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THE PRODUCTION AND MICROBIAL QUALITY OF FERMENTED DRY POULTRY
SAUSAGE FROM THIGH TRIMS

BY

MOHAMAD AZIZ BIN TAIB
(D.V.M., MALAYSIAN AGRICULTURAL UNIVERSITY)

Department of Poultry Science
The West of Scotland College
Auchincruive
Ayr KA6 5HW

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The University of Glasgow
Scotland, The United Kingdom

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INDEX

LIST OF DIAGRAMS, TABLES AND FIGURES	i
ACKNOWLEDGEMENT	ii
ABBREVIATIONS	iv
SUMMARY	v
INTRODUCTION	1
CHAPTER ONE: LITERATURE REVIEW	
1.1 Fermented sausages	4
1.2 Inclusion of poultry meat into fermented sausages	5
1.3 Starter culture bacteria in fermented sausages	8
1.3.1 Methods of fermenting meat	11
1.3.2 Microbial and chemical changes of starter cultures and their roles in fermented sausages	13
1.4 Processing factors and their contributions to product and microbial qualities during fermentation and drying	15
1.4.1 Internal factors	16
1.4.1.1 Meat	16
1.4.1.2 Fat	18
1.4.1.3 Salt	19
1.4.1.4 Nitrate/Nitrite	19
1.4.1.5 Carbohydrate	20
1.4.1.6 Condiments	22
1.4.1.7 Functional additives- soy isolate	22
1.4.2 External factors	25
1.4.2.1 Temperature	25
1.4.2.2 Relative humidity	26
1.4.2.3 Casing diameter, air velocity and smoking	26

1.5	Sensory quality of fermented sausages	28
1.5.1	Texture	28
1.5.2	Colour	29
1.5.3	Flavour	30
1.6	Safety aspects of fermented poultry sausages	32
1.6.1	Amines and nitrosoamines	32
1.6.2	Polycyclic aromatic hydrocarbon	35
1.6.3	Microbial quality of poultry meat used in the formulation.	36
1.6.4	Microbial quality of fermented sausages	39
1.6.4.1	<u>Salmonella</u> spp	43
1.6.4.2	<u>Listeria monocytogenes</u>	44
1.6.4.3	<u>Staphylococcus aureus</u>	46
1.6.4.4	<u>Clostridium perfringens</u> and <u>Cl. botulinum</u>	48
CHAPTER TWO: MATERIALS AND METHODS		
2.1	Experiment One	50
2.1.1	Aim of experiment	50
2.1.2	Materials and Methods	50
2.1.2.1	Materials	50
2.1.2.2	Sausage Formulation	51
2.1.2.3	Preparation of Fermented Sausage	51
2.1.3	Sampling and Recording	54
2.1.4	Chemical Methods	54
2.1.4.1	Moisture	54
2.1.4.2	Fat	55
2.1.4.3	Unbound fat	55

2.1.4.4 Lipid separation	56
2.1.4.5 Fatty acid determination and gas liquid chromatography	58
2.1.4.6 Protein	59
2.1.4.7 pH	60
2.1.4.8 Titratable acidity	60
2.1.4.9 Salt	60
2.1.4.10 Water activity	61
2.1.5 Objective measurements	61
2.1.5.1 Weight loss	61
2.1.5.2 Texture	62
2.1.6 Sensory analysis	62
2.2 Experiment Two	63
2.2.1 Aim of experiment	63
2.2.2 Materials and Methods	64
2.2.3 Sampling and recording	64
2.2.4 Confidence limits for colony counts	64
2.2.5 Microbial Methods	65
2.2.5.1 Preparation and dilution of the food homogenate	65
2.2.5.2 Plating	66
2.2.5.3 Total Viable Counts	67
2.2.5.4 <u>L. pentosus</u>	67
2.2.5.5 <u>S. carnosus</u>	67
2.2.5.6 <u>S. aureus</u>	67
2.2.5.7 <u>Cl. perfringens</u>	68
2.2.5.8 Enumeration of coliforms and <u>E. coli</u> by Most Probable Numbers Method (MPN)	68

2.2.5.9 Isolation of <u>Salm. enteritidis</u>	70
a. Determination of <u>Salm. enteritidis</u> total count	70
b. Detection and confirmation of <u>Salm.</u> <u>enteritidis</u>	71
2.2.5.10 Isolation of <u>Listeria monocytogenes</u>	72

CHAPTER THREE: RESULTS

3.1 Results of Experiment One	75
3.1.1 Chemical analysis	75
3.1.1.1 Effect of soy isolate on moisture, fat and proteins	75
3.1.1.2 Effect of soy isolate on titratable acidity and pH	81
3.1.1.3 Effect of soy isolate on salt and water activity	83
3.1.2 Objective Analysis	85
3.1.2.1 Weight loss	85
3.1.2.2 Texture	88
3.1.3 Sensory Analysis	88
3.1.4 Lipid Analysis	90
3.1.4.1 Unbound fat	90
3.1.4.2 Lipid composition of the fermented sausages	90
3.1.4.3 Fatty acid composition of the fermented sausages	90
3.2 Results Experiment Two	94
3.2.1 Microbiological analysis	94

3.2.1.1	Effect of soy isolate and smoking on growth of mesophiles	94
3.2.1.2	Effect of soy isolate and smoking on growth of <u>Lactobacillus pentosus</u>	105
3.2.1.3	Effect of soy isolate and smoking on growth of <u>S. carnosus</u>	106
3.2.1.4	Effect of soy isolate and smoking on coliforms	106
3.2.1.5	Effect of soy isolate and smoking on psychrophiles	107
3.2.1.6	Growth and inhibition <u>Salm. enteritidis</u>	107
3.2.1.7	Microbial quality of the fermented sausages	109
3.2.2	Chemical analysis	111
3.2.2.1	Moisture	111
3.2.2.2	pH and titratable acidity	114
3.2.2.3	Salt content	119
3.2.3	Objective analysis	122
3.2.3.1	Moisture loss	122

CHAPTER FOUR: DISCUSSION

4.1	Effects of soy isolate on the chemical analysis of the fermented dry poultry sausage	125
4.2	Effects of soy isolate on the sensory characteristics and objective analysis of the fermented dry poultry sausage	129
4.3	Effects of soy isolate on the microbiological analysis of fermented dry poultry sausage	131
4.4	Microbiological quality of the fermented dry poultry sausage	134

4.5	Effects of smoke on the overall analysis of the fermented dry poultry sausage	136
4.6	Fermented sausage- source of fat in human diet	138
	REFERENCES	139

LIST OF TABLES, DIAGRAMS AND FIGURES

TABLES

1.....	10
2.....	51
3.....	80
4.....	93
5.....	108
6.....	110

DIAGRAMS

1.....	53
2.....	73
3.....	74

FIGURES

1.....	41
2.....	42
3.....	77
4.....	78
5.....	79
6.....	82
7.....	84
8.....	86
9.....	87
10.....	89
11.....	92
12.....	96
13.....	97
14.....	98
15.....	99
16.....	100
17.....	101
18.....	102
19.....	103
20.....	104
21.....	112
22.....	113
23.....	115
24.....	116
25.....	117
26.....	118
27.....	120
28.....	121
29.....	123
30.....	124

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ABBREVIATIONS

a_w	= Water activity
cfu/g	= Colony forming unit per gram
<u>et al.</u>	= And others
cm	= Centimetre
g	= Gram
h	= Hour
kg	= Kilogram
LAB	= Lactic acid bacteria
l	= Litre
mm	= Millimetre
ml	= Millilitre
min	= Minutes
ppb	= Parts per billion
ppm	= Parts per million
P_o	= Oxidation-reduction potential
μ l	= Microliter
μ g	= Microgram
μ mol/g	= Micromoles per gram
$^{\circ}$ C	= Degree Centigrade
$^{\circ}$ F	= Degree Farenheit
dia	= Diameter
%	= Percentage
d	= Day
<	= Less than
>	= More than
sec	= Second

ABSTRACT

Fermented dry poultry sausage from chicken thigh trims was successfully produced with different levels of soy isolate.

Sausage mixtures consisting of chopped thigh trims, salts, sugar, water and with or without soy isolate were blended with starter culture bacteria consisting of Lactobacillus pentosus ($0.2 * 10^{10}$ cfu/g) and Staphylococcus carnosus ($2 * 10^{10}$ cfu/g). After initial fermentation of 2 d, the sausage mixtures were stuffed into the casing and then dried under predetermined temperature and relative humidity in the cold room and the drying of the sausages was stopped when the moisture content of about 35 % was attained. Some of the sausages were subjected to smoking.

Addition of soy isolate reduced the greasiness and weight loss of the final product. At the end of the drying period, the texture of the fermented sausage produced with 4 % soy isolate was firmer than those produced with 2 and 0 % soy isolate.

Non-smoked sausages had a lower composition of moisture at the end of drying period than those smoked. The moisture loss during drying appeared to be affected significantly by the fall in pH during the initial fermentation period. Sausage mixture with a pH at the end of the initial fermentation period of 5.24 took 12 more days to reach the 'dry' stage than those with pH below 5.

The fall in pH was the result in the production of lactic acid. Sausage with 4 % soy isolate in the formulation had a

higher percent of lactic acid as shown by the higher percent of titratable acidity and the higher titratable acidity was matched by a rapid increase in the growth of the L. pentosus. This finding suggested that soy isolate stimulated the growth of the lactobacilli.

During fermentation the rapid fall in pH was followed by the decrease in the numbers of the mesophiles, L. pentosus, psychrophiles, the coliforms and E.coli. The numbers of these microorganisms fell further after smoking of the sausages. However S. carnosus appeared to be resistant to the effect of lactic acid, smoking and other intrinsic factors, especially during the drying period.

High lactic acid, low pH and a_w were thought to be responsible for the death of of Salm. enteritidis, S. aureus and Cl. perfringens. However, the sausage was not free from L. monocytogenes.

These findings suggest that soy isolate is not only responsible for the changes in the chemical compositions, sensory qualities and objective measurements of the fermented dry poultry sausages produced, it is also important from the microbial quality of the finished products. But the presence of L. monocytogenes warrants further research in the future.

INTRODUCTION

The consumption of poultry meat has been on a tremendous increase and the main contributing factors are high production efficiency resulting in relatively low prices of the meat, rigorous development and production of convenience, further processed and value-added products and the healthy image portrayed by the meat itself.

Besides the production of value-added and convenience products such as chicken nuggets, chicken franks, rolled smoked breast and chicken fresh or cooked sausages and chicken hams, to name a few, poultry meat is also suitable for the production of fermented sausages. Baran et al. (1973) reported the preliminary result on the production of dry fermented turkey sausages and according to their results sausages smoked at more than 47°C for 5 h gave a strong and undesirable acid-taste. Nedeljkovic et al. (1987) revealed that the fermented sausages from breast meat had a better water holding capacity than that from the thigh meat and sausages from poultry meat and fat had 'a specific and pleasant flavour'. However, their latter result was in disagreement with Dawson (1970) who suggested that fermented sausages from poultry or turkey meat 'were organoleptically worse than those produced with addition of beef'.

The meat component of the fermented sausage can be either derived from the expensive cuts such as breast or thigh or the cheaper meat such as the mechanically separated poultry meat (MSPM). Apparently, no study has been made to utilise other

cheaper source of meat such as thigh trims in the production of fermented sausages. The idea of utilising thigh trims in the production of poultry sausages serves three purposes. Being high in myoglobin content, the fermented sausage from thigh trim is expected to have a better curing colour and the fat available from the trims is adequate for the production of fermented sausages. Also, inclusion of cheaper trims into value-added product raw, such as in fermented sausage may generate better income to the processor.

The problem of oiliness or greasiness has been reported by Acton and Dick (1975) and Holley et al. (1986). Beef kidney fat and beef back fat were used by both authors to reduce the problem. There is also no report to reduce the 'oily' problem of fermented sausages made wholly from poultry meat and fat tissue. Although soy and other plant proteins have been used extensively as meat extenders (Rao et al., 1984) and emulsifiers (Thompson et al., 1984), work on the utilisation of soy protein products on fermented sausages for fat binding has been rarely reported (Lee et al., 1989).

It is also realised that relatively few works have been carried out to evaluate the inhibition of food borne pathogens such as Salmonella spp, Staphylococcus aureus, Clostridium perfringens and Listeria monocytogenes, which are inherent in poultry meat, on the poultry fermented sausages. Baran and Stevenson (1975) reported that under high dose of different pathogens, the presence of inhibitory factors such as low pH and reduced a_w would not guarantee the total inhibition of the

Salmonella spp., C. perfringens and S. aureus. However, Holley et al. (1986) reported that both S. aureus and Salmonella spp. were undetected after 14 days of ripening in smoked sausages from MSPM. Heat treatment to an internal temperature of 60°C, salt and sodium nitrite in combination with lactic acid gave the largest reduction of the population of S. aureus (Raccach and Baker, 1979).

Therefore the purpose of the research is to produce and evaluate the fermented dry poultry sausage produced from thigh trims incorporated with different levels of soy isolate. Chemical compositions, objective measurements and sensory qualities will be assessed. The research will also be looking into the growth and development of the mesophiles, lactobacilli, S. carnosus, coliforms and psychrophiles in the smoked and unsmoked sausage prepared with different levels of soy isolate and survival of the foodborne pathogen Salmonella enteritidis spiked into the sausages. Finally, the microbial quality of the product at the end of the drying period would also be examined.

The information available could help the processors to utilise more poultry meat and fat, particularly the cheaper parts such as thigh trims and to understand the effect of soy isolate in the production of fermented poultry dry sausage. It could also help to reveal the survival or the elimination of one of the major food-borne pathogen Salmonella enteritidis and the microbial quality of the final product.

LITERATURE REVIEW

Fermentation of meat is a branch of biotechnology whereby starter culture bacteria such as lactic acid bacteria (LAB) are being exploited to produce and preserve the finished meat products. Campbell-Platt (1987) defined fermented foods, including the fermented sausages, as those foods which have been subjected to the actions of microorganisms or enzymes so that desirable biochemical changes cause significant modification to the foods. By fermentation the food may be more nutritious, more digestible, safer or have better flavour.

1.1 Fermented sausages

Fermented sausages have been known for centuries and are increasingly liked since they are 'naturally' preserved and stabilised by a reduced water activity (a_w) and or pH (Leistner, 1987).

American Meat Institute (AMI) (1989) defines dry and semi-dry sausages as fermented meat products that are dried to remove 25-50 % and 10-15 % of the moisture, respectively with moisture/protein ratios of dry and semi-dry sausages not to exceed 2.3:1 (AMI, 1982) and 3.1:1 (AMI, 1989), respectively. The moisture/protein ratio of the final product indicates the extent of drying (Bacus, 1984).

Another factor that helps to differentiate the dry and semi-dry sausages is the fact that the semi-dry sausage is usually

fully cooked in the smoked house to aid in drying, during which time active fermentation occurs (Pederson, 1971). Smoking the sausage improves its flavour but this step is not an important factor in the production of dry sausage.

Depending on the final moisture content of the fermented sausages and whether they are subjected to smoking or not, different types of fermented sausages are available to the consumers. They include semi-dry cervelat, German salami and pepperoni, which are smoked, and dry Italian salami and chorizo, which are not. There are also cooked products such as mortadello, kochsalami and thuringer, which are less common. Other fermented sausages include Lebanon bologna, summer sausages, bruhwurst, kochwurst, rohwurst and mettwurst. Poultry fermented sausages include chicken dry sausage, turkey dry sausage, summer-styled turkey sausage, etc.

1.2 Inclusion of poultry meat into fermented sausages

The inclusion of poultry meat into the fermented sausages commonly comes from two main sources; the expensive cuts, from the breast and thigh, and the cheaper meat or the mechanically seperated poultry meat (MSPM) from racks, backs and necks. The utilisation of the poultry meat up to more than 50 % in combination with meat from other species has been the subject of a number of studies by Keller and Acton (1974); Acton and Dick (1975); Baran et al. (1973); Dhillon and Maurer (1974); McMahon and Dawson (1976) and Raccach and Baker (1979).

Baran et al. (1973) reported the preliminary production of a

dry fermented sausages from turkey meat. The most acceptable sausages were produced using the following smoking treatments: 27°C for 3 h; 32°C for 4 h and 46°C for 5h. Too long a smoking time gives an undesirable sharp-acid flavour. A final internal heating temperature of 71°C rather than 46°C gave a pronounced cooked flavour and an unacceptable dry texture.

Nedeljkovic et al. (1987) reported that the fermented sausages from chicken breast meat had a better water holding capacity than sausages from thigh meat. The author also revealed that the sausages of poultry meat with chicken fat had a 'specific and very pleasant flavour'. However, his latter finding was in disagreement with the result obtained by Dawson (1970) on the production of semi-dry and dry fermented sausages that contain poultry or turkey meat and in combination with 25 or 50 % beef. The sausages from poultry or turkey meat 'were organoleptically estimated as worst than those produced with addition of beef'.

With the advent of mechanical deboners, more MSPM has been produced. Due to the nature of the deboning process, the meat is in a puree form , higher in fat and lower in protein than hand deboned meat. Essary (1989) found that the protein content of MSPM was from 3.6 % after removing the breast and leg meat to 17 % in the MSPM from whole carcasses and the fat content was 18.5% in samples both breast and leg removed. Therefore, the MSPM is less able to maintain structural stability in comminuted meat product (Froning, 1970). The structural stability of sausages from MSPM could be enhanced by centrifugation, addition of other meat sources and polyphosphate salts. Froning and Johnson (1973)

found that centrifugation of MSPM improved its function and stability of the sausage. Dhillon and Maurer (1974) produced sausages formulated from MSPM / Beef in the proportions of 15/85, 35/65 and 50/50 and the sausages were found equally acceptable and possessed a desirable texture, sliceability and firmness. However, firmness decreased with the increased addition of MSPM. Holley et al. (1986) reported that by adding 10 % of MSPM to other meat sources such as pork, beef and beef back fat, it would not affect the final organoleptic quality of the final fermented product.

The inclusion of polyphosphate salts to the meat mixture prior fermentation, such as sodium salts of polyphosphates, has been shown to bind poultry and other red meats (Spencer and Smith, 1962; Froning, 1966; Vadehra and Baker , 1970). Phosphates improved binding by increasing the amount of soluble protein exudation (Furia, 1972). The salt soluble protein, especially myoglobin, has been found to be responsible for the greater binding of the meat (Fukazawa et al., 1961). McMahon and Dawson, (1975) reported that the use of 0.5 % phosphate salt in fermented turkey sausage from MSPM improved binding and tear value. Addition of that amount of phosphate permitted incorporation of up to 35 % MSPM in the fermented turkey sausages. Dhillon and Muarer (1974) also reported that by adding phosphates and functional additives such as soya protein in the proportion of 2 and 3.5 % respectively into 50/50 sausage emulsions containing MSPM and ground beef improves the firmness and sliceability and reduced smoke house loss of the summer sausage as compared to

those without the additives. However, there was no significant difference in the sensory evaluation of the finished product in both with or without the additives.

1.3 Starter culture bacteria in fermented sausages

Smith and Palumbo (1983) defined meat starter culture bacteria as viable microorganisms added directly to meat to improve the keeping quality, improve the safety and enhance consumer acceptability of the meat product.

The use of starter culture for meat fermentation was first introduced by Jensen and Paddock in 1940 and the first bacteria proposed were a number of Lactobacilli spp.. The other bacteria in use later on were Micrococcus urantiacus (Niinivaara, 1955), followed by Pediococcus cerevisiae (lately P. acidilactici). P. acidilactici was popular as it could withstand the lyophilisation process (Everson, 1971). Later on starter cultures such as Lactobacillus plantarum and P. acidilactici were available as frozen concentrate. The frozen cultures were being widely accepted because they improved sausage uniformity and shortened fermentation cycles from 32-40 h using lyophilised culture form and to 18-24 h using the frozen concentrate (Everson et al, 1970). Keller and Acton (1974) reported that a lyophilised culture of P. acidilactici needs a longer lag phase than the frozen concentrate starter. The 12-16 h lag phase of the lyophilised form prior to acid production was necessary to permit the bacteria to absorb moisture and return to a vegetative state. This rehydration period may be critical in sausage production, especially in the event the meat mixture contained putrefactive

or pathogenic microorganisms. Currently, starter cultures are available in freeze-dried form and this makes transportation to distant places very much easier as a freezing temperature is no longer required during a journey lasting a few days.

All lactic acid bacteria (LAB) used as starter cultures in meat products should be homofermentative (ie. they ferment glucose and other sugars used in the making of raw sausages into mainly lactic acid). Lucke and Hechelmann (1987) revealed that each and every LAB has a different optimum temperature. Pediococcus acidilactici ferment most quickly at temperatures above 40°C and will not grow below 15°C; the optimum temperature for P. pentosus and L. plantarum is between 30-35°C; whilst L. sake and L. curvatus are more suited to even lower temperature of 20-22°C. The optimum temperature for L. pentosus is 30°C. Pediococci do not grow at temperature below 8°C. The choice of LAB is also determined by the available carbohydrate added into the sausage mixtures. The spectrum of fermentable sugar is the widest in the case of L. plantarum, while L. sake seldom ferments maltose and L. curvatus rarely ferment lactose and saccharose.

In the production of semi-dry and dry fermented sausages from poultry and other meat sources, single species of P. acidilactici, P. pentosaceus, P. plantarum or mixtures of L. plantarum and P. acidilactici and Micrococcus varians have been used as in Table 1.

Table 1: Microorganisms used as starter cultures in some of the fermented sausages

Lactic Acid Bacteria	Type of fermented sausages
<u>Pediococcus cerevisiae</u>	<ul style="list-style-type: none"> A. Semi-dry fermented sausage <ul style="list-style-type: none"> a. Summer sausage b. Cervelat c. Thuringer d. Summer-styled turkey sausage B. Dry fermented sausage <ul style="list-style-type: none"> a. Dry sausage b. Dry turkey sausage c. Salami d. Pepperoni e. Hot bar sausage
<u>P. pentosaceum</u>	<ul style="list-style-type: none"> A. Semi-dry fermented sausage <ul style="list-style-type: none"> a. Summer sausage B. Dry fermented sausage <ul style="list-style-type: none"> a. Pepperoni b. Genoa
<u>Lactobacillus plantarum</u>	<ul style="list-style-type: none"> A. Semi-dry fermented sausage <ul style="list-style-type: none"> a. Summer sausage B. Dry fermented sausage <ul style="list-style-type: none"> a. Salami b. European-type dry sausage
Mixtures of <u>P. cerevisiae</u> and <u>L. plantarum</u>	<ul style="list-style-type: none"> A. Semi-dry fermented sausage <ul style="list-style-type: none"> a. Lebanon bologna b. Summer sausage c. Cervelat B. Dry fermented sausage <ul style="list-style-type: none"> a. Pepperoni b. Dry turkey sausage
Mixtures of <u>P. cerevisiae</u> and <u>Micrococcus varians</u>	<ul style="list-style-type: none"> A. Dry fermented sausage <ul style="list-style-type: none"> a. Genoa b. Dry sausage

Source: Smith and Palumbo (1983).

Species of the family Micrococcaceae such as Micrococcus varians have been used as part of the starter culture bacteria. Recently, organisms such as Staphylococcus carnosus and S. xylosus which are able to reduce nitrate into nitrite, grow in 10 % salt and only grow poorly in anaerobic condition (Hammes et al., 1985) have been used. The last characteristic explains why these organisms are frequently found more on the outer part of the fermented sausages.

The minimum temperature for the growth of these Micrococcaceae strains currently available is 10°C or slightly below. During the ripening process, the microorganisms inoculated into the sausage mixture as starter culture grow little or not at all (Reuter, 1972), though they are metabolically active. Thus for successful colour and flavour development it is therefore important to add them at a sufficiently high level and 10^6 - 10^7 cells/g emulsion is recommended (Lucke and Hechelmann, 1987).

1.3.1 Methods of fermenting meat

Fermentation of meat can be accomplished by the lactic acid bacteria present as a part of the natural meat flora, introduced from equipment or introduced by adding back part of a freshly fermented meat batch (Deibel et al., 1961; Everson, 1971). However the most reliable method is by introducing large numbers of desirable LAB microorganism (10^7 to 10^9 cell /g sausage mixture). The growth and development of starter culture bacteria and Micrococcaceae in 'natural' and 'rapid' methods are illustrated in Figures 1 and 2 as in pages 41 and 42.

Since lactobacilli, one of the lactic acid bacteria, form an essential component of the microbial population in meat and meat products (Morishita and Shiromizu, 1986), there is a small number of fermented sausages producers who still depend on 'chance inoculation' to effect the fermentation of meat. Since there is no starter culture being added into the meat mixture, if relied upon requires a longer holding time (Daly et al., 1973). Raccach and Baker (1978) found that salted MSPM (3 % salt) stored at 5°C promoted growth of lactobacilli to a level of 10^6 cells/g after 12 days and MSPM attained pH of 4.7 after 5 days of fermentation.

Another method which is still in use is called 'back-slopping'. The meat reserved from a previous successful fermentation is held at 38°C for 24 h than added to sausage mixture (Daly et al., 1973). However, failures may occur in the aforementioned methods especially if heterofermentative or non-fermentative microorganisms predominate. This will result in production failures such as the development of off-flavour and casing 'explosions'.

Besides using microorganisms to bring about fermentation of the meat, chemical acidulation has also been used to achieve a desired acidification in the manufacture of some sausage products. The commonly used chemicals are glucono-delta-lactone (GdL) and citric acid whereby both of them are either used alone or in combinations to acidify the sausage. GdL is hydrolysed to gluconic acid in the presence of water and during hydrolysis it yields an abrupt drop in pH and causes an excessive reduction of water content , which is difficult to control (Daly et al.,

1973).

1.3.2 Microbial and chemical changes of starter cultures and their roles in fermented sausages

Smith and Palumbo (1981); Bacus and Brown (1985a and 1985b) and Vignolo et al. (1989) reviewed the role of LAB as a food additive, which in turn preserve the final product. After the preparation of the sausage batter, the a_w is about 0.97 - 0.96 and the low oxygen tension in the sausage results in a shift in microflora population towards the LAB and Micrococcaceae (Hoffmann and Scharner, 1980).

Under anaerobic condition the major activity of homofermentative LAB is the conversion of sugars, usually glucose or sucrose to lactic acid by the Embden-Meyerhof pathway. Deketelaere et al. (1974) found that the lactic acid was the main acid formed during the fermentation of carbohydrates, with minor components of acetic acid, generally about 10 to 1 of lactic to acetic acid. They also found small amounts of butyric and propionic acid. Both L- and D-lactic acids may be produced in a ratio which is determined by the microorganisms used, the fermentation conditions (pH, temperature, P_o), reaction time and substrate (Wagner, 1981). Acton et al. (1972) reported that fermentation time was a significant factor affecting both the rate of decrease in pH and the rate of increase in percent lactic acid.

Not only does the lactic acid imparts a tangy flavour to the fermented sausage but the reliable acidification activity of LAB

brings down the pH of fresh meat 6 - 6.5 to 5 and below. This exerts a favourable effect on the consistency and drying of the final product, which must be firm and chewy in texture so that it can be sliced. The low pH will denature the muscle proteins, making them gel-like and thus permitting aggregation of particles so the mass of the product becomes compact. This activity is largely responsible for the texture associated with fermented sausages (Smith and Palumbo, 1983; Vignolo et al., 1988).

Since pH is an important consideration for the drying of fermented sausage, Kramlich (1971) recommended that the pH of sausages after initial fermentation and before drying should be near 5.1 to ensure satisfactory removal of moisture from the sausages on drying. The results of Townsend et al. (1975) producing dry salami products with the pH chemically-adjusted to 6.6 and 5.5 and compared these with a control sausage of 5.9 showed no practical differences in weight loss over a 24 hour period. Palumbo et al. (1976) worked with peperoni of pH values ranging from 6.1 to 4.7 could show no direct dependence of weight loss with pH. However a study by Acton and Keller (1974) did show that summer sausages subjected to drying with a pH in the range of 5.9- 5.5 lost weight significantly slower than sausages in a pH range of 4.8-4.6. It has been found that the water holding capacity decreased to a minimum at pH 5.2. It has also been suggested that the higher water holding capacity of sausage mixes at a pH lower than 5.2 was due to the fact that some protein does remain functional to bind moisture and was not completely denatured.

During fermentation some LAB produce hydrogen peroxide. Hydrogen peroxide has been shown to possess both bacteriostatic and bacteriocidal effects in food associated with LAB. Dahiya and Speck (1968) found that 6 ug of hydrogen peroxide were bacteriostatic to S. aureus and a range of 25-35 ug of hydrogen peroxide/ml was bacteriocidal to the same organism. Levels lower than 12 ug/ml were bacteriocidal to Pseudomonas organism (Price and Lee, 1970). However, the chemical causes a defect in pink colour formation during the fermentation stage. The hydrogen peroxide will bleach red nitric oxide myochromogen to green porphyrines (Tjaberg et al., 1969).

The problem of hydrogen peroxide could be solved satisfactorily if the starter culture bacteria also contain suitable Micrococcaceae. Not only are these bacteria responsible for colour formation but they also produce catalase; an enzyme which is able to decompose hydrogen peroxide in meat and meat products by microbial action or oxidative reaction (Smith and Palumbo, 1983). Otherwise these peroxides would oxidise the fat causing a rancid taste. However, these bacteria are poor acid producers.

1.4 Processing factors and their contributions to product and microbial qualities during fermentation and drying

Production of fermented sausages involves four major stages namely formulation, stuffing, fermentation or ripening and drying. Some workers ferment and bring down the pH of the sausage mixture to the desired levels first before filling into the

casing, while others prefer to stuff the sausage mixtures first and let the fermentation happen in the casing and later follow by drying.

During fermentation the drop in pH is more important than a drop in water activity and moisture content. However, after the desired pH is achieved the gradual decrease of moisture and water activity is pertinent in determining the quality characteristics of the sausage such as shelf-life and nutritional safety and sensorial quality such as texture, colour and flavour (Demeyer et al., 1986) and appearance of the fermented sausages.

The change in moisture, water activity and pH over period of fermentation and drying is controlled by both external factors such as temperature, relative humidity and air velocity and also by internal control factors such as carbohydrates, salts, nitrites/nitrates, meat and starter cultures (Rodel and Steibing, 1988; Bacus and Brown, 1985a ; Lucke, 1985).

1.4.1 Internal factors

The internal factors are the ingredients used in the formulation. Besides the main ingredient such as meat, salts, fat, curing salts, the minor ingredients include phosphates, GdL and citric acid.

1.4.1.1 Meat

The meat is the main component of the fermented sausage. As the lactic acid bacteria operate in water phase, the greater the proportion of lean meat in the sausage mixture the higher the amount of available water, hence a faster pH drop during

fermentation. Acton and Dick (1975) suggested that the higher amount of lean turkey meat was a major factor in determining the total acidity in finished sausages. This helps to explain why freeze-dried meat has a low initial fermentation rate due to reduced moisture (Bacus and Brown, 1985a).

There are other meat factors that could affect the rate of fermentation. Although meat factors such as buffering capacity, DFD (dry, firm and dark) and PSE (pale, soft and exudative) and the proportion of frozen meat used in the formulation of fermented poultry sausages have not been investigated, the effect of those meat factors have been studied in fermented sausages prepared from beef and pork. Bacus and Brown (1985a) reported that beef has a higher buffering capacity than pork and this explained why pork has a higher fermentation rate than beef sausages.

A pH range of 5.9 to 6.3 at 15 minutes of post-mortem was considered normal in broiler muscle (Kijowski and Niewiaroeicz, 1978). Chicken breast meat has been classified as PSE or DFD on the basis of post-mortem decline in pH when measurements are made 15 min after slaughter (Kijowski and Niewiaroeicz, 1978; Ristic, 1977). At this time the PSE meat will have pH value as low as 5.7 and DFD meat a value of 6.4 and above. Wirth (1972) recommended that not more than 20 % of PSE pork could be incorporated into fermented sausages. But Townsend et al. (1978) found that Genoa salami could be produced from PSE pork without a major problem. DFD meat has been found to be unsuitable for dry fermented sausage because it binds water more tightly and spoils more

rapidly than meat of normal pH (Newton and Gill, 1981).

