AN INVESTIGATION OF FACTORS CONTRIBUTING TO EXTENDED STORAGE LIFE OF MEAT PRODUCTS FOR THE TROPICS

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ABSTRACT

A combination of factors which could extend the shelf-life of minced mutton meat during storage under warm conditions (30 °C) while still maintaining a low cost product with acceptable sensory and microbiological properties was investigated. The combination of factors studied involved pH values from 5.5 to 3.5 achieved by the addition of acetic acid, water activity (a_w) values from 0.99 to 0.91 achieved by the addition of sorbitol and the reduction in moisture contents, heat treatments from 30 to 50 °C, and the use of different packaging materials (cellulose, polyethylene and aluminium foil films).

It was expected (from reported work) that stability would be achieved by reducing a_w to 0.95 and pH to 5.2, but at 30° C the shelf-life was less than one week. Within the limits of the values that were considered, the control of pH was the only significant factor in extending the shelf-life of minced mutton meat stored at 30° C. To achieve a commercial acceptable shelf-life of 8 weeks, a pH of 4.1 or below was required. Minced mutton with pH of 4.1 can be organoleptically acceptable with the addition of the right combinations of spices and seasonings suited to specific localities and countries. The practical value of lowering the pH of meat products below 4.1 must be questioned because no additional microbiological protection will be obtained and the increase of the acid level will only decrease the flavour acceptance. Combinations of low acid and spices and seasonings would appear to be a development area of greater promise for tropical countries than intermediate moisture meats.

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CHAPTER 1

INTRODUCTION

The history of food preservation closely parallels the great advances in civilization. Man needed a stable source of food, readily available before he could turn his attention to the development of his culture and society. During the early period of recorded history, dried products were the major stored food items to offset shortages of fresh products. These foods were preserved mainly by removing water but other factors were involved. With a growth of understanding of these other factors producing stability it is now possible to design reliable preservation techniques.

One of the more recently developed areas of food preservation that is showing considerable promise for commercial development in the reasonably near future uses the concept called "hurdle technology". The concept of hurdle technology is being developed mainly as an application for meat products. The preservation of meats by the hurdle concept is based on a combination of factors such as high temperature (F), low temperature (t), water activity (a_w), acidity (pH), redox potential (Eh), preservatives (e.g. nitrite or smoke), and competitive microorganisms (e.g. lactic acid bacteria) (Leistner, 1985). Each of the factors is used at an activity level that individually is sub-lethal to spoilage organism, but in combination provides the required protection. Each factor provides a "hurdle" to the growth of microorganisms.

The development and the design of new meat products using the hurdle concept is based on each specific need. In developed countries, the use of the hurdle concept is stimulated for example by the necessity for a reduction of nitrite addition to cured meat products or to save energy during storage of meats.

In developing countries it is possible that the hurdle concept could also be used to design meat products equivalent to existing products in price and able to be distributed and displayed for sale without the need for refrigeration facilities or the use of high priced packaging materials. Hurdle technology may be a suitable technology for the processing of meat in such countries where refrigeration facilities are scarce and income levels of the population are low.

Intermediate Moisture Food (IMF) and Shelf Stable Products (SSP) are both examples of food preservation techniques based on hurdle technology. The production of IMF is an extension of the traditional drying of foods. That is instead of removing the bulk of the water in the product, just enough water is removed or tied up through the addition of humectants to insure that the growth of microorganisms is prevented or greatly reduced. Intermediate moisture foods which have a_ws in the range of 0.90 to 0.60 are able to be stored without refrigeration and have been thought of possible benefit in applications particularly in tropical countries. Newly developed IMF based on meat, however, have not provided the break-through expected and have encountered difficulties in introduction for general use. The reasons for these are that the newly developed IMF often have an unfamiliar taste (sweet or bitter), contain too many additives ("chemical overloading" of the food), and pose legal problems in the need to obtain approval of new additives (Leistner, 1985).

To overcome the above difficulties a new generation of meat products was introduced based on the principle firstly explored by Leistner and Karandjundic (1970) named Shelf Stable Products (SSP) for high moisture food products with a_w in the range of 0.95 to 0.90. The a_w of SSP is only depressed below 0.95 and above 0.90 which is possible by legally approved additives in relatively low concentrations

or by removal of water by a slight drying process. SSP are mildly heated (70-90° C core temperature) in sealed containers (can, glass, jar, pouch, casing, etc.), but sufficient to inactivate non-sporing microorganisms. Recontamination after heat processing is avoided because of sealed containers and therefore only spores of bacilli and clostridia are of concern. The growth of surviving spores however, is inhibited by sufficient decrease of a_w, pH and Eh. For instance, in processed meats adjusted by salts or sugars or by the removal of water to an a_w below 0.95, growth of bacilli and clostridia does not occur, and the number of viable spores present even declines during storage (Leistner, *et al.*, 1979).

According to Leistner (1985) SSP meats offer the following advantages:

1) Mild heat treatment, which improves the sensory and nutritional properties (e.g. retention of vitamins) of the product and saves energy.

2) No refrigeration required, which simplifies the distribution of the products and saves much energy during storage.

3) Reduced addition of nitrite (< 50 ppm), since nitrite is necessary only for odour and flavour of the products, but not for their preservation.

Leistner (1985) further claimed that at present, for industrialized countries, meat products of the SSP type are more feasible than IMF, because the reduction of micro-organism, except for spores of bacilli and clostridia, by the heat treatment makes the stabilization of SSP much easier than that of IMF. If the a_w is decreased below 0.95, SSP should be stable (Leistner *et al.*, 1979), whereas the a_w of IMF must be decreased to 0.85 if fungistatic substances are added, or even below 0.70 without addition of such substances. In most of this experimental work, ambient temperatures are assumed to be around 20°C.

It would therefore be interesting to evaluate the potential and the feasibility of SSP meat products for application in developing countries which are characterized by warm and humid conditions.

The purpose of this research topic is to investigate some of the problems and look for answers to the vastly complex and challenging area of hurdle technology. In particular to identify the combination of hurdles (F, a_w, pH, Eh) which would ensure stability in meat products to be distributed in tropical countries such as the Philippines where cost factors are also important. It is always important and also a responsibility of the food technologist to make food of high nutritional value such as meat products, more easily available to the people in a densely populated country in order to combat not only hunger but also malnutrition.

The specific objective of this study was to investigate practical combinations of hurdles (F, a_w, pH, Eh) which could extend the shelf-life of minced mutton meat stored at 30^{o}C while still maintaining a product that has acceptable sensory properties.

CHAPTER 2

REVIEW OF LITERATURE

2.1 SPOILAGE OF MEATS AND MEAT PRODUCTS

2.1.1 Definition

The broad definition of spoilage includes changes caused by microbial proliferation and physio-chemical changes in the meat. Microbial proliferation includes the growth of pathogenic as well as spoilage microorganisms. The growth of spoilage microorganisms could result in changes to meat odour, flavour, or appearance and in the case of pathogens bring harm to the consumers.

Physico-chemical changes include the reactions undergone by the meat to form off-flavours such as rancidity and changes in texture. These changes are due to enzymatic and oxidative activity.

Meat can be defined as primarily the edible muscular tissue of healthy animals but can include various organs ("offals") such as liver, heart, lungs, tripe, etc.. The line is being drawn at somewhat different points in different countries (Ingram and Simonsen, 1980).

Manufactured meats are usually diverse, ranging from those consisting wholly of meat (e.g., pure ground beef, salami) through those with admixtures of non-meat protein (e.g., casein or soya protein) or of carbohydrates (e.g., sausage rusk) to those in which the meat is only a minor element (e.g., certain kinds of meat pie or vegetable and meat stews) (Ingram and Simonsen, 1980). There are widely different views about what should or should not be regarded as manufactured meats. For this study manufactured meats are those consisting almost entirely of meat which has been salted, or salted and cured and which may either be raw or cooked. Fresh pork sausage, roast beef, luncheon meats, and frankfurter sausages are examples of manufactured meats.

For this study fresh meats are defined as raw, uncooked meats which may be either chilled or frozen.

2.1.2 Microbial Spoilage of Meats

Meat is nutritious for man but can also be a substrate for the growth of microorganisms. Meat has a high water content (Table 2.1), corresponding to a water activity (a_w) approximately 0.99, which is suitable for growth of most microorganisms and the chemical composition is also favourable to many microorganisms.

Table 2.1: Approximate composition of adult mammalian muscle after rigor mortis[a]

Component		% Wet weight	
Water			75
Protein			
Structural connective		2.0	
Myofibrillar		11.5	19
Sarcoplasmic		5.5	
Fat			2.5
Carbohydrate			
Glycogen		0.1	
Glucose + phosphates		0.2	1.2
Lactic acid		0.9	
Miscellaneous soluble			
Nitrogenous:	amino acids	0.35	
	creatine	0.55	1.65
	minor ingredients	0.75	
Inorganic:	K	0.35	
	P	0.2	0.65
	others	0.1	
Vitamins: most B-vitamins present in useful amounts			

[a] Based on Lawrie (1975)

2.1.2.1 Important Properties of Meat

1) Meat has a high moisture content

Meat contains about 75% water, in which is dissolved a variety of major growth substrates and supporting nutrients. This makes meat a very good medium for growing a wide variety of microorganisms, particularly bacteria, which are favoured by wet conditions (Ingram and Simonsen, 1980).

2) The redox properties of meat have a major influence on microbiological growth

According to Ingram and Simonsen (1980), the central factor is tissue respiration that continues to consume oxygen (if present) and produce CO_2. In life, the demand is more than compensated for by oxygen transported in the blood, and the oxygen tension and the redox potential in living muscle are high (except in some muscles in periods of violent exercise). With the cessation of blood supply at death, oxygen content and redox potential in the muscle gradually fall, leading to anaerobic production and accumulation of lactic acid. The acidity developed may greatly diminish tissue metabolism, which nevertheless continues for several days at a rate - even at low temperature - exceeding that at which oxygen can diffuse into meat for more than a few millimeters. Therefore, the bulk of meat becomes anaerobic within a few hours *post mortem* and remains so except for an aerated surface layer a few millimeters thick that is indicated by its brighter red colour and which does not develop acid. When frozen, cooked or salted, the tissue respiration is inhibited; nevertheless, sufficient reducing activity normally remains to maintain anaerobic conditions within any piece of meat more than approximately 10 mm thick. Consequently, although an aerobic flora develops on the surface, only anaerobes or facultative anaerobes

can grow within the meat. (Some of the latter grow relatively slowly). Because few of these anaerobic organisms grow readily at low temperatures, they scarcely develop within the bulk of chilled meat, even after long periods. When meat is minced, it is reaerated throughout, but if it is then packed together again, anaerobic conditions are gradually established within its mass. If numerous microorganisms (10^6 /g) are present, their respiration augments the tissue respiration.

3) The pH of meat varies between approx 5.5 - 6.5

Most meat products fall in the pH range of 5.0 to 7.0. A pH near neutrality (pH of 7.0) is nearly optimal for most pathogenic and spoilage bacteria. In general, values approaching 5.0 are unfavourable in themselves to the growth of most of the important bacteria, and in combination with other unfavourable factors such as low temperature may almost prevent bacterial growth (Ingram & Simonsen, 1980).

According to Ingram & Simonsen (1980), the pH of fresh meat is inversely proportional to the amount of lactic acid developed by muscular glycolysis following death: pH 7.0 corresponding to almost none; pH 5.5 to approximately 1%. The amounts of lactic acid depend, in turn, on the amount of glycogen in the muscle at death. This may be low if the muscle has been exercised before slaughter, in which event the ultimate pH will be relatively high and the muscle dry and firm in texture and dark in colour (DFD condition). In a muscle not exercised at slaughter, all or most of the glycogen will be converted gradually after death to give a low ultimate pH near 5.5 with normal appearance and texture. If however, such as non-exercised muscle is stimulated just before death as readily occurs in stress-susceptible pigs, the largely unused reserve of glycogen is rapidly converted before the tissues have had

time to cool; this denatures sacroplasmic proteins, aggravating an apparent whitening of the tissues and a loss of water-holding capacity, called the pale soft exudative (PSE) condition (Ingram and Simonsen, 1980).

2.1.2.2 Factors Affecting Microbial Spoilage

1) Temperature

Temperature is the most important single factor influencing the growth of microorganisms. Broadly, the higher the temperature the greater is the rate of growth. Many meat microorganisms will grow to some extent at all temperatures from below $0^{o}C$ to above $65^{o}C$, but, for a given organism, vigorous growth occurs in a more limited temperature range (Lawrie, 1979). It is customary to classify meat spoilage organisms in three categories based on this temperature range for growth but the distinction is by no means absolute. Psychrophiles grow well at temperatures of 0^{o} to $20^{o}C$. Examples are *pseudomonas* bacteria and some yeasts and moulds. Mesophiles grow well at temperatures between 20^{o} and $45^{o}C$. Most pathogenic bacteria belong to this group. *Clostridium botulinum* (types A and B), *C. perfringens*, *Salmonella*, and *Staphylococcus aureus* are examples. Thermophiles grow at high temperatures between 45^{o} and $65^{o}C$. Examples are *C. nigrificans*, *C. thermosaccarolyticum*, and *Bacillus steanothermophilus*.

Temperature not only determines the rate of bacterial growth that can take place on meat substrate but also influences the nature of the flora which becomes dominant. A majority of meat products - fresh, cured and pasteurized, and vinegar-packed - are held under refrigeration. These meats generally develop a

psychrophilic bacterial flora (Lechowich, 1971). However, mesophilic microorganisms such as the above mesophilic pathogens present prior to refrigeration can generally survive for extended periods at these temperatures, and any significant temperature abuse would allow them to re-initiate growth (Buchanan, 1986).

Thermophilic bacteria are seldom encountered in meat products. However, improperly cooled commercially heat-processed canned meats, such as larger cans of canned beef hash., could support the growth of thermophiles (Lechowich, 1971).

2) Degree of acidity or alkalinity (pH)

The pH (hydrogen ion concentration) is an important factor in the growth and metabolism of microorganisms. Various organisms tolerate different pH ranges, the pH of the food determining the microorganisms present. For example, most proteolytic and pathogenic spoilage organisms have a pH optimum at about the neutral value (pH 7.0).

Three groups of foods can be distinguished based on their pH values (Stiebing, 1985).

a) low-acid to neutral with a pH >4.5.
b) acid foods with a pH of 4.0-4.5.
c) high-acid foods with a pH <4.0.

This grouping is particularly important in relation to the heat-inactivation of microorganisms. For instance, it has been known for a long time that preserved fruits and pickles with a pH below 4.5 only need mild heat treatment (pasteurizing) (~90°C) and remain microbiologically stable without refrigeration. In products of this kind vegetative bacteria and moulds are inactivated by heat and, because of their low pH, the heat resistance of *Clostridium* and *Bacillus* spores is reduced and the germination of the spores inhibited.

Meat products with the exception of those fermented or acidified are typically low-acid having a pH near 6 (Table 2.2). This pH is well within the range that permits the growth of most bacteria, yeasts and moulds (Table 2.3) (Jay, 1978; Ayres *et al.*, 1980), and it would be expected that a wide range of microbial species could effectively compete in such an environment.

In the case of fermented or acidified meats, the typical pH of the meat system is 5.0 or below. This is outside the range that will support the growth of most bacterial species, so the microflora of these products would be expected to consist predominantly of yeasts, moulds, and acid-tolerant bacteria, such as the lactic acid bacteria (Buchanan, 1986).

3) Moisture Content

Microorganism responsible for the spoilage of food depend on moisture for growth. But not all the moisture present in a food is available to microorganism as it may be bound (chemically or physically) in the food (Stiebing, 1985). Water activity (a_w) is a measure of unbound moisture available to microorganism. The water activity is the quotient of the vapour pressure of the water in the food and the vapour pressure of pure water at the same temperature.

Microorganisms, depending on their type, genus and species need a certain minumum water activity for their life processess. If this free moisture is not available in the food, microorganisms are unable to cause spoilage (Stiebing, 1985). Different kinds of microorganisms have varying abilities to grow at defined minimum a_w values (Table 2.4). Most spoilage bacteria for example, require an a_w of 0.91 or greater for growth while

Table 2.2: Average pH and a_w values for meat and meat products[a]

Product	pH	a_w
Fresh meat, hot	7.2	0.99
Beef, ripened	5.4 - 5.8	0.98
Pork, ripened	5.6 - 6.0	0.98
Frankfurter-type sausage, fresh	6.0 - 6.4	~0.97 - 0.98
Long-life frankfurter-type sausage	6.0 - 6.4	~0.91 - 0.95
Cooked ham	6.0 - 6.4	~0.98
Dry sausage, firm	4.9 - 5.2	~0.85 - 0.93
Dry, sausage, spreadable	5.0 - 5.8	~0.93 - 0.95
Raw ham	5.3 - 5.8	~0.90 - 0.93
Blood sausage	6.2 - 7.0	~0.96 - 0.98
Liver sausage	6.0 - 6.5	~0.95 - 0.97
Brawn sausage	4.5 - 5.7	~0.97 - 0.98

[a] From Stiebing (1985)

TABLE 2.3: The limits of pH allowing initiation of growth by various microorganisms, in laboratory media adjusted with strong acid or alkali[a]

	Minimum pH	Maximum pH
Gram-negative bacteria		
Acetobacter acidophilum	2.8	4.3
Alcaligenes faecalis	6.4	9.7
Escherichia coli	4.4	9.0
Klebsiella pneumoniae (aerogenes)	4.4	9.0
Proteus vulgaris	4.4	9.2
Pseudomonas aeruginosa	5.6	8.0
Salmonella paratyphi	4.5	7.8
Salmonella schottmuelleri	4.5	8.0
Salmonella typhi	4.0-4.5	8.0-9.6
Thiobacillus thiooxidans	1.0	9.8
Vibrio parahaemolyticus	4.8	11.0
Gram Positive bacteria		
Bacillus cereus	4.9	9.3
B. subtilis	4.5	8.5
B. stearothermophilus	5.2	9.2
Clostridium botulinum	4.7	8.5
Clostridium sporogenes	5.0	9.0
Enterococcus spp.	4.8	10.6
Bifidobacterium bifidum (Lactobacillus bifidus)	3.8	7.2
Lactobacillus spp.	3.8-4.4	7.2
Serratia marcescens	4.0	9.0
Micrococcus sp.	5.6	8.1
Staphylococcus aureus	4.0	9.8
Streptococcus faecium	4.4-4.7	9.2
Streptococcus lactis	4.3-4.8	9.2
Streptococcus pyogenes	6.35	9.2

Continued

Table 2.3 : Continued

	Minimum pH	Maximum pH
Yeasts		
Candida pseudotropicalis	2.3	8.8
Hansenula canadensis	2.15	8.6
Saccharomyces cerevisiae	2.35	8.6
Saccharomyces fragilis	2.4	9.05
Saccharomyces microellipsoides	2.2	8.8
Saccharomyces pastori	2.1	8.8
Saccharomyces exiguus	1.5	-
Schizosaccharomyces octosporus	5.45	7.05
Candida krusei	1.5	-
Hanseniaspora melligeri	1.5	-
Rhodotorula mucilaginosa	1.5	-
Moulds		
Aspergillus oryzae	1.6	9.3
Penicillium italicum	1.9	9.3
Penicillium variabile	1.6	11.1
Fusarium oxysporum	1.8	11.1
Marasmius foetidus	2	6.8
Phycomyces blakesleeanus	3.0	7.5

[a] Assembled by Dr Martyn Brown

From Christian (1980)

TABLE 2.4 : Approximate minimum levels of water activity (a_w) permitting growth of microorganisms at temperatures near optimal[a]

	a_w
Moulds	
Alternaria citri	0.84
Aspergillus candidus	0.75
A. conicus	0.70
A. flavus	0.78
A. fumigatus	0.82
A. niger	0.77
A. ochraceous	0.77
A. restrictus	0.75
A. sydowii	0.78
A. tamarii	0.78
A. terreus	0.78
A. versicolor	0.78
A. wentii	0.84
Botrytis cinerea	0.93
Chrysosporium fastidium	0.69
C. xerophilum	0.71
Emericella (Aspergillus) nidulans	0.78
Eremascus albus	0.70
E. fertilis	0.77
Erotum (Aspergillus) amstelodami	0.70
E. carnoyi	0.74
E. chevalieri	0.71
E. echinulatum	0.62
E. herbariorum	0.74
E. repens	0.71
E. rubrum	0.70
Monascus (Xeromyces) bisporus	0.61
Mucor plumbeus	0.93
Paecilomyces variotii	0.84
Pencillium brevicompactum	0.81
P. chrysogenum	0.79

Continued

Table 2.4: Continued

	a_w
P. citrinum	0.80
P. cyclopium	0.81
P. expansum	0.83
P. fellutanum	0.80
P. frequentans	0.81
P. islandicum	0.83
P. martensii	0.79
P. palitans	0.83
P. patulum	0.81
P. puberlum	0.81
P. spinulosum	0.80
P. viridicatum	0.81
Rhizopus nigricans	0.93
Rhizoctonia solani	0.96
Stachybotrys atra	0.94
Wallemia seki (Sporendonema epizoum)	0.75
Yeasts	
Debaryomyces hansenii	0.83
Saccharomyces bailii	0.80
S. cerevisiae	0.90
S. rouxii	0.62
Bacteria[b]	
Bacillus cereus	0.95
B. megaterium	0.95
B. stearothermophilus	0.93
B. subtilis	0.90
Clostridium botulinum type A	0.95
C. botulinum type B	0.94
C. botulinum type E	0.97
C. perfringens	0.95
Enterobacter aerogenes	0.94

Table 2.4 : Continued

	a_w
Escherichia coli	0.95
Halobacterium halobium	0.75
Lactobacillus viridescens	0.95
L. plantarum	0.94
Microbacterium sp.	0.94
Paracoccus (Micrococcus) halodenitrificans	0.86
Micrococcus luteus (lysodeikticus)	0.93
Pediococcus cerevisiae	0.94
Pseudomonas fluorescens	0.97
Salmonella sp.	0.95
Staphylococcus aureus	0.86
Vibrio costicolus	0.86
V. parahaemolyticus	0.94

[a] From Troller and Christian (1978)

[b] a_w adjusted with salts

spoilage moulds can grow as low as 0.80. The a_w requirements can also vary considerably within bacterial species. With *Clostridium botulinum*, for instance, the limit for cold tolerant (psychrotrophic) clostridia is 0.97 but mesophilic clostridia can grow up to an a_w of 0.95. These minimum a_w values should only be regarded as a guide however, as they can be affected by small changes in pH, temperature or nutrient content (Jay, 1978).

Fresh meats have an a_w which is frequently about 0.99, and they are thus liable to spoil through the growth of a wide range of organisms (Scott, 1957).

One of the general characteristics of manufactured meat products is a reduction in the level of biologically available water as a result of the addition of sodium chloride and other humectants. Thus, while fresh meats typically have a water activity ≥ 0.99, a cured product with 4-5% aqueous-phase salt would be expected to have an a_w of approximately 0.97 (Lechowich, 1971). This relatively small reduction in a_w will prevent the growth of a few particularly salt-sensitive species such as *Campylobacter jejuni* (Doyle and Roman, 1982; Palumbo, 1984), but most microorganisms can tolerate this level of available water. However, their growth rates will typically be diminished, and those species more able to tolerate the water stress will have a competitive edge. For example, Blickstad and Molin (1983) observed that the predominant species on fresh pork loins aerobically stored at 0 °C was *Pseudomonas* (~90%), while cured product containing 3% NaCl had a mixture of 60% *Brochotrix* (formerly *Microbacterium thermosphactum*), 20% *Moraxella*, and 20% *Pseudomonas*. In general, decreasing the a_w of a meat system by addition of NaCl and other humectants or by partial dehydration shifts the microflora to more salt-tolerant bacteria (halophiles) such as *Lactobacillus*, *Streptococcus*, *Micrococcus*, *Pediococcus*, *Staphylococcus*

and *Vibrio*, and/or allows the growth of various yeasts which tolerate high osmotic pressures (osmophiles) and moulds which tolerate dry conditions (xerophiles) (Jay, 1978).

4) Oxidation - Reduction Potential (O/R, Eh)

According to Jay (1978), it has been known for many years that microorganisms display varying degrees of sensitivity to the oxidation-reduction potential of their growth medium. The O/R potential of a substance may be defined generally as the ease with which substrate loses or gains electrons. The potential, measured in millivolts (mv) with an electrode, is pH-dependent and is given as the Eh value. Rising Eh values correspond to increasing oxidation and vice-versa.

The redox-potential depends greatly on the chemical composition and also on the retention of oxygen from the air by the food (Stiebing, 1985). In meats, for example, chemicals containing -SH groups help to maintain reducing conditions while in fruits and vegetables ascorbic acid and reducing sugars have the same effect (Jay, 1978).

Microorganisms are traditionally subdivided into four categories based on their Eh requirements: anaerobes, acrobes, microaerophiles, and facultative anaerobes. Anaerobes such as the genus *clostridium* require reduced conditions for growth initiation (Eh of about - 200 mv). Aerobes such as the genus *Bacillus*, pseudomonads and micrococci require a positive Eh for growth. Some aerobes actually grow better under slightly reduced conditions and these organisms are referred to as microaerophiles. The microaerophilic group are also important in relation to meat products since they grow best in the presence of minute quantities of free oxygen.

Examples of microaerophiles are streptococci, pediococci and lactobacilli. Some microbes have the capacity to grow under either aerobic or anaerobic conditions. These are referred to as facultative anaerobes such as the staphylococci and coliform bacteria. Most moulds and yeasts encountered in and on meats are aerobic though a few tend to be facultative anaerobes (Jay, 1978).

In regard to the Eh of meat products, the interior of solid pieces of meat (post-rigor) have Eh values around -200 mv. While in loosely packed minced meats the Eh value is generally around +200 mV (Brown and Emberger, 1980). The low redox potential in the former would encourage the growth of anaerobic bacteria such as clostridia while the latter would support the growth of aerobes such as pseudomonads.

With respect to the Eh of pre-rigor as opposed to post-rigor muscles, Barnes and Ingram (1955, 1956) undertook a study of the measurement of Eh in muscle over periods of up to 30 hr post mortem and its effect upon the growth of anaerobic bacteria. These authors found that the initiation of growth by clostridia in horse muscle was affected by its redox potential. Immediately after death (pre-rigor) the redox potential was +250 mV, and in muscle free from bacteria this fell to about -130 mV at 30 hr post mortem. When bacteria were present in the sample the redox fell to -250 mV. Clostridia grew in the latter only when the redox potential fell below -36 mV, suggesting that anaerobes naturally present in meat do not grow until the onset of rigor mortis because of high redox potential in pre-rigor meat.

Manufactured meats are typically highly reduced, and accordingly the oxygen content can vary considerably within the product. An example is the redox potential of frankfurters which varies, depending on the comminution

conditions (oxygen content) and the recipe, from +20 to -100 mV (Stiebing, 1985). Generally, in most cured meats and sausage-type products, the surface of the meat will be highly aerobic favouring the growth of aerobes and facultative anaerobes. However, a short distance beneath the surface, the amount of available oxygen is greatly decreased, and microaerophiles, anaerobes, and facultative anaerobes would be expected to predominate. It could be anticipated that more aerobically oriented genera such as *Moraxella* or *Brochothrix* would predominate on the surface, while microaerophiles such as *Lactobaccilus* as well as some anaerobes and facultative anaerobes would thrive in the interior.

5) Gaseous Environment

It is implicit in the concept of oxidation-reduction potential when meat is exposed to the air the oxygen content contributes in determining the growth of surface spoilage organisms on meat and meat products. When the oxygen content of a product is altered, the microflora will change to reflect those species best adapted to the new oxygen level.

The type of packaging employed can significantly alter the gaseous environment surrounding meats and meat products. Packaging methods have been shown to have a strong influence on the types of microorganisms occurring in both fresh meats (Hanna *et al.*, 1977; Blickstad and Molin, 1983; Simard *et al.*, 1984) and cured meats (Egan *et al.* 1980; Blickstad and Molin, 1983; Kemp *et al.*, 1983; Laleye *et al.*, 1984). Typically, the use of a barrier that reduces the oxygen content of the atmosphere surrounding the product retards the growth of aerobic or noncompetitive facultatively anaerobic species (e.g., *Pseudomonas*, *Brochothrix*, and

Moraxella), while fostering the growth of microaerophiles such as Lactobacillus and yeast. This process of microbial selection can be enhanced further by flushing the product with inhibitory gases. For example, flushing with carbon dioxide retards the competitive growth of yeast and moulds in cured meats (Blickstad and Molin, 1983).

6) Nutrient content

Microorganisms, like all living systems, have specific carbon, nitrogen, mineral and vitamin requirements. Meats and meat products are generally considered a rich source of nutrients. Accordingly, they can support the growth of a wide variety of microbial species.

Bacteria grow on fresh meat at the expense of some low molecular weight soluble components. The concentrations of several of these microbial nutrients alter considerably during the development of rigor (Table 2.5), with the major changes resulting from the degradation of reserve glycogen via glycolysis. The concentration of these substrates, and the order in which they are attacked by different groups (Table 2.6), can affect the course of spoilage. Glucose concentration has been assumed to be the prime nutrient which is a determinant of the time of spoilage onset in meat (Gill, 1986).

In meat products, the addition of specific nutrients to meat formulations has been shown to influence the physiological characteristics of contaminating microorganisms. For example, the addition of certain spices to fermented meat formulations stimulates bacterial lactic acid production (Zaika and Kissinger, 1979; Nes and Skjelkvale, 1982). This stimulatory effect is due to the spices acting as a supplemental source of manganese, a trace mineral that is cofactor for lactic

acid production in *Lactobacillus* and *Pediococcus* and that is generally limiting in meat products (Zaika and Kissinger 1984; Raccach and Marshall, 1985).

Table 2.5: Concentrations of the main low molecular weight soluble components of beef before and after completion of rigor [a]

Substance	Concentration (mg gm)	
	Prerigor	Postrigor
Creatine phosphate	3.0	-
Creatine	4.5	6.5
Adenosine triphosphate	3.0	-
Inosine monophosphate	0.2	3.0
Glycogen	10.0	1.0
Glucose	0.5	0.1
Glucose 6-phosphate	1.0	0.2
Lactic acid	1.0	9.0
Amino acid	1.0	3.5
Dipeptides (carnosine, anserine)	3.0	3.0
pH	7.2	5.5

[a] From Gill (1986)

Table 2.6: Order of utilization of individual low molecular weight components of meat for bacterial growth[a]

Substrate	Aerobic growth				
	Pseudomonas	*Acinetobacter*	*Enterobacter*	*Brochothrix thermosphacta*	*Alteromonas putrifaciens*
Glucose	1	-	1	1	1
Glucose 6-phosphate	-	-	2	-	-
Amino acids	2	1	3	2 (glutamate only)	1 and 2 (serine, cysteine)
Lactate	3	2	4	-	3

Substrate	Anaerobic growth			
	Lactobacillus	*Enterobacter*	*Brochothrix thermosphacia*	*Alteromonas putrefaciens*
Glucose	1	1	1	1
Glucose 6-phosphate	-	2	-	-
Amino acids	2 (arginine only)	-	-	1 (serine cysteine)

[a] Data from Gill and Newton 1977; Newton and Gill 1978

7) Anti-microbials

Microbial growth in meats and meat products is also influenced by inhibitory compounds that are introduced either purposely, as a normal constituent of an ingredient, or through a biological process such as fermentation. The best known antimicrobial employed in meat products is sodium nitrite. This compound is well known for its activity against clostridial species such as *C. Botulinum* and *C. perfringens*. Other ingredients such as liquid smoke (Donnelly *et al.*, 1982) and spices (Shelef *et al.*, 1980; Ueda *et al.*, 1982) have antimicrobial activity, although they appear to be of minor importance at the concentrations employed in most meat products.

2.2 PRESERVATION OF MEATS BY INTERMEDIATE MOISTURE FOOD TECHNOLOGY

An intermediate moisture food (IMF) is one that is moist enough to be eaten without rehydration, and yet is shelf stable without refrigeration or thermal processing. It is characterized by a water activity low enough to prevent the growth of bacteria, and by conditions minimizing the potential for growth of other microorganisms. IMF usually have moisture contents in the range of 25-50% (wet basis) and water activity in the range of 0.60-0.90. This depression of a_w can be achieved by adding soluble substances to the aqueous phase of the food. Several solutes which include salts, sugars, and polyols have been incorporated in IMF to lower the a_w to the desired range. The compounds most used to depress a_w are sodium chloride, glycerol, propylene glycol, sucrose, corn syrup, sorbitol and dextrose.

IMF have been the subject of numerous technical and scientific studies in the last decade (Heidelbaugh and Karel, 1975; Flink, 1978; Davies *et al*, 1976; Troller, 1979). These and various other studies covered several aspects such as microbial and chemical stability at reduced water activity, the technology of IMF, and the selection of humectants for depressing water activity.

The object of this survye, however, is to assess the state of knowledge on the technology of IMF with specific reference to shelf-stable intermediate moisture meat products. The interest in the exploitation of the potential of intermediate moisture meat products is of importance because there is seemingly a growing need to look for alternatives or combination of means of preserving meat besides the traditional refrigeration, drying and canning. This is brought about by the ever increasing cost of energy and the necessity to look for a technology which will use minimal energy and less highly priced packaging materials. It is possible that the IMF technology has the potential for application in tropical countries like the Philippines.

This section attempts to review initially the concept and principle of the lowering water activity as a means or as a way of preserving food, specifically meat. This is because the concept of water activity occupies a key role in the formulation of intermediate moisture foods and factors relating to a_w are of central importance in understanding the principles that underlie IMF technology. A discussion therefore is also given on the factors affecting water activity lowering e.g. effect of solutes; and the problems associated with a_w lowering i.e., effects of a_w lowering to other deteriorative reactions of food like enzymatic reactions, non enzymatic browning reactions, lipid oxidation. It is also attempted to relate a_w lowering with other forms of preservation; e.g. interaction with pH; interaction with antimetabolities; interaction of heat processing and a_w.

2.2.1 Water Activity

2.2.1.1 Definition of Water Activity

Water activity is simply defined as the equilibrium vapour pressure a food exerts (P_{H_2O}) divided by the vapour pressure of pure water (P_o) at the temperature of the food. Water activity can also be defined as the relative humidity of air (% RH)) at which a food if held would neither gain nor lose moisture. In equation form this become:

$$a_w = \frac{P_{H_2O}}{P_o} = \frac{RH}{100}$$

The a_w defines the degree to which the water present in foods is tied up or bound and thus unavailable for certain reactions.

From the viewpoint of the food microbiologist water activity indicates the amount of water in a food which is available for microorganims. This is not the total water content of the food because a proportion of it maybe bound by water soluble salts, proteins and carbohydrates; such bound water is not available to microorganisms (Liestner and Rodel, 1976).

2.2.1.2 Measurement of Water Activity

A review of various water activity determination techniques has been made by Smith (1971), Labuza (1975), Leistner and Rodel (1976), Troller and Christian (1978), and Prior (1979). However, the water activity methods which may be of use and/or have been tried for determination of a_w of meats and meat products are described below:

1) Graphic Interpolation. Landrock and Proctor (1951) were the first to propose this method and they found it to be a practical means of a_w measurement. Bem and Leistner (1970) have applied this graphic method to meats and have proved this method to be quite accurate, provided the a_w of product was 0.98. However, they found the procedure a little cumbersome and time-consuming.

2) Manometry. Another method for measuring the a_w is the direct measurement of the vapour pressure of water in the vapour space surrounding the food by manometric techniques. This method was first reported by McKower and Myers (1943). Refinements of this instrument were subsequently described by Taylor (1961) and Labuza

et al. (1972).

The sample to be measured is ground and introduced into a flask, which is attached through a trap to a simple manometer. The manometer assembly (Fig. 2.1) is then evacuated, and during this time the sample chamber is maintained at approximately -80°C. Following evacuation, the sample is warmed to room temperature, while one side of the manometer is maintained at essentially zero pressure. The fluid level in the sample arm of the manometer is then deflected by the increase in pressure caused by the vapor pressure of the sample itself.

Troller and Christian (1978) have described manometers to have good precision and are relatively inexpensive. However, they continued that instruments capable of measuring wide vapor pressure differences are cumbersome and extremely fragile. Also, absolutely uniform temperatures and a high degree of thermometric accuracy are required. This instrument holds considerable promise for routine a_w analyses if these shortcomings can be overcome.

