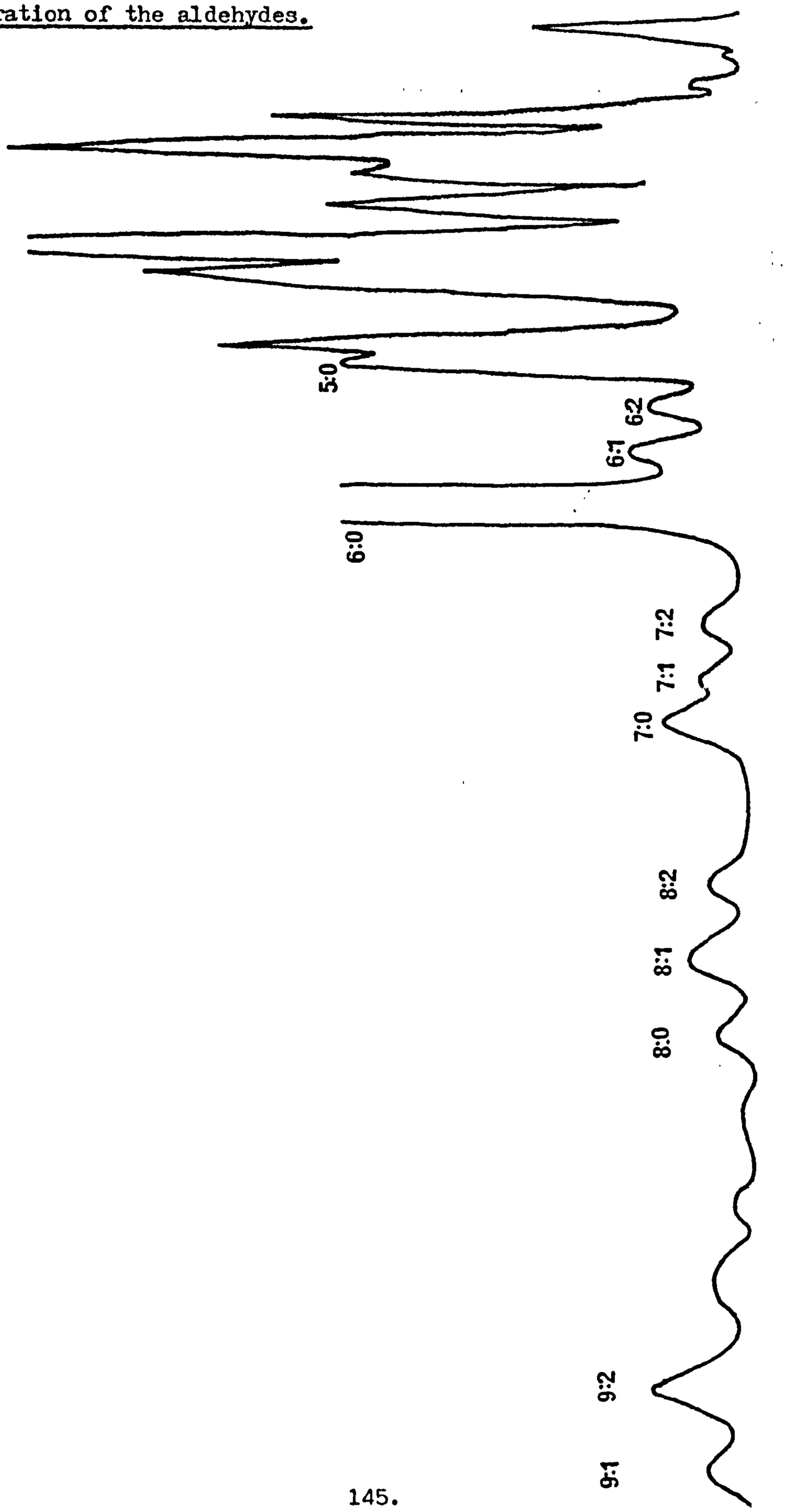


Table 5.10.

Concentration (proportional to peak height) of Aldehydes in the meat filling containing no rusk.

Aldehyde	CONTROL				NO RUSK			
	Storage Time (Days)							
	1	7	14	21	1	7	14	21
5:0	32	25	14	41	30	17	20	86
6:0	70	8	32	17	25	1	-	4
7:0	-	-	-	-	6.5	1.5	-	3
8:0	2	-	-	7	-	-	-	-
9:0	-	-	-	14	7	3.5	3	11
6:2	9	3	5	2	2	-	-	-
7:2	1.5	-	-	3.5	6	2	2	2.5
8:2	-	-	-	-	2.5	-	-	-
9:1	-	-	-	6	3	2	-	-
9:2	-	-	-	14	7	3.5	3	11

"-" not detected.

Values expressed as peak height (cm) to concentration.

Table 5.11

Concentration (proportional to peak height) of Aldehydes in the meat filling containing no seasoning mix, and no rusk or seasoning mix.

Aldehyde	Control				No seasoning mix				No rusk or seasoning mix			
	Storage Time (Days)											
	1	7	14	21	1	7	14	21	1	7	14	21
5:0	43	14	51	65	14	3	11	46	20	8	57	33
6:0	73	25	15	56	72	35	135	88	49	46	138	183
7:0	-	-	-	-	-	-	-	-	-	1.5	-	-
8:0	2	2	2	-	-	-	-	1	-	1	-	-
9:0	-	4	3	6	2	-	-	5	2	6	6	6
6:2	11	2	5	50	17	5	2	5	7	8	75	5
7:2	-	-	5	4	-	-	-	4	-	1.5	45	5
8:1	1	1	1	1	1	1	0.5	-	-	-	-	-
9:2	-	2	3	-	1	-	-	-	1	2	2	2

"-" not detected

Values expressed as peak height (cm)

with histidine. Whatever the cause any quantitative analysis must be suspect, but if one assumes that the losses (recovery) of each aldehyde are affected similarly at each sampling, expression of the concentration of each aldehyde as a function of the total concentration should yield information regarding the relative rates of production of these compounds. Thus all results have been expressed as a percentage of the total aldehyde concentration in Tables 5.12, 5.13 and 5.14 (where Table 5.12 records aldehyde changes without rusk, Table 5.13 is without the seasoning mix, and Table 5.14 is without rusk or seasoning).

All the control pies showed an increase in the pentanal (5:0) levels (as a % of total aldehyde) and a decrease in hexanal levels. The meat filling without rusk (Table 5.12) and those without seasoning mix (Table 5.13) also showed this pattern. In the filling devoid of rusk and seasoning mix (Table 5.14) the relative level of hexanal was found to increase with storage, but little change was observed in the pentanal levels (Table 5.14).

The relative levels of 2, 4-heptadienal (7:2) and 2,4-nonadienal (9:2) rose during storage. These aldehydes are cited as being involved in rancid flavours by Kochar and Meara (1975). The levels of octanal and 2-octanal (8:0 and 8:1) varied throughout storage in all the samples, which was probably due to the fact that they can decompose to form aldehydes of shorter chain length relatively rapidly (Schieberle and Grosch, 1981).

Table 5.12

Changes in Aldehyde profiles[a] the meat filling of pork pies devoid of rusk.

Stored at 4°C, 80% .

Aldehyde	Sample							
	CONTROL				NO RUSK			
	Storage Time (Days)							
	1	7	14	21	1	7	14	21
5:0	27.9	18.5	27.4	35.80	46.8	58.6	74.0	79.0
6:0	61.1	59.2	62.7	53.76	3.9	3.4	-	3.7
7:0	-	-	-	-	10.2	5.1	-	2.8
8:0	1.7	-	-	2.7				
9:0	-	-	-	7.5	10.9	12.1	11.1	10.1
6:2	7.8	1.22	9.8	1.07	3.1	-	-	-
7:2	1.3	-	-	1.8	9.3	6.8	7.4	2.3
8:0	-	-	-	-		-	-	-
9:1	-	-	-	3.1	4.6	6.8	-	-
9:2	-	-	-	2.9	7.8	6.8	7.4	1.84

a. expressed as % of total aldehyde content.

All values are expressed as mean of triplicate determinations

"-" not detected.

Table 5.13.

Changes in Aldehyde profiles[a] in the meat filling of pork pies devoid of seasoning mix, stored at 4°C, 80% rh.

Aldehyde	CONTROL				No seasoning mix -			
	Storage Time (Days)							
	1	7	14	21	1	7	14	21
5:0	33.3	28.5	38.5	40.9	13.2	6.9	41.5	30.8
6:0	56.5	51.0	33.4	35.9	67.9	81.3	50.9	59.0
8:0	1.5	4.0	3.1	-	-	-	-	0.7
9:0	-	8.16	4.7	3.3	1.9	-	-	3.4
6:2	8.5	4.0	7.8	17.6	16.0	11.6	7.5	3.3
7:2	-	-	7.8	2.2	-	-	-	2.7
8:1	0.4	0.1	-	0.1	0.01	0.4	0.07	-
9:2	-	4.0	4.68	-	0.9	-	-	-

a. expressed as % of total aldehyde content.

All values are means of triplicate determinations.

"-" not detected.

Table 5.14.

Changes in Aldehyde profiles[a] in meat filling of pork pies devoid of rusk and seasoning mix, stored at 4°C, 80% rh.

Aldehyde	CONTROL				No rusk or seasoning mix			
	Storage Time (Days)							
	1	7	14	21	1	7	14	21
5:0	30.2	28.7	38.6	41.0	25.3	11.1	21.9	17.5
6:0	58.4	53.0	38.5	34.7	62.0	65.7	70.4	73.0
7:0	-	-	-	-	-	2.1	-	-
8:0	1.5	1.2	2.1	1.1	-	1.4	-	-
9:0	-	4.1	4.6	4.2	2.5	2.5	2.3	3.2
6:2	8.2	7.6	9.8	10.1	8.7	11.4	2.9	2.6
7:2	1.8	2.0	1.7	1.7	-	2.1	1.7	2.6
8:1	-	-	-	1.7	-	-	-	-
9:2	-	3.4	4.6	4.5	1.2	2.8	0.7	1.0

a. expressed as % of total aldehyde.

All values are means of triplicate determinations.

"-" not detected.

When no rusk or seasoning mix was used in the meat filling, the relative levels of hexanal rose but this was not found in the other systems. Thus rusk and the seasoning mix could either act to reduce hexanal formation, or when formed, react with this aldehyde. This is of importance as hexanal has been used to indicate rancidity development (Karel and Labuza, 1968; Buttery et al., 1961) prior to the onset of off-odours and off-flavours (Fritsch and Gale, 1977).

Other authors (Legendre et al., 1979; Bailey et al., 1981) have considered levels of hexanal, pentanal and 2,4-decadienal in relation to rancidity. They found strong inverse correlations with flavour scores of the oxidized components in oils (as did Dupuy, 1967; 1977).

Warner et al., (1978) established regression coefficients of 0.96 and 0.90 between taste panel scores and hexanal and pentanal levels respectively in vegetable oils. They concluded that either hexanal or pentanal levels could be used as indicators of rancidity. However due to the complexity of meat filling, which are not pure systems like vegetable oils, it would be unwise to place too much reliance solely on one index (pentanal or hexanal).

Estimation of pentanal in the meat fillings indicated that levels of 3×10^{-5} g/g were present in the fillings having TBA values of 5 (Table 5.15). Thus it would appear that once a level of 30ppm was recorded, the product was organoleptically unacceptable (rancid). For hexanal the levels were 5×10^{-5} g/g meat, or 50ppm. As the levels

Table 5.15.

Day of storage when rancidity was detectable

in the meat fillings used in the aldehyde determinations.

Pie	Detected rancidity (TBA value of 5) Day of storage
CONTROL	15/16
NO RUSK	9
NO SEASONING MIX	10
NO RUSK OR SEASONING MIX	5/6

for both pentanal and hexanal are above the threshold values in oil for detection by trained taste panels (0.15ppm for each aldehyde; according to Kochar and Meara, 1975) other compounds in the filling must be masking their rancid taste and odours. This view had been expressed by Labuza 1971, but is not based on firm scientific findings.

Thus, sensory rancidity can be related to 30ppm of pentanal or 50ppm of hexanal in fillings where no rusk and seasoning mix is used.

Chapter 6.

Moisture migration in pork pies.

Chapter 6. Moisture migration in pork pies.

The findings reported in Sections 4.1 and 4.2 indicated that the softening of pastry texture, which is a major factor in limiting the shelf life of pork pies, is due to its absorption of moisture from the jelly in the pies' interior and from the atmosphere externally.

The possibilities of preventing or limiting such migration of moisture depends on first identifying the distribution of moisture within the pies and, thereafter on defining the upper threshold of moisture content beyond which the texture of the pastry deteriorates to an unacceptable level.

6.1. A_w of the components of pork pies stored at 4°C and 80% r.h.

The available water (a_w) in each component part of the pork pie was studied as a function of storage. This was to record the different a_w's in the pie, and see how moisture migrated. As it was expected that moisture would migrate from the areas of high a_w to areas of low a_w in an attempt to establish an equilibrium (with regard to moisture) within the pie.

6.1.1. Experimental design.

The pies were manufactured using a single pastry mix, and one meat filling mix, in which the age of the back fat and shoulder meat were constant (2 days post slaughter) (Section 3.1). Following baking, jellying and cooling the wrapped pies were stored at 4°C and 80% r.h.

After 1, 7 and 23 days storage, 6 or more pies were removed (Section 3.3.1.) for a_w determinations, being separated into pastry, meat filling and jelly.

As the outer (brown) pastry layer appeared to be responsible for crispness, its properties were separately assessed by removing from it the white inner pastry layer.

6.1.2. Results and discussion.

The a_w of each pie component during storage is recorded in Table 6.1. As expected there was a marked decline in the a_w of the jelly, and a concomitant increase in the a_w of the crisp (brown) pastry layer, as moisture migrated into it. These trends were also reflected when a comparison was made with the % moisture content results (cf Section 4.1). The changes observed in both parameters cannot be fully explained by the loss in jelly moisture (or by the fall in a_w) alone. This indicates that moisture must also be migrating into the pastry from the environment.

The a_w of the whole pastry remained unchanged during storage, leading to the hypothesis that it is in the outer brown pastry layer where the changes in moisture and subsequent loss of texture occurred. The same hypothesis was put forward by Butcher (1981). Clearly this area merited particular study, in all future work, with regard to moisture migration.

From Table 6.1, it can be seen that the a_w of the meat filling remained virtually constant, (as did its moisture content, Sections 4.1 and 4.2), indicating that it does not play a part in moisture migration in the pie.

Table 6.1.

A_w of the components of pork pies stored at 80% r.h. and 4°C.

COMPONENT	STORAGE TIME (DAYS) 1	7	23
GELATINE JELLY	0.970	0.963	0.864
	0.976	0.960	0.874
	0.977	0.963	0.869
	0.975	0.964	0.864
	0.972	0.961	0.869
	0.972	0.962	0.864
	0.973	0.961	0.867
BROWN PASTRY LAYER	0.671	0.817	0.897
	0.670	0.816	0.897
	0.678	0.817	0.887
	0.684	0.816	0.886
	0.683	0.810	0.862
	0.672	0.814	0.889
	0.674	0.815	0.888
WHOLE PASTRY	0.976	0.966	
	0.970	0.967	
	0.971	0.965	
	0.978	0.971	NOT
	0.977	0.970	DETERMINED
	0.975	0.967	
	0.977	0.967	
MEAT FILLING	0.988	0.987	0.974
	0.988	0.987	0.972
		0.988	
	0.994	0.971	0.973
	0.991	0.988	0.970
	0.991	0.970	0.969
	0.990	0.971	0.966
	0.991		0.971

The changes in a_w as a function of storage support the moisture content results (Sections 4.1 and 4.2) in that moisture migrates from the jelly in the pie, and from the exterior atmosphere, into the pastry, especially into the outer brown layer.

6.2. Relationships between texture, moisture content and organoleptic assessment of pastry stored at 4°C and 80% r.h.

The migration of moisture into the brown pastry layer (Section 6.1.2)results in an increase in moisture content and decrease in texture (crispness) of the pastry. After sufficient moisture has been absorbed, the pastry will be deemed unacceptable due to its loss of crispness. It was thus desirable to be able to relate the texture readings and moisture content with the level of acceptability and thus to establish values for these parameters, which will limit consumer satisfaction.

6.2.1. Experimental design.

As described earlier (Section 3.1) the pies were made from a single pastry mix and a single meat filling in which the age of the back fat and shoulder meat was 2 days post slaughter.

The pies were baked, jellied and cooled and stored at 4°C and 80% r.h. On a daily basis at least six pies were removed from storage, and the brown pastry layer assessed for moisture content (Section 3.3.4.), texture (Section 3.3.5.) and organoleptic assessment (Section 3.3.5.2.).

6.2.2. Results and discussion.

The moisture content and texture reading (g) of the brown pastry layer are given in Figure 6.1. It shows that, as storage proceeded, the moisture content of the brown pastry layer rose, while the texture readings declined as did the organoleptic scores. A correlation coefficient (r) of -0.89 was found between moisture content and organoleptic scores, (Figure 6.2). This was found to be statistically significant ($P < 0.05$) (Section 3.3.1.5.).

Figure 6.3 shows the correlation between texture readings and organoleptic score, where r = 0.84. Again, a significant ($P < 0.05$) result. The organoleptic assessments indicated that below a score of 1½ the pastry was unacceptable due to loss of crispness. From Figure 6.2 this score of 1½ corresponds to a moisture content of 12%.

Applying this to figure 6.3, an organoleptic score of 1.5 is seen to equate to a texture reading of 160g. Thus the threshold values for acceptable moisture content and texture are below 12% and above 160g respectively.

Figure 6.4 confirms the relationship between texture and moisture content, where r was found to be -0.76 (which is significant $P < 0.05$). A moisture content of 12% appears to correspond to about 9 days storage (Figure 6.1). This is the shelf life estimated for pies by the manufacturer.

These threshold values for acceptability were used to evaluate the effectiveness of proceedures designed to prevent or retard moisture migration in the pie.

FIGURE 6.1

Changes in the Moisture Content (■—■), Texture Reading (▲—▲) and Organoleptic Assessment (◿—◿) of the outer brown pastry layer of pork pies stored at 4°C and 80% r.h.

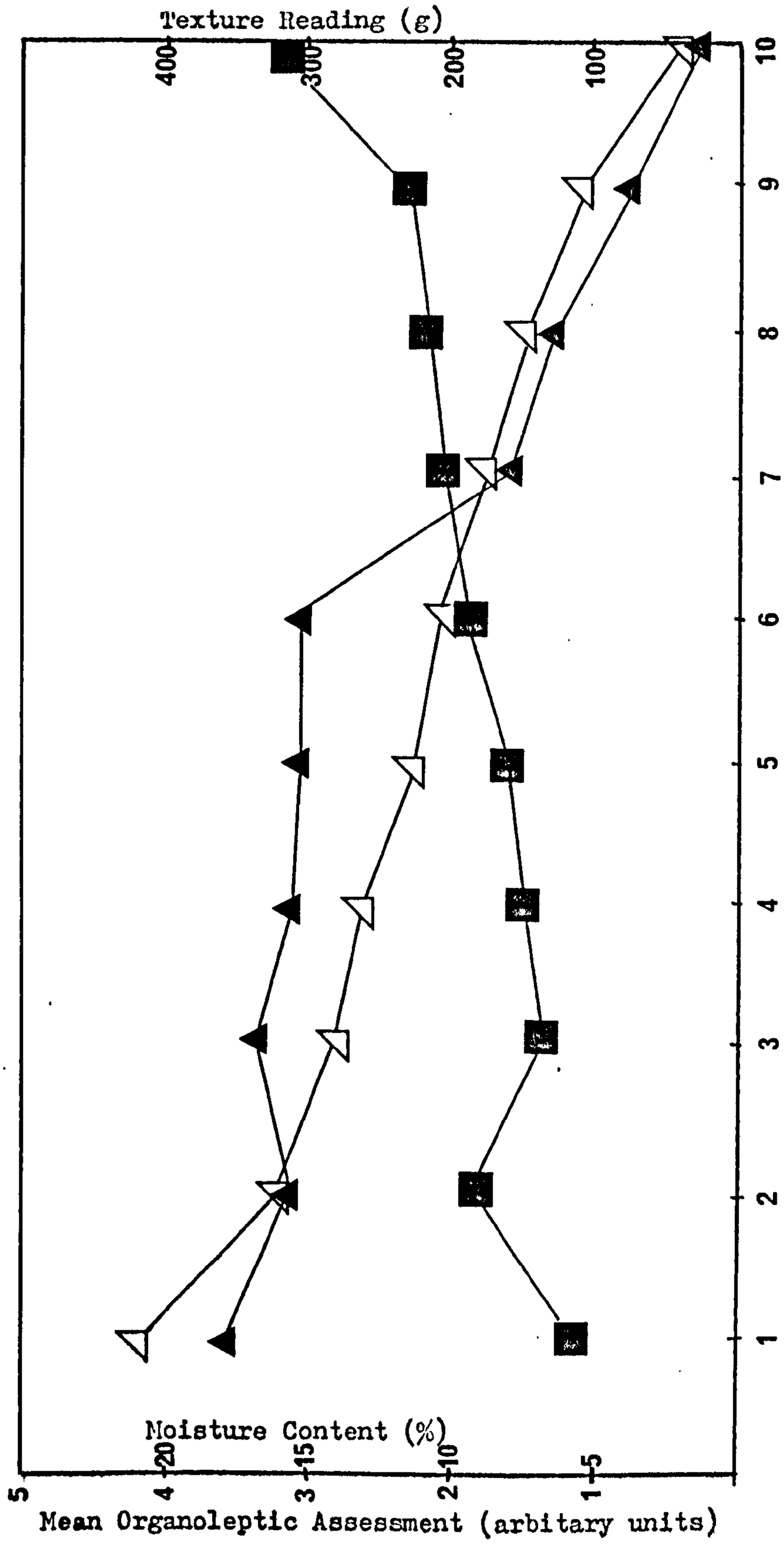


FIGURE 6.2

Correlation between the Moisture Content and the Organoleptic assessment of the brown pastry layer of pork pies stored at 4°C and 80% r.h.