The bacterial load of the meat prior to handling has also an influence on fermentation. A high level of LAB may actually increase the rate of fermentation, while undesirable organisms such as Pseudomonads and yeast will cause spoilage, off-flavour and retard the pH drop.

1.4.1.2 Fat

Fat tissues constitute about 30 - 40 % of the sausage mixture. As the moisture level of the sausage decreases with time during drying, a final fat content of 40-45 % is not unusual. It has been reported that the level of fat affects significantly the microbial growth in fermented sausages. Microbial numbers decreased as percent fat increased and this is also true in poultry sausages. Acton and Dick (1975) showed that when the level of poultry fat was raised to more than 30 %, it lowered the amount of lactic acid in the final sausage product.

The incorporation of poultry fat into fermented sausages has often been associated with greasiness or oiliness and rancidity problems. The greasiness of raw ripened poultry sausages is due to the fact that more than 70 % of poultry fat is made up of unsaturated fatty acids and the melting points of these fats are relatively low. The relatively high level of linoleic and linolenic acids at 30 and 3 % respectively, made the poultry fat more prone to autoxidation. Thus Lucke (1985) suggested that for high quality dry sausages pork back-fat should be used instead. Beef tallow and fat tissues of other ruminants can also

be used but they are less acceptable organoleptically when used in high proportions.

1.4.1.3 Salt

Fermented sausages are formulated with 2 to 3.5 % salt so that the initial water activity is 0.97 to 0.96. This water activity level will inhibit or delay the growth of many undesired or dangerous microorganisms and favour the growth of LAB and micrococci (Lucke, 1985). However Bacus and Brown (1985a) observed that as the initial concentration of salt went up to more than 3 % it began to lengthen the fermentation time.

From a food technology stand point, salt will react with the myofibrillar protein structures and solubilise the proteins which later form a sticky film of gel around meat particles for them to aggregate. This activity is important to the final texture, firmness and sliceability of the finished products. In the finished product, salt contributes to flavour and preserves the sausage, particularly in combination with a low moisture content and a_w .

1.4.1.4 Nitrate/Nitrite

Although the inclusion of nitrate/nitrite are not the limiting factor in determining the rate of fermentation in fermented sausages, nitrate or nitrite is important for the desired pink colour and flavour of raw sausages (Kramlich *et al.*, 1980). About 50 mg sodium nitrite per kg sausage is sufficient for the desired flavour and colour (Wirth, 1973).

A high level of nitrite at the beginning of fermentation will suppress the microorganism active in synthesizing flavour (Wirth, 1983). He also recommended that the addition of nitrate along with a low amount of nitrite results in a dry sausage frequently tastes better than those made with the usual amount of nitrite only.

From the microbial point of view, the nitrate cure does not improve the stability of fermented sausages; it might even encourage the growth of Gram-negative bacteria, such as salmonellae at the beginning of ripening stage as demonstrated by Hechelmann et al. (1974). Collins-Thompson et al. (1984) also showed that nitrite had little or no effect in controlling the growth of Stapylococcus aureus, Salmonella spp and Clostridium sporogenes. Cl perfringens and S. aureus have also been shown to survive the nitrite and sorbate salts in thuringer, cervelat and frankfurters (Hallerbach and Potter, 1981).

1.4.1.5 Carbohydrate

The glucose content of fresh, post rigor beef, pork and chicken is of the order of 4.5, 7 and 8 $\mu\text{mol/g}$ fresh weight respectively (Dalrymple and Hamm, 1975; Kastenschmidt et al., 1968; Fisher and Augustini, 1977; Grey et al., 1974). These levels are inadequate to allow significant reduction in pH. Therefore, a fermentable carbohydrate is added to raw sausage mixtures.

The rate of pH decline and the increase in lactic acid concentration depends on the variety and amount of carbohydrate

added into the sausage mixtures. Carbohydrates like glucose, sucrose and maltose will produce a higher rate of lactic acid production than lactose, starch and dextrin (Klettner and List, 1980). This observation reflects the biochemical ability of the LAB involved. If large amounts of rapidly metabolisable carbohydrates such as dextrose and glucose are added, the pH drop can be so rapid that acid-sensitive bacteria which contribute to the curing colour and flavour of the sausages are suppressed. On the other hand, addition of too little carbohydrate or carbohydrate which is degraded only slowly (eg. oligosaccharides) usually stops the fermentation at a higher pH value because the water activity decreases during prolonged fermentation and becomes inhibitory to the lactic acid formation at a_w values about 0.91 (List and Klettner, 1978).

For high quality dry sausages, it is recommended that a slower rate of lactic acid production is desirable. This allows the acid-sensitive bacteria Micrococcaceae to contribute to colour and flavour by enabling them to 'keep up' with the LAB to some extent (Lucke, 1985). Usually 0.4-0.8 % carbohydrate proves sufficient (Coretti and Tandler, 1965; Pyrez and Pezacki, 1981). The lactic acid production will then lower the pH to about 5.0 to 4.8 which ensures satisfactory microbial stability and a rapid increase in firmness (Klettner and Rodell, 1978). Lower amounts of 0.2 to 0.3 % of fermentable sugar are recommended if nitrate rather than nitrite is used as the curing agent (Tandler, 1963) as in the manufacture of Italian and Hungarian salamis. In USA, up to 2 % fermentable sugar is sometimes used, especially in

the production of semi-dry fermented sausages. However the pH may drop to 4.5 and this produces such a 'tangy' sausage that it may not be readily acceptable to some consumers.

1.4.1.6 Condiments

Spices which are commonly added into the sausage mixtures are ground pepper, paprika, garlic, mace, pimento and cardamon. Ground pepper is usually present in all types of fermented sausages at the 0.2-0.3 % level. Some spices such as red pepper, mustard and mace have been found to stimulate the rate of lactic acid formation (Zaika et al., 1978) due to the presence of manganese in the spices. Manganese is required by the LAB for various enzymatic activities including the enzymes of glycolysis and fructose 1, 6-diphosphate aldolase (Kandler, 1982). Besides contributing to the flavour of the finished products, some spices contain powerful antioxidants and this extends the shelf-life of dry sausage (Hammer, 1977).

1.4.1.7 Functional additive - soy proteins

Soy and other plant proteins have been widely used as meat extenders (Rao et al., 1984) and emulsifiers (Thompson, et al., 1984). The emulsifying properties of soy proteins have long been utilised as a processing aid in comminuted meat production. However, studies related to the use of soy protein in fermented sausages have been scarce (Lee et al., 1989). Soy products that are most frequently used in comminuted meat products include soy flours, grits, concentrates and isolates. The protein contents of soy flour and grits, concentrate and isolates are 40-60, 70 and

90 % respectively.

Although sausage mixture sometimes has been referred to as an oil-in-water emulsion, it is more appropriate to classify it as a gel-type emulsion in which fat is dispersed uniformly in a continuous protein matrix. In this type of emulsion the fat droplets are physically confined within the protein matrix and thus the shape of the fat droplets is not necessarily globular as in an oil-in-water emulsion. They may coalesce together and form somewhat larger droplets but they cannot escape from the matrix to produce a single phase. It is thus assumed that the stability depends largely on the rigidity of the gel and the distribution of fat droplets at the beginning of a gel matrix formation. Lee et al. (1989) found that emulsion stability is determined largely by low chopping temperature and the type of fat present in the emulsion. Meat emulsion might be destabilised not only by increasing chopping temperature beyond the softening point of fat, but also the presence of 'soft' fat. The authors also observed that emulsion of beef prepared by maintaining the chopping temperature at 16°C was more stable as fat was dispersed uniformly throughout a protein matrix without showing a fat coalescence.

In the coarsely-comminuted products, binding between the meat particles requires the formation of a concentrated emulsion between meat pieces. Upon heating, there appears to be interaction between dissolved myosin formed during chopping, and myosin filaments located within muscle cells and near the surface of the pieces of meat (Schmidt et al. 1981). Myosin appears to be

the only muscle protein that forms a rigid heat-set gel and specifically the heavy chain portion of the myosin molecule can form a stable, filamentous three-dimensional network matrix that gives processed emulsion meats the desired bind and texture (Schmidt, 1979).

A number of studies have shown that soy protein will also form gel upon heating (Aoki and Sakurai, 1969; Catsimpoilas and Maeyer, 1970 and Hashizume et al., 1975). However, Aoki et al., 1980 showed that proteins 7 S and II S, the main components of soy protein, would have a low emulsifying capacity when they were heated at more than 80°C. Their work also showed that the solubility of the 7 S and 11 S proteins was at the lowest point when the pH of the medium was about 4.5. Schut (1976) suggested that soy proteins probably do not act as emulsifying agents in meat systems but they do fortify the matrix of meat emulsion by immobilising water within a stable gel that forms upon heating. There is also a possibility that soy proteins protect the matrix when gel is formed during acidification of fermented sausages.

By protecting the meat emulsion matrix, less water and fat is mobilised and this property is responsible for the lower fat released and higher water retention in the fermented sausages extended with soy proteins (Rao et al., 1984 and Thompson et al., 1984). High water retention is responsible for the high water activity in fermented sausages with incorporated soy products and this has led to the faster growth of LAB and concurrent higher concentration of lactic acid. Rao et al. (1984) suggested that soy proteins might increase the availability of carbohydrate

for LAB which results in faster growth and higher numbers of the microorganism.

1.4.2 External factors

1.4.2.1 Temperature

Deviation of fermentation temperatures from the temperature optima of the particular type of LAB will slow down the rate of fermentation. Acton et al. (1972) reported that by incorporating L. plantarum and P. acidilactici, fermentation of summer sausages at either 22, 30 or 37°C did not significantly affect product flavour, although less lactic acid was produced at 22°C than 30 or 37°C. However, under chance inoculation fermenting the sausage at a relatively high temperature of about 25°C is unfavourable because the slow drop in pH will enable the undesirable organisms such as Salmonella spp. and Staphylococcus aureus to multiply in the sausage. Addition of starter culture bacteria will ensure a rapid drop in pH and the problem of food poisoning is satisfactorily solved.

Nitrite instead of nitrate should be used if high fermentation temperature is the choice because too rapid a pH drop will not allow sufficient time for the Micrococcaceae to reduce the nitrate to nitrite. In many instances, slower fermentation at a lower temperature of 20-24°C is more desirable in better controlling colour, flavour and other product characteristics and microbial quality.

Once the desired pH is obtained, the ripening temperature is reduced. A temperature of 15°C or even lower reduces the

metabolic activity of the lactobacilli, thus preventing the lactic acid bacteria producing too much lactic acid. Eventhough, the Micrococcaceae will not actively grow at this temperature the slight reduction in pH helps in stable development of pink, curing colour.

1.4.2.2 Relative humidity

High relative humidity favours more rapid fermentation since any degree of drying of the product reduces the availability of water. Since water activity of fermented sausages at the beginning of fermentation is around 0.96, a relative humidity of 90 % at 20°C is more appropriate than 95 % because the latter will result in little or no moisture loss because the difference between room humidity and the a_w in the sausage is too small (Steibing and Rodell, 1988). The author also suggested that as the ripening process continued, the temperature and relative humidity of the cold room should be reduced so that from the fourth day, for example, a gradual water loss should be obtained at temperature and relative humidity of 18°C and 80-90 %, respectively.

1.4.2.3 Casing diameter, air velocity and smoking

The diameter of casings has a great influence on weight loss during ripening. As the diameter of the casing increases weight loss over the same period of time becomes smaller. It is for this reason that dry sausage stuffed into a bigger diameter casing is less inclined to wrinkle (Rodel and Klettner, 1981). Air velocity is an important consideration in the drying stage. It

is also important if the sausage mixture is stuffed prior to fermentation. The recommended air velocity of the cold room should be 0.1-0.4 m/sec during ripening and drying in air-conditioned stores (Steibing and Rodel, 1988a).

Smoke helps to preserve meats and their products by acting as an antioxidant, a bactericidal agent, a bacteriostatic agent and by providing a protective film on the products surfaces. A number of different compounds are liberated from slow and incomplete burning or pyrolysis of wood. Typical compounds in smoke from oak wood include phenols, formaldehyde, higher aldehydes, ketones, formic acid, acetic and higher acids, methyl alcohol, tar and water (Draudt, 1963). Phenols appear to play a three-fold role during and after smoking: a) they act as antioxidant, b) they contribute to the colour and flavour of smoked products, c) they have a bacteriostatic effect that contributes to preservation (Kramlich et al., 1980). The colour and flavour development during smoking is however dependent on time, temperature, density of smoking and the type of wood used. The same conditions must be considered if the bacteriostatic and bactericidal effects are to be effective. The bactericidal action of smoking is due to the combined effect of heating, drying and chemical components of the smoke. Smoke components such as acetic acid, formaldehyde and creosote prevent the growth of microbes such as Staphylococcus aureus (Bacus, 1984). Liquid smoke has also been found to contain antimicrobial compounds such as the phenols which have strong antilisterial activity (Messina et al., 1988)

1.5 Sensory quality of fermented sausages

Basically there are three sensory qualities or characteristics of fermented sausage. They are texture, colour and flavour as suggested by Demeyer et al., 1986).

1.5.1 Texture

The texture of fermented sausage is mainly due to the effect of salt on the myofibrillar proteins, the reduction of pH and the thermal energy provided during fermentation. During this phase, salt solubilises and denatures mainly the myofibrillar proteins and partly the sarcoplasmic proteins (Acton, 1977). As the pH of the sausage emulsion drops progressively and coupled with the heat provided during fermentation, it increases the rate of myofibrillar proteins solubilisation and causes them to coagulate and form a gel around the meat masses and bind them (Palumbo et al., 1976). Klement et al. (1973) had showed that there was no firmness developed in non-fermented control sausages subjected to the same conditions as fermenting sausages without heat.

After the solubilisation and denaturation of meat proteins, the reduction of pH to the isoelectric point of meat proteins (pH 5.2-5) minimises the water holding capacity of the proteins and this allows the water to be removed more freely and rapidly during the drying phase. In the drying phase there is a continual development of the textural characteristics of firmness or hardness of meat particles. Shear values during drying in various studies have shown a correlation to drying time (Acton and Keller, 1974 and Keller et al., 1974) and sausage moisture content (Wardlaw et al., 1973).

1.5.2 Colour

Demeyer et al. (1986) described the formation of characteristic curing colour of fermented sausage. The pinkish-red stable colour of sausage is due to the formation of nitrosomyo-chromogen from added sodium or potassium nitrate and/or sodium nitrite. Nitrite is responsible for the colour and nitrates can be converted by bacteria such as Micrococceae, and the nitrite is, in turn, converted to nitric oxide which reacts with myoglobin to form red nitric oxide myoglobin (NoMb). The protein moiety of NoMb may be denatured and the nitric oxide myochromogen (NoMc) formed improves colour stability. However at low pH (<4.8) and redox potentials, NoMc may be attacked by hydrogen peroxide produced by lactobacilli, bleaching the colour to grey, brown and green (Demeyer et al., 1986 and Tjaberg et al., 1969).

For proper development of curing colour, Monagle et al. (1974) observed that two conditions have to be made available. They include high hydrogen ion concentration and input of heat during the processing. During fermentation the reduction of pH to 5.5-4.5 increases the overall rate of the nitric oxide pigment or NoMb (Fox and Thompson, 1963) and the rate of conversion of the pigment in frankfurter is progressively increased as product temperature rose from 49 to 60°C (Fox et al., 1967). Since not all types of fermented sausages receive heat treatment up to 60°C, addition of GdL has been proposed. The chemical will bring down the pH further to 4.8 and it has been shown that additional acidity from GdL promoted greater conversion of the total heme

pigments to nitric oxide pigment (NoMb). Too low a pH may cause the flavour to be too 'tangy' and dry sausage with initial pH of 5.5 had a more intense cured colour than those having initial values of 5.9 or 6.6 (Townsend et al., 1975).

1.5.3 Flavour

The characteristic flavour of fermented sausages is derived from lactic acid, nitrate and/or nitrite, spicing combination, residual sugar, the amount of smoking practised and salting (Deibel, 1974). In Italian salami, the mold growth on the surface is essentially for flavour development and it also serves as a natural antioxidant. Flavour of the product is also determined by a series of end products of bacterial metabolism of fats, proteins and carbohydrates such as free fatty acids, carbonyl compounds and peptides (Demeyer et al., 1974).

The lactic acid flavour is more predominant in the semi-dry and undried products which are sold after only a few days of ripening. Fiszer (1970), found that other substances such as aldehydes, ketones and ethanols are present during the early phase of ripening as a result of carbohydrate metabolism. Besides lactic acid, the presence of other volatile fatty acids, particularly acetate, propionate and butyrate does contribute to the flavour of the finished products (DeKetelaere et al., 1974 and Halvarson, 1973). Recently the acid taste of fermented sausage has been associated with excessive amounts of D-lactate (Burchard et al., 1984).

Fat has also been found to be responsible for flavour development of fermented sausages and this is due in part to the hydrolytic and oxidative changes that occur in the lipid fraction during ripening. The hydrolytic changes in fat are brought about by the action of bacterial lipases, resulting in variable quantities of fatty acids and glycerol. Micrococci have been found responsible for hydrolysis of fats in dry sausages (Cantoni et al., 1967), but studies also show that some species of lactobacilli produce very active lipase at 20, 25 and 37°C (Stoychev et al., 1972; Sanz et al., 1988 and Coretti, 1965). These lipases preferentially hydrolyse the outer fatty acids of a triglyceride molecule (Alford et al., 1971). Hydrolysis of beef and pork lipids generally results in the production of long-chain fatty acids which do not contribute to off-flavours unless fatty acid oxidation also occurs (Bennion, 1972). During ripening of an Italian salami from pork, Cerise et al., 1973 reported that oleic acid was the principal fatty acid found in the lipid fraction. However, Demeyer et al. (1974) reported that linoleic acid was the major fatty acid found in the dry sausage containing predominantly pork fat.

During drying, the long fatty acids formed earlier from the breakdown of triglycerides were oxidised by peroxides to yield short chain fatty acids and carbonyls. Carbonyl compounds such as ethanal, propanal, propanone, 2-methyl and 3-methyl butanal are extremely important flavour contributors in dry sausage. Formaldehyde and acetaldehyde made up the greater portion of carbonyls (Langner et al., 1970). Carbonyls are also formed

during carbohydrate fermentation (Cerise et al., 1973 and Demeyer et al., 1974).

Bacterial proteolysis , mainly by lactobacilli is responsible for the hydrolysis of proteins into substances such as peptides, amino acids, nucleosides, nucleotides and amines. The composition and concentration of these substances, to a large extent influence the aroma and flavour of traditionally ripened dry salami (Dahl, 1970).

Dierick et al. (1974) studied the changes on the concentration of ammonia, total and free amino acids, total peptides, nucleosides and nucleotides of dry sausages during ripening. It was found that the amount of free amino acids increased as peptides decreased when starter culture bacteria were used. Over a 36 day period, the level of all amino acids increased with an exception of glutamic, histidine, tyrosine and ornithine. The low levels of the four exceptional amino acids were due to the decarboxylation of the amino acids with an approximate 10-fold increase of histamine, tyramine and putrescine. The major free amino acids which increased in concentrations were alanine, leucine, valine, serine, glycine and proline.

1.6 Safety aspects of fermented poultry sausages

1.6.1 Amine and Nitrosoamines

Besides contributing to flavour, some of the amino acids in fermented sausages are decarboxylated by microorganisms to form amines which in high amounts are harmful to man after ingestion.

Various amines such as histamine, putrescine, tyramine, cadaverine and 2-phenylethylamine have been reported in dry fermented sausages (Rice et al., 1975 and Vanderkerckhove, 1977). The factors that govern the formation of amines include the availability of free amino acids, presence of microorganisms that can decarboxylate the amino acids, and favourable conditions both for the growth of the microorganisms and for the production of the decarboxylase enzymes. The small amounts normally present in lactic fermented or aged products are harmless to man but problems arise when large numbers of decarboxylating bacteria are present, which through improper handling or processing would give them the chance to produce appreciable amounts of histidine and tyrosine decarboxylase in the finished products.

Of all the amines, special consideration has been given to histamine and tyramine since they are important to man. Histamine and tyramine are produced from the decarboxylation of histidine and tyrosine. Clinically, tyramine is the major cause of hypertensive crisis in patients treated with monoamine oxidase inhibitors which block the pathway for catabolism and inactivation of the amine after ingestion. Symptoms of hypertensive crisis include high blood pressure, headache, fever, sometimes perspiration and vomiting, while histamine is more related to allergy.

In fresh meat, the amount of histamine and tyramine is low with 1-2 $\mu\text{g/g}$ and 4-5 $\mu\text{g/g}$ respectively. Under normal circumstances the low levels of histamine and tyramine do not pose any public health hazard. From a survey conducted by Rice

et al. (1975), semi-dry sausage products contained an average histamine concentration of 3.59 µg/g compared to an average of 2.87 µg/g in dry sausages. Average tyramine concentrations were 244 and 85.8 µg/g in dry and semi-dry sausages respectively. Since ingestion of relatively large amounts (70-1000 mg) of histamine is required to produce moderate intoxication symptoms, the histamine present in the sausages under survey was of no significance from a toxicity viewpoint. As far as tyramine is concerned, a maximum value of 1000 µg/g have been recorded but this will not elicit symptoms in individuals having normal activity of monoamine oxidase (Luthy, 1981).

Rice and Kochler (1976) found that L. plantarum and P. cerevisiae did not display appreciable decarboxylase activity. Mixtures of L. plantarum and P. cerevisiae were also unable to produce significant amounts of these amines. However in the same experiment they found high amounts of tyramine was produced by Streptococci spp. From these findings it has been suggested that formation of large amounts of the amines is due to gross mishandling or processing problems.

Fermented products, including the sausages have also been shown to allow the formation of carcinogenic nitrosamine. Nitrosamine is formed principally from the reaction of naturally occurring amine with nitrite that may be added into the food or produced from bacterial reduction of nitrate. Eventhough the formation of nitrosamine is unavoidable due to the presence of both precursors; amines and nitrites, Kuhne and Mirna (1981) reported only about 2 µg/g of nitrosamine in 14 types of

fermented sausages manufactured in West Germany. Kueper and Trelease (1974) and Dethmers et al. (1975) did not detect any nitrosamine at all in summer sausage and thuringer respectively. Although nitrite is present in the sausages, its level is too low to form the nitrosamine and this is due to the fact that residual nitrite is reduced to ammonia by sausage microflora (Wirth, 1983).

1.6.2 Polycyclic aromatic hydrocarbon

Besides containing phenol, eugenol, guaicol, alcohol, carbonyl and terpenes, to name a few, smoke has also been found to contain polycyclic aromatic hydrocarbons (PAH). These PAH particularly the 3,4-benzopyrene are undesirable because of their carcinogenic action. These substances get onto the smoked products, such as fermented sausages during smoking (Potthast, 1978).

During smoking by pyrolysis of wood, sawdust or wood shavings, the smouldering temperature is about 400-500°C and at that smoke production temperature the production of PAH is unavoidable. But the content of PAH is relatively low compared with smoke generated at 600°C or higher (Hamm, 1977; Potthast, 1978). However, Engst and Fritz (1977) found that the level of 3,4-benzopyrene of smoked meat products and sausages in West Germany was between 0.05 and 1.62 ug/kg. Higher level of the carcinogen was detected from hot -smoked than cold-smoked products. They also observed that the soot from a hot-smoking chamber contained, apart from other PAH compounds, about the four-fold quantity of 3,4-benzopyrene compared to that of a cold-smoking chamber.

The permitted level of 3,4-benzopyrene in smoked products in West Germany since 1973 is 1 ppb (1 µg/kg) and this level was hardly ever exceeded in meat products smoked by using wet, friction and liquified smokes (Potthast, 1978; Daun, 1979). The level of the carcinogen could also be reduced by the casing of the sausages (Engst and Fritz, 1977). All processes which cause a removal of the larger smoke particles (soot) will cause a reduction of 3,4-benzopyrene and other PAH (Hamm, 1977). Therefore, if smoking by smouldering wood is the choice, the level of soot can be reduced by separating the smoking chamber from the smoking generator by some distance from one another. This will allow cooling traps, sprays or filters to be built in between the smoke generator and the smoking chamber.

1.6.3 Microbial quality of poultry meat

Broadly, there are two types of microorganisms of importance found on any type of meat, including poultry meat. They are the spoilage and pathogenic bacteria. The spoilage of poultry meat stored in aerobic refrigerated conditions is largely due to the growth of Gram-negative, aerobic bacteria (Griffiths and Mead, 1986; McMeekin, 1982). Under the aforementioned storing conditions, pseudomonads are invariably the predominant microorganisms of spoilage (Patterson, 1980). The other groups of bacteria that are grown on poultry meat under the same conditions are the psychrotrophic Enterobacteriaceae and Gram-positive organisms including lactic acid bacteria.

Barnes and Impey (1968) showed that the leg muscle was more

prone to spoilage organisms than the breast meat. The two muscles differ in their ability to support the growth of certain strains of poultry spoilage bacteria. Although pigmented and non-pigmented Pseudomonas spp. grew equally well at 1°C when added to minced breast (pH 5.7 to 5.9) or leg (pH 6.4 to 6.7), strains of Acinetobacter/Morexella spp. grew well in leg but not in breast, whilst Altermonas putrefaciens always grew faster in leg muscle. These findings suggest that the pH values of the muscles could influence both shelf life and spoilage microorganisms under chilled and aerobic conditions.

The other major danger inherent in the use of poultry meat for sausage manufacture is the threat posed by Staphylococcus aureus, Salmonella spp., and Clostridium perfringens. Data for England and Wales attribute 50 % of poultry outbreaks to Salmonella, 25 % to S. aureus and 25 % to Cl. perfringens (Gilbert, 1979). Zottola and Busta, 1971 sampled 50 different further-processed poultry products from eight different processors and found that 35 of the raw products all contained coliforms, 25 contained S. aureus, 19 had E. coli, 7 contained Cl. perfringens, while only 3 samples contained Salmonella spp.. Swaminathan et al., 1978 found salmonella in 7.3 % of retail poultry sampled, while Bentley and Pettit (1982) reported 51 % of raw eviscerated poultry to be salmonella positive during 1979 - 1980 survey conducted in Canada.

The presence of low numbers of S. aureus in poultry meat is not unusual because the microorganism is carried on the skin and naso-pharynx by a proportion of all healthy birds reaching

slaughter age (Devriese et al., 1975). It has also been demonstrated that some strains may become established in plant machinery (Gibbs et al., 1978). Further processing of the carcasses also allows staphylococcal contamination (Hagbert et al., 1973).

Since Salmonella spp. and Cl. perfringens are both enteric microorganisms, contamination and cross contamination of these organisms to poultry meat can not be avoided. While a high scalding temperature of 60°C readily inactivated Salmonella spp. (Notermans et al., 1975), the same treatment will not destroy the sporing Cl. perfringens because the spores can withstand any of the scalding treatments used. Although, Cl. perfringens is frequently present on the skin of birds, sometimes in relatively high numbers, the numbers of the microorganism after processing are not high (Mead and Impey, 1970). High numbers of vegetative cells are needed to cause food poisoning, therefore multiplication of the organism is necessary before a poultry-borne outbreak occurs. Although, the spores of Cl. botulinum are widely distributed, the presence of Cl. botulinum in poultry meat is rarely detected.

Being less resistant to a factor like temperature, salmonella do not multiply when poultry meat is properly frozen and stored at temperatures below 4°C and their presence at the retail level should reflect the situation at the end of the processing line. However, during further preparation contamination may take place (Schothorst and Notermans, 1980).

The other type of bacteria which receives much attention currently is the presence of Listeria monocytogenes in poultry meat and products, including fermented sausages. Kwantes and Isaac (1971) isolated L. monocytogenes from 57 % of the fresh and frozen poultry sampled. The number of organisms in refrigerated, retail poultry have been observed to increase during storage by up to 2 log₁₀ in 10 days; freezing appears to have no detrimental effect on the microorganism (WHO, 1988).

Campylobacter fetus has also been recognised as an important causative agent of gastro-intestinal disturbance and poultry is considered to be one of the reservoirs of this microorganism (Schothorst and Notermans, 1980). However the significance of the organism in poultry meat and fermented sausages is not going to be discussed in this review.

1.6.4 Microbial quality of fermented sausages

In both semi-dry and dry fermented sausages interaction of several factors such as low pH due to the high amount of lactic acid produced, high salt and nitrite contents, low moisture and water activity and low redox-potential inhibit the growth and destroy the spoilage microflora and food-borne pathogens. When salt and nitrate / nitrite are added to the meat mixture, they bring down the water activity to 0.97 - 0.96. During fermentation process the oxygen level is rapidly depleted and anaerobic conditions prevails. These conditions are detrimental to Pseudomonads which require oxygen and are usually sensitive to salt and nitrite (Hechelmann et al., 1977) and are inactivated.

Similarly, the competitiveness of Enterobacteriaceae is reduced at low oxygen tension, low pH (Grau, 1981; Gill, 1982) and in the presence of salt (Ingram and Kitchell, 1967). If the Pseudomonads are still surviving under the stated conditions, the production of lactic acid will inhibit them. Raccach and Baker (1978) demonstrated that LAB have their greatest repressive action against the Pseudomonas spp.. The effect of starter cultures on growth of some microorganisms such as streptococci and gram-negatives during both 'natural' and 'rapid' production methods is illustrated in Figures 1 and 2, respectively.

Even though raw sausages are considered to be safe, though not perfect, there have been infrequent reports associating them with foodborne disease. S. aureus has been associated with gastroenteritis caused by the consumption of Genoa salami (Anon, 1971), Italian dry salami (Anon, 1975) and pepperoni (Anon, 1978). A case of salmonellosis was attributed to S choleraesuis in Italian dry salami (Morazza and Crespi, 1963).

In 1987, Warburton et al. proposed microbial guidelines for two classes of fermented sausages; heat treated and raw manufactured under good hygienic practices in Canada. The author suggested that the maximum number of microorganism allowed per unit (ml or g) for E. coli was 100 cfu/g and 2,000 cfu/g for heated and raw products, respectively. A higher number of S. aureus of 500 cfu/g for heat treated and 10,000 cfu/g for non-heated fermented sausages was allowed but the products should be free from Salmonella, Campylobacter and Yersinia.