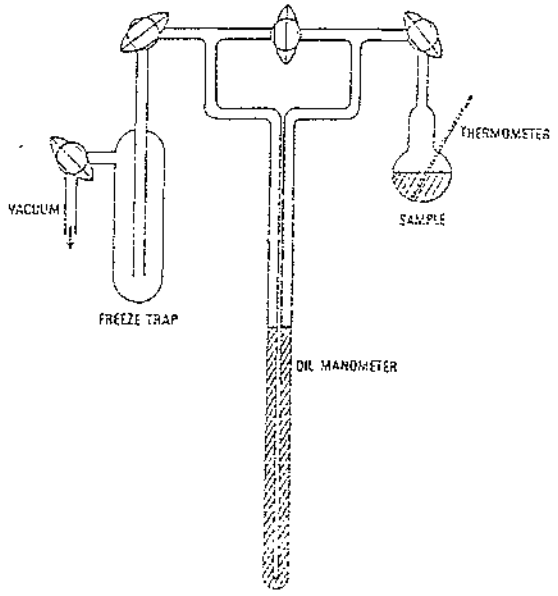


Figure 2.1: Vapor pressure manometer (From Troller and Christian, 1978)

3) Hair Hygrometry. This precedure relies on the hygroscopicity of human hair and the ability of this material to stretch when hydrated. Hair, usually three or more strands braided, is fixed at one end and attached to a sensitive level arm at the other. The level arm is connected to a recorder pen or dial that reads directly in percent relative humidity.

Hair hygrometers are reasonably priced but not very accurate for research purposes. However, the accuracy is improved when human hair is replaced by selected, artificially aged, plastic threads which have a sensitive response in the humidity range of interest (Leistner and Rodell, 1976).

An Aw-Wert-Messer-device was developed in West Germany by Firma LUFFT, for measuring the a_w of meat and meat products. This instrument is based on hair hygrometry and was found suitable for quality control and routine examination of meats (Leistner and Rodell, 1976).

This instrument is made of a stainless steel box with a lid containing the artificially aged plastic threads (polyamide thread) and a capillary thermometer for temperature compensation. In this method, the sample is placed in the box, the lid is closed and in about 3-5 hr the result is read on an a_w scale.

4) Electric Hygrometry. According to Troller and Christian (1978), two basic types of electric hygrometers are used in food-related applications. The first is based on the measurement of conductivity or resistance of an hygroscopic salt in equilibrium with an ambient atmosphere. As water is absorbed or desorbed by the salt, its ability to carry current (conductance) is measurably altered.

The second type has been referred to as an electrolytic hygrometer. The operation of this instrument requires that an alternating current be passed through a saturated LiCl solution suspended onto an inert carrier such as glass wool. A current potential difference of 25V, which heats the cell, is applied across the solution. The water vapor pressure (w.v.p.) of the solution rises, and upon reaching the w.v.p. of the environment, water evaporation occurs. The dried LiCl residue remaining after evaporation no longer conducts current, and heating ceases. As the residue cools, water is once again taken up from the environment, and the cycle is repeated at a reduced amplitude. Eventually, a temperature is reached at which the w.v.p. of the solution is equal to the w.v.p. of the environment. This temperature is then measured and related to the w.v.p. of the saturated LiCl solution and hence, the environment. From this, the equilibrium relative humidity of the environment can be calculated.

The former of these two types of instruments, requiring the measurement of conductance and resistance across a hydrated material, such as LiCl, is probably the instrument used most frequently to measure the water activity of foods. These instruments are described to respond rapidly to changes in relative humidity and have the advantage of being portable and easy to use. Reported limitations, however, are the loss of accuracy due to sensor aging, eventual poisoning by absorbed volatiles such as glycols, hysteresis effects at high a_w levels, and need of frequent calibration. Electric hygrometers have been shown to have good accuracy over a wide range of a_w values. Labuza *et al.* (1976) showed an accuracy of 0.02 a_w units over the 0.32 to 0.97 a_w range for several electric hygrometers. Troller (1977) reported an accuracy of 0.005 a_w units over the 0.75 - 0.97 a_w range for a Sinascope, with a coefficient of variation not exceeding 1%.

5) Dew Point Methods. The basic principle involved in dew point determinations is that air may be cooled without change in water content until saturation is reached. The temperature at which this saturation is achieved can be determined by observing condensation on a smooth, cooled surface such as a mirror or sight glass. This dew point temperature is related to vapour pressure, relative humidity, and water activity by reference to psychrometric charts. Numerous instruments are available for determining dew point temperatures. Various refinements of these devices have been developed, most of which relate to obtaining the exact point at which condensation first appears.

Rodel and Leistner (1972) as cited by Troller and Christian (1978) have modified a dew point hygrometer of EG and G, Cambridge Systems, Mass., U.S.A.. With this instrument, the a_w of small quantities of saturated salt solutions of known a_w, and also, meats and meat products were measured with considerable precision. Water activity measurements of different sausages revealed close agreement with those obtained using a commercially available electric hygrometer.

Dew point sensing devices are reported to give an accuracy of 0.003 a_w units in the 0.75 - 0.99 a_w range, as reviewed by Prior (1979). At lower a_ws their accuracy falls off because their is not enough vapour in the headspace to cover the mirrored surface and change its reflectivity.

The two types of electric hygrometers and the dew point methods are seemingly suited for use in research purposes for determination of a_w of meats and meat products while hair hygrometers and manometer for routine examinations. However, when using an instrument it is important to understand the fundamental scientific principle on which it is based and the characteristics

of the sensor which is being used...

2.2.2 Factors Responsible for Lowering of Water Activity

2.2.2.1 Solute-Water Interactions (Effect of Solutes)

The basic factor lowering a_w when a solute such as sodium chloride is dissolved in water is that the solute associates with the water to form a hydration shell. Depending on the amount present, the availability and vapor pressure of water is decreased according to equation:

$$a_w = \gamma \frac{N_{H_2O}}{N_{H_2O} + N_{solute}}$$

where:

N_{H_2O} = Moles of water in solution

N_{solute} = moles of dissolved solute species in a solution of N_{H_2O} moles of water, and

γ = activity coefficient = 1 for ideal solute.

The γ value is a measure of the nonideality of a dissolved species. For large molecules like gums, starches, and protein, γ is very small and thus the large molecules decrease a_w much more than do ideal solutes like sugars or salt. However, since their molecular weight (MW) is very large, the total a_w decrease is small. Electrolytes decrease the a_w more than expected on a molecular weight basis because they dissociate. For example, 1 mole of NaCl is actually two moles in solutes, Na^+ and Cl^-.

Tables 2.7, 2.8 and 2.9 present data relating the water activity of water to concentrations of various solute. If ideal relations are followed, then water activity should be numerically equal to the mole fraction of water in solution. Deviations from ideality may arise from several causes:

1) Not all water in food is capable of acting as a solvent.
2) Not all of the solute is in actual solution. (Some may, for instance, be bound to other insoluble food components, as in the case of salts bound to proteins).
3) Interactions between solute molecules may cause deviations from ideality.

Minimum activities achievable by common solutes present in foods are shown in Table 2.10.

Table 2.7: Water activity of selected solutions[a]

Water activity	Molality (moles solute/litre pure H_2O)			
	Ideal solute	NaCl	Sucrose	Glycerol
0.90	6.17	2.83	4.11	5.6
0.80	13.9	5.15	-	11.5

[a] From Karel (1975)

Table 2.8: Concentrations of sodium chloride and sucrose at various values of water activity (a_w) at 25°C[a]

	NaCl			Sucrose		
a_w	m	%, w/w	°Salometer	m[b]	%, w/w (°Brix)	°Baume
1.000	0	0	0	0	0	0
0.995	0.150	0.88	3.32	0.272	8.52	4.75
0.990	0.300	1.74	6.57	0.534	15.45	8.59
0.980	0.607	3.43	12.94	1.03	26.07	14.43
0.960	1.20	6.57	24.79	1.92	39.66	21.79
0.940	1.77	9.38	35.40	2.72	48.22	26.34
0.920	2.31	11.90	44.91	3.48	54.36	29.57
0.900	2.83	14.18	53.51	4.11	58.45	31.69
0.880	2.32	16.28	61.43	4.93	62.77	33.90
0.860	3.80	18.18	68.60	5.58	65.63	35.36
0.840	4.26	19.94	75.25			
0.820	4.71	21.59	81.47			
0.800	5.15	23.13	87.28			
0.753	6.16	26.5	100.00			

[a] Based on data of Robinson and Stokes (1959)

[b] Molality, the number of gram moles per kilogram of water.

Table 2.9: Concentration of glucose and glucose-containing syrups at various levels of water activity (a_w) at 25°C[a]

				Glucose syrups[c] with D.E.[d] values of			
	Glucose		Inverted sugar[b]	32.8	42.0	55.0	83.4
a_w	m[e]	%,w/w	%,w/w	%,w/w	%,w/w	%,w/w	%,w/w
0.995	0.259	4.45	2.05	1.74	1.67	1.57	1.36
0.990	0.542	8.90	4.11	3.48	3.34	3.15	2.73
0.980	1.04	15.74	8.22	6.95	6.68	6.30	5.45
0.960	2.21	28.51	16.43	13.90	13.36	12.59	10.90
0.940	3.38	37.83	24.65	17.33	20.03	18.89	16.35
0.920	4.31	43.72	32.87	20.86	26.71	25.19	21.80
0.900	5.24	48.54	41.09	34.77	33.39	31.49	27.25
0.880	6.27	53.05	49.30	41.73	40.07	37.78	32.70
0.860	7.81	58.45	57.52	48.68	46.75	44.08	38.15
0.850	9.00	61.84	61.63	52.16	50.09	47.23	40.88

[a] Based on data of Taylor and Rowlinson (1955)

[b] Based on data of Grover (1947)

[c] Based on data of Cleland and Fetzer (1944)

[d] D.E., dextrose equivalent.

[e] Molality, the number of gram moles per kilogram of water.

2.7: Minimum activities of solutions (at room temperature)[a]

	Solubility limit (% w/w)	Minimum activity
Sucrose	67	0.86
Glucose	47	0.915
Invert sugar	63	0.82
Sucrose + invert sugar (Sucrose, 37.6%; invert sugar, 62.4%)	75	0.71
NaCl	27	0.74

[a]From Karel (1975)

2.2.2.2 Capillary Effect

A second effect that depresses water activity is the capillary effect. The vapor pressure of water above a curved liquid meniscus is less than that of pure water because of changes in the hydrogen-bonding between water molecules as a result of the surface curvature. Since foods have a myriad of capillaries, some lowering of the a_w should result. The Kelvin equation predicts this lowering by:

$$a_w = \exp - \frac{2\ \gamma_S\ \mathrm{CoS}\theta\ \bar{V}_L}{rRT}$$

Where: γ_S = surface tension of liquid in a pore
θ = wetting angle
$\bar{V}_L$ = molar volume of liquid in cm^3/mole,
r = capillary radius,
R = gas constant (8.314×10^7 ergs/^oK mole), and
T = ^oK

Most pores in foods are in the 10-300 µm range. Assuming complete wetting ($COS\theta = 1$) and pure water (γ_S = 72.3 dyne/cm) in the pores, the Kelvin equation predicts an a_w in the

range of 0.989 - 0.999. Thus, the a_w is lowered very little by capillarity. However, 5-7% of the pore volume in foods is for pores of 0.01 - 0.001 µm, which lowers the a_w above the vapor space to values of 0.899 - 0.34. Thus, smaller capillaries have a greater effect on lowering the a_w and those of small diameter will be the last to empty on drying. In terms of water binding, this phenomenon is used successfully in the Bauman device to measure water uptake of gums, fibers and starches. Note that materials that swell will take up more water because the spore space increases. Yet the a_w will be higher according to the Kelvin equation. This means that the water is less tightly bound on the average as moisture content increases ($a_w \rightarrow 1.0$). The desired ingredient would have a high moisture content at lower a_w (Labuza, 1984).

2.2.2.3 Effect of Hysteresis

A third factor responsible for lowering of water activity can account partially for the fact that hysteresis occurs. A different path is followed depending on whether the isotherm is approached by adsorption or desorption. An example for meat is shown in Fig. 2.3. The desorption isotherm is prepared by incremental drying, and the adsorption isotherm, which is displaced from the desorption isotherm, is prepared by incremental moistening of a previously dried sample. The fact that the two isotherms are not superimposable is an indication that some of the changes during drying irreversibly alter the water solute relationships in the sample. The shape of the isotherms in Fig. 2.2 is qualitatively typical of most foods.

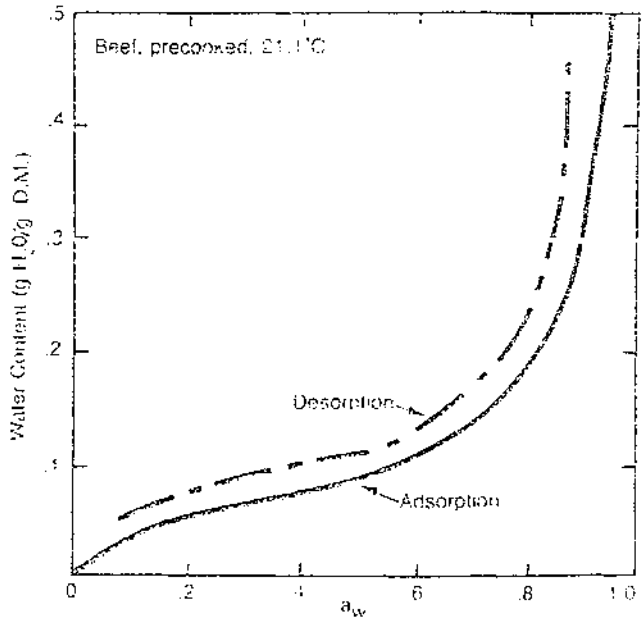


Figure 2.2: Relationship between water content and water activity in meat (From Palnitkar and Heldman, 1971)

2.2.2.4 Effect of Interaction of Water with Solid Surfaces as well as with High Molecular Weight Colloidal Systems

Water molecules usually interact with the polar groups on surfaces and are held very tightly. The energy to remove these water molecules is greater than the energy to vaporize a water molecule from the surface of pure water. If one starts from a dry state, a moisture content-a_w is approached where there is one water bound per polar group to form a monolayer (McLaren and Rowen, 1952). This occurs close to the inflection on the isotherm which gives a monolayer value of about 0.2 a to 0.3 a_w for most foods. Water above this monolayer is usually thought of as being the same as pure water, but long-range effects do occur which structure the water in such a fashion that it is also "bound" to a certain degree to have a lower a_w. Ling (1965) has studied this effect in living tissues and Nemethy (1968) calculated the effect of large molecules such as proteins on structuring water in solutions. Duckworth (1972) demonstrated that

large molecules by themselves can prevent water from freezing at temperature down to -20°C. This was shown to be directly related to the a_w lowering effect on the water (Labuza, 1975).

2.2.3 Water Activity and Microbial Growth

The water activity of a food influences the multiplication and metabolic activity (including toxin production) of microorganisms; also their survival and resistance. This is true not only for organisms that cause spoilage and food-poisoning, but also for those which are desirable for the fermentation of certain foods, such as fermented sausages. Microbial spoilage, food poisoning and fermentation take place if the a_w of the substrate is favourable for the multiplication and metabolic activity of the organisms involved.

The classic article of Scott (1957) summarized the water relations of microorganisms where most of the principles suggested are still valid. These include the following:

1) Water activity, rather than water content, determine the lower limit of available water for microbial growth. Most bacteria do not grow below a_w = 0.91 and most moulds cease to grow below a_w = 0.80. Some xerophilic fungi have been reported to grow at water activities of 0.65, but the range of 0.70 - 0.75 is generally considered their lower limit.

2) Environmental factors affect the level of water activity required for microbial growth. The general principle which often applies is that the less favourable the other environmental factors (nutritional adequacy, pH, oxygen pressure, temperature) the higher becomes

the minimum water activity at which microorganisms can grow.

3) Some adaptation to low activities occurs, particularly when water activity is depressed by addition of water soluble substances (principle of intermediate moisture foods), rather than by water crystallization (frozen foods) or water removal (dehydrated foods).

4) When water activity is depressed by solutes, the solutes themselves may have effects which complicate the effects of water activity *per se*. For instance, at a given water activity, microbial growth is less effectively depressed by glycerol than by sodium chloride.

Subsequent research, especially with freeze-dried and intermediate-moisture foods, have resulted in the following major additional findings (Karel, 1975):

1) Water activity modifies sensitivity of microorganisms to heat, light and chemicals. In general, organisms are most sensitive at high water activities (i.e., in dilute solution) and minimum sensitivity occurs in an intermediate-moisture range.

2) Minimum water activities for production of toxins are often higher than those for microbial growth. This phenomenon may represent an important safety factor in the distribution of dehydrated and intermediate-moisture foods.

Leistner and Rodel (1976) had compiled the minimum water activities below which common microorganisms associated with meats and meat products are inhibited in growth (Table 2.11).

Table 2.11: Minimal a_w for multiplication of micro-organisms associated with meat and meat products

a_w	Bacteria	Yeasts	Moulds
0.98	*Clostridium (1), Pseudomonas**	-	-
0.97	*Clostridium (2)*	-	-
0.96	*Flavobacterium, Klebsiella, Lactobacillus*, Proteus, Pseudomonas*, Shigella*	-	-
0.95	*Alcaligenes, Bacillus, Citrobacter, Clostridium (3), Enterobacter, Escherichia, Proteus, Pseudomonas, Salmonella, Serratia, Vibrio*	-	-
0.94	*Lactobacillus, Microbacterium; Pediococcus, Streptococcus*, Vibrio**	-	-
0.93	*Lactobacillus*, Streptococcus*	-	*Rhizopus, Mucor*
0.92	-	*Rhodotorula, Pichia*	
0.91	*Corynebacterium, Staphylococcus (4), Streptococcus**	-	-
0.90	*Lactobacillus*, Micrococcus, Pediococcus, Vibrio**	*Hansenula Saccharomyces*	
0.88	-	*Candida, Debaryomyces. Hanseniaspora, Torulopsis*	*Cladosporium*
0.87	-	*Debaryomyces**	
0.86	*Staphylococcus (5)*	-	*Paecilomyces*
0.80	-	*Saccharomyces**	*Aspergillus, Penicillium*
0.75	*Halophilic bacteria*	-	*Aspergillus**
0.70	-	-	*Eurotium*
0.62	-	*Saccharomyces**	*Eurotium**

* Some strains; (1), *Clostridium botulinum* type C; (2), *Cl. botulinum* type E and some strains of *Cl. perfringens*; (3) *Cl. botulinum* type A and B and *Cl. perfringens*; (4), anaerobic; (5), aerobic.

Table 2.12 lists the minimal and maximal as well as the modal a_w of fresh meat and representative meat products.

Table 2.12: The a_w range of fresh meat and representative continental meat products[a]

Product	Minimal	Maximal	Modal
Fresh meat	0.98	0.99	0.99
Bologna sausage	0.93*	0.98	0.97
Liver sausage	0.95	0.97	0.96
Blood sausage	0.86+	0.97	0.96
Raw ham	0.80++	0.96	0.92
Dried beef‡	0.80	0.94	0.90
Fermented sausage	0.65 §	0.96 §§	0.91

* Italian Mortadella; + Speckwurst; ++ Country cured ham;
‡ Bundner Fleisch; § Hard sausage: §§ Frische Mettwurst.

[a] From Leistner and Rodel (1976)

It is necessary to have information and/or to update existing information on the water activities at which the different groups of microorganism important for meats are able to multiply or to form toxins, and on the a_w of meat and meat products. This is because based on this information, the a_w of meats could be adjusted and the stability and safety of the products could be improved.

Salting, curing, drying and freezing are traditional processes used to adjust the a_w of meats; and some of these processes are applied in combination.

Inhibition of microorganism in a food is frequently not caused solely by decrease in a_w, but may be influenced by pH, Eh, temperature, preservatives or competitive microflora.

However, one process is briefly described below that is the addition of additives to possibly adjust the a_w of high moisture meats. This process has gained an increased interest in recent decades with the resurgence of interest in IMF food which is brought about by the successful introduction of IMF processing in the world pet food markets.

Leistner and Karan-Djurdjic (1970) (as cited by Leistner & Rodel, 1975) investigated some additives for meats to decrease the a_w. They empirically added different substances to ground meat. Table 2.13 shows the depression of the a_w of meat caused by addition of 1% of additives. The 1% value can be used to calculate the resulting depression of a_w when these additives are used in the usual amount in meat manufacture.

The data in Table 2.13 indicate that sodium chloride was the most effective approved substance for the depression of the a_w of meat. But the present trend to make meat products low in NaCl, conflicts with the approach of adding NaCl to adjust the a_w to an optimal level.

Subsequent research has shown that the possibilities of adjusting the a_w of IMF with most food additives in use today are quite limited. Various less conventional additives have also been explored by various workers looking for compounds which may exhibit strong a_w lowering abilities (Benmergui *et al*., 1979; Chirife *et al*. 1980; Chirife and Ferro Fontan, 1980 a,b). But many of these results have not been successful and still the selection of additives for depressing water activity remains an important area of research.

Table 2.13 Depression of the a_w of meat caused by additives

1% value*	Additive	Depression of a_w caused by using additives at (%)							
		0.1	0.3	2.0	3.0	5.0	10	30	50
0.0100	Lithium chloride+	0.0010	0.0030	-	-	-	-	-	-
0.0062	Sodium chloride	0.0006	0.0019	0.0124	0.0186	-	-	-	-
0.0061	Polyphosphate	0.0006	0.0018	-	-	-	-	-	-
0.0050	1.2-Propylene glycol+	0.0005	0.0015	0.0100	-	-	-	-	-
0.0047	Sodium citrate x 5.5 H_2O	0.0005	0.0014	-	-	-	-	-	-
0.0041	Ascorbic acid	0.0004	-	-	-	-	-	-	-
0.0040	Glucono-δ-lactone	0.0004	0.0012	-	-	-	-	-	-
0.0037	Sodium acetate, cryst.	0.0004	-	-	-	-	-	-	-
0.0033	Sodium hydrogen tartrate	0.0003	-	-	-	-	-	-	-
0.0030	Glyerol+	0.0003	0.0009	0.0060	0.0090	0.0150	0.0300	0.0900	
0.0026	Potassium sorbate+	0.0003	0.0008	-	-	-	-	-	-
0.0024	Glucose	0.0002	0.0006	-	-	-	-	-	-
0.0022	Lactose	0.0002	0.0006	0.0044	0.0066	-	-	-	-
0.0019	Sucrose	0.0002	0.0006	-	-	-	-	-	-
0.0013	Milk protein	0.0001	0.0004	0.0026	0.0039	-	-	-	-
0.00062	Fat	0.0001	0.0002	0.0012	0.0019	0.0031	0.0062	0.0186	0.0310

* Depression of a_w caused by 1% additive. + Not legally permitted in West Germany.

2.2.4 Interaction of Water Activity with Other Factors

It is tempting to consider the influence of water activity as an isolated factor. In reality the a_w of a food interacts with any number of factors in the environment to produce additive microbial inhibition. Generally, as the minimal a_w for growth of the organism is approached, it becomes more sensitive to other inhibitors and inhibitory conditions that might be present in its environment (Troller, 1986).

2.2.4.1 Heat Heat interacts directly with a_w, which is to say that a higher a_w level will limit growth as a minimal or maximal temperature for growth is approached. In terms of lethality, however, a different picture emerges because very low a_w levels are protective as opposed to heating at more elevated a_w levels. In this context, the terms "wet heat" and "dry heat" are used to describe the relative humidity existing at the time of heating. *Salmonella* species are especially heat-resistant in the absence of unbound water (low a_w), especially if sucrose is employed to reduce a_w (Troller, 1980).

Like salmonellae, staphylococci become more heat resistant as the a_w is lowered until at an a_w level between 0.70 and 0.80, resistance begins to decline (Troller, 1986).

2.2.4.2 pH The interrelationships between a_w and pH are shown in Figure 2.3. Generally as the a_w of a food is lowered, the pH limits within which growth will occur are narrowed. These effects have been described by Ohye and Christian (1967) for *C. perfringens* and by Troller (1973) for *S. aureus*. Similar effects occur with yeasts and molds.

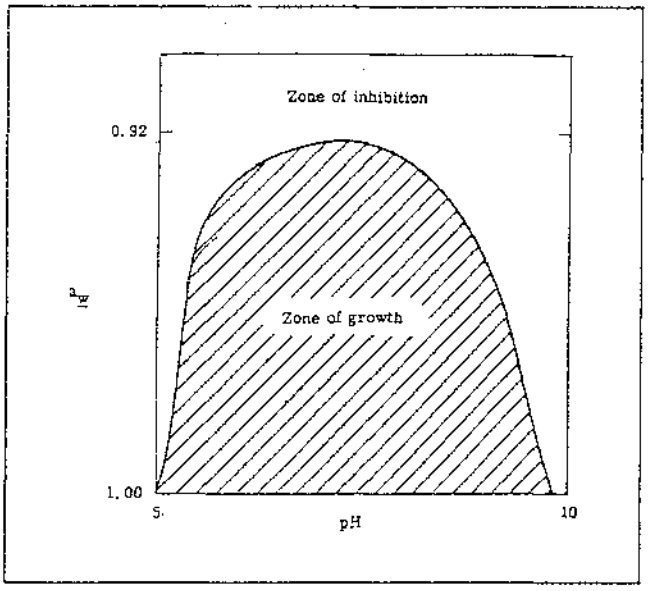


Figure 2.3: Effect of a_w and pH on growth of microorganisms (from Mossell, 1976)

Figure 2.4 represents specifically the stability of meat and meat products as defined in terms of a_w and pH. The figure implies that the combination of a_w and pH is a better predictor of meat stability than a_w alone. Knowing the pH and a_w of a meat product, one can with ease determine from Fig. 2.4 the storage conditions that are most suitable.

It is apparent that any Aw above 0.91, reducing the pH to a value below 5.0 - 5.2 greatly enhances the stability of meat.

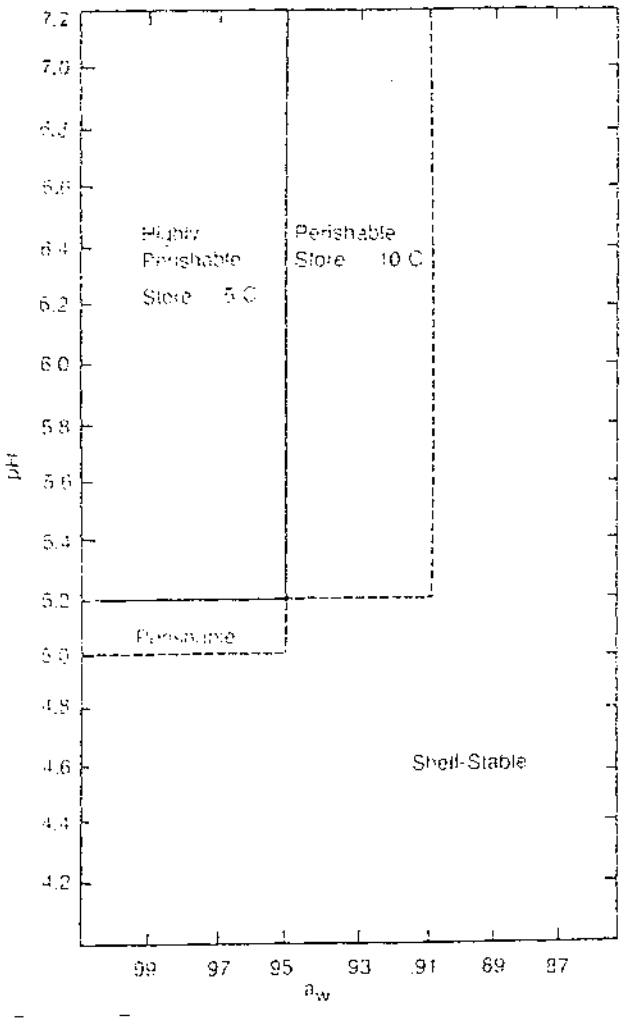


Figure 2.4: Effect of water activity and pH on the stability of meat products (from Leistner and Rodel, 1975)

2.2.4.3 Atmospheres. The minimal a_w at which growth will occur is lower under aerobic than under anaerobic conditions for those organisms capable of growing facultatively. For example, Scott (1953) demonstrated that the minimal a_w at which growth of *S. aureus* occurred was 0.86 under aerobic conditions of 0.92 a_w under anaerobic conditions.

Another example, Silvermann *et al.* (1983) tested an oxygen concentration of 5.5% (comparable to about 50 mmHg of vacuum) for suppression of inoculated populations of *S. aureus* in canned, pre-cooked bacon. They found that the minimal a_w in the inoculated cans was 0.87 at 37°C and 0.91 at 20°C at the decreased O_2 level. This was intermediate to results obtained by Lee *et al.* (1981) when the same strain was grown aerobically (about 20% oxygen) in canned bacon and anaerobically (no oxygen present) in canned bacon. With anaerobic incubation, the minimal a_w for growth was 0.90, whereas an aerobically incubated strain was able to grow at 0.84 a_w.

Blickstad (1984) studied the relationship of reduced water activity and gas headspace composition on the microflora of a minced, cured, raw meat product during storage. The water activity was reduced with NaCl or glycerol + NaCl and the products stored in air, N_2 or CO_2 at 4°C. The results showed that the lower the a_w, the lower was the growth rate of the total microflora except when a_w was reduced below 0.98 with glycerol and products were stored in CO_2, when the growth rate was independent of a_w. In air the final microflora of products stored at $a_w \leq 0.96$ consisted primarily of yeasts while at 0.98 a_w, both *Lactobacillus* (62%) and yeasts (38%) were found. In CO_2 and N_2 Lactibacillus predominated at 0.94 - 0.98 a_w when 2% NaCl or NaCl + glycerol were used. In general, an increase in the shelf-life of minced cured pork is obtained, at least with regard to microbial spoilage, by reducing the a_w of the product particularly for storage in N_2 -

and CO_2 - atmospheres. When combining reduced a_w with CO_2 - storage the type of solute chosen to regulate a_w is important, since the solute may affect the solubility of CO_2. However, CO_2 gives a longer shelf-life than N_2 at similar a_w levels.

2.2.4.4 Chemical preservatives. The role of combined nitrite and reduced Aw (added NaCl) in preserving meat by inhibiting the growth of *C. botulinum* is shown in Figure 2.5. Varga *et al.* (1979) have found that spoilage of salt minced cod which occurs above Aw 0.71 can be halted at 35°C if 0.3% sorbic acid is added to the fish.

The interaction between chemical preservatives and Aw appears not to have been extensively documented to date.

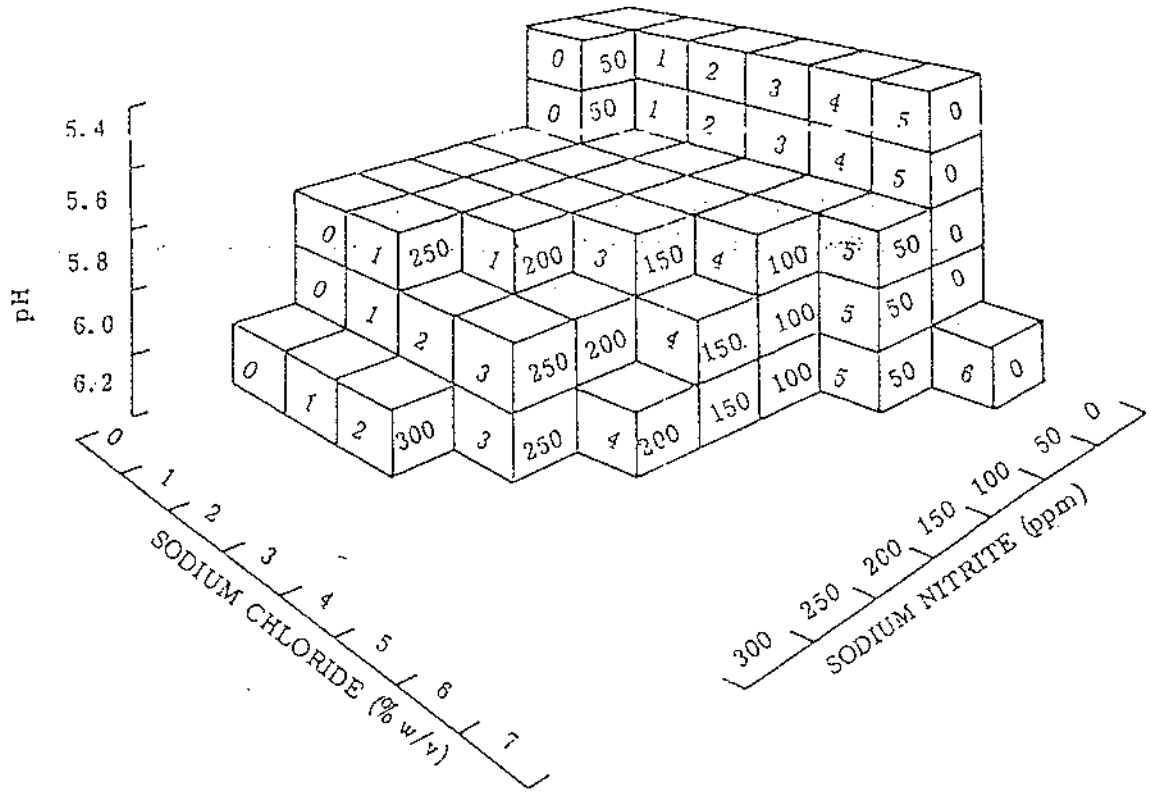


Figure 2.5: Effects of pH, sodium chloride, and sodium nitrite on the growth of Clostridium batulinum (from Roberts and Ingram, 1973)

2.2.5 Effect of Aw on the Chemical Deterioration of Foods

If microbiological problems are eliminated by control of a_w, the storage life of food becomes limited due to chemical reactions. Water influences the chemical stability of foods by serving both as a reactant and a solvent in which dissolved materials are allowed to diffuse and react with each other.

The level of a_w determines the rates of enzymatic reactions, nonenzymatic browning, fatty acid hydrolysis, autoxidation, and other reactions that occur in foods. Reactions depending on solution are retarded at very low a_w and accelerate as a_w is increased due to the increased availability and mobility of reactants. At very high a_w, however, these reactions are retarded again due to the dilution of reactants by the high moisture (Gailani and Fung, 1986).

2.2.5.1 Enzymatic Reaction

Enzymatic reactions can occur in low and intermediate moisture foods when the enzymes have not been inactivated by heating. It has been shown that there is a correlation between the activity of enzymes and the water content of food. Although this correlation is complex, it is best expressed as a function of a_w rather than moisture content (Acker, 1969; Multon and Guilbot, 1975).

That enzymatic reactions can occur even at very low a_w can be explained by the following; first, on molar bases, water is not limiting even in dry foods, therefore reactions that require water as a reactant can proceed under these conditions. Secondly, it has been shown that even under dry conditions, all soluble compounds have substantial mobility (Duckworth and Smith, 1963), therefore reactions that require water as a vehicle for substrate mobility can also proceed under low a_w.

2.2.5.2 NonEnzymatic Browning

Nonenzymatic browning is specially important in IMF because the rate of this reaction usually has a maximum in the range of a_w corresponding to IMF; e.g., 0.65 - 0.85 (Labuza, 1972; Loncin *et al.*, 1968). Nonenzymatic browning through the Maillard Reaction is a reaction of reducing sugars under the influence of either free amino acid or protein side chains leading to darkening, off flavours and loss of solubility of proteins. For long term storage, this also means a reduction in the biological value of the food, as lysine, an essential amino acid, becomes bound to the pigment produced in the reaction and not available for digestion.

The effects of water on this reaction are complex because water can act in one or more of the following roles: a) as a solvent for reactants and for products, (b) as a product of reaction, and (c) as a modifier of the catalytic or inhibitory activities of other substances. Studies of Toribio *et al*. (1984) showed that a maximum nonenzymatic browning rate occured in apple juice concentrate between a_w values of 0.53 to 0.55.

2.2.5.3 Lipid Oxidation

The effect of a_w on the kinetics of lipid oxidation have been reviewed by Labuza *et al*. (1969) and Labuza (1971, 1975).

The major pathways of lipid oxidation are shown in Figure 2.6 and the general influence of a_w is illustrated in Fig. 2.7. As seen, lipid oxidation follows a unique pattern on its dependence on a_w. It is restricted to a minimum rate at moderate a_w values and is accelerated at both too high or too low a_w. At high a_w, lipid oxidation increases because of increased catalyst mobility and because new catalytic

surfaces are exposed as the matrix swells (Fennema, 1976). Decreasing the moisture content beyond a minimum level results in an increased lipid oxidation because water has some antioxidative effects which will be lost upon reducing the moisture content (Labuza *et al.*, 1972).

