# DETERIORATION OF INTERMEDIATE MOISTURE MEATS DURING STORAGE AT TROPICAL TEMPERATURES

ZAK A. OBANU

FOOD SCIENCE AND TECHNOLOGY, DEPARTMENT OF FOOD AND HOME SCIENCES, UNIVERSITY OF NIGERIA, NSUKKA

## INTRODUCTION

The recent commercial success of intermediate moisture (semi-moist) pet foods (Pinkos, 1968) has stimulated interest in the extension of intermediate moisture food (IMF) technology to human food production. The major attractive features of these intermediate moisture (IM) foods is their apparent shelf-stability without sophisticated packaging (Brockmann, 1970) despite their 20-50% moisture content(Potter, 1970), and the ease and cheapness of their production especially by desorption (Hollis et al., 1968), rather than adsorption, processing. These features make IMF technology particularly attractive in the hot humid tropics for cheaply and effectively stabilizing the scarce, highly perishable foods such as meat and fish. With this in view the present study was undertaken to evaluate the storage behaviour at tropical temperatures of intermediate moisture meats processed from common farm-animals.

## MATERIALS AND METHODS

The longissimus dorsi of eight cattle, two sheep, two goats, and one pig as well as the goat leg muscles and chicken breast muscles were studied post rigor. These muscles were trimmed free of visible fat and connective tissues, cut roughly into $1cm^3$ pieces and sealed in cans containing 1.5 times their weight of infusing solutions consisting of NaCl (9.5%), potassium sorbate (0.5%) and pre-determined amounts of glycerol and water to give the desired post-equilibration water activities 0.80-0.86. The cans were heated in 77°C water bath to an internal temperature of 70°C and held for 15 minutes to arrest enzymic and microbial deterioration. They were then equilibrated overnight at room temperature, after which the surfaces of the meat pieces were dried, packaged in Cryovac impermeable PVDC bags (W.R.Grace Ltd., London) and stored thermostatically controlled hot-air ovens at 28°C and 38°C which approximate mean and maximal tropical temperatures. In each study, part of the raw meat was blast-frozen and stored at -10°C for use as controls. At each sampling aliquots were thawed, cut into $1cm^3$ pieces and cooked at 70°C for 15 minutes.

Samples were taken at three week intervals, frozen in liquid $N_2$, milled in solid $CO_2$ and kept in a refrigerator for the $CO_2$ to evaporate off. Duplicate aliquots of these milled samples were used to follow changes in the collagen, myoglobin and protein sloubility and molecular size while the solid $1cm^3$ pieces were used to evaluate the texture, colour and refectance spectrophotometry of the meats during storage.

Collagen was evaluated through hydroxyproline determination. The milled meat samples were heated in ¼-strength Ringer's solution at 77°C for 70 minutes (Herring, Cassens and Briskey, 1967). Both the supernatant and the residue were digested with 6N HCl in a retort at 16-19lb pressure (Goll, Bray and Hoekstra, 1963). The hydroxyproline contents of both fractions were determined by the method of Woessner (1961).

Texture was determined by shearing across the fibres of $1cm^3$ meat pieces using Volodkevich Shear Jaw Tenderometer (Volodkevich, 1938; Grunewald, 1957).

Myoglobin was studied through reflectance spectra of the intact $1cm^3$ meat pieces scanned against a MgO standard through 700 to 340 nm at 60 nm/min. (Targladgis, 1962: Ledward, 1971). A Perkin-Elmer Model 124 double-beam spectrophometer was used. The state and reactivity of the haematin component of myoglobin were followed by solubility changes in 40% ($\frac{v}{v}$) pyridine (Lemberg and Legge, 1949; Ledward, 1971). The filtrate was scanned through 700-370 nm in a Unicam SP 800 double-beam Spectrophometer using a pathlength of 1cm.

The state of the proteins in the meat pieces was studied by polyacrylamide gel electrophoresis, and solubility in 3% sodium dodecyl sulphate (SDS) solution containg 1% β-mercaptoethanol. For electrophoresis non-protein materials were removed from the milled meat by the method of Parsons and Lawrie (1972) and 50mg of the resulting powder was dissolved in 10ml of 3% SDS-plus-1%-β-mercaptoethanol (Penny and Hofmann, 1971). The dissolved proteins were resolved in vertical gel slabs as described by Young and Lawrie (1974). Changes in solubility of the proteins in the 3% SDS-plus-1%-β-mercaptoethanol were assessed by macro-Kjeldahl determination of the nitrogen contents of both the clear supernatant (or filtrate) and the residue.