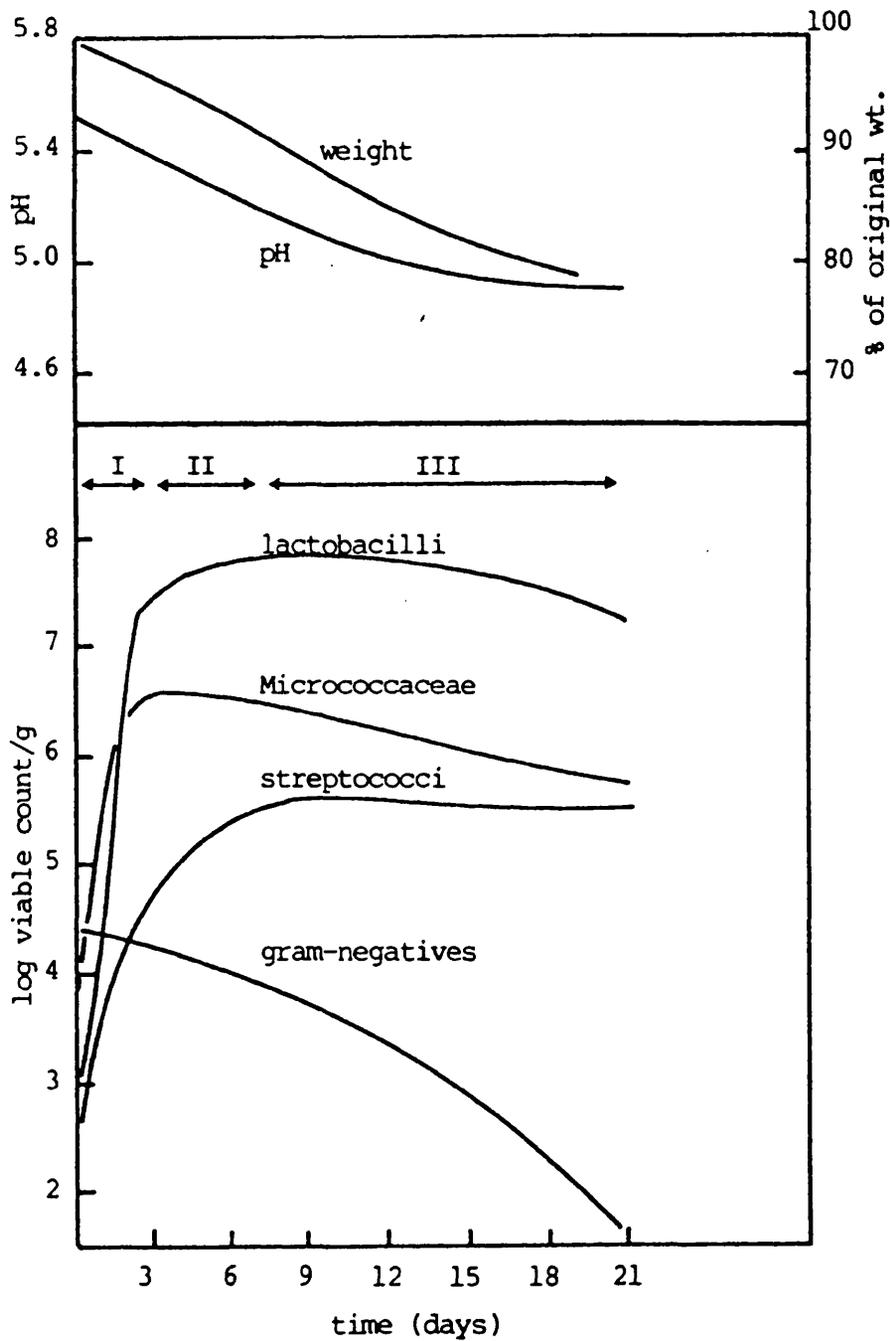


Fig. 1. Development of the microflora (log colony-forming units/g fresh weight) during ripening of salami-type sausage by the 'natural' method (use of nitrate, low amounts of sugar, low fermentation temperature). Ripening conditions: (I) 18-20°C; (II) 20-22°C smoke; (III) 15-17°C. Data of Nurmi (1966).

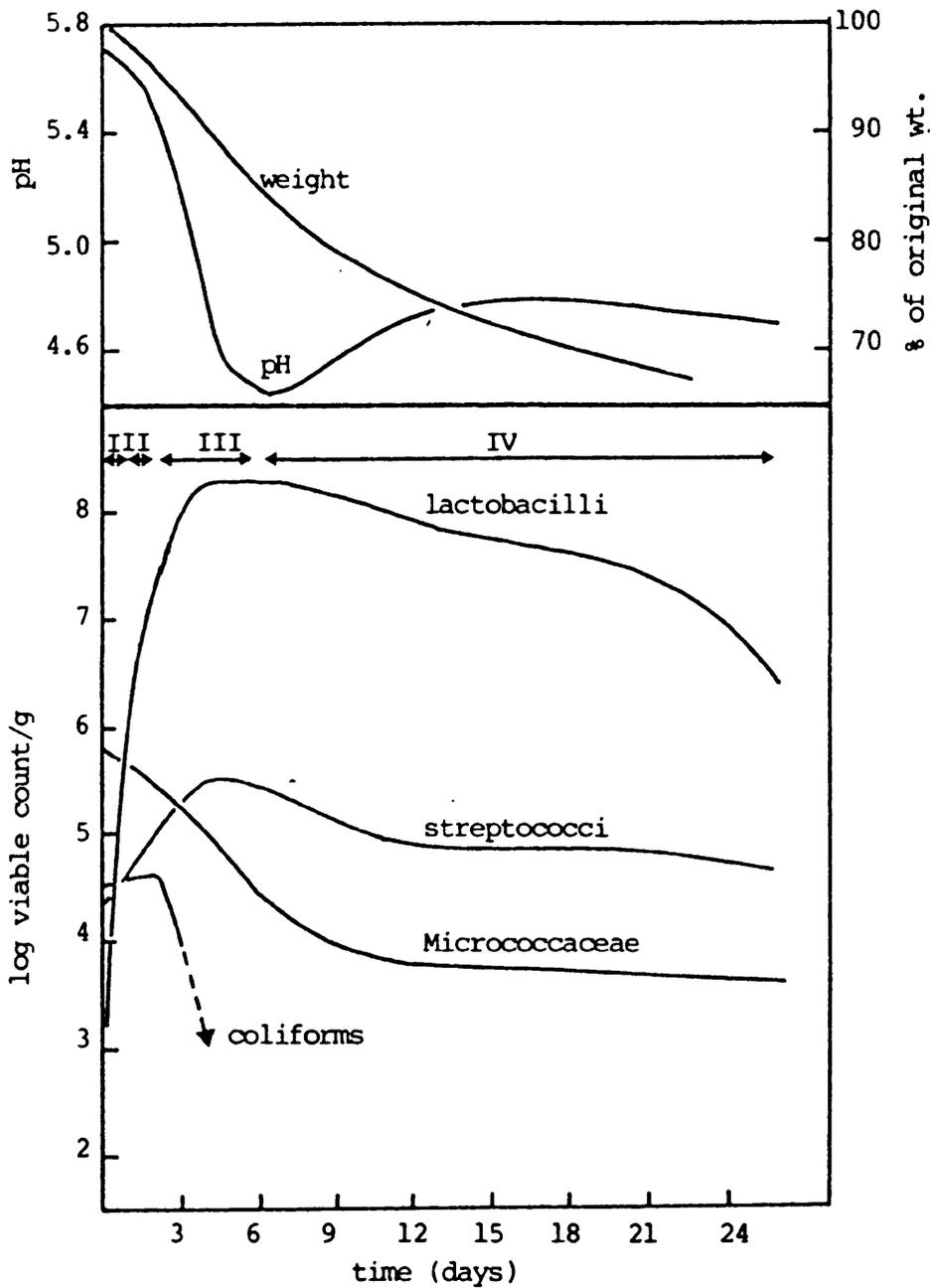


Fig. 2. Development of the microflora (log colony-forming units/g fresh weight) during ripening of salami-type sausage by the 'rapid' method (use of nitrite, high amounts of sugar and elevated fermentation temperature). Ripening conditions: (I) 4°C: (II) 18°C: (III) 24°C: (IV) 18-20°C. Data of Reuter et al. (1968).

1.6.4.1 Salmonella spp.

During the initial fermentation the Salmonella spp. count usually remained constant or increased, at most by one log-cycle. During drying salmonellae are slowly inactivated (Scharner, 1968; Smith et al., 1975b and b; Stecchini et al., 1982).

Factors which favour the growth of salmonellae during sausage fermentation and thereby increase the risk of salmonellosis include a high initial water activity, a high initial pH value, a low concentration of fermentable carbohydrate, low numbers of lactobacilli in the fresh sausage mixture, the use of nitrate or very low levels of nitrite as curing agent (Leistner et al., 1973; Sirvio et al., 1977) and high ripening temperatures.

Salmonella has been found to survive in the fermented sausages. By using Pediococcus cerevisiae as the fermenting organism in dry fermented turkey sausages, Baran and Stevenson, (1975) found that at an initial inoculum of 10^6 cells / g, Salm. pullorum and Salm. seftenberg were not totally eliminated either during fermentation or subsequent processing of the sausage. It seems to suggest that the species differences and the level of inoculum are also important for the inhibition of salmonellae. But Weber et al. (1976) and Schmidt (1983) noted that Enterobacteriaceae, including Salmonella spp. could be effectively controlled if the sausage was formulated with at least 2.5 % salt, some carbohydrate or small amounts of GdL. This finding was in parallel with the result of Goepfert and Chung (1970) in which the combination of acidity and salt was the principal reason for the demise of salmonellae during

fermentation. Avnat and Spangental (1986) observed that it was possible to inhibit salmonella growth in mettwurst containing up to 10^3 cells/g by using lactobacilli (10^7 cells/g) as starter culture, provided that the sausages were kept at 0-4°C for at least 7 days. In their paper, Masters et al. (1981) observed that elimination of salmonella was dependent upon the initiation level, serotype involved, rate of fermentation and processing temperature. Heating bologna to 51.7°C or above led to destruction of Salm. typhimurium and Salm. dublin and smoking also appeared to contribute to destruction of salmonella (Smith et al., 1975a).

1.6.4.2 Listeria monocytogenes

Earlier literature suggested that L. monocytogenes survived poorly in an acidic environment and work by Seeliger (1961) mentioned that the microorganism cannot grow below pH 5.6. But, Conner et al., (1986) revealed that the bacteria can still grow at pH 4.8. The effect of lactic acid to the inhibition of L. monocytogenes is still questionable because the various results from different workers are inconsistent. The possible tolerance of the bacteria to lactic acid has turned the attention of several workers to factors like salt and nitrite concentration, high heating temperatures, low storage temperature or other factors in combinations.

Farber et al. (1988) found that 15 % of dry fermented sausages were positive for L. monocytogenes after fermentation, but not after the drying period. However, if the numbers of L.

monocytogenes in the batter were $>10^3$ cfu/g , the organism can survive during fermentation, drying and refrigerated storage of hard salami, but at a reduced level (Johnson et al., 1988). A similar observation was made by Glass and Doyle (1989a) about the fate of L. monocytogenes during the manufacture and refrigerated storage of pepperoni. In another work by Glass and Doyle (1989b), they also observed that the organism only survived but did not grow on summer sausage after refrigerated storage for 12 weeks. The author indicated that the organism grew well on meats near or above pH 6 and poorly or not at all on products near or below pH 5. Studies with pepperoni revealed that heating sausages to an internal temperature of 51.7°C (125°F) for 4 h after fermentation but before the drying cycle killed most of L. monocytogenes, but the organism was occasionally detected in samples after drying. However, the organism was not in any sausage that was stored refrigerated after drying. Perhaps, the combined stress induced by acid produced during fermentation, heating at 51.7°C and drying sufficiently injure the bacterium so that the additional stress of cold storage prevents recovery.

Although, L. monocytogenes is tolerant to high level of salt and able to survive at 37°C for 15 days in 10.5 % salt and 5 days in 20 - 30 % salt, Shahamat et al. (1980) revealed that the antimicrobial activity can be achieved by 100 ppm sodium nitrite in the presence of at least 3 % salt and a pH <5.5 at 5°C ; warmer temperatures may decrease the inhibitory effect. The other factor that can be used to destroy the organism is smoking. Messina et al., (1988) found that some liquid smoke preparations have

antimicrobial activity against L. monocytogenes and the author suggested that the antilisterial activity might be due to phenol present in the liquid smoke preparation.

1.6.4.3 Stapylococcus aureus

S. aureus can grow rapidly and produce enterotoxin during the first 1 to 3 days of fermentation (Daly et al., 1973) in the outer 1/8 inch of the product (Bacus and Brown, 1981). Being salt tolerant and able to grow in anaerobic conditions and low pH, S. aureus is an important organism in fermented sausage production.

The combined effect of pH, water activity and certain additive (NaNO_2 , NaNO_3 , polyphosphate, wine and pepper) on growth of S. aureus has been studied by Martinez et al. (1986). Fermented at 25°C on model salami, it was found that the combination of a_w 0.925 - pH 5.00, or a_w 0.915 - pH 5.50, or a_w 0.900 - pH 6.00, together with addition of additives resulted in synergistic growth inhibition of S. aureus. Sodium nitrate and nitrite played an important role in the inhibition, while the others had no important effect in controlling the growth of the organism.

The successful inhibition of S. aureus has been reported by several workes. P. cerevisiae has been reported to inhibit 99.9 % of S. aureus at 24 h of fermentation incubated at 37°C. The combination of P. cerevisiae and Lactobacillus plantarum could inhibit more than 99 % at the same time and temperature (Daly et al., 1973). However, a higher initial population will cause the degree of inhibition to be reduced (Baran and Stevenson, 1975).

It has also been observed that a fermentation temperature of 21°C did not inhibit the growth of S. aureus significantly and this could be due to the slower rate of lactic acid production at 21°C Raccach and Baker (1979) reported that the lactic acid producing organisms should outnumber the S. aureus population by 10^5 to 10^6 cells to suppress the pathogen.

As S. aureus is also responsible for the production of enterotoxins, several studies had been carried out to detect their presence in fermented sausages. It has been reported that S. aureus concentrations of over 10^6 cells/ g are required before enterotoxin is produced in quantities detectable in 100-g samples of food (Barber and Deibel, 1972; Genigeorgis, 1976; Niskanen and Nurmi, 1979). However several works by Tatini et al. (1976) and Metaxopoulos et al. (1981a and 1981b), showed that enterotoxin of S. aureus are not detected even in salami inoculated at up to 10^8 cells/g. The latter author suggested that the lack of enterotoxin production at a detectable level might be due to the combined effect of processing conditions (pH, salt, NaNO_2 , a_w) and the effect of LAB. Haines and Harman (1973) indicated that although lactobacilli alone may not inhibit the growth of S. aureus, they can exert an inhibitory effect on enterotoxin production. Without the starter culture and thus effective competition, Lee at al. (1977) were able to demonstrate enterotoxin A but not B production in Genoa salami prepared under laboratory conditions.

During fermentation, the production of lactic acid injures the S. aureus. Smith and Palumbo (1978) found that this was due to

lactic acid. The injury was more pronounced at the lower pH and a high concentration of glucose. In the absence of glucose or starter culture no injury was observed. When sausages containing S. aureus injured by fermentation at 35°C were incubated at 5°C, the count remained constant.

1.6.4.4 Cl. perfringens and Cl. botulinum

There are many factors that influence the formation of botulinal toxin in meats. They are the oxidation-reduction potential, pH, a_w , salt, nitrite, moisture level and temperature as suggested by several authors (Baird-Parker and Freame, 1967; Johnston et al., 1969; Christiansen et al., 1973; Roberts and Ingram, 1973; Bowen and Deibel, 1974 and Collin-Thompson et al., 1974). It is a well known fact that nitrite alone adds protection to the cured meats (Hustad et al., 1973) but Jacobsen and Trolle (1979) suggested low a_w as the most important factor for the inhibition of clostridia.

The behaviour of Cl. botulinum during manufacture of summer sausage and thuringer has been studied by Christiansen et al. (1975) and Tanaka et al. (1980). Botulinum toxin was only detected if no glucose was added and the pH did not fall below 5.5. The rapid drop in pH has been found to be effective in preventing toxin formation. Some investigators had postulated that the nitrite level may be safely lowered in meat products if lactic acid and carbohydrates are added (Tanaka et al., 1980). In a review, Wagner (1986) even suggested the nitrite level can also be reduced by addition of certain phosphates such as sodium

acid pyrophosphate.

Nordal and Gudding (1975) and Lucke et al. (1983) suggested that the growth and toxin formation of Cl. botulinum was even less likely in European-style fermented sausages. Cl. botulinum is controlled by low pH and low a_w , irrespective of nitrite addition. The same appears to be true for Cl. perfringens (Hallerbach and Potter, 1981).

CHAPTER TWO

2.1 EXPERIMENT ONE

2.1.1 AIM OF EXPERIMENT

The experiment was carried out to evaluate the effect of three different levels of soya isolate (ie. 0, 2 and 4 per cent) on chemical compositions, objective measurements and sensory qualities of the fermented dry sausages from thigh trims. It involves the determination of moisture, fat, protein, a_w , pH, lactic acid, texture, weight loss and greasiness of the products.

2.1.2 MATERIALS AND METHODS

2.1.2.1 Materials

Chicken thigh trims were used throughout as the meat component in the experiment and they were collected from D. B. Marshall Ltd., Newbridge, Edinburgh. Upon arrival at the College, they were kept in a deep-freezer at -20°C before being used in the sausage production. Soya isolate and soya concentrate were obtained from The British Arkady Co. Ltd., Old Trafford, Manchester M16 0NJ. Synthetic casings (30 mm diameter and other sizes) were supplied by Devro Ltd., Moodiesburn, Glasgow G69 0JE and the starter culture bacteria SL100 from Christiansen-Hensen Laboratorium A/S, Copenhagen, Denmark. All the ingredients and the casings were given free of charge by the respective companies and organisations.

2.1.2.2 Sausage formulation

The fermented dry sausages from thigh trims were formulated using the various ingredients such as salt, sugar, sodium nitrite, starter culture bacteria and three different levels of soya isolate (ie. 0, 2 and 4 %) as in Table 2. The starter culture bacteria were enumerated and found to contain 0.2×10^{10} cfu/g of Lactobacillus pentosus and 2×10^{10} cfu/g of Staphylococcus carnosus. Each gram of soy isolate was found to have a Total Viable Count and coliforms count of <100 and <4 cfu, respectively.

Table 2: Thigh trims fermented sausage formulation

Ingredients	Amount (g / kg thigh trims)
1. Sodium chloride	30.00
2. Sucrose	15.00
3. Sodium nitrate	1.00
4. Starter culture	1.00
5. Soya isolate	
a) 0 %	-
a) 2 %	20.00
b) 4 %	40.00
6. Water	250.00 ml

2.1.2.3 Preparation of fermented sausage

After thawing overnight, 12 kg of thigh trims were chopped by using Moulinex Masterchef 65a for one minute. During the process, care was taken to ensure that the trims were properly chopped so that all particles were about the same size. The chopped meat was placed inside a pail and mixed by hand before a kilogram of chopped meat was weighed and other ingredients were added to it. The other non-meat ingredients such as salt,

nitrate, sucrose, and soya isolate were placed inside a liquidizer containing 250 ml of water. These ingredients were blended for 2 minutes and later poured into the bowl containing a kilogram of chopped thigh trims. Starter culture was mixed with 50 ml of water before adding in the mixture and all the ingredients were later blended with blunt plastic blades for another one minute for proper mixing of the sausage mixture. The same procedure was repeated for the control but without the soya isolate. The 4 kg batter from each treatment was then placed inside 4 sterilisable plastic bags; a kg each and they were vacuumed and sealed. All the plastic bags containing the batter was placed inside an incubator at 23°C for fermentation to take place. The pH of the batter was monitored every day and once the pH was below 5.3, the batter was taken out from the incubator and was ready to be stuffed into synthetic casings.

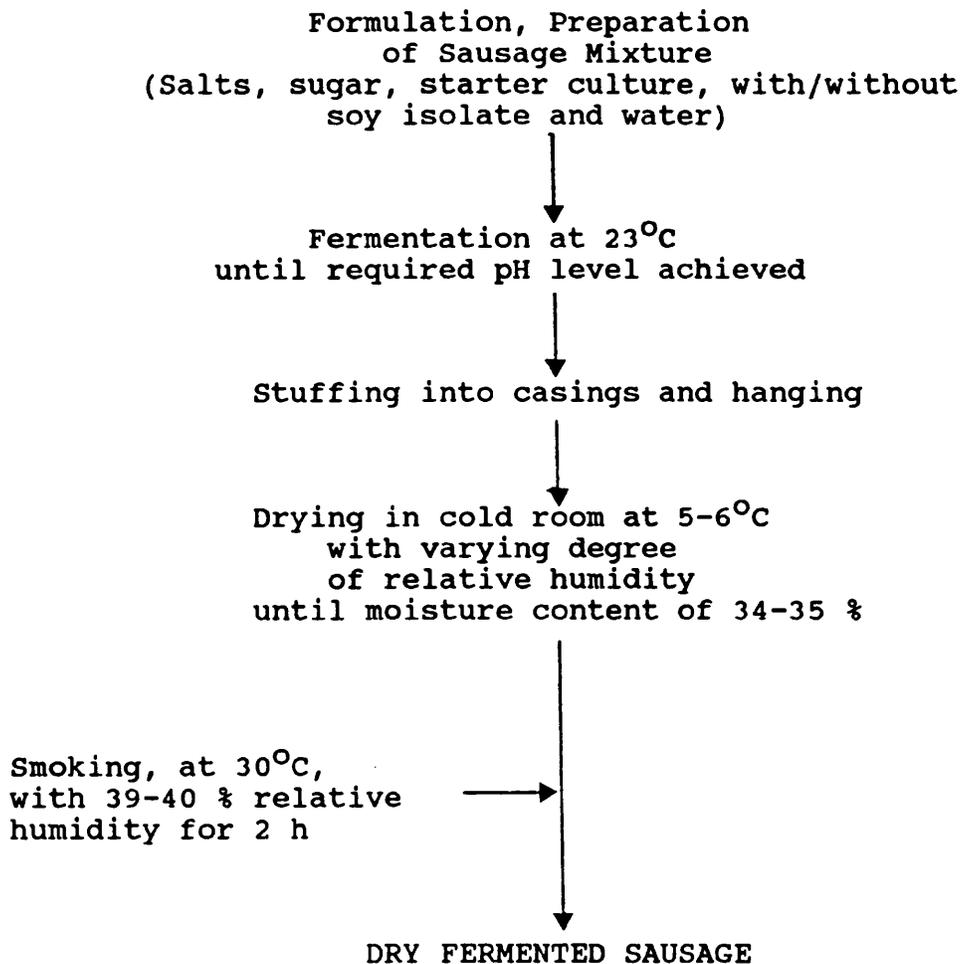
Stuffing of the sausages was carried out with the help of Kenwood KM201 Chef Mixer and the mixture was filled into synthetic casings (30 mm dia), with each sausage stick about 15 cm long. Once the required length was achieved, the stuffed sausage was twisted several times and tied with rope so that each stick of sausage was separated from the other. During stuffing care was taken to make sure that the presence of air was brought to a minimum.

Once stuffing had been completed, the sausages were hung inside a cold room and left to dry. The temperature of the cold room was controlled at 5-6°C and the relative humidity was

gradually reduced from 90-95 % to a lower value to affect drying.

The smoking process was carried out in the smoking kiln 1 day after the sausages were hung inside the cold room. The temperature of the smoke kiln was adjusted to 30°C and smoke was obtained by smothering of wood chips. During smoking sausages were hung inside the kiln and the process lasted for 2 h with the relative humidity inside the kiln being recorded as 39-40 %. All processes involved in the preparation of fermented sausages are shown in Diagram 1.

Diagram 1: Preparation of Fermented Dry Sausage From Thigh Trims



2.1.3 SAMPLING AND RECORDING

Duplicate samples were collected and minced through the 5 mm plate (Maulinex, Jeannette). Titratable acidity, and pH determinations were carried out immediately after mincing and the rest of the sample was kept in air-tight bottles before analysis. In the case of stuffed sausages the casings were first peeled off before the mincing. All samples for chemical analysis were collected at the following stages: a) day 0, ie. after proper blending of sausage batter, b) days 1 and 2 , c) days 5, 8, 11, 14, 17 and 20. Analysis of texture and weight loss only involved stuffed sausages. Samples were taken out of the cold room and after recording all whole sausages were hung again in the cold room.

2.1.4 CHEMICAL METHODS

2.1.4.1 Moisture

Duplicate 5 g samples were weighed on Mettler AE 166 and a thin glass rod was placed in each aluminium dish containing 10-15 g oven-dried acid washed sand. The sample and the sand were mixed together with glass rod and placed in an oven set at 101°C for 6-8 h. It was then cooled in a dessicator and weighed.

Percentage of moisture:

$$\frac{\text{Wt of dish before heating} - \text{Wt of dish after heating}}{\text{(Sample, sand and rod) (sample, sand and rod)}} \times 100$$

Wt of sample

2.1.4.2 Fat

The dried samples from the determination of moisture were placed into an extraction thimble. The extraction of fat was carried out in Soxhlet extraction apparatus for 3-4 h using 160-200 ml of petroleum ether (boiling point 40-60°C). The solvent in the round bottom flask was evaporated in Thermostat Vacuum Oven (Townson & Mercer Ltd., Croydon, England) at 80°C and the content was dried at 101°C for 1 h before being cooled and weighed. The flask was then cleaned and dried at 101°C for another hour before its weight was determined. Up to six samples could be assessed at one time during the extraction process on the Soxhlet extractor.

Calculation of percentage of fat:

$$\frac{\text{Wt of round flask with fat} - \text{Wt of round flask}}{\text{Wt of sample}} * 100$$

2.1.4.3 Unbound lipid

The percentage of unbound lipid was determined by extracting the fat in the sausage samples by using chloroform:methanol (2:1, v/v) and diethyl ether:hexane (1:1, v/v). Extraction with chloroform:methanol (2:1, v/v) would extract the total lipid present, whilst diethyl ether:hexane (1:1, v/v) was used to give some indication of the more easily extractable or unbound fat in the sausages. Both extractions were performed concurrently so that results could be used to calculate the percentage of unbound fat.

Two 1-g freeze-dried samples from the same treatment were chopped into smaller particles and placed inside two different test tubes and 50 ml of chloroform:methanol or diethyl ether:hexane were added. The samples were then shaken for 1 min and the homogenates filtered through Whatman No 41 filter papers. Both residues were washed thoroughly with the respective solvents to remove any residual lipid. The filtrates were dried down under vacuum on a rotary evaporator at 40°C. 4-5 ml of chloroform was added into the flasks and the solubilised lipid transferred into 10 ml flasks by means of a pasteur pipette. The volume was made to 10 ml by further addition of chloroform. 5 ml of this solution was transferred into a pre-weighed 15 ml round-bottomed flask and evaporated again under vacuum on a rotary film evaporator. Following complete evaporation of solvent, the flasks were removed from the evaporator and placed in an oven (100°C) for half an hour to remove any residual moisture that might be present. The weights of lipid associated with two solvent extractions were then used to determine the percentage of unbound fat according to the relationship,

$$\text{Percentage of unbound fat: } \frac{\text{Unbound fat}}{\text{Total fat}} * 100$$

2.1.4.4 Lipid separation

The major lipid classes were separated by thin layer chromatography. Dry glass plates (20 cm * 20 cm) were arranged in a standard manner on a commercial plate holder (FSA Laboratory Supplies, Loughborough, Leicestershire, England). The plates were

wiped clean with hexane before a suspension of 22.5 gm Kieselgel 60 G (Merck, A.-G, Darmstadt, West Germany) in 50 ml distilled water was evenly spread on the glass plates to a thickness of 0.25 mm using aluminium spreader. The plates were air-dried until they lost their glossiness and then activated by heating in an oven at 110°C for a minimum of 2 h. Separation of the lipids was performed using a freshly prepared eluting mixture of hexane:diethyl ether:formic acid (80:20:1, v/v/v). Solvent saturation within the chromatographic tank was maintained by lining one side with tissue paper soaked in the solvent.

A suitable aliquot of the lipid from the sample extracted by chloroform:methanol (2:1, v/v) was applied carefully as a discrete band, in chloroform, at the base of the thin layer chromatographic plate using a 100 µl microsyringe. Development time for each plate was around 30 min, by which time the solvent front had reached approximately to the top of the plate. After a brief period of air-drying to remove excess solvent, the separated lipids were sprayed with a charring agent in order to facilitate the identification of each of the separated lipid bands. The charring was performed using a charring reagent of 3 % cupric acetate (w/v) in 8 % (w/v) of ortho-phosphoric acid and heating in a force-draft oven at 180°C for 15 min. The major lipids resolved were triglyceride, free fatty acids, free cholesterol, cholesterol esters and phospholipid, which in each case were identified by their retention times relative to standards.

2.1.4.5 Fatty acid determination and gas-liquid chromatography

To the remaining 5 ml of the extract, 100 μ l of the solution was added to a solution containing 0.322 mg of a pentadecanoic acid in methanol. The mixture was dried on a rotary evaporator at 40°C. 4 ml of a methylating agent (anhydrous methanol:toluene:sulphuric acid 20:10:1, v/v/v) was added and refluxed at 66°C for 45 min. After cooling, the fatty acid methyl esters were extracted by partitioning between equal volumes of hexane and water. The top hexane layer containing methyl esters was carefully transferred to tubes, a small amount of drying agent ($\text{Na}_2\text{SO}_4:\text{NaHCO}_3$, 4:1 w/v) added, and after 30 min the hexane was decanted off into small tapered tubes. The solvent was removed under a stream of nitrogen and esters were then redissolved in 100 μ l of hexane for injection into the gas-liquid chromatograph (Model 4500; Pye Philips Ltd, Cambridge, England). The column packing material was 15 % methyl silicon-treated ethylene glycol succinate (EGSSX) on a Gaschrom P solid support (Chrompack, Middleburg, the Netherlands). The carrier gas was nitrogen at a flow rate of 40 ml per min. The column temperature was 200°C. One μ l of the fatty acid methyl ester extract was injected onto the column and analysis time was chosen for methyl ester that was sufficient to include all fatty acids up to docosahexaenoic. The relative proportions of individual separated fatty acid was determined using electronic intergration and the relative amounts of the individual lipid fraction quantified by referring the separated fatty acid to the known pentadecanoic acid internal standard.

2.1.4.6 Protein

In the determination of protein, the amount of Nitrogen was analysed by Kjeldahl method. A duplicate of 2 g samples and each was added with 5 Kjeldahl copper catalyst tablets, which contains 0.5 g copper sulphate (BDH Limited, Poole, England) as catalyst and 25 ml of 98 % (w/w) concentrated sulphuric acid for digestion. The sample was then placed into a digestion tube and later digested into a digestion block (Techne Dri-Block, DB-4) preset at 450°C for 2 h and was further heated 90 min at the above temperature after clarification. Blanks were also prepared and received the same treatment, but without the sample. Two blanks were prepared for every 4 samples tested.

Once digested, distilled water was then added to prepare the solution for titration. The blue solution was then transferred into a 100 ml-volumetric conical flask and filled with distilled water until the 100-ml mark. 10 ml of aliquot was transferred into the titration tube and ready for the titration. The excess of the acid was titrated with standard sodium hydroxide solution using a Kjeltac Auto 1030 Analyser (Tecator AB, Box 70, Hoganas, Sweeden), the digital read-outs of blanks and samples were recorded and later used in the calculation.

Calculation of protein content:

$$\frac{(\text{Titre (sample)} - \text{Titre (blank)}) * (14.007 * 6.25 * 2)}{\text{Wt sample in milligram}} * 10$$

2.1.4.7 pH

The pH of sample was determined by first preparing a slurry from an equal amount of sample and distilled water. A 10 g sample was mixed with 10 ml of water in a 50 ml beaker and it was then stirred with a spatula to form a slurry. The electrode of a calibrated Philips PW9420 pH meter was placed into the beaker containing the slurry and the pH value was then recorded. Buffer solutions of pH 4 and 7 were used for the calibration.

2.1.4.8 Titrable acidity

In this determination the developed acidity was assumed to be due to lactic acid production. A sample of 10 g was put inside a 200 ml beaker and placed on a weighing machine and later brought to 100 g by adding warm distilled water. Constant stirring of the slurry was maintained by magnetic stirrer. The initial pH value of the homogenate was recorded. Sample slurry was then titrated with N/9 NaOH to an end point of pH 8.30. The volume of NaOH solution used was divided by 10 to give the figure of the acidity as percentage lactic acid.

2.1.4.9 Salt

Duplicate 2-g samples were weighed on Mettler PL200 and placed inside a 250-ml conical flask and added into the flask were 10 ml of distilled water, 25 ml of 0.05 N silver nitrate and 10 ml of nitric acid before the flask was heated at 180°C. Heating was carried out for 15-20 min until the sample digested and later left for few min to cool. Upon cooling, 50 ml of distilled water, 3 g of urea and 2 ml of iron alum (as indicator) were added into

the flask and titrated with 0.05 N potassium thiocyanate (containing 0.00292 g NaCl) to the end point ie. until the colour of the yellowish solution turned into a brick-red discolouration. The blank titration was determined at 25.4 and the correction factor was determined at 0.00257.