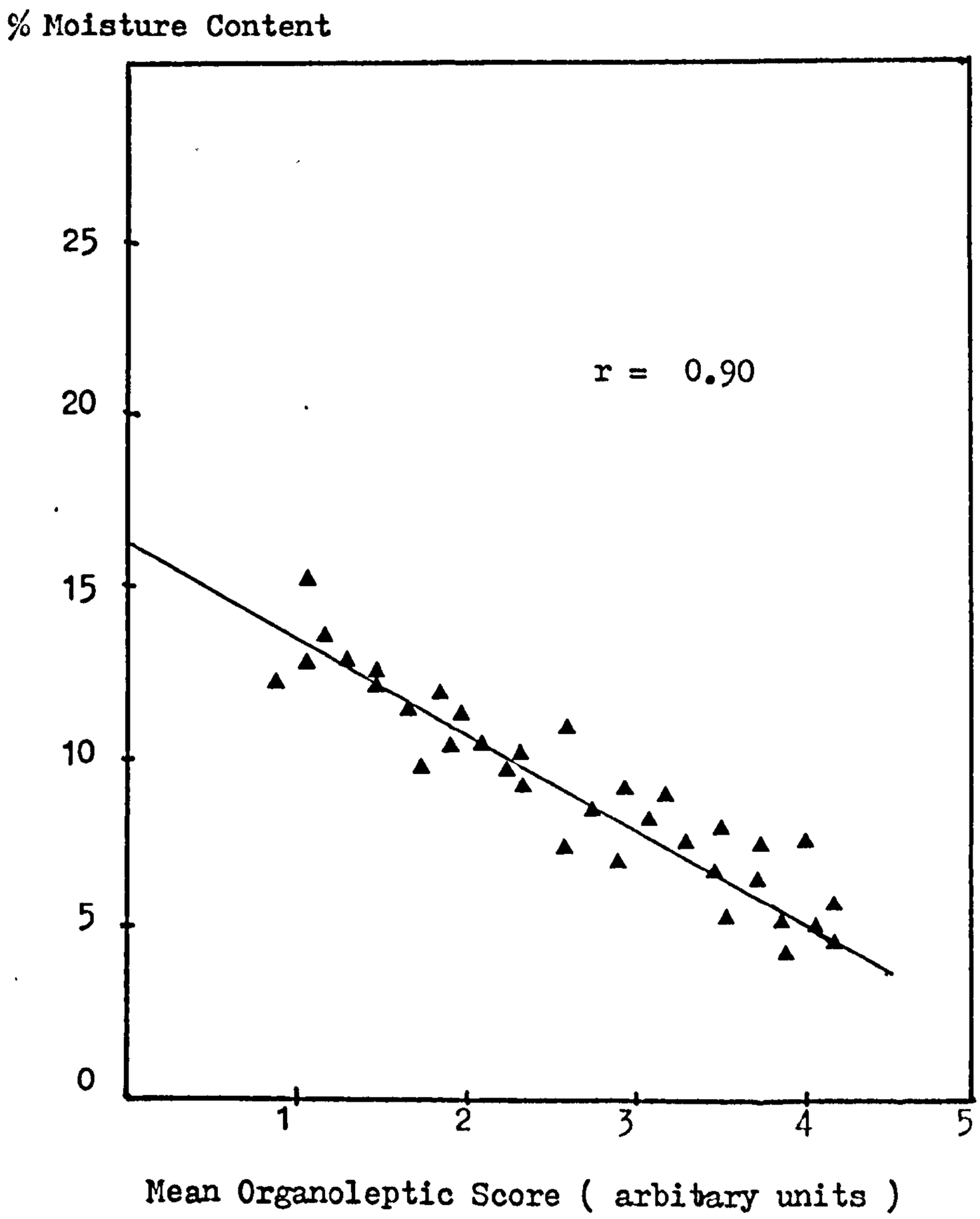


FIGURE 6.3

Correlation between the Objective and Subjective Assessments of Texture of the brown pastry layer of pork pies stored at 4°C and 80% r.h, over a 10 day period.

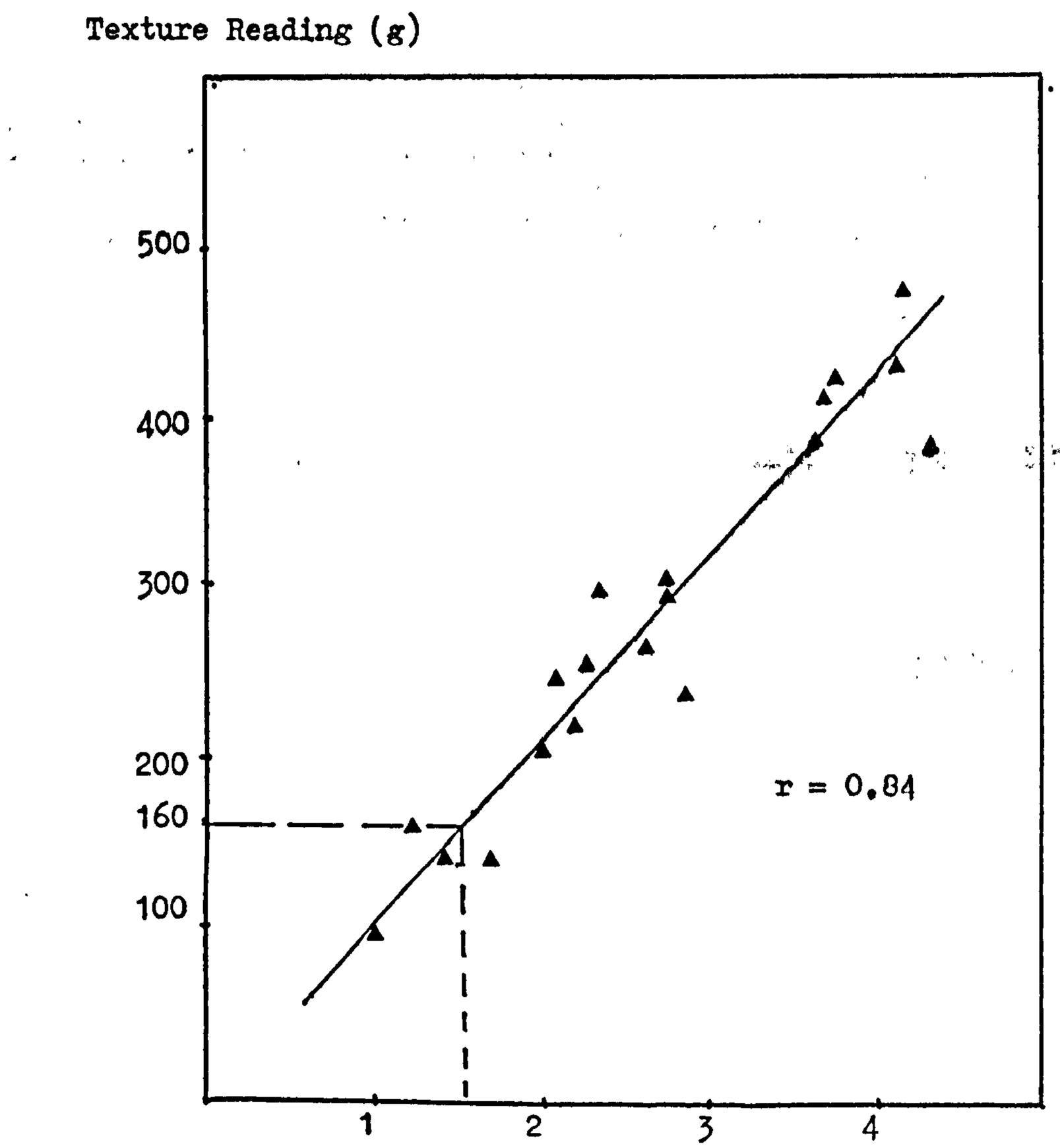
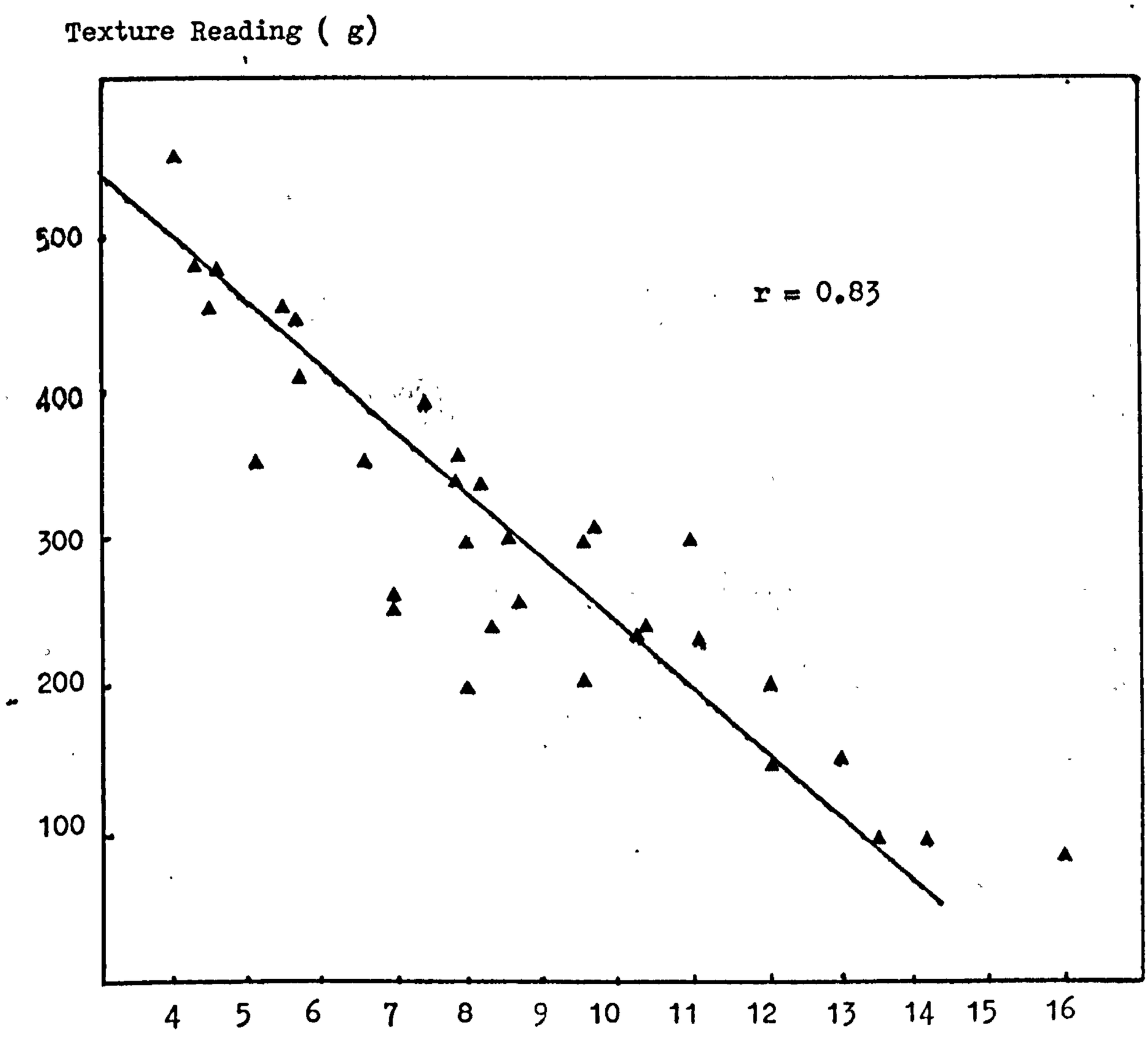


FIGURE 6.4

Correlation between Moisture Content and Texture Reading of the brown pastry layer of pork pies stored at 4°C and 80% r.h.



6.3. Effect of relative humidity (r.h.) and jelly a_w on moisture migration in pork pies stored at 4°C.

It has already been established that moisture migration occurs between the jelly, the atmosphere and the pastry, especially the brown crisp layer, resulting in its loss of crispness (texture) (Section 6.1.2). The threshold values for acceptability were a moisture content of 12% and a texture value of 160g (Section 6.2). These criteria were next employed in this investigation, to evaluate the effects of the relative humidity (r.h.) of the atmosphere, and of the a_w of the jelly on moisture migration, in the hope of controlling these parameters, to prevent or sufficiently retard moisture migration and to promote considerable extension in shelf life, in so far as loss of texture was the main factor determining the latter.

6.3.1. Effect of relative humidity (r.h.) and jelly a_w on the moisture content and texture of pastry (brown layer) of pies stored at 4°C.

As established in Sections 6.1 and 6.2 the possibility of controlling moisture migration in pies requires a knowledge of the r.h. of the atmosphere and of the jelly a_w at which little or no moisture is free to migrate into the pastry. These values were sought, and then applied to pork pies, in the hope of achieving a substantial extension of shelf life.

6.3.1.1. Experimental design.

In order to establish the r.h. at which no moisture migration occurred between the atmosphere and pastry, pies were made as described in Section 3.1.

After baking, jellying and cooling, the pies were numbered for identification purposes. The meat filling, jelly and white pastry layer were dissected out, leaving a brown pastry layer 'shell', which was divided into two, one half of the 'shell' was stored at 4°C in a box having a relative humidity of 0, 34, 58, 67 or 100% (Appendix 1). At least 10 'shells' were stored at each r.h. for 8 days. The corresponding half of each stored shell was analysed immediately for initial moisture content (Section 3.3.4.) and initial texture value (Section 3.3.5.).

The stored halves of the shells were analysed for moisture content and texture value and these results were compared with the initial values obtained from the corresponding unstored halves.

In order to establish the jelly a_w at which moisture did not migrate into the pastry, jellies of different a_w were made by replacing the water content with glycerol in varying quantities (0-90%). Brown pastry shells were obtained from pies made as described in Section 3.1. Half of each shell was completely covered in a jelly containing glycerol in varying amounts. The other half (the control) had moisture content and texture value determinations performed on it immediately.

After 8 days storage at 4°C in the jelly, the brown pastry moisture content and texture was recorded, and compared with its corresponding initial values.Using the results obtained from the first two studies (on r.h. and jelly a_w) Pies were made as described in Section 3.1 and jellied

using the glycerol jelly found to stop moisture migration from the jelly (i.e. 70% glycerol). The pies were stored in a controlled r.h. (76%, which had been found to stop moisture migration from the atmosphere) at 4°C, for 35 days. Moisture content and texture determinations on the brown pastry layer of the pies was performed daily for 14 days, and then at 35 days storage at 4°C. (All determinations used a minimum of six pies).

6.3.1.2. Results and discussion.

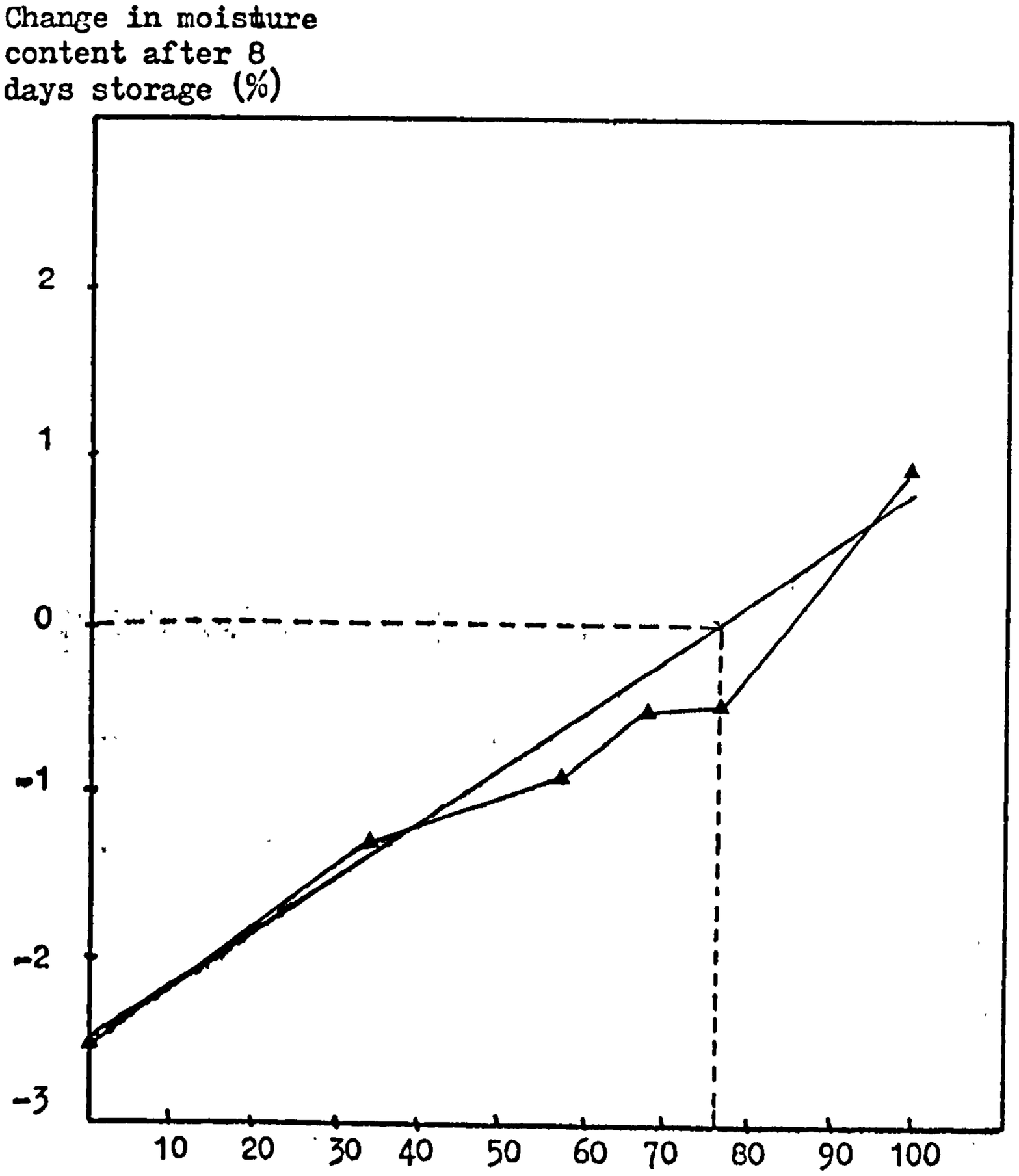
The effect of r.h. on moisture migration (as measured by change in moisture content of the brown pastry layer) is shown in Figure 6.5, from which it appears that, at a relative humidity of 87%, no moisture migrates from the atmosphere into the brown pastry (i.e. there is no change in the brown pastry moisture content). However, this r.h. value of 87% is doubtful, since it has already been shown that moisture migration occurs at 80% r.h. (Sections 4.1 and 4.2). Linear regression of the data in Figure 6.5 if performed, indicates that an atmosphere of 76% r.h, would prevent moisture migrating from the atmpsphere into the pastry and loss of crispness in the latter.