RESULTS

At all water activities all meat samples studied showed marked increases in soluble hydrxyproline (OHP) as storage advance indicating progressive collagen breakdown. Fig. 1 illustrates the results obtained with the l. dorsi of beef animals of different sexes and ages processed to water activity ($a_w$) of 0.85 and stored at 38°C. Similar increases in soluble OHP with storage were obtained with the l. dorsi of other farm animals and other muscles (goat hindleg and chicken breast muscles) as shown in Fig. 2. Comparison of storage at 28°C and at 38°C of pig l. dorsi and chicken breast muscles in Fig. 2 shows the OHP release to be strongly temperature dependent being remarkably faster at 38°C than at 28°C ($P < 0.001$). Nevertheless at both temperatures OHP release was generally faster early in storage with the rates declining after the first 3-6 weeks.

The collagen breakdown was detectable by finger-feel and mouth-feel. Within the first 3-6 weeks at 38°C the IM meat pieces showed considerable decrease in the cohesiveness and springiness of the component fibres. Samples were consequently brittle resulting in marked increase in tenderness as assessed by chew count. However, with the advance storage at 38°C the meat pieces became progressivesly drier, harder and tougher. These textural changes were picked up by the Volodkevich Shear Jaw Tenderometer as initial reduction in shear resistance within the first 3-6 weeks at 38°C followed later in storage by progressively greater shear resistance (Obanu et al., 1975b, 1976).

The reflectance spectra of all IM meats prior to storage were similar to the spectra of their cooked meat controls with their typical distinct minima (i.e. absorption maxima) at about 415,540 and 640 nm. However, with storage the IM meat spectra became less distinctive with the minima at 540 and 640nm disappearing within three weeks' storage at 38°C. Simultaneously the Soret peak at about 415nm became broader (less distinct) and shifted to lower wavelength as previously reported (Obanu and Ledward, 1975; Obanu et al., 1976). These changes indicate that the myoglobin is being broken down quite fast and early in storage. This was subjectively detectable in the myoglobin-rich beef, mutton and goat muscles as progressive fading in colour from the dark grey colour of the cooked meat to very pale yellow within 3-6 weeks at 38°C. In the white muscles of pork and chicken instead of further lightening in colour the meat became progressively browner. This browning was observed in the myoglobin-rich muscles later in storage, after about 6-9 weeks in mutton and goat meat stored at 38°C and about 12 weeks in beef at 38°C. The browning of IM meats during storage was markedly dependent on storage temperature, being very much slower at 28°C than at 38°C.

Although only limited solubility of the meat pigments was achieved by 40% ($\frac{v}{v}$) pyridine, the spectra of the solutions were typical of a pyridine haemichrome with an absorbance peak at about 406nm. The optical densities of the extracts at 406nm mirrored the visual changes in colour - declining in intensity within the first 3-6 weeks at 38°C and later increasing in intensity as the meat pieces browned. Fig. 3, which shows the trend with storage at 38°C for IM beef ($a_w$ 0.85) processed from the l. dorsi of cattle of different sexes and ages, typifies this phenomenol change observed in all the stored IM meats irrespective of the species of the meat-animal (Obanu et al., 1976).

3%-SDS-plus-1%-β-mercaptoethanol, which is commonly used in electropphoresis for the quantitative solubilization of denatured proteins (Shapiro et al., 1967; Scopes and Penny, 1971), was found to be moderately effective in solubilizing the proteins in intermediate moisture meats. However, during storage the percentage of protein solubilized decreased progressively as shown in Fig. 4 which summarizes observations for beef l. dorsi, from animals of different sexes and ages, processed to $a_w = 0.85$ and stored at 38°C. There was a frequent tendency for low protein insolubility early in storage (first three weeks at 38°C) with consequent high solubility percentage during the period as illustrated by the IM meat of the young bull in Fig. 4. Nevertheless as storage advanced all IM meats showed progressive insolubility in 3%-SDS-plus-1%β-mercaptoethanol. This phenomenon was observed in all species and muscles studied (Fig. 5). However, the rate of protein insolubilization was significantly ($P < 0.001$) lower in the avian (chicken) meat than in the mammalian meats (Fig. 5). Also the protein insolubilization was remarkably faster at 38°C storage than at 28°C (Fig. 5).