Calculation of percentage of salt:

$$\frac{(\text{Blank} - \text{Titration for sample}) * 0.00257}{\text{Wt of sample}} * 100$$

2.1.4.10 Water activity

Water activity of the samples were determined by the formula derived from Demeyer, 1979 and De Jaeger *et al.*, 1984.

$$a_w = 1.0014 - 0.6039 \left(\frac{\% \text{ Salt } s}{\% \text{ Moisture } s} \right) \left(1 + \frac{0.0189 \% \text{ Moisture } b}{\% \text{ Salt } b} \right),$$

where the index s refers to sausage and b to initial batter.

2.1.5 OBJECTIVE MEASUREMENTS

2.1.5.1 Weight loss

Six sausages from each treatment were marked and weighed after the stuffing process completed. The same sausages were weighed every 3 days after stuffing to determine the amount of water loss during the drying period. The weight loss for day 2 for example, was determined by difference in weight between days 1 and 2 and divided by the weight of the sample on day 1 as shown below.

below.

Calculation of weight loss:

$$\frac{\text{Wt of sausages (day 1)} - \text{Wt of sausages (day 2)}}{\text{Wt of sausage (day 1)}} * 100$$

2.1.5.2 Texture

All tests were performed on Stevensons-LFRA Texture Analyser (C. Stevensons & Sons Ltd., St. Albans, Hertfordshire). All samples after taken out of the cold room were left at room temperature for 5 h before the analysis. Only stuffed sausages were subjected to the texture test. Before the test, the respective sausage was cut longitudinally into 5-6 cm in length and placed vertically on the table of the analyser. The tests were done with a TA 9 needle probe driven at the centre of the sausage to 10 mm at a speed of 0.5 mm/sec. The amount of force in grams was then recorded from the digital read-out.

2.1.6 SENSORY ANALYSIS

In the experiment only colour, firmness and greasiness of the sausage produced with the three different levels of soy isolate were determined with the help of the supervisor. No taste panel was established because of the presence of Listeria monocytogenes. All sausages to be tested were kept in room temperature for 5 hours after taken out from the cold room.

2.2 EXPERIMENT TWO

2.2.1 AIM OF EXPERIMENT

The experiment was carried out with four main objectives. Firstly, it was intended to evaluate the growth and inhibition of: starter culture bacteria, coliforms, mesophiles and psychrotrophs; at processing, during fermentation and during the drying period. This was evaluated in sausages with two different levels of soya isolates (0 % and 2 %), and between smoked and unsmoked sausages. Secondly, to observe the survival of the food-borne pathogen Salmonella enteritidis during sausage production. Thirdly, to evaluate the microbial quality of the final product with respect to the presence and number of the food-borne microorganisms E. coli, S. aureus, L. monocytogenes and C. perfringens. The last objective was to evaluate the effect of the pH of the sausage mixture during fermentation to the overall rate of drying of the fermented sausages. This fourth objective was aimed to investigate the problem of water retention observed in sausages without soy isolate in Experiment 1.

These four objectives were all carried out in four different sausage types namely: mixture containing no added soy isolate both unsmoked and smoked and mixture containing 2 % added soy isolate both unsmoked and smoked.

Eventhough three different levels of soy isolate was used in Experiment 1, the Experiment 2 only utilised 0 and 2 % soy isolate. The two levels of soy isolate were chosen because there was an observable difference of results from various chemical,

and objective analysis from the previous experiment.

2.2.2 MATERIALS AND METHODS

Materials, sausage formulation and sausage preparation were similar to 2.1.2.1-2.1.2.3, except that only 2 levels of soy isolate was used (0 % and 2 %). During drying period, the temperature of the cold room was still at 5-6°C throughout the drying period but the relative humidity was adjusted so that from day 1 - 12, the relative humidity was at 85 % and later reduced to 72-75 % until the end of drying period.

2.2.3 SAMPLING AND RECORDING

First samples were immediately collected after processing of sausage mixtures was completed. Later on samples were collected at the following times: a) fermentation period; day 1 and 2, and b) drying period; day 7, 11, 15 , 19 and 23. Determination of moisture, pH, titrable acidity and salt was also carried out during these stages of sausage preparation. The methods for the chemical analysis and determination of weight loss were as in Experiment 1. The processing of samples for microbiological evaluation started 1-2 hours after collecting the samples.

In this experiment smoking was carried out on day 3 which was one day after the stuffing of the sausages into the casings.

2.2.4 CONFIDENCE LIMITS FOR COLONY COUNTS

Bacterial counting in the experiment involves only a very small fraction of the total numbers of the microorganism present

in the bulk sausage sample and it satisfies the Poisson's distribution or the Law of small Probabilities. Confidence limits (CL) which describe the upper and lower values of a range within which the true population mean lies are used since they are easier to interpret. The determination of the 95 % confidence limit is as follows,

$$95 \% \text{ CL} = \bar{x} + 2 \sqrt{\frac{s^2}{n}}$$

As the mean (\bar{x}) is approximately equal to variance (s^2) in Poisson's distribution, the 95 % CL can then be calculated according to the relationship,

$$95 \% \text{ CL} = \bar{x} + 2 \sqrt{\frac{\bar{x}}{n}}, \text{ where } n \text{ is the number of plates or}$$

sampling units.

2.2.5 MICROBIAL METHODS

2.2.5.1 Preparation and dilution of the food homogenate

Preparation of samples firstly involved homogenising with appropriate diluent before they were used in the various determinative and enumerative procedures. The homogenisation of the sample was carried out so as to obtain a homogenous suspension of sample and microorganisms which could be pipetted for further dilution and plating. Throughout the microbiological analysis, 0.1 % peptone solution was used as the diluent.

Except in Salmonella enteritidis and L. monocytogenes isolations, the homogenate was prepared by weighing 10 ± 0.1 g of

sample inside a sterile plastic bag placed on top of a weighing balance and into which 90 ml of diluent was added. The sample and diluent in the plastic bag were placed inside a stomacher and stomached for 2 min to give a dilution of 10^{-1} . The homogenate was then transferred into a sterilised bottle.

Further ten-fold dilution of the homogenate was prepared by transferring 1 ml of the homogenised material into another 9 ml 0.1 % peptone solution to give a 10^{-2} dilution. This process was repeated for the next dilution and so forth. Care was taken to ensure that the bottle was shaken vigorously to disperse the organisms evenly before each dilution was prepared.

In the case of the isolation of Salmonella spp. and L. monocytogenes, Buffered Peptone Water (Gibco, 152-05300 M) and UVM (Oxoid, CM863), were respectively used as pre-enrichment media. A sample weighing 25 g was weighed and then placed into a sterile bag containing 225ml medium to give a dilution of 10^{-1} . It was then homogenised in the stomacher for 2 min. The blended homogenate was then transferred into a sterile bottle for incubation.

2.2.5.2 Plating

Plating of the solution containing the microorganisms was carried out on the plates containing appropriate media after proper preparation and dilution of the sample. The spread plate technique with 0.1 ml aliquot was used throughout the plating procedure. All plates were properly dried before being plated out. Only plates containing 20-200 colonies were counted.

2.2.5.3 Total Viable Count

The homogenate and its dilutions were enumerated on the Plate Count Agar (Gibco, 152-04470 M). The plates were later incubated at 30 °C for three days. All colonies were taken into consideration during counting.

2.2.5.4 Lactobacillus pentosus

The plating of the homogenate with the different dilutions was carried out on Rogosa medium (Oxoid, CM627). The plates were then placed inside an anaerobic jar with an Oxoid Gas Generating Kit sachet (Br 38) containing tablets that released carbon dioxide and hydrogen upon addition of water. The anaerobic jar was incubated at 30 °C for 3 days. The Rogosa medium would allow the growth of lactobacilli and all colonies on the plates were counted as L. pentosus.

2.2.5.5 Staphylococcus carnosus

The homogenate was plated on Kranep medium (Oxoid, CM441) and the plates were incubated at 37 °C and readings were taken after 24 and 48 h. All round white colonies were counted as S. carnosus.

2.2.5.6 Staphylococcus aureus

The same method was employed as 2.2.5.5 but on the plates with Baird-Parker medium (Oxoid, CM275). The plates were incubated at 37°C for 24 and 48 h. To inhibit the growth of Proteus, 0.055 gm of sulfamezathine was added to a liter of

molten agar before it was sterilised and poured into the plates. Only black colonies with halo surrounding them and positive to coagulase test were counted and presumptively identified as S. aureus.

2.2.5.7 Clostridium perfringens

The homogenate was plated on the Tryptose Sulfite Cycloserine agar (TSC) (Oxoid, CM587) and overlaid with TSC-Yolk Free Medium and incubated at 37°C for for 24 h. Only black colonies surrounded by an opaque halo were counted and presumptively identified as Cl. perfringens.

2.2.5.8 Enumeration of Coliform and E. coli by Most Probable Method (MPN)

The 3 tubes MPN method was used to enumerate the coliform and E. coli in all the samples collected at the different stages of preparation and ripening. The MPN for coliform was carried out in universal bottles containing 10 ml of MacConkey (Oxoid, CM5a), single strength broth and an inverted Durham fermentation tube. The confirmation of E. coli, however was done in universal bottles containing Brilliant Green Bile Broth (BGBB) (Oxoid, CM31) with an inverted Durham tube. The other test carried out for E. coli confirmation was Indole test. In this experiment the MPN of coliform was done from the beginning of sausage preparation and the MPN of E. coli was only performed to the sausages at the end of the drying period.

All samples were prepared and diluted as in 2.2.5.1. 1 ml of

the decimal dilutions of sample homogenate was pipetted into each of the three tubes of MacConkey broth. The tubes were incubated at 35-37°C for 24 and 48 h. After 24 h, tubes showing colour change from pink to yellow and gas production was recorded and the non-gas forming tubes were again incubated for another 24 h. After 48 h tubes containing gas were recorded as before.

For the enumeration of the coliform, the least diluted set in which all the 3 tubes showed positive reaction and the next two dilutions were selected. For example, if the dilution in which all three tubes were positive was 10^{-2} , the MPN would be obtained from the result of the 10^{-2} , 10^{-3} and 10^{-4} dilutions and the numbers of positive tubes in each dilution were 3, 1 and 0 respectively, the result was $10^{-2}=3$, $10^{-3}=1$ and $10^{-4}=0$. By using an appropriate table, the MPN of coliforms for the sample was recorded as 700/g.

If no dilution contained three positive tubes, the three least dilute sets containing positive tubes were selected. In the case where the last four positives were 10^{-1} , 10^{-2} , 10^{-3} and 10^{-4} and the numbers of positive tubes in these dilutions were 2, 2, 2 and 1 respectively, the result $10^{-1}=2$, $10^{-2}=2$, $10^{-3}=2$ would be used and the MPN of coliform would be recorded as 200. The MPN of E.coli was determined as for the coliforms except tubes containing BGGB were incubated at 44°C for 18-24 h. The new MPN sets were inoculated with 1-2 drops from each tube previously positive for coliforms. Those tubes with gas production were considered to be presumptively positive. A confirmatory test was performed by adding 1-2 drops from samples showing positive

reaction into Bijou bottles containing 3-4 ml of Tryptone Water (Oxoid, CM87) for indole test. This assesses for indole production from the breakdown of tryptophan by E. coli. The Bijou bottles were incubated at 44°C for 18-24 h and for the indole assessment. 0.2-0.3 ml of Kovac's reagent was added into the Bijou bottle and was shaken and let to stand for 2 minutes before result was taken. A dark red colour in the amyl alcohol surface layer indicated a positive test and a negative result was indicated by an orange colour. Only tubes which showed both gas production and a positive indole test were considered as E. coli and the MPN was then determined as above.

2.2.5.9 Isolation of Salmonella enteritidis

2.2.5.9.a Determination of Salm. enteritidis total count

A pure culture of Salm. enteritidis was inoculated into a flask containing Nutrient Broth, incubated shaken at 37°C for 24 h and the turbidity of the suspension was determined using a nephelometer. The nephelometer was calibrated with turbidity standards on each occasion before the turbidity of the suspension was read.

Several ten-fold dilutions of the suspension were plated out onto Plate Count Agar, incubated at 37°C for 24 h. From the resulting colony count the viable count of salmonella cells in the suspension was calculated and used to calibrate the nephelometer turbidity measurement. Several colonies on the plates were inoculated into Nutrient Broth and incubated as before. The turbidity reading from the second suspension was

taken and the number of Salm. enteritidis/ml suspension was estimated from the nephelometer turbidity measurement. The actual viable count of this suspension was also determined as before.

In order to achieve the required number of cell of Salm. enteritidis per g of sausage mixture, the latter suspension was serially diluted into 0.1 % peptone and added with proper amounts into the sausage mixture so that every sausage batch contained about 100 cell/g.

2.2.5.9.b Detection and confirmation of Salm. enteritidis

The time taken to isolate the salmonella organism was made shorter by the use of the Oxoid Selective Motility Enrichment Technique (SMET) as described by Holbrook et al. (1989). The SMET , which is based on the migration of salmonella from elective to selective media was selected because the technique was found to be rapid, simple and highly specific.

Initially, 25 g sample was pre-enriched into Buffered Peptone Water and incubated at 37°C for 24 hr. The next stage is to add 1 ml of the pre-enrichment into a culture vessel. However, certain preparation has to be done to the culture vessel before the pre-enrichment solution could be added into the vessel.

The culture vessel consists of two tubes each of which contain two different dehydrated enrichment media in their lower compartment and dehydrated selective media in their upper. The media was first rehydrated by adding sterile distilled water and

later a specially formulated salmonella elective medium and a disc containing novobiocin before 1 ml of pre-enrichment was added into the vessel. The vessel was incubated at 41°C (\pm 0.5°C) and the change of colour was observed. If the colour change was positive, a loopful of culture was further tested with the Oxoid Salmonella Latex Test and Salmonella coloured Latex Test. The whole procedure is illustrated as in Diagram 2.

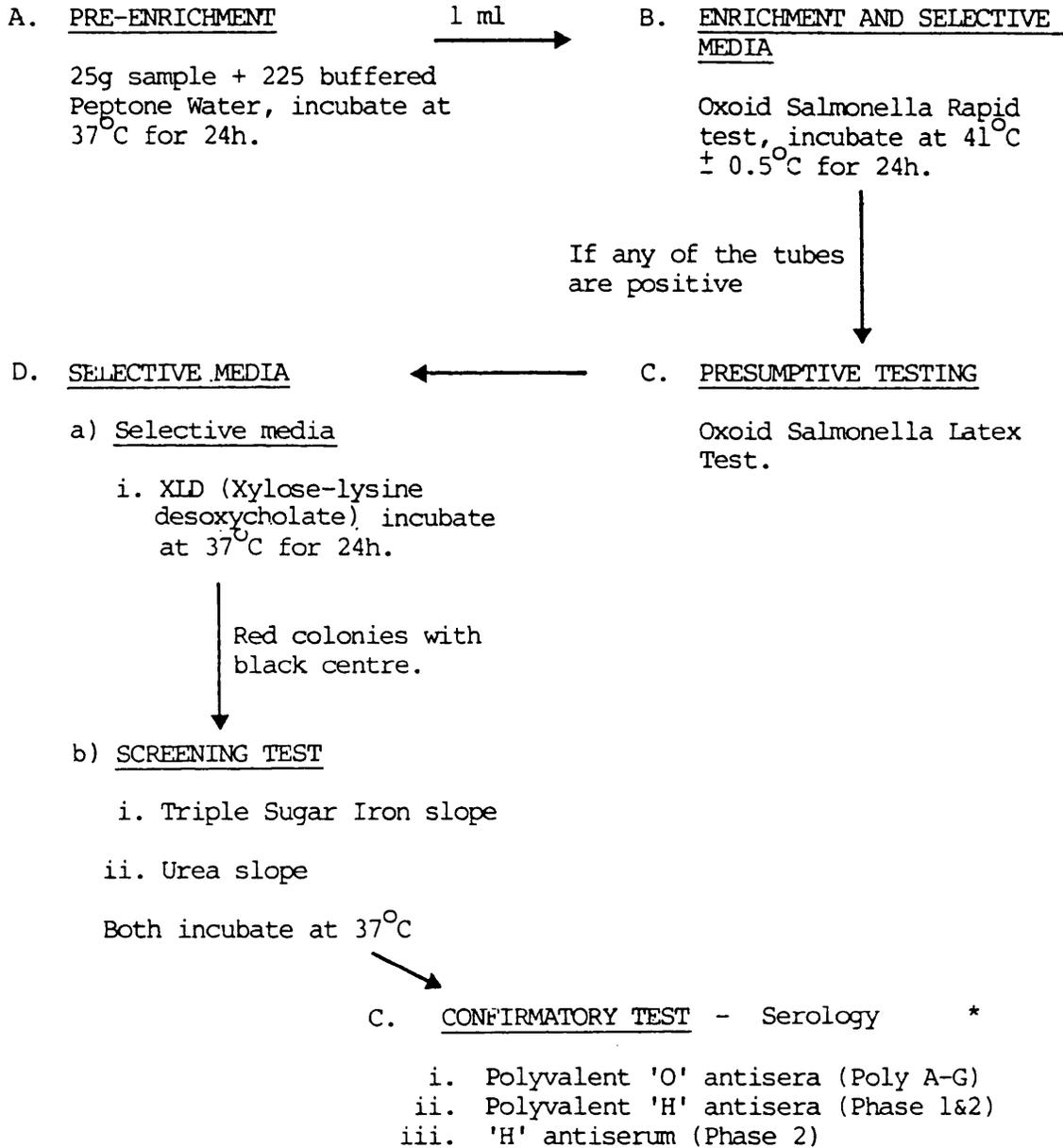
2.2.5.10 Isolation of *Listeria monocytogenes*

Isolation of *Listeria monocytogenes* involved various stages of pre-enrichment, enrichment, selective plating and purification as in Diagram 3.

For the confirmation of *L. monocytogenes*, the microorganism should haemolyse the the Horse Blood Agar and be positive with CAMP Test on Sheep Blood Agar and rhamnose sugar fermentation, but negative with xylose fermentation.

DIAGRAM 2: DIAGRAMATIC SCHEME FOR ISOLATION OF

SALMONELLA SPP



* Salm. enteritidis is positive with polyvalent 'O' antisera, positive with polyvalent 'H' antisera, phase 1 & 2 but negative with 'H' antiserum, phase 2.

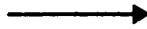
DIAGRAM 3: DIAGRAMATIC SCHEME FOR ISOLATION OF

LISTERIA MONOCYTOGENES

A. PRE-ENRICHMENT

25g sample + 225 UVM,
incubate at 30°C for 24h.
Repeat at 7 days.

0.1ml



B. ENRICHMENT

10ml Fraser Broth with
0.1 0.125% acriflavin
and 0.1ml 5% ferric
ammonium citrate,
incubate at 35°C for
26± 2h.

If turns black, a
loopful onto each



D. PURIFICATION

a) Brain Heart Infusion
or Trypticase Soy
Yeast Agar, incubate
at 30°C for 24 and
48 h.

Check for blue colour
and catalase reaction
(3% H₂O₂) if both
positive.



b) Brain Heart Infusion
Broth, incubate at
20-25°C for 24h.

check for
tumbling motility



if positive

Check for B-haemolysis
if positive.

C. SELECTIVE PLATING

i. Oxfords Medium (37°C/
48h.)
ii. Modified McBrides
(30°C/48h.)

E. CONFIRMATION TEST

a) Horse Blood Agar,
incubate at 37°C.



Also check

b. CAMP reaction. (37°C for
48h.)
c. Umbrella motility
(20-25°C for 7d.)
d. Rhamnose fermentation
(35°C for 48h.)
e. Xylose fermentation
(35°C for 48h.)

* If colonies in Horse Blood
Agar are non-haemolytic,
the colonies are recorded as
non-haemolytic *Listeria* spp.,
other than *L. monocytogenes*.

CHAPTER THREE

RESULTS

3.1 RESULTS OF EXPERIMENT ONE

3.1.1 CHEMICAL ANALYSIS

3.1.1.1 Effect of soy isolate on moisture, fat and protein

The initial moisture content of the sausage mixtures with 0, 2 and 4 % soy isolate was 64.29, 63.01 and 61.91 % respectively. By keeping the amount of water and other ingredients constant, addition of 2 and 4 % soy reduced the amount of moisture in the respective sausage mixtures. But, the protein content of the sausage mixture with 4 % soy isolate was the highest among the other group. The same result was also observed for the fat content of the mixture. During active fermentation period (ie. day 0 to day 2), the different levels of moisture, fat and protein remained constant. However during drying, there was a general increase in the fat and protein levels, followed by the decrease in the moisture content of the fermented sausages. The changes were more pronounced in sausage groups with 2 and 4 % soy isolate. At the end of the drying period, the moisture content of sausage groups 2 and 4 % was at the 'dry' stage and sausage without soy isolate needed another 12 more days of drying to achieve the 'dry' stage with moisture content of about 35 %.

Although the sausage without soy isolate had some difficulty in drying, sausage with 4 % soy isolate had been found to have a slightly higher protein and a lower fat content at the end of drying period. The changes in moisture, fat and protein contents

of all the sausage groups are illustrated in Figures 1, 2 and 3.

From Table 3, results of chemical analysis show that by prolonging the time of drying of the sausages, the proportions of water to protein (moisture:protein ratio) is decreasing.

After drying for 18 days, the final moisture contents of the three sausage groups of 0, 2 and 4 % soy isolate were determined as 43.8, 35.8 and 37.6 % respectively. The overall moisture loss in grams per day from day 0 to the end of the drying period was highest in sausage with 2 % soy, being 1.36 g/d, followed by 1.17 g/d for the sausage with 4 % soy and only 1.02 g/d for the sausage without soy isolate. It appears that less moisture was removed from sausage prepared with 4 % soy than that prepared with 2 % soy, if sausage without soy was not taken into consideration.

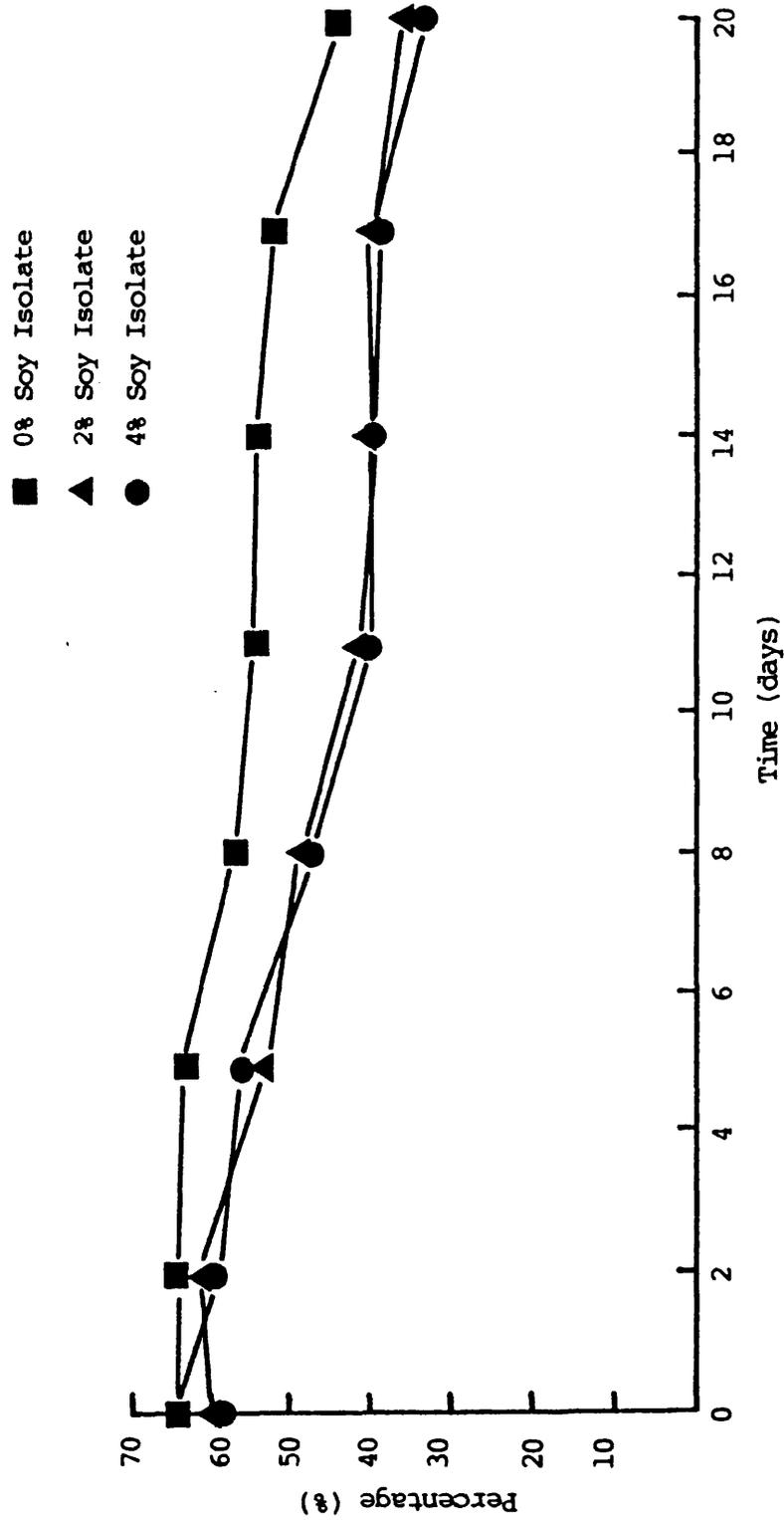


Figure 3: Moisture Content of the Smoked Fermented Poultry Sausages

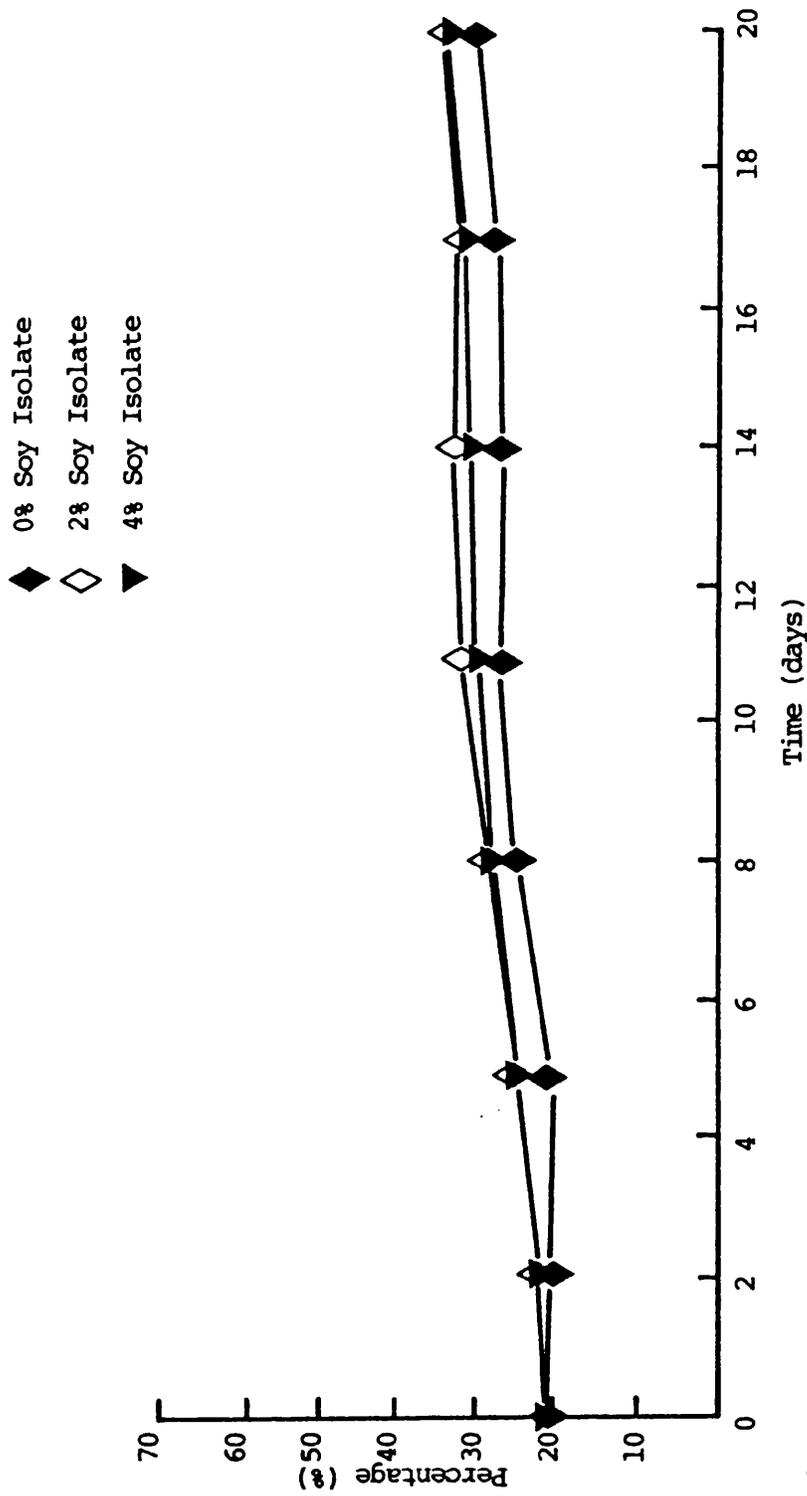


Figure 4: Fat Content of the Smoked Fermented Poultry Sausages

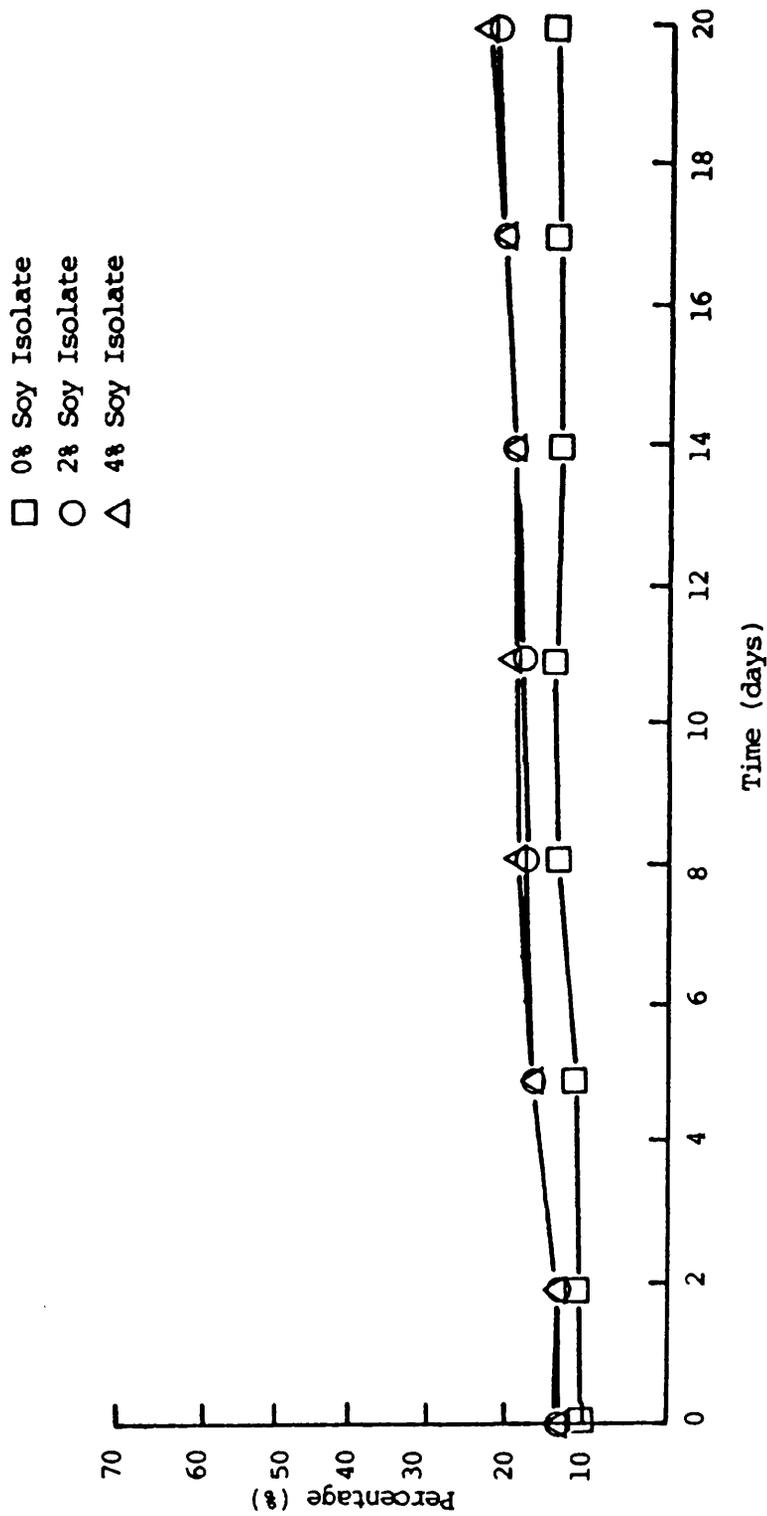


Figure 5: Protein Content of the Smoked Fermented Poultry Sausages

TABLE 3: CHEMICAL CHANGES OF FERMENTED POULTRY SAUSAGES WITH 0, 2 and 4% SOY ISOLATE

No. of days	Level of Soy Isolate (%)	Moisture	Fat	Protein	$\frac{\text{Moisture}}{\text{Protein}}$	a_w	pH
0	0	64.29 ± 0.04	21.03 ± 1.03	11.36 [±] 0.18	5.66:1	0.97	6.3
	2	63.01 ± 2.04	20.64 ± 0.82	13.81 [±] 0.06	4.56:1	0.97	6.28
	4	61.91 ± 0.28	21.57 ± 0.32	14.59 [±] 0.28	4.17:1	0.97	6.29
2	0	64.51 ± 1.35	21.03 ± 0.21	11.62 [±] 0.21	5.55:1	0.97	5.24
	2	62.63 ± 0.59	22.21 ± 0.65	14.08 [±] 0.20	4.56:1	0.97	4.92
	4	61.41 ± 1.71	20.62 ± 0.80	14.22 [±] 0.69	4.32:1	0.97	4.75
11	0	54.15 ± 1.51	28.03 ± 1.22	14.71 [±] 0.09	3.75:1	0.95	4.83
	2	40.03 ± 0.23	31.83 ± 0.82	19.28 [±] 0.32	2.08:1	0.92	4.64
	4	41.42 ± 0.64	29.51 ± 0.61	19.93 [±] 0.69	2.07:1	0.92	4.55
20	0	43.81 ± 1.01	31.65 ± 1.22	16.49 [±] 1.72	2.65:1	0.95	4.83
	2	35.81 ± 0.60	36.38 ± 0.81	23.15 [±] 0.86	1.54:1	0.89	4.65
	4	37.61 ± 0.75	34.56 ± 1.02	24.13 [±] 1.28	1.60:1	0.90	4.55

3.1.1.2 Effect of soy isolate on titratable acidity and pH

All sausage mixtures from the three different groups had a pH between 6.28 and 6.30 initially. After 24 hours of fermentation there was a slight fall in pH in all the groups so that on day 1 the pH of all sausage mixtures was recorded at 5.7. However, the rapid drop in pH was observed on day 2, particularly to the sausages with 4 % soy isolate. The drop in pH during this active period of fermentation, ie. between days 1 and 2 was 0.36, 0.49 and 0.81 units in the sausages with 0, 2 and 4 % respectively. However, the decline in pH was observed to be rather slow and towards the end of drying period the pH was seen to rise slightly, especially from day 18 onwards.