From Figure 6.6., it may be seen that an atmosphere o. 67% r.h. prevented moisture migrating into the pastry from the atmosphere, thus an r.h. of 67% or less is required to stop loss of the brown pastry crispness (texture).

The effect of the a_w of the jelly on the moisture content and texture of the brown pastry layer, are shown in Figures 6.7 and 6.8 respectively. In order to maintain

FIGURE 6.5

Change in Moisture Content of the brown pastry layer as a function of r.h, after 8 days storage at 4°C.

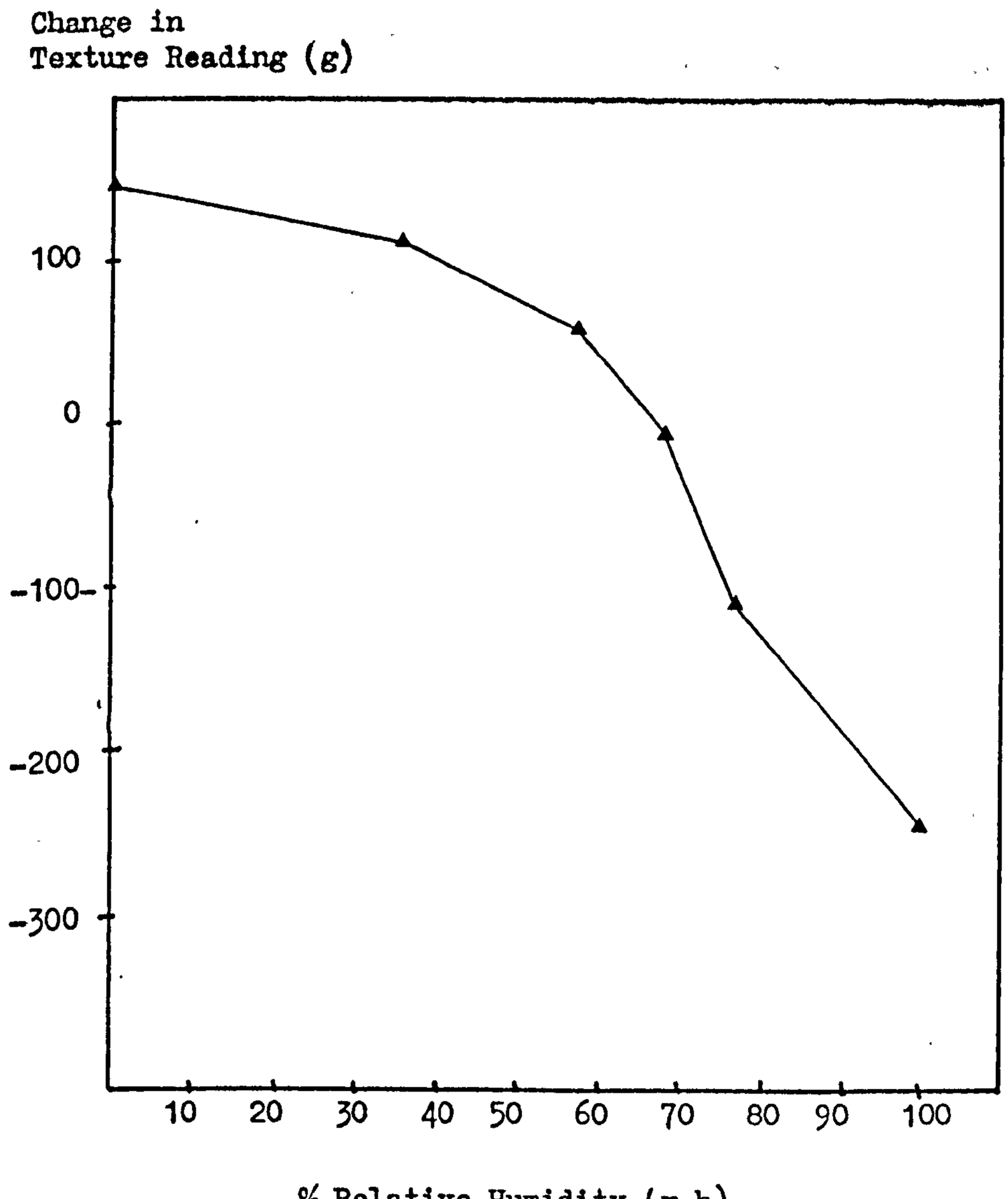


Linear regression analysis gives a best line fit which intercepts the Y axis at -2.52, with a slope of 0.033. A Y value of 0 gives a X value (r.h) of 76%. r= 0.97.

% r.h obtained using saturated salt solutions (appendix 1).

FIGURE 6.6

Change in Texture Reading of the brown pastry layer stored at 0 - 100% r.h. for 8 days at 4°C.



%r.h obtained using saturated salt solutions (appendix 1).

FIGURE 6.7

Effect of jelly a_w on the Texture Reading of the brown pastry layer stored at 4 C for 8 days.

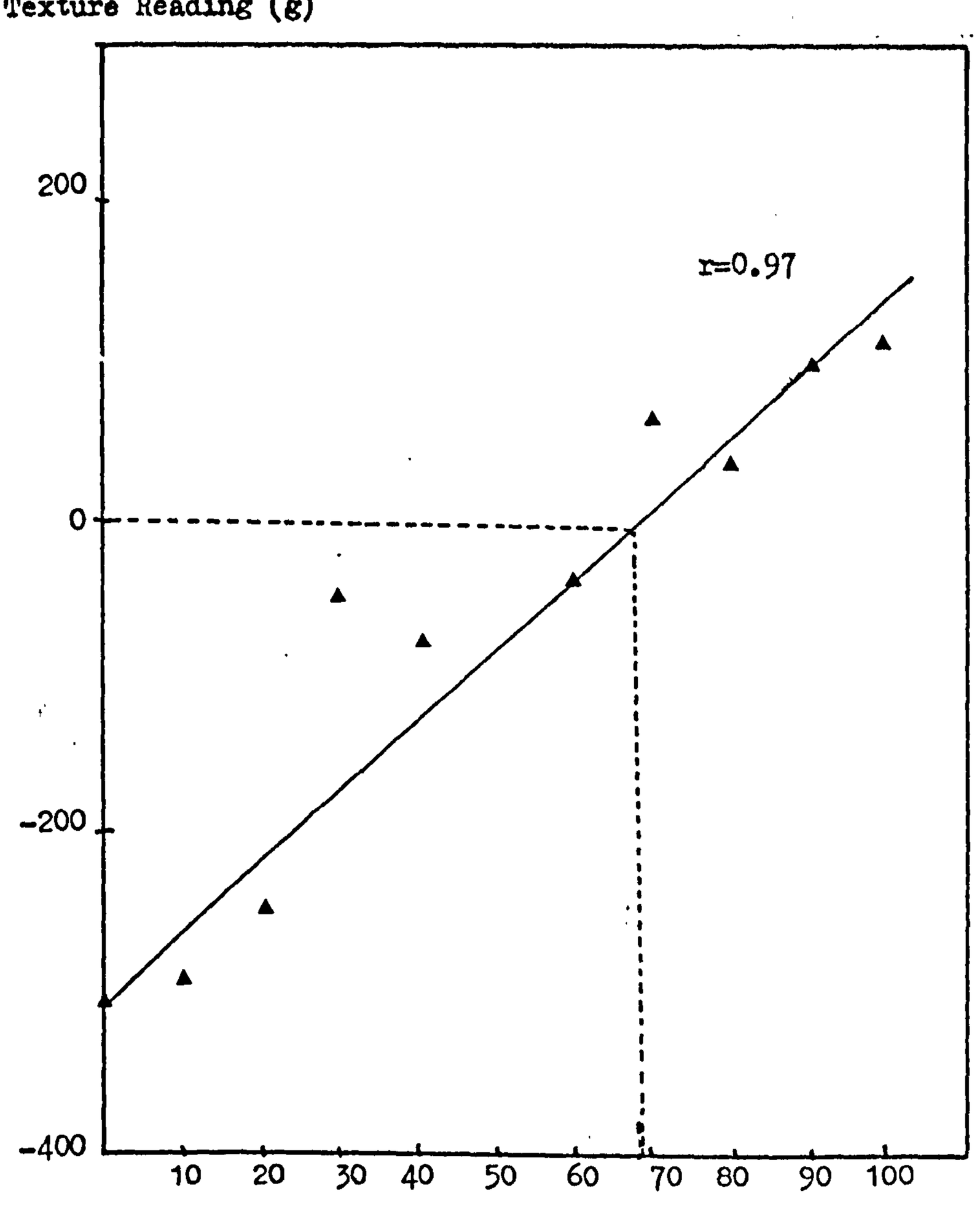
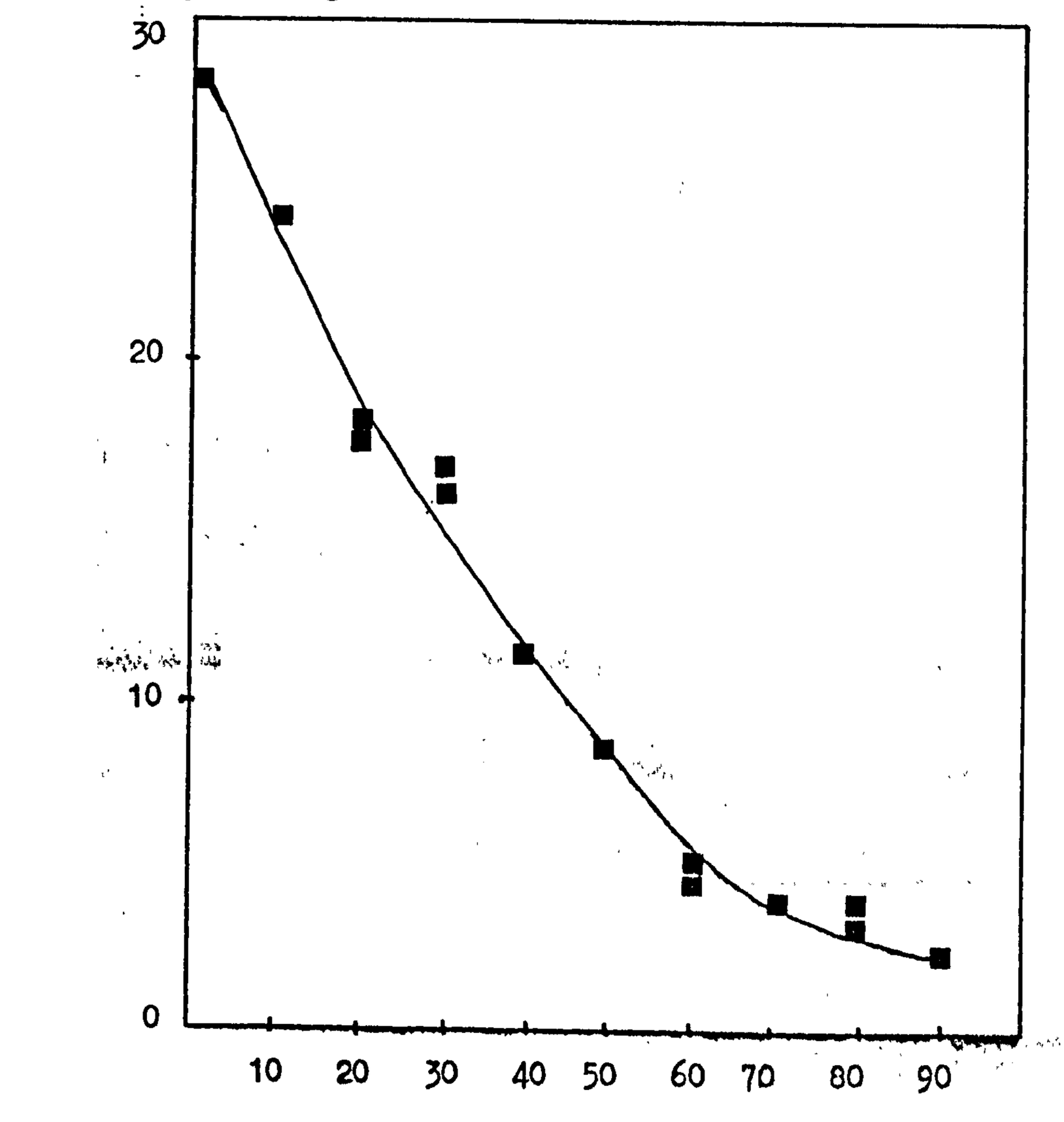


FIGURE 6.8

Effect of the jelly a_w on the Moisture Content of the brown pastry layer after 8 days storage at 4°C.



the texture at its initial value (no change in texture in Figure 6.7) the % of glycerol in the jelly was found, by linear regression analysis, to be 68.8%. From Table 6.2 this represents an a_w of approximately 0.55. It was found that higher levels of glycerol in the jelly (lower jelly a_ws) resulted in improved texture (increased crispness) of the pastry, presumably due to moisture migrating into the jelly (due to its lower a_w) from the brown pastry.

Figure 6.8 shows the increase in actual moisture content of the brown pastry layer as a function of replacing water with glycerol in the jelly. As the level of glycerol in the jelly increased (and its a_w decreased, Table 6.2) the increase in the moisture content of the brown pastry layer became progressively less marked.

Since the initial a_w of the brown pastry layer was found to be 0.67 (cf Section 6.1): - and this equates to 60% glycerol in the jelly (from Table 6.2).it would be expected that no moisture migration would occur, because the jelly and pastry were in equilibrium with each other. With a higher % of glycerol in the jelly the even lower a_w would result in moisture loss from the pastry to the jelly so leading to an improvement in pastry texture. This was found to occur (Figure 6.7), but at glycerol levels of above 70%.

To reiterate it thus appeared that a relative humidity of 76% was needed to retain the moisture content of the brown pastry layer at its initial value; whereas, for good texture, the r.h. should be no more than 67%. Similarly,

Table 6.2.

Effect on A_w of replacing water in the pie jelly with glycerol. A_w recorded 24 hrs after manufacture.

sample	A_w
4% GELATINE JELLY	0.994
Replacing water with:	
10% Glycerol	0.977
20% Glycerol	0.939
30% Glycerol	0.918
40% Glycerol	0.849
50% Glycerol	0.773
60% Glycerol	0.655
70% Glycerol	0.556
80% Glycerol	0.484
90% Glycerol	0.320

for these purposes the a_w of the jelly should be between 0.65 and 0.55 (60 and 70% glycerol levels).

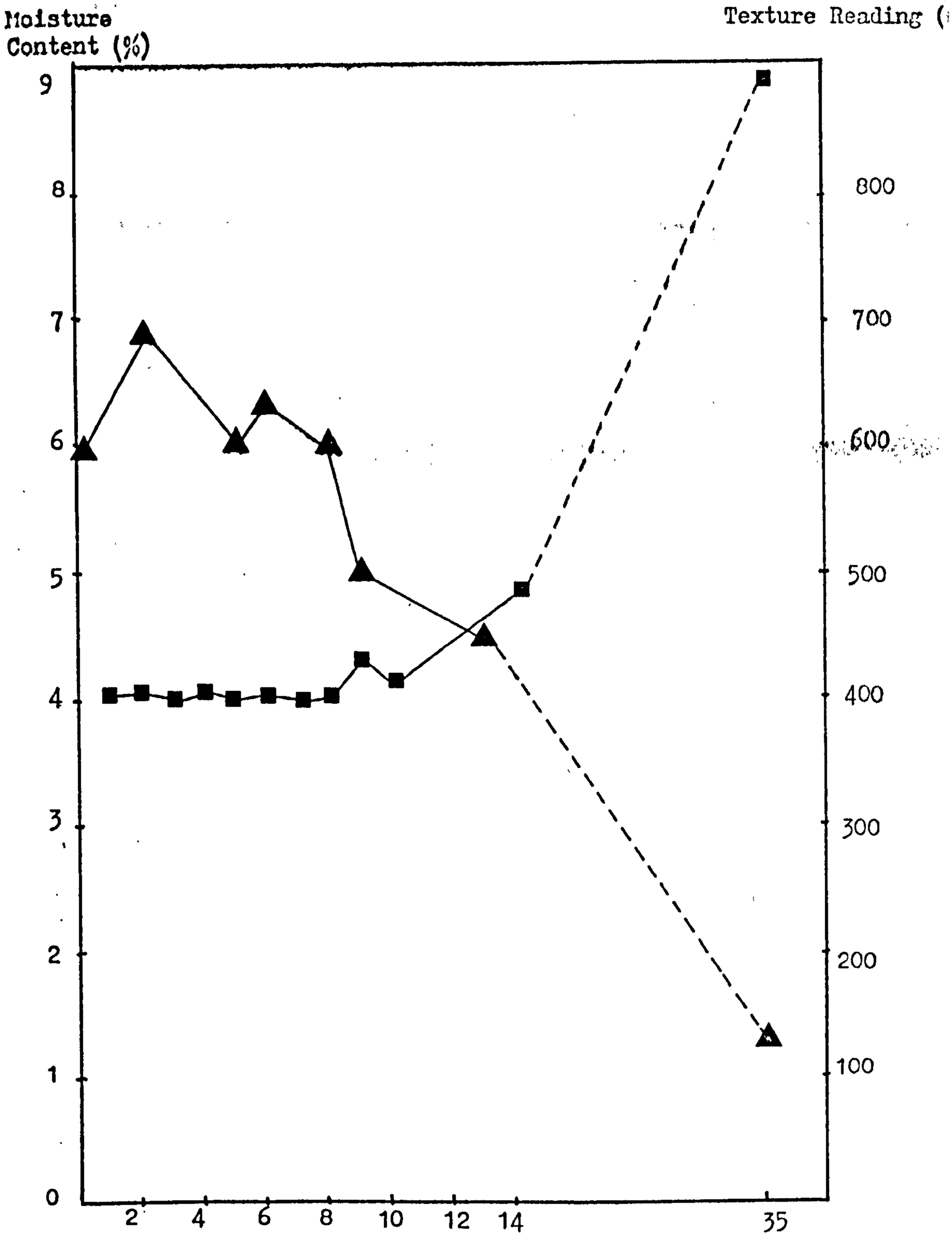
Accordingly, pies were made containing 70% glycerol jelly and stored at 76% r.h., to see if moisture migration from the jelly and atmosphere into the brown pastry still occurred. Figure 6.9 records the effects of 76% r.h. and 70% glycerol jelly (0.55 a_w) on the brown pastry layer's moisture content and texture.

The moisture content remained virtually constant for 14 days; and even by 35 days storage, the unacceptable value of 12% moisture was not attained. The preservation of texture was not so marked; losses occurring by 9 days of storage. The texture value of 160g (Section 6.2) was reached by 35 days storage. The eventual increase in moisture content, and subsequent loss of texture, is most likely due to the migration of glycerol from the jelly into the pastry (detected organoleptically). This would raise the jelly a_w, and allow moisture to then follow the glycerol into the pastry. Even so, there was a considerable extension of pie shelf life (approximately 30 days) compared with the 8/9 days (cf Section 6.2) normally found (when the moisture has risen to 12% and texture fallen to 160g - and unacceptable pies result).

Thus manipulation of r.h. and of jelly a_w (using glycerol to achieve the reduction) considerably extends the shelf life of the pies (using loss of crispness as the controlling factor). Unfortunately the use of glycerol results in pork pies with an unacceptable taste; and it is

FIGURE 6.9

Change in Moisture Content (■—■) and Texture (▲—▲) of the brown pastry layer of pork pies stored at 4°C 76% r.h, containing "70 % glycerol" jelly.



uneconomical to use. Alternative cost effective means of lowering the jelly a_w were thus sought.

6.3.2. Effect of jelly a_w on moisture losses from gelatine gels.

Previous work had shown (Section 4.1) that the increase in the moisture of the content of the pie's pastry could not be totally accounted for by the moisture loss of the jelly. An attempt was thus made to assess the theoretical loss/gain of moisture by the jelly as a function of its a_w, and thus its possible contribution to moisture migration into the pastry.