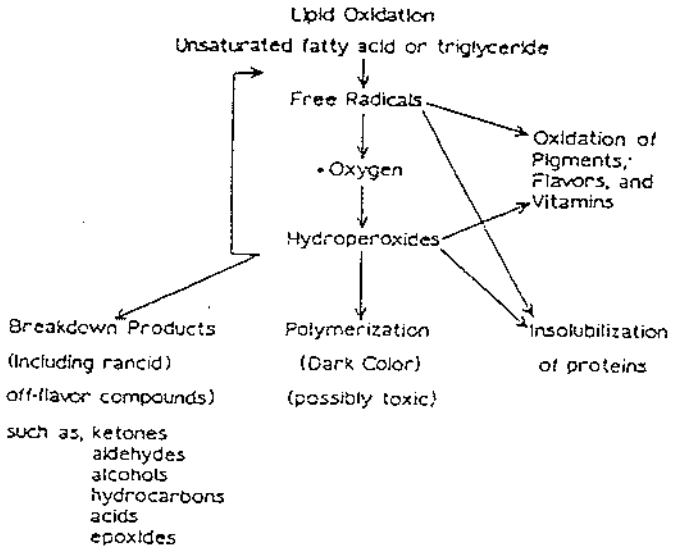


Figure 2.6: Lipid oxidation pathway (from Labuza, 1975)

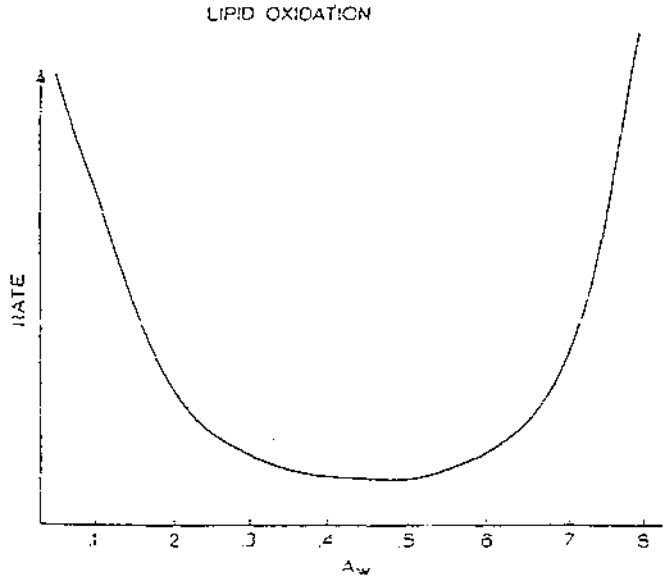


Figure 2.7: Effect of a_w on lipid oxidation rate (from Labuza, 1975)

Over all, as illustrated in Figure 2.8, a stability map can be drawn which directly relates the stability of a food to its Aw (Labuza, 1975).

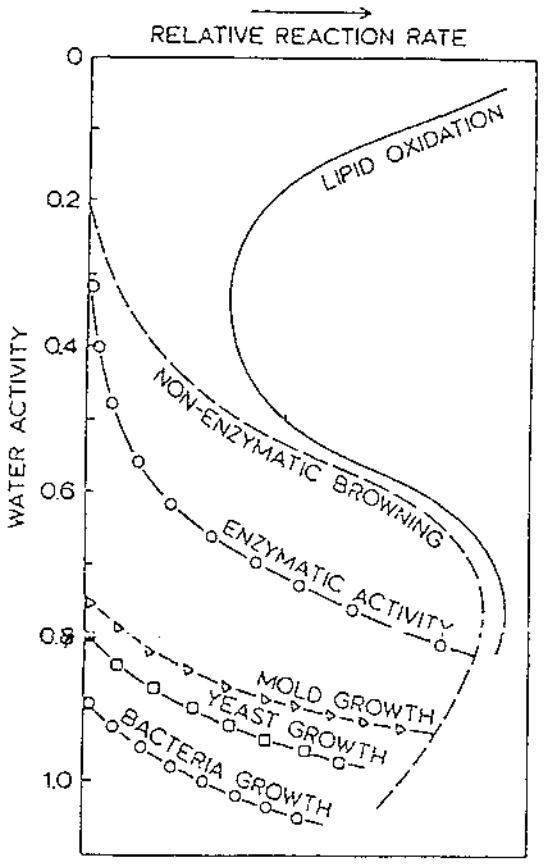


Figure 2.8: Stability map for dehydrated and intermediate moisture foods (from Labuza, 1975)

2.2.6 Predictive Equations for Estimating Water Activity

Sloan and Labuza (1976) studied the applicability of several operations that have been used to predict the final a_w of some model food systems. Of those studied they found that the method suggested by Ross (1975) was a good predictor of final a_w. The Ross equation is of the following form:

$$A_f = A_i \times A_{H1} \times A_{H2} \times A_{H3}, \text{ etc}$$

where A_f is the final a_w of the system, A_i the initial a_w of the food (0.993, for meat, Scott, 1957) and A_{H1}, A_{H2}, etc. are the water activities of the individual humectants at the total water content of the system. Thus the moisture content/unit weight of each humectant is given as:

$$\frac{\text{Weight water in meat + weight water in infusing solution}}{\text{Weight humectant in infusing solution}}$$

and from the desorption isotherm for each individual humectant A_{H1}, A_{H2}, etc. can be determined.

Webster *et al.* (1979) have reported on the reproducibility of the relationship between a_w and water content for lean meats processed by cook-soak equilibration in glycerol/salt solutions and on how well the data fit the Ross equation. They have also described an empirical procedure based on the method first proposed by Grover (1947) for confectionery products that accurately predicts the final a_w of these meats from the composition of the infusing solution.

Grover (1947) suggested that the a_w could be estimated from the relationship:

$$Aw = \frac{ERH}{100} = 104 - 10\ E_s + 0.45\ (E_s)^2$$

In this technique the sucrose equivalent, E_s, of the humectants is found from the equation:

$$E_s = E_1X_1 + E_2X_2 + \text{---} E_nX_n$$

where E_1, E_2, etc are empirical constants for each specific component and

$$X_1,\ X_2,\ \text{etc} = \frac{\text{weight of component in system}}{\text{weight of } H_2O \text{ in the system}}$$

It is apparent that crucial to the success of this equation must be the values assigned to the constants E_1, E_2, E_n.

In the work of Webster *et al.* (1979), they have used E values of 9.0 for NaCl + 2.2 for glycerol in glycerol/salt systems and their results showed excellent agreement between the experimental values of a_w and those predicted by the Grover equation (as shown in Table 2.11). This is within the experimental error associated with the direct measurement of a_w with the Sina - equihygroscope ($\pm$ 0.02).

Webster *et al*. (1979) have noted that in this study all the foods were prepared by desorption and it is well known that adsorption isotherms are often very different from desorption isotherms. Thus for foods prepared by adsorption different E values will apply. They (Webster *et al*, 1979) continued that the final a_w of a system may vary with the method of preparation which explain why Sloan & Labuza (1976) found the Ross equation to be a good predictor of the final a_w of an IM pet food prepared by blending the ingredients while their study shows it to be a poor predictor for cook-soak equilibrated meats.

However, they concluded that provided the above limitations are borne in mind, the modified Grover method can be used with confidence in predicting final a_w values of cook-soak equilibrated meats. They concluded further that the method was

Table 2.14: Comparison of experimentally determined values of a_w for several glycerol infused intermediate moisture foods and the values calculated by the Ross equation and Grover method [a]

Food	% Glycerol in infusing soln.	% Salt in infusing soln.	Ratio Food Soln.	Measured a_w	Calc. a_w 'Ross'	Calc. a_w 'Grover' modified
Beef L. dorsi	-	9.5	0.67	0.95	0.94	0.96
Beef L. dorsi	10	9.5	0.67	0.94	0.91	0.95
Beef L. dorsi	20	9.5	0.67	0.92	0.88	0.93
Beef L. dorsi	30	9.5	0.67	0.91	0.86	0.90
Beef L. dorsi	40	9.5	0.67	0.87	0.83	0.88
Beef L. dorsi	50	9.5	0.67	0.84	0.79	0.84
Beef L. dorsi	60	9.5	0.67	0.79	0.75	0.79
Beef L. dorsi	70	9.5	0.67	0.75	0.69	0.74
Beef L. dorsi	80	9.5	0.67	0.70	0.63	0.68
Beef L. dorsi	90	9.5	0.67	0.62	0.57	-
Beef L. dorsi	36	9.5	0.67	0.88	0.84	0.89
Beef L. dorsi	35	9.5	0.67	0.90	0.83	0.89
Beef L. dorsi	35	0	0.67	0.95	0.93	0.95
Beef L. dorsi	35	5	0.67	0.92	0.89	0.92
Beef L. dorsi	35	10	0.67	0.88	0.84	0.89
Beef L. dorsi	35	15	0.67	0.84	0.80	0.85
Beef L. dorsi	35	20	0.67	0.80	0.76	0.80
Beef L. dorsi	45	0	0.67	0.92	0.92	0.91
Beef L. dorsi	45	5	0.67	0.89	0.87	0.90
Beef L. dorsi	45	10	0.67	0.86	0.82	0.86
Beef L. dorsi	45	15	0.67	0.81	0.77	0.81
Beef L. dorsi	45	20	0.67	0.75	0.72	0.75
Ewe L. dorsi	35	9.5	0.67	0.87	0.83	0.89
Sow L. dorsi	35	9.5	0.67	0.87	0.83	0.89
Goat L. Dorsi	35	9.5	0.67	0.87	0.83	0.89
Lamb	44	7.4	0.57	0.86	0.84	0.89
Ham	39	7.3	0.30	0.85	0.81	0.85
Peas	49	4.9	0.41	0.83	0.83	0.86
Onion	47	6.3	0.45	0.85	0.83	0.86
Green pepper	68	6.0	0.53	0.82	0.76	0.80
Egg noodle	47	6.3	0.49	0.84	0.81	0.85

[a] From Webster *et al.* (1979)

equally applicable to propylene glycol (E value 3.2)/salt systems and sorbitol (E value 1.0)/salt systems.

2.2.7 Intermediate Moisture Food (IMF) Production Techniques

Karel (1976) and Heidelbaugh and Karel (1975) have reviewed the methods used for producing IMF products. Basically they can be divided into three areas i.e., moist or solute infusion, dry infusion, and blending.

2.2.7.1 Moist or Solute Infusion

In moist or solute infusion method, solid food items are soaked and/or cooked in a solution of appropriate concentration so that the final product will have taken up solutes and lost water in amounts to give the desired water activity level and anti-mycotic levels.

Some raw materials which have been processed using this method include: beef, pork, tuna, carrots, macaroni, and celery (Brockman, 1970). The infusion solution for such foods usually contains a combination of glycerol, water, salt and potassium sorbate.

2.2.7.2 Dry Infusion

In dry infusion method, solid food pieces are first dehydrated (generally freeze dried) and then infused by soaking in a solution containing the desired osmotic agents.

Some IMF which have been made by this method include bite-size ready-to-eat cubes of roast beef, roast pork, barbecue beef, barbecue chicken, chicken a la King, beef stew, corned beef, chili with beans, sausages and ham. These products

were prepared by freeze dehydration of the solid ingredients followed by their blending in a low speed mixer and subsequent infusion. The formula of one of these product is shown in Table 2.15. Each product required specialised infusion techniques in regards to sequence of addition and method of addition of ingredients. In general, it was found that 5-10% glycerol, about 5% gelatin and about 3% sorbitol in the infusion solution and 7-12% fat in the dry product provided excellent physical binding properties. This formulation also resulted in an acceptable texture and minimum need for sugar with resultant reduction in browning on storage at 38 ^{o}C. The resulting moisture content and water activities of these products are summarised in Table 2.16 (Karel, 1976).

Table 2.15: Formula of ready-to-eat intermediate moisture roast beef cubes [a]

Ingredients	% by weight
Beef, cooked, ground, freeze-dried	51.00
Water distilled	12.00
Water as steam	4.941 5
Glycerol	6.00
Pregelatinised starch	5.00
Gelatin (100 Bloom)	5.00
Non-dairy coffee whitener	3.50
Sorbitol, dry	3.00
Soup and gravy base, beef flavour	2.50
Sucrose	2.00
Salt	2.00
Hydrolysed vegetable protein, beef	2.00
Onions, dehydrated	0.50
Monosodium glutamate	0.25
Sorbic acid	0.20
Ascorbic acid	0.045
Black pepper	0.040
Ribotide	0.020
Citric acid	0.003 5
Total	100.000 0

[a] From Karel (1976)

Table 2.16: Analysis of examples of intermediate moisture foods

Cubes	Water content (%)	Average percent salt	pH	Water Activity
Roast beef	22.2	3.0	5.75	0.79
Barbecue beef	16.2	2.7	5.05	0.66
Roast pork	22.4	3.6	5.70	0.74
Barbecue chicken	19.7	4.0	5.20	0.70
Chicken a la King	14.9	3.6	5.90	0.61
Beef stew	17.3	3.7	5.80	0.65
Corned beef	16.2	5.4	5.85	0.62
Chili with beans	13.9	2.6	5.65	0.79
Sausage	24.2	4.5	4.90	0.78
Ham	19.9	4.5	5.90	0.72

From Karel (1976)

However, most of the examples cited were formulated specifically for the US defence and space programmes (Erikson, 1982) and had been prepared from freeze-dried solid ingredients. It is obvious that with the freeze-dehydration process, this IMF production technique will be high energy requiring and possibly too complicated for many developing countries to adapt.

2.2.7.3 Blending

In blending technique, the components of the final system are weighed, blended, cooked and/or extruded to give a product of desired water activity.

The blending technique represents the principal method of production of the economically successful intermediate moisture pet foods. A typical intermediate moisture pet food will contain: chopped meat by-products 30-40%, cereal 30-40%, sucrose 15-20%, plus 1-2% of each of such ingredients as: dicalcium phosphate, dried non-fat milk solids, propylene glycol, tallow, mono- and diglyceride, salt, bacteriocides and nutritional supplements. Potassium sorbate is usually added to inhibit yeast and moulds. Colours and flavours are also commonly used. The fresh or frozen meat products may by pasteurized separately or together with sugar, propylene glycol or potassium sorbate prior to addition of the remaining ingredients. An alternative method is to blend all ingredients at one time and pasteurise. The product may be extruded in the form of chunks. A typical ingredient formula for a common intermediate moisture animal food is shown in Table 2.17.

Table 2.17: A typical formula for 'texturised' soft-moist (intermediate moisture) pet food[a]

Ingredients	Percentage
Meat and/or meat by-product	30-70
Sodium caseinate	7.5-25
Sugar	15-30
Propylene glycol	2-10
Starch	0.5-10
Nutritional supplements	1-5
Flavour and colour	as desired

[a] From Karel (1976)

The blending technique has the potential to be developed as the least energy requiring process with its precise levels of moisture (weighed in at the desired final level) and the short-time extrusion cooking. And in this method, ingredients (e.g. additives and humectants) can be mixed uniformly into products at predetermined levels which will be difficult to do in diffusion dependent methods (Flink, 1977).

CHAPTER 3

MATERIALS AND METHODS

3.1 EXPERIMENT 1

The practical combinations of factors that were studied in this experiment were heat treatment (F), pH, water activity (a_w), and redox potential (Eh). Mild heat treatment was chosen because the available literature has indicated that high moisture food products ($a_w > 0.90$) which were the objective of this study, require a mild heat treatment as one of the major controlling factors for stability (Fox and Loncin, 1982). Their work was done in a model product i.e., nutrient broth. The a_w, pH and Eh was chosen because the sufficient decrease of these factors could inhibit the growth of surviving spores of bacilli and clostridia after heat treatment. This could provide the stabilization needed by the product during storage of the product without refrigeration. To be able to identify the practical levels of combinations of these factors was the aim of this experiment.

3.1.1 Design of Experiment

3.1.1.1 Selection of Ingredient

3.1.1.1.1 Humectants

The literature was surveyed to select suitable humectants that could be used in the minced meat mixture to lower the a_w. Criteria for selection were the efficiency of the humectant in lowering the a_w, their taste and cost. Among the humectants considered, sorbitol was chosen because it had the most acceptable taste, had a high level of efficiency in lowering the a_w, and is relatively cheap.

In this experiment the levels of sorbitol that were tried were 5%, 10% and 15%. These levels were chosen following tests which showed that the minced meat product would have a_ws of 0.96, 0.95 and 0.94, respectively. The above levels of sorbitol were also found to have a limited effect on taste in the preliminary sensory evaluation test. The actual a_w achieved in the main experimental samples was variable, due to other factors. This is discussed later.

3.1.1.1.2 Acids

The available acids that can be used to adjust the pH of the mixture are somewhat limited as shown by the literature survey. This is because the amounts and type of acids permitted are carefully controlled by governmental agencies responsible for food safety. Acetic acid was chosen mainly because of its acceptable taste, solubility, and the unrestricted amount that can be used (Appendix 1). Acetic acid is widely known for food preservation particularly in pickling meat, fish and vegetables (Baird-Parker, 1980).

In this experiment the selected pH values were pH 5.5, 5.0 and 4.5. These pH values represent a range of values found in many manufactured meats (see Table 2.2) and go down to the maximum amount of acid which is considered acceptable with respect to taste of this product.

The amount of acetic acid added was calculated from figure 3.1 to give the desired values of pH.

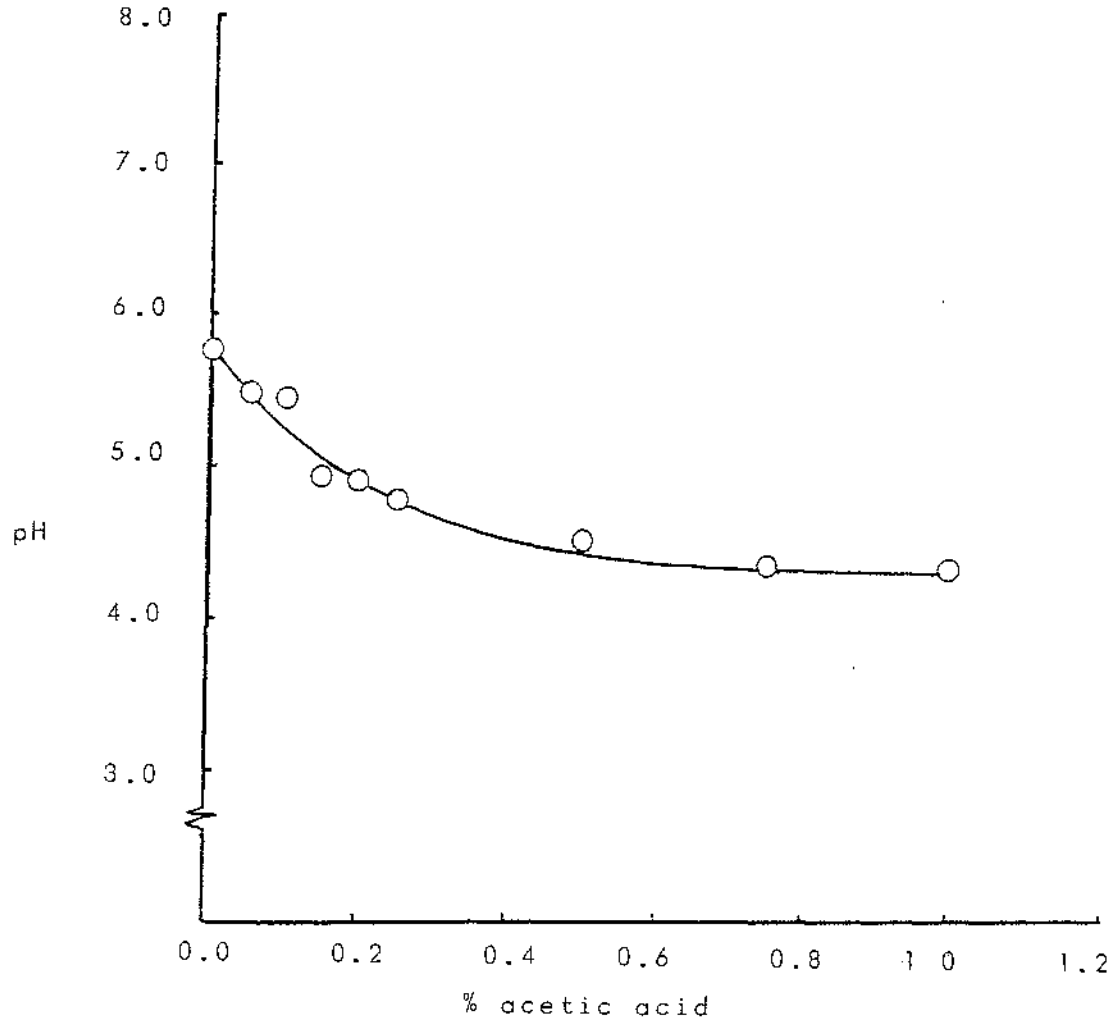


Figure 3.1: Reduction of pH of minced mutton meat by direct addition of glacial acetic acid

3.1.1.2 Factorial Design

To analyse the combinations of factors that could be used for the development of a shelf-stable minced mutton meat product the experimental design selected was a full factorial on 5 variables plus a half-fraction factorial using even higher levels as (x) (Table 3.1).

The experiment was divided into four run-experiments done in blocks per day. Only 4 runs were done as only four water activity readings could be done in a day with four water activity meters available. Each block to be prepared each day was randomized using the random numbers.

The procedures for the preparation of samples with the combinations of factors is summarized in Appendix 3 and an example is shown in Figure 3.2. The treatments that were given are described in detail in the succeeding sections. The figures are shown to illustrate the sequence of treatments which were applied to the minced mutton meat.

Table 3.1: Factorial design

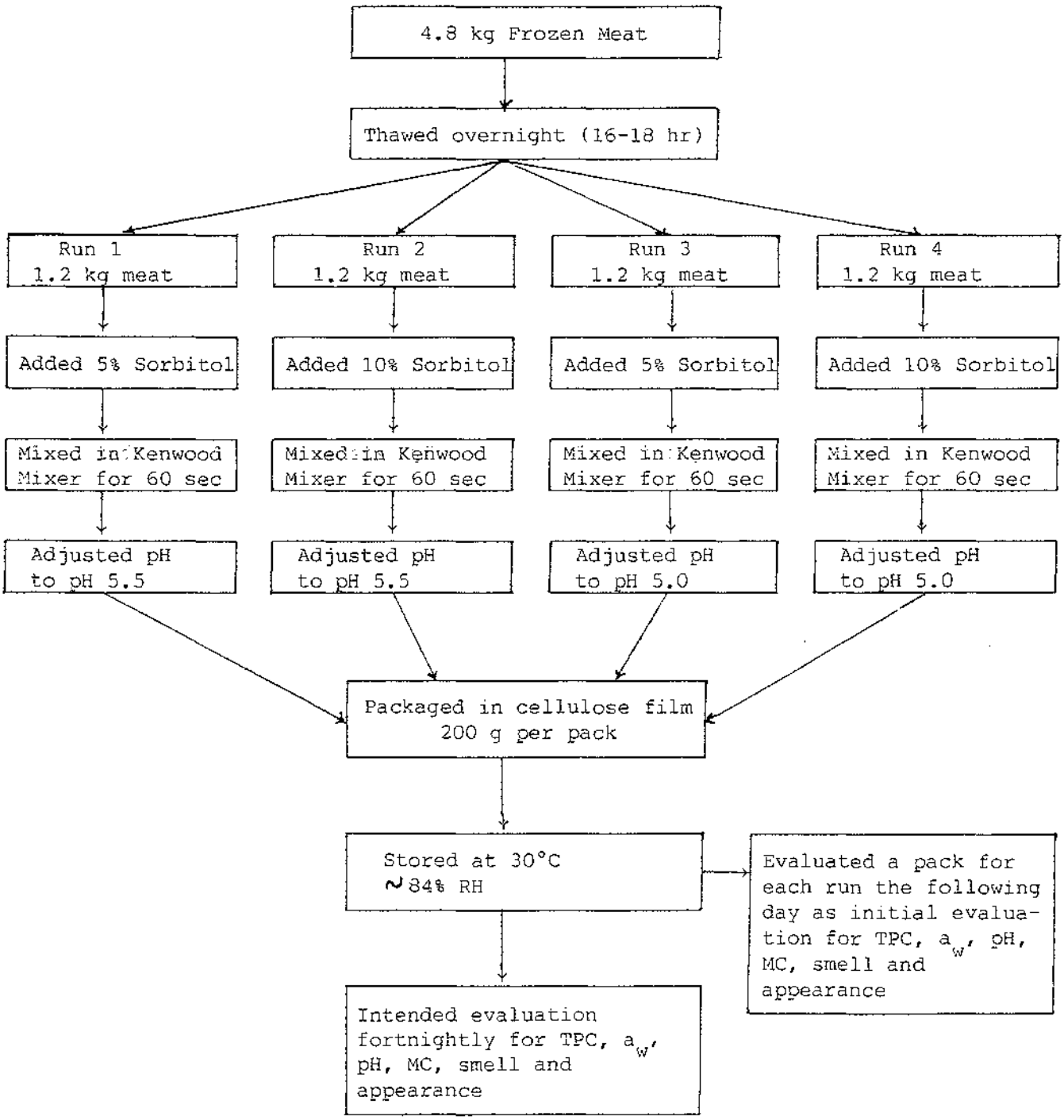
	Variable	− (low level)	+ (high level)	x (higher level)
1.	Level of Humectant (Sorbitol)	5%	10%	15%
2.	pH (Acetic Acid)	5.5	5.0	4.5
3.	Heat Treatment	30°C	50°C	75°C
4.	Moisture Content (Drying)	68-70%	58-60%	48-50%
5.	Packaging Materials	low barrier film (cellulose)	medium barrier film (polythylene)	high barrier film (Aluminium foil)

Run	Variable 1	2	3	4	5
1	−	−	−	−	−
2	+	−	−	−	−
3	−	+	−	−	−
4	+	+	−	−	−
5	−	−	+	−	−
6	+	−	+	−	−
7	−	+	+	−	−
8	+	+	+	−	−
9	−	−	−	+	−
10	+	−	−	+	−
11	−	+	−	+	−
12	+	+	−	+	−
13	−	−	+	+	−
14	+	−	+	+	−
15	−	+	+	+	−
16	+	+	+	+	−

Run	Variable 1	2	3	4	5
17	−	−	−	−	+
18	+	−	−	−	+
19	−	+	−	−	+
20	+	+	−	−	+
21	−	−	+	−	+
22	+	−	+	−	+
23	−	+	+	−	+
24	+	+	+	−	+
25	−	−	−	+	+
26	+	−	−	+	+
27	−	+	−	+	+
28	+	+	−	+	+
29	−	−	+	+	+
30	+	−	+	+	+
31	−	+	+	+	+
32	+	+	+	+	+

Run	Variable 1	2	3	4	5
33	−	−	−	−	x
34	x	−	−	−	−
35	−	x	−	−	−
36	x	x	−	−	x
37	−	−	x	−	−
38	x	−	x	−	x
39	−	x	x	−	x
40	x	x	x	−	−
41	−	−	−	x	−
42	x	−	−	x	x
43	−	x	−	x	x
44	x	x	−	x	−
45	−	−	x	x	x
46	x	−	x	x	−
47	−	x	x	x	−
48	x	x	x	x	x

Figure 3.2: An example of the order of sequence for the preparation of samples, Block 1 Day 11 (Runs 1 to 4)



3.1.2 Preparation of Samples

3.1.2.1 Mincing of Meat

Eighty one kg of chilled boneless mutton meat was obtained from Waitaki International Ltd., Feilding and was transported to Massey University Food Plant under ambient conditions. Visible fat was removed manually using a sharp knife. Meat was chopped manually using a sharp knife into smaller pieces (approximately 10 cm by 15 cm) so that it could be minced. The meat was ground in a Berry Meat Master Junior mincer (Henry Berry Ltd., Palmerston North, New Zealand) and was then mixed for 3 minutes in a Kenwood mixer. Three batches were done as the mixer has the capacity of only 15 kg. About a third of meat from each batch was pooled and this was mixed again for 3 minutes.

Fat content of the three different batches after the second mixing was determined by the modified Babcock method developed MIRINZ (1986) for rapid determination of free fat (Appendix 3). The average fat contents obtained were 6.5%, 6.0%, and 7.5%. This indicated that the batches of minced meat were adequately mixed and were comparatively homogeneous in composition.

The minced meat was then vacuum-packaged in 3 kg portions and was stored frozen at -18^{o}C until required.

3.1.2.2 Thawing of Minced Meat

Individual 3 kg packs of frozen minced mutton meat depending on the amount of meat needed for the day were removed from the freezer. Thawing was done at ambient conditions over-night (16-18 hours) prior to the day of preparation of samples.

3.1.2.3 Addition of the Ingredients

3.1.2.3.1 Sorbitol

Sorbitol was added first directly into a 1.2 kg portion of minced mutton meat and was mixed with a K paddle attachment in a Kenwood Food Processor for 30 seconds. Scraps of meat on the wall of the container and on the paddle were scraped and mixed into the bulk of the meat. The meat was then mixed again for 30 seconds. Although it was realized that the a_w of meat was important to control at the prescribed level of the treatment, this was not possible because of very long time (7 hours) before equilibration of the a_w meter and hence a stable a_w reading. If a_w had to be readjusted, it would take another day before continuing the succeeding treatment. This would greatly affect the microbial load of the meat at the start of storage. Whatever a_w was observed at the level of sorbitol that was added, this was reported.

3.1.2.3.2 Acetic Acid

Acetic acid in the required amount of distilled water for each run was then added directly into the minced meat in one lot. (Addition of distilled water was to maintain the same liquid addition for each run.) The meat sample was mixed in the Kenwood Mixer with a K paddle attachment for 30 seconds. Meat scraps from the walls of the container and paddle were cleaned off and mixed into the bulk of the meat. Another mixing of 30 seconds was done. The pH measurements were made by direct insertion of electrodes. If the pH obtained was with $\pm$ 0.2 pH units of the desired level, no adjustment was made. Otherwise, the pH was adjusted by carefully adding undiluted acetic acid or adding more meat.

3.1.2.4 Drying of Meat

The meat was dried in a tray cabinet dryer at 30°, 50° and 75 °C being the temperatures which correspond to the heat treatments required. The meat was spread about 2cm thick on nylon mesh screen (1.5 mm diameter) on the trays. The meat was mixed twice during drying. The approximate times of drying to reach the required moisture contents under different drying temperatures are shown below. The dryer was set to the same air velocity throughout. The meat temperature was measured by using a Jenway thermometer (Model 1001 with thermocouple attachment (sensor - NiCr/NiAl; Range = -30°C to +450°C)).

Drying Temperature	Required Moisture Content	Approximate Hours of Drying
30°C	58-60%	7
30°C	48-50%	8
50°C	58-60%	4
75°C	48-50%	3

3.1.2.5 Heat Treatment

Meat which needed drying was considered to have received the required heat treatment. Meat which did not need drying (i.e., samples with moisture content of 70%) was heat treated in a water bath of 50°C or 75°C. The meat was stuffed into 130 mm diameter impermeable synthetic casings, sealed by tying with a string, and then heated to achieve a centre temperature of 50° C or 75° C. The meat was heated for 4 and 3 hours to 50° and 75°C core temperatures, respectively. Once the internal temperature reached the prescribed temperature it was heated at that temperature for another 5 min.

The centre temperature was measured by using a Jenway thermometer (Model 1001 with thermocouple attachment (sensor = NiCr/NiAl; Range = -30° C to +450° C)). After heating the meat was cooled by immersing in crushed ice.

The meat samples requiring a 30° C treatment were unheated as all meat samples would be stored at 30° C in the walk-in constant temperature room.

3.1.2.6 Packaging

Meat was packed into 200 g packs in cellulose, polyethylene and aluminium foil packaging films. The meat was heat sealed using a Lindgren vacuum sealer (Lindgren, Sydney, Australia).

3.1.3 Storage Methods

3.1.3.1 Storage Conditions

To simulate ambient conditions in the Philippines, the meat samples were placed inside 6 controlled-humidity cabinets (see Figure 3.3) were stored in a 30 $\pm$ 2^{o}C walk-in constant temperature room. The humidity cabinets were made of stainless steel and measured 30 x 40 x 25 cm in width, length and height, respectively. The front panel of the cabinet which served as the sliding door was made of hard transparent plastic material. The relative humidity inside the cabinet was 84%. Saturated solution of potassium chloride (Appendix 4) was used to control the relative humidity in the cabinets. The actual humidity was checked by putting the lid of the water activity meter ("Aw-Wert Messer" manufactured by Firma, LUFFT, Stuttgart, West Germany) inside the cabinets. The samples were equilibrated (16-18 hr) before the humidity readings were taken.

3.1.3.2 Testing of Samples

The meat samples were to be evaluated fortnightly for pH, moisture content (MC), water activity (a_w), smell, appearance and total plate counts (TPC).

3.1.3.2.1 pH Measurement

For pH measurement, ten grams of minced meat samples were blended with 100 ml distilled water in a waring blender for 60 seconds. Measurements of pH of the homogenate were made using a digital-pH electrode (ORION Research Model 701A/digital ionalyzer). The pH meter was calibrated with buffers at pH 4.0 and 7.0 before measurements.

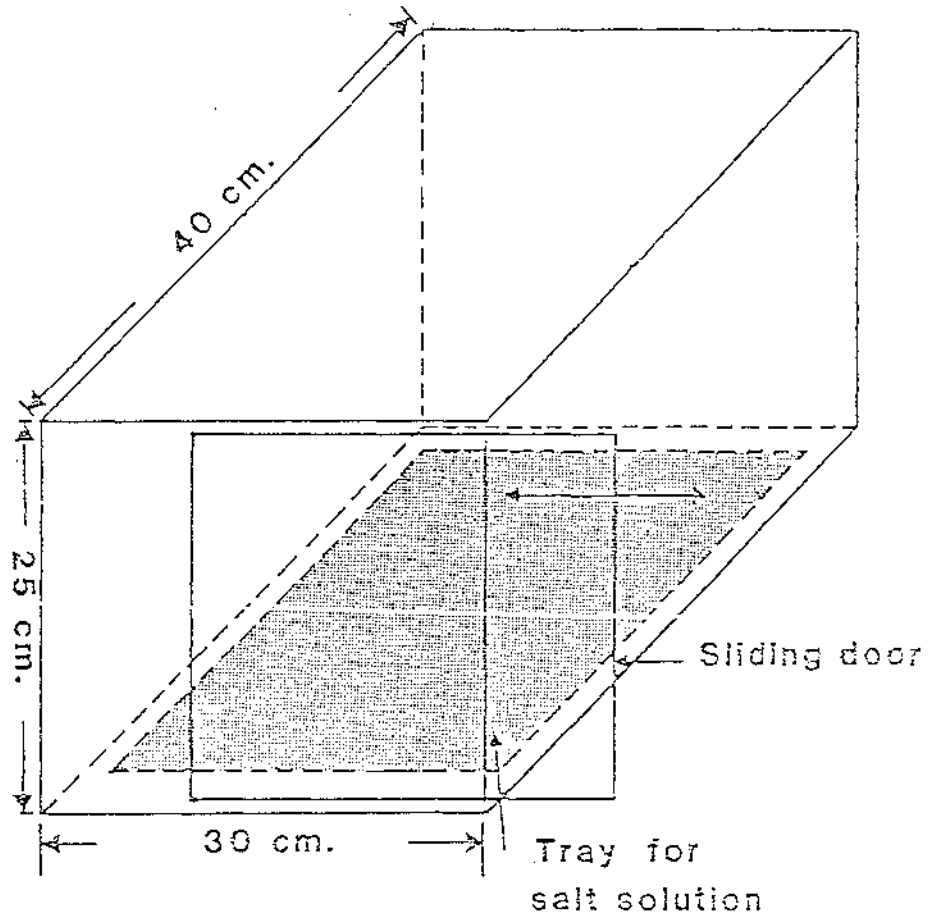


Figure 3.3: Controlled humidity cabinet

3.1.3.2.2 Moisture Content Determination

The AOAC (1980) method -24.003(a) for the determination of moisture content (%) of the meat samples was followed.

3.1.3.2.3 Water Activity (a_w) Determination

Meat samples were equilibrated at 30° C overnight (16-18 hours) before measurement with a dial-type polyamide thread hygrometers, ("a_w-Wert Messer" manufactured by Firma, LUFFT, Stuttgart, West Germany.) According to the manufacturer's recommendations, calibration is performed using a saturated solution of $BaCl_2$ (a_w = 0.89 at 30° C) and adjusting the indicator to this value with a set screw. However, in order to improve the accuracy of a_w measurements, calibration was also performed with other saturated salt solutions. The instruments were checked against ammonium sulphate, potassium chloride, and potassium sulphate solutions with a_ws of 0.79, 0.84, and 0.97, respectively at 30° C. Calibration curves for the four available instruments were obtained as shown in Figures 3.4 to 3.7.

The instruments were kept in a constant walk-in temperature room at 30° C ± 2° C. The instruments were calibrated back with a saturated solution of $BaCl_2$ after each water activity reading of the meat samples.