From Figure 6, it is very clearly shown that throughout the fermentation and drying periods, the sausages without soy isolate had a higher pH than the other two groups.

During the fermentation process a fall in pH was brought about by the concurrent increase in the production of lactic acid. From day 3 to the end of drying period, more lactic acid had been produced from the groups with 4 % soy isolate than 0 and 2 % even though there was a general increase in the pH during that period of time. The different levels of lactic acid over the whole fermentation and drying periods from the three different groups of sausages is also shown in Figure 6.

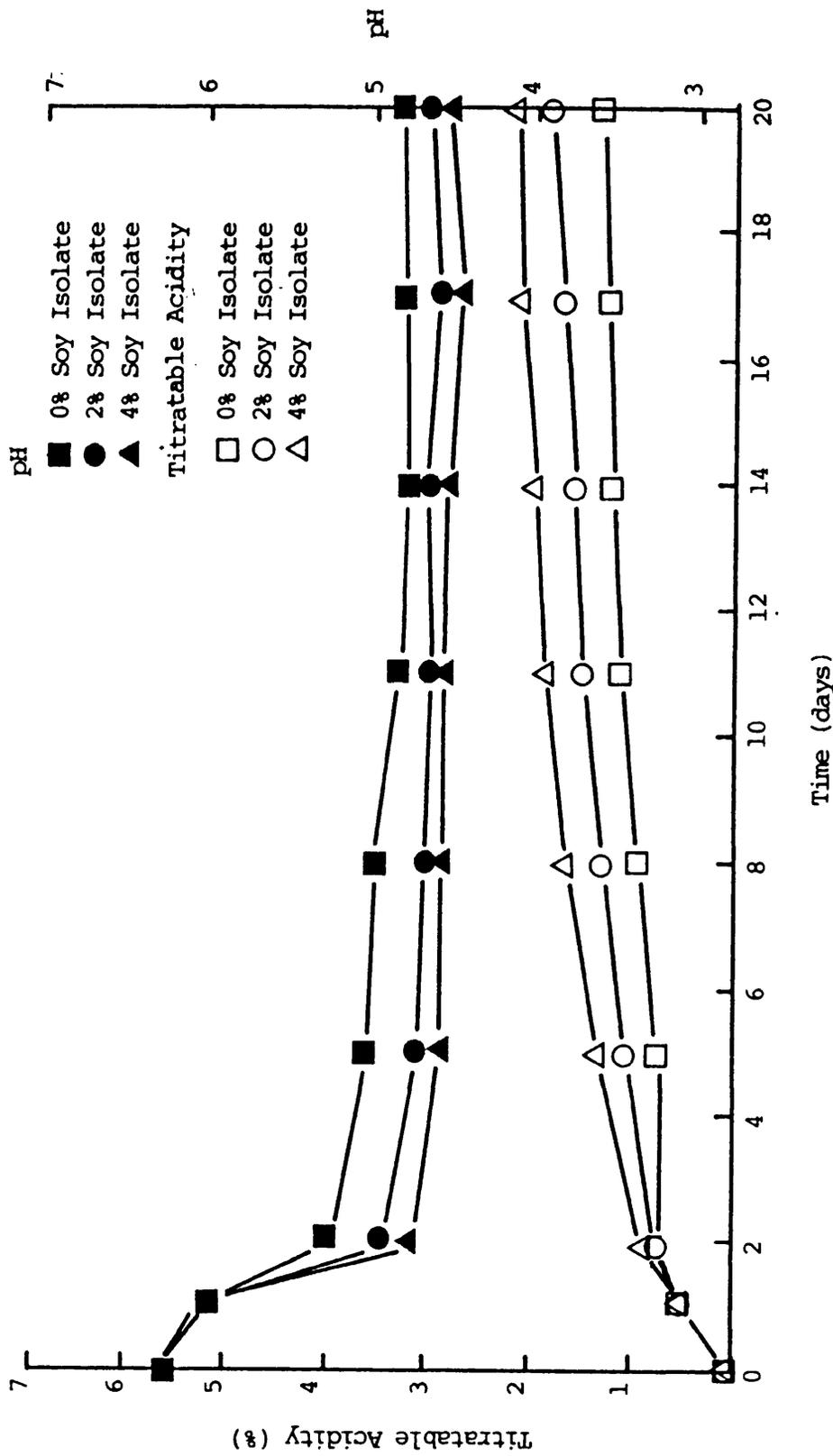


Figure 6: pH and Titratable Acidity of the Smoked Fermented Poultry Sausages

3.1.1.3 Effect of soy isolate on salt and water activity

Figure 7 shows the changes in the salt content of sausages with three different levels of soy isolate. The amount of salt in the sausages were affected by the level of moisture present in the sausages themselves, both at the times of preparation of mixtures and during the test being carried out. The higher the amount of moisture the lower is the salt content in the sausages and vice versa. During the initial fermentation (ie. days 0-2), the salt content of all the sausages remained constant.

In the experiment, the initial a_w of the sausage emulsions was determined at 0.97. It remained constant throughout the fermentation period but gradually reduced to a lower level thereafter, except in sausage without soy isolate, during the drying period. Sausage with 2 % soy isolate was shown to have a lower a_w , followed by the sausages with 4 and 0 % soy isolate, even though the a_w of all the sausages was almost constant from days 0 to 5. The final a_w of the sausages with 0, 2 and 4 % soy isolate was 0.95, 0.89 and 0.9 respectively.

■ 0% Soy Isolate
 ● 2% Soy Isolate
 ▲ 4% Soy Isolate

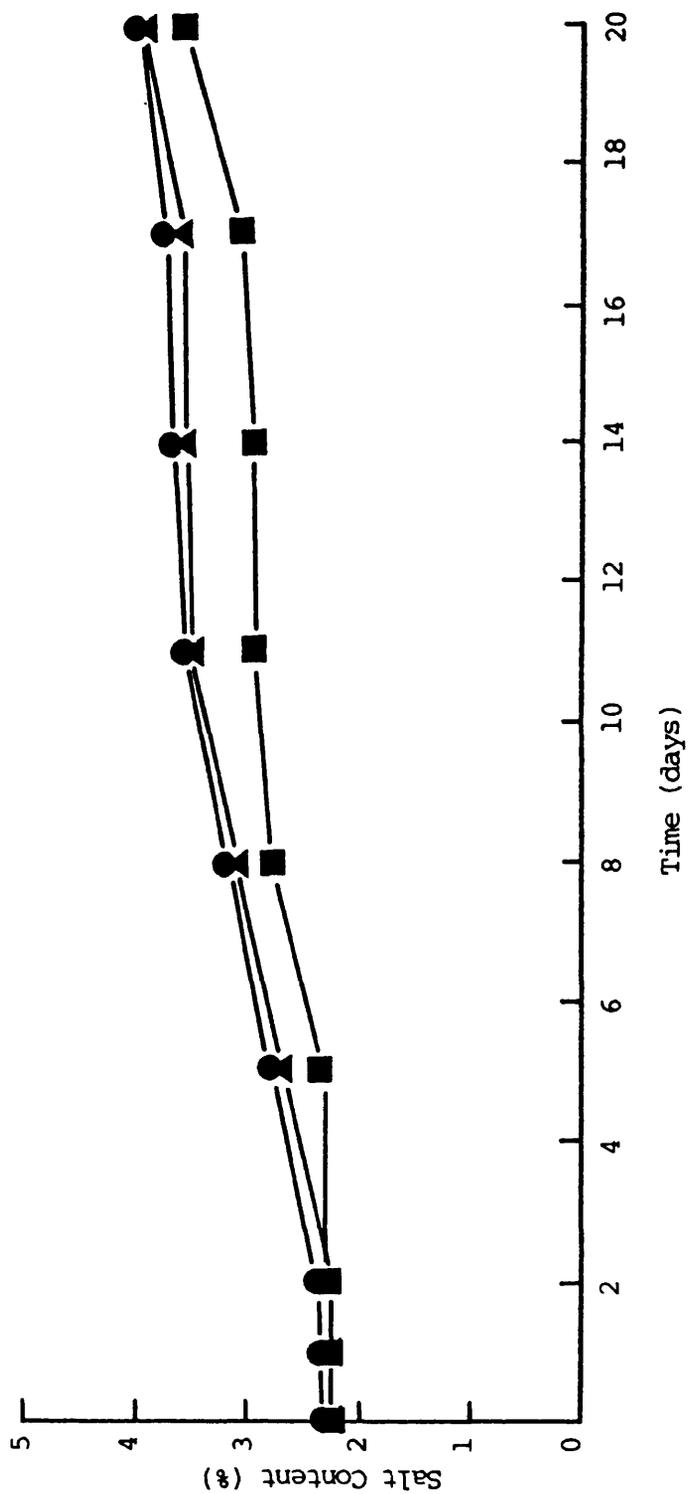


Figure 7: Salt Content of the Smoked Fermented Poultry Sausages

3.1.2 OBJECTIVE ANALYSIS

3.1.2.1 Weight loss

Figure 8 shows the cumulative weight loss of the three groups of fermented sausages. Sausages without soy isolate had a lower weight loss than sausages with 2 and 4 % soy isolate. It shows that there was a retention of moisture during the production of the sausage. The figure also indicates that the sausages with 2 and 4 % soy isolate had almost the same percent cumulative weight loss over the drying period, but the sausage with 2 % soy isolate had a slightly higher cumulative weight loss than the sausages with 4 % soy isolate. However, the sausages without the soy isolate was found to have a lower rate of moisture loss/day and this had resulted in a higher moisture content at the end of drying period. Overall rate of weight loss/day of those sausages with 0, 2 and 4 % soy isolate after stuffing into casings was 2.17, 3.11 and 2.91 g, respectively.

Figure 9 shows the relative size of the three sausages produced from three different levels of soy isolate. Sausage without soy had to be dried 12 days longer in order to achieve almost the same size and moisture level as the other two sausages produced with soy isolate.

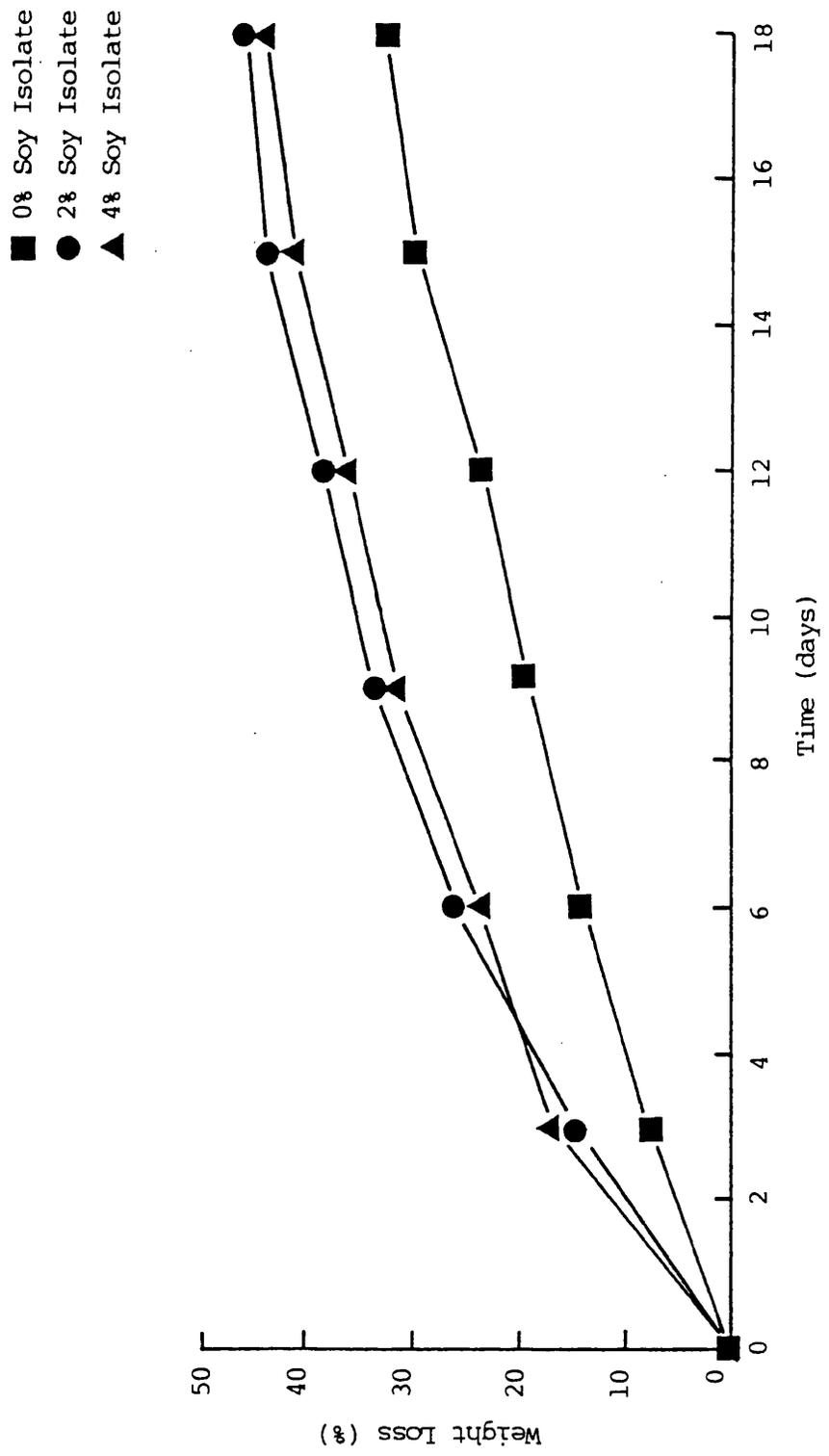


Figure 8: Cumulative Weight Loss of Smoked Fermented Poultry Sausages During Drying

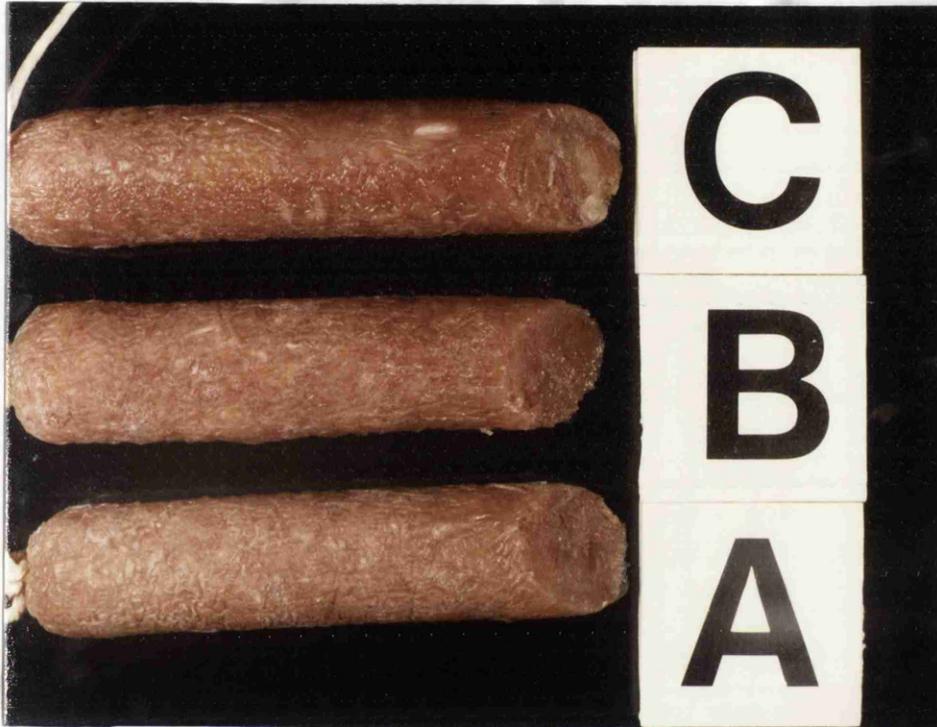


Figure 9: Relative Sizes of the Smoked Fermented Poultry Sausages
(A, 0 % Soy Isolate which took 12 d longer to the 'dry'
stage; B, 2 % Soy Isolate and C, 4 % Soy Isolate)

3.1.2.2 Texture

The texture of fermented sausages was very soft a few days after drying and this reflects the the higher amount of moisture in all sausages after being stuffed into the casings. As drying progressed, the firmness of the sausages was found to increase gradually and to be high particularly in those sausages with 4 % soy isolate. Also from Figure 10, the texture of the sausages without soy isolate is low compared to the other two groups. High amounts of moisture would also reduce the ability of the gel to cause a stronger aggregation of meat particles.

3.3.3. SENSORY CHARACTERISTICS

There was no observable difference in the colour of all the sausages with or without the soy isolate. All the sausages had the characteristic pink-red colour of the cured meat products.

The effect of soy isolate on the firmness of the sausages was also being examined and it was observed that the sausage without soy isolate was softer than those with 2 and 4 % soy isolate. Sausages with 4 % soy isolate was firmer than the 2 % group. Generally all sausages, even those with 4 % soy isolate was less firm than those sausages made from beef or pork.

With respect to the level of lipid on the surface of the sausages (greasiness), sausages without soy isolate showed a high degree of greasiness, addition of 2 % soy isolate reduced the problem. In fact, sausages with 4 % soy isolate had a lower degree of greasiness than those with 0 and 2 % soy isolate.

- 0% Soy Isolate
- 2% Soy Isolate
- △ 4% Soy Isolate

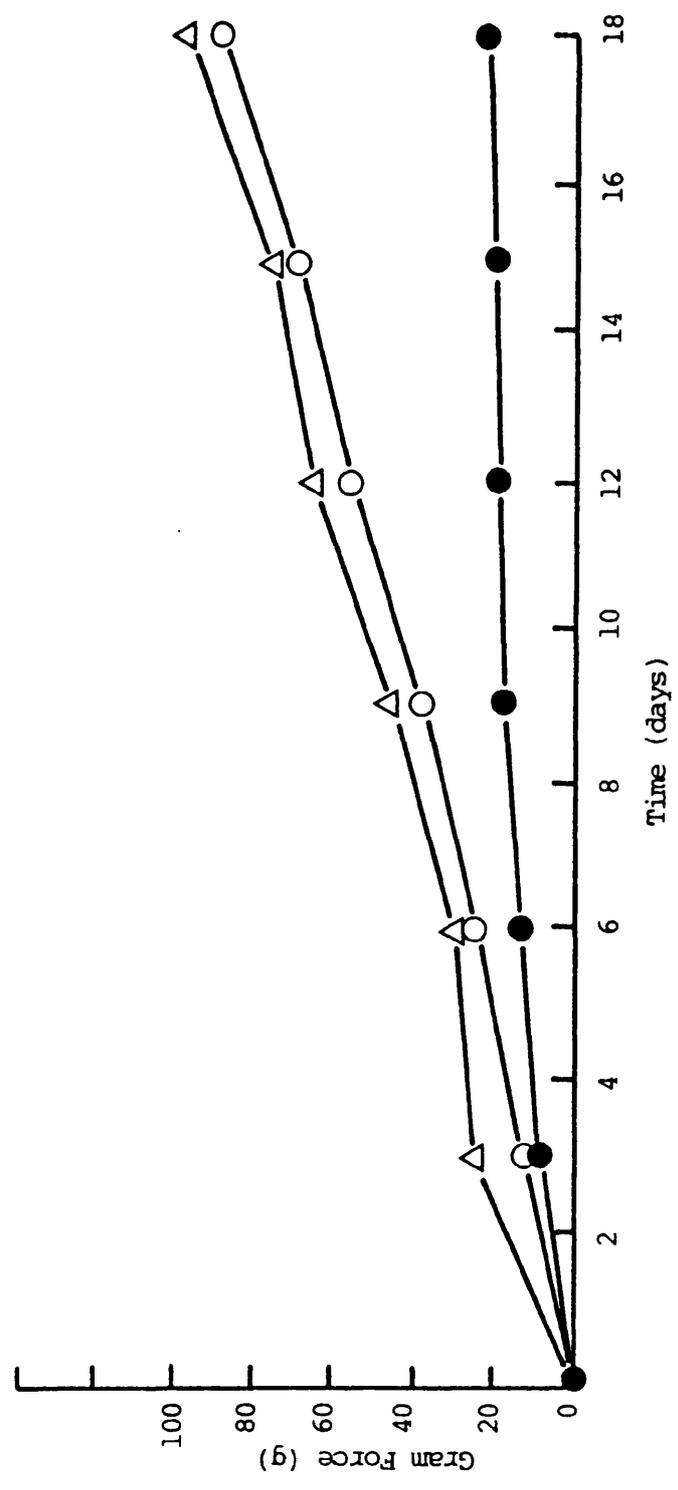


Figure 10: Texture of the Smoked Fermented Poultry Sausages

3.1.3 LIPID ANALYSIS

The problem of water retention in the sausages without soy isolate was a stimulus for the undertaking of lipid analysis. Theoretically, fermented sausages without soy isolate should have a higher amount of water loss/day than those with soy isolate because there is nothing to bind the water and thereby hinder the release of moisture from the sausages. The investigation involving both lipid and fatty acid compositions was performed with the aim of trying to find out if differences in water loss were in any way associated with the lipid and fatty acid compositions of the sausages.

3.1.3.1 Unbound fat

All the sausage displayed more than 99 % of unbound fat (see section 2.1.3.3) at the end of the drying period.

3.1.3.2 Lipid composition of the sausages

Figure 11 shows the chromatographic separation of the lipid present in the sausages. There was no significant difference with respect to relative proportions of triglycerides, free fatty acids, free cholesterol, partial glycerides and phospholipids in sausages with or without the soy isolate.

3.1.3.3 Fatty acid composition of the sausages

There was also no significant difference in the relative proportions of major fatty acids in the sausages. Table 4 shows the mean values obtained for the fatty acid compositions of the three groups of sausages. The result indicated that the main saturated fatty acid was palmitic acid, 22.5 to 23.5 %; the level of stearic acid was considerably lower, 4.9 to 5.3 %. Of

the total fatty acid present , the proportion of unsaturated fatty acids in all sausages was about 71-73 % and the main unsaturated fatty acid was oleic acid, followed in descending order by linoleic, palmitoleic and linolenic acids. The proportions of other unsaturated fatty acids such as eicosatrienoic, arachidonic, docosapentaenoic and docosahexaenoic acids were relatively low in all the sausages investigated. The relative proportions of the polyunsaturated and saturated fatty acids (P/S ratio) in the sausages containing 0, 2 and 4 % soy isolate were 0.74, 0.69 and 0.75, respectively.

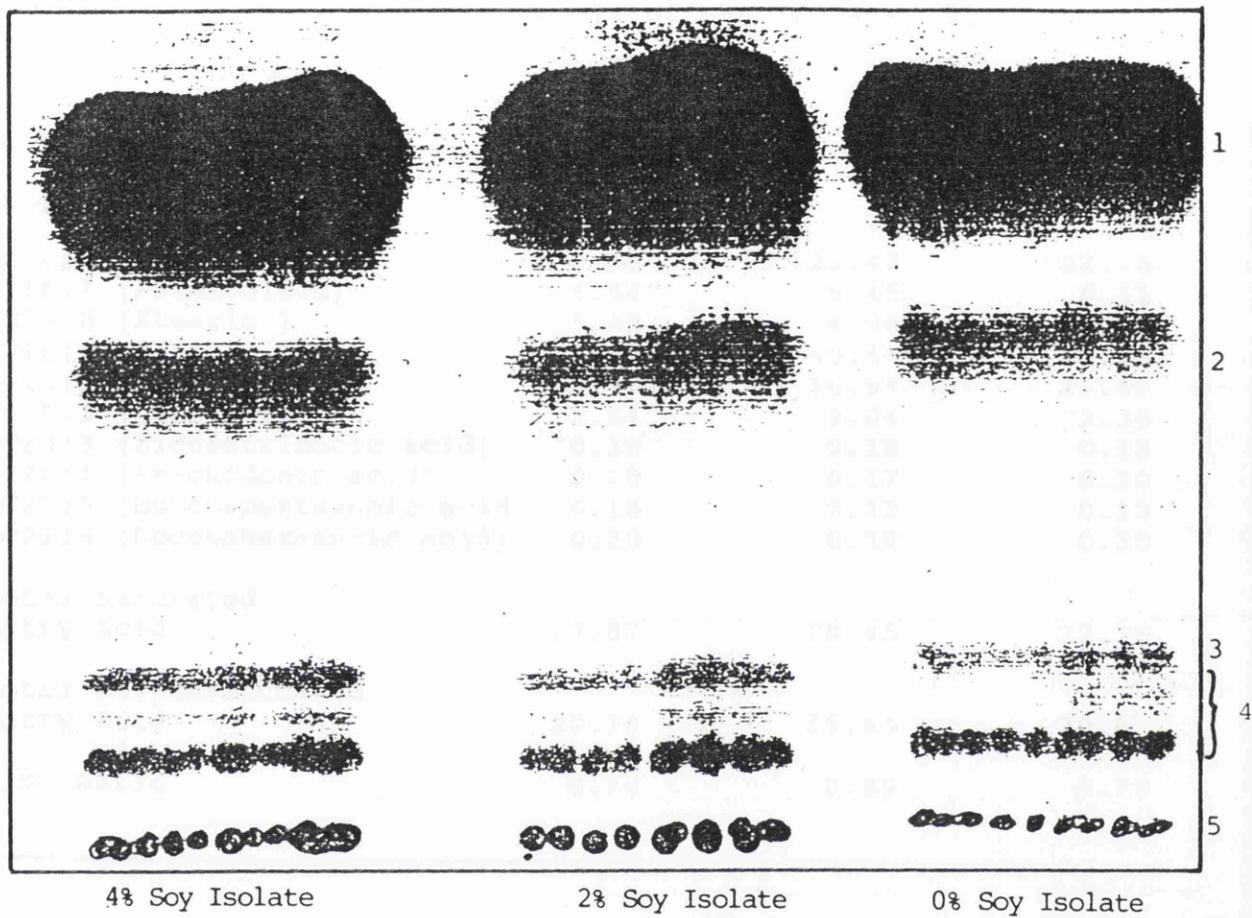


Figure 11: Chromatographic Separation of Lipids

- (1, Triglyceride; 2, Free Fatty Acids;
 3, Free cholesterol; 4, Partial Glycerides;
 5, Phospholipids)

Table 4: Percentage of fatty acids composition (weight percentage of total) of the sausages

	Levels of soy isolate (%)		
	0	2	4
Fatty Acids			
C16:0 (Palmitic)	22.58	23.47	22.76
C16:1 (Palmitoleic)	6.54	6.45	6.41
C18:0 (Stearic)	5.29	4.98	4.94
C18:1 (Oleic)	44.83	45.44	45.05
C18:2 (Linoleic)	17.46	16.97	17.69
C18:3 (Linolenic)	2.64	2.04	3.36
C20:3 (Eicosatrienoic acid)	0.10	0.18	0.12
C20:4 (Arachidonic acid)	0.20	0.17	0.20
C22:5 (Docosapentaenoic acid)	0.16	0.12	0.13
C22:6 (Docosahexaenoic acid)	0.20	0.18	0.30
Total Saturated Fatty Acid	27.87	28.45	27.70
Total Polyunsaturated Fatty Acid	20.76	19.66	20.8
P/S Ratio	0.74	0.69	0.75

3.2 RESULTS OF EXPERIMENT TWO

3.2.1 MICROBIOLOGICAL ANALYSIS

The growth and development of L. pentosus, S. carnosus, coliforms and psychrophiles were studied during fermentation and ripening of unsmoked and smoked dry sausages prepared from thigh trims with 0 and 2 % soy isolate. The growth and development of the microorganisms, including the mesophiles was as in Figures 12 to 20.

The survival of Salm. enteritidis inoculated at 100 cells/g sausage mixture during sausage preparation was also investigated. At the end of the drying period, determination of microbial quality of fermented sausages was carried out and special attention was given to assessing important food-borne microorganisms such as S. aureus, E. coli, Cl. perfringens, L.monocytogenes and Salm. enteritidis.

3.2.1.1 Effect of soy isolate and smoking on growth of mesophiles

The initial level of mesophiles as represented by the Total Viable Count (TVC) in sausages with 0 and 2 % soy isolate was approximately the same being $1.6 \pm 0.3 * 10^7$ cfu/g and $2.1 \pm 0.3 * 10^7$ cfu/g, respectively (Figure 16). There was rapid growth of the mesophiles during the first 24 h of fermentation and the same was true during the 24 to 48 h period for the sausage with 2 % soy. However, the growth of mesophiles in sausage without soy isolate was less between 24 and 48 h so that after 48 h of

fermentation, the number in sausage with soy was 17 times that of sausage without soy isolate; being $6.8 \pm 0.2 * 10^9$ and $4.0 \pm 0.2 * 10^8$ cfu/g, respectively.