6.3.2.1, Experimental design.

For conditions to be comparable to those actually found in pies, 16.5ml gelatine jelly were set in 88mm dia. petri dishes. This procedure had been found to give the same depth of jelly as that between the meat filling and pastry in the pies, (Drury, 1984). 4% gelatine solutions (w/v) were made containing 0, 10, 30, 50, 55, 65 and 70% glycerol. The petri dishes were stored in relative humidity boxes at 67% r.h. (determined by saturated sodium nitrate solution). This r.h. was chosen since it corresponded to the initial a_w of 0.67 of the brown pastry layer (Section 6.1). Thus any loss of moisture from the jelly (measured by the decrease in weight of the petri dish) could be assumed, theoretically to affect the extent of moisture migration in pies from the jelly into the pastry.

The petri dishes were weighed at regular intervals (and the weight gain or loss recorded).

6.3.2.2. Results and discussion.

The weight gains and losses, as a function of jelly a_w, are shown in figure 6.10. Not unexpectedly, the largest loss was recorded from the gel containing no glycerol and the least from gel containing 55% glycerol (losses from the gels containing 10-50% glycerol were intermediate).

The 65% and 70% glycerol gels both exhibited an increase in weight, although these were found not to be significant increases ($P > 0.05$).

Extrapolating from Figure 6.11 the 65% glycerol gel had an a_w of 0.66 after 1 day from manufacture (0.63 from Table 6.2). Since this a_w was the same as that of fresh pastry (0.67), the use of 65% glycerol gel in a pie should prevent marked moisture migration between the phases, provided the meat filling does not lose water. The meat filling has an a_w of 0.98 (from Table 6.1) and it might be expected that moisture would migrate from the meat into the jelly. The results in Section 6.1, showed only a slight decrease in a_w of the meat filling during 23 days, and, therefore, one can conclude that the meat filling plays virtually no part in moisture migration in pork pies. In actual experimental conditions the 65% glycerol gels were found to have an a_w slightly less than that of the brown pastry and thus these gels tend to pick up moisture from the pastry as seen by the increase in weight (Figure 6.10). 55% glycerol gels have an a_w of 0.78 (from extrapolations in figure 6.11) and so lose moisture to the pastry. Thus the glycerol level of a_w required to prevent moisture migration into the pastry

FIGURE 6.10

Change in weight of the jellies containing 0% (▲—▲), 10% (■—■), 30% (□—□), 50% (◁—◁), 55% (◢—◢), 65% (○—○) and 70% (△—△) glycerol, stored at 20°C and 67% r.h.

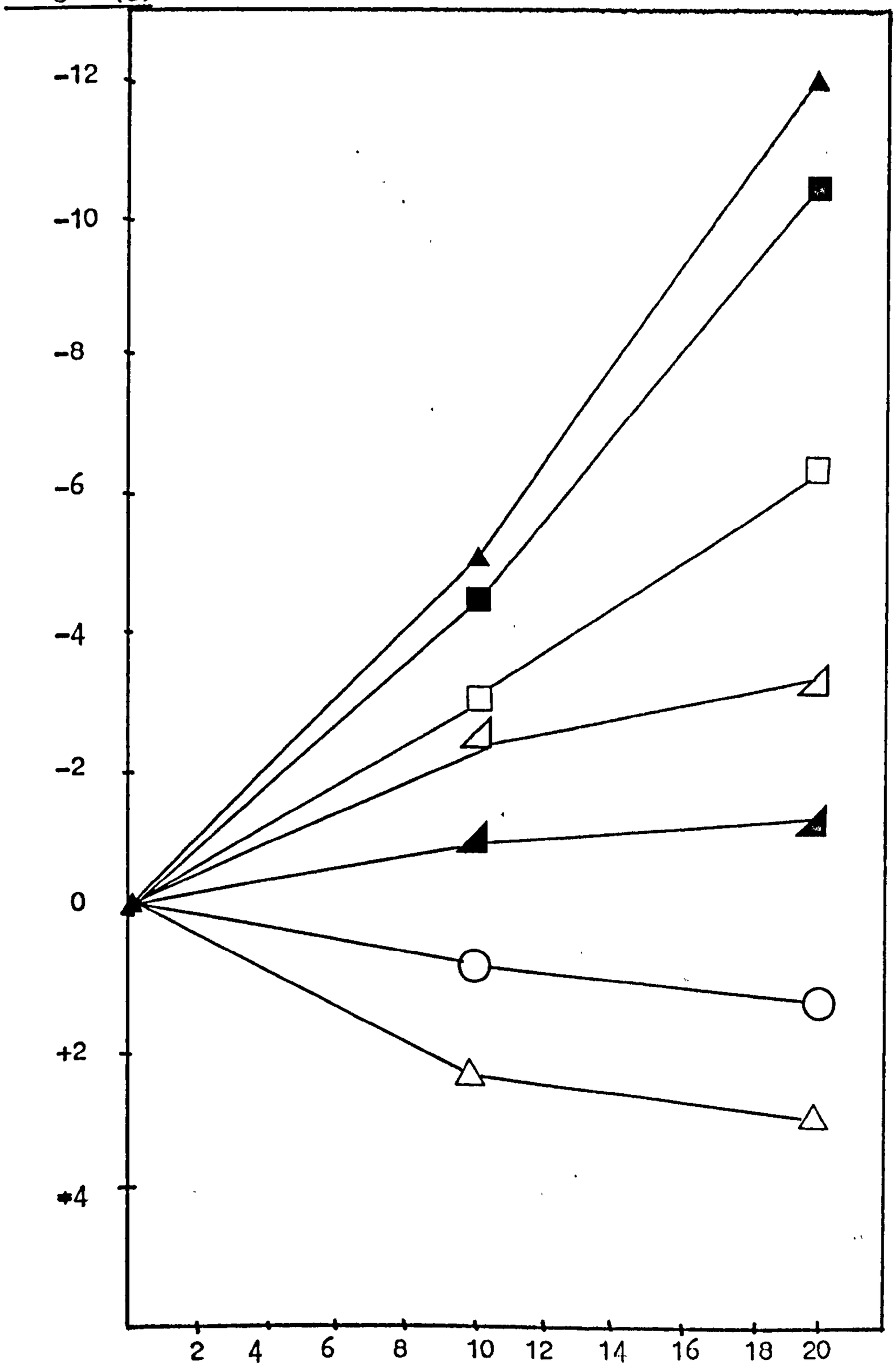
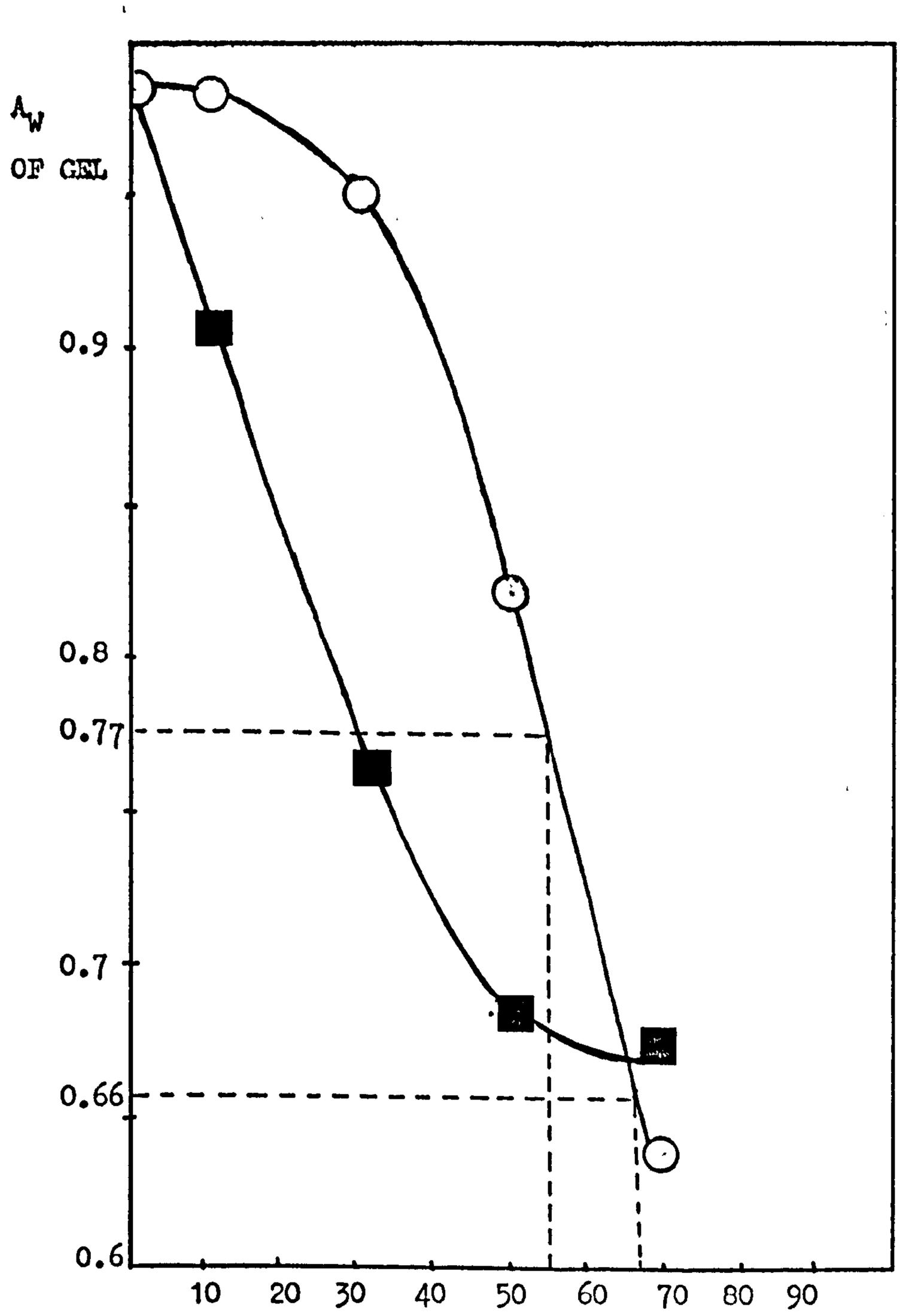


FIGURE 6.11

A_w of the gelatine gels containing 0 to 70% glycerol after 1 days storage (○—○), and after 22 days storage (■—■) at 20°C and 67%r.h.



of the pies lies between 65 and 55% glycerol (a 60% glycerol was obtained in Section 6.3.1.).

Using the straight line regions of each relationship in Figure 6.10, the rates of moisture loss over 10 days were calculated for the 0 to 55% glycerol gels, (65 and 70% glycerol gels gained moisture) and are given in Table 6.3.

Table 6.3.

Moisture loss (g/10 days) for 0-55% glycerol gels.

% glycerol	a_w	moisture loss (g/10 days)
0	0.97	7
10	0.96	6
30	0.91	4
50	0.82	2.5
55	0.78	1

Moisture loss may be expected to obey a relationship

where rate of loss, $(x) = K [\Delta a_w]^n$ (I)

Thus, Log X =

$$\text{Log of rate} = n \log [\Delta A_w^1] + \text{Log } K \quad \text{(II)}$$

where

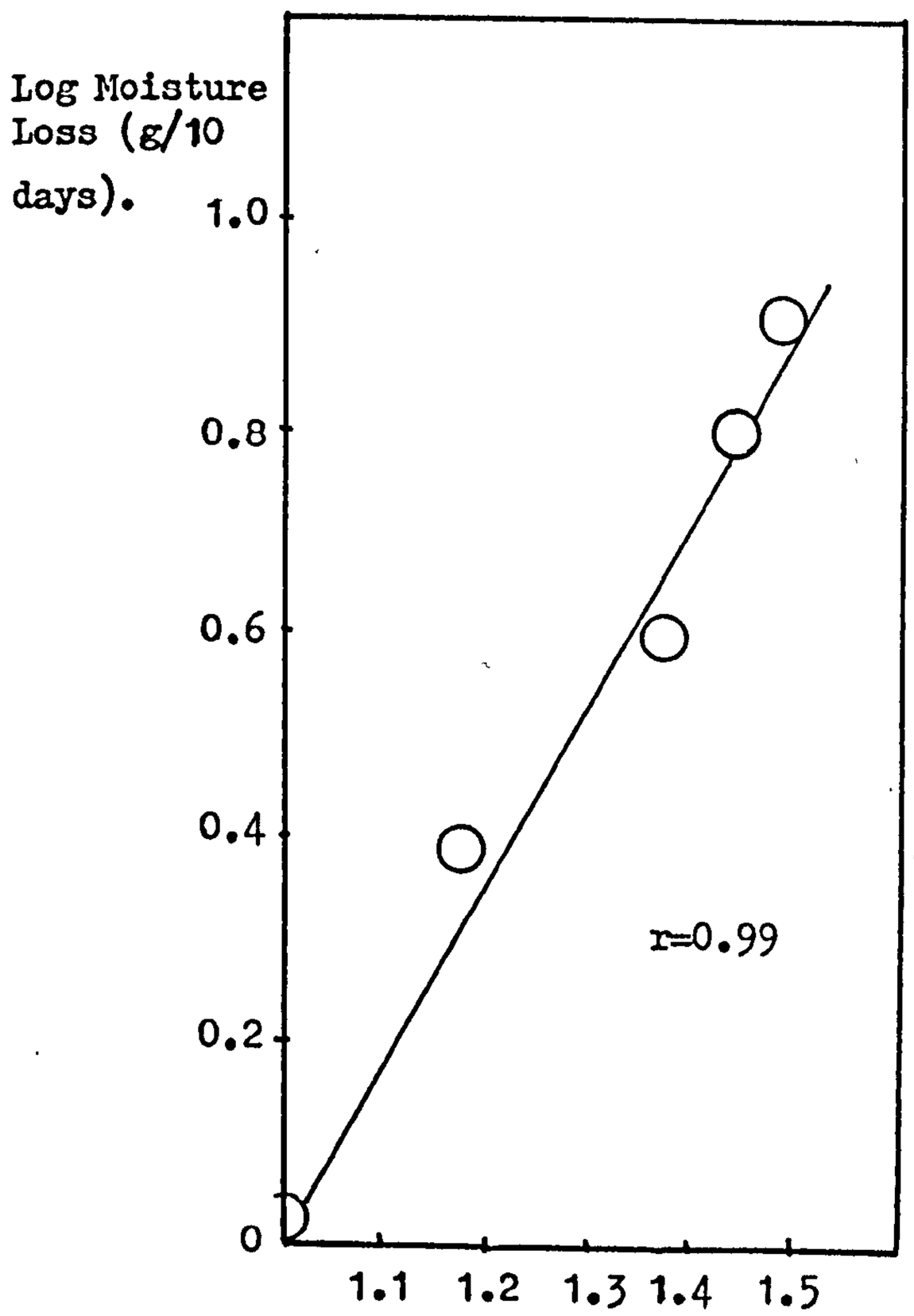
n = order of reaction

a_w = difference between gel and pastry water activity

K = proportional constant.

FIGURE 6.12

Log rate of Moisture loss (g/10 days) from 0, 10, 30, 50, 65 and 70% glycerol gels stored at 67%r.h, against Log$_{\Delta}$r.h (difference in r.h between gel and the environment).



A linear correlation (r) of + 0.99 was recorded (Figure 6.12) when the log of the rate was plotted against $\text{Log}[\Delta a_w]$. Thus equation II can be expressed as

$$\text{Log (rate)} = 1.65 \log[\Delta \text{ r.h.}] + -1.62$$

where 1.65 is the slope and -1.62 the intercept of the line of best fit in figure 6.12.

The moisture loss (g/10 days) from the gels was used to estimate the theoretical loss of moisture from the total jelly in the pie to the pastry. These values were compared with the moisture gain for the brown pastry layer (cf Figure 6.8 and Appendix 2), and are presented in Table 6.4.

Table 6.4.

Moisture loss by the gels, and moisture gain by the brown pastry layer.

gel a_w	moisture loss from petri dish (g/10 days)	calculated loss from jelly in pie (g/10 days)	moisture gain by brown pastry layer (g/10 days)
0.97	6.7	16.2	28.3
0.96	6.3	15.2	23.0
0.91	4.7	11.4	14.0
0.82	2.2	5.3	7.0

It is seen that the loss of moisture from the gel does not match the gain in moisture by the brown pastry layer. This was also found in Sections 4.1 and 4.2, and 6.2,where it was stated that the atmosphere must also contribute to the moisture increase in the pastry.

It is of interest to note that the nearer the a_w of the jelly approaches that of the environment (0.67), the amount of moisture gained from the atmosphere by the brown pastry layer becomes proportionally less than that from the jelly. This would be expected,as once the atmosphere and brown pastry layer are in equilibrium, the moisture must come from the jelly.

6.4. Attempts to control moisture migration from the jelly of pork pies.

As the use of glycerol to reduce the a_w of the jelly is limited by its unacceptably sweet taste, alternatives were sought, the effects of various gelatin jellies and of binders and humectants in the jelly, were investigated.

6.4.1. The water holding capacity (W.H.C.) of various gelatine gels.

One way to assess the potential use of a jelly in pork pies is to establish its water holding capacity (W.H.C.). This is a measure of the amount of water that is released when an external force is applied to it (Hamm, 1960; Jaur egui et al., 1981). The higher this W.H.C. value, the less is the amount of water in the test gel which is freely available to migrate into the pastry, cause its

softening, and thus limit its shelf life.

The W.H.C. of some commercially available gelatine gels was investigated.

6.4.1.1. Experimental design.

4% gelatine solutions in distilled water were made from the standard and from "Hi Set" gelatines supplied by Leiner Gelatine Ltd., and from blends 855, 856, 858 and 859 supplied by Gelatine Products Ltd. These were set in glass tubes (10 mm diameter, 60mm tall). These were stoppered to avoid evaporative losses. The gels were examined after 1, 2, 4, 7 and 9 days storage at 4°C (80% r.h.), and the water holding capacity of each gel was determined in triplicate by the method of Jauregui et al., (1981) (Section 3.3.7.), and recorded as % expressible moisture, the lower this is, the better the W.H.C.

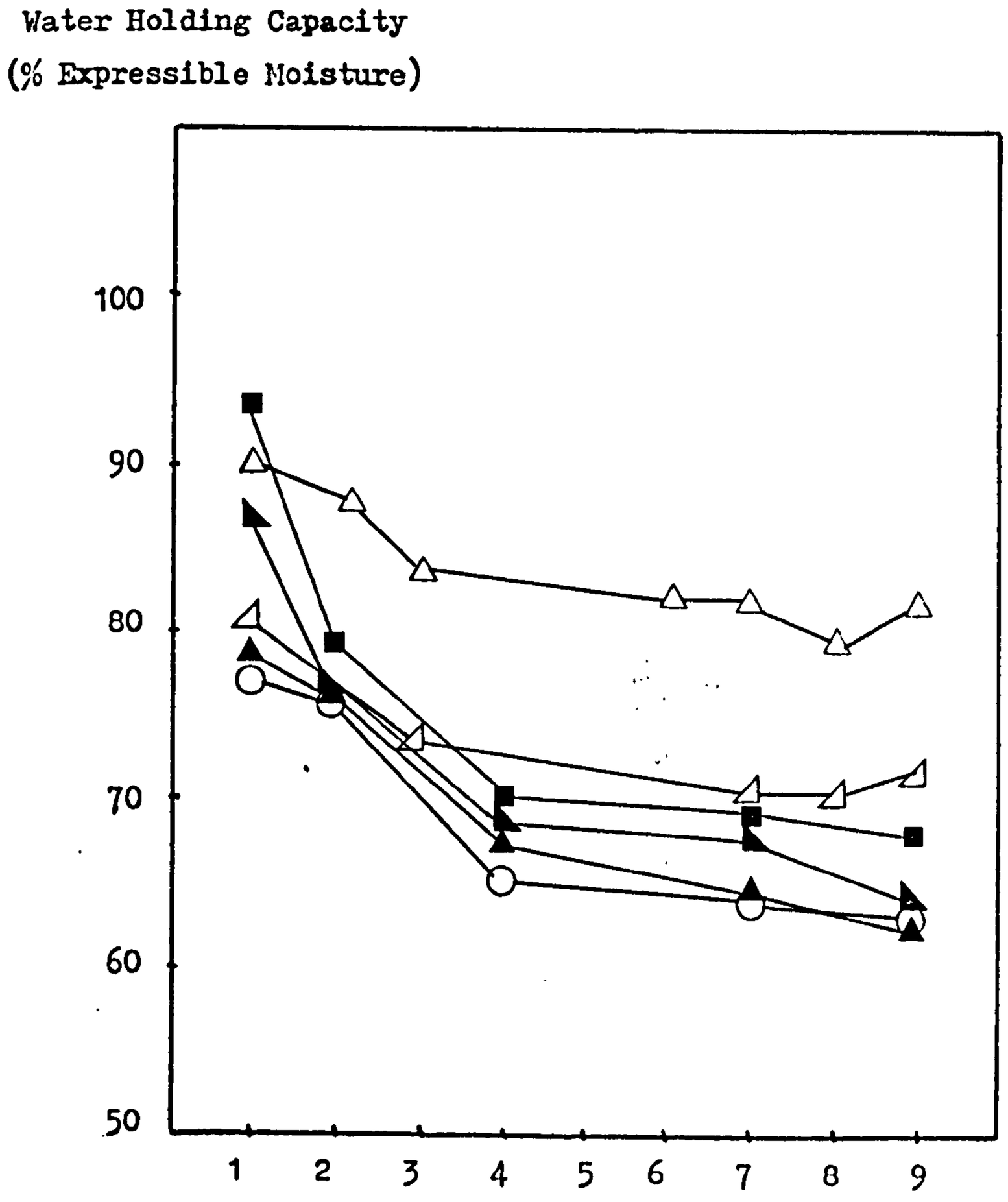
Pies were also manufactured from a single pastry mix and one meat filling block as described in Section 3.1, the age of the back fat and shoulder meat was 2 days. These pies were jellied using the gelatine under test, and examined (after 6, 8 and 15 days storage at 4°C, 80% r.h.) by a trained taste panel of six members.