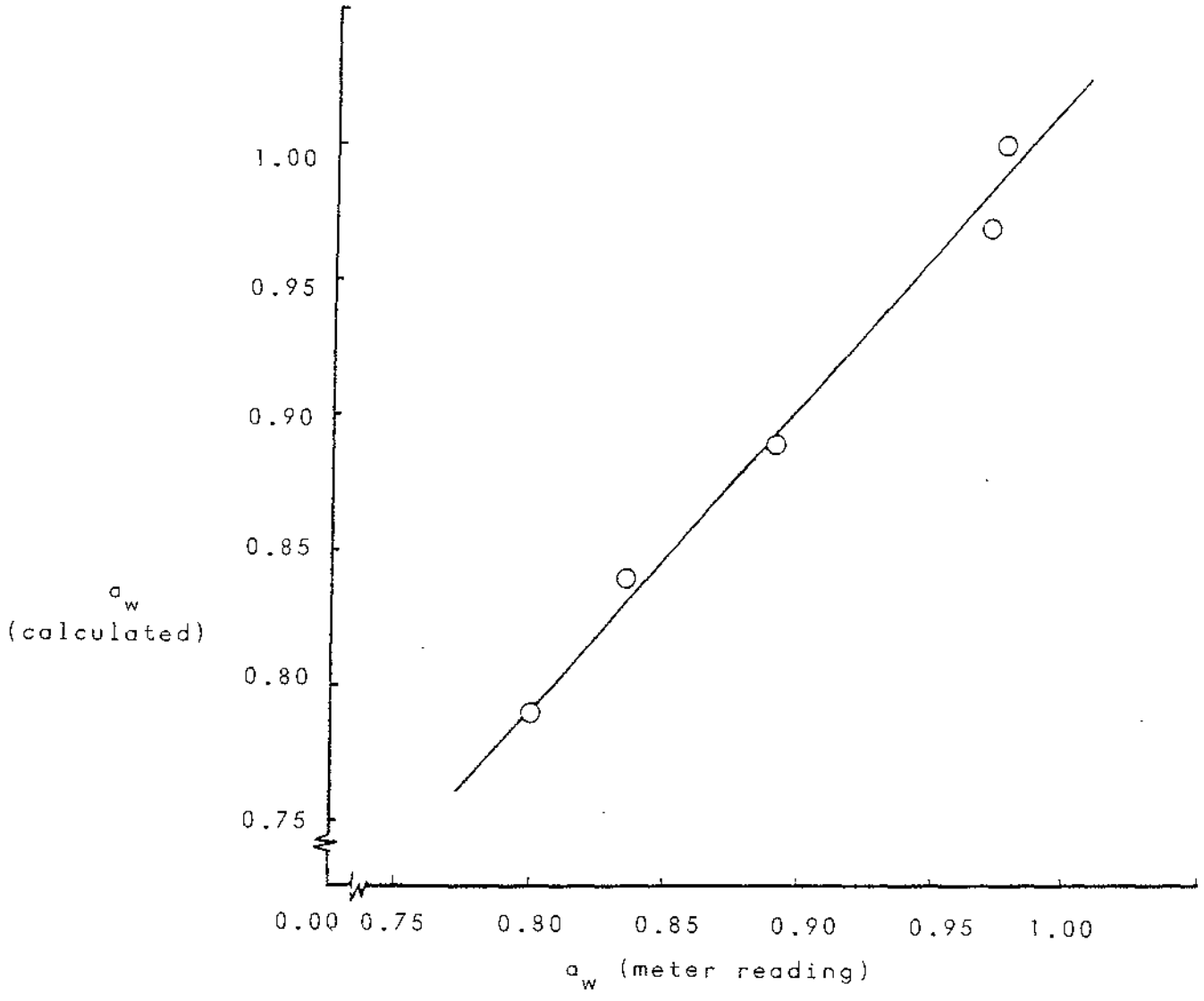


Figure 3.4 : Calibration curve for a_w meter no. 1446 at 30°C

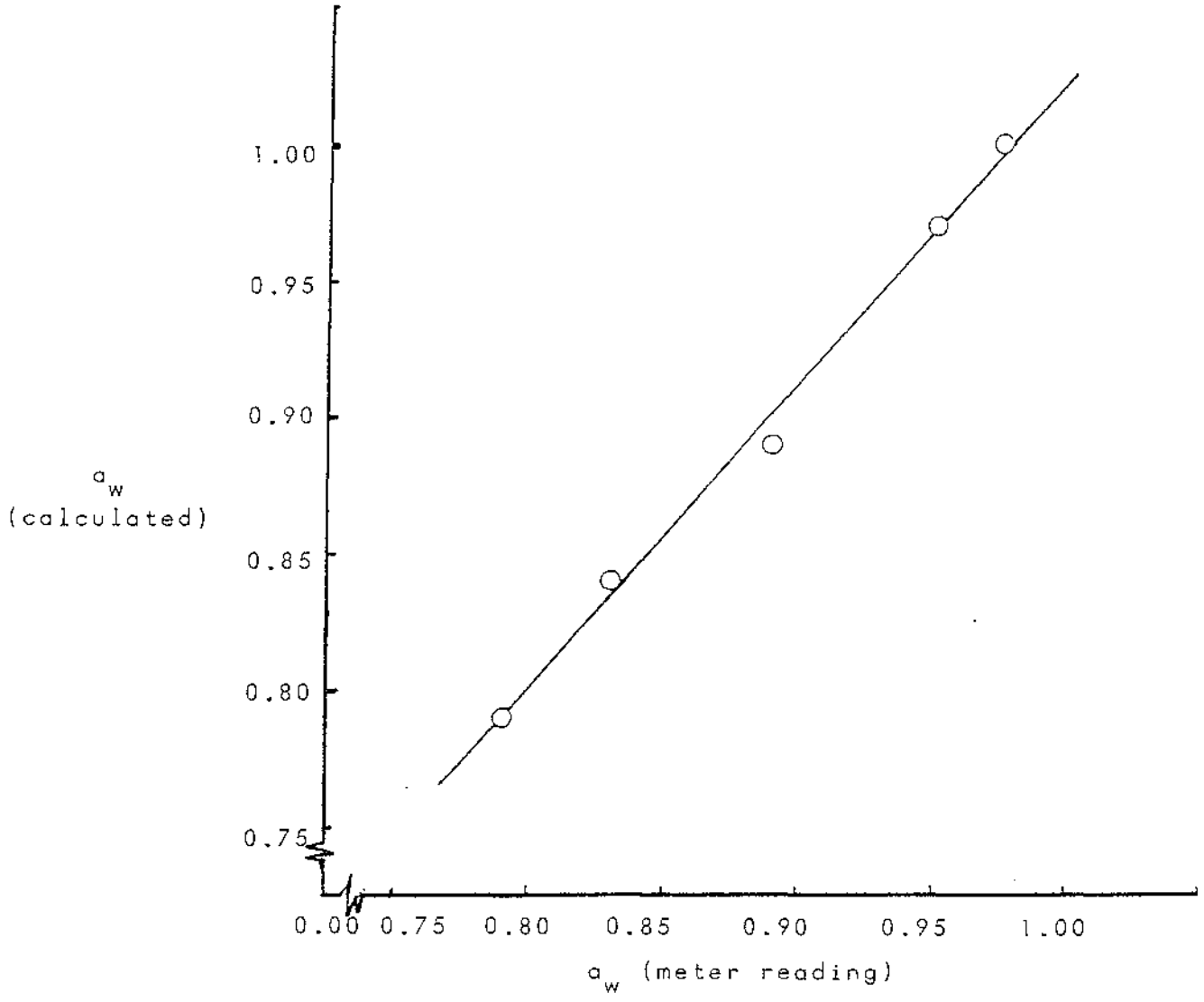


Figure 3.5: Calibration curve for a_w meter no. 1454 at 30°C

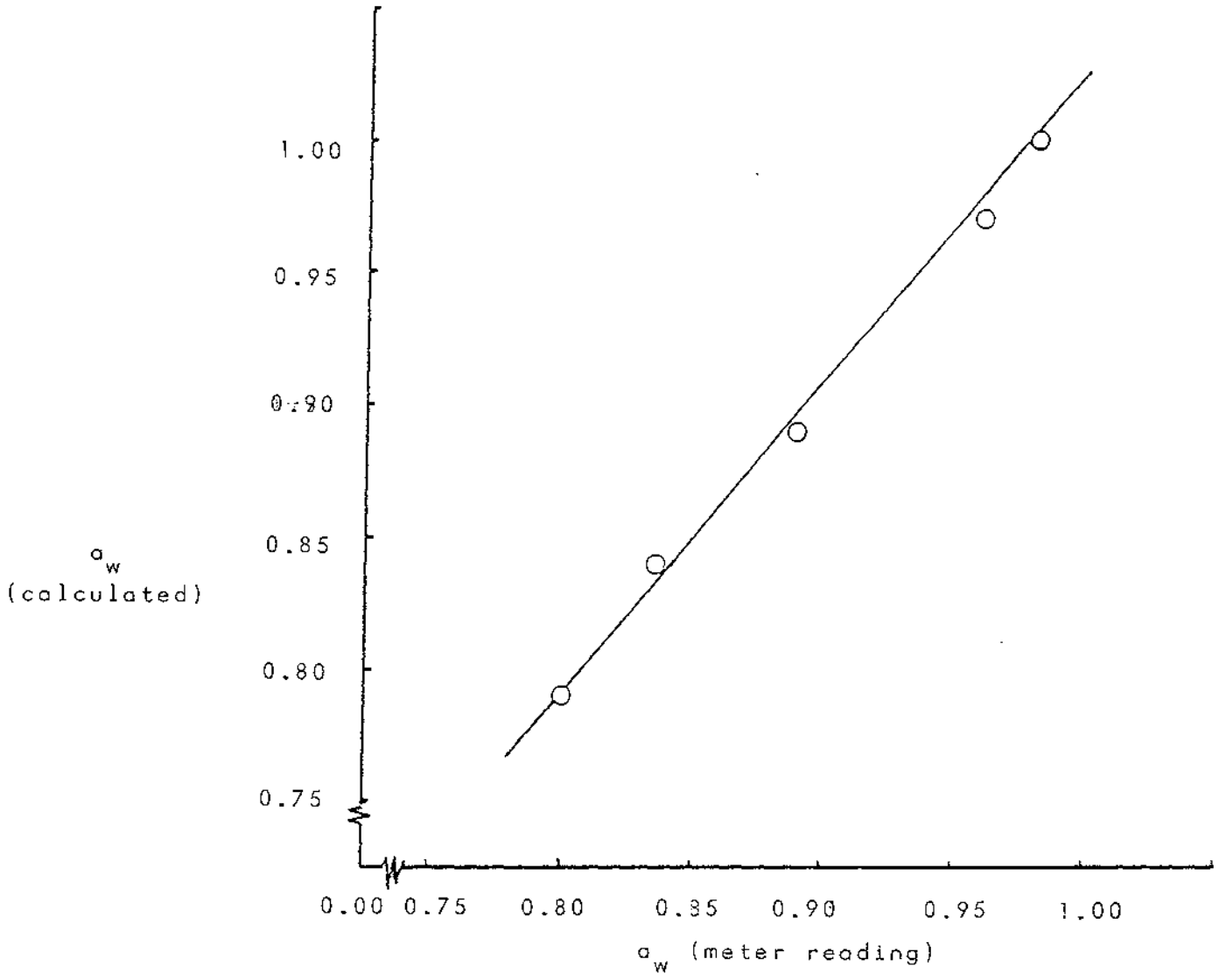


Figure 3.6: Calibration curve for a_w meter no 1433 at 30°C

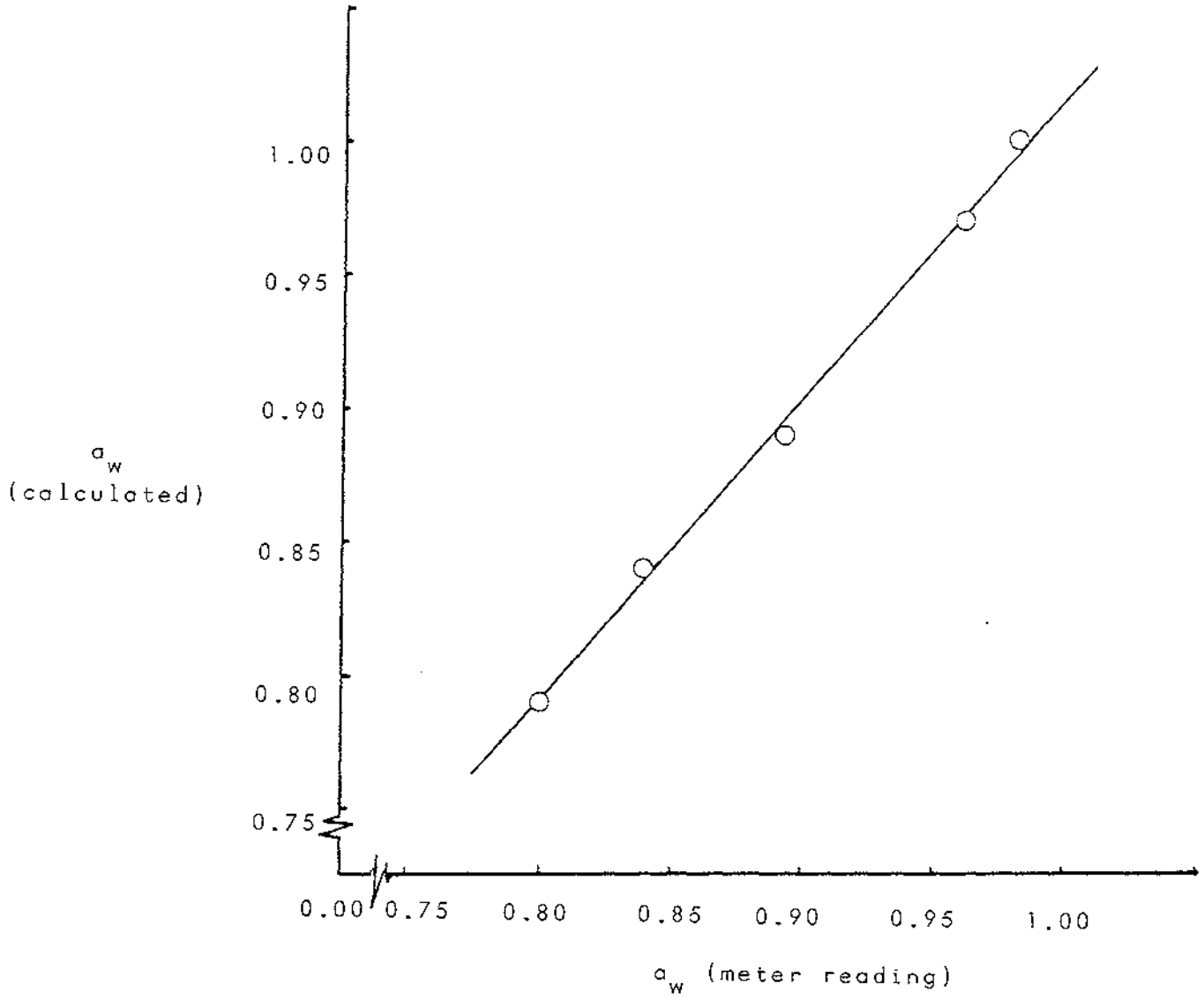


Figure 3.7 : Calibration curve for a_w meter no 1434 at 30°C

3.1.3.2.4 Total Aerobic Counts

Ten grams of minced meat were aseptically weighed into a sterile stomacher bag. Ninety mls of sterile (0.1% (w/v)) peptone were added to the samples. Samples were blended in a Colworth Stomacher (model 400, A.J. Seward, Blackfriars Rd, London, England) for 60 sec and serially diluted in sterile 0.1% peptone.

To determine total numbers of bacteria, samples were plated with molten (45-50^{o}C) plate count agar (PCA) and incubated at 37^{o}C for 48 hr. Analysis was performed in duplicate petri dishes. The peptone used was obtained from BDH Chemicals Ltd., Palmerston North, New Zealand and the plate count agar from BBL Microbiology Systems, Cockeysville, Maryland, U.S.A.

3.1.3.2.5 Smell and Appearance

If any off-odours and/or slime were observed at the time of testing of samples, they were noted.

3.2 EXPERIMENT 2

3.2.1 Design of Experiment

Another 2 blocks of 4-experimental runs were done to verify the limited trends observed in Experiment 1 about the major controlling factors and the practical levels that would extend the shelf-life of minced mutton meat stored at 30°C with about 84% RH.

Based on the results of Experiment 1, pH levels and moisture contents were the major controlling factors responsible for the microbial stability of minced mutton meat. This was examined in greater depth in this experiment. The first block of this experiment was done to investigate the effect of two levels of pH (4.5 and 4.0) and two levels of moisture content (70% and 50%) in extending the shelf-life of minced mutton meat. The possible controlling effect of reducing the a_w below 0.95 by the addition of more (20%) sorbitol had not been established. Addition of more sorbitol was considered because the levels used had not been successful in consistently lowering the a_w below 0.95. 20% sorbitol was set as the maximum level that could be added without producing two sweet a meat product which would be less acceptable. Hence in this block, 20% sorbitol was added in all samples.

The second block of this experiment investigated the effect of two storage temperatures (30° and 20°C) and two pH values (pH = 4.5 and 4.0) and on the shelf-life of minced mutton meat. 0.1% potassium sorbate was added to all samples because initial results obtained in Experiment 1 and in the first block of this experiment showed that the growth of moulds interfered in the evaluation of the effect of the variables being considered. All samples had 20% sorbitol and 50% moisture content.

3.2.2 Preparation of Sample

3.2.2.1 Minced Meat

Minced meat samples were taken from the same lot of meat prepared in Experiment 1.

3.2.2.2 Thawing of Meat

The same thawing procedure as in Experiment 1 was followed.

3.2.2.3 Addition of the Ingredients

Sorbitol and acetic acid were added in the same manner as in Experiment 1. In the second block of this experiment, potassium sorbate was added at the same time as sorbitol.

3.2.2.4 Drying of Meat

The same procedure for drying the meat as in Experiment 1 was followed except that drying was only done at 30° C temperature to reach 48 - 50% moisture content.

3.2.2.5 Packaging

Meat was only packaged in polyethylene bags in 200 g packs.

3.2.3 Storage Methods

The same storage methods as in Experiment 1 were applied in this experiment except that the testing of samples was done every week instead of every fortnight. This was because it was observed in the first experiment that most of the meat samples had deteriorated at some period less than two weeks.

3.3 EXPERIMENT 3

3.3.1 Design of Experiment

A 3 x 5 full factorial design based on 2 storage temperatures (30°C and 20°C) was used. Five levels of acetic acid (1.0, 2.5, 5.0, 7.5 and 10.0%), and three levels of sorbitol (0, 15 and 20%) were used.

Levels of acetic acid instead of pH values were considered in this experiment in order to achieve the same number of mixing and mixing time for every sample. This was considered to be as important in obtaining the same microbiological level at the start of storage. However, it was found that the pH values obtained corresponding to the different levels of acetic acid used were within acceptable limits. This is shown in Table 3.2.

Table 3.2: Lowering of pH of minced mutton meat by addition of acetic acid

	Level of Acetic Acid (%)				
Replicate	1.0	2.5	5.0	7.5	10.0
1	4.38	4.08	3.76	3.63	3.54
2	4.36	4.03	3.78	3.60	3.49
3	4.38	4.10	3.77	3.60	3.54
4	4.39	4.05	3.80	3.68	3.56
5	4.47	4.14	3.85	3.63	3.54
6	4.46	4.08	3.83	3.61	3.52
Mean ($\bar{X}$)	4.41	4.08	3.80	3.62	3.53
Range (R)	0.11	0.11	0.09	0.08	0.07
Standard Deviation (σ_{n-1})	0.05	0.04	0.04	0.03	0.02

3.3.2 Preparation of Sample

3.3.2.1 Mincing of Meat

The same procedure as in Experiment 1 was followed but instead of chilled meat, frozen boneless mutton meat was used. This was the only available meat at the time of purchase from Waitaki International Ltd., Feilding.

3.3.2.2 Thawing of Meat

The same procedure as in Experiment 1 was followed.

3.3.2.3 Addition of Ingredients

The same procedure was followed in adding sorbitol but in the addition of acetic acid, no attempt was made to readjust the pH levels. This was done to have consistent mixing for all samples. Hence, whatever the pH values obtained for the particular levels of acetic acid considered, this was reported.

3.3.2.4 Packaging

Meat was packaged in polyethelene bags in 200 g packs. Only polyethylene film was used in this experiment as it was shown in Experiment 1 that the use of high barrier film such as aluminium foil film did not appear to extend the shelf-life of minced mutton meat. Packages were heat sealed by Lindgren vacuum sealer (Lindgren, Sydney, Australia).

3.3.3. Storage Methods

In this experiment, two storage temperatures (30^{o} and 20^{o}C) were considered. Meat samples were stored at either 30 $\pm$ 2^{o}C or 20 $\pm$ 2^{o}C walk-in constant temperature rooms.

The same testing procedures as in Experiment 1 were done except for a_w measurements and periods of sampling. The a_w measurements were not taken in this experiment because the water activity meters broke down. Sampling was performed at 0, 14, 28 and 56 days of storage.

3.3.4 Taste Panel Test

Taste panel tests were designed to determine the acceptability of minced mutton meat with reduced pH with regards to its sensory properties. Minced mutton meat was prepared with and without sorbitol, salt and spices.

Four or five Filipino panelists, depending on their availability, were asked to taste the products on two separate occasions. They were initially briefed on the terms and scales that were to be used in the questionnaire and sensory values to assess. They were informed of the objective of the test which was to be able to describe the flavour of an acidified meat product and to determine its acceptability. Descriptive scoring was used in this test. The questionnaire used is included in the Appendix 5.

Samples for flavour evaluations were prepared in accordance with AMSA guidelines (1978). Uniform patties were formed by shaping 80.0 $\pm$ 0.5 g of minced mutton meat in standard size (60 mm x 15 mm) disposable petri dishes. After forming, samples were pan fried in an electric skillet which was preheated and maintained at the same temperature setting during cooking. Patties were cooked for 4 min on each side (well done), cut into 8 bite size wedges and served immediately on white china plates. Panelists were instructed to rinse their mouth with water in between samples when needed.

The panelists were presented with two coded samples on each occasion. In the first evaluation, the samples presented were mutton meat patties with two levels of acetic acid i.e., 1.0% and 2.5% corresponding to pH = 4.4 and 4.1, respectively. In the second evaluation, the samples presented were mutton meat patties with the same two levels of acetic acid but other ingredients were added. The other ingredients added were 20% sorbitol, 1.75% salt, 1.0% garlic powder, and 1.0% ground black pepper. The panelists were

asked to indicate on the questionnaire their description of the sour flavour intensity and the acceptability of the samples. A 5-point scale was used for the assessment - 5: very acceptable, 4: acceptable, 3: slightly acceptable, 2: unacceptable, 1: very unacceptable. Scores for sour flavour intensity were 5: none, 4: slightly noticeable, 3: recognizable, 2: pronounced, 1: very pronounced.

After evaluation the scores were added and averaged. An average score of 3 for both criteria was considered an acceptable product.

CHAPTER 4

RESULTS

4.1 EXPERIMENT 1

Tables 4.1 and 4.2 show the summary of the effect of various combinations of preservative factors on the shelf-life of minced mutton meat stored at 30 ^{o}C with about 84% relative humidity (RH). The combinations of factors studied were three levels of sorbitol (5.0, 10.0 and 15.0%), three pH values (5.5, 4.5 and 4.0), three heat treatments (30^{o}, 50^{o} and 75^{o} C), three levels of moisture content (70, 60 and 50%) and three types of packaging material (cellulose, polyethylene and aluminium foil films). The standards for end of shelf-life were based on an increase in number of microorganisms (TPC), changes in pH and development of off-odours. Samples were to be evaluated fortnightly but this proved to be too long for samples had spoiled, as indicated by either presence of mould growth and/or detection of foul smell (putrefied odour), even before the second evaluation. Exceptions were runs 42, 43, 47 and 48. Because of the obvious putrefaction and/or presence of mould growth on samples, it was unnecessary to do all the testing methods (TPC, a_w, pH, moisture content) on all samples. Hence, the shelf-life determination was initially based on physical observations of the meat samples.

A shelf-life of more than 14 days but less than 28 days was observed in runs 42, 43, 47 and 48. The specific combinations of factors involved in these runs are enumerated below (extracted from Table 4.2).

Table 4.1: Shelf-life of minced mutton meat during storage at 30°C with various combinations of factors

Run	Combination of Treatments					TPC (37°C) (log_{10} colonies per gram After 16-18 hr storage at 30°C	Shelf-life (days)
	Level Sorbitol (%) (a_w)	pH Required (Actual)	Heat Treatment (°C)	Moisture Content (%) Required (Actual)	Packaging Material		
5	5 (0.96)	5.5 (5.31)	50	70 (74.3)	Cellophane	-	< 7
6	10 (0.97)	5.5 (5.31)	50	70 (74.4)	Cellophane	-	< 7
7	5 (0.95)	5.0 (4.78)	50	70 (74.6)	Cellophane	3.67	< 7
8	10 (0.96)	5.0 (4.76)	50	70 (72.5)	Cellophane	6.72	< 7
9	5 (0.99)	5.5 (5.26)	30	60 (63.4)	Cellophane	5.52	< 14
10	10 (0.96)	5.5 (5.28)	30	60 (63.1)	Cellophane	4.92	< 14
11	5 (0.97)	5.0 (4.83)	30	60 (63.9)	Cellophane	3.28	< 14
12	10 (0.96)	5.0 (4.92)	30	60 (62.8)	Cellophane	3.38	< 14
13	5 -	5.5 (5.50)	50	60 (58.8)	Cellophane	6.38	< 14
14	10 (0.99)	5.5 (5.49)	50	60 (58.6)	Cellophane	6.40	< 14
15	5 -	5.0 (5.23)	50	60 (60.4)	Cellophane	5.00	< 14
16	10 (0.98)	5.0 (5.18)	50	60 (58.5)	Cellophane	4.78	< 14
21	5 (0.97)	5.5 (5.27)	50	70 (70.8)	Polyethylene	4.92	< 14
22	10 (0.97)	5.5 (5.29)	50	70 (72.4)	Polyethylene	4.88	< 14
23	5 (0.97)	5.0 (4.79)	50	70 (73.8)	Polyethylene	3.34	< 14
24	10 (0.97)	5.0 (4.78)	50	70 (71.5)	Polyethylene	2.93	< 14
25	5 (0.97)	5.5 (5.28)	30	60 (59.3)	Polyethylene	3.92	< 7
26	10 (0.97)	5.5 (5.06)	30	60 (59.6)	Polyethylene	3.87	< 7
27	5 (0.97)	5.0 (4.99)	30	60 (59.6)	Polyethylene	3.84	< 7
28	10 (0.97)	5.0 (4.94)	30	60 (60.0)	Polyethylene	3.99	< 7
29	5 (0.96)	5.5 (5.63)	50	60 (57.4)	Polyethylene	6.00	< 7
30	10 (0.96)	5.5 (5.56)	50	60 (56.6)	Polyethylene	5.20/	< 7
31	5 (0.96)	5.0 (5.24)	50	60 (57.4)	Polyethylene	5.04	< 7
32	10 (0.96)	5.0 (5.24)	50	60 (57.0)	Polyethylene	5.04	< 7

(-) not determined

Table 4.2: Shelf-life of minced mutton meat during storage at 30°C with various combinations of factors

Run	Combination of Treatments					TPC (37°C) (log_{10} colonies) per gram After 16-18 hr storage at 30°C	Shelf-life (days)
	Level Sorbitol (%) (a_w)	pH Required (Actual)	Heat Treatment (°C)	Moisture Content (%) Required (Actual)	Packaging Material		
33	5 (0.96)	5.5 (5.33)	30	70 (69.5)	Al foil	4.61	< 7
34	15 (0.98)	5.5 (5.32)	30	70 (67.8)	Cellophane	4.08	< 7
35	5 (0.98)	4.5 (4.35)	30	70 (70.6)	Cellophane	2.40	> 7 <14
36	15 (0.97)	4.5 (4.30)	30	70 (70.0)	Al foil	2.00	> 7 <14
37	5 (0.96)	5.5 (5.26)	75	70 (72.8)	Cellophane	6.18	<14
38	15 -	5.5 (5.28)	75	70 (68.0)	Al foil	3.63	<14
39	5 (0.98)	4.5 (4.47)	75	70 (72.2)	Al foil	2.00	<14
40	15 -	4.5 (4.41)	75	70 (70.6)	Cellophane	3.75	<14
41	5 (0.97)	5.5 (5.28)	30	50 (50.8)	Cellophane	4.96	<14
42	15 (0.95)	5.5 (5.32)	30	50 (50.5)	Al foil	5.49	>14 <28
43	5 (0.96)	4.5 (4.58)	30	50 (51.7)	Al foil	5.26	>14 <28
44	15 (0.95)	4.5 (4.44)	30	50 (51.7)	Cellophane	5.23	> 7 <14
45	5 (0.96)	5.5 (5.68)	75	50 (49.1)	Al foil	5.34	> 7 <14
46	15 (0.95)	5.5 (5.71)	75	50 (48.7)	Cellophane	5.54	<14
47	5 (0.96)	4.5 (4.76)	75	50 (49.0)	Cellophane	3.23	>14 <28
48	15 (0.93)	4.5 (4.79)	75	50 (48.5)	Al foil	3.23	>14 <18

Run	Combination of Treatment					Shelf-life (days)
	Level of Sorbitol (a_w)	pH	Heat Treatment (^{o}C)	Moisture Content (%)	Packaging Material	
42	15(0.95)	5.32	30	50.5	Al foil	>14 < 28
43	5(0.96)	4.58	30	51.7	Al foil	>14 < 28
47	5(0.96)	4.76	75	49.0	Cellophane	>14 < 28
48	15(0.93)	4.79	75	48.5	Al foil	>14 < 28

A shelf life of more than 7 but less than 14 days were observed in runs 35, 36, 44, and 46. The specific combinations of factors involved in these runs are enumerated below (extracted for Table 4.2).

Run	Combination of Treatment					Shelf-life	
	Level of Sorbitol (a_w)	pH	Heat Treatment (^oC)	Moisture Content (%)	Packaging Material		
35	5(0.98)	4.35	30	70.6	Cellophane	>7	<14
36	15(0.97)	4.30	30	70.0	Al foil	>7	<14
44	15(0.95)	4.44	30	51.7	Cellophane	>7	<14
46	15(0.95)	5.71	75	48.7	Cellophane	>7	<14

However, it is possible that those runs (possibly 37 to 41, and 45) which were evaluated only after 14 days had a shelf-life also in between 7 and 14 days. Nevertheless, the above observations were still given to show the combination of factors which were already identified in this experiment that would give a shelf-life of more than 7 but less than 14 days.

The runs which had a shelf-life of less than 7 days were Runs 5 to 8, and 25 to 34. All of these runs except Runs 33 and 34 had combinations of factors at levels which were referred here as at "low" (-) and "high" (+) levels (see Table 3.1). Runs 33 and 34 had one variable at a level which was referred here as at a "higher" level (x). The specific combinations of factors and the values involved are shown in Tables 4.1 and 4.2.

Based on above results it was unnecessary to do the last 2 blocks of the experiment (Runs 1 to 4 and 17 to 20) which would have the same levels of treatments as Runs 5 to 8 and 25 to 32 but only with different combinations of factors.

However, multiple linear regression analysis was done to describe the relationship between the total plate count (37^oC) after 16-18 hours in storage of minced mutton meat

at 30°C and the different preservative factors being considered. This was done to determine which among the various factors had the greatest effect on the microbiological stability of minced mutton meat during storage at 30°C. Hence, these factors would be worth retaining to examine in greater depth in succeeding experiments. The TPC was used as the dependent variable as it was the only quantitative data taken for all the samples after they had been in storage for the same fixed of time.

The results of the regression analysis from the data extracted in Tables 4.1 and 4.2 are given in Tables 4.3 and 4.4, respectively. Table 4.3 shows that TPC's were highly affected by pH and heat treatment among the factors that were considered. Table 4.4 shows that TPC's were most affected by pH and moisture content.

Table 4.3: Multiple linear regression analysis describing the relationships between the total plate count (37°C) after 16-18 hours of storage of minced mutton meat at 30°C and the various preservative factors (n = 22)[a]

Predictor (Preservative Factor)	Coefficient	Standard deviation of coef	T-ratio = Coeff/S.D.
Constant	4.3904	0.2162	20.31
Level of sorbitol	0.0545	0.1673	0.33
pH	-0.5027	0.1712	-2.94*
Heat treatment	0.6950	0.1962	3.54*
Moisture content	0.3946	0.2162	1.83
Packaging material	-0.3394	0.1712	-1.98

[a] Data extracted from table 4.1

* Significant at 5% level

R^2 = 60.9%

Table 4.4 Multiple linear regression analysis describing the relationships between the total plate count (37°C) after 16-18 hours of storage of minced mutton meat at 30°C and the various preservative factors (n = 16)[a]

Predictor (Preservative Factor)	Coefficient	Standard deviation of coef	T-ratio = Coeff (S.D.)
Constant	4.1831	0.2496	16.76
Level of sorbitol	-0.0644	0.2496	-0.26
pH	-0.7956	0.2496	-3.19*
heat treatment	-0.0706	0.2496	-0.28
moisture content	0.6019	0.2496	2.41*
packaging material	-0.2381	0.2496	-0.95

[a] Data extracted from Table 4.2

* Significant at 5% level

R^2 = 63.0%

Table 4.5 (extracted from Tables 4.1 and 4.2) summarizes the water activities of minced mutton meat which were observed based on the three different levels of sorbitol that were added. The addition of 5, 10 and 15% sorbitol resulted average a_w's of 0.967, 0.967 and 0.955, respectively. However, the water activity range obtained for each sorbitol level was observed to be large. The addition of 5% sorbitol gave a range of water activities from 0.95 to 0.99; 10% sorbitol from 0.96 to 0.99; and 15% sorbitol from 0.93 to 0.98.

Table 4.5: Water activity of minced mutton meat based on three levels of sorbitol addition

Level of Sorbitol (%)	No. of Sample	Mean	Minimum	Maximum	Standard Deviation ($_{n-1}$)
5	18	0.967	0.95	0.99	0.0097
10	12	0.967	0.96	0.99	0.0097
15	6	0.955	0.93	0.98	0.0176

4.2 EXPERIMENT 2

The end of shelf-life of the minced mutton meat treated with a combination of two pH values (4.5 and 4.0) and two moisture contents (70 and 50%) stored at 30°C was indicated by an increase in total plate count (TPC) and/or the development of off-odours as shown in Table 4.6. Minced meat with pH values of 4.4 and 4.6 spoiled within seven days, while those with pH values of 4.1 and 4.3 were stable within 21 days of storage, irrespective of the two levels of moisture content tried in this experiment. An increase of 2 to 3 log units in TPC was observed in the former samples, while small (increase of about 1 log units) or no increase or even a decrease in TPC was observed in the latter samples.

Slight rancidity was detected in the meat samples with pH values of 4.1 and 4.3 at 21 days of storage but this was not considered their end of shelf-life. However, the putrefied smell was considered objectionable and was only observed in meat samples with pH of 4.4 and 4.6 at 21 and 14 days of storage, respectively.

No positive effect from reduction in moisture content within the limits of this experiment was evident.

The presence of moulds was only observed in meat samples with pH of 4.6 and 48% moisture content.

Table 4.7 shows the summary of the effect of two pH values (4.5 and 4.0) on the shelf-life of minced mutton meat stored at 30° or 20°C. The difference in the treatments of this block from the former (refer to Table 4.6) was that 0.1% potassium sorbate was added in all the meat samples. This was done to remove the interfering effect of mould growth which was observed previously (Table 4.6 and in Experiment 1).

Table 4.6: Total plate count (37°C), pH, moisture content and a_w of minced mutton meat[a] with combination of two pH values and two moisture contents during storage at 30°C.