After stuffing and storage at 5-6°C in the cold room the mesophile numbers declined in both unsmoked samples, reaching about $1-3 * 10^8$ cfu/g by day 7.

The effect of smoking was to bring about a reduction in the mesophile numbers as can be seen from the results on day 7 where the TVC of the smoked samples was about 20-50 % of the unsmoked samples. After day 7 however, there was a recovery in mesophiles numbers and by days 11-15 the TVC of all samples was of the order of 10^8 cfu/g. Thereafter there was a slow decline in mesophile numbers in all the samples to $2-3 * 10^7$ cfu/g by the end of the drying period. The TVC of unsmoked sausages without and with 2 % soy isolate was $2.9 \pm 0.1 * 10^7$ and $2.6 \pm 0.3 * 10^7$ cfu/g, respectively and the mesophile count of smoked sausages without soy isolate was $1.9 \pm 0.3 * 10^7$, virtually identical to the $2.0 \pm 0.3 * 10^7$ cfu/g in sausages with 2 % soy isolate at the end of the drying period.

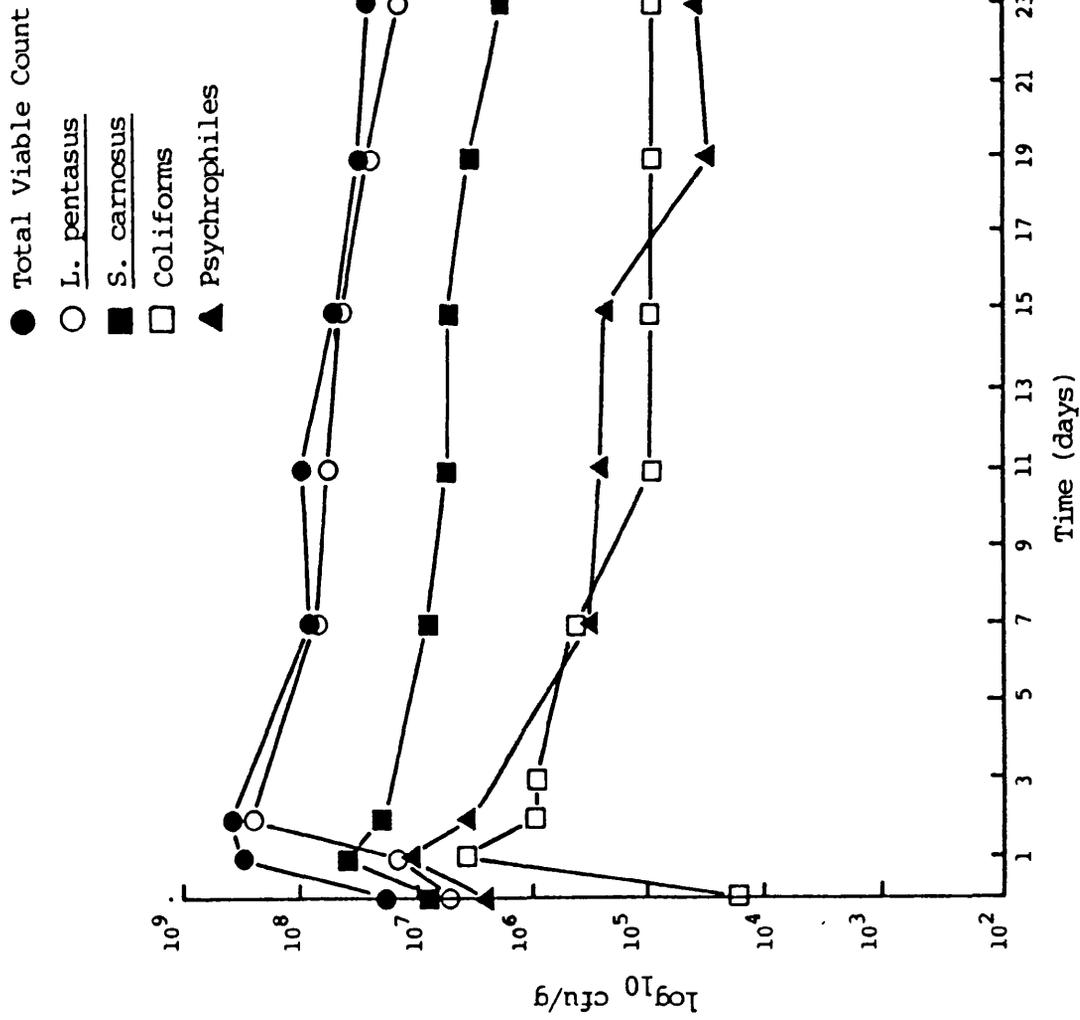


Figure 12: Growth and Development of Microbial Populations in Unsmoked Fermented Poultry Sausages Without Soy Isolate.

● Total Viable Count

○ L. pentasus

■ S. carnosus

□ Coliforms

▲ Psychrophiles

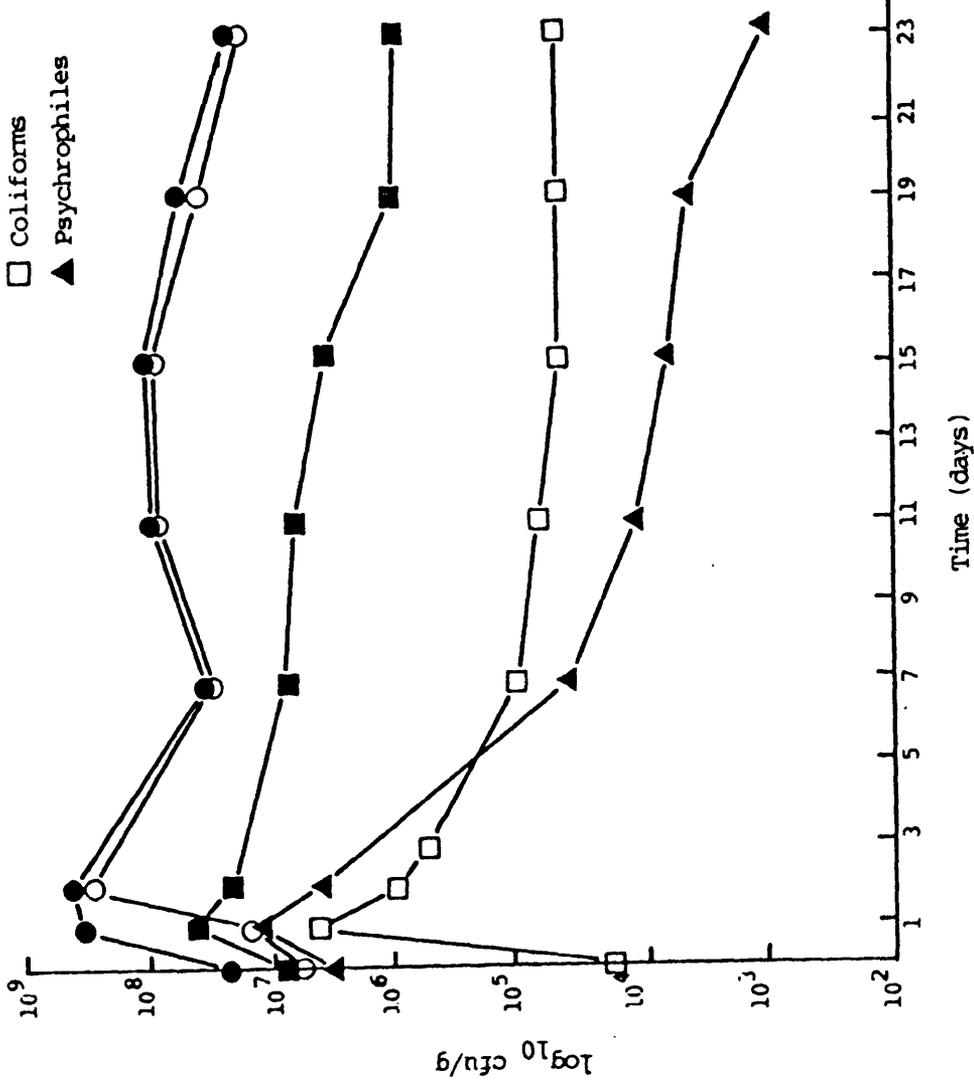


Figure 13: Growth and Development of Microbial Populations in Smoked Fermented Poultry Sausages Without Soy Isolate

Total Viable Count

- *L. pentasus*
- *S. carnosus*
- Coliforms
- Psychrophiles
- ▲ Total Viable Count

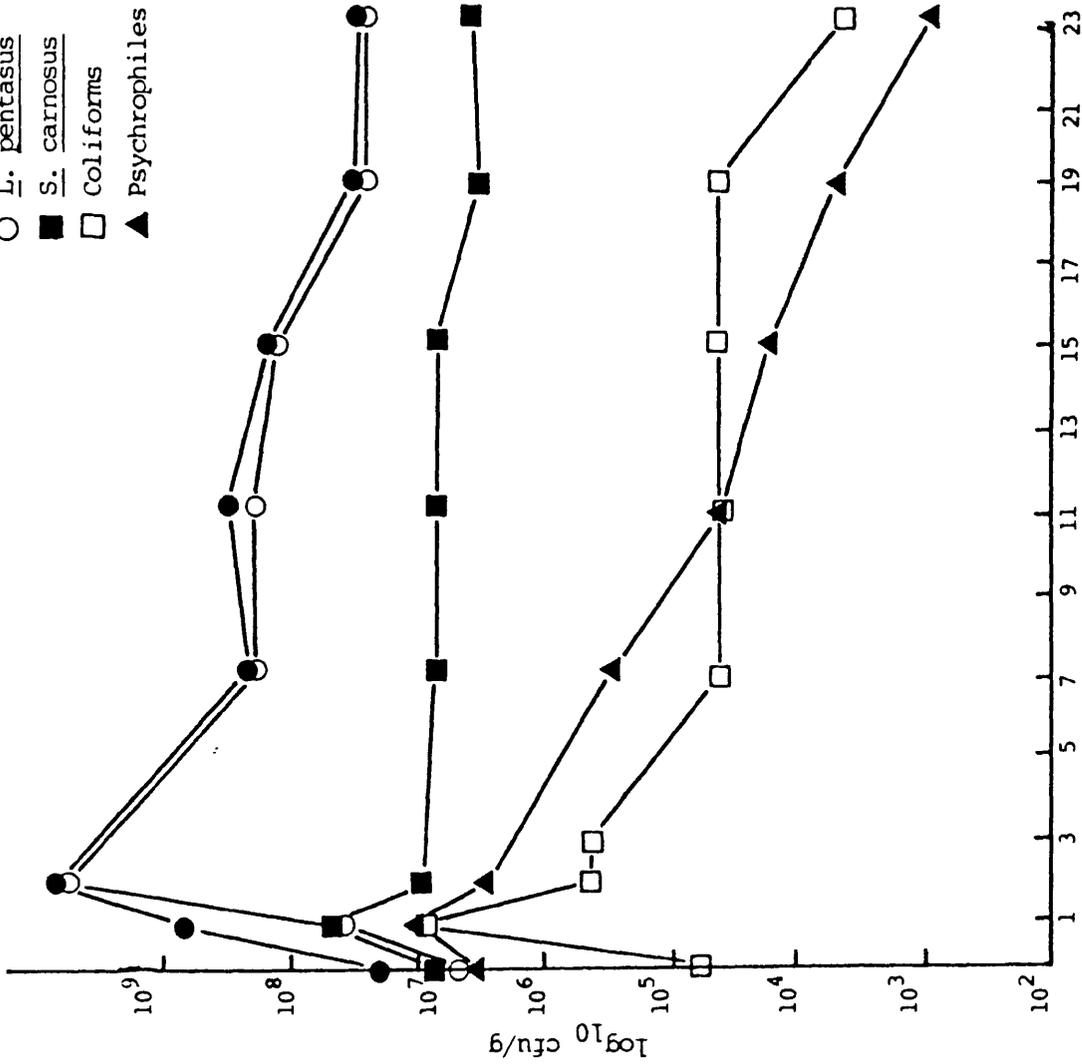


Figure 14: Growth and Development of Microbial Populations in Unsmoked Fermented Poultry Sausages With 2% Soy Isolate.

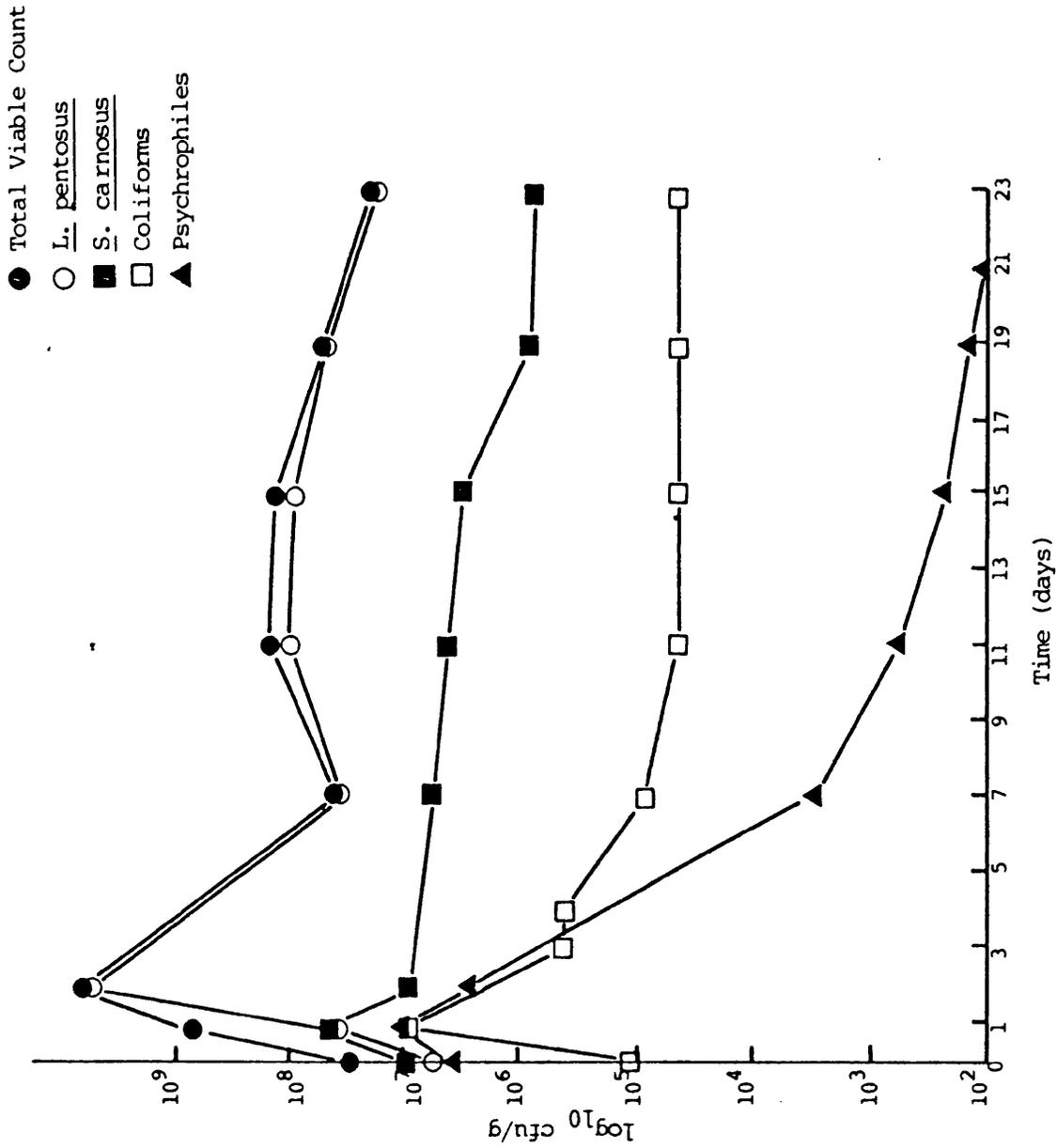


Figure 15: Growth and Development of Microbial Populations in Smoked Fermented Poultry Sausage With 2% Isolate

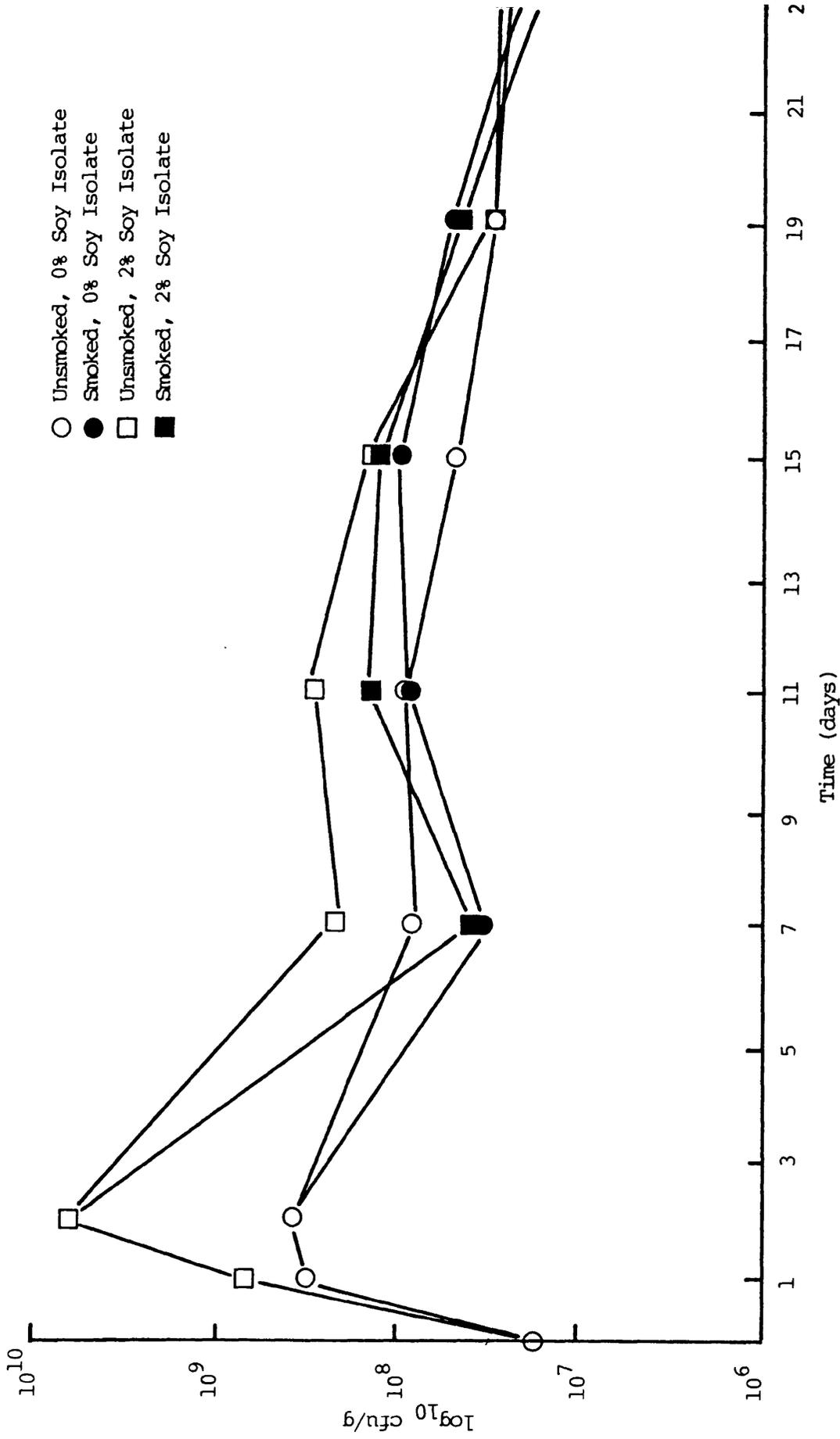


Figure 16: Total Viable Counts of Fermented Poultry Sausages

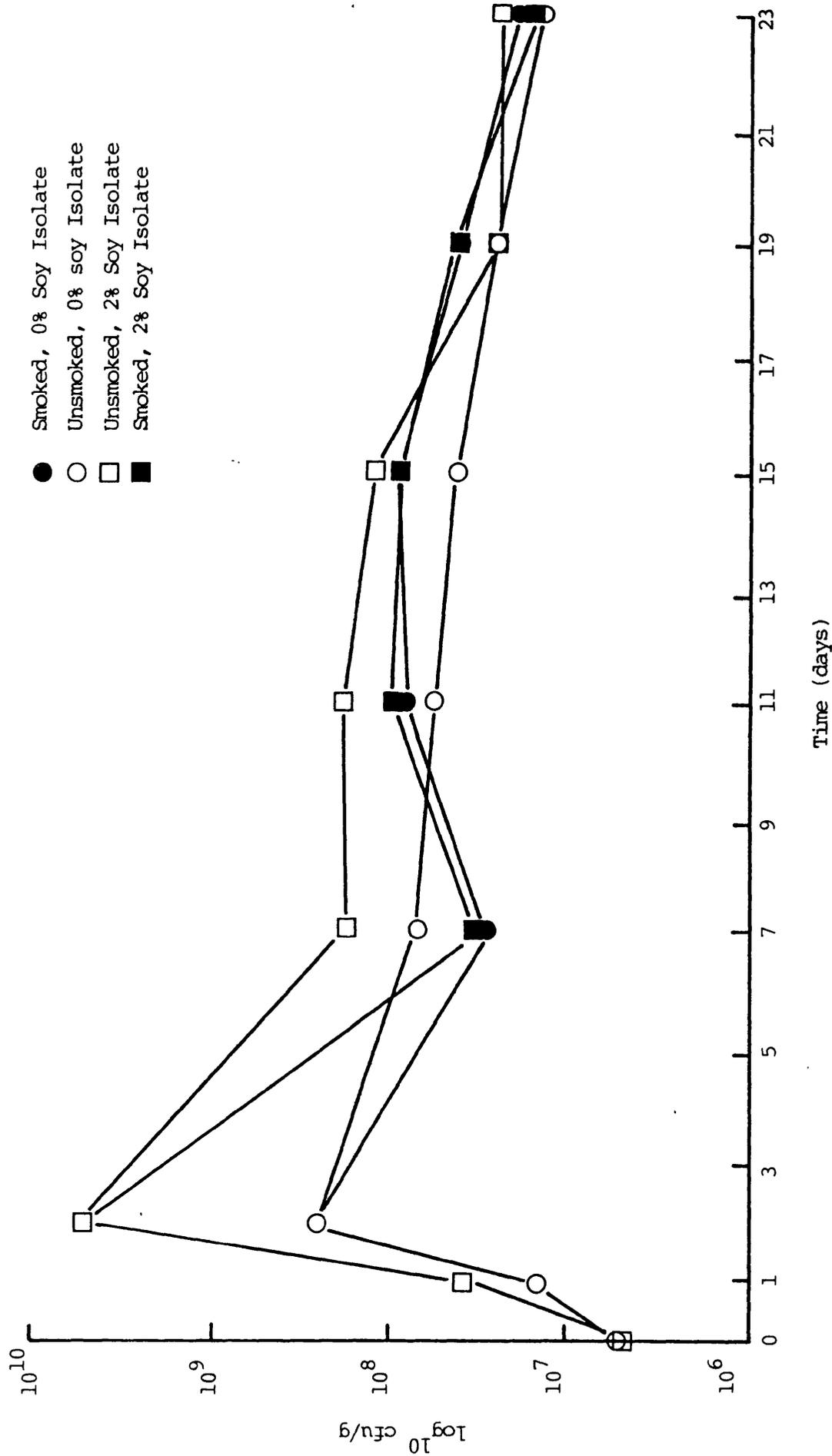


Figure 17: Growth and Development of *Lactobacillus pentosus* in Fermented Poultry Sausages

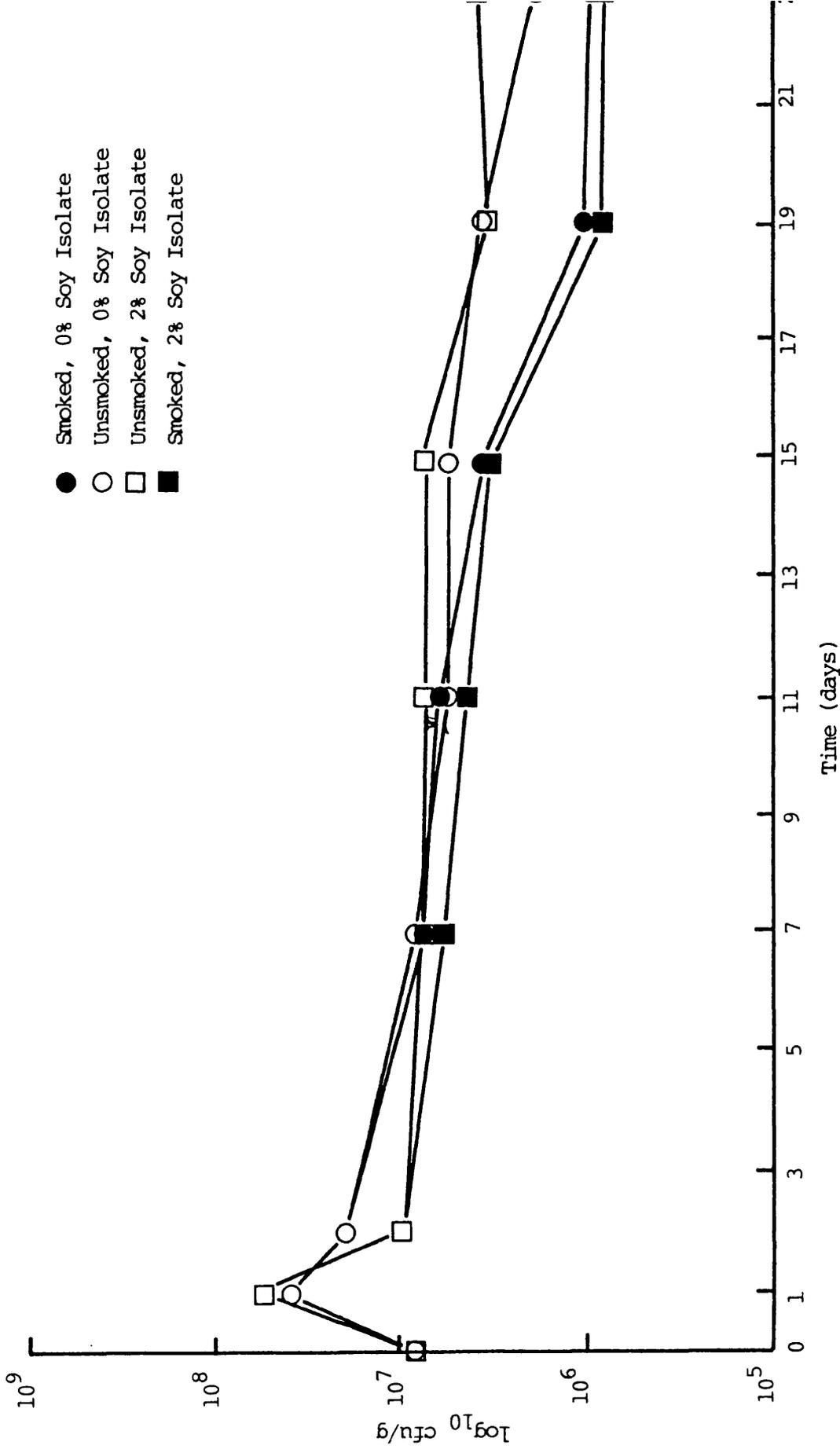


Figure 18: Growth and Development of Staphylococcus carnosus in Fermented Poultry Sausages

- Smoked, 0% Soy Isolate
- Unsmoked, 0% Soy Isolate
- Unsmoked, 2% Soy Isolate
- Smoked, 2% Soy Isolate

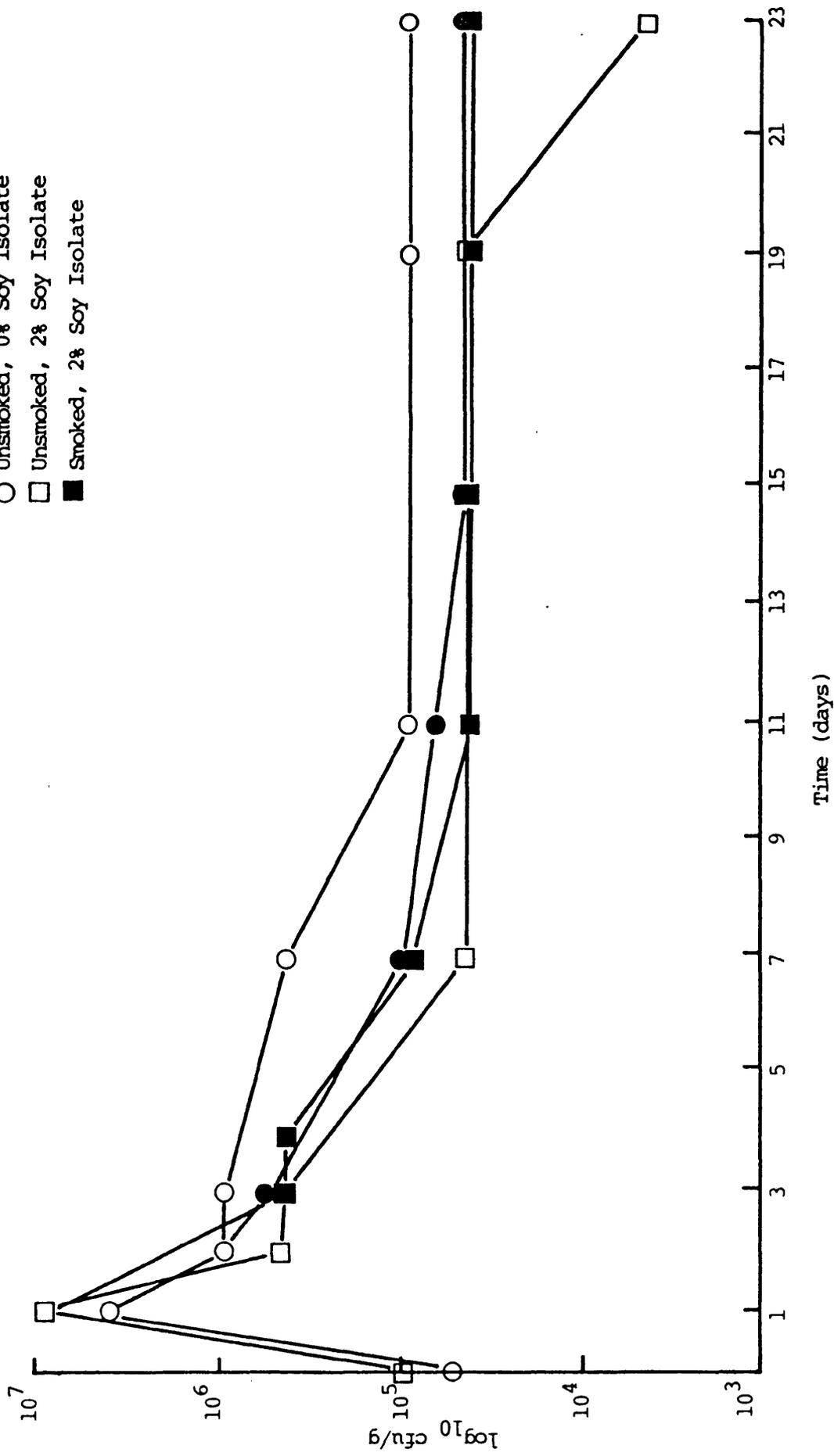


Figure 19: Growth and Development of Coliforms in Fermented Poultry Sausages

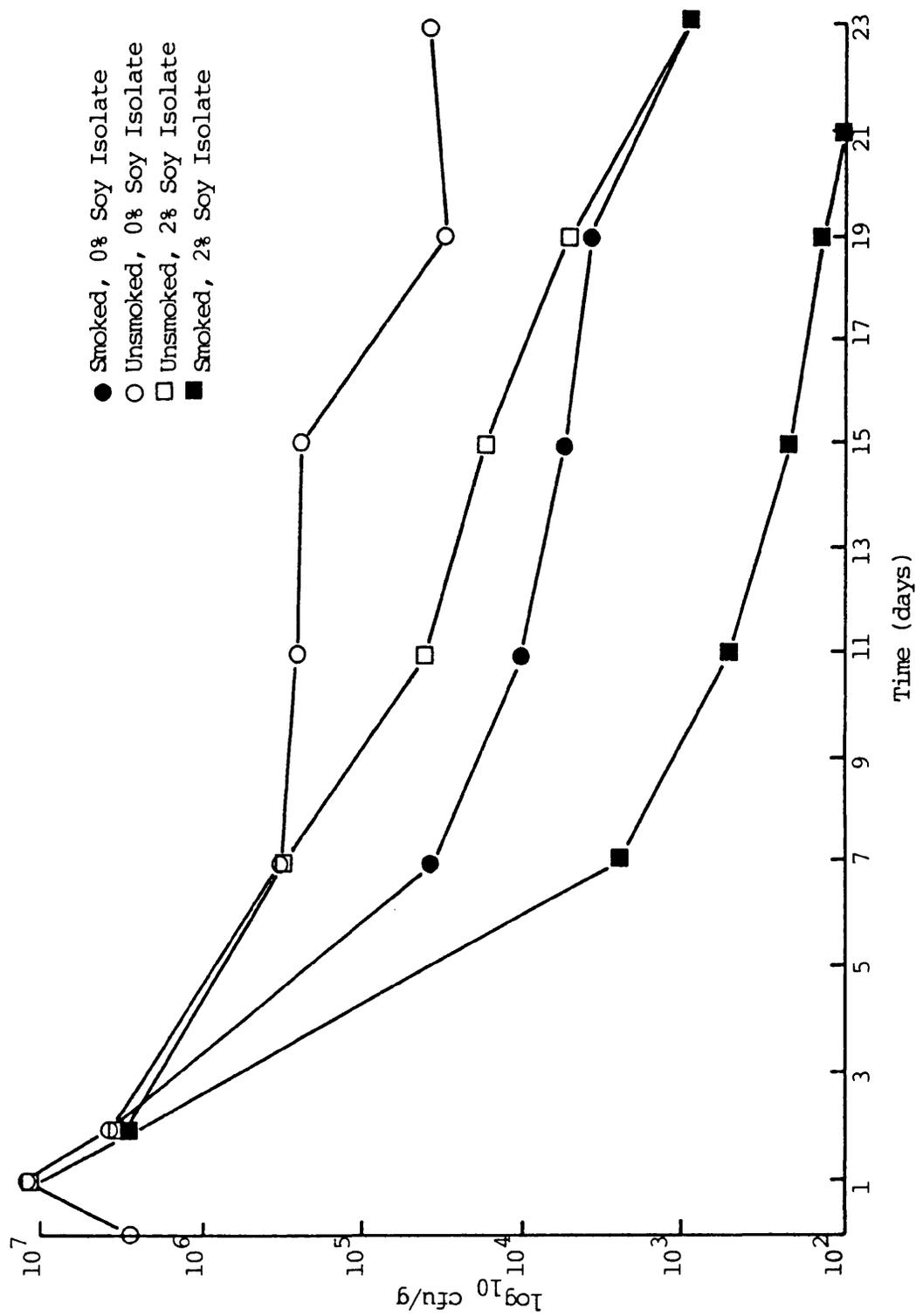


Figure 20: Growth and Development of Psychrophiles in Fermented Poultry Sausages

3.2.1.2. Effect of soy isolate and smoking on growth of Lactobacillus pentosus

The numbers of L. pentosus during preparation of sausage mixtures with 0 and 2 % soy isolate were determined as $5.3 \pm 0.3 * 10^6$ cfu/g and $4.7 \pm 0.4 * 10^6$ cfu/g, respectively. In both sausage mixtures there was a rapid growth of the lactobacillus, especially after 24 h of fermentation. By 48 h the maximum numbers were reached with an overall increase of about 2 \log_{10} cycles for the sausages without soy and around 3 \log_{10} cycles for those with 2 % soy.