6.4.1.2. Results and discussion.

Figure 6.13 shows the W.H.C. values (% expressible moisture), as a function of storage time for the gelatine gels under investigation. As can be seen the W.H.C. of all the gels improves (shown by a decrease in % expressible moisture) as storage proceeds. This is due to the internal

FIGURE 6.13

Water Holding Capacity (W.H.C) of the Standard gelatine jelly (▲–▲), Hiset gelatine jelly (■–■), gelatine blends 855 (○–○), 856 (◣–◣), 858 (◿–◿) and 859 (△–△) stored at 4°C.



reorganization of bonds and structure which occur as the gel matures (Pouradier, 1973).

The gelatine blend 859 had the poorest W.H.C. This could have been expected because it has a lower bloom strength than the other gels (see Appendix 3). Blend 858 had a better W.H.C. than blend 859 but not as good as the other gels. Unexpectedly, the "Hi Set" gel, with a 300 bloom strength (Appendix 3) did not have as good W.H.C. as blend 855, and was similar to 856. These had been modified by the supplier (Gelatine Products Ltd.) with carrageenan to help stop thermal reduction of gel strength during the jelly procedure. The "Hi Set" was developed by Leiners Ltd. to reduce loss of gel strength, by starting with an initially high gel strength in Boucher units (British Standard 1975; Marrs and Weir, 1977). Thus the carrageenan appears to improve the W.H.C. of gels 855 and 856 in some manner. The W.H.C. of 855 was not significantly different ($P > 0.05$) from that of the standard gelatine (Appendix 3), but was significantly different ($P < 0.05$) (Section 3.3.15) to the W.H.C. of the "Hi Set" gelatine.

From Table 6.5 it can be seen that all the gels showed separation from the meat filling and pastry after 6 days storage. This is due to moisture migration into the pastry from the jelly. Thus the jelly shrinks away from the pastry and filling, eventually becoming rubbery and unpalatable (Glicksman, 1979). By day 8 of storage although all the pastry was soft that used with the standard and 855 gelatins did not become unacceptably soft until day 15 of storage.

Table 6.5.

Organoleptic and visible assessments of pork pies jellied using different gelatine jellies, stored at 4°C, 80% r.h.

SAMPLE	COMMENTS DAY OF STORAGE 6	8	15
STANDARD	Slight jelly separation from meat. Good jelly levels.	Slight jelly separation. Pastry soft Similar to 855.	Soft pastry Marked jelly separation.
"Hi-Set"	As Standard.	Soft pastry.	As Standard.
855	As Standard. Good mouth feel.	Soft pastry. Not as soft as 856.	Jelly separation. Soft pastry.
856	Separation of jelly Soft pastry Good mouth feel.	Jelly separation from meat and jelly. Very soft pastry Chewy jelly.	As 855
858	Marked jelly, Separation, less than 859. Reasonable mouthfeel.	Marked jelly, separation. Pastry not as soft as 859.	Not assessed. Pastry very, very soft.
859	Marked jelly, Separation. Reasonable mouthfeel.	Marked separation. Pastry very soft.	As 858.

Thus it appeared that blend 855, and the standard gelatine, yielded gels with the best W.H.C., and also with the best performance in pies, as determined by taste panels (Table 6.5).

6.4.2. Use of ancillary agents in the control of moisture migration.

It was established that a jelly a_w of 0.55, in an atmosphere of 76% r.h. (Section 6.3.1.), would severely retard moisture migration; and that a jelly a_w of 0.84 (cf Figure 6.8) would retard moisture migration sufficiently to keep the moisture content of the brown pastry layer below 12% (and the texture value above 160g, Section 6.2) for a longer storage time than pies with jelly of 0.98 a_w :- the standard gelatine jelly a_w.

The standard gelatine jelly (supplied by Leiners Ltd.), had been shown to have the best W.H.C., and therefore the most effect on retarding moisture migration from the jelly into the pastry (Section 6.4.1.). This gelatine was thus used (in combination with humectants, pH, binders, alginate and cetyl alcohol) in an attempt to achieve a jelly a_w of 0.84, without the use of glycerol.

6.4.2.1. Experimental design.

4% gelatine solutions were used (4% is the gelatine level used by the pie manufacturers in their jelly), with the addition of one of the following: i) 50% (w/v) sodium tripolyphosphate; ii) 20% (w/v) sodium tripolyphosphate, iii) 5 and 10% (w/v) of a 1:1:1 mix of sodium tripolyphosphate: sodium citrate: sodium chloride, iv) 5% (w/v) carrageenan

v) 10-40% (v/v) ethanol, vi) 50% (v/v) propylene glycol.

The effect of pH on a_w was studied using 4% gelatine solutions with varying pH adjustments made using 10% HCl or 10% NaOH to give pH values of 6 to 12 (before heating the solution). All the solutions were cooled, and set in 30 mm x 30 mm dia. pots, lidded and stored at 4°C. After 24 hours the a_w of each gel was determined in triplicate.

The effect of alginate on the W.H.C. of the pie jelly was investigated using 4% gelatine solutions of the standard gelatin as used by the manufacturer, (or of blend 855). 1.5% sodium alginate (code Manncol DH, supplied by Alginate Industries Ltd.), was added to the gelatine solutions. The solutions were set in glass tubes 60 mm x 10 mm dia., and stoppered to avoid evaporation. Samples of each gel were taken for daily W.H.C. determinations (Section 3.3.7.).

Since cetyl alcohol is used in the meat industry as a edible barrier against moisture loss, a 50 ppm dispersion of cetyl alcohol in a 50% 50% glycerol: water solution (Anderson, 1961; Daniels, 1973) was brushed on the inside wall of the pie pastry before addition of the meat filling. Pastry from the same batch was used to make the untreated (control) pies. Both sets of pies were stored at 4%, and 80% r.h. Moisture and texture determinations of the brown pastry layer were recorded daily for the untreated and treated pies.

6.2.2. Results and discussion.

The effects that the humectants and binders had on the jelly a_w are recorded in Table 6.6. On the assumption that the desired jelly a_w was 0.84 (Section 6.4.1. 6.3.1.), only

Table 6.6.

Effect of some humectants and binders incorporation into 4% gelatine jellies on the a_w determinations of the jelly.

Sample	a_w	Comment
4% GELATINE JELLY	0.990	
PLUS:-		
5% (w/v) Sodium Tripoly Phosphate (STPP)	0.987	
20% (w/v) STPP.	-	No gel formed. Two phase system with gelatine on to formed.
5% (w/v) 1:1:1 Sodium citrate : STPP : Sodium chloride.	0.970	
10% (w/v) 1:1:1 Sodium citrate : STPP : Sodium chloride.	0.950	Unacceptable organoleptically.
5% (w/v) Carrageenan	0.98	
10% (v/v) Ethanol (replacing 10% water)	0.97	
20% (v/v) Ethanol	0.93	Unacceptable organoleptically.
30% (v/v) Ethanol	0.91	"
40% (v/v) Ethanol	0.90	"
50% (v/v) Propylene glycol.	0.80	"
50% 50:50 (v/v) Ethanol : propylene glycol.	0.56	"

50% (v/v) propylene glycol was effective. This humectant however imparted a bitter taste to the jelly, and thus was organoleptically unacceptable. All the other humectants were unsuitable, since they did not reduce the jelly a_w to 0.84.

Figure 6.14 shows the effect of pH on the jelly a_w: as can be seen, the higher the pH of the jelly in the range 7 to 12 the lower is its a_w. Even so an a_w of 0.84, was not achieved.

The incorporation of 1.5% alginate into 4% gelatine solutions of the standard gelatine, or of blend 855, (Figure 6.15) results in an improved W.H.C. In comparison with those of the standard and blend without alginate, the improvement in W.H.C. was found to be significant ($P < 0.05$) (Section 3.3.15). However attempts to increase the level of alginate in the jelly (in order to increase its W.H.C.) were unsuccessful due to the formation of alginate lumps which could not be easily dispersed.

The effect of the use of acetyl alcohol moisture barrier on the moisture content of the pastry (brown layer) is shown in Figure 6.16. There was found to be no significant difference between the moisture content of the pastry of the treated and untreated pies. Thus cetyl alcohol had no effect on pastry moisture content. This was also found for the texture values obtained (Figure 6.17).

From the results obtained it could be concluded that cetyl alcohol, when used in this manner, has no effect on moisture migration in pork pies, and overall, the use of various ancillary factors to reduce the moisture losses from the jelly in pork pies was unsuccessful.

FIGURE 6.14

Effect of pH on the a_w of 4% gelatine gels stored at 4°C and 80%r.h.

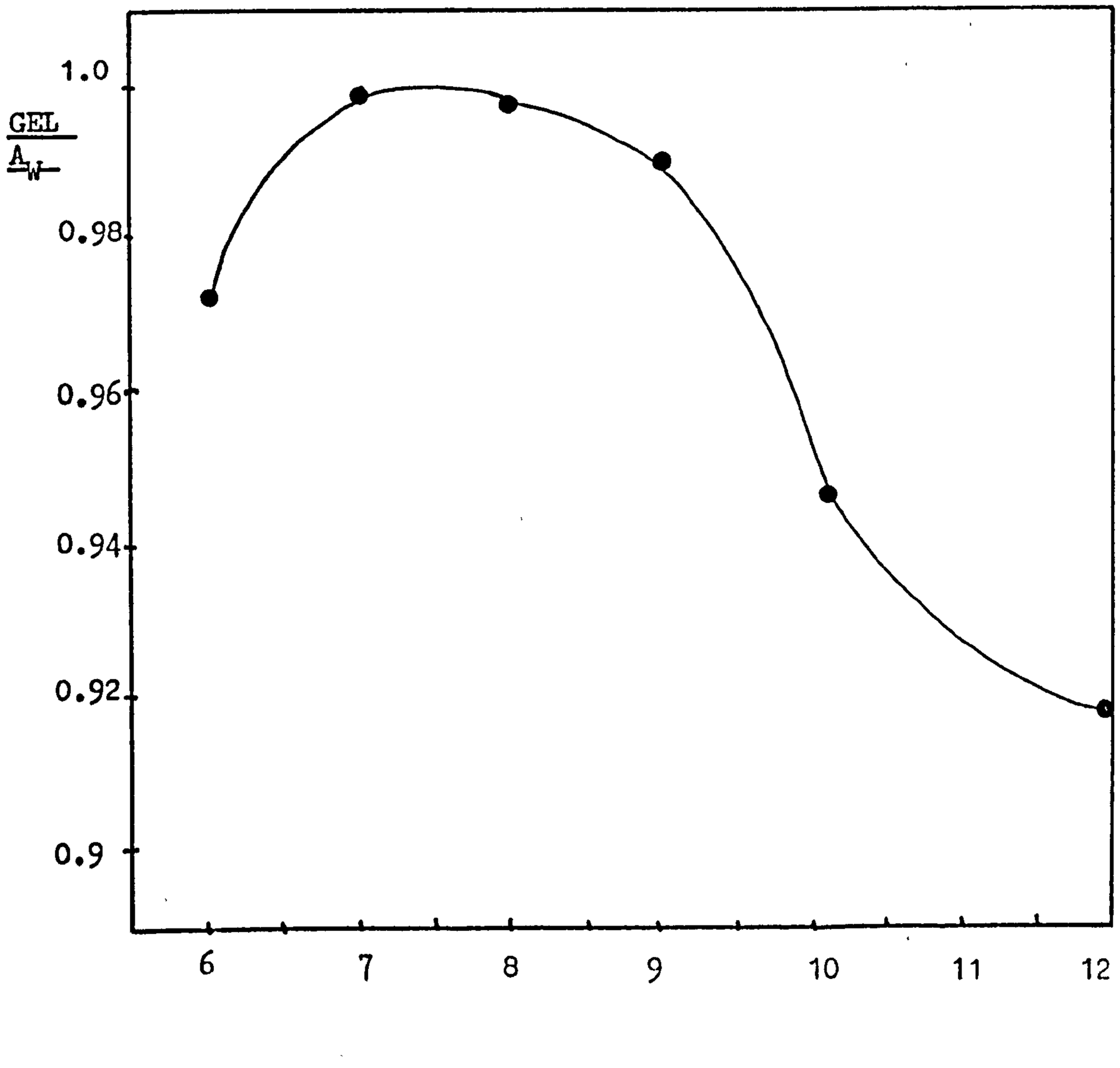
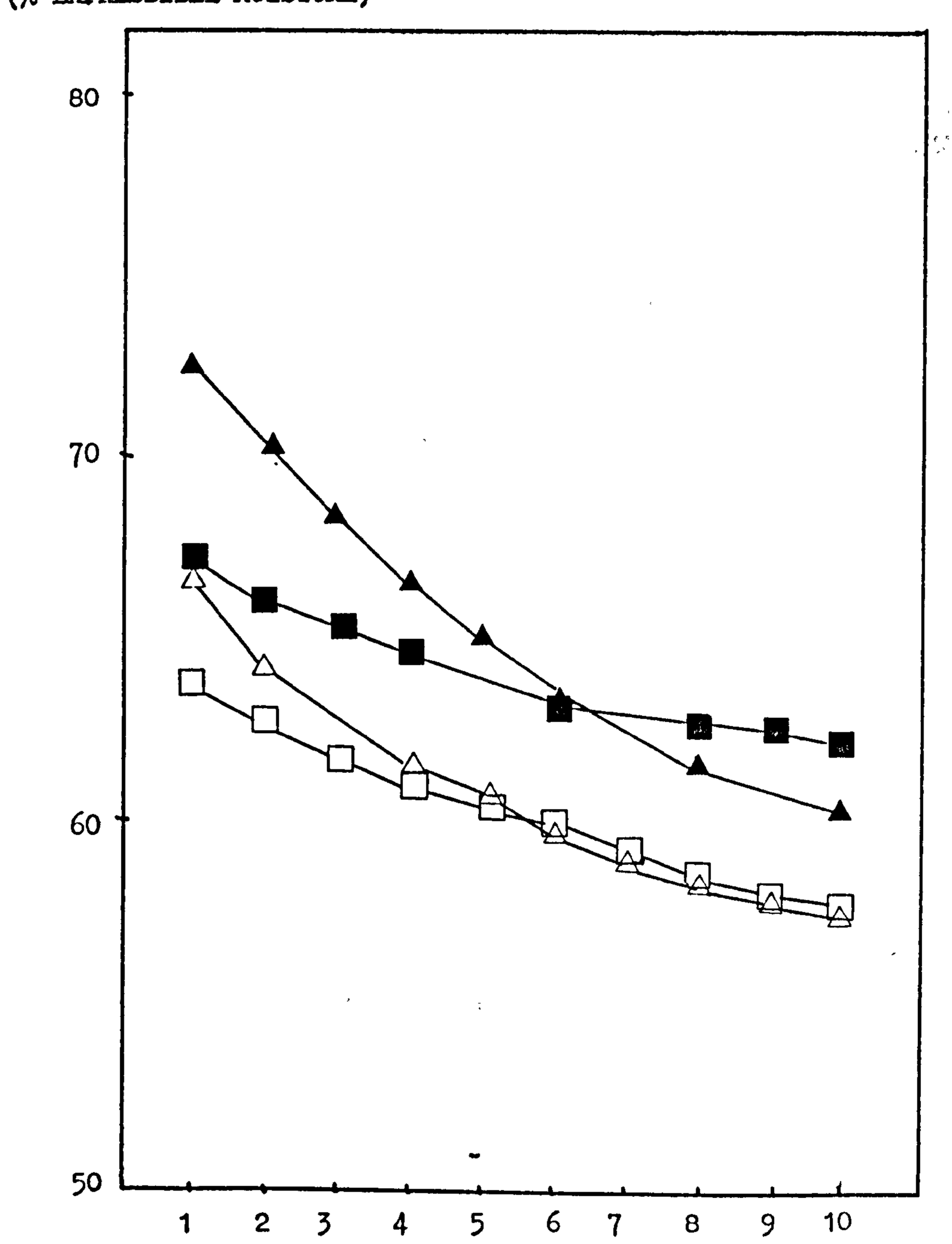


FIGURE 6.15

The influence of 1.5% alginate on the WHC of the Standard gelatine gel, and the gelatine blend 855.



(▲—▲) is the gelatine blend 855

(△—△) is the gelatine blend 855 with 1.5% alginate.

(■—■) is the 'standard' gelatine

(□—□) is the 'standard' gelatine with 1.5% alginate.

FIGURE 6.16

Effect of Cetyl Alcohol as a moisture barrier in pork pies, on the brown pastry layer moisture content of pies with the cetyl alcohol film (□-□), and pies without the cetyl alcohol film (■-■), stored at 4°C and 80%r.h.