All quality parameters studied suggest initial protein degradations during storage of IM meats at tropical temperatures, the degradative reactions being progressively superceded by protein insolubilizing (i.e. complexing) reactions as storage advanced. These gross changes in the proteins of IM meats stored at 38°/28°C were monitored by SDS polyacrylamide gel electrophoresis as general loss in intensity of the slow-moving high-molecular weight protein bands close to the origin (-ve) with simultaneous increase in the size, number and speed of the fast-moving low-molecular weight protein bands close to the bottom (+ve) of the electrophoretograms (Obanu et al., 1975a).

## DISCUSSION

Although freshly processed IM meats were similar to fresh cooked meat, differences were apparent in the colour and texture of the meat upon storage at tropical temperatures around 28°C or 38°C the rate of changes being markedly dependent on the storage temperature. These changes are apparently traceable to two contrasting but concurrent series of deteriorative changes in the meat proteins; namely - proteolysis and crosslinking. Proteolysis, but not crosslinking occurs to a limited extent in stored cooked meat (Sharp, 1963; Obanu et al., 1975a,b). Early in storage, first 3-6 weeks at 38°C, considerable proteolysis occurred in the glycerol-infused (IM) meats. However, as storage advanced a fraction of the protein became less soluble even in SDS-plus-$\beta$-mercaptoethanol suggesting that stable crosslinks were being formed. Both deteriorative processes were almost insensitive to $a_w$ changes in the range 0.80-0.86. Thus any slight variations in water activity emanating from the differences in residual moisture contents of the IM meats during storage may not be an important factor in explaining or controlling these changes. It seems more likely that the non-enzymic browning, which was visible in the less pigmented meats (pork and chicken) early in storage and later in the darker meats (beef, mutton and goat-meat), was responsible for some of the crosslinking and protein insolubilization. There is evidence that the glycerol in IM meats may actively accelerate such protein crosslinking (Sheppard and Sweet, 1921; Bello and Bello, 1976; Obanu et al., 1977). Also, oxidation of the meat lipids, readily catalyzed by haematin compounds life myoglobin (Tappel et al.,1961; Kendrick and Watts, 1969) and maximal at intermediate water activities (Labuza, 1972, 1976; Labuza et al., 1971), is known to induce protein crosslinking reactions like protein-protein interactions and lipid-protein interactions (Obanu et al., 1980) and produces also reactive carbonyls such as malonalddryde which reacts with collagen to form stable crosslinks (Svadlenka et al., 1975).

## CONCLUSION

It is evident from the results that during storage at temperatures around 28°C/38°C the proteins in IM meats undergo marked changes which affect the organoleptic quality, and hence acceptability, of the meats. There is need to understand the nature and mechanisms of the causative deteriorative reactions in order to arrest them and ensure not only stability but also the acceptability and usefullness of intermediate moisture meats (and other muscle-foods) under tropical conditions.

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Fig. 1 Effect of Storage at 38°C on Collagen Breakdown in Some IM (0.85 $a_w$) Beef Samples

Collagen breakdown measured by the release of soluble hydroxyproline (OHP)
OHP release (%) = Soluble OHP x 100/Total OHP

● = Young Bull (11 mth) ○ = Old Bull (12 yr)
□ = Cow ( 6 yr) ■ = Heifer ( 2 yr)

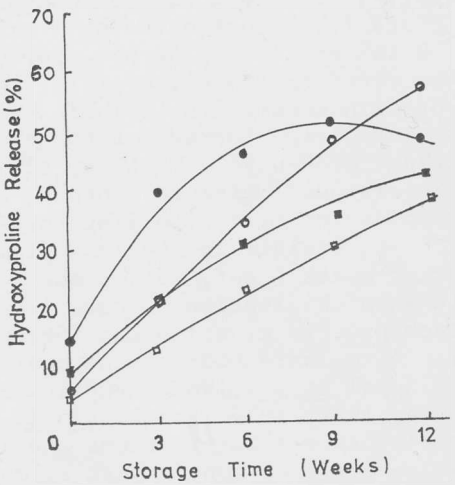



Fig. 2 Release of Soluble Hydroxyproline from Collagen Breakdown During Storage at 28°C and/or 38°C of IM (0.85 $a_w$) Meats from Different Species of Farm Animals