After peaking the numbers of lactobacillus fell during drying in the cold room. The number fell more rapidly in sausages with soy isolate than those without so that by day 7 the counts for both samples were in the range of $1-2 * 10^8$ cfu/g. The lactobacillus count in samples with soy remained slightly higher although the numbers continued to decline slowly in both samples until by the end of drying period the count for both samples was similar at $2-3 * 10^7$ cfu/g. The effect of smoking on the lactobacillus was virtually identical to its effect on the mesophiles with a definite killing effect during smoking followed by a recovery stage so that after day 11 the smoked and the unsmoked counts were not very different.

3.2.1.3 Effect of soy isolate and smoking on growth of S. carnosus

There was a general increase of about $0.5 \log_{10}$ cycle for the sausages with and without soy isolate during the first 24 h of fermentation, with the count in both samples peaking at about $5-7 * 10^7$ cfu/g so that there was no significant difference in the maximum level of S. carnosus. As fermentation progressed the numbers of S. carnosus started to fall, particularly from 24-48 h of fermentation. After this the decline in numbers of S. carnosus was gradual.

Although after smoking the numbers of S. carnosus were almost the same in smoked and unsmoked sausages without soy isolate, the reduction in S. carnosus was later found to be greater in smoked sausages, particularly from day 15 onwards.

3.2.1.4 Effect of soy isolate and smoking on coliforms

During the first 24 h of fermentation coliforms numbers increased rapidly in all samples from about $6 * 10^4$ cfu/g by about two \log_{10} cycles to approximately $6 * 10^6$ cfu/g. However, after 48 h the numbers in sausage with soy fell more rapidly reaching by day 23 $4 * 10^3$ cfu/g compared with $9 * 10^4$ cfu/g in sausage without soy.

Although smoking brought a more rapid decline in coliforms numbers between days 3 and 10 the effect of smoking was not found to be pronounced by the end of the drying period. As shown in Figure 6 almost none of the coliforms were identified as Escherichia coli by the end of the drying period.

3.2.1.5 Effect of soy isolate and smoking on psychrophiles

The initial counts of psychrophiles were about $3.0 \pm 0.2 \times 10^6$. In all samples there was some growth during the first 24 h of incubation with numbers increasing by about 0.5 \log_{10} cycle. After day 1 psychrophile numbers fell, but the reduction was more sustained in sausage with soy isolate, reaching $8 \pm 0.3 \times 10^2$ cfu/g by the end of drying period whereas numbers were about 2 \log_{10} cycles higher in sausage without soy isolate.

Smoking had a pronounced effect on psychrophile numbers causing a more rapid decline until day 11. In smoked sausage with soy isolate no psychrophiles were detected by the end of the drying period. In smoked sausage without soy only a few survived until the end of the drying period.

3.2.1.6 Elimination of Salm. enteritidis

The results for the survival of Salm. enteritidis are shown in Table 5. As expected all sausage mixtures on day 0 were positive and the same positive results were observed after smoking and a few days after drying. However by day 11, both smoked and unsmoked sausages prepared with 2 % soy had a negative result and the same result continued until the end of drying period. With respect to the unsmoked and smoked sausages without soy isolate, total elimination of Salm. enteritidis was only achieved on the last day of drying.

Table 5: Survival of Salmonella enteritidis in Fermented Poultry Sausages

NO. OF DAYS	0 % SOY ISOLATE		2 % SOY ISOLATE	
	UNSMOKED	SMOKED	UNSMOKED	SMOKED
0	+		+	
1	+		+	
2	+		+	
3	+	+	+	+
7	+	+	+	+
11	+	+	-	-
15	+	+	-	-
19	+	+	-	-
23	-	-	-	-

(+ Present and - Absent)

3.2.1.6 Microbial quality of the fermented sausages

Table 6 shows the microbial quality of fermented sausages at the end of drying period. With low levels of S. aureus, Cl. perfringens, E. coli and the absence of Salm. enteritidis, the products appear safe. However, the presence of Listeria monocytogenes showed that the organism still had the ability to survive with sausages which had been subjected to fermentation, smoking, low a_w and low pH.

**Table 6: The Microbial Quality of Fermented Poultry Sausages
At the End of Drying Period**

PATHOGEN	0 % SOY ISOLATE		2 % SOY ISOLATE	
	UNSMOKED (cfu/g)	SMOKED (cfu/g)	UNSMOKED (cfu/g)	SMOKED (cfu/g)
<u>Staplylococcus aureus</u>	<100	<100	<100	<100
<u>Clostridium perfringens</u>	<100	<100	<100	<100
<u>Escherichia coli</u>	25	4	<4	<4
<u>Salmonella enteritidis</u>	-	-	-	-
<u>Listeria monocytogenes</u>	+	+	*	+

* L. ivanovii was isolated being positive with rhamnase sugar fermentation but negative with xylose sugar fermentation and CAMP test.

(+ Present and - Absent)

3.2.2 CHEMICAL ANALYSIS

3.2.2.1 Moisture

From Figures 21 and 22, unlike in the previous experiment, there was no difficulty in removing the moisture in sausage without soy isolate. The pH during fermentation on day 2 was brought to 4.88. At this low pH, it is expected that all the proteins were denatured and the water holding capacity was at its lowest causing the moisture to be released during drying more easily. Although in this experiment sausage without soy maintained a relatively lower moisture content during drying the difference was slight being 2 % by the end of the drying period. Sausages with soy had been found to have higher moisture contents. The average moisture loss/day of unsmoked sausage to the end of the drying period was higher in that without soy, being 1.68 g, compared with only 1.51 g for the sausage with 2 % soy.

Smoking of sausages brought a faster removal of moisture, particularly from days 4 to 11. But towards the end of drying period the moisture contents of all smoked sausages were slightly higher than the unsmoked ones. The changes in the moisture content of the sausages are shown in Figures 21 and 22.

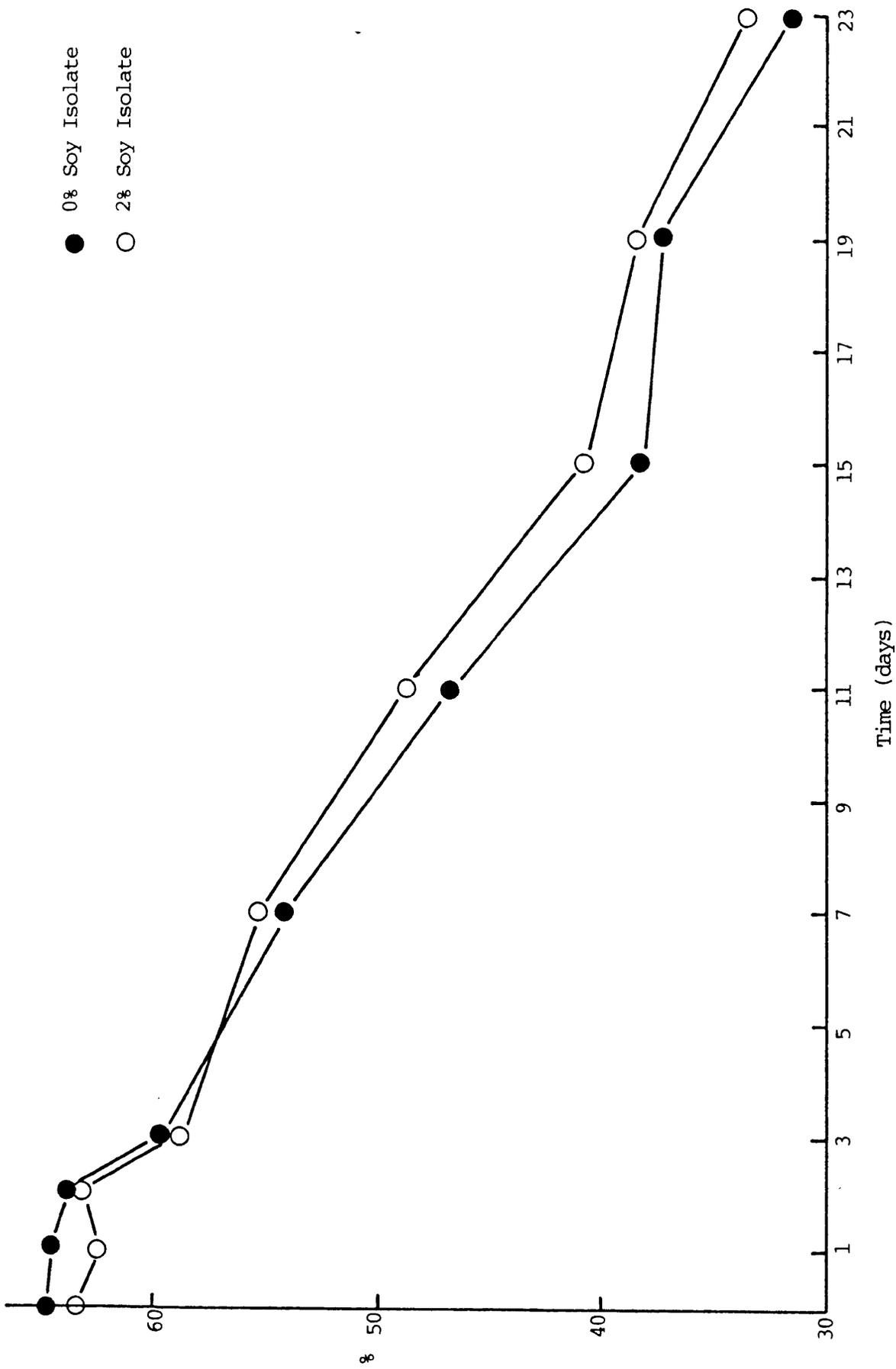


Figure 21: Moisture Content of Unsmoked Fermented Poultry Sausages

- 0% Soy Isolate
- 2% Soy Isolate

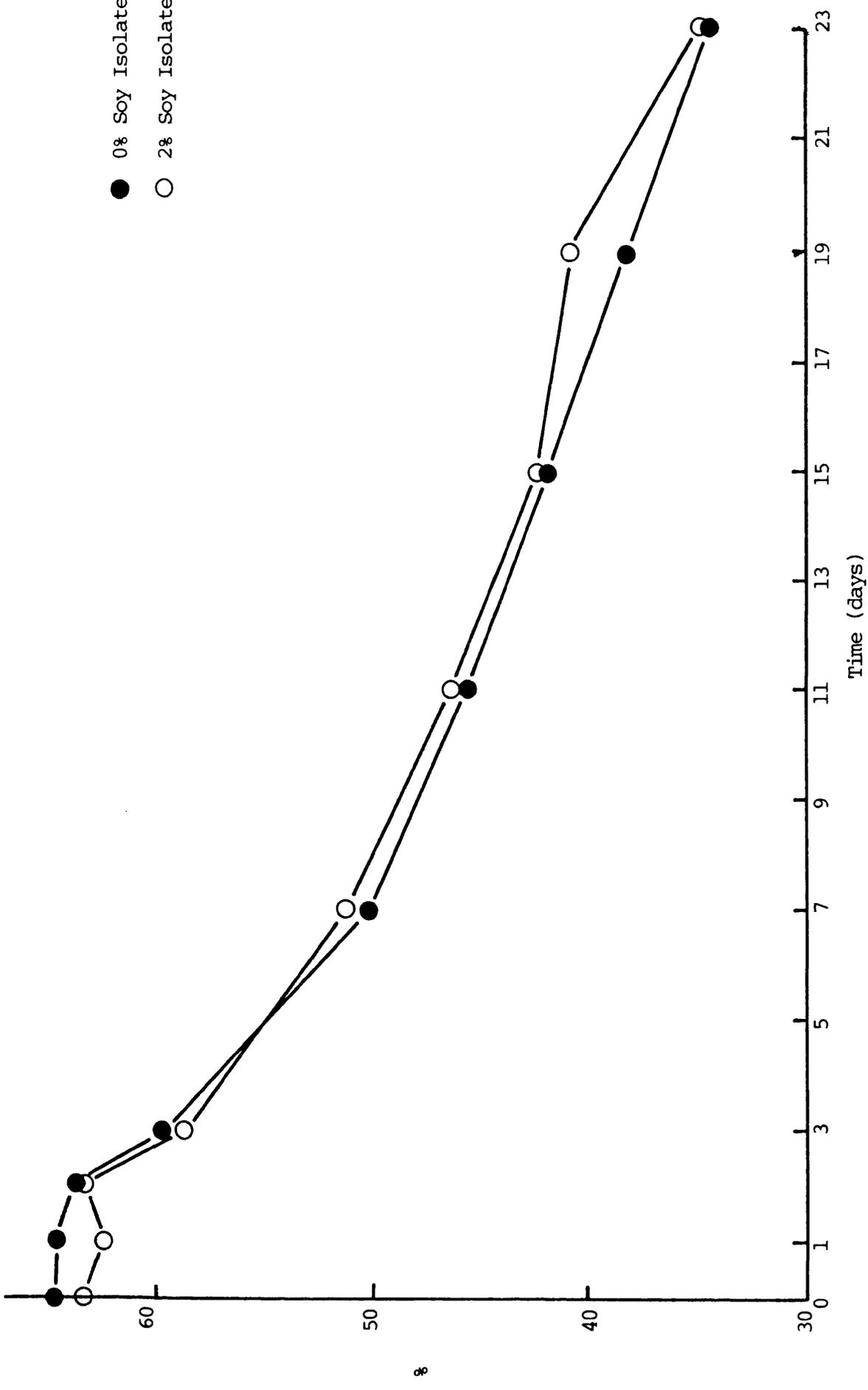


Figure 22: Moisture Content of Smoked Fermented Poultry Sausages

3.2.2.2 pH and Titratable acidity

As shown in Figure 23 the pH value fell rapidly during the first 48 h of fermentation from the initial value of 6.3. The minimum pH value was reached in sausages both with and without soy on day 3 being pH 4.38 and 4.51, respectively.

The rapid fall in pH was accompanied by a converse rapid rise in lactic acid content (as shown in Figure 25) from 0.21 % to 1.22 % and 1.06 % in the sausage with and without soy isolate respectively. During drying the lactic acid content of those sausages continued to increase slowly reaching 2.04 % and 1.64 %, respectively by day 23. However the pH value slowly increased during this period of time in both sausages with and without soy as shown in Figure 23 reaching pH 4.40 and 4.54 respectively. As can be seen from Figures 23 and 24, smoking had little effect on the pattern of change of the pH values.

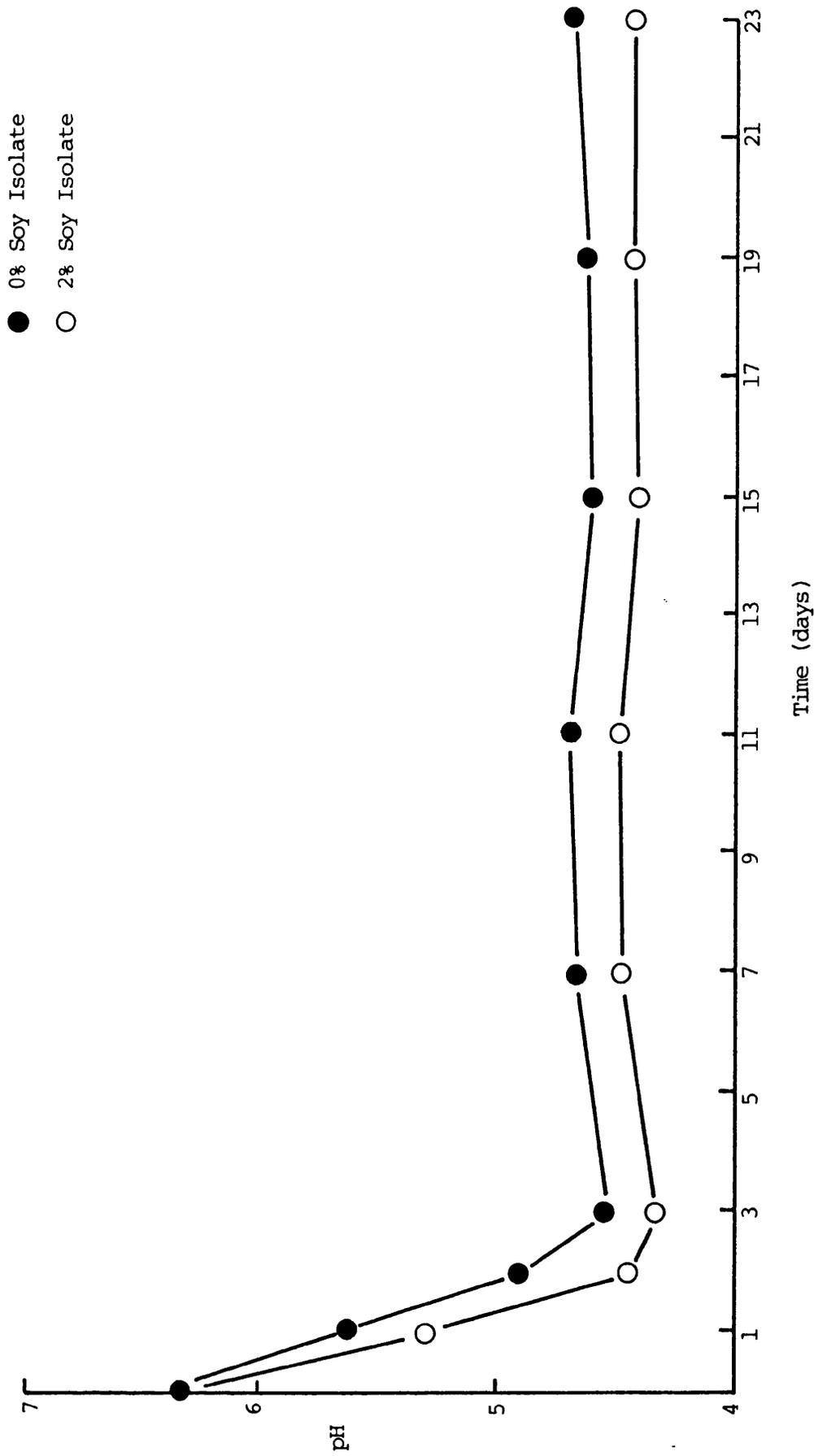


Figure 23: pH of Unsmoked Fermented Poultry Sausages

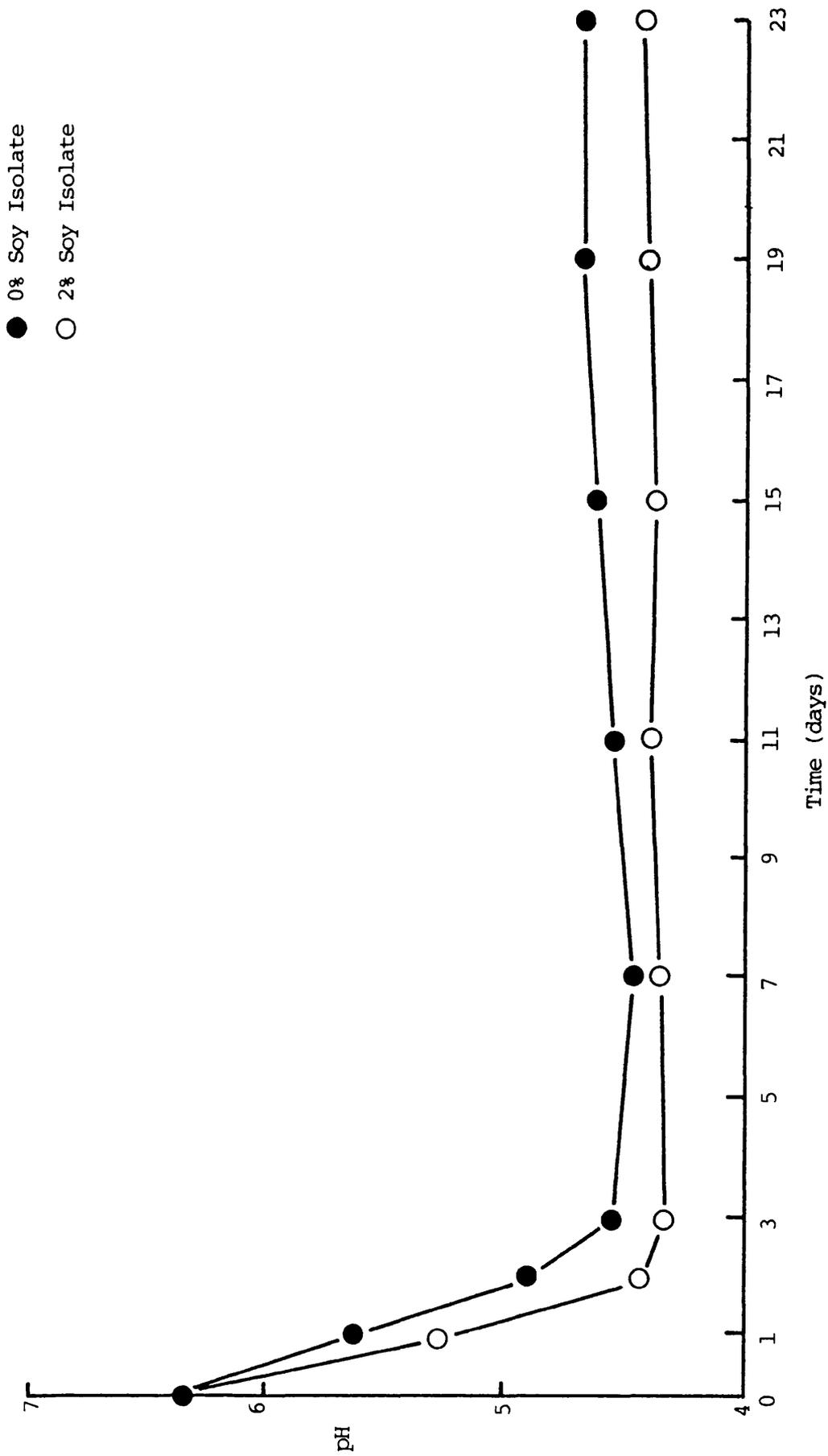


Figure 24: pH of Smoked Fermented Poultry Sausages

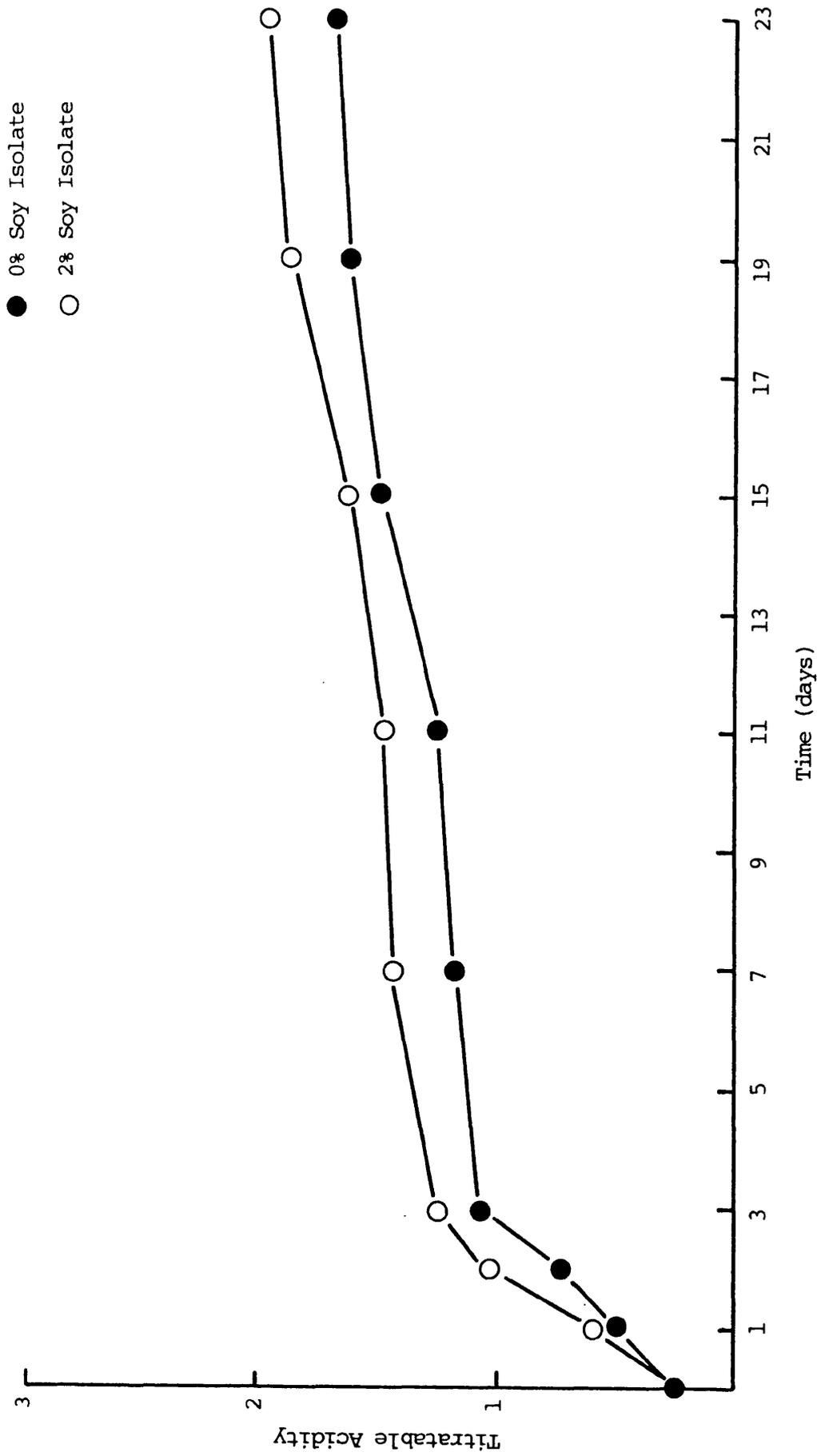


Figure 25: Titratable Acidity in Unsmoked Fermented Poultry Sausages

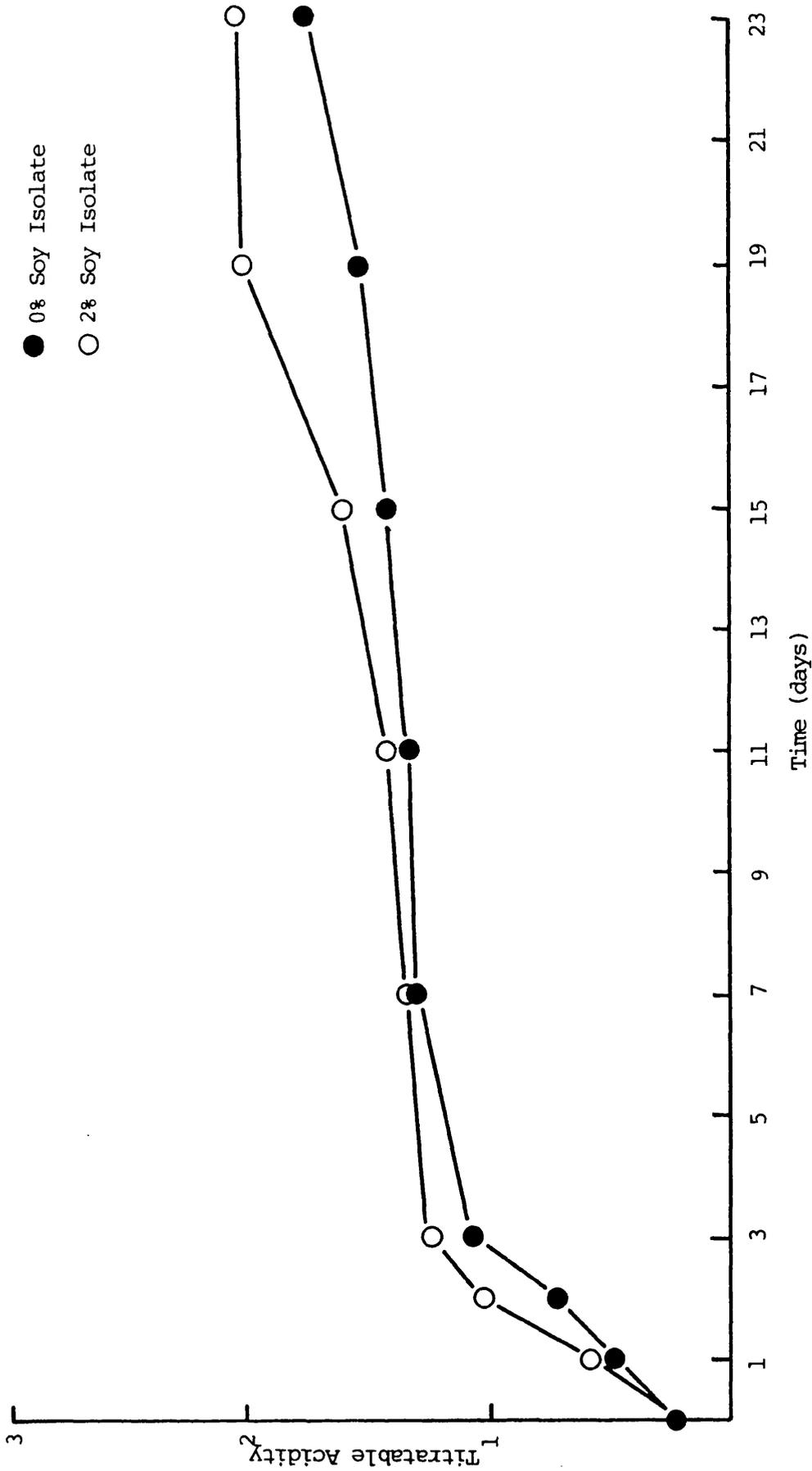


Figure 26: Titratable Acidity in Smoked Fermented Poultry Sausages

3.2.2.3 Salt content

The salt content of smoked and unsmoked sausages with and without soy isolate is shown in Figures 27 and 28. The initial levels of salt in sausage mixtures without soy isolate was 2.26 %; whilst the salt content of the sausage with soy isolate was slightly different being 2.24 %.