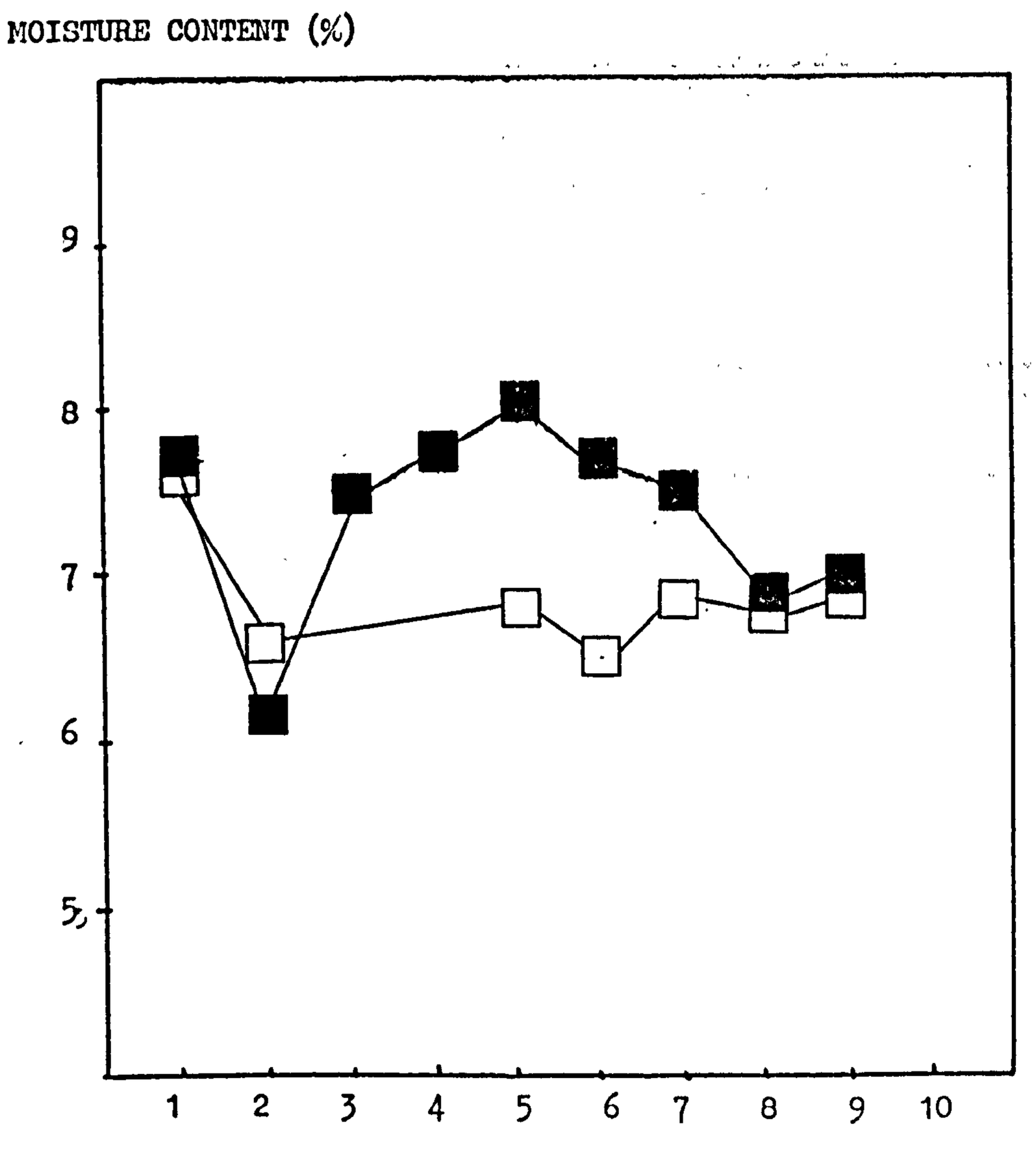
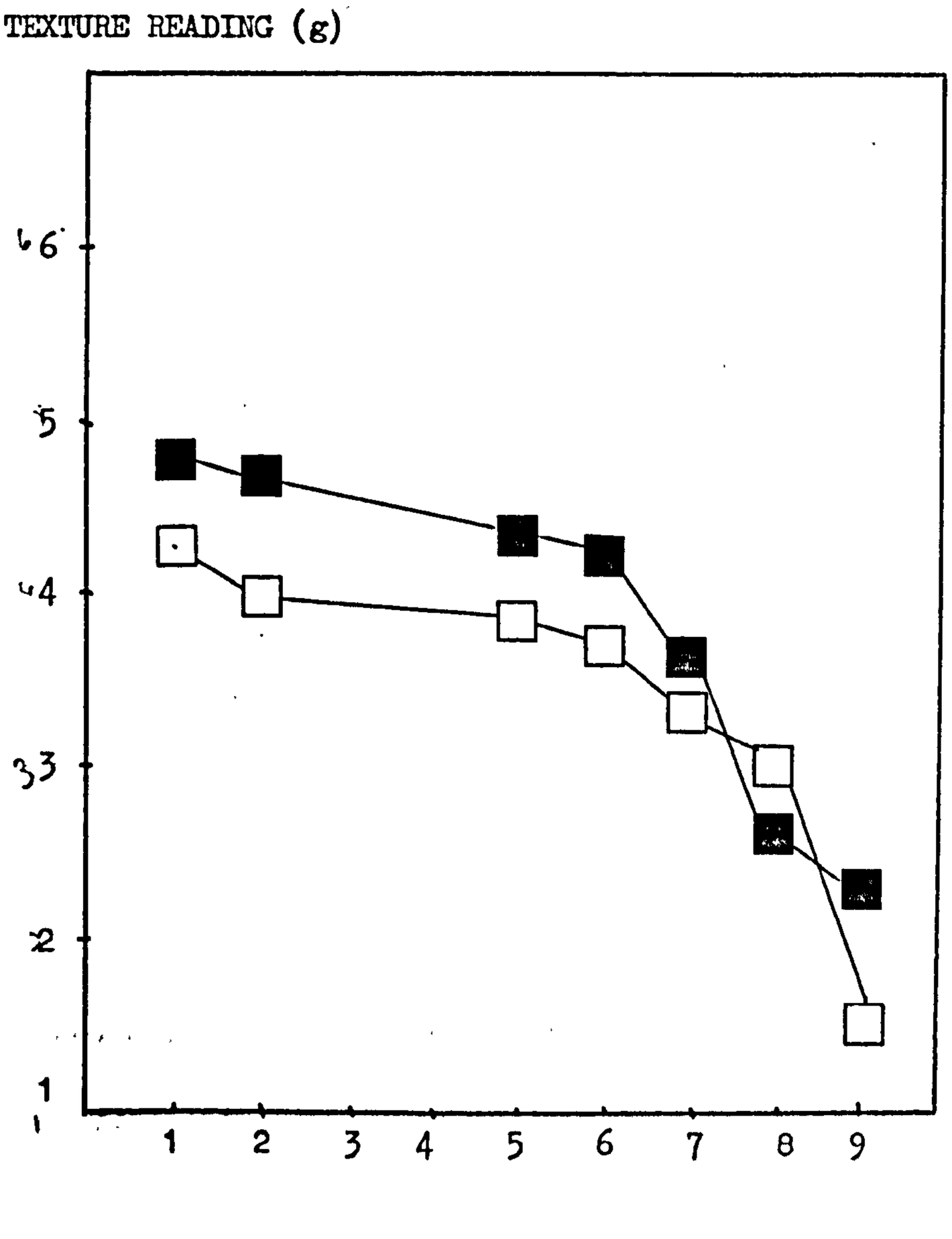


FIGURE 6.17

Effect of cetyl alcohol as a moisture barrier in pork pies on the texture of the brown pastry layer of pies treated with cetyl alcohol (□-□), and those untreated with cetyl alcohol (■-■), stored at 4°C and 80% r.h.



6.5. The role of lactein-gelatine cogels in the control of moisture migration in pork pies.

Work described in the previous Sections (6.1 to 6.3) had shown that lowering the jelly a_w or improving its water holding capacity (W.H.C.) could reduce or prevent moisture migration within the pie; and some of the possible additives which might have had the desired effect were considered.

Lactein appeared to be another possible additive. Its manufacturers claim that "it ensures effective binding of water ...". Lactein is available in two forms, Lactein 27 and Lactein 75, which contain 27 and 75% protein respectively. (supplied by Dairy Crest, M.M.B.)
It is manufactured from "hard pressed" cheese whey, by centrifugation, pasturization and ultrafiltration. Lactein 75 also undergoes diafiltration to remove lactose and thus increase the protein concentration (Marshall, 1982).

Lactein 27 contains approximately 27% protein, 58% lactose, 7% ash and 2% fat. Lactein 75 contains 75% protein and 6% fat, it is termed a whey protein concentrate (W.P.C.) and Lactein 27, a whey protein isolate (W.P.I.) according to Marshall (1982).

6.5.1. Effect of temperature and pH on whey protein (lactein) gels.

Whey protein (α-lactalbumin, β-lactoglobulin and serum albumin) have the ability to form heat-induced irreversible gels, and can be used to stabilize foams and emulsions (Philips, 1977). This ability is known to be dependant on pH and on protein concentration, which vary according to the mode of manufacture of the whey protein

(Morr, 1979; 1982). It was decided to assess how pH, temperature and protein concentration affected the ability of whey proteins (Lactein 27 and 75) to form gels.

6.5.1.1. Experimental design.

Solutions of 8, 10 and 12% protein (w/v) were made from Lactein 27 by slowly mixing it with water, and allowing the mixture to stand for 1 hour. The initial pH was adjusted to 6 using 10% NaOH or 10% HCl. 1 ml solutions were pipetted into 10 mm x 150 mm test tubes in water baths heated to 60° - 90°C. After an initial 30 second "heating up" period (during which the tubes were gently agitated), 6 tubes were removed after a further 30 seconds and, thereafter, at 1 minute intervals. Gelation was regarded as the condition when the tube could be inverted without loss of the contents. Coagulation was regarded as the condition when the tube contents increased in viscosity, but were lost on inversion of the tube.

The experimental design was repeated, using pH values of 4.5 to 10, with 12% protein (w/v) solutions of Lactein 27. Similarly 12% protein (w/v) solutions of lactein 75 were heated to temperatures of 60-80°C at pH ranging from 6 to 8.

6.5.1.2. Results and discussions.

The effects of temperature on the gelation of Lactein 27 at pH 6 are shown in Table 6.7. It is seen that a minimum of 10% protein was necessary to allow coagulation (Gelation did not occur) this only occurring at 90°C. Heat-induced gelation did occur, however, with Lactein 27 (12% protein w/v)

Table 6.7.

Gelation and Coagulation of Whey Proteins at

8, 10 and 12% protein concentrations of Lactein 27 at

temperatures of 60°C to 90°C, at pH 6.

	% PROTEIN (w/v)		
TEMPERATURE 0°C	8	10	12
60	-	-	-
70	-	-	C
80	-	-	C
90	-	C	C

- : NO GELATION, NO COAGULATION.

C : NO GELATION, BUT COAGULATION OCCURRED.

Table 6.8.

Gelation of Lactein 27 (12% Protein) at 70°C

and various pHs.

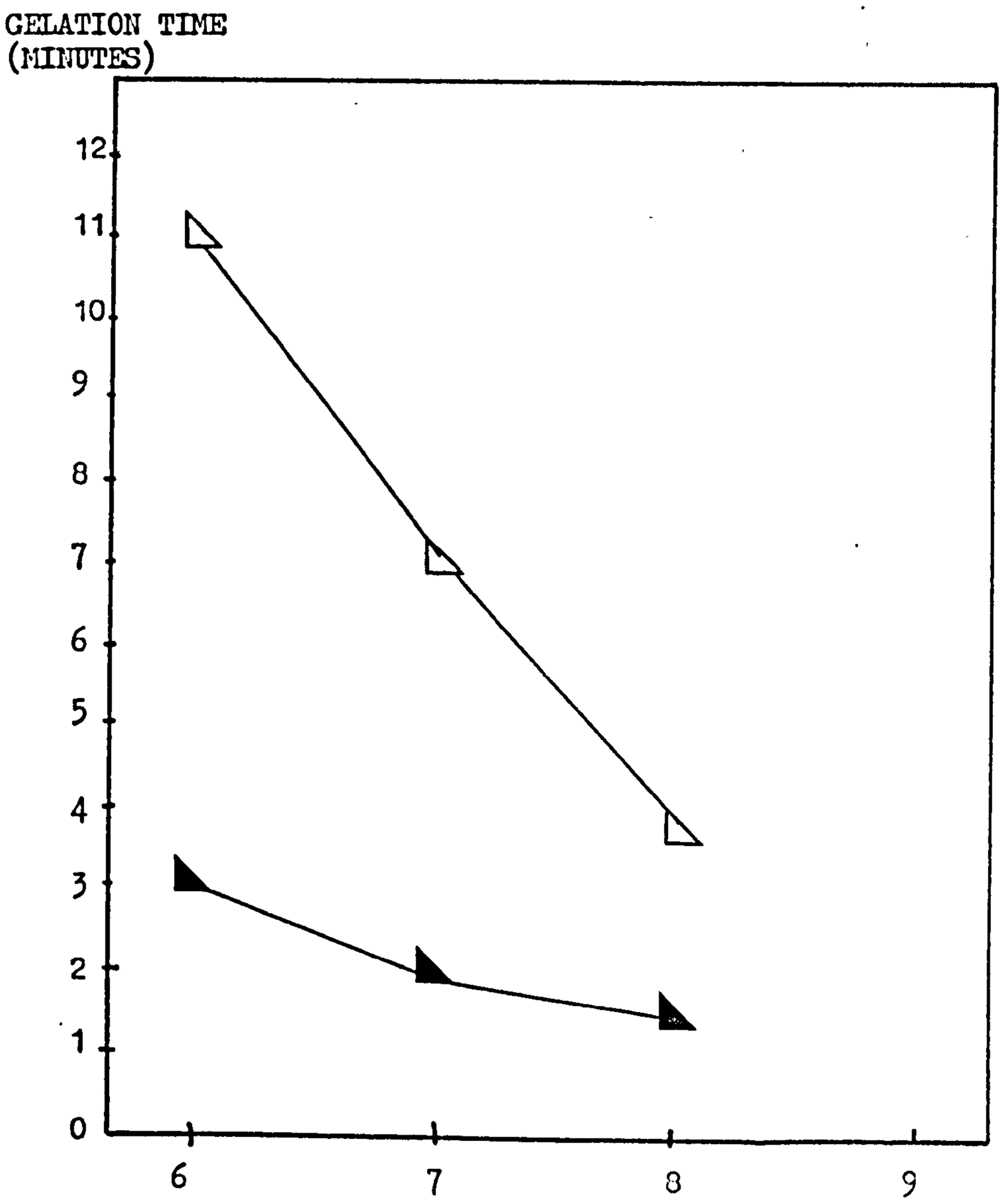
pH	GELATION
4.5	-
6	-
7	+
8	+
9	+
10.5	+

- : NO GELATION

+ : GELATION

FIGURE 6.18

Effect of pH and temperature on the gelation time of 12% protein concentration of Lactein 75.



Where the solutions were heated to 70°C (◺—◺), and 80°C (◣—◣) in water baths.

at pH 7 and above, when temperatures of 70°C were used (Table 6.8).

Welsby et al., (1982) claimed that gelation of whey proteins occurred at 12% protein (w/v), concentration pH 4.5, and temperatures of 60-70°C. Whereas, in the present work, no gelation of Lactein 27 was observed to occur below pH 7 - only coagulation. Green (1982) (reporting on the work by Hillier at N.I.R.D.), stated that in water gelation only occurred above pH 7. This is because, below pH 7, coagulation occurs, due to proximity to the isoelectric point of β-lactoglobulin (pH 5.2). It is possible, therefore that Welsby et al., (1982) observed coagulation rather than a gelation in their whey proteins.

Figure 6.18 illustrates the effect of pH and temperature on the gelation of Lactein 75 (12% protein). Like Lactein 27, this whey protein did not form a gel at pH 6, and at 60°C. It did gel at 70°C and 80°C; and raising the pH of the system from 6 to 8 resulted in more rapid gelation. Lactein 75 is thus pH-sensitive, with regard to gelation. This agrees with the findings of Burgess and Kelly (1979). Hillier and Cheeseman (1979) also found that, as the pH of the system was raised, the gelation time decreased, and that the effect of pH was less as the temperature increased. This observation held true for the relationship between gelation time, pH and temperature for Lactein 75.

The best gelation was achieved using 12% protein concentrations at pH 7-8 heated to 70°C. Higher temperatures resulted in rapid gelation. This was not desirable since such gelation would block the pie jellying system.

6.5.2. Establishment of a lactein-gelatine cogel.

It seemed that there might be some benefit in establishing a cogel of Lactein and gelatine incorporating the claimed water binding properties of the former, with the gel strength of the latter. A dispersion of lactein in gelatine was unsuccessful since the two compounds were clearly visible as two phases.

Lactein produces gels which are heat-induced (by heating to above 60°C). While gelatine produces gels by a "cold set process" (setting point ≏ 32°C). A method was thus sought to establish a gel using both lactein and gelatine.

6,6.2,1. Experimental design.

Six methods were used in trying to establish a homogeneous gel of Lactein and gelatine.

Method a). A 13% protein solution in water of Lactein 27 was made (using half the final volume of water). It was left to stand for 45 minutes. The pH was adjusted to 9 using 10% NaOH solution. The gelatine representing a final concentration of 4% was dissolved in the remaining water, heated to 90°C and cooled to 45°C, the Lactein solution mixed in, and the mix heated to 70°C.

Method b). Lactein (13% protein w/v) and gelatine (4% w/v) were mixed together in water, and the pH adjusted to 9 using 10% NaOH solution. The solution was heated to 70°C.

Method c). As method a, but the gelatine solution cooled to 50°C.

Method d). As method a, using 12% protein solution of Lactein 75.

Method e). Using Lactein 75 (to give a 12% protein) and method a, adjusting the pH to 8 with 10% NaOH solution.

Method f). Gelatine (4%) was dissolved in 25% of the final volume of water. Lactein 75 (12% protein concentration) was dissolved in the remaining water, and left to stand for 45 min., when the pH was adjusted to values of 7.5 - 9.

The gelatine solution was heated to 90°C, then cooled to 45°C. The lactein solution was heated to 45°C, the gelatine solution was mixed in, and the mixture then heated to 65°C.

6.5.2.2. Results and discussion.

The observations made during the establishment of the cogels produced by the six different methods are summerized in Table 6.9. It was noted that, when the gelatine was added to the lactein solution at temperatures of 50°C and above, the lactein formed a visible precipitate. If the gelatine was added at a temperature below 40°C, it underwent gelation, and so was visible as agglomerations in the lactein gel network. thus gelatine must be added to the lactein solution at temperatures of 45-50°C.

Heating the combined solutions (Methods a, c, d, e, and f) was important. Heating above 80°C resulted in a two phase system gel. pH affected the gelation time, (Rapid gelation occurring at pH 9, a feature which would cause blockages in the pie jellying system). Gels formed using

Table 6.9.

Observations made during cogel formation

by different methods.

Method	Observations
a.	The gel formed with time upon standing at Room temperature. It was firm but opaque and creamy in appearance. The two phases were visible but not separating out.
b.	The gel was formed very quickly, with a soft texture, it was opaque in appearance.
c.	The gel again formed with time. It was creamy and opaque. The protein had been precipitated out, but was held in the gel matrix by the gelatine.
d.	The gel formed very quickly but was homogeneous. It was cream and opaque in appearance.
e.	The gel formed over time remaining fluid at 50°C, but gelling at 4°C. The gel was homogeneous, creamy and opaque with an a_w of 0.96.
f.	At pH 9 a heat-induced gel formed almost immediately whilst at pH 8 the lactein underwent some precipitation and the

continued/....

Table 6.9 continued/....

Method	Observations
f	resultant gel was opaque and granular in appearance. The best gelation occurred at pH of less than 8. The product was homogeneous and opaque in appearance a_w of 0.95.

pH values below 8 were suitable. In method F, pH values of 8 or 9 were unsuitable, since rapid gelation occurred. pH 7.5 was acceptable however.

Method F yielded the most satisfactory cogels, at pH 7.5. It was thus employed as the standard procedure for making cogels in the remainder of the study.

6.5.3. Effect of pH and temperature on gelation of cogels of Lactein 75 and gelatine.

Due to the low density of Lactein 27 a large volume was required to make a 12% (w/v) protein solution. It was thus discarded, and only the dense Lactein 75 was used in the remainder of this study.