Selected Combination of Treatments							
pH	Moisture Content (%)	Days in storage	Total Plate Count 37°C ($\log_{10}$ colonies per gram)	Actual pH during storage	Actual Moisture Content (%) during storage	a_w	Off Odours/ Moulds
4.5±0.2	68-70	0	3.00	4.38	68.18	0.94	No
		7	6.59*	4.45	67.64	0.94	No
		14	7.83	4.42	65.87	0.93	Yes (Sl. rancid)
		21	6.96	4.16	66.60	0.95	Yes (putrid)
	48-50	0	4.28	4.59	48.13	0.94	No
		7	6.28*	4.78	46.70	0.94	Yes (rancid) (molds)
		14	4.95	4.68	48.26	0.94	Yes (putrid)
4.0±0.2	68-70	0	2.60	4.06	68.68	0.95	No
		7	<2.00	4.10	70.07	0.95	No
		14	<2.00	4.15	68.86	0.95	No
		21	3.20	4.22	67.37	0.92	Yes (Sl. rancid)
		28	6.87*	4.11	67.40	0.95	Yes (Sl. rancid but not putrid)
	48-50	0	3.73	4.26	48.39	0.92	No
		7	2.48	4.35	49.76	0.95	No
		14	2.30	4.35	48.47	0.93	No
		21	<2.00	4.40	48.04	0.92	Yes (Sl. rancid)
		28	6.08*	4.37	48.86	0.92	Yes (Sl. rancid but not putrid)

[a] All samples had 20% sorbitol and packaged in polyethylene bags
* Stars indicate end of shelf-life

Table 4.7: Total plate count (37°C), pH, moisture content and a_w of minced mutton meat[a] with two pH values and two storage temperatures

Selected Combination of Treatments							
pH	Storage Temperature (°C)	Days in storage	Total Plate Count (37°C) (log_{10} colonies per gram)	Actual pH during storage	Moisture Content (%)	a_w	Off Odours
4.5±0.2	30±2	0	3.95	4.59	48.27	0.94	No
		7	4.76	4.69	48.62	0.93	Yes (Sl. rancid)
		14	3.70	4.78	49.08	0.92	Yes (Sl. rancid)
		21	2.60	4.73	46.78	0.93	Yes (Sl. rancid)
		35	7.74*	4.75	47.06	-	Yes (Sl. rancid)
	20±2	0	4.04	4.63	47.85	0.95	No
		7	3.08	4.69	47.13	0.94	No
		14	<4.00	4.73	47.99	0.91	No
		21	-	-	-	-	-
		35	5.51	4.72	49.34	-	No
4.0±0.2	30±2	0	3.23	4.21	47.96	0.94	No
		7	<2.00	4.26	48.55	0.93	No
		14	<2.00	4.33	49.14	0.91	No
		21	<2.00	4.33	48.22	0.92	No
		35	<2.00	4.33	48.06	-	Yes (Sl. rancid)
	20±2	0	3.28	4.21	47.64	0.92	No
		7	2.40	4.23	47.00	0.95	No
		14	<2.00	4.34	47.50	0.92	No
		21	2.00	4.28	46.42	0.95	No
		35	<2.00	4.31	48.64	-	No

[a] All samples had 20% sorbitol, 48-50% Moisture Content, 0.1% Potassium Sorbate and packaged in polyethylene bags

(-) Not determined

* Stars indicate end of shelf-life

An improvement in shelf-life of minced mutton meat was observed with the addition of potassium sorbate at all pH values. Minced meat with a pH of 4.6 and moisture content of 48% showed an extension of shelf-life from less than 7 to 21 days during storage at 30°C. Minced meat with the same pH and moisture content had a shelf-life of 35 days at 20°C. The minced meat with a pH of 4.2 and moisture content of 48% also had 35 days of shelf-life at 30°C and 20°C. Shelf-life in this experiment can be assumed only up to 35 days as subsequent tests were not taken.

Slight rancidity was detected only in meat samples stored at 30°C. Slight rancidity was noted in meat samples with a pH of 4.6 at seven days of storage and in meat samples with a pH of 4.2 at 35 days of storage.

4.3 EXPERIMENT 3

Tables 4.8 and 4.9 show the summary of the effect of combinations of 5 levels of acetic acid and 3 levels of sorbitol on the shelf-life of minced mutton meat stored at 30°C and 20°C. The levels of acetic acid selected were 1.0, 2.5, 5.0, 7.5, and 10.0%. The pH values obtained based on the above addition of acid levels were 4.4, 4.1, 3.8, 3.6 and 3.5, respectively. The levels of sorbitol selected were 0, 15, and 20%. The standards for the end of shelf-life were based on an increase in total plate count (TPC) and/or the detection of putrefied odours. The delineation between a stable and an unstable product was quite apparent by the substantial increase (more than 2 log units) in TPC and the subsequent development of putrid odours after another one or two weeks in storage. This was established based on the data obtained from Experiment 2.

To allow the data from Tables 4.8 and 4.9 to be more readily analyzed, they are presented graphically in Figures 4.1 to 4.6. The figures show more specifically the greatest individual effect of pH on the microbiological stability of the minced meat product. It was consistently shown that minced mutton meat samples with a pH of 4.4 only had less than 14 days of shelf-life compared to meat samples at pH 4.1 or below which had up to 56 days shelf-life irrespective of storage temperatures (30° and 20°C) and levels of sorbitol (0, 15, and 20%). Exceptions were the minced meat products with pH of 4.1 and 15% sorbitol at 30° and 20°C, which were assumed to have a shelf-life of only more than 28 days but less than 56 days. An increase of 4 log units in TPC was observed at the final test conducted on the above mentioned meat samples, although no putrefied odours were detected.

Putrefied odours were only detected in minced meat samples with a pH of 4.4 beginning at 14 days in storage irrespective

Table 4.8: Total plate count (37°C) of minced mutton Meat[a] with combinations of 5 pH values and 3 levels of sorbitol during storage at 30° and 20°C

Level of Acetic Acid (%)	Days in storage	Total Plate Count (37°C) (log_{10} colonies per gram) 30°C Storage Temp 0% Sorbitol	30°C Storage Temp 15% Sorbitol	30°C Storage Temp 20% Sorbitol	20°C Storage Temp 0% Sorbitol	20°C Storage Temp 15% Sorbitol	20°C Storage Temp 20% Sorbitol
1.0 (pH=4.4)	0	3.53	3.95	3.64	3.59	3.75	5.92
	14	8.49	6.92	6.73	8.15	7.74	7.61
	28	8.78	7.20	6.85	7.11	7.91	7.46
	56	8.08	8.00	6.97	7.11	7.26	7.85
2.5 (pH=4.1)	0	< 2.00	3.52	2.54	2.65	2.18	2.18
	14	< 2.00	< 2.00	< 2.00	< 2.00	< 2.00	5.36
	28	3.66	< 2.00	< 2.00	< 2.00	2.54	< 4.00
	56	< 2.00	6.78	< 2.00	< 2.00	6.74	< 2.00
5.0 (pH=3.8)	0	2.40	2.40	< 2.00	< 2.00	< 2.00	< 2.00
	14	< 2.00	< 2.00	< 2.00	< 2.00	< 2.00	3.59
	28	2.70	< 2.00	< 2.00	< 2.00	< 2.00	< 2.00
	56	< 2.00	< 2.00	2.85	< 2.00	2.86	< 2.00
7.5 (pH=3.6)	0	< 2.00	2.30	< 2.00	< 2.00	< 2.00	< 2.00
	14	3.04	2.74	2.18	3.71	2.30	3.11
	28	2.00	2.00	< 2.00	3.78	< 2.00	< 2.00
	56	< 2.00	< 2.00	2.65	< 2.00	< 2.30	2.30
10.0 (pH=3.5)	0	< 2.00	< 2.00	< 2.00	< 2.00	< 2.00	< 2.00
	14	< 2.00	2.18	< 2.00	2.48	< 2.00	< 2.00
	28	< 2.18	< 2.00	< 2.00	3.23	< 2.00	< 2.00
	56	< 2.00	< 2.00	2.48	< 2.00	< 2.00	< 2.00

[a] All samples had 0.1% Potassium sorbate and packaged in polyethylene bags.

Table 4.9: Development of off-odours in minced mutton meat[a] with combinations of five pH values and 3 levels of sorbitol during storage at 30°C and 20°C

Level of Acetic Acid (%)	Days in Storage	Off-Odours					
		30°C Storage Temp.			20°C Storage Temp.		
		0% Sorbitol	15% Sorbitol	20% Sorbitol	0% Sorbitol	15% Sorbitol	20% Sorbitol
1.0 (pH=4.4)	0	-	-	-	-	-	-
	14	x	x	x	x	x	x
	28	x	x	x	x	x	xx
	56	x	x	xx	xx	x	xx
2.5 (pH=4.1)	0	-	-	-	-	-	-
	14	-	-	-	-	-	-
	28	-	-	+	-	-	+
	56	+	-	++	+	-	+
5.0 (pH=3.8)	0	-	-	-	-	-	-
	14	-	-	-	-	-	-
	28	+	+	+	-	-	-
	56	+	+	+	+	-	-
7.5 (pH=3.6)	0	-	-	-	-	-	-
	14	-	-	-	-	-	-
	28	-	-	-	-	-	-
	56	-	-	-	-	-	-
10.0 (pH=3.5)	0	-	-	-	-	-	-
	14	-	-	-	-	-	-
	28	-	-	-	-	-	-
	56	-	-	-	-	-	-

[a] all samples had 0.1% Potassium sorbate and packaged in polyethylene bags

(-) No off-odours
(+) Sl. rancid
(++) rancid
(x) Sl. putrid
(xx) putrid

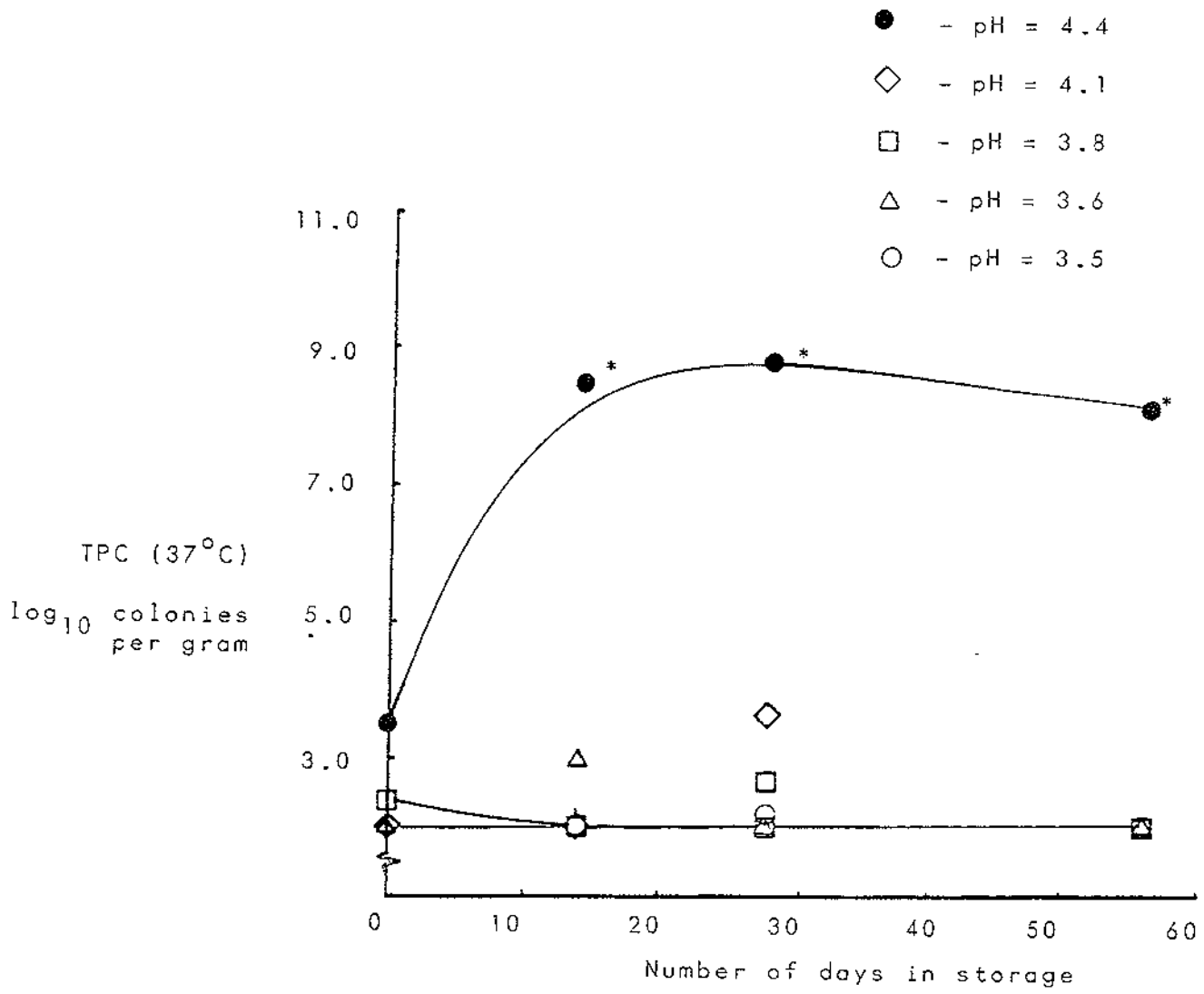


Figure 4.1: Total plate count (37°C) of minced meat with combinations of 5 pH values and 0% sorbitol stored at 30°C. (* - Stars indicate that putrefied odours were detected)

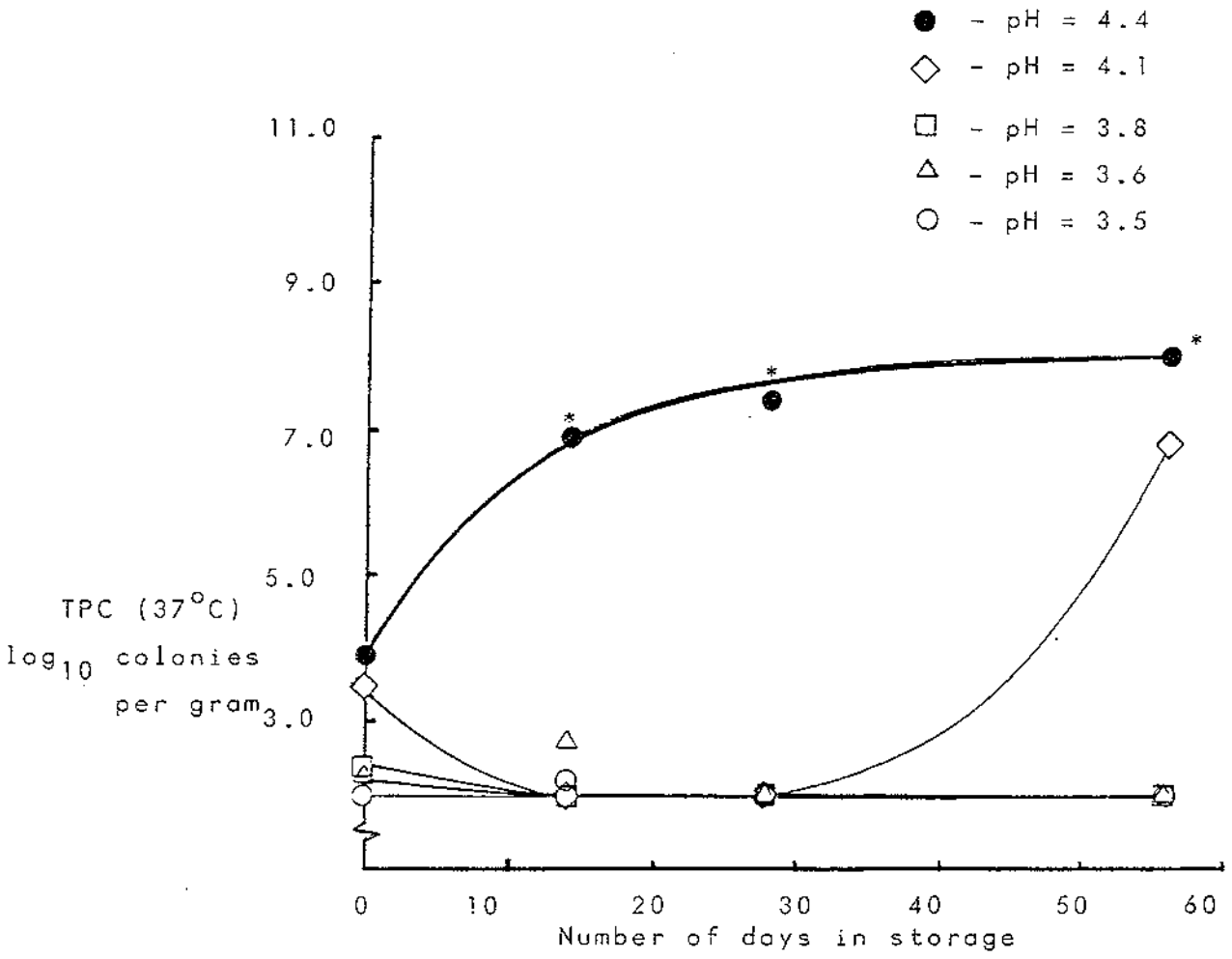


Figure 4.2: Total plate count (37°C) of minced mutton meat with combinations of 5 pH values and 15% sorbitol stored at 30°C. (* - Stars indicate that putrefied odours were detected.)

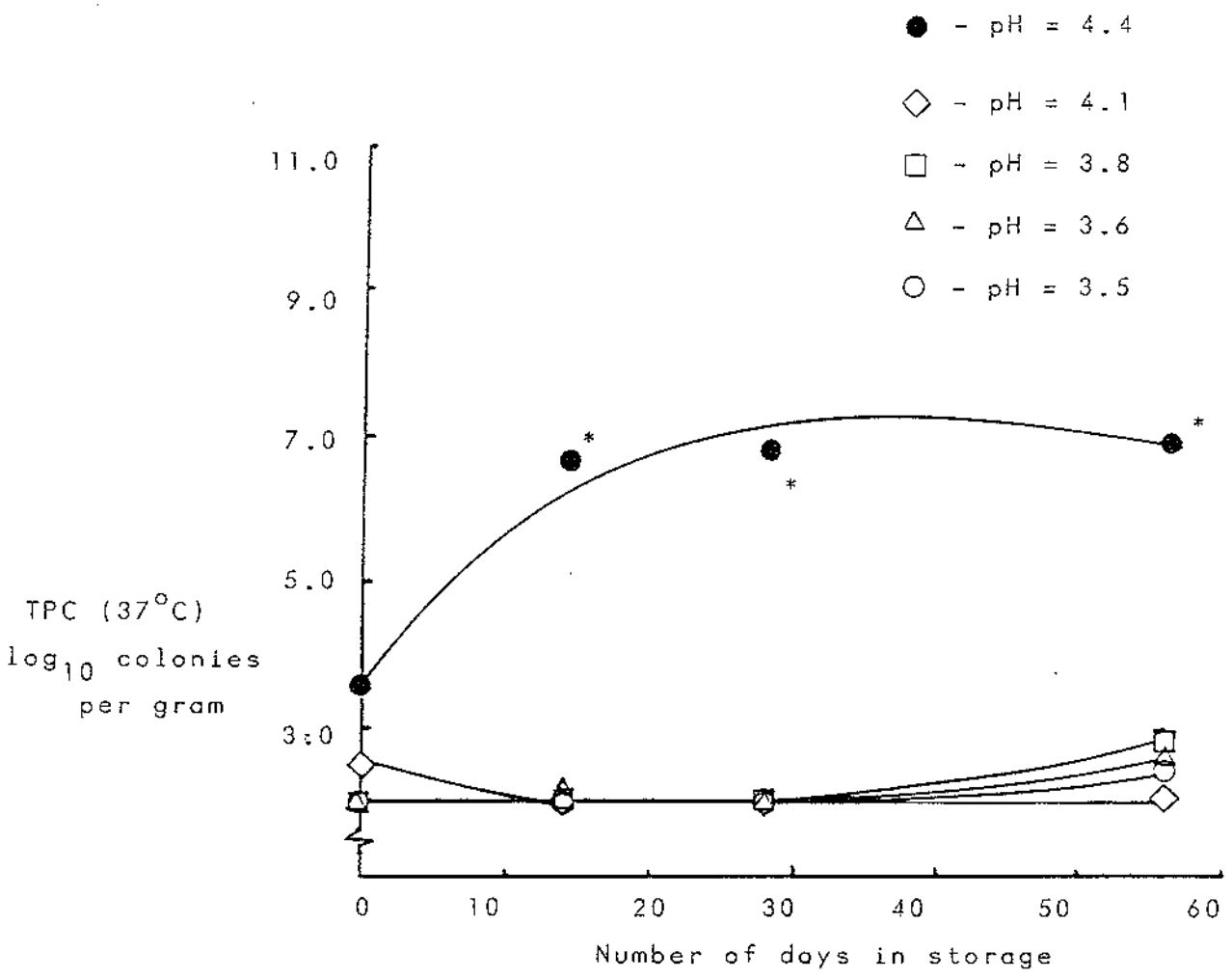


Figure 4.3: Total plate count (37°C) of minced mutton meat with combinations of 5 pH values and 20% sorbitol stored at 30°C (* - Stars indicate that putrefied odours were detected)

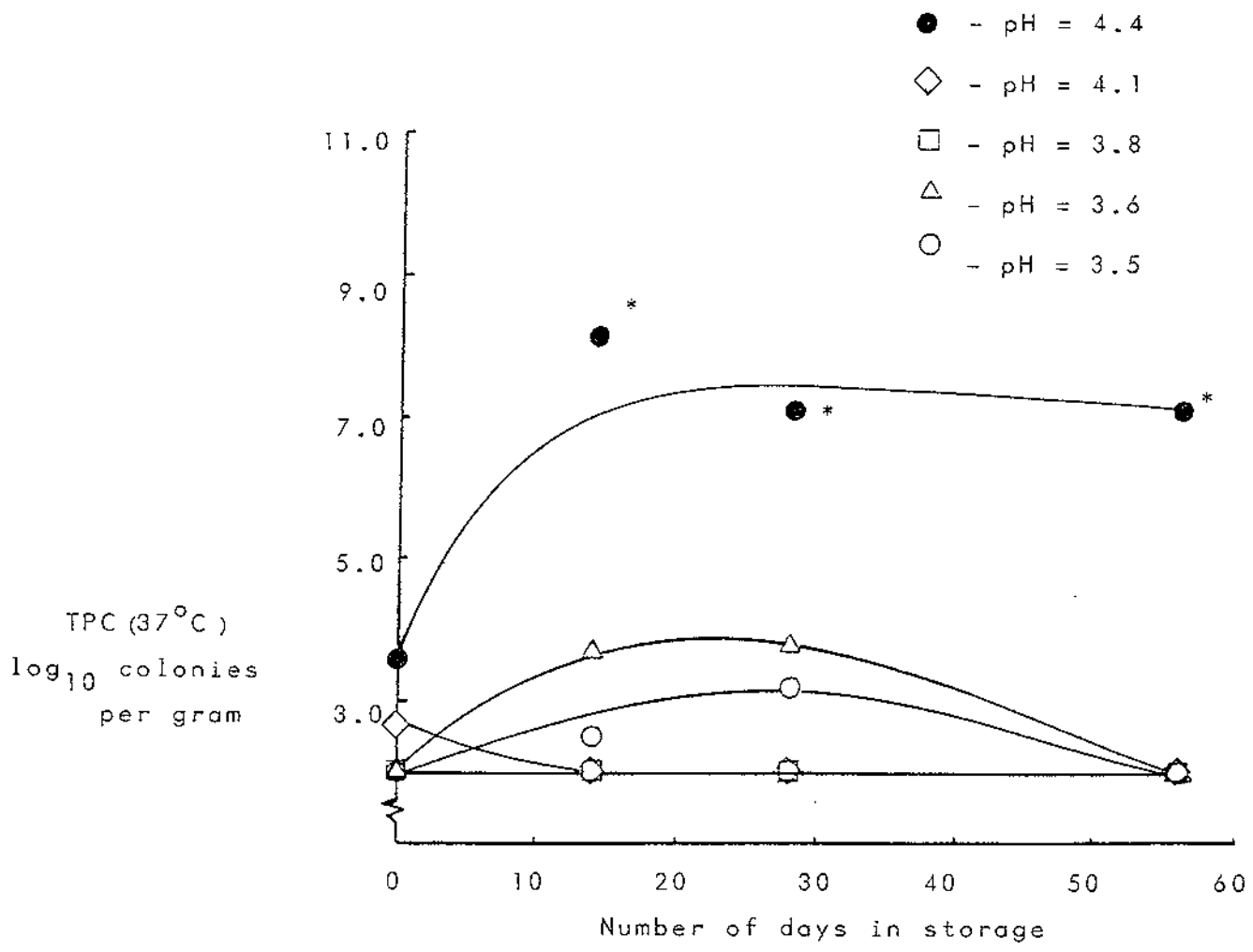


Figure 4.4: Total plate count (37°C) of minced mutton meat with combinations of 5 pH values and 0% sorbitol stored at 20°C (* - Stars indicate that putrefied odours were detected)

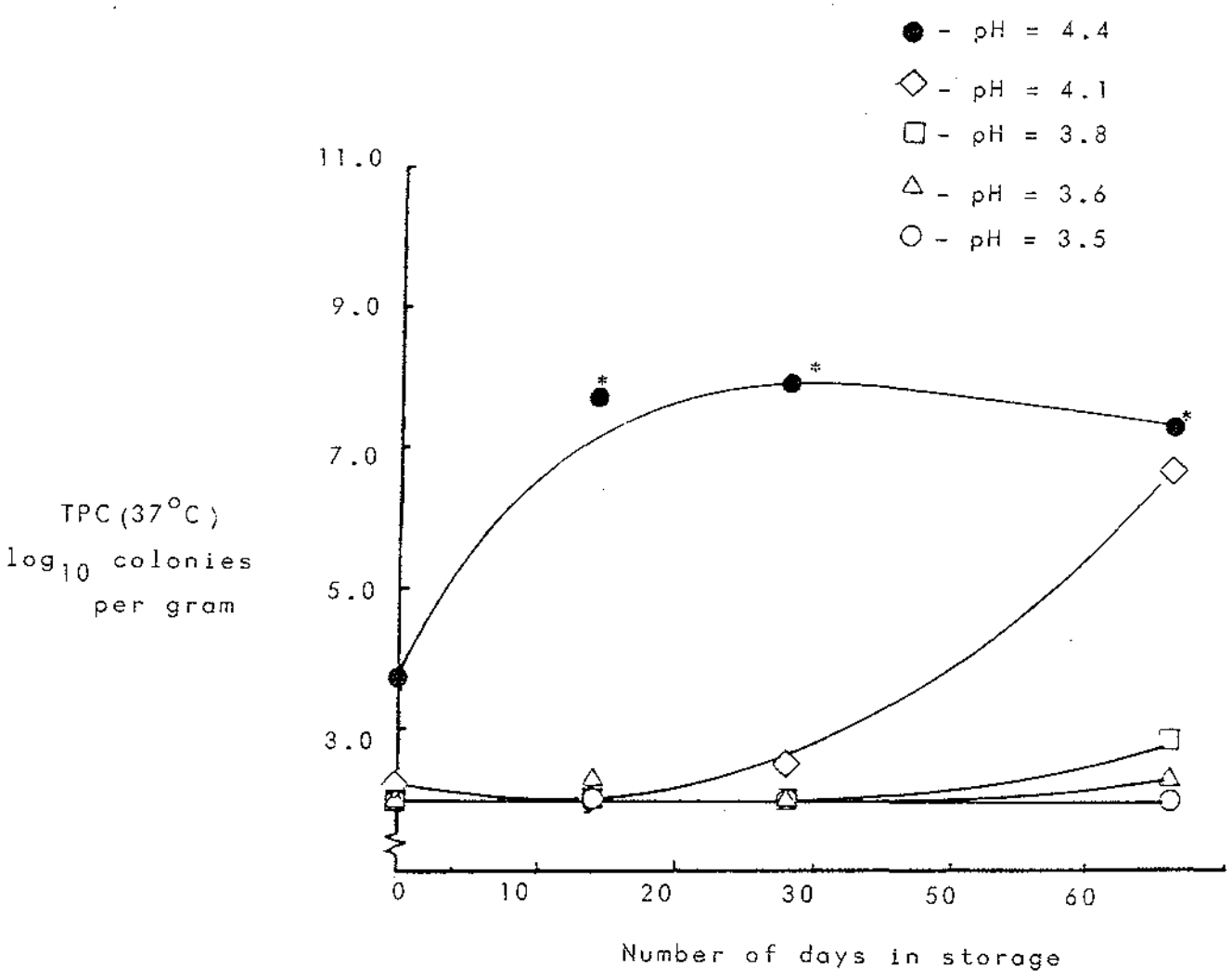


Figure 4.5: Total plate count (37°C) of minced mutton meat with combinations of 5 pH values and 15% sorbitol at 20°C (* - Stars indicate that putrefied odours were detected)

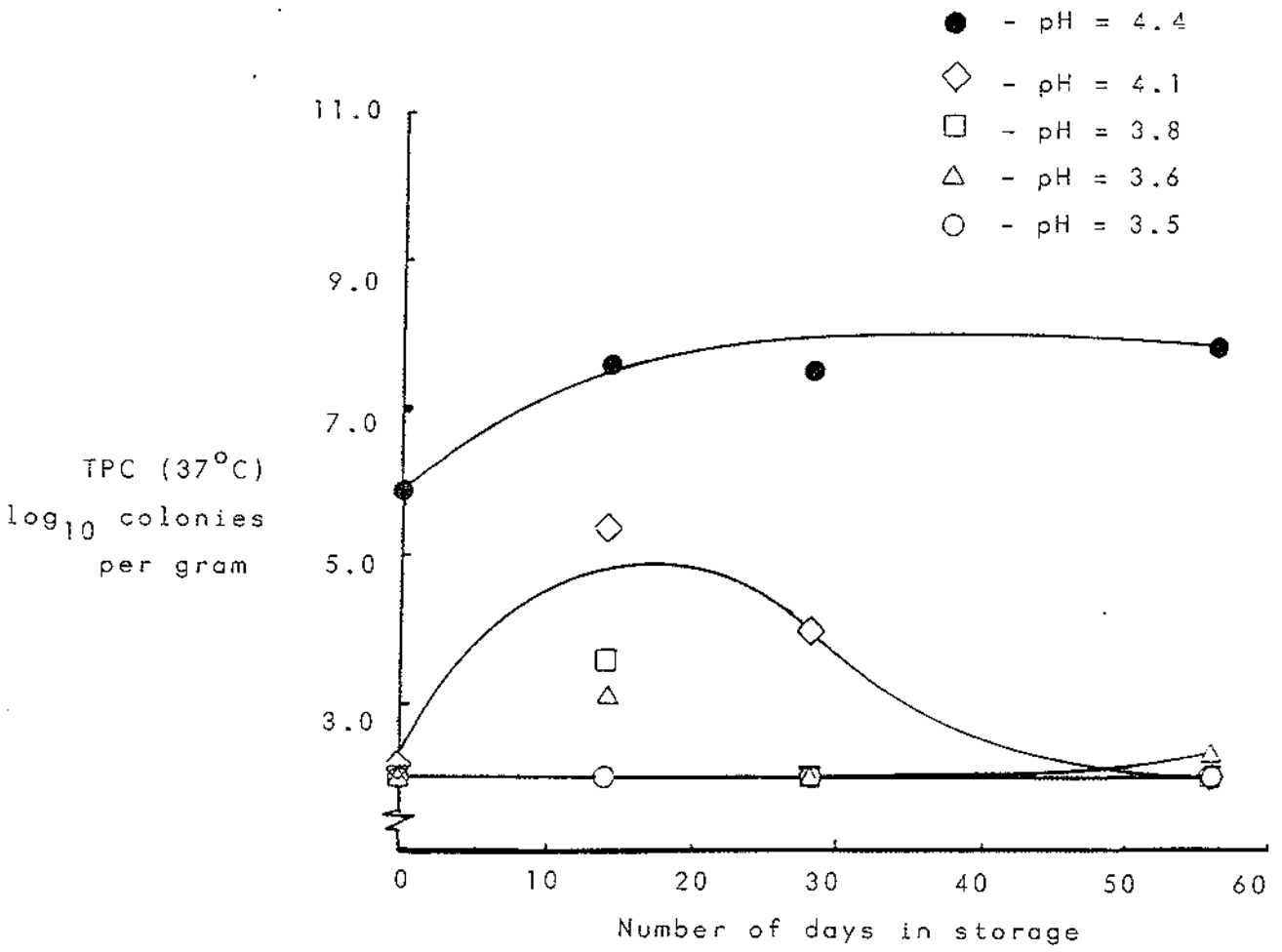


Figure 4.6: Total plate count (37°C) of minced mutton meat with combinations of 5 pH values and 20% sorbitol stored at 20°C (* - Stars indicate that putrefied odours were detected)

of storage temperatures and levels of sorbitol which were tried in this experiment (Table 4.9).

Slight rancidity was detected at 28 and 56 days of storage in meat samples with pH of 4.1 and 3.8 but not at all levels of sorbitol (Table 4.9). More meat samples (9 packets) stored at 30°C developed rancidity than those stored at 20°C (4 packets).

Rancidity was hard to detect in samples with higher levels of acetic acid because of the pronounced acid (vinegar-like) smell observed in these samples.

Table 4.10 shows the pH changes in minced mutton meat with combinations of five levels of acetic acid (1.0, 2.5, 5.0 7.5 and 10%) and 3 levels of sorbitol (0, 15, and 20%) during storage at 30° and 20°C. To clearly illustrate the trend in changes in pH from the data in Table 4.10, these are presented graphically in Figures 4.7 to 4.12. The figures show that it was only the samples with 1.0% level of acetic acid which had substantial increase or decrease (depending on levels of sorbitol addition and storage temperatures) in pH during storage.

In minced meat with no sorbitol added, there was an increase in pH observed during storage at 30° and 20°C. At a 15% sorbitol level, a decrease in pH was observed at both storage temperatures. However, in minced meat with 20% sorbitol added, different trends (depending on storage temperatures) were observed in changes in pH. A decrease in pH was observed at 30°C storage temperature and an increase was observed at 20°C.

Table 4.11 shows the moisture content of minced mutton meat with combinations of 5 pH values (4.4, 4.1, 3.8, 3.6 and 3.5) and 3 levels of sorbitol (0, 15, and 20%) during storage at 30°C and 20°C. Moisture content for all samples did not substantially vary during storage.