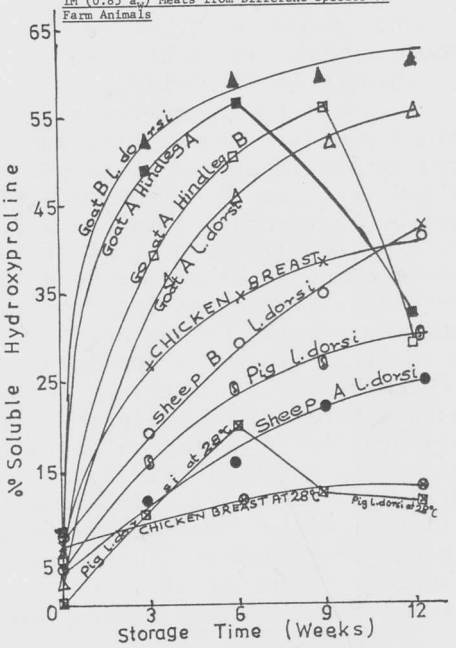



Fig. 3 Extractibility by Pyridine (40%V/v)of the Haematin Complexes in Some IM (0.85 $a_w$) Beef Samples Stored at 38°C

● = Young Bull(11 mth) ○ = Old Bull(12 yr)
□ = Cow ( 6 yr) ■ = Heifer ( 2 yr)

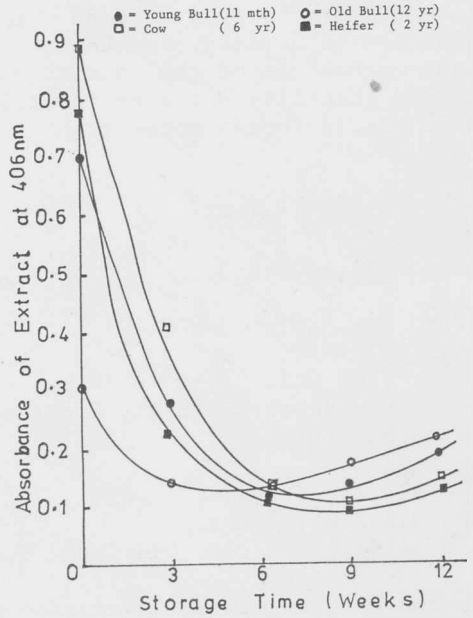



Fig. 4 Solubility in SDS-plus-β-mercaptoethanol of the Nitrogen in Some IM (0.85 $a_w$) Beef Samples

● = Young Bull (11 mth) ○ = Old Bull (12 yr)
□ = Cow ( 6 yr) ■ = Heifer ( 2 yr)

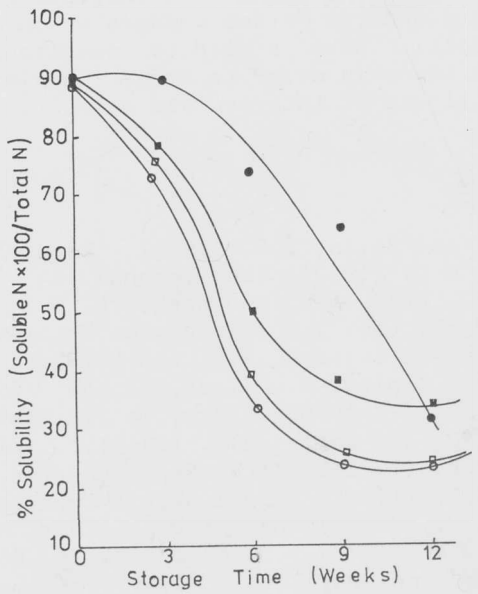
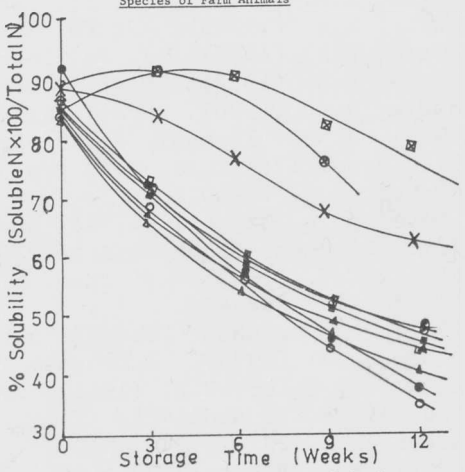



Fig. 5 Solubility in SDS-Plus-B-mercaptoethanol of IM (0.85 $a_w$) Meats from Different Species of Farm Animals




△ = Goat A 1 dorsi ▲ = Goat B 1 dorsi
■ = Goat A Hindleg A □ = Goat A Hindleg B
● = Sheep A 1 dorsi ○ = Sheep B 1 dorsi
⊗ = Pig 1 dorsi X = Hen Breast
⊠ = Pig 1 dorsi at 28°C ⊗ = Hen Breast at 28°C