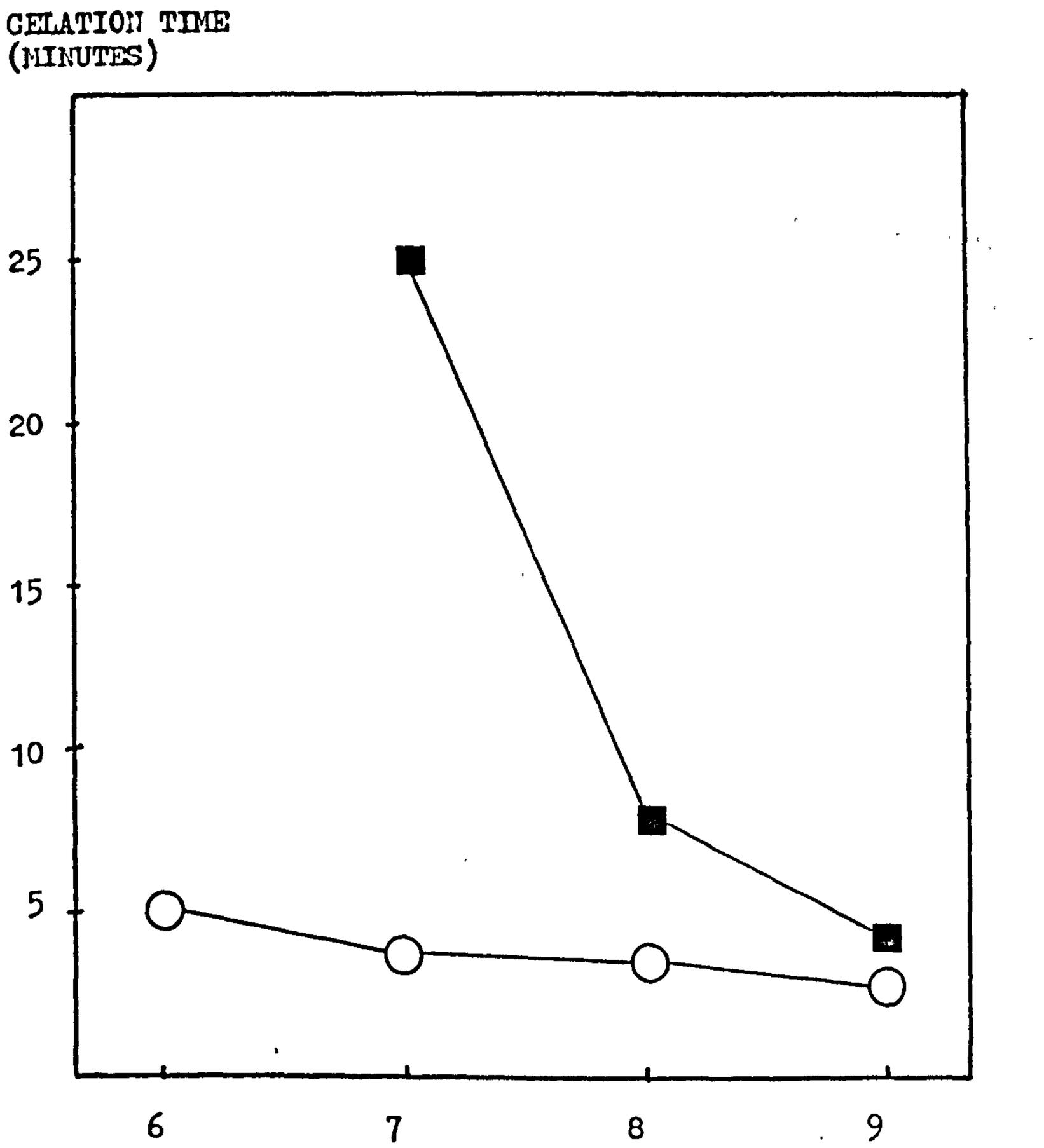
6.5.3.1. Experimental design.

Gelatine(4% w/v) was dissolved in 25% of the final volume of water. The lactein (12% protein w/v) was dissolved into the remaining water, and left to stand for 45 minutes. The pH was adjusted to 6 using 10% NaOH solution. The gelatine was heated to 90°C then cooled to 45°C. Lactein was heated to 45°C, gelatine added, and the mixture heated to 60°C in 10 mm x 150 mm test tubes in a water bath. After a 30 second "warm up" period, 6 tubes were removed at 1 minute intervals. Gelation or coagulation in the tubes was determined as described in Section 6.5.1.1.

This experimental design was repeated, adjusting the lactein solution to pH values of 7, 8 and 9 and heating the combined solutions to 70°C and 80°C in water baths.

FIGURE 6.19

Effect of pH and temperature on the gelation time of Lactein 75 (12% protein concentration) and 4% gelatine cogels.



Where the cogel solution was heated to 70°C (■—■) and to 80°C (○—○).

6.5.3.2. Results and discussions.

The effect of pH and temperature on the gelation time for cogels is illustrated in Figure 6.19. There was no heat-induced gelation after heating at 60°C at any pH. Upon cooling the gelatin formed a gel.

After heating at 70°C, there was no heat-induced gelation at pH 6, but this did occur at pH values between 7 and 9, with rapid gelation at pHs 8 and 9. Heating at 80°C resulted in heat-induced gelation at all test pHs, which was rapid in all cases.

What is required is a relatively slow heat-induced gelation gel and the gelatineforming a firm set gel on cooling. The cogel which met these requirements was that in which the lactein solution had a pH of 7.5, and in which the two solutions had been heated together to 70°C. Thus, with these final adjustments to method F (Section 6.5.3.2.) a method for cogel manufacture was established. Some of its properties in pork pies were examined in the following Section (6.5.4.).

6.5.4. The water holding capacity (W.H.C.) of the cogel and its use in pork pies in comparison with the 'standard' gelatine jelly.

The final stage of the cogel development was to compare its physical properties with those of the standard gelatin jelly.

6.5.4.1. Experimental design.

4% gelatinesolution was made and pipetted into 10 mm x 700 mm glass flat bottom tubes, stoppered to prevent evaporative losses. Further sets of tubes were prepared using the cogel and lactein 75 (12% w/v protein concentration). All the tubes were stored at 4°C. The W.H.C. of each set was determined, in triplicate, by the method described in Section 3.3.7. The results were assessed as % expressible moisture (the lower the % expressible moisture the higher the W.H.C. of the gel).

The distribution of the cogel and gelatine gels was compared in pork pies which had been manufactured as in Section 3.1. After baking, each pie was separated into two batches. The whole pastry and meat filling were detached, each weighed, and then the pies were reassembled. One batch was jellied with the cogel, the other with the gelatine jelly. After cooling at 4°C for 2 hours, each pie was dissected into whole pastry, jelly and meat filling; and each weighed. The percentage distribution of cogel or of gelatinejelly in the whole pastry and meat filling was determined by the increase in weight of these components.

A single pie batch (Section 3.1) was made, and after baking half jellied with the cogel, the other with gelatire jelly. The pies were stored at 4°C and 80% r.h. Daily moisture content and texture determinations of the brown pastry layer were performed. Organoleptic assessment of the pastry was also carried out.

6.5.4.2. Results and discussion.

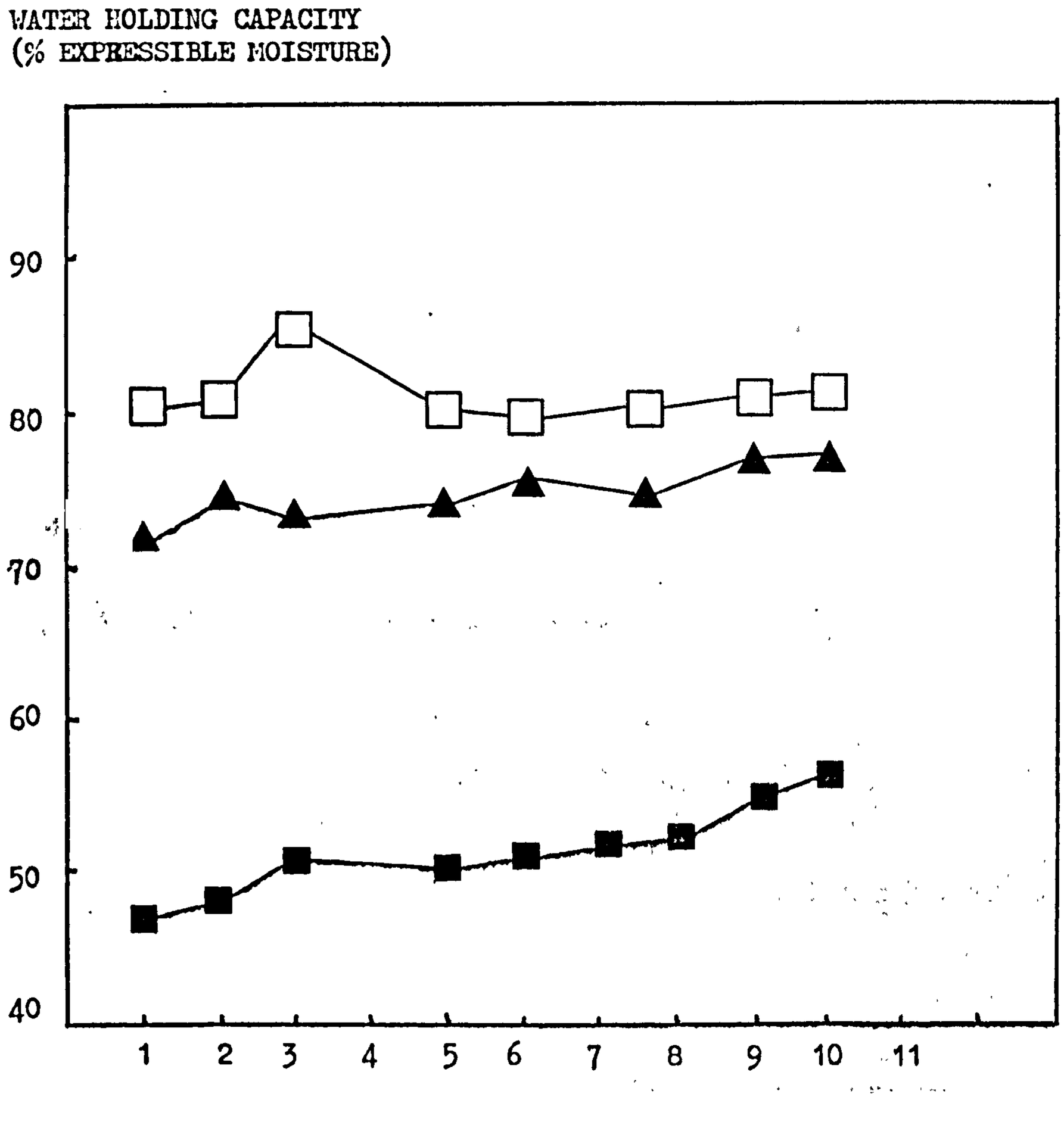
The percentage expressible moisture of the gels are presented in Figure 6.20. Significant differences ($P < 0.05$, Section 3.3.15) between the cogel and gelatinejelly, and between the gelatinejelly and Lactein 75 gel were established. The cogel had lowest percentage expressible moisture and the Lactein 75 gel the highest values. Thus the cogel had the best W.H.C., the Lactein 75 the poorest W.H.C., with that of the gelatinejelly being intermediate between the two.

The better W.H.C. of the cogel may be due to the fact that lactein forms a heat-induced gel which entraps moisture/water in its structure. In order to form gels, there needs to be a balance between protein - protein and protein - solvent interactions. If the balance is swung towards protein - protein interactions (by alkali and salt), strong local interactions between proteins will occur, resulting in dense regions in the gel network. These in turn 'squeeze' out the "free water" entrapped in the gel matrix, thus reducing its W.H.C. Gelatine probably acts by keeping the protein - protein balance correct, preventing local interactions. It also will bind water that is not involved in the lactein network. Thus there are two modes of entrapping water, which could account for the better W.H.C. of the cogel compared to Lactein 75 and gelatinegels (Figure 6.2Ø).

The distribution of the cogel and of the gelatinejelly are presented in Tables 6.10 and 6.11 respectively. It can be seen from the tables that, with both gels, most of

FIGURE 6.20

The Water Holding Capacity (WHC) of the 'standard' gelatine gel, the Lactein-gelatine cogel and of the Lactein 75 gel.



Where (□–□) are the values for the Lactein 75 gel,

(▲–▲) for the standard gelatine gel,

(■–■) for the Lactein-gelatine gogel.

Table 6.10.

Distribution of cogel in 11oz pork pies.

Pre-Jellying Weight (g)		After Jellying weight (g)			Total jelly added	% Distribution of Jelly.		
meat filling	entire pastry case	Meat	entire pastry case	Visible jelly		In meat filling	pastry case	Visible
104	132	111	140	15	38	23.8	27.3	48.8
112	129	116	135	14	35	14.7	24.7	60.0
100	126	107	131	12	29	16.1	25.3	58.5
112	133	120	139	16	40	25.9	19.8	54.2
113	126	117	131	17	40	17.0	19.0	63.0
113	128	117	133	14	31	16.1	21.9	61.8
114	134	121	141	20	27	20.6	19.4	59.8
				Average distribution.		19.3	22.4	58.0

Table 6.11.

Distribution of gelatin jelly (4%) in 11oz pork pies.

Pre-Jellying Weight (g)		After Jellying weight (g)			Total jelly added	% Distribution of Jelly.		
meat filling	entire pastry case	Meat	Entire pastry case	Visible jelly		In meat filling	pastry case	Visible
106	166	111	181	19	39	12.8	38.5	48.5
108	160	112	174	20	38	10.5	36.8	52.6
107	166	111	182	20	38	12.2	36.8	50.0
110	174	115	187	19	34	14,7	38.2	47.1
			Average distribution.					
						12.5	37.5	49.5

the gel remains as visible jelly, and that more is present in the pastry than in the meat filling. Less of the cogel entered the pastry walls than the gelatin jelly (Tables 6.10 and 6.11), this could be important since, to achieve the same level of pastry softening (due to moisture migration from the jelly) more moisture would have to migrate from the visible cogel jelly. In addition less moisture is available in this cogel (higher W.H.C.) to migrate. Thus it follows that the moisture content of the pastry will remain below the critical level of 12% (Section 6.2) for far longer, leading to an extended shelf life when compared to products containing the standard gelatine jelly.

At all storage times the moisture content of the pastry (brown layer) of the pies containing cogel were significantly less than in the pastry of the gelatine jelly pies (Figure 6.21). The brown pastry layer of the standard jelly pies reached the critical 12% moisture content after 9 days storage, while in the brown pastry layer of the cogel pies the value was only reached after 15 days storage (Figure 6.21). The results suggested that the lower moisture content of the pastry, allied to the greater W.H.C. of the jelly in the pies containing the cogel, would lead to an extension of shelf life of about 40% at 4°C.

Figure 6.22 illustrates that in the pie containing cogel, the brown pastry layer retained its texture (crispness) for longer than those containing only 4% gelatin. A texture value of 160g for the brown pastry layer has been deemed as the value below which the pastry is unacceptable (Section 6.2). This value of 160g was

FIGURE 6.21

Change in the Moisture Content of the brown pastry layer of pies containing the standard gelatine jelly (▲–▲) and pies containing the Lactein-gelatine cogel (■–■) stored at 4°C and 80% r.h.

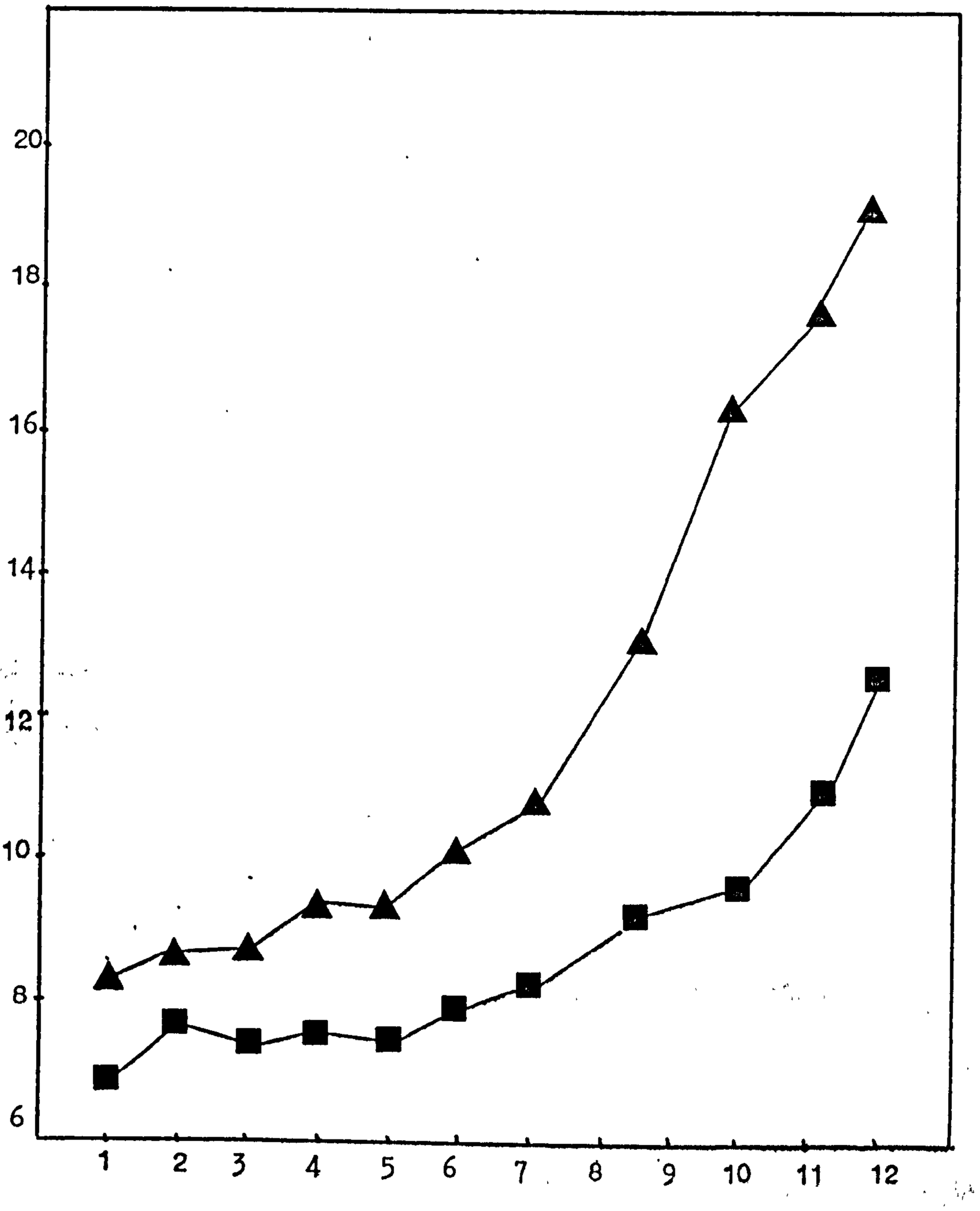
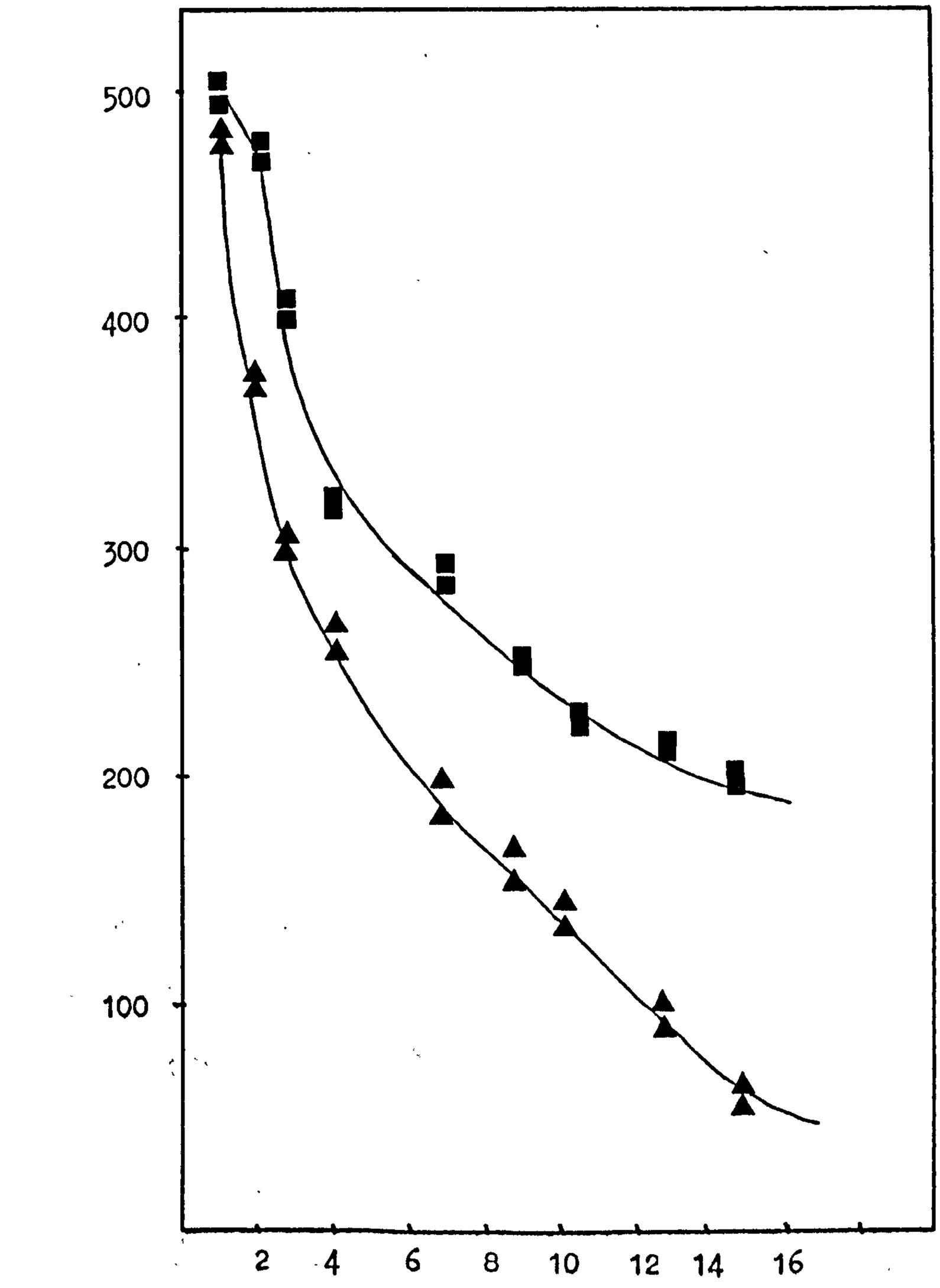


FIGURE 6.22

Change in Texture Readings of the brown pastry layer of pies containing the standard gelatine jelly (▲—▲) and pies containing the Lactein-gelatine cogel (■—■), stored at 4°C and 80%r.h.



reached by the brown pastry layer of the pies containing cogel in 15/16 days, while in those using the standard jelly the brown pastry layer reached this value by 8/9 days of storage indicating that the cogel in pies leads to an extension in pie shelf life at 4°C.