During drying there was a gradual increase in the level of salt in both sausages. Fermented sausages without soy isolate had a slightly higher level of salt than those with soy, the values being 4.20 % and 4.14 %, respectively. After smoking and a few days of drying, the level of salt in smoked sausages was slightly higher than the unsmoked ones, especially from days 4 to 12. However, towards the end of drying period, starting from day 15 this changed and the unsmoked sausages showed a higher salt content by 0.06 and 0.15 % than the smoked sausages.

- 0% Soy Isolate
- 2% Soy Isolate

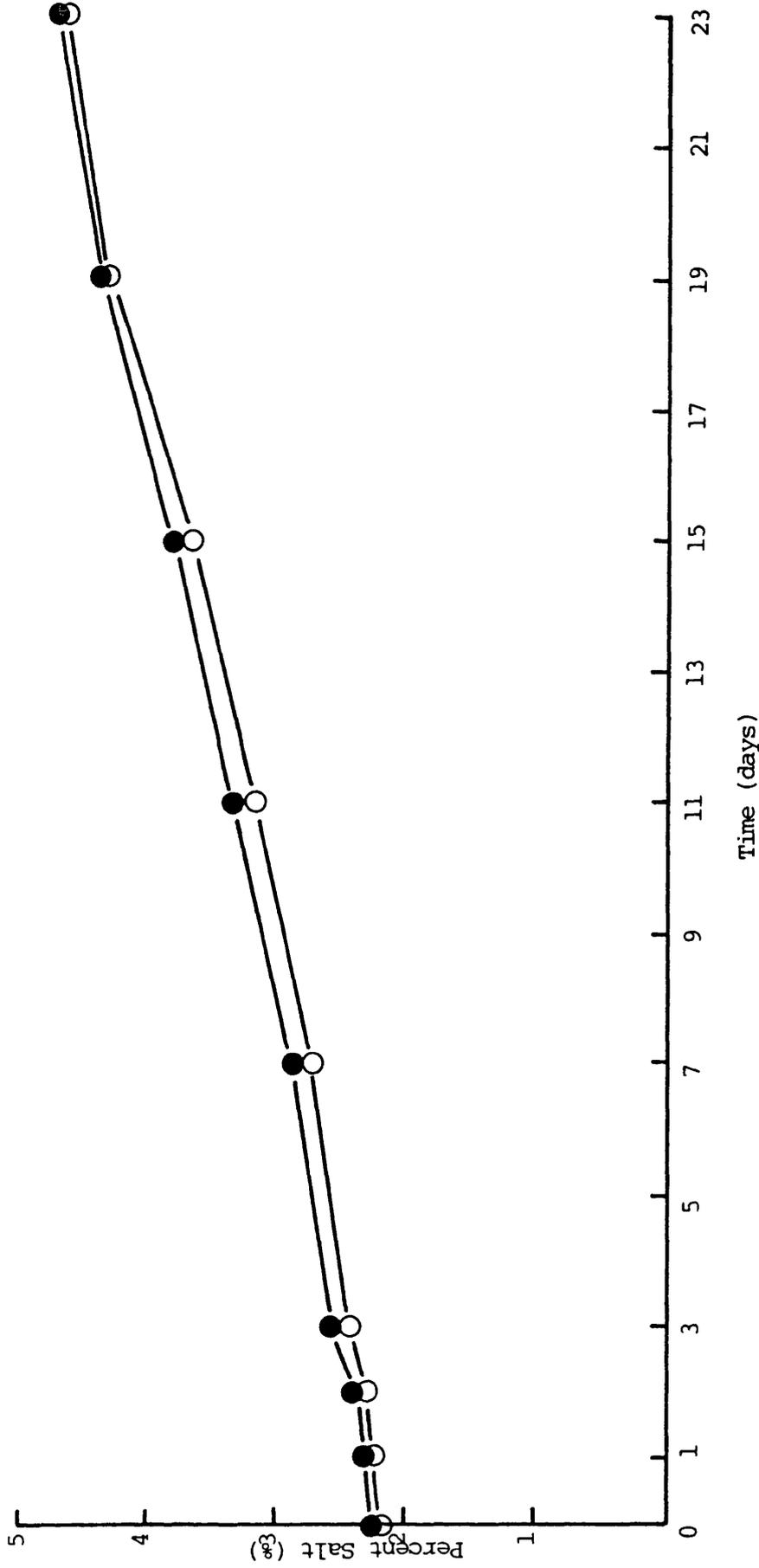


Figure 27: Salt Content in Unsmoked Fermented Poultry Sausages

- 0% Soy Isolate
- 2% Soy Isolate

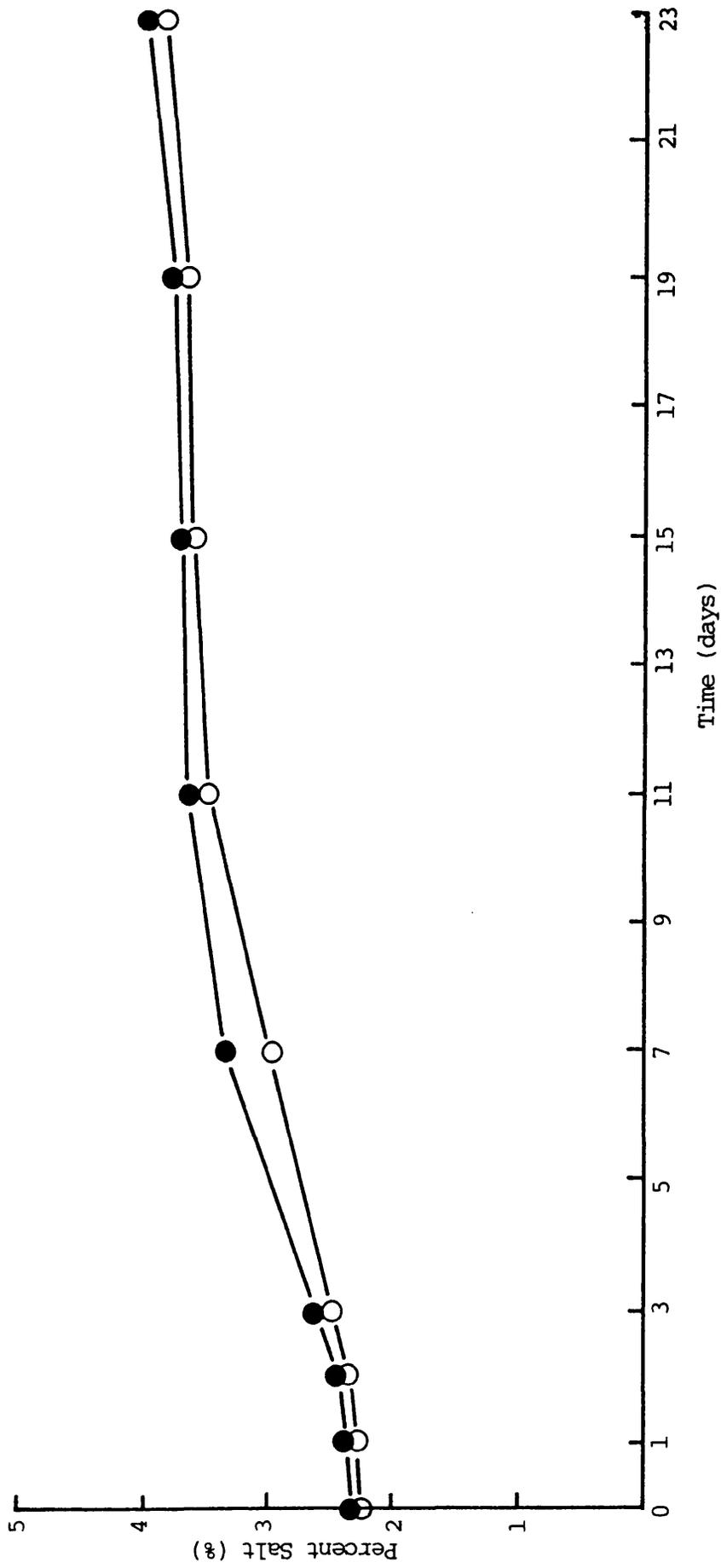


Figure 28: Salt Content in Smoked Fermented Poultry Sausages

3.2.3 OBJECTIVE ANALYSIS

3.2.3.1 Weight loss

Figure 29 shows the cumulative weight loss of the smoked sausages prepared without and with soy isolate added at a rate of 2 %; measured from day 2 (when they were stuffed into casings) until the completion of the drying period. The weight loss was always slightly lower for the sausages with 2 % soy isolate throughout the drying period.

The average weight loss/d of smoked sausages with 0 and 2 % soy isolate during drying was 2.09 and 2.04 g, respectively. Figure 30 shows the relative size of the sausages after 21 days of drying. Although the difference in weight loss was small, the sausage with 2 % soy always had a lower total weight loss and less shrinkage.

- 2% Soy Isolate
- 0% Soy Isolate

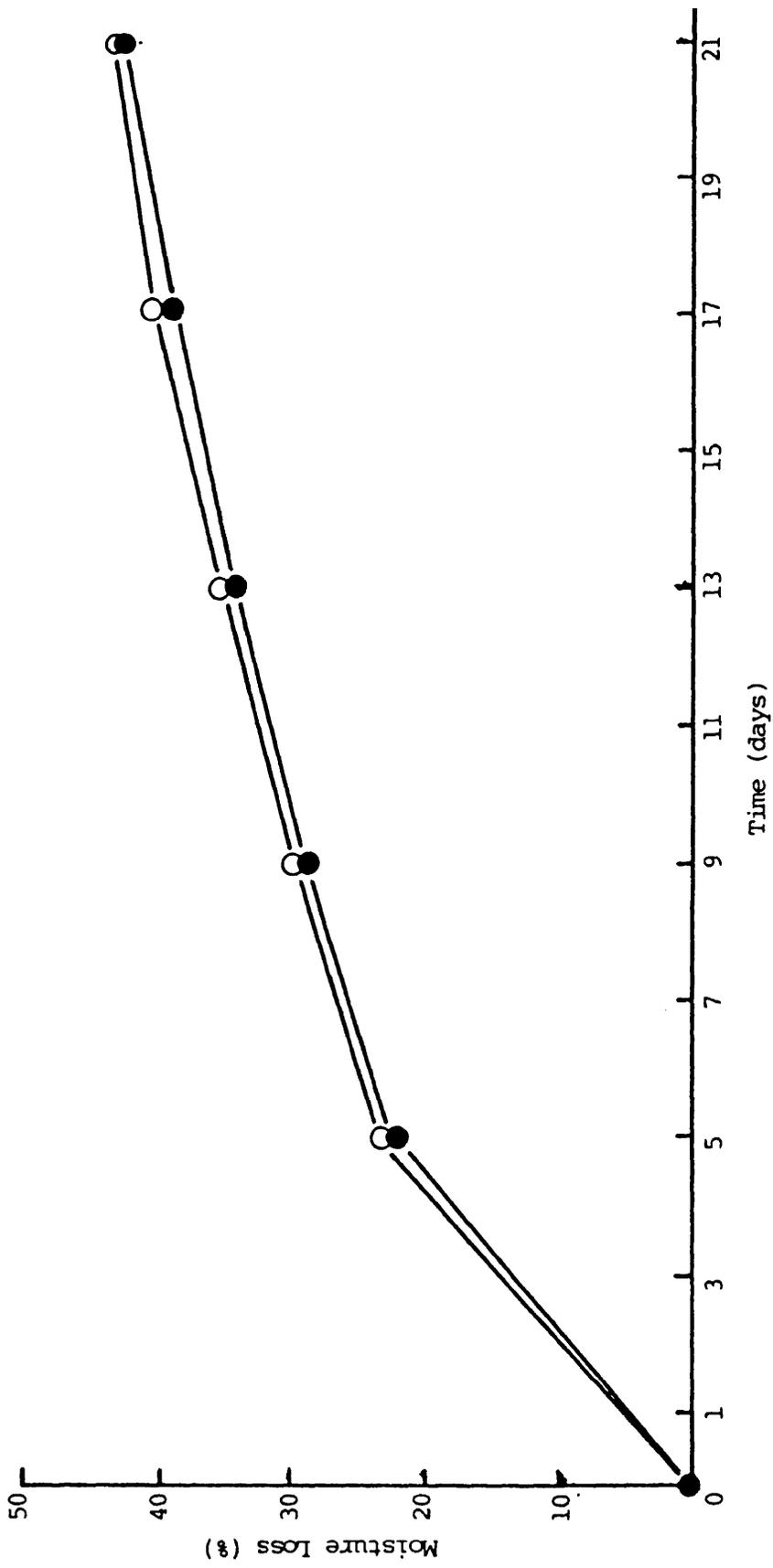


Figure 29: Cumulative Weight Loss of the Smoked Fermented Poultry Sausages



Figure 30: Relative Sizes of the Fermented Poultry Dry Sausage
(A, 0 % Soy Isolate and B, 2 % Soy Isolate)

CHAPTER FOUR

DISCUSSION

4.1 Effects of soy isolate on the chemical composition of the fermented dry poultry sausage

From the results of these two experiments, fermented dry poultry sausage can be produced from a cheaper poultry meat source; the thigh trims. The successful production of the sausage relies upon several contributing factors, namely: a) normal pH of the thigh trims determined at pH 5.29-5.30, b) adequate amount of moisture and fat of the trims being 64-65 % and 20-21 %, respectively, c) thigh trims with an acceptable microbial quality, and d) efficient and rapid drop in pH during fermentation brought about by the L. pentosus. The importance of these factors had also been suggested by Bacus and Brown (1985a).

During the initial preparation of the product, sausage mixture with 4 % soy isolate displayed a higher level of protein and fat and lower level of moisture than those produced with 2 and 0 % soy. The higher level of protein and fat was contributed by the protein (93 %) and fat (0.2 %) and the low moisture content (4.5 %) of the soy isolate.

During the initial fermentation period (from days 0 to 2), the protein, fat and moisture compositions of all the types of sausages remained almost constant since no drying took place as the sausage mixtures were vacuum-packed inside the plastic bags.

The levels of salt and a_w also remained constant during this period. However, as drying of the sausages progressed the levels of fat, protein and salt increased followed by a concurrent decrease in the a_w values. These changes were brought about by the gradual loss of moisture from those sausages and are in agreement with previous reports by Acton and Dick (1975; Keller et al. (1974) and Keller and Acton (1974).

The initial moisture:protein ratios of all the sausages were high relative to thigh meat because water was added into the formulation for a better blending of the soy isolate, starter culture, salts and sugar into the chopped meat. As drying continued, the moisture:protein ratios fell gradually and the lower the ratio, the drier the sausage since this ratio is a measure of the degree of dryness in fermented sausage as suggested by Bacus (1984). In Experiment 1 sausage with 2 % soy isolate was found to have a lower moisture:protein ratio since there was a retention of moisture in the sausage produced without soy. However in Experiment 2, sausage without soy dried faster and displayed a lower moisture:protein ratio than that produced with 2 % soy.

From both experiments, sausages with addition of soy had a higher moisture:protein ratio and hence a higher moisture level at the end of the drying period. Sausage with 4 % soy isolate displayed a higher moisture content than that produced with 2 % soy. In Experiment 2, sausage without soy was drier than that with 2 % soy. The higher level of moisture in sausages with added soy isolate could be due to the effective binding of the moisture

by the soy isolate. The higher the amount of soy added then the greater is the amount of moisture trapped inside the sausage.

In Experiment 1, sausage without soy isolate displayed a reluctance to reach a 'dry' stage over the course of the drying period. Although the problem was first thought to be due to the different fat and fatty acid compositions of the various sausages, the problem was later found due to the high pH value of 5.24 at fermentation stage. In Experiment 2, when the pH of the sausage without soy was purposely lowered to below 5, the rate of drying was slightly faster than that produced with addition of 2 % soy. Lowering the pH to less than 5.1 favours the denaturation of the myofibrillar protein and the isoelectric point of the actomyosin was reported to be at pH 5.2 as suggested by Acton and Keller (1974). At the isoelectric point of the actomyosin, the proteins are denatured and hence less moisture is attracted to the proteins thereby releasing the moisture more freely during the drying period.

Results of lipid and fatty acid compositions of all the sausages with three different levels of soy isolate show that the ability to remove moisture by drying in the sausage without soy isolate was not influenced by the different proportions of fat or their fatty acids compositions. Phospholipids are known to have a higher emulsifying capacity than other lipids and could thus affect water retention. However, since the sausage without soy displayed the same lipid composition as the other two types of sausage, the drying parameters displayed in the absence of soy could not be due to higher proportion of phospholipids. Also, the

overall fatty acid composition of the sausages displayed no features that could not be explained by the known lipid components of the sausages.

The time spent to dry the fermented sausage is of paramount importance because a longer drying period means a higher amount of energy is required which results in a higher production cost. A pH value of 5.3 before drying has been recommended by the AMI (1982) and AMI (1989) for the safety against S. aureus. However the results of Experiment 1 and 2 showed that a better and faster removal of moisture from the sausage under predetermined temperature and relative humidity occurs by lowering the pH to below 5.. In Experiment 1, a period of about 1 d would be required to lower the pH of sausage without soy from 5.24 to 5 or even lower and this extra time appears very important for effective and efficient removal of moisture because it would reduce the time for drying the sausage. But the extra time of 1 d is not necessary if the sausage has added 2 or 4 % soy isolate since the soy protein has been shown responsible for the lowering of pH well below 5 on day 2 of the sausage production in both experiments.

Addition of soy isolate into the sausage enhanced the fall in pH and the subsequent increase in the lactic acid as displayed by the rise in the percent of titratable acidity over the fermentation period. Sausage with 4 % soy had a higher titratable acidity than that produced with either 2 or 0 % soy. This finding suggests that soy isolate had stimulated the rapid growth of the lactobacilli. The ability to bind moisture results in better

growth of the lactobacilli in sausage with added soy isolate since lactobacilli operates in the water phase (Bacus and Brown , 1985a; Thompson et al. 1984).

From fermentation to the end of the drying period, there was a gradual increase in the concentration of the lactic acid. This resulted in the lowering of the pH. However, the rise in the pH toward the end of the drying period could be brought about by ammonium ions released by the breakdown of proteins, peptides and amino acids as suggested by Lois et al. (1987), and Dierick et al. (1974). The rise in pH could also be due to the increase in the buffering substances such as amines produced during the ripening of the sausage (Kormendy and Gantner, 1962).

The higher amount of lactic acid produced by the L. pentosus in sausages with soy isolate was matched by a significant increase in the numbers of the lactobacilli, particularly during the fermentation period.

4.2 The effects of soy isolate on the sensory characteristics and objective analysis of the fermented dry poultry sausage

Various sensory characteristics and objective analyse of the sausages such as texture, firmness, greasiness and weight loss appeared to be affected by the addition of soy isolate into the sausage.

During the lactic acid fermentation, salt solubilises myofibrillar proteins which coagulate and form a rigid gel surrounding the coarse-comminuted meat particles (Schmidt et al.

1981). Soy protein has also been shown to form gel upon heating (Aoki and Sakurai, 1969; Catsimpoolas and Maeyer, 1970 and Hashizume et al. 1975. Addition of soy protein together with the myofibrillar proteins thus would provide more protein and hence a thicker and denser gel should be formed upon acidification. Aoki et al. (1980) showed that lowest emulsion solubility of the soy occurred at pH 4.5 to 4.6 and the highest emulsion capacity when heated to a temperature less than 80°C. Since the fermentation pH and temperature of the sausage were about 4.7-4.8 and 23°C respectively in sausage with 2 and 4 % soy isolate, the high firmness and texture was possibly due to the thicker and denser gel formed. Soy isolate resulted in a better aggregation of the meat particles by the gel and this explains the higher force required by the needle probe to penetrate the sausage and thereby giving rise to a higher texture value and firmness in sausage with 4 % soy isolate.

As suggested by Rao et al. (1984) and Thompson et al. (1984), the formation of gel resulted in a greater binding of moisture and fat. Less moisture was removed in sausage with high soy isolate content and this explains the lower rate of moisture loss per day in sausage with high level of soy isolate.

Although analysis showed that the fat of all the three types of sausages was loosely bound, sensory characteristic evaluation indicated that lipid level (greasiness) was lowest in the sausage with 4 % soy isolate. McWilliams (1989) suggested that chopping the sausage into smaller particles might break the gel and release the fat and thereby giving rise to the above perception.

However the results of the present study indicate that a better binding of fat was achieved when the higher level of soy isolate was added into the formulation, thereby giving rise to a low level of perceived greasiness.

4.3 Effects of soy isolate on the microbiological analysis of the fermented dry poultry sausage

The growth of the mesophiles corresponded to that of L. pentosus and this suggests that the major proportion of the mesophiles was L. pentosus.

The increase of L. pentosus by about 2 log₁₀ cycles for the sausage without soy in the experiment has also been observed by Wardlaw et al. (1973) and Acton et al. (1972). The rapid growth of the lactobacilli by more than 3 log₁₀ cycles explained the higher production of lactic acid in sausage with 2 % soy isolate. Busta and Schroder (1971) suggested that soy proteins might contain some unidentified factor(s) that could enhance the lactobacilli growth over that observed in sausage without soy. However the higher and faster growth of the lactobacilli in sausage with 2 % soy might be attributed to the increased availability of carbohydrate from the soy isolate as suggested by Rao et al. (1984).

After day 3, the numbers of lactobacilli started to fall and the lower counts of the microorganism might have been due to the depletion of carbohydrate source from the soy as suggested by Vignolo et al. (1989) and probably the effect of lactic acid.

There was a small difference in the growth of S. carnosus after day 1 of fermentation in sausage with and without soy; the growth being slightly higher in sausage with 2 % soy. This result suggests that the 2 % soy isolate has little influence over the growth of S. carnosus during fermentation and this corresponds with the result of Reuter (1972). The author suggested that the Micrococccaceae inoculated into the dry sausage mixture as starter culture grew little or not at all during ripening. There was a drop in the numbers of S. carnosus after 48 h of fermentation. However Lucke and Hechelman (1987) found that a level of 10^6 - 10^7 cells/g sausage mixture during preparation was sufficient to reduce the nitrate into nitrite for the curing colour formation.

The rapid decline in the coliform counts after 2 days of fermentation, particularly in the sausage with 2 % soy suggests that the higher concentration of lactic acid and the lower pH were responsible for the death of this organism. In the unsmoked sausages the numbers of the coliforms remained constant over the drying period, although there was a reduction of about 1-1.5 \log_{10} cycles after the growth peaked on day 1. Low moisture and low a_w over the drying period did not further reduce the numbers of the coliforms however. Further research in this area may help to elucidate whether the coliforms which are present in the fermented sausage are resistant to the lactic acid.

The pattern of growth and survival of the psychrophiles was similar to that of the coliforms, particularly during the fermentation period. The decline in the psychrophile numbers from

day 1 of fermentation period was more pronounced in sausage prepared with 2 % soy. The higher concentration of lactic acid was again responsible for the low counts. The decline in the psychrophile after day 1 of fermentation was also observed by Gokalp and Ockerman (1985). Raccach and Baker (1978) demonstrated that lactobacilli have a repressive effect against Pseudomonas spp. Pseudomonas spp. forms the major portion of the psychrophiles in the sausage since it is the main psychrophile in chicken meat (Patterson, 1980).

With respect to the absence of the Salm. enteritidis in all sausages at the end of the drying period, the death of the organism has been brought about by the interaction of several factors. These include the low pH, the reduced a_w and the high concentration of lactic acid, working together during the course of the fermentation and drying periods. Salm. enteritidis in sausage without soy isolate took a longer time to be totally removed than in sausage with soy. This finding indicates that the concentration of lactic acid was the main factor responsible for the killing of the organism. If high salt concentration and a lower a_w were the major factors, Salm. enteritidis should have been eliminated from the sausage without soy earlier. The salt concentration of the sausage without soy was always higher and the a_w lower because of a faster removal of moisture from the sausage. However, the death of the organism was expected because all the ingredients necessary for the killing of salmonella as identified by Schmidt (1988) were included in the formulation. Schmidt (1988) recommended that for maximum control of salmonella

in fermented sausages following conditions must be achieved in the formulation: a) 2.5 % nitrite curing salt, b) lactic acid bacteria as starter culture, c) ripening at 25°C or less, d) addition of 0.3 % glucano-delta-lactone (GdL) or 0.4 % sugar should be included in the sausage formulation. The source of carbohydrate in sausage with 2 % soy should be higher than that obtained directly from 1.5 % sugar alone as used in the formulation.

4.4 Microbial quality of the fermented dry poultry sausage

During the manufacture of the fermented dry poultry sausage from chicken thigh trims, factors such as proper and adequate removal of moisture, correct moisture:protein ratio, the reduction of pH to 5.3 or below during fermentation phase and general hygiene as outlined in Good Manufacturing Practices (GMPs) recommended by the American Meat Institute (AMI) (1982) and AMI (1989) were observed. These recommendations are based on the assumption that the environment for S. aureus is effectively controlled at pH 5.3 or less. Smoking and incubation temperature of less than 25°C had also been shown to reduce the likelihood of the growth of the coagulase-positive staphylococci.

The absence of Cl. perfringens in all types of sausages was also expected because the organism has been found to be removed in sausages in which there is a rapid drop in pH to below 5 during fermentation and low a_w irrespective of nitrite addition (Christiansen et al. (1975); Tanaka et al. (1980); Jacobsen and Trolle (1979) and Hallerbach and Potter (1981).

During fermentation there was a depletion of oxygen followed by the rapid growth of lactobacilli. The high numbers of the lactobacilli and reduced oxygen potential reduced the competitiveness of coliforms and E. coli. Baran and Stevenson (1975) and Warburton et al. (1987) found that there was a reduction of about 2 log₁₀ cycles in the numbers of E. coli during the fermentation phase. During drying further growth of E. coli was unlikely because the bacteria need a high a_w. The low a_w attained in the experiment combined with the other factors already described produced an adverse environment, thus resulting in the low numbers of E. coli by the end of drying period. A high E. coli count is an indication of unhygienic production conditions or the use of meat of unacceptable quality.

L. monocytogenes had not been totally eliminated in the experiment. This was due to its ability to survive at pH as low as pH 4.8 and salt content as high as 10.5 % as demonstrated by Conner et al. (1986); Farber et al. (1988) and Shahamat et al. (1980). Furthermore the chance of survival was found to be higher if the numbers of L. monocytogenes was more than 10³ cfu/g (Johnson et al. 1988). They also observed that heating to an internal temperature of 51.7°C (125°F) for 4 h and refrigerated storage of 12 weeks could not guarantee the total destruction of L. monocytogenes in sausage. Since L. monocytogenes is ubiquitous, total elimination of the microorganism in fermented sausage is difficult. A suitable pasteurisation treatment to the meat prior to the fermentation stage should be looked into. But the treatment should be efficient to destroy the microorganism

and not significantly affect the organoleptic quality of the sausage.

4.5 Effects of smoke on the overall analysis of the fermented dry poultry sausage

The effects of smoke on the chemical and microbiological analysis were observed only in the Experiment 2. While the lactic acid and pH appeared not to be affected by smoke, the heat generated from smouldering of wood chips and the low relative humidity of 39-40 % in the smoke kiln helped to remove substantial amounts of moisture from the sausages. This explains why there was a lower level of moisture in smoked sausages a few days after smoking. The heat from smoking also liquified the fat and upon cooling in the cold room, the fat solidified and this might block partially or fully the pores of the sausage casings. This hinders the release of moisture from the smoked sausages resulting in a slower drying of these sausages compared with the unsmoked ones, especially from the middle of the drying period. The lower level of moisture in unsmoked sausages caused a higher concentration of salt compared with the smoked sausages.

With respect to the microbiology of the fermented sausages, smoking the sausages on day 3 generally reduced the numbers of all the tested microorganisms. However, smoking did show a pronounced bacteriocidal effect particularly to the mesophiles, L. pentosus, coliforms and psychrophiles. Not only does smoking depletes oxygen, it also releases phenols, creosote, formaldehyde and acetic acid which are responsible for the bacteriostatic and

bacteriocidal effects as suggested by Kramlich et al. (1980).

As S. carnosus did not show a pronounced effect immediately after smoking, the organism might be able to withstand the smoke better. However, smoking in the presence of high lactic acid and low a_w appeared to have a greater bacteriostatic effect than just the smoke alone and this might explain the lower counts of the organism after 15 d of drying.

Smoke was also responsible for the death of the S. aureus since this organism has been found more readily on the outer surface of the sausages as suggested by Bacus (1984). But smoking the sausages had no distinct killing effect on the Salm. enteritidis. The salmonella was eliminated at the same period of time irrespective whether the sausages had been smoked or not. With respect to the total elimination of Salm. enteritidis it took 21 d of drying in smoked and unsmoked sausages without soy isolate and only 9 days for the smoked and unsmoked sausages with 2 % soy .

Although smoking has been shown to have an anti-listerial activity as suggested by Messina et al. 1988, the death of the organism was dependent on a longer smoking time of up to 72 h and the types of liquid smoke used. The presence of L. monocytogenes might suggest that the concentration of chemicals such as phenol was too low for a lethal effect after the smoking at 30°C was stopped after only 2 h. This finding should stimulate further research to make sure that the other fermented sausages available to the consumers are free from the organism.

4.6 Fermented sausage- source of fat in human nutrition

With respect to the polyunsaturated relative to saturated fatty acid (the P/S ratio), the P/S ratios of 0.69 to 0.75 displayed by the sausages were significantly better than those which have been described for other major items in the human diet, eg. chicken thigh meat (0.58), egg yolk (0.59) and ox liver (0.24) (Noble, 1987 and Murray, 1989). In the United Kingdom the most recent value for the P/S ratio of the diet is 0.28 and was suggested the P/S value of 0.32 or 0.4 should be aimed for (NACNE, 1983 and DHSS, 1984). From present results, therefore the provision of the sausage with a P/S ratio of 0.69 to 0.75 would be more acceptable for those members of the society who are particularly health conscious with respect to fat consumption.

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