Table 4.10: Changes in pH of minced mutton meat with combinations of 5 pH values and 3 levels of sorbitol during storage at 30°C and 20°C

Level of Acetic Acid (%)	Days in Storage	pH 30°C Storage Temp 0% Sorbitol	30°C Storage Temp 15% Sorbitol	30°C Storage Temp 20% Sorbitol	20° Storage Temp 0% Sorbitol	20° Storage Temp 15% Sorbitol	20° Storage Temp 20% Sorbitol
1.0	0	4.46	4.38	4.38	4.47	4.39	4.36
	14	4.79	4.63	4.63	4.53	4.45	4.52
	28	4.92	4.39	4.37	4.66	4.63	4.80
	56	4.93	4.21	4.22	4.61	4.18	4.79
2.5	0	4.08	4.10	4.08	4.14	4.05	4.03
	14	4.22	4.14	4.05	4.17	4.12	4.15
	28	4.22	4.19	4.06	4.17	4.16	4.15
	56	4.20	4.16	4.15	4.16	4.17	4.14
5.0	0	3.83	3.77	3.76	3.85	3.80	3.78
	14	3.91	3.89	3.87	3.88	3.83	3.92
	28	3.90	3.92	3.87	3.88	3.89	3.91
	56	3.86	3.93	3.91	3.86	3.88	3.89
7.5	0	3.63	3.60	3.63	3.61	3.68	3.60
	14	3.81	3.76	3.75	3.70	3.68	3.73
	28	3.76	3.76	3.71	3.75	3.74	3.72
	56	3.78	3.75	3.73	3.72	3.73	3.75
10.0	0	3.54	3.54	3.54	3.52	3.56	3.49
	14	3.70	3.66	3.60	3.63	3.61	3.58
	28	3.63	3.66	3.58	3.66	3.62	3.65
	56	3.65	3.69	3.70	3.60	3.61	3.67

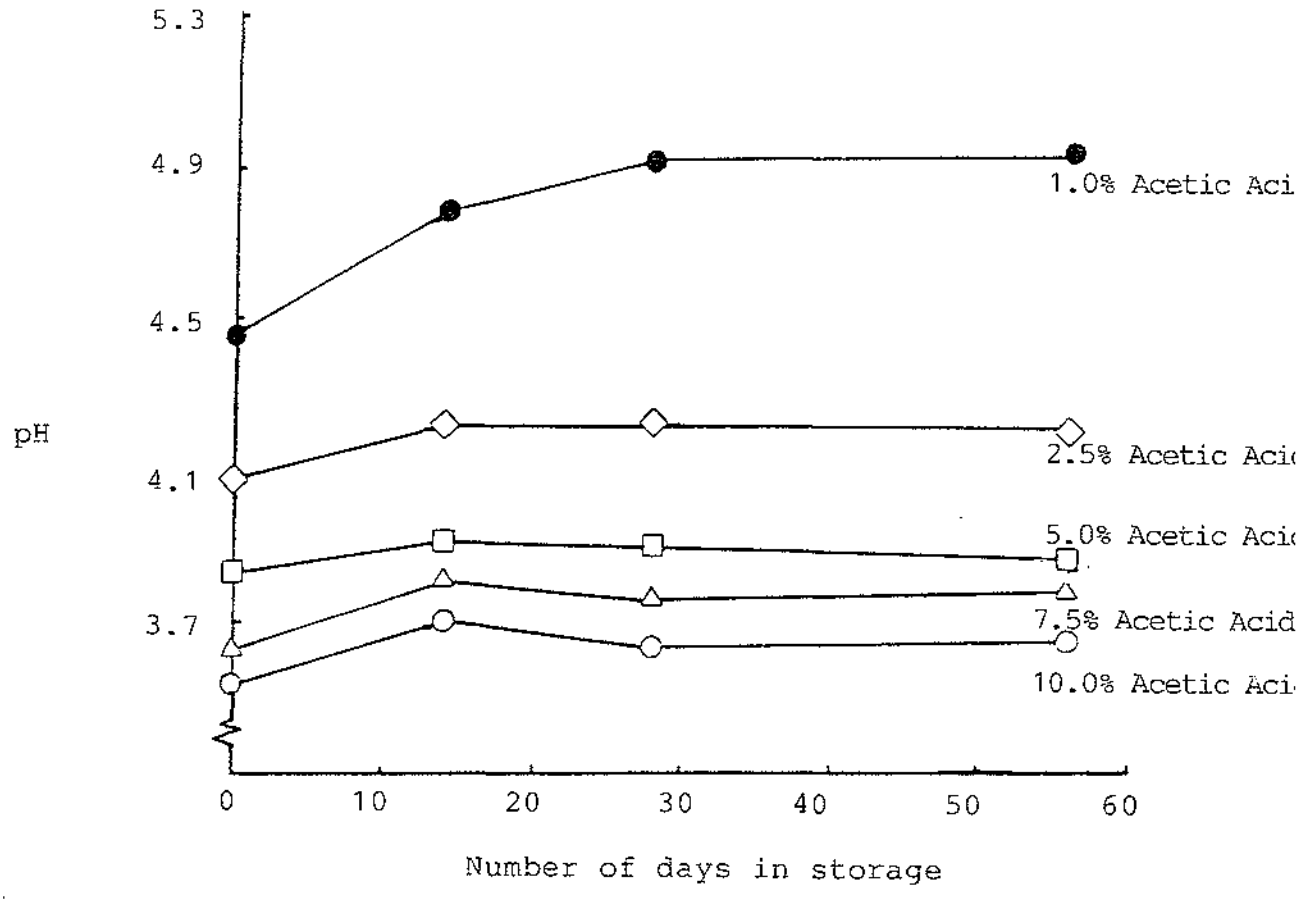


Figure 4.7: Changes in pH minced mutton meat with combinations of 5 levels of acetic acid and 0% sorbitol during storage at 30°C

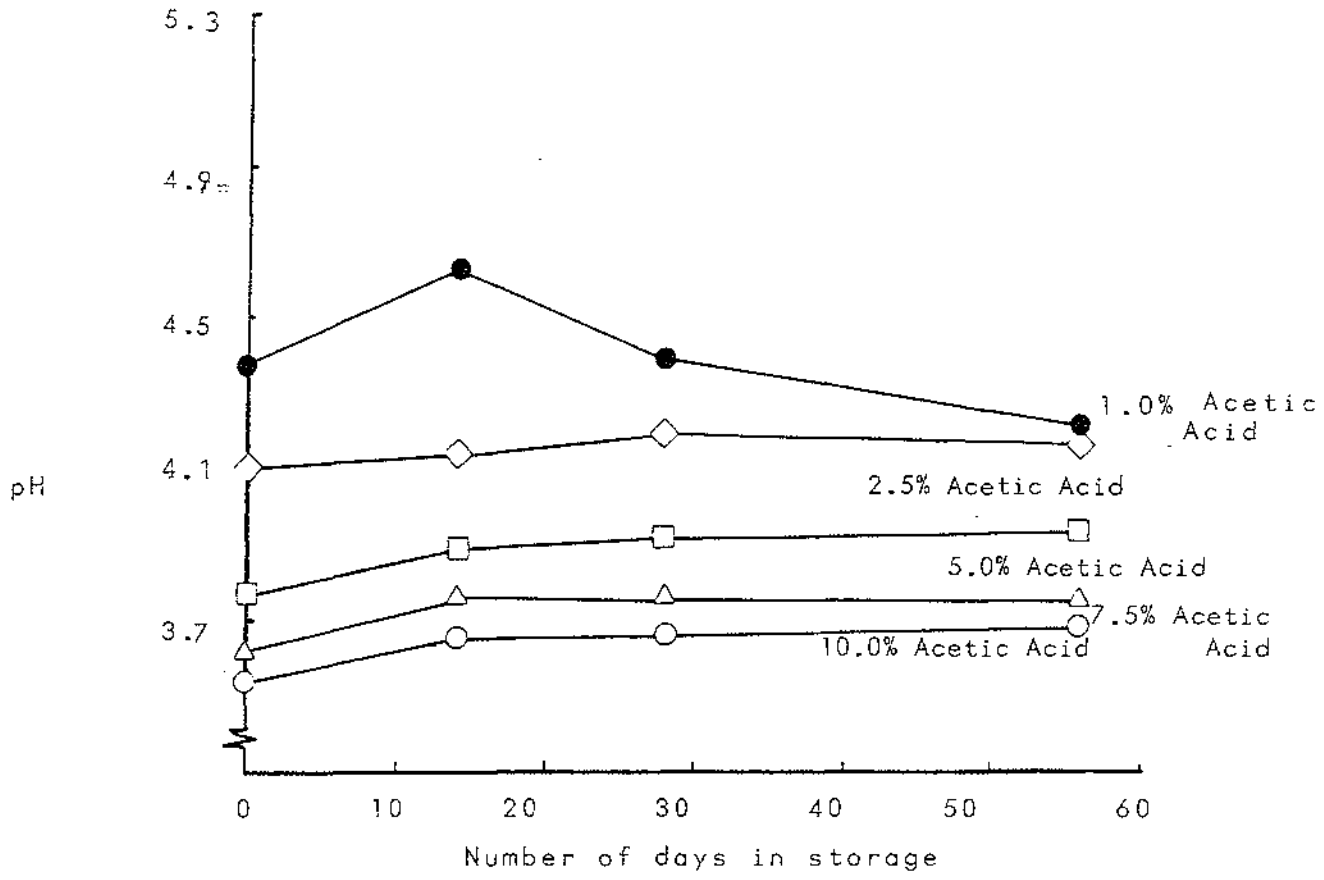


Figure 4.8 : Changes in pH minced mutton meat with combinations of 5 levels of acetic acid and 15% sorbitol during storage at 30°C

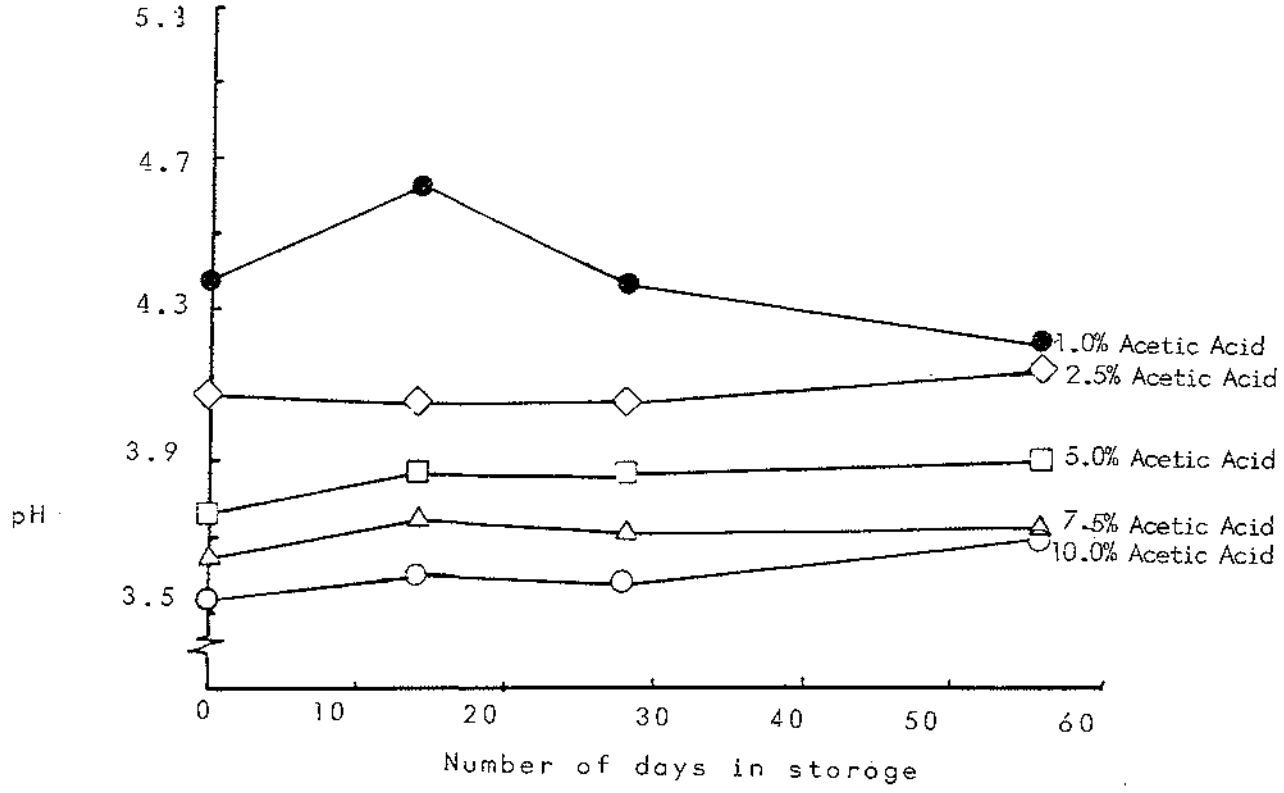


Figure 4.9: Changes in pH minced mutton meat with combinations of 5 levels of acetic acid and 20% sorbitol during storage at 30°C

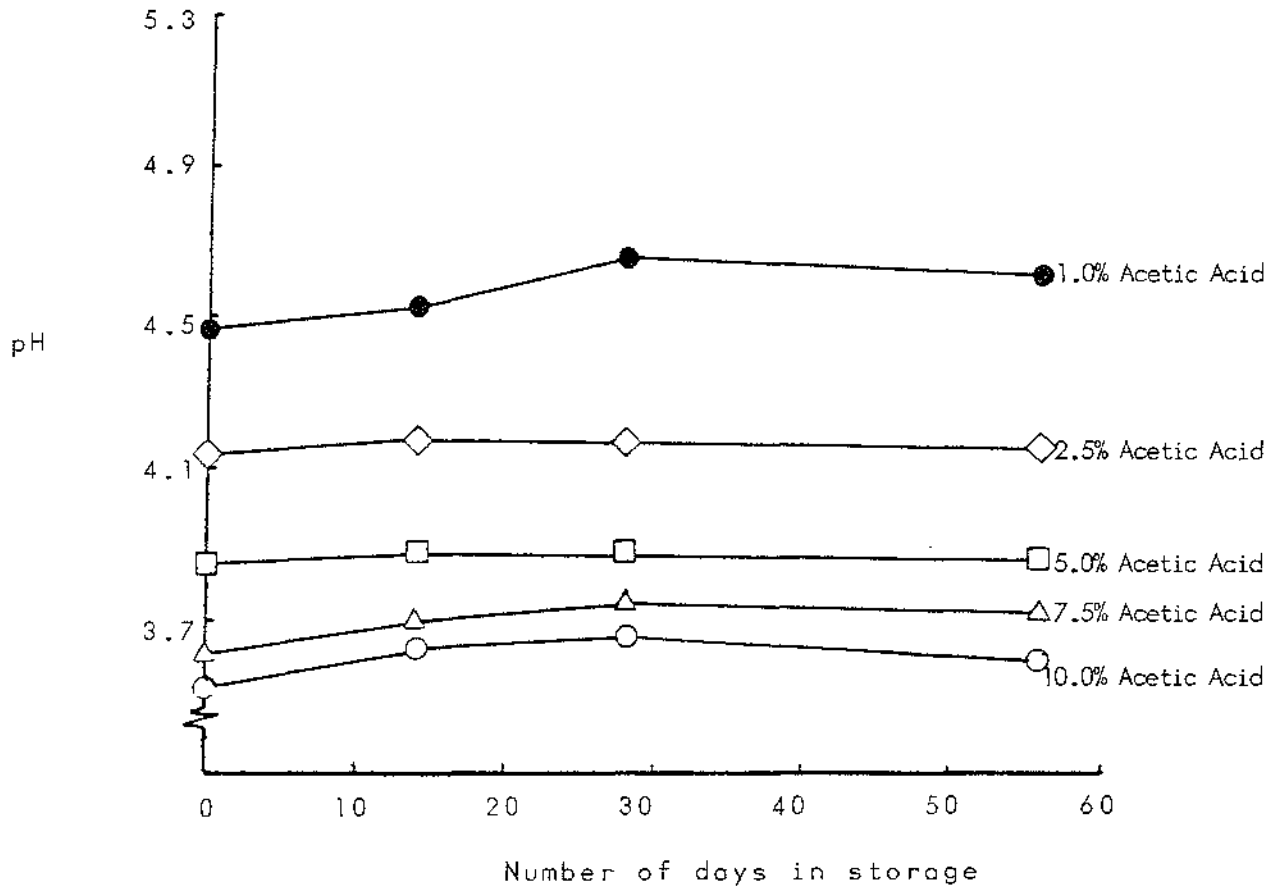


Figure 4.10: Changes in pH minced mutton meat with combinations of 5 levels of acetic acid and 0% sorbitol during storage at 20°C

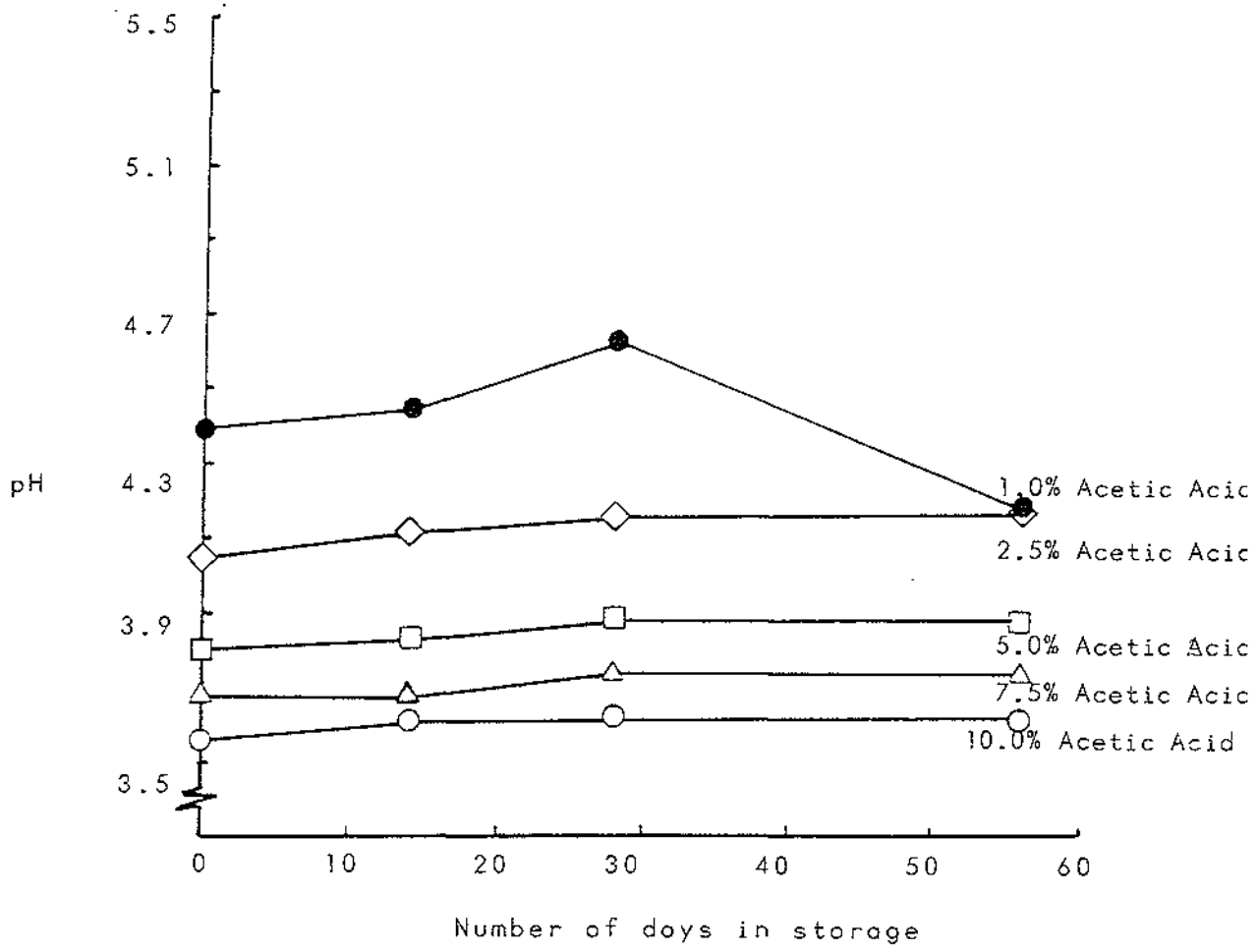


Figure 4.11: Changes in pH minced mutton meat with combinations of 5 levels of acetic acid and 15% sorbitol during storage at 20°C

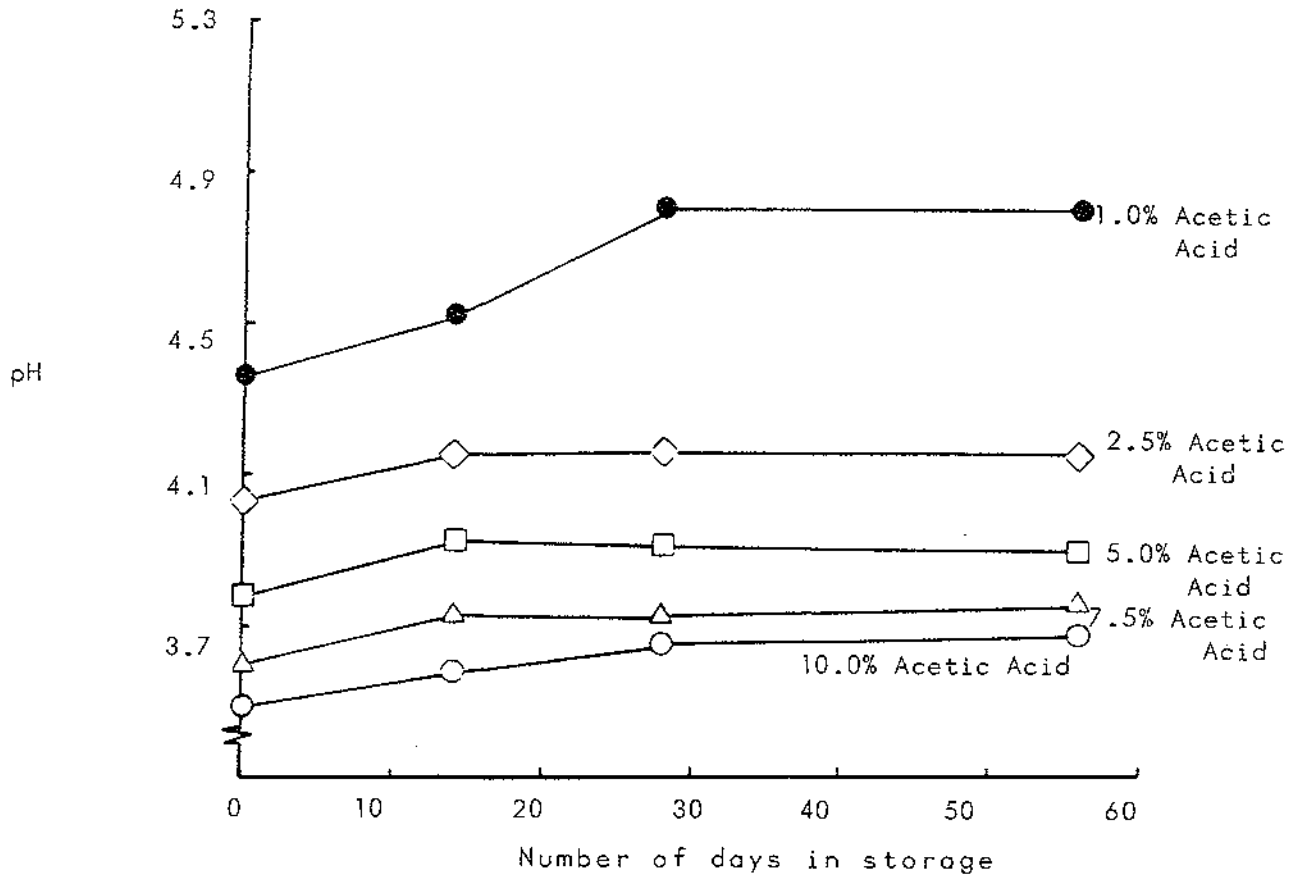


Figure 4.12: Changes in pH minced mutton meat with combinations of 5 levels of acetic acid and 20% sorbitol during storage at 20°C

Table 4.11: Moisture content of minced mutton meat with combinations of 5 pH values and 3 levels of sorbitol stored at 30°C and 20°C

Level of Acetic Acid (%)	Days in Storage	Moisture Content (%)					
		30°C Storage Temp			20°C Storage Temp		
		0% Sorbitol	15% Sorbitol	20% Sorbitol	0% Sorbitol	15% Sorbitol	20% Sorbitol
1.0 (pH=4.4)	0	73.1	71.4	71.3	73.0	70.9	70.9
	14	69.9	68.7	70.6	72.4	72.3	70.6
	28	70.5	69.3	69.6	69.2	67.2	70.7
	56	70.9	69.8	69.9	70.0	68.8	67.8
2.5 (pH=4.1)	0	71.0	70.0	71.0	70.2	71.4	70.7
	14	68.0	70.6	72.4	68.4	70.2	71.2
	28	68.0	69.6	72.8	69.3	71.0	70.7
	56	68.5	68.4	70.8	69.5	68.8	70.8
5.0 (pH=3.8)	0	71.8	71.7	70.8	71.0	71.0	71.0
	14	69.5	71.8	70.1	70.4	71.7	69.8
	28	68.6	70.7	71.5	69.3	70.3	70.4
	56	70.1	69.1	70.9	68.1	71.9	70.9
7.5 (pH=3.6)	0	71.6	71.2	69.7	72.9	69.6	70.2
	14	72.1	71.5	70.3	71.5	71.5	70.4
	28	71.3	71.4	72.0	70.8	70.9	71.0
	56	70.5	70.5	70.2	69.9	70.7	71.6
10.0 (pH=3.5)	0	71.8	72.6	68.3	71.8	71.9	71.5
	14	71.3	69.0	71.9	72.1	70.4	72.4
	28	71.1	70.9	72.1	70.9	71.3	71.4
	56	70.6	69.9	68.9	69.2	71.0	69.8

The results of the taste panel evaluation is given in Table 4.12. Minced mutton meat with a pH of 4.4 was acceptable in sourness and in general acceptability with or without the spices and seasonings. Samples without the seasonings and spices were given average panel scores of 3.6 and 3.0 for the sour flavour and general acceptability, respectively. Those with seasonings and spices were given scores of 4.2 and 4.0. Meat products with an average score of 3 or more for both characteristics were considered to have acceptable sensory properties.

Minced mutton meat with a pH of 4.1 was only acceptable with the addition of seasonings and spices. Samples without the seasonings and spices were given average panel scores of 2.6 and 2.4 for the sour flavour and general acceptability, respectively. Those with seasonings and spices were given scores of 3.5 and 4.0.

The results indicated that the reduction of pH below 4.4 had great consequence on the flavour and acceptability of the meat products as shown by the decrease in panel scores. However, the addition of seasonings and spices had masked the pronounced sourness in the meat product and had created an acceptable product which was described by the panelists as having a balanced sweet and sour flavour.

Table 4.12: Average taste panel scores for acidified mutton meat patties (pH 4.4 and 4.1) with and without seasonings and spices (for sour flavour intensity: 5 = none, 1 = very pronounced; for general acceptability: 5 = very acceptable, 1 = very unacceptable)

Level of Acetic Acid	Seasoning and spices	Average Scores	
		Sour Flavour	General Acceptability
1.0% (pH=4.4)	without [a]	3.6	3.0
	with [b]	4.5	4.0
2.5% (pH=4.1)	without [a]	2.6	2.4
	with [b]	3.5	4.0

[a] 5 panelists evaluated the samples

[b] 4 panelists evaluated the samples

CHAPTER 5

DISCUSSION

5.1 EXPERIMENT 1

In this study, the microbiological stability of minced mutton meat was determined in relation to the combination effects of pH, moisture content, level of sorbitol (a_w), mild heat treatment, and packaging material (Eh). This information is of importance as there is little known about the effect of the combinations of these factors on the stability of meat products particularly during storage under warm conditions (30° C). The experiment was done in minced meat product because of its simplicity to prepare and ease to subject with the intended processing treatments. Minced meat is also considered a versatile meat base for different meat dishes and formulations. Minced meat can be used in sausages, meat balls, patties or can be made into wontons, spring rolls, mixed with vegetables and fried rice, curry and meat sauce for spaghetti. Mutton meat was used because of its availability and low cost.

The pH values investigated in this study were 5.5, 5.0 and 4.5. The levels of sorbitol investigated were 5, 10 and 15%. The heat treatments done were 30°, 50° and 75° C. The meat had 70, 60 and 50% moisture contents. The meat was packed in cellulose, polyethylene and aluminium foil films.

The effect of the various combinations of factors investigated were not evident in the initial results obtained. The microbial stability expected was not observed except in four combinations of treatments (or runs as they will be referred here for ease of discussion) which gave a shelf-life of more than 14 but less than

28 days (Table 4.2). The other four runs (Table 4.2) which gave a shelf-life of more than 7 but less than 14 days could not only be considered to have that shelf-life because some other runs were evaluated only after 14 days and some of these might have had the same stability.

However, all of the runs were analyzed by multiple linear regression analysis (the total plate count (TPC) obtained after 16-18 hours of storage at 30 ^{o}C against various preservative factors). This was found of use in giving an indication which among the five factors considered had the greatest individual effect on microbiological counts of meat samples.

The results of the regression analysis gave an indication that pH, heat treatment and moisture content were the significant factors that may be responsible for the microbiological stability of minced mutton meat during storage at 30^{o}C.

The above findings agree with those of Fox and Loncin (1982) who evaluated four process parameters (heat treatment, a_w, pH, Eh) in 250 combinations in a model product (nutrient broth). They also found that heat treatment and pH were the most important significant factors among the process parameters studied on stability.

Although heat treatment could have been considered to have the greatest individual effect on stability, its advantage would be useless without considering the sealed containers needed to protect the product from recontamination. Incidentally, these packaging materials, such as cans, glass, jars, aluminium pouch and casings, are expensive.

It was decided therefore not to consider any further the heat treatment factor but to concentrate in examining in greater depth the following combinations of factors: pH, moisture content and a_w.

5.2 EXPERIMENT 2

5.2.1 Part 1

Based on the results of Experiment 1, it was decided to investigate the combined effects of pH, a_w and moisture content in greater depth. Two levels of pH (4.5 and 4.0) and two levels of moisture content (50 and 70%) were examined. Moisture content was not lowered further as it was the objective of this study to be able to produce a shelf-stable meat product possibly with reduced moisture content but without changing the texture by drying. The pH was reduced to 4.0. In addition, the level of sorbitol was increased to 20% for all samples. The possible controlling effect of reducing the a_w below 0.95 by the addition of the maximum sorbitol consistent with acceptable flavour and texture had not been established.

Microbiological stability was observed in minced meat samples with pH of 4.0 $\pm$ 0.2 units for 21 days of storage at 30^{o}C for both 50 and 70% moisture contents. This indicated that pH had the greatest individual effect on stability of minced mutton meat during storage at 30^{o}C. Inspection of minimum pH values required by microorganisms for growth reported in the literature (although most of these results were obtained using laboratory media) showed that most of the bacteria can not support growth below pH 4.4. Extrapolation of these data to meat in a practical situation although needing to be applied with caution, nevertheless indicated that the data obtained in this experiment agrees with the literature findings.

Mould growth were observed in samples with pH 4.6 and 48% moisture content. These observations were also noted in Experiment 1. Moulds were observed to grow more often in samples with lower moisture content. Moulds have not often been observed to grow at high moisture content

probably because of competition with bacteria. The reduction of moisture content removes this competitive condition and favours their growth (Gailani and Fung, 1986). The presence of moulds in meat samples is not surprising as the literature values for the minimum pH growth requirement of moulds as well as yeast indicated that they are capable of growing even in extremely low pH (Table 2.3).

However, the problem of mould growth could be checked by addition of mycostatic agents such as potassium sorbate. In manufactured meats, potassium sorbate has been used as a dip for preventing mould growth during ripening of dry sausages such as Italian dry salami (Holley, 1986).

In part 2 of this experiment it was decided to add potassium sorbate to remove the complication of mould growth masking microbial spoilage.

The results of this experiment also had established that samples of minced meat which reached a total plate count of 10^6 colonies per gram during storage at 30°C would develop putrefied odours within one or two weeks in storage. Minced meat had initial counts of 10^3 to 10^4 colonies per gram. Hence, a log increase of 2.0 or more was considered to be the end of its shelf-life. While minced meat with small (less than 2.0) or no log increase in microbial counts were considered to have achieved microbial stability. This value was arbitrary as it was only based on the empirical data obtained from this experiment. However, minced meat containing 10^6 to 10^7 total plate count (37° C) would be considered by many governmental agencies to be already unacceptable (Wehr, 1982).

The expected additional effect on stability of reducing the a_w below 0.95, which was obtained by the addition of 20% sorbitol, (Table 4.3) was not observed at higher pH

values investigated (pH 4.5 to 5.0) (Results of this experiment and Experiment 1). These results tend to disagree with the proposed storage categories of Leistner and Rodel (1975) for meat products based on a_w and pH values with respect to their stability. They proposed that meat products with an a_w at or below 0.95 and a pH at or below 5.2; or a pH below 5.0; or an a_w below 0.91 should need no refrigeration and should be considered "shelf-stable". However, they (Leistner and Rodel) did not specify storage temperatures. It was assumed that they were referring to the room conditions in West Germany i.e., about 20°C.

The assumed difference in storage conditions could explain the disagreement with the results obtained in this experiment and that of Leistner and Rodel (1975).

It was decided as well therefore, to investigate the effect of two storage temperatures (i.e., 20° vs 30°C) on the shelf-life of minced mutton meat in part 2 of this experiment.

5.2.2 Part 2

In this experiment, the effect of 2 storage temperatures (30° and 20° C) and two levels of pH (4.5 and 4.0) on the stability of minced mutton meat was determined. All minced meat was prepared with 20% sorbitol, 50% moisture content and 0.1% potassium sorbate. The same levels of pH were tried as with part 1 of this experiment to be able to compare the effect of the addition of 0.1% potassium sorbate. The meat was reduced to 50% moisture content since the previous results tended to show that mould growth was favoured by a reduced moisture content. Hence, a condition was created where in the effect of the addition of potassium sorbate could be easily seen. The same amount

of sorbitol (20%) was added to be able to compare the effect of storage temperatures on stability with respect to pH and a_w values.

Microbial stability of minced mutton meat was increased from less than 7 days to 21 days at pH 4.6 with the addition of 0.1% potassium sorbate in both storage temperatures (30° and $20^{\circ}C$). At pH 4.2, an increase in shelf-life from 21 days to 35 days was observed. The anti-microbial effect of potassium sorbate had also been shown by the works of Pisanu (1982) on Thai fermented sausage (Sai Krok Prew) and Holley (1981, 1986) on Italian dry salami. However, both workers had used potassium sorbate as dips for the sausages. In this study, potassium sorbate was directly blended into the meat. This partly removes the problem of the potassium sorbate dripping from the surface of treated sausages as was observed by Holley (1981) in treated salami as soon as the meat was hung in the drying rooms.

The amount of potassium sorbate (0.1%) used in this experiment seemed to indicate that this was adequate to prevent mould growth. This level is less than the approved (U.S.D.A.) application level of 2.5% sorbate.

This study has indicated that microbial stability of minced mutton meat stored at $30^{\circ}C$ is achieved by lowering the pH below 4.4. The observation suggests that there is a dividing line between a stable and unstable meat product particularly those which would be stored at warm conditions ($30^{\circ}C$) based on pH alone. This was examined therefore, in greater depth in Experiment 3.

The a_w was reduced to below 0.95 by the addition of 20% sorbitol (Tables 4.4 and 4.5). The values obtained ranged from 0.92 to 0.95 in both parts of this experiment. It had not been established however, if there was an interactive effect between the a_w values and the pH values being considered on stability of minced mutton meat. This was also examined further in Experiment 3.

5.3 EXPERIMENT 3

In this experiment, the effect of five pH values (4.4, 4.1, 3.8, 3.6 and 3.5), three levels of sorbitol (0, 15 and 20%) and two storage temperatures (30° and 20°C) on stability of minced mutton meat was examined.

A significant difference in the microbiological stability of minced mutton during storage at 30° and 20°C was established based on the level of pH. This showed that at a pH of 4.1 or lower the meat is expected to have a shelf-life of 2 months at both 20° and 30°C. The difference observed between the two storage temperatures was on the development of rancidity rather than on microbial growth. More batches developed slight rancidity at 30°C than at 20°C. No positive interactive effect between the sorbitol levels and the various pH values on microbiological stability was established.

Extending the shelf-life of meat products based primarily on the influence of pH alone or in combinations with other preservative factors particularly at pH levels studied in this experiment had not been extensively studied. The available literature indicates that most work had been done on either nutrient media or mostly on fruits and vegetables. The major reason why pH of meat products had not been lowered below 4.5 is taste (Leistner, 1985).

However, sensory evaluation test conducted in this experiment showed that minced meat with 2.5% acetic acid (pH = 4.1), 20% sorbitol and spices (1.75% salt, 1.0% garlic powder and 1.0% pepper) were acceptable compared to minced meat with only the acetic acid. The results of the sensory tests suggest that with the right combinations of spices and seasonings suited to specific localities or countries, an acceptable meat product could be produced with reduced pH of 4.1. It is outside the scope of this study to investigate different combinations

of spices and seasonings that would give an acceptable product based on meats with pH of 4.1.

The addition of 20% sorbitol, although it had not given additional microbial stability to the product, had improved the taste and texture of the minced meat. Minced meat with 2.5% acetic acid alone (pH = 4.1) was sour in taste but the addition of sorbitol masked the sourness and resulted in a meat product with an acceptable sweet and sour taste. The addition of sorbitol also produced a jelly like texture compared to a "crumbly" texture without the sorbitol. The jelly-like texture was found to be also desirable in terms of moulding the product into patties.

The meat product produced above could be similar to the brawns of West Germany (Leistner, 1985), although this has pH of 5.0 - 4.5. Both products are similar because both rely mainly in the control of pH for stability by the addition of acetic acid. Leistner (1985) reported that brawns can be stored without refrigeration, if recontamination after heat processing is avoided.

CHAPTER 6

SUMMARY AND CONCLUSIONS

The available literature has shown that there is insufficient research work done to quantify the effect of combinations of factors on the stability of meat products during storage under warm conditions (30° C). The results of this study provides some initial information on the combination of hurdles (F, a_w, pH, Eh) which would ensure stability in meat products to be distributed in tropical countries such as the Philippines where cost factors are also important.

Three experiments were done. In experiment 1, the combination of factors studied involved three pH values (5.5, 5.0 and 4.5), three levels of a_w achieved by three sorbitol levels (5, 10 and 15%) and three moisture content levels (70, 60 and 50%), three heat treatments (30°, 50° and 75° C) and three packaging materials (cellulose, polyethylene and aluminium foil films). Within the limits of the values that were considered, no significant effect of the various combinations of factors investigated was evident. However, the results did indicate that both pH and heat treatment and pH and moisture content were the combinations of factors that should be investigated to improve the microbiological stability of minced mutton meat during storage at 30°C.

As neither heat treatments could be increased nor moisture content decreased without adversely affecting the cost structure, experiment 2 considered decreasing the pH by additional acid and decreasing the a_w by increasing the sorbitol. An additional storage temperature of 20° C was used to allow comparison with results obtained from the literature.

Experiment 2 was divided into two sections. Initially, two levels of pH (between 4.6 and 4.1) and two levels of moisture content (70 and 50%) were examined. The level of sorbitol added was increased to 20% for all meat samples. The possible controlling effect of reducing the a_w below 0.95 by the addition of the maximum sorbitol consistent with acceptable flavour and texture had not been established. All meat samples were packaged in polyethylene bags. Microbiological stability was found for minced meat samples with pH of 4.3 and 4.1 for 21 days of storage at 30 ^{o}C irrespective of moisture contents. Minced meat samples with pH of 4.6 and 4.4 had spoiled within seven days as shown by the increase in total plate counts (log increase of 2 to 3 units) as well as the development of mould growth for samples with moisture content (MC) of 48%. The expected additional effect of reducing the a_w below 0.95 which was obtained by the addition of 20% sorbitol on stability particularly at higher pH values investigated (pH ~ 4.5) was not observed.