In respect of the organoleptic assessment, 19 out of 20 panelists did not notice the cogel in the pie. However it should be noted that the cogel appears to merge in with the white/cream pastry layer making the pastry appear slightly thicker than is usual.

To summarize the results, it was found that the cogel had a better W.H.C. than the standard gelatine gel. When used in pies, less of the cogel was found in the pastry. Taken together, these findings indicate that using the cogel should result in less moisture migration from the jelly into the pastry and lead to a longer shelf life. This hypothesis was proved correct, as the use of the cogel in pies kept the brown pastry layer moisture content below 12% and its texture above 160 for a significantly longer period than the standard gelatine jelly. Therefore use of the cogel in pork pies instead of gelatine jelly results in an extension of shelf life (as determined by texture - crispness of the pastry).

6.5.5. <u>Potential of lactein as an addition/alternative to the gelatin jelly.</u>

The work outlined in Sections 6.5.1., 6.5.2. and 6.5.3. indicated how a method for preparing lactein/gelatine cogels for incorporation into pies could be developed.

The procedure is not absolute, because, the type of cogel produced can be expected to vary for several reasons:-

1) Type of cheese whey used. As the whey protein concentrates (W.P.C.) from cottage cheese whey produce stronger gels than those from cheddar cheese whey (McDonough et al., 1974).

2) Method of manufacture. The W.P.C.s produced by dialysis are different in appearance and texture from those produced by ultrafiltration. This is probably due to different protein (ion and protein) lactose ratios in the W.P.C.s (Morr, 1979).

3) Lipid content. If the mode of manufacture allows the whey lipids to be concentrated along with the proteins, then a reduction in gel strength will occur (Sternberg et al., 1976) due to the lipids competing for the hydrogen bonding sites involved in protein - protein association i.e. gelation (Burgess and Kelly, 1979). The lipids can be removed by ion exchange celluloses (Palmer, 1977) or by diafiltration.

4) Water quality. Since the presence of calcium ions influences gel strength (Schmidt et al., 1979) W.P.C.'s produce gels of increasing strength as the Ca^{2+} concentration increases to 11 mM (but at levels higher than 14 mM reductions in gel strengths are observed (Schmidt et al., 1979).

For the cogel to be use in a commercial operation it must be capable of withstanding abuse without loss of its functional properties. From Figure 6.19 it is seen that cogels can be produced by heating to 70°C for 15 min at

pH 7, followed by cooling. Thus the gelatin/W.P.C. mixture could be held at this temperature and pH in heated tanks prior to jellying the pies. Use of more alkaline pH's or higher temperatures leads to rapid gelation; and thus holding of the jelly in the system would not be possible. However if the temperature and pH are closely monitored it would be possible to use the cogel in pies in place of the standard gelatine jelly, thus extending the pastry's shelf life.

A problem however with the cogel is its opacity. Fortunately it blends in with the inner pastry layer and consumer testing may well indicate that it is acceptable. If it is not acceptable it could be alleviated by inhibiting the meat shrinkage that occurs on cooking, as this results in a smaller gap between the pastry and meat. Thus less jelly is required. A further advantage of using less gel is that less moisture is available to migrate from the jelly into the pastry resulting in a further extension of shelf life.

The use and performance of the lactein gelatin cogel in pork pies extends the pastry shelf life. What must now be decided is can it be used in pies, or does the opacity mean this problem has to be solved before its full potential can be realized.

Chapter 7.

General Discussion.

Chapter 7. General Discussion.

The aim of this thesis was to establish the changes which occur during the storage of pork pies. From the initial investigations, two factors appeared to have a predominant influence on the shelf life of the pies, namely lipid oxidation and moisture migration. Factors of less importance were colour changes and protein cross-linkages, pH had little effect on pie shelf life.

Lipid oxidation is the reaction between oxygen and unsaturated fatty acid moieties, which causes the formation of many end products, including aldehydes, ketones, alcohols and short chain fatty acids. It is the latter, with the aldehydes which are responsible for the off-flavours and odours that make foodstuffs unacceptable to consumers (Labuza, 1971). Lipid oxidation can be influenced by a number of factors (discussed in Section 2.3.3.), which included haem and non-haem iron (Tappel, 1961; Eriksson et al., 1971; Eriksson and Vallentin, 1973) and other catalysts present in the product e.g. enzymes (Veldink et al., 1977); tocopherols (Labuza, 1971; Cillard et al., 1980 a,b) and plant constituents (Metha and Sesharidi 1959; Jeney et al., 1960; Herrmann, 1976; Hudson and Mahgoub, 1980).

Additional factors influencing rancidity development were found during the course of the present investigation.

Thus the time after slaughter of the animals at which the shoulder meat and back fat (components of the pies) are used is important for their shelf life. The longer the time after slaughter, the shorter is the time until a Thiobarbituric acid

(TBA) value of 5 is attained. This value corresponds to the upper limit of acceptability. The TBA value is a measure of the oxidized lipids in the meat filling. These seep out onto the baking tray during cooking, and subsequently re-enter the pastry of the pie, during the cooling phase of manufacture, thus shortening the shelf life of the whole pie.

Oxidation of the lipids in the back fat, if these are used some days post mortem, is affected markedly by the temperature of storage, further deterioration being rapid at 20°C. Thus use of "old" back fat in the pie meat filling would markedly reduce the pie shelf life.

Cooking accelerated rancidity development in the meat filling of the pies (and therefore in the whole pork pie); and also in other products such as pork burgers. This acceleration of rancidity in both pies and burgers was not so rapid however, as to be termed "warmed over flavour" (WOF, Igene et al., 1979a,b).

According to Gandermer et al., (1983) cooking changes the lipid profiles, with a particular loss of C18:2 (linoleic acid) and a general reduction in the concentration of all polyunsaturated fatty acids (PUFA's). Unfortunately these workers did not examine the lipid changes in fatty acids of chain length less than C14:0, otherwise they might have noted a concomitant increase in short chain fatty acids. The latter were detected when the lipid changes were studied in more detail (Sections 5.5.1., 5.5.2. and 5.5.3.). It was found that, as storage proceeded, the levels of C18:1, the major fatty acid and of C18:2 and C16:1 (as a % of the total fatty acids) declined. Concomitantly the levels of short chain fatty acids, especially C6:0, increased

(a % of the total fatty acids). These changes occurred in both the phospholipid and neutral lipid fractions but at a much faster rate in the former. This observation is supported by the findings of El-Gharbawi. and Dugan (1963) who noted that lipid oxidation occurs first in the phospholipids, and then in the neutral lipids. Igene et al., (1980) and Igene and Pearson (1979) reported a relationship between oxidation of the phospholipid PUFA's and rancidity development as did (18:3)/Igene et al.,(1980;)Gokalp et al.,(1983).

The increase in C6:0 (hexanoic acid) in the phospholipid fraction of the lipids, observed in the present investigation, was found to be matched by the decrease in product acceptability (as determined by the attainment of a TBA value of 5). When rusk or the seasoning mix were omitted from the meat filling, there was an increase in the rate of rancidity development, which shortened the shelf life of the pies below normal. Lipid analysis showed that, in these circumstances the production of hexanoic acid occurred at a faster rate. This rate was even greater when rusk and seasoning mix were simultaneously omitted. A TBA value of 5 was obtained by 5-6 days of storage at 4°C compared with 9-10 days when either rusk or seasoning were omitted and 15 days when both were present.

Although no positive correlation between the level of hexanoic acid and TBA value was established, the rusk and seasoning mix was observed to have antioxidant activity, limiting the production of hexanoic acid and the rise in TBA values. The exact mode of action of the rusk and seasoning mix on lipids was not investigated in great detail.

In the seasoning mix there is starch, monosodium glutamate, salt and white pepper. The first two were found to have no effect on rancidity development, whereas salt was found to be pro-oxidative (in keeping with reportsby Rhee et al., 1983; Tappel, 1961). White pepper was found to be antioxidative and this activity was found to reside in the ethanol soluble fraction; - the oleoresin. This agrees with the view of Palizsch et al., (1969); (1974) and Saito et al., (1976).

The major constituent of the oleoresin is piperine (Nambudiri et al., 1970; Pintauro, 1971). This compound was found to have antioxidant activity, but not to be totally responsible for the antioxidant action of the oleoresin. The effect of other compounds present (e.g. the volatile oils), clearly needs to be investigated. Since the piperine molecule contains a methyl interrupted double bond structure (Govindarajan, 1977), as found in unsaturated fatty acids, it may be that the piperine is oxidized in preference to the fatty acid; or else, it could be involved in a reaction which inhibits oxidation of fatty acids. It might also prevent, or block, the oxidation reaction by acting as a free radical scavenger. If so, this would lengthen the induction period, and decrease the rate of oxidation.

Piperine (piperinic acid piperidide) contains a 3, 4 methylene diophenyl group (Ladenburg and Scholtz, 1984), and phenol and the phenyl groups have been reported to have some antioxidant activity (Thumann and Herrmann, 1980). Any of these reasons could account for the antioxidant activity of piperine. This also merits further study.

Maillard browning products have been claimed to have antioxidant properties (Hugyebaert et al., 1982). They can be formed during the manufacture of rusk. In this work, trials showed that the antioxidant activity, developed during rusk manufacture was enhanced by prolonged heating (80 mins at 230°C). Such rusk had good antioxidant activity, but unfortunately, this had poor water binding capacity. since the purpose of adding rusk to the meat mix (Wilson, 1981) is to exploit its (normally high) capacity to bind water, The rusk was unsuitable for use in meat products. As salt has been shown to be proxidative, the effect of reducing its level in the seasoning mix could well justify study, not only in terms of rancidity, but in relation to current views implicating salt in the development of high blood pressure.

Other compounds which are known to reduce rancidity could be used in the seasoning mix. Thus soya flour and soya bean extracts help lower TBA values (Pratt, 1972; Sato et al., 1973; Bowers and English, 1975), and, also reduce shrinkage of the meat filling (Judge et al., 1974). The reason for the antioxidant activity of soya is the presence of 6, 7, 4′-trihydroxyl isoflavone, which can be oxidized to an aglycone, a strong antioxidant (Sangor and Pratt, 1974). Vegetable extracts containing flavone aglycones could also be used at low levels (1% vegetable extract) to reduce rancidity development (Younathan et al., 1983; Pratt and Watts, 1964; Scarborough and Watts, 1959) in the meat filling, by acting as free radical acceptors (Pratt and Watts, 1964), thus reducing the rate of lipid oxidation.

When studying the lipid fatty acid profile changes in a model system, the phospholipid fraction was found to develop rancidity but only when isolated from the neutral

lipids. This suggests that in the presence of neutral lipids, the phospholipids are in some way protected from very rapid oxidation. Alternatively, it may be that when the phospholipids were extracted, some protective substance (piperine?) originally present remained in the residue.

Aldehydes are the major end product of lipid oxidation. Their production as a function of storage was followed. Due to their volatile nature, the potential use of pentanal and hexanal as indices of rancidity is not entirely reliable. Nevertheless it was noted that when levels of 30 p.p.m. pentanal and 50 p.p.m. hexanal were obtained they corresponded closely with sensory rancidity.

As the aldehydes are volatile the use of headspace analysis (above the meat filling, and inside the pie wrap), together with the aldehyde analysis of the meat filling itself may reveal a correlation between hexanal and pentanal levels and sensory rancidity.

Moisture migration in pork pies was found to proceed from both the atmosphere and jelly, into the pastry (and mainly into the brown outer layer) as an equilibrium in moisture status tended to develop between these three environments. In the brown pastry layer the a_w was originally 0.65, in the jelly 0.97 and in the atmosphere ca 0.80. The migration of moisture resulted in an increase in the moisture content of the pastry (brown layer) and to its subsequent loss of crispness (texture).

A moisture content of 12% in the brown pastry layer, and a texture reading of 160g, were found to be the

threshold values above and below which, respectively, the pastry was deemed unacceptable by taste panels. They also correlated with the shelf life given by the manufacturer. These threshold values were used to determine pastry shelf life throughout the rest of the investigation.

Moisture migration from the atmosphere could be controlled by altering the relative humidity. Below 76% r.h. the brown pastry layer lost moisture to the atmosphere : the reverse was true above 76% r.h.

Reducing the jelly a_w to 0.56 and storing the pies at 76% r.h. virtually stopped moisture migration for 9 days. After this time movement of the humectant (used to lower the jelly a_w) into the pastry was sufficient to raise the a_w of the jelly. Therefore moisture migrated into the pastry, at a very reduced rate, which meant the threshold values of 12% moisture and 160g texture were reached after 35 days storage.

Reducing the a_w of the jelly to 0.84 was found to keep the texture value and moisture content, above 160g and below 12% respectively for longer (by 2 days) than the manufacturer's 8 day shelf life for the pie. Thus use of a jelly with an a_w of 0.84 would result in an extension of the shelf life of the pie. With this criterion, humectants, binders and pH were investigated in an attempt to develop a jelly with an a_w of 0.84 but there was little success. It is possible that the use of L-amino acids (Anderson and Witter, 1982) could be used to produce a jelly of a_w 0.84; without the use of glycerol and its unacceptable taste.

The possibility of using cetyl alcohol as a moisture barrier (Anderson, 1961; Daniels, 1973) between the pastry and jelly was considered, but it failed to prevent moisture migrating from the jelly into the brown pastry layer. It is feasible that the use of fatty acid suspensions (Daniels, 1973) may be successful in this context.

The role of the jelly in moisture migration in pork pies was studied in a model system. This revealed that loss of moisture by the jelly did not account fully for the gain in moisture by the pastry (brown layer), confirming that the atmosphere also plays a part in moisture migration in pork pies.

The investigation also showed that the gelatine used by the manufacturer was the best of various preparations in retarding gel moisture losses. It could be improved, however, by the formation of a cogel with Lactein 75, a whey protein concentrate (Marshall, 1982). This cogel was found to be dependent on both pH and temperature. Once a method had been devised for its formation, it was found to extend the shelf life of the pastry of pies containing it. Thus, provided the problem of its opaque nature can be overcome, the extension of pastry shelf life is feasible using the lactein-gelatine cogel.

Most of the further work on the storage changes in pork pies which the present investigation has shown to be desirable has been suggested above as well as in the other relevant sections. There are additional questions, however, which may merit study. These include: the role of the wrapping material in moisture migration. Does it increase

or retard moisture gains from the atmosphere? Butcher (1981) found them to increase moisture gains. Other interesting areas involve the relationship between meat colour changes, protein cross linkaging and lipid oxidation. The use of moisture barriers within and outside the pie and the role of packaging material on rancidity development (Igbinedion et al., 1983).

Finally it should be noted that any attempt to improve the shelf life of pork pies by reducing lipid oxidation and moisture migration obviously should not make the pie any less acceptable to the consumer, either in quality or otherwise.

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Appendices.

APPENDIX 1

THE SATURATED SALT SOLUTIONS USED IN SECTION 6.3, AND THEIR RELATIVE HUMIDITY (r.h) VALUES.

SALT	% r.h
Silica gel	0
Magnesium chloride ($MgCl_2$)	34
Magnesium nitrate ($Mg(NO_3)_2$)	58
Copper chloride ($CuCl_2$)	67
Sodium nitrate ($NaNO_3$)	67
Sodium chloride (NaCl)	76
Distilled water	100

APPENDIX 2

DATA USED IN THE PLOTTING OF FIGURE 6.8

% GLYCEROL IN THE GEL.	% MOISTURE CONTENT OF BROWN PASTRY LAYER		ACTUAL CHANGE IN % MOISTURE CONTENT
	AFTER		
	1 day	8 days storage	
0	5.31	34.14	28.83
	5.00	33.74	28.74
10	4.75	29.18	24.43
	4.60	27.93	23.33
20	6.35	24.06	17.71
	6.15	23.98	17.85
30	3.35	20.16	16.81
	3.44	19.14	15.70
40	3.97	15.34	11.37
	4.42	12.52	8.10
50	4.65	11.99	7.34
	4.82	12.77	7.95
60	6.57	11.00	4.43
	6.58	10.89	4.31
70	5.13	9.87	4.14
	5.06	9.54	4.48
80	4.54	8.45	3.91
	4.51	8.86	3.35
90	4.70	5.80	1.05
	4.28	5.41	1.13
100	4.55	6.12	1.07
	4.35	6.33	1.08

APPENDIX 3

BLOOM STRENGTH CHARACTERISTICS OF THE GELATINE JELLIES USED IN SECTION 6.4.1.

GELATINE GEL'	SUPPLIER	BLOOM STRENGTH	OTHER DETAILS [a]
STANDARD GELATINE	LEINER GELATINE LTD, TREFOREST MID GLAMORGAN.	210	
HISET GELATINE	LEINER GELATINE LTD TREFOREST MID GLAMORGAN	300	
BLEND 855	GELATINE PRODUCTS LTD, RUNCORN CHESHIRE.	200	MODIFIED WITH CARRAGEENAN FOR A HIGHER MELTING POINT.
BLEND 856	GELATINE PRODUCTS LTD, RUNCORN CHESHIRE.	200	LESS CARRAGEENAN THAN 855, AND WITH A SOFTER TEXTURE.
BLEND 858	GELATINE PRODUCTS LTD, RUNCORN, CHESHIRE.	200	SUPPLIERS STANDARD 200 BLOOM STRENGTH GELATINE.
BLEND 859	GELATINE PRODUCTS LTD, RUNCORN, CHESHIRE.	LESS THAN 200	WITH CARRAGEENAN TO GIVE A SIMILAR TEXTURE AS 858.

a: details supplied by supplier Gelatine Products Ltd.

ORGANOLEPTIC SCORES FOR THE BROWN PASTRY LAYER , AND MEAN SCORE USED IN FIGURE 6.1

MEAN SCORE	ORGANOLEPTIC SCORES
4.25	4.5 4.5 4.5 4 4 3.5 4 4.5 4.5 4.5
3.2	3.5 3.5 3 3 4 3.5 3 3.5 2.5 3.5
3.0	3 3 3 3 3 3.5 2.5 3 3.5 3.5
2.8	2.5 2.5 2.5 2.5 2.5 2.5 3 3 3.5 3.5
2.5	2 2 2.5 2.5 2.5 2.5 3 3.5 1.5 2.5
2.0	2 2 2 2 1.5 2 1.5 2 2.5 2.5
1.9	1.5 1.5 1.5 1.5 2.5 2 2 2 2 2
1.7	1.5 1.5 1 1 2 2 2.5 1.5 2 2
1.5	1.5 1.5 1.5 2 1 1 1.5 1.5 2 1.5
0.9	1 1 1 1 1 0.5 0.5 0.5 1.5 1