Following the first part of experiment 2, the effect of two pH values (4.6 and 4.2) and two storage temperatures (30^{o} and 20^{o}C) on the shelf-life of minced mutton meat was examined. All meat samples had 20% sorbitol, 50% MC, 0.1% potassium sorbate and packaged in polyethylene bags. Potassium sorbate was added to remove the complication of mould growth masking microbial spoilage. The shelf-life of minced mutton meat was increased from less than 7 to 21 days at pH 4.6 with the addition of 0.1% potassium sorbate irrespective of storage temperatures. At pH 4.2, an increase in shelf-life from 21 to 35 days was observed.

The results of experiment 2 had indicated that microbiological stability of minced mutton with a_w s $\geq$ 0.91 and $\leq$ 0.95, 0.1% potassium sorbate packaged in polyethylene and stored at 30^{o}C is achieved by lowering the pH below 4.4. The observation suggests that within these

experimental limits there is a dividing line between a stable and an unstable meat product stored under warm conditions (~ 30 C) based on pH alone.

In experiment 3, the effect of five pH values (4.4, 4.1, 3.8, 3.6 and 3.5), three levels of sorbitol (0, 15 and 20%) and two storage temperatures (30° and 20°C) on the stability of minced mutton meat was examined. All meat samples had 0.1% potassium sorbate and were packaged in polyethylene bags. A significant difference in the microbiological stability of minced mutton during storage at 30° and 20°C was established based on level of pH. This showed that with a pH of 4.1 or lower the meat is expected to have a shelf-life of 2 months at both 20° and 30°C. The difference observed in the two storage temperatures was the development of rancidity rather than microbial growth. No positive interactive effect between the sorbitol level and the various pH values on the microbiological stability was established. However, the addition of sorbitol had improved the taste and texture of the minced meat. The sourness had been balanced and a jelly-like texture compared to a "crumbly" texture without the sorbitol had been produced.

It was concluded in this study that within the limits of the values for combinations of factors that were considered, the control of pH was the only significant factor in extending the shelf life of minced mutton meat stored at 30°C. However, the practical value of lowering the pH of meat products below 4.1 must be questioned because no additional microbiological protection will be obtained and the increase of the acid level will only decrease the flavour acceptance. But minced mutton meat with pH of 4.1 can be organoleptically acceptable with the addition of the right combinations of spices and seasonings suited to specific localities and countries.

BIBLIOGRAPHY

Acker, L. 1969. Water activity and enzyme activity. Food Technol. 23: 1257-1270.

A.O.A.C. 1980. Official Methods of Analysis. (13th ed.). Association of Official Analytical Chemist, Washington D.C.

A.M.S.A. 1978. Guidelines for Cookery and Sensory Evaluation of Meat. Am. Meat Sci. Assn. and Natl. Live Stock and Meat Board, Chicago, Il.

Ayres, J.C., Mundt, J.O., and Sandine, W.E. 1980. Microbiology of Foods. W.H. Freeman & Co., San Francisco, p. 391.

Baird-Parker, A.C. 1980. Organic Acids. In Microbial Ecology of Foods Vol. 1. Factors Affecting Life and Death of Microorganisms. ICMSF. Academic Press, New York. pp. 126-135.

Barnes, E.M. and Ingram, M. 1955. Changes in the oxidation reduction potential of the sternocephalicus muscle of the horse after death in relation to the development of bacteria. J. Sci. Food Agric. 6: 448-455.

Barnes, E.M. and Ingram, M. 1956. The effect of redox potential on the growth of *Clostridium welchii* strains isolated from horse muscle. J. Appl. Bacteriol. 19: 117-128.

Bem, Z. and Leistner, L. 1970. As cited in: Leistner, L. and Rodel, W. 1976. Inhibition of microorganisms in food by water activity. In Inhibition and Inactivation of Vegetative Microbes. Skinner, F.A. and Hugo, W.B. Academic Press, New York. p. 223.

Benmergui, E.A., Ferro Fontan, C., and Chirife, J. 1979. The prediction of water activity in aqueous solutions in connection with intermediate moisture foods. 1. a_w prediction in single aqueous electrolyte solutions. J. Food Technol. 14: 625-637.

Blickstad, E. 1984. Influence of water activity on microbial development in minced, cured pork, in relation to gas headspace composition. Food Microbiol. 1: 177-185.

Blickstad, E. and Molin, G. 1983. Carbon dioxide as a controller of the spoilage of pork, with special reference to temperature and sodium chloride. J. Food Protect. 46: 756.

Bone, D. 1969. Water acticity: Its chemistry and applications. Food Prod. Devel. 3. 81-94.

Brockmann, M.C. 1970. Development of intermediate moisture foods for military use. Food Technol. 24: 60-64.

Brown, M.H., and Emberger, O. 1980. Oxidation-Reduction Potential. In Microbial Ecology of Foods. Vol. 1 Factors Affecting Life and Death of Microorganism. ICMSF. Academic Press, New York. pp. 112-125.

Buchanan, R.L. 1986. Processed meats as a microbial environment. Food Technol. 40: 134-138.

Chou, H.E., Acott, K. and Labuza, T.P. 1973. Sorption hysterisis and chemical reactivity: Lipid oxidation. J. Food Sci. 38: 316-319.

Chirife, J. and Ferro Fontan, C. 1980a. A study of the water activity lowering behaviour of polyethylene glycols in the intermediate moisture range. J. Food Sci. 45: 1717-1719.

Chirife, J. and Ferro Fontan, C. 1980b. The prediction of water activity in aqueous solutions in connection with intermediate moisture foods. Experimental investigation of a_w lowering behaviour of sodium lactate and some related compounds. J. Food Sci. 45; 802-804.

Chirife, J., Ferro Fontan, C., and Scorza, O. 1980. A study of the water activity lowering behaviour of some amino acids. J. Food Techol. 15: 383-387.

Christian, J.H.B., and Waltho, J.A. 1962. The water relations of Staphylococci and micrococci. J. Appl. Bacteriol. 25: 369-377.

Cleland, J.E., and Fetzer, W.R. 1944. As cited in: Christian, J.H.B. 1980. Reduced Water Activity. In Microbial Ecology of Foods. Vol. 1 Factors Affecting Life and Death of Microorganisms. ICMSF. Academic Press, New York, pp 70-91.

Donnelly, L.S., Ziegler, G.R., and Acton, J.C. 1982. Effect of liquid smoke on the growth of lactic acid starter cultures used to manufacture fermented sausage. A research note. J. Food Sci. 47: 2074-2075.

Doyle, M.P. and Roman, D.J. 1982. Response of *Campylobacter jejuni* to sodium chloride. Appl. Environ. Microbiol. 43: 561-565.

Duckworth, R.B. 1972. As cited in: Labuza T.P. 1975. Sorption Phenomena in Foods: Theoretical and Practical Aspects. In Theory, Determination, and Control of Physical Properties of Food Materials. Chokjun, Rha (ed.) Reidel Pub. Co., Dondrecht, Holland. pp 197-219.

Duckworth, R.B., and Smith, D.M. 1968. Diffusion of solutes at low moisture levels. In Recent Advances In Food Science, Vol. 3. Leith, J.M. and Rhodes, D.N. (eds.). Butterworths, London.

Dymsza, H.A. and Silverman, G. 1979. Improving the acceptability of intermediate-moisture fish. Food Technol. 14: 55-53.

Eichner, K. 1975. The influence of water content on non-enzyamic browning reactions in dehydrated food and model systems and the inhibition of fat oxidation by browning intermediates. In Water Relations of Foods. Duckworth, R.B. (ed.). Academic Press, New York. pp. 417 - 434.

Egan, A.F., Ford, A.L. and Shay, B.J. 1980. Comparison of *Microbacterium thermosphactum* and lactobacilli as spoilage organisms of vacuum - packed sliced luncheon meats. J. Food Sci. 45: 1745 - 1748.

Erickson, L.E. 1982. Recent developments in intermediate moisture foods. J. Food Protect. 45: 484-491.

Fennema, O.R. 1976. Principle of Food Science. Part 1 Food Chemistry. Marcel Dekker, New York.

Fennema, O. and Carpenter, J. 1984. Water activity in muscle and related tissues. Reciprocal Meat Conference Proceedings. 37: 19-23.

Flink, J.M. 1978. Intermediate moisture food products in the American marketplace. J. of Food Proc. and Preservation. 2: 324-339.

Gailani, M.B. and Fung, D.Y. 1986. Critical review of water activities and microbiology of drying of meats. CRC Critical Reviews in Food Science and Nutrition. 25: 159-183.

Gill, C.O., 1986. The control of microbial spoilage in fresh meats. In Advances in Meat Research Volume 2. AVI Publishing Co. pp 44-88.

Gill, C.O. and Newton, K.G. 1977. The development of aerobic spoilage flora on meat stored at chill temperatures. J. Appl. Bacteriol. 43: 189-195.

Grover, D.W. 1947. The keeping properties of confectionery as influenced by its water vapour pressure. J. Soc. Chem. Ind. 66: 201-205.

Hanna, M.O., Stewart, J.C., Carpenter, Z.L. and Vanderzant, C. 1977. Effect of packaging methods on the development of *Yersinia enterocolica* on beef steaks. J. Food Safety 1: 29.

Heidelbaugh, N.D. and Karel, M. 1975. Intermediate moisture food technology. In Freeze Drying and Advanced Food Technology. Goldblith, S.A., Rey, L. and Rothmayr, W.W. (eds.) Academic Press, London. p. 618-667.

Heldman, D.R., Reidy, G.A. and Palnitkar, M.P. 1973. Texture stability during storage of freeze-dried beef at low and intermediate moisture contents. J. Food Sci. 38: 282-285.

Holley, R.A. 1981. Prevention of spoilage mold growth on Italian dry sausage by natamycin and potassium sorbate. Applied Environ. Microbiol. 45: 422-429.

Holley, R. 1985. Beef jerky: Viability of food-poisoning microorganisms on jerky during its manufacture and storage. JFood Protect. 48: 100-106.

Holley, R.A. 1986. Effect of sorbate and pimaricin on surface mold and ripening of Italian Dry salami. Lebensm-Wiss. u-Technol. 19: 59-65.

Iglesias, H.A. and Chirife, J. 1976. Equilibrium moisture contents of air dried beef. Dependence on drying temperature. J. Food Technol. 11: 565-573.

Ingram, M. and Simonsen, B. 1980. Meat and meat products. In Microbial Ecology of Foods Vol. II Food Commodities. The International Commission on Microbiological Specifications for Foods (ICMSF). Academic Press, New York. pp. 333-409.

Jay, J.M. 1978. Modern Food Microbiology. (2nd ed.) D. Van Nostrand Co., New York. p. 29.

Kaplow, M. 1970. Commercial development of intermediate moisture foods. Food Technology. 24: 53-57.

Karel, M. 1975. Free radicals in low moisture system. In Water Relations of Foods. Duckworth, R.B. (ed.). Academic Press, New York.

Karel, M. 1976. Technology and application of new intermediate moisture foods. In Intermediate Moisture Foods. Davies, R., Birch, G.G. and Parker, K.J. (eds.). Applied Science Publishers, London. pp. 4-31.

Karel, M. and Labuza, T.P. 1968. Nonenzymatic browning in model systems containing sucrose. J. Agric. Food Chem. 16: 717.

Karmas, E. 1980. Techniques for measurement of moisture content of foods. Food Technol. 34: 52-59.

Karmas, E. and Chen, C.C. 1975. Relationship between water activity and water binding in high and intermediate moisture foods. J. Food Sci. 40: 800-801.

Kemp, J.D., Langlois, B.E. and Fox, J.D. 1983. Effect of potassium sorbate and vacuum packaging on the quality and microflora of dry-cured intact and boneless ham. J. Food Sci. 48: 1709-1714.

Konstance, R.P. and Panzer, C.C. 1985. Air drying bacon slices to reduce a_w: An anti-clostridial alternative to sodium nitrite. J. Food Sci. 50: 862-868.

Labuza, T.P. 1968. Sorption phenomena in foods. Food Technol. 22: 15.

Labuza, T.P. 1971. Kinetics of lipid oxidation in foods. CRC Crit. Rev. Food Technol. 2: 355-405.

Labuza, T.P. 1975. Oxidative changes in foods at low and intermediate moisture levels. In Water Relations of Foods. Duckworth, R.B. (ed.) Academic Press, New York. p. 455.

Labuza, T.P. 1975. Sorption phenomena in foods: Theoretical and practical aspects. In Theory, Determination and Control of Physical Properties of Food Materials. Chokyun, R. (ed.) Reidel Pub. Co., Dordrecht-Holland. pp 197-219.

Labuza, T.P. 1984. Moisture Sorption: Practical Aspects of Isotherm Measurement and Use. American Association of Cereal Chemists, St. Paul, Minnesota. pp. 150.

Labuza, T.P., Tsuyaki, H. and Karel, M. 1969. Kinetics of Lipid oxidation in model systems. JAOCS 46: 409.

Labuza, T.P., Tannenbaum, S.R. and Karel, M. 1970. Water content and stability of low moisture and intermediate moisture foods. Food Technol. 24: 543-550.

Labuza, T.P., Cassil, S. and Sinskey, A.J. 1972. Stability of intermediate moisture foods. 2. Microbiology. J.Food Sci. 37: 160-162.

Labuza, T.P., McNally, L., Gallagher, D., Hawkes, J. and Hurtado, F. 1972. Stability of intermediate moisture foods. 1. Lipid oxidation. J.Food Sci. 37: 154-159.

Labuza, T.P., Acott, K., Tatini, S.R., Lee, R.Y. and Flink, J.M. 1976. Water activity determination: A collaborative study of different methods. J. Food Sci. 41: 911-917.

Laleye, L.C., Lee, B.H., Simard, R.E., Carmichael, L. and Holley, R.A. 1984. Shelf-life of vacuum- or nitrogen-packed pastrami: Effects of packaging atmospheres, temperature and duration of storage on microflora changes. J. Food Sci. 49: 827-831.

Lawrie, R.A. 1975. Meat components and their variability. In Meat. Cole, D.J.A. and Lawrie, R.A. (eds.). Butterworth, London. pp 249-268.

Lawrie, R.A. 1979. Meat Science. (3rd ed.). Pergamon Press, Oxford. pp 163-299.

Landrock, A.H. and Proctor, B. 1951. A new graphical interpolation method for obtaining humidity equilibria data, with special reference to its role in food packaging studies. Food Technol. 5: 332-337.

Lee, R.Y., Silverman, G.J. and Mumsey, D.T. 1981. Growth and enterotoxinA production by *Staphylococcus aureus* in precooked bacon in the intermediate moisture range. J. Food Sci. 46: 1687-1692, 1700.

Lechowich, R.V. 1971. Microbiology of Meat. In The Science of Meat and Meat Products. Price, J.F. and Schweigert, B.S. (eds.). W.H. Freeman and Company, San Francisco, pp 230-286.

Leistner, L. 1985. Hurdle technology applied to meat products of the shelf-stable product and intermediate moisture food types. In Properties of Water in Foods in Relation to Quality and Stability. Simato, D. and Multon, J.L. (eds.). Martinus Nijhoff Publishers, Dondrecht, The Netherlands. pp. 309-329.

Leistner, L. and Karan-Djurdic, S. 1970. As cited in: Leistner, L. 1985. Hurdle technology applied to meat products of the shelf-stable product and intermediate moisture food types. In Properties of Water in Foods in Relation to Quality and Stability. Simato, D. and Multon, J.L. (eds.) Martinus Nijhoff Publishers, Dondrecht, The Netherlands. pp. 309-329.

Leistner, L. and Rodel, W. 1975. The significance of a_w for microorganisms in meats. In Water Relations of Foods. Duckworth R.B. (ed.) p. 309.

Leistner, L. and Rodel, W. 1976. Inhibition of microorganisms in food by water activity. In Inhibition and Inactivation of Vegetative Microbes. Skinner, F. A. and Hugo, W.P. (eds.). Academic Press, New York. pp. 219-235.

Leistner, L., Shin, H.K., Hechelmann, H. and Lin, S.Y. (1984) Microbiology and technology of Chinese meat products. EMMRW (6): 279-280.

Leistner, L., Wirth, F. and Vukovic, I. 1979. Fleisch-wirtschaft 59: 1313.

Ling, G. 1965. As cited in: Labuza, T.P. 1975. Sorption phenomena in foods. Theoretical and practical aspects. In Theory, Determination and Control of Physical Properties of Food Materials. Rha, C. (ed.). Reidel Pub. Co., Dordrecht-Holland. pp. 197-219.

Loncin, M., Bimbenet, J.J., and Lenges, L. 1968. Influence of the activity of water on the spoilage of food-stuffs. J. Food Technol. 3: 131-142.

Mossel, D.A.A. 1975. Water and microorganisms in foods - A synthesis In Water Relations of Foods. Duckworth R.B. (ed.). Academic Press, New York. p. 347-364.

Multon, J.L., and Guilbot, A. 1975. Water activity in relation to the thermal inactivation of enzymatic proteins. In Water Relations of Foods. Duckworth, R.B. (ed.). Academic Press, New York p. 379-396.

McLaren, A.D. and Rowen, J.W. 1952. As cited in: Labuza, T.P. 1975. Sorption phenomena in foods: Theoretical and practical aspects. In Theory, Determination and Control of Physical Properties of Food Materials. Rha, C. (ed.). D. Reidel Publishing Co., Dondrecht-Holland. pp. 197-219.

McKower and Myers. 1943. As cited in: Troller, J.A. 1983. Water activity measurements with a capacitance manometer. J.Food Sci. 48: 739-741.

MIRINZ 1986. Recommended Methods for the Analysis of Meat and Meat Products. Technical Report 841. Meat Industry Research Institution of New Zealand Inc., Hamilton, New Zealand.

Nemethy, G. 1968. The structure of water and aqueous solutions in low temperature biology foodstuff. Rec. Adv. Food Sci. 4: 1.

Nes, I.F. and Skjelkvale, R. 1982. Effect of natural spices and oleorecins on *Lactobacillus platarum* in the fermentation of dry sausages. J. Food Sci. 47: 1618-1621, 1625.

Newton, K.G and Gill, C.O. 1978. The development of the anaerobic spoilage flora of meat stored at chill temperatures. J. Appl. Bacteriol. 44: 91-95.

Obanu, Z.A., Biggin, R.J., Neale, R.J., Ledward, D.A. and Lawrie, R.A. 1976. Iron availability from intermediate moisture beef. J. Food Technol. 11: 583-588.

Obanu, Z.A., Biggin, R.J., Neale, R.J., Ledward, D.A. and Lawrie, R.A. 1976. The protein of intermediate moisture meat stored at tropical temperature. IV Nutritional quality. J. Food Technol. 11: 575-581.

Obanu, Z.A. and Ledward, D.A. 1975. On the nature and reactivity of the haematin complexes present in intermediate moisture beef. J. Food Technol. 10: 675-680.

Obanu, Z.A., Ledward, D.A. and Lawrie, R.A. 1975. The protein of intermediate moisture meat stored at tropical temperature. I. Changes in solubility and electrophoretic pattern. J. Food Technol. 10: 657-666.

Obanu, Z.A., Ledward, D.A. and Lawrie, R.A. 1975. The protein of intermediate moisture meat stored at tropical temperature. II. Effect of protein changes on some aspects of meat quality. J. Food Technol. 10: 667-674.

Obanu, Z.A., Ledward, D.A. and Lawrie, R.A. 1980. Lipid-protein interactions as agents of quality deterioration in intermediate moisture meats: An appraisal. Meat Sci. 4: 79-88.

Ockerman, H.W. and Kuo, J.C. 1982. Manufacture and acceptability of an Oriental dried pork product. EMMRW: 230-233.

Paltnikar, M.P. and Heldman, D.R. 1971. Equilibrium moisture characteristics of freeze dried beef components. J. Food Sci. 36: 1015-1018.

Palumbo, S.A. 1984. Heat injury and repair *Campylobacter jejuni*. Applied Environ. Microbiol. 48: 477-480.

Prior, B.A. 1979. Measurement of water activity in foods: a review. J. Food Protect. 42: 668-674.

Raccach, M. and Marshall, P.S. 1985. Effect of manganese ions on the fermentative activity of frozen-thawed lactobacilli. J. Food Sci. 50: 665-668.

Robinson, R.A. and Stokes, R.H. 1959. As cited in: Christian, J.H.B. 1980 Reduced water activity. In Microbial Ecology of Food Vol. 1. Factors Affecting Life and Death of Microorganism. ICMSF. Academic Press, New York. pp. 70-91.

Rockland, L.B. and Nishi, S.K. 1980. Influence of water activity of food product stability. Food Technol. 34: 42-51, 59.

Rodel, W. and Leistner, L. 1972. As cited in: Troller, J. and Christian, J.H.B. 1978. Water Activity and Food. Academic Press, New York.

Ross, K.D. 1975. Estimation of water activity in intermediate moisture foods. Food Technol. (March): 26-34.

Scott, W.J. 1957. Water relations of food spoilage microorganisms. In Adv. Food Res. 7: 83-127.

Scott, W.J. 1953. As cited in: Scott, W.J. 1957. Water relations of food spoilage microorganisms. In Adv. Food Res. 7: 83-127.

Shelef, L.A., Naglik, O.A. and Bogen, D.W. 1980. Sensitivity of some common food-bonne bacteria to the spices, sage, rosemary and allspice. J. Food Sci. 45: 1042.

Silverman, G.J., Munsey, D.T. and Ebert, E. 1983. Interrelationship between water activity, temperature and 5.5 percent oxygen on the growth and enterotoxinA secretion by *Staphylococcus aureus* in precooked bacon. J. Food Sci. 48: 1783.

Simard, R.L., Zee, J. and L'Heureux, L. 1984. Microbial growth in carcasses and boxed beef during storage. J. Food Protect. 47: 773.

Sloan, A.E. and Labuza, T.P. 1976. Prediction of water activity lowering ability of food humectants at high a_w J. Food Sci. 41: 632-645.

Smith, P.R. 1971. The determination of equilibrium relative humidity or water activity in foods - A literature review. BFMIRA Scientific and Technical Survey No. 70.

Smith, D.R. 1984. Shelf-stable intermediate moisture meat product. CSIRO Food Research Quarterly 44: 12-14.

Stiebing, A. 1985. Frankfurter-type sausages: Heat treatment and shelf-life. Fleischwirtshaft 65: 1087-1091.

Taylor, J.B. 1961. Determination of moisture equilibrium in hydrated foods. Food Technol. 15: 536-537.

Taylor, J.B. and Rowlinson, J.S. 1955. As cited in: Christian, J.H.B. 1980. In Microbial Ecology of Food Vol. I Factors Affecting Life and Death of Microorganisms. ICMBF. Academic Press, New York. pp 70-91.

Toribio, J.L., Nunes, R.V. and Lozano, J.E. 1984. Influence of water activity on the non-enzymatic browning of applie juice concentrate during storage. A research note. J. Food Sci. 49: 1630-1631.

Troller, J.A. 1973. The water relations of food-bonne bacterial pathogens. J. Milk Food Technol. 36: 276-288.

Troller, J.A. 1977. Statistical analysis of a_w measurements obtained with the Sina scope. J. Food Sci. 42: 86-90.

Troller, J.A. 1979. Food spoilage by microorganisms tolerating low water activity environments. Food Technol. 33: 72-75.

Troller, J.A. 1983. Methods to measure water activity. J. Food Prot. 46: 129.

Troller, J.A. 1983. Water activity measurements with a capacitance manometer. J. Food Sci. 48: 739-741.

Troller, J.A. 1986. Water relations of foodborne bacterial pathogens - an updated review. J. Food Protect. 49(9): 656-670.

Troller, J.A. and Christian, J.H.B. 1978. Water Activity and Food. Academic Press, New York.

Ueda, S., Yamashita, H., Nakajima, M. and Kuwabara, Y. 1982. As cited in: Buchanan, R.L. 1986. Processed meats as a microbial environment. Food Technol (April): 134-138.

Van Ardel, W.B. 1963: Food Dehydration. AVI Pub. Co. Westport, Conn.

Varga, S., Sims, G.G., Michalik, P. and Regier, L.W. 1979. Growth and control of halophilic micro-organisms in salt minced fish. J. Food Sci. 44: 47-50.

Webster, C.E.M., Wood, R.M. and Ledward, D.A. 1979. Prediction of water activity, a_w, in cook-soak equilibrated intermediate moisture meats. Meat Sci. 3: 43-51.

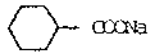
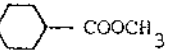
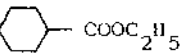
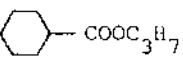
Wehr, H.M. 1982. Attitudes and policies of governmental agencies on microbial criteria for foods - An update. Food Technol. 36: 45-54, 92.

Zaika, L.L. and Kissinger, J.C. 1979. Effects of some spices on acid production by starter cultures. J. Food Protect. 42: 572-576.

Zaika, L.L. and Kissinger, J.C. 1984. Fermentation enhancement by spices: Identification of active component. J. Food Sci. 49: 5-9.

APPENDICES

Appendix 1: Data on the pH, solubility, and the usage of organic acids and esters as food preservatives (From Baird-Parker, 1980)

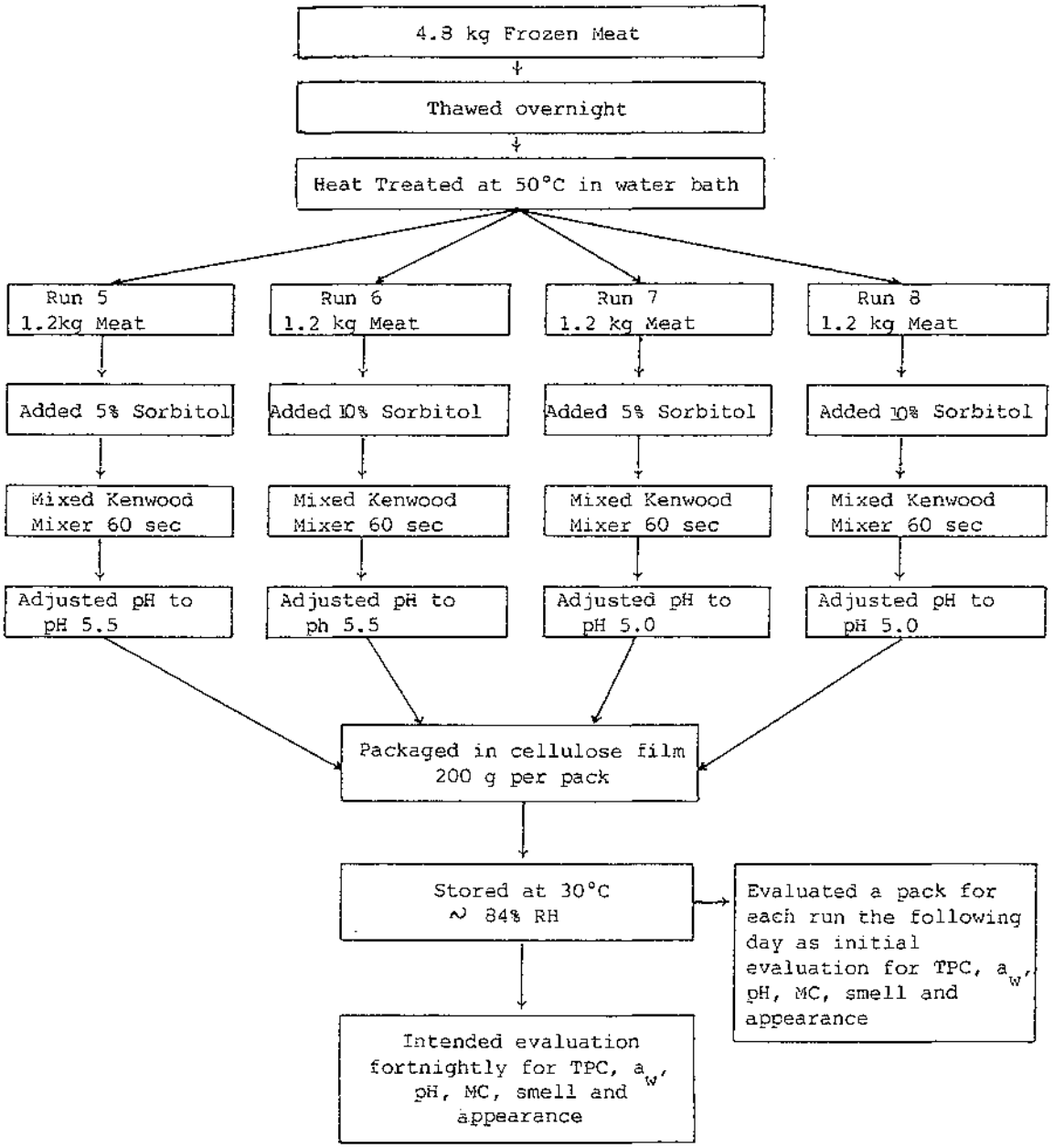
		pK_a	Solubility[b] (gm/100gm)	ADI[c] (mg/kg body weight)	Typical maximum use concentration (mg/kg)	Examples of usage
Acetic acid	CH_3COOH	4.75	Very soluble	Not limited	No limit	Pickling of meat, fish, and vegetable products
Sodium diacetate	$CH_3COONaCH_3COOH\frac{1}{2}H_2O$	4.75	Very soluble	Not limited	4000	Bread and bakery products
Sodium benzoate		4.2	50 (25°C)	5	1-3000	Pickles, acid sauces and salads, semipreserved fish, fruit juices, soft drinks, jams, margarines
Citric acid	$(COOH)CH_2C(OH)(COOH)CH_2COOH$	3.1	Very soluble	Not limited	No limit	Soft drinks
Lactic acid	$CH_3CHOHCOOH$	3.1	Very soluble	Not limited	No limit	Salad creams and mayonnaise
Methyl paraben[a]		8.5	0.16 (15°C)	10	1-2000	See sodium benzoate
Ethyl paraben[a]		8.5	0.08 (15°C)	10	1-2000	
Propyl paraben[a]		8.5	0.023 (15°C)	10	1-2000	
Sodium propionate	$CH_3CH_2COON_a$	4.9	Very soluble	10	1-3000	Bread, bakery, and cheese products
Sorbic acid (including K salt)	$CH_3CH=CHCH=CHCOOH$	4.8	0.16 (20°C)	25	1-2000	Fresh and processed cheese, dairy products, bakery products, fruit juices, acid sauces and salads, jams, jellies, soft drinks margarines, semi-preserved fish, and meat products

[a] Paraben = p-hydroxybenzoic acid [b] Solubility in water (temperature) [c] Acceptable Daily Intake (FAO/WHO, 1973).

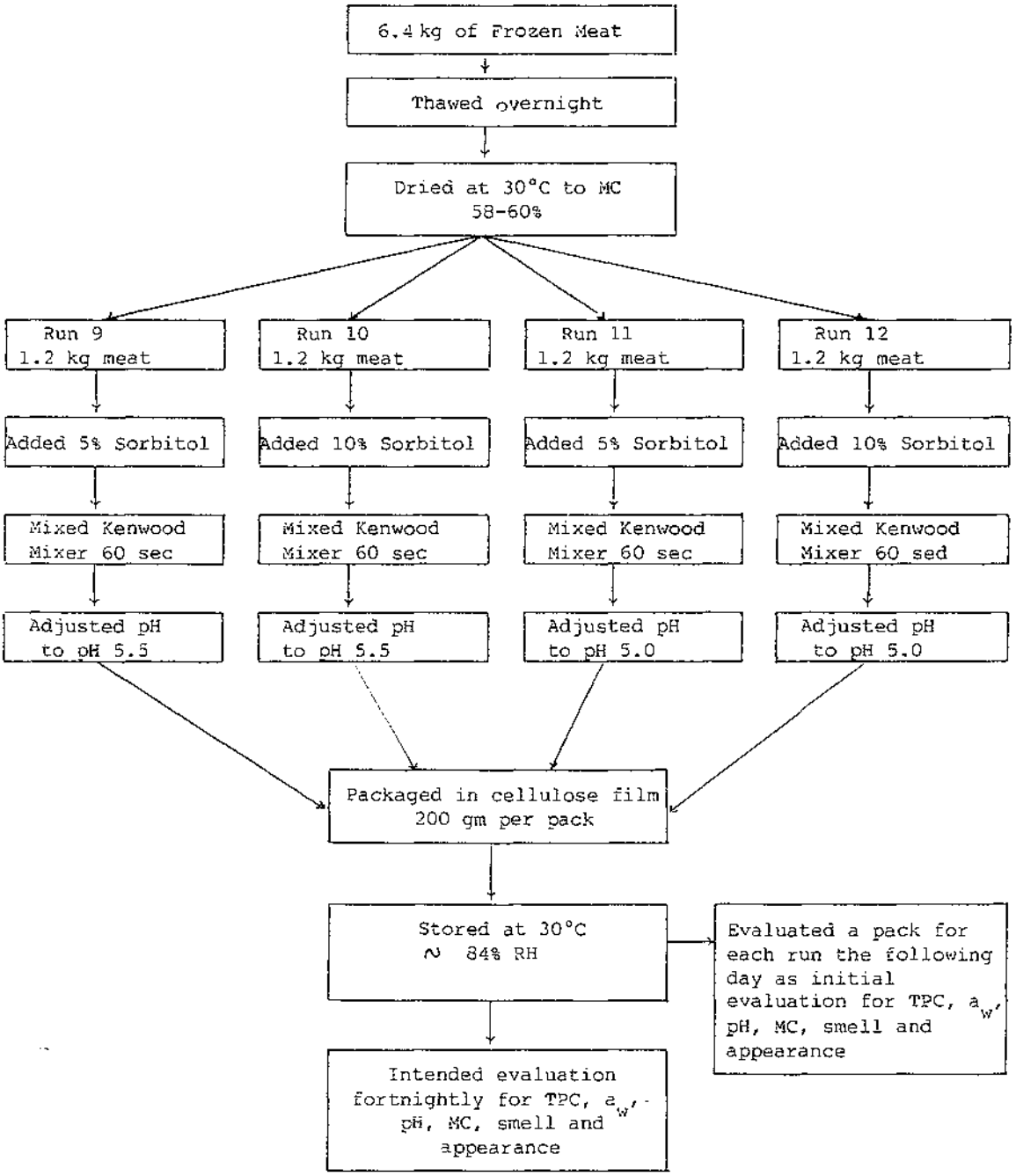
[d] Typical values; actual values will vary with product and from country to country. Food Additive Lists should be consulted for specific limits for a country.

Appendix 2: Procedures for the preparation of samples with various combination of factors

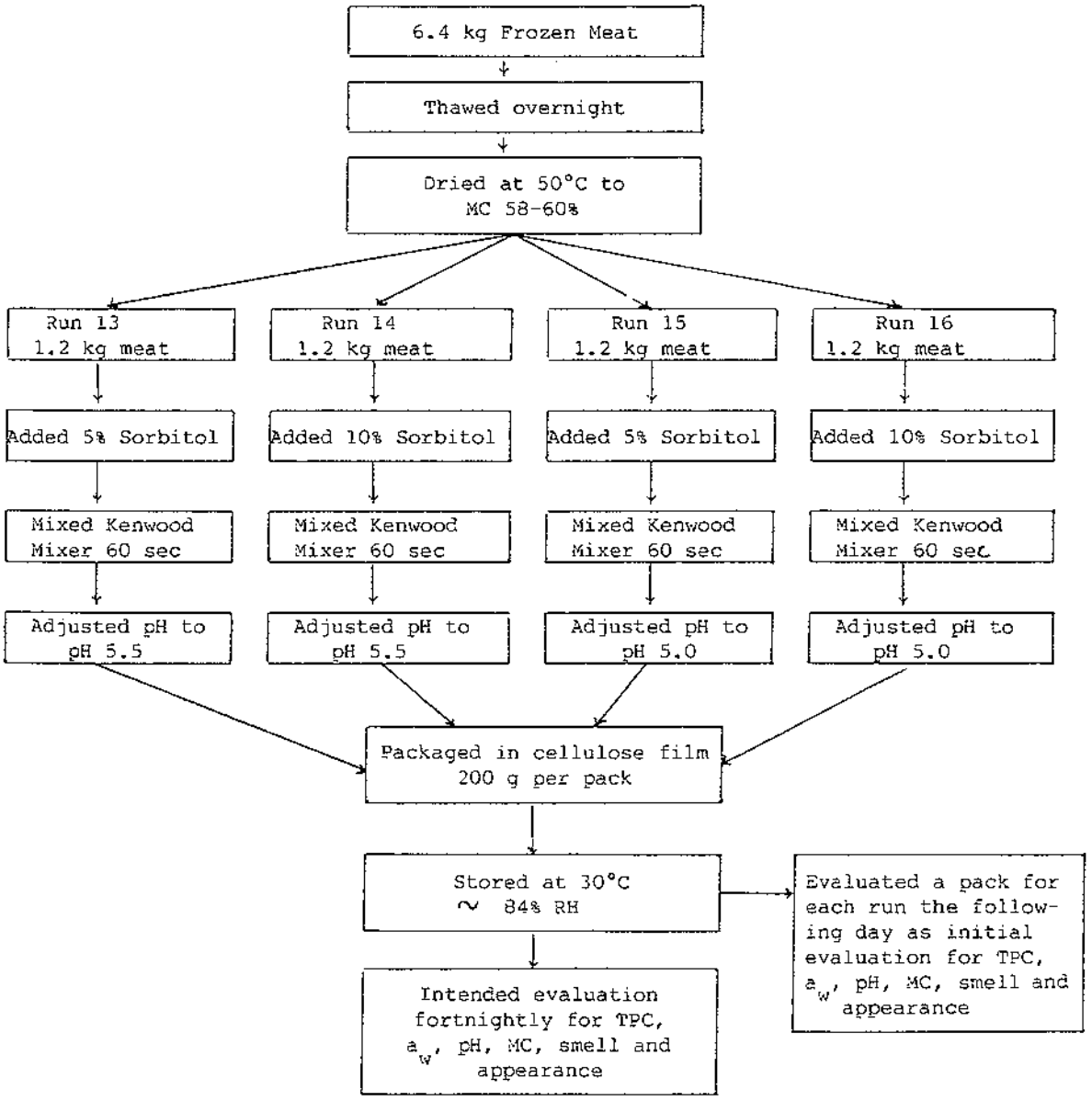
Appendix 2.1: Order of Sequence for the preparation of samples for Block 2 Day 6 (Runs 5 to 8)



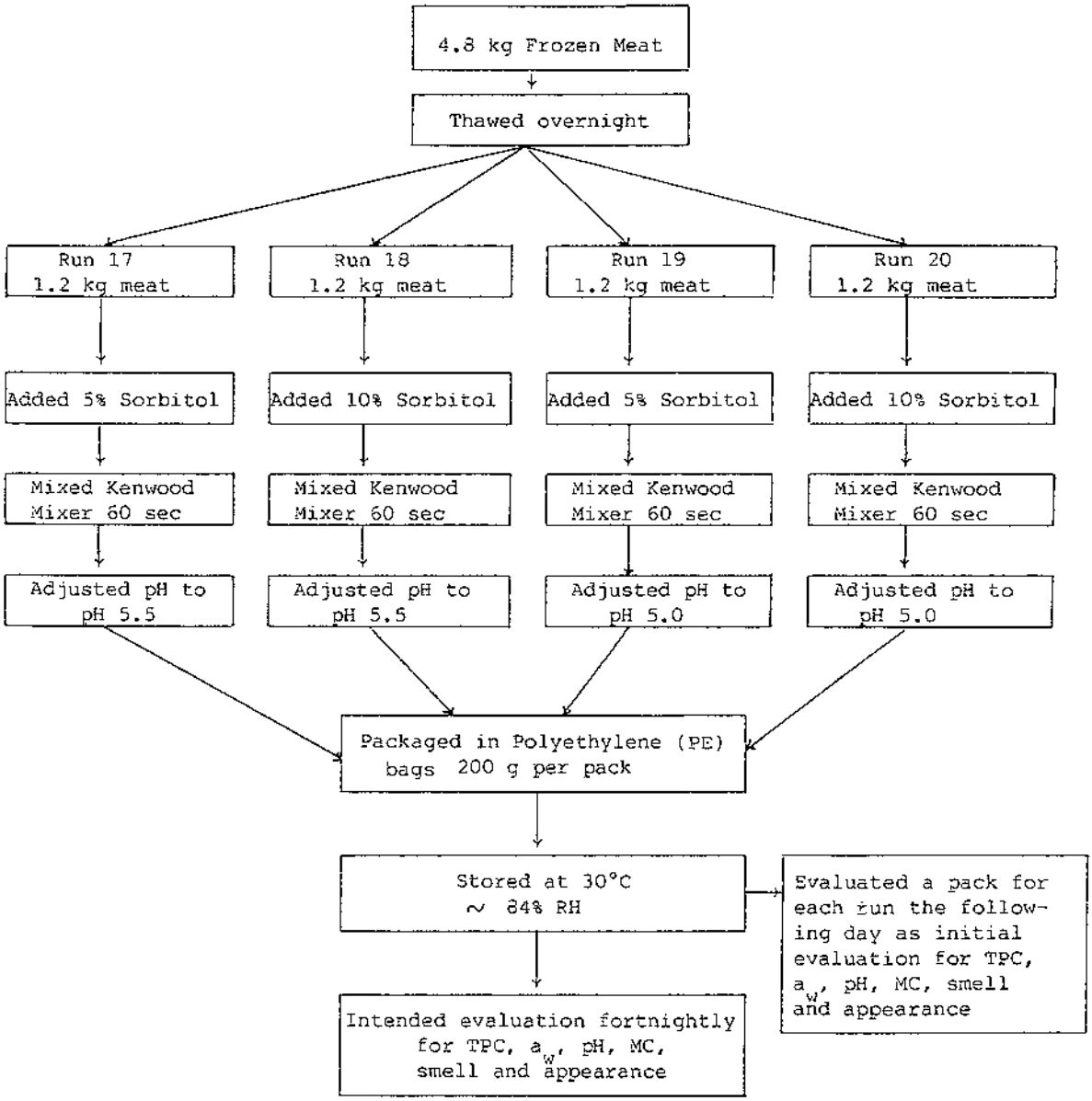
Appendix 2.2: Order of sequence for the preparation of samples for Block 3 Day 3 (Runs 9 to 12)



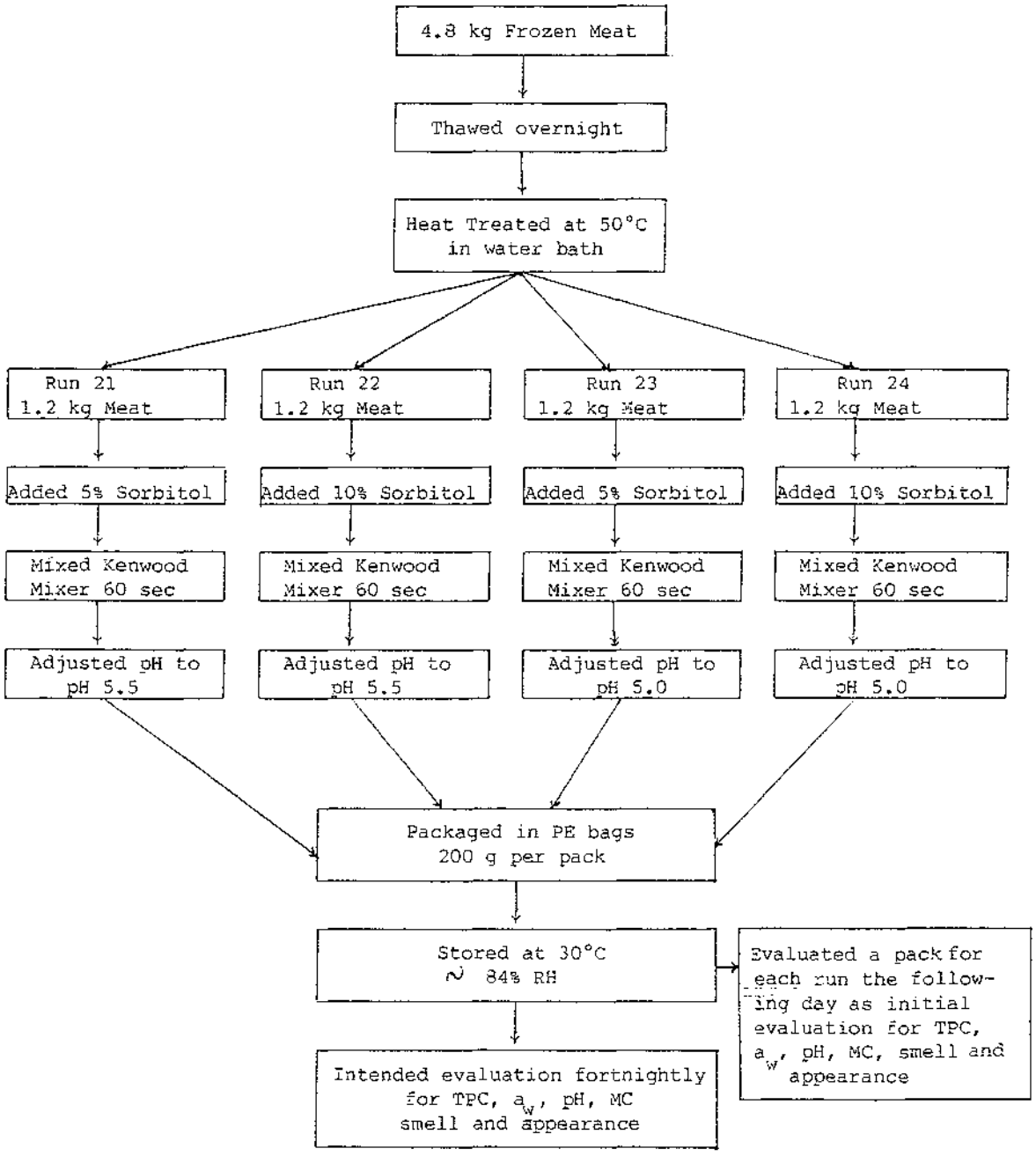
Appendix 2.3: Order of sequence for the preparation of samples for Block 4 Day 8 (Runs 13 to 16)



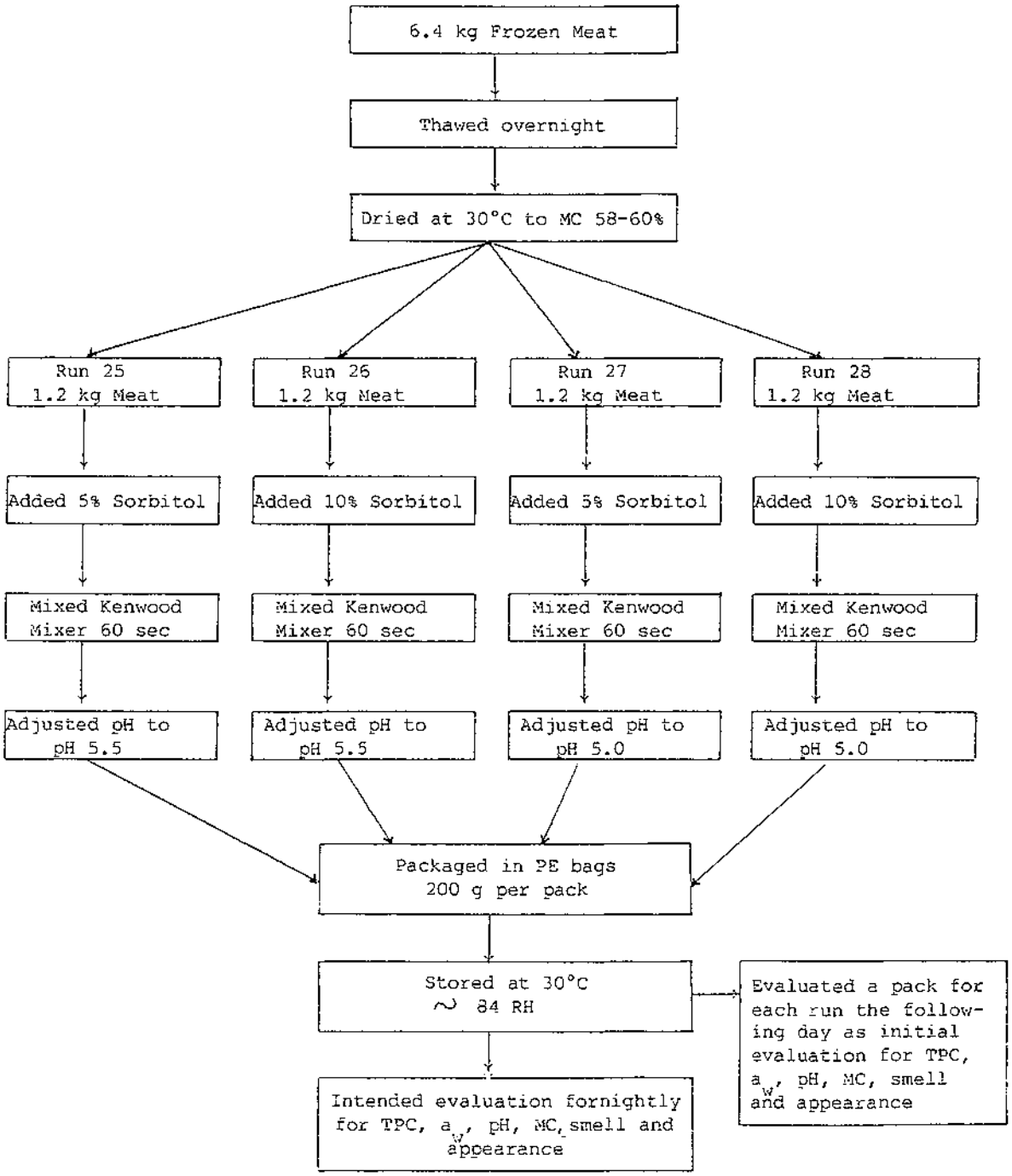
Appendix 2.4: Order of sequence for the preparation of samples for Block 5 Day 12 (Runs 17 to 20)



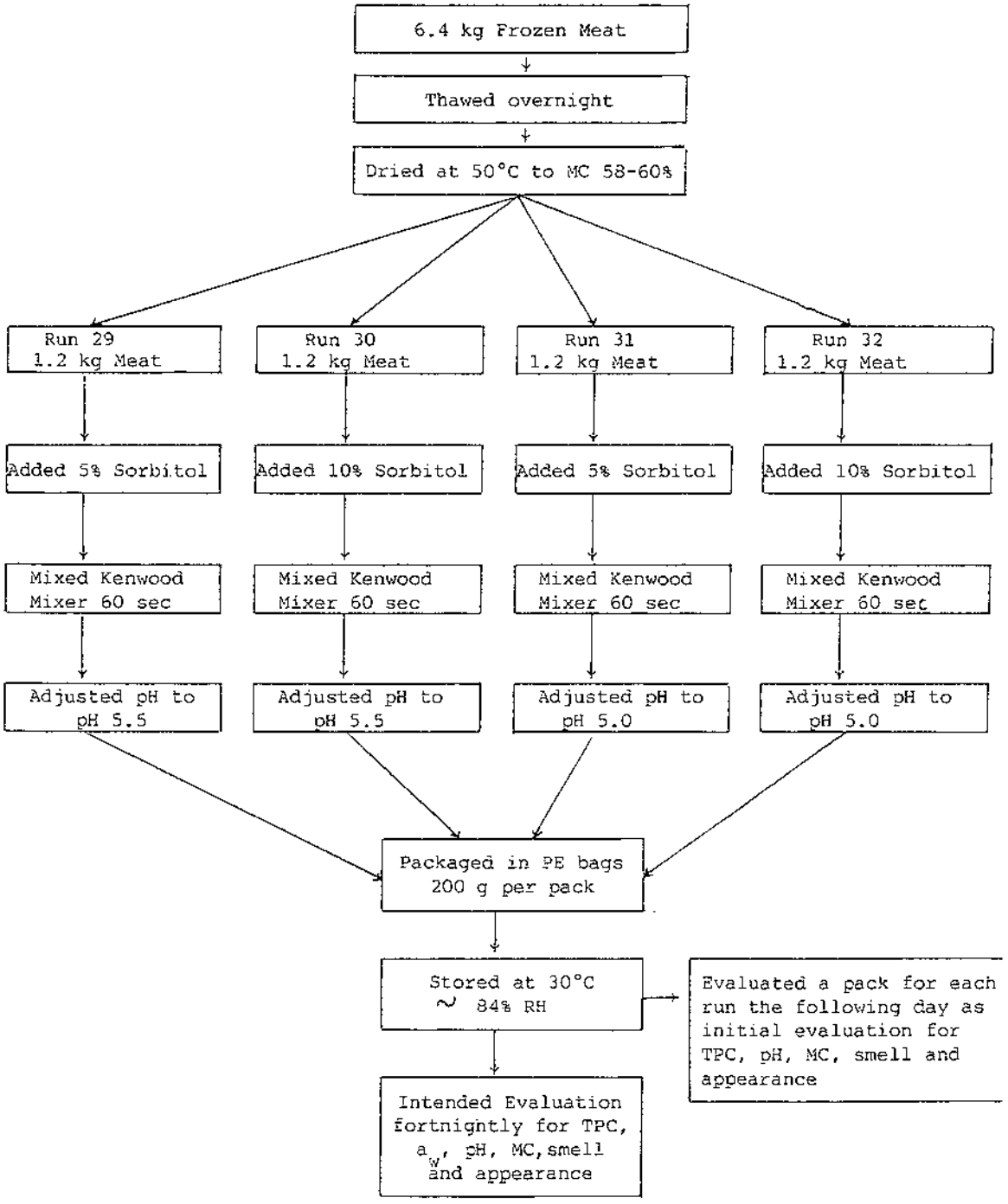
Appendix 2.5: Order of sequence for the preparation of samples for Block 6 Day 4 (Runs 21 to 24)



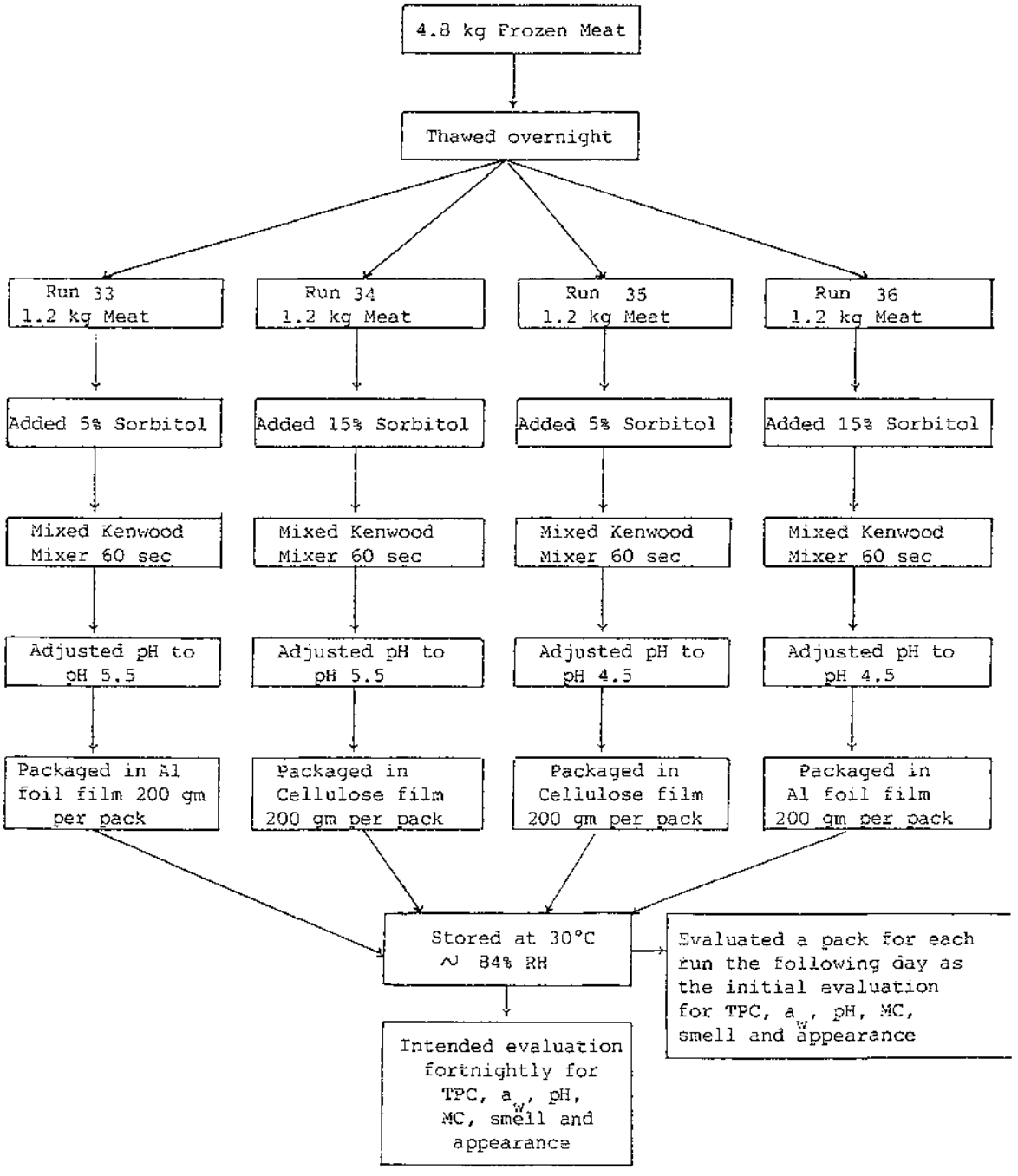
Appendix 2.6: Order of sequence for the preparation of samples for Block 7 Day 13 (Runs 25 to 28)



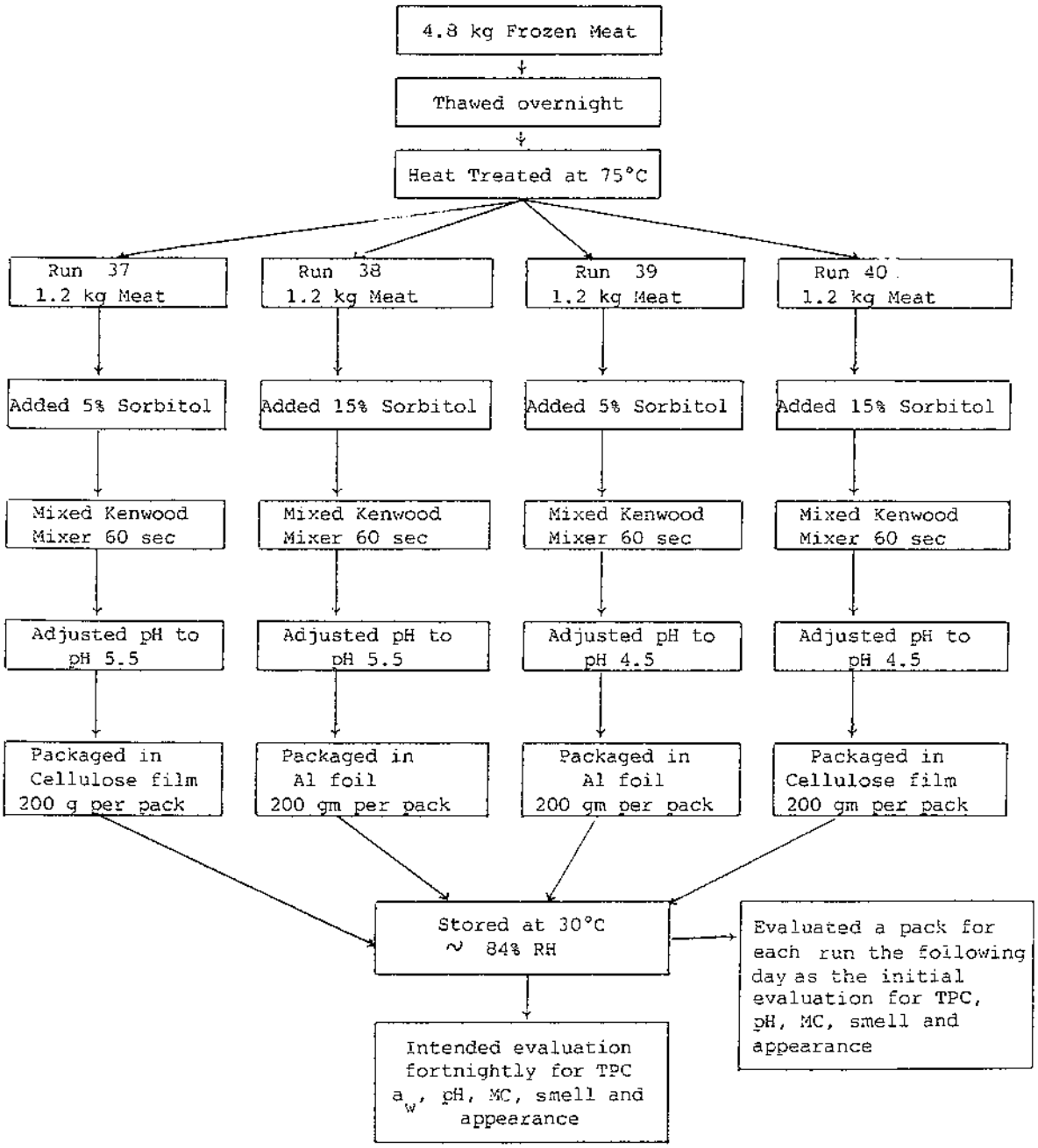
Appendix 2.7: Order of sequence for the preparation of sample for Block 8 Day 9 (Runs 29 to 32)



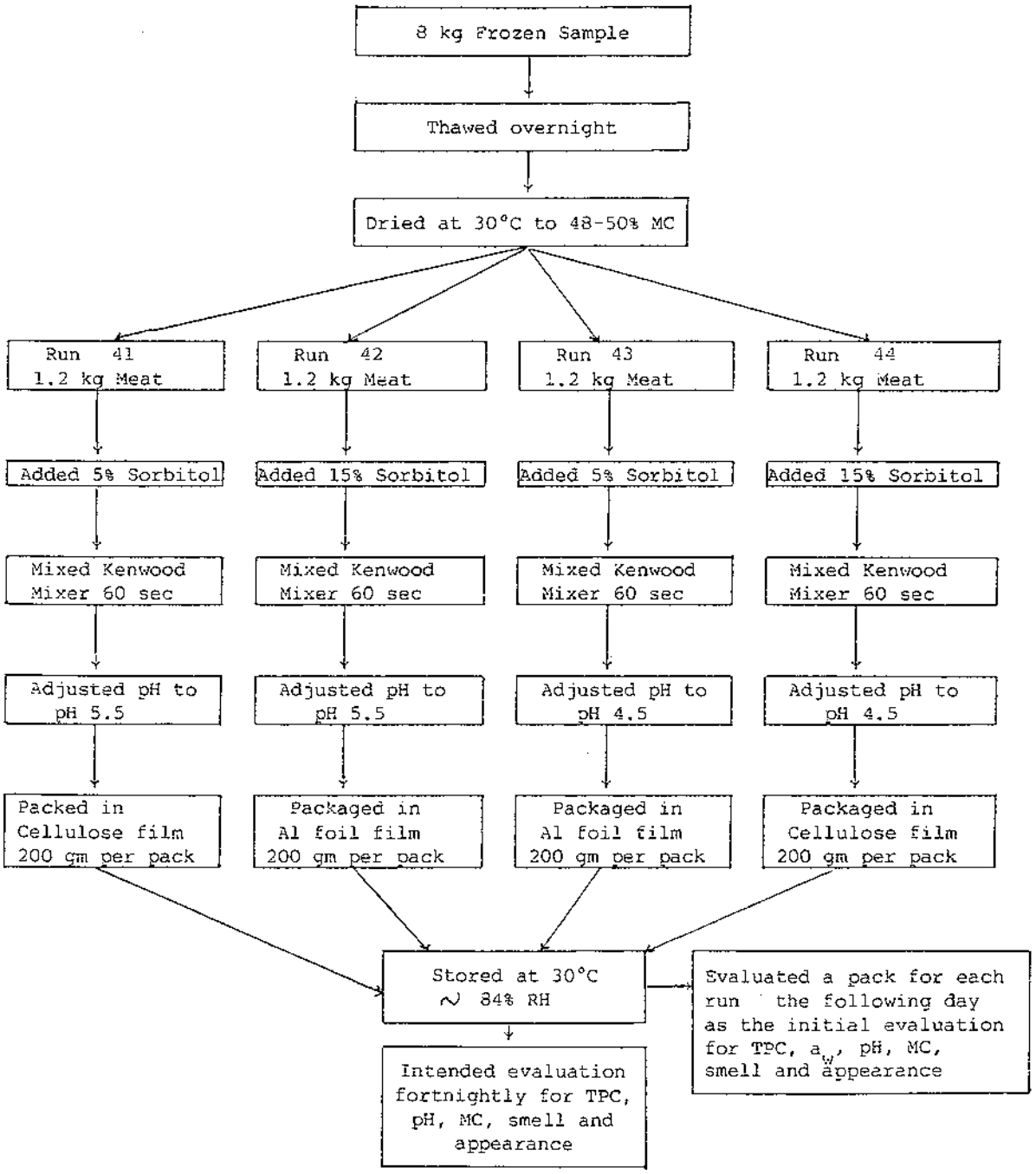
Appendix 2.8: Order of sequence for the preparation of samples for Block 9 Day 10 (Runs 33 to 36)



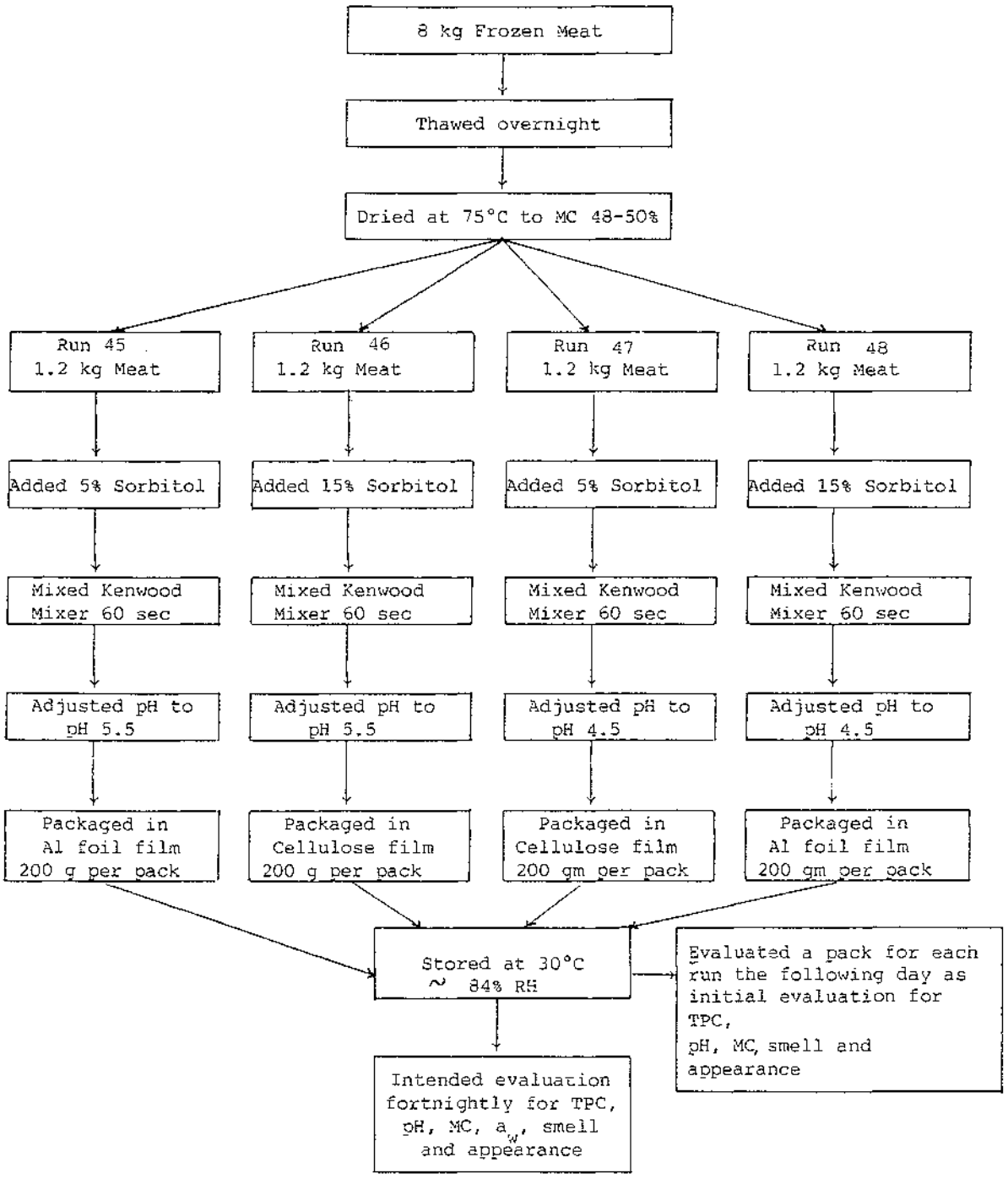
Appendix 2.9: Order of Sequence for the preparation of samples for Block 10 Day 5 (Runs 37 to 40)



Appendix 2.10: Order of sequence for the preparation of samples for Block 11 Day 2 (Runs 41 to 44)



Appendix 2.11: Order of sequence for the preparation of samples for Block 12 Day 1 (Runs 45 to 48)



Appendix 3: Rapid method (modified Babcock method) for free fat determination (From MIRINZ, 1986)

EQUIPMENT

Usual laboratory equipment plus -
Paley bottles (Kimble No. 2085 or equal).

REAGENTS

Mix equal volumes of glacial acetic acid and 60% perchloric acid.

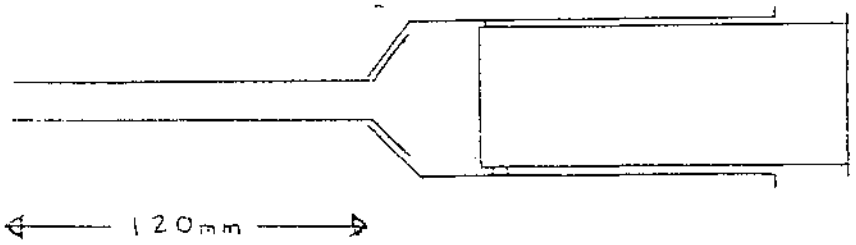
Note: Perchloric acid must be handled and disposed of with care.

PROCEDURE

Weigh accurately 9.0 g of sample into a Paley bottle. The use of a modified 50 or 100 cc syringe is convenient (sketch below). Add about 30 ml of acetic acid/perchloric acid reagent, mix, and place in a boiling water bath for about 10 min. Shake the bottle occasionally until sample is dissolved. Remove from bath and add more acetic/perchloric acid reagent until the column of fat is within the graduated range. Centrifuge if necessary (2 min at 600 rev/min). Read the percentage of fat from the graduated scale, which is calibrated for a 9.0 g sample.

Modified 50 cc syringe

The modification consists of the insertion of a flared stainless steel tube of the dimensions shown into the body of the plastic syringe.



s/s about 10 mm OD
and about 8 mm ID

50 cc plastic syringe

Millipore XXII-050-05
or similar

Appendix 4 : Suitable salts together with their corresponding relative humidities in the 5° - 40°C range (From Rockland, 1960)

Salt	Relative Humidity % at deg. C							
	5	10	15	20	25	30	35	40
Lithium chloride	16	14	13	12	11	11	11	11
Potassium acetate	25	24	24	23	23	23	23	23
Magnesium bromide	32	31	31	31	31	30	30	30
Magnesium chloride	33	33	33	33	33	32	32	31
Potassium carbonate	-	47	45	44	43	42	41	40
Magnesium nitrate	54	53	53	52	52	52	51	51
Sodium bromide	59	58	58	57	57	57	57	57
Cupric chloride	65	68	68	68	67	67	67	67
Lithium acetate	72	72	71	70	68	66	65	64
Strontium chloride	77	77	75	73	71	69	68	68
Sodium chloride	76	75	75	75	75	75	75	75
Ammonium sulphate	81	80	79	79	79	79	79	79
Cadmium chloride	83	83	83	82	82	82	79	75
Potassium bromide	-	86	85	84	83	82	81	80
Lithium sulphate	84	84	84	85	85	85	85	81
Potassium chloride	88	87	87	86	86	84	84	83
Potassium chromate	89	89	88	88	87	86	84	82
Sodium benzoate	88	88	88	88	88	88	86	83
Barium chloride	93	93	92	91	90	89	88	87
Potassium nitrate	96	95	95	94	93	92	91	89
Potassium sulphate	98	97	97	97	97	97	96	95
Disodium phosphate	98	98	98	98	97	96	93	91[b]
Lead nitrate	99	99	98	98	97	96	96	95
Group B								
Zinc nitrate	43	43	41	38	31	24	21	19
Lithium nitrate	61	59	55	49	41	31	19	11
Calcium nitrate	-	66	60	56	54	51	48	46
Cobalt chloride	-	-	73[a]	67	64	62	59	57
Zinc sulphate	95	93	92	90	88	86	85	84

[a] 18°C

[b] 38°C

Appendix 5: Questionnaire for descriptive evaluation of minced mutton meat with reduced pH

NAME: _____ DATE: _____

QUESTIONNAIRE

Instructions: Please evaluate the two coded samples of meat patties based on sour flavour intensity and general acceptability. Check the descriptive term which best describes the product. Please do not hesitate to give comments.

Sour Flavour	Sample Code		Acceptability	Sample Code	
none	5	___ ___	very acceptable	5	___ ___
slightly noticeable	4	___ ___	acceptable	4	___ ___
recognizable	3	___ ___	slightly acceptable	3	___ ___
pronounced	2	___ ___	unacceptable	2	___ ___
very pronounced	1	___ ___	very unacceptable	1	___ ___

COMMENTS: _____

_____

Thank you