NUTRITIVE VALUE OF INTERMEDIATE MOISTURE MEATS STORED AT TROPICAL TEMPERATURES

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INTRODUCTION

Meat is an excellent source of essential amino acids and, to a lesser extent, of certain minerals particularly iron. The high incidence of protein-energy malnutrition and iron-deficiency anaemia in the developing (tropical) countries, especially in children and women, makes it mandatory for any preservation/processing technique for meat in these countries to protect the nutritional value of meat proteins and iron. Thus intermediate moisture food (IMF) technology for meat preservation in the tropics, with all its technical merits (Obanu, Ledward and Lawrie, 1975a), can only be adopted if it preserves not only the organoleptic quality and acceptability of meat but also the nutrients in the meat. This necessitates evaluation of the effects of IMF processing (Hollis et al., 1968; Obanu et al., 1975a) and subsequent storage under tropical conditions on the nutritive value of the preserved meat, more so as the gross changes in the nature of proteins in intermediate moisture (IM) meats (Obanu, Ledward and Lawrie, 1975a,b, 1976a) may well be of great importance with regard to the nutritional quality of the meat. In the present study the biological value of the iron and proteins in IM beef samples were assessed immediately after IMF processing and during several weeks of storage at 38°C - a maximal tropical temperature - and compared with values determined for the same beef samples after cooking in ordinary water at the same heat regime as in the IMF processing.

MATERIALS AND METHODS

The longissimus dorsi of beef animals (bulls and steers) were used post rigor. The muscles were trimmed of visible fat and connective tissues, cut into roughly $1cm^3$ pieces and processed in sealed cans containg 1.5 times their weight of infusing solution composed of NaCl (9.5%), potassium sorbate (0.5%) and pre-determined amounts of glycerol and water to give the desired post-equilibration water activity (a_w) of 0.85 (Obanu et al., 1975a). The resulting IM beef samples were packaged in Cryovac impermeable PVDC bags (W.R. Grace Ltd., London) and stored in thermostatically controlled hot-air ovens at 38°C. In each study, part of the raw beef was blast-frozen and stored at -10°C for use as controls. At each sampling aliquots of these control samples were thawed, cut into $1cm^3$ pieces and cooked at 70°C for 15 min. as for the IM samples. Sampling in all cases was at three weeks intervals.

Automated Amino Acid Analysis - 0.5g of the IM beef stored at 0,3,6,9 and 12 weeks at 38°C and 0.5g of the frozen control beef stored for nine weeks were digested with 6N HCl at 100°C for 16 hr. and the hydrolysate made up to 500ml. To 50ml aliquots of the hydrolysate 2ml of standard Nor-leucine solution (1.0μM/ml) was added and evaporated almost to dryness, under vacuum at 37°C. This was made up to 10ml with sodium citrate or lithium citrate buffer (pH 2.2).

The acidic and neutral amino acids were eluted using 0.1ml aliqouts on a 30cm column of locarte cationic resin (Locarte Co., London) at pH 2.58 and 34°C; the pH was changed to 3.65 after valine had eluted. For basic amino acids, 0.1ml was eluted on a 10cm column of locarte cationic resin at 34°C and pH 4.19. For both runs the Locarte automatic amino acid analyser was used. From the concentrations of amino acids eluted, chemical scores were determined using the control cooked beef as standard.

Chemical Assay of Protein Quality - Available (ε -) lysine was determined at each sampling period by Carpenter's method (Carpenter, 1960) based on the Sanger reaction of ε-amino groups with fluoro-dinitrobenzene (FDNB). The solubility of the meat proteins in a solution of 3% sodium dodecyl sulphate (SDS) and 1% β-mercaptoethanol - a mixed solvent known to monitor the protein quality of intermediate moisture meats (Obanu et al., 1975a, 1976b) - was determined as previously described (Obanu et al. 1975a).

Bioassay of Protein Quality - The protein efficiency ratio (PER) (Osborne and Mendel, 1917) and net protein utilization (Miller and Bender, 1955) of the IM meats after 0,3,12 and 24 weeks of storage at 38°C and of the frozen (24 weeks) control beef were determined using male and female fats of the Wistar strain. The meat samples replaced part of the maize starch in the basal protein-free diet (Table 1) such that all diets, apart from the basal protein-free diet, contained 100g protein/kg diet. Since the crude protein contents of the IM meats (49.0-

51.1%) were lower than that of the cooked control beef (81.6%) the levels of the inclusion of the IM beef samples were usually much greater (194.3-204.0g/kg diet) than that of the cooked beef (122.6g/kg diet). A casein diet, also of 100g protein/kg, supplemented with DL methionine at 2.5g/kg was included for comparison.

Table 1: Composition (g/kg) of Basal Protein-free Diet

D-glucose	150
Arachis oil	150
Salt mixture*	50
Vitamin mixture*	11
Maize starch	639

The rats were sorted into males and females and caged in equal weight groups of three. Thus all data obtained were for three rats all of the same sex. The feeding trial followed the standard procedure of Miller (1963) and lasted 10 days after which the rats were killed and the carcasses dissolved completely in concentrated H_2SO_4. The nitrogen contents of aliquots of the carcasses, food and faeces were determined by the macro-kjeldahl technique. PER was calculated as body weight gain (g)Protein intake (g) while NPU% = $\frac{Bt - (Bk - Ik) \times 100}{It}$

where Bt = total body nitrogen of rats fed on the protein diets
It = total intake of food nitrogen of rats fed on the protein diets
Bk = total body nitrogen of rats fed on protein-free diets
Ik = total intake of food nitrogen of rats fed on protein-free diets

*Composition as described by Payne and Stewart (1972)

Bioassay of Iron Availability - Two feeding trials were carried out with 0.85 a_w beef. In the first trial the availability of the IM beef iron after 0,3, 6 and 9 weeks storage at 38°C and of the cooked control beef was evaluated with normal iron-replete rats by the ability of the meat iron to increase the iron contents of the haemoglobin and iron storage organs. In the second feeding trial iron availability from IM beef after 0,3,12 and 24 weeks storage at 38°C and of the cooked control beef was assessed by the ability of the meat iron to regenerate haemoglobin in anaemic rats.

A basal protein-free, iron-free diet similar to that of Table 1 was formulated using an iron-free mineral mix. The IM and cooked beef diets were formulated by using the meat to raise the protein content of the basal diet to 100g/kg and the iron contents to 21.8mg/kg in the first trial and20.0mg/kg in the second trial. In both trials casein diet supplemented with finely ground $FeSO_4.7H_2O$ to give the same levels of iron as in the meat diets was included for comparison.

In the first feeding trial specific pathogen-free (SPF) Wistar male rats were used. These were caged in equal weight groups of three and duplicate groups were assigned to each experimental diet. An extra group of six rats were killed immediately for the assessment of the initial iron contents of the storage organs: liver, spleen and heart. In the second feeding trial equal number of male and female (non-SPF) Wistar rats were used. The rats were split into pairs of the same sex and weight and each experimental diet was fed to a pair of male and a pair of female rats. In both trials rats were fed ad lib for 10 days with free access to distilled water.

At the beginning and end of both trials the haemoglobin (Hb) concentrations of the blood from the tip of the tail of all rats were determined by the cyanmethaemoglobin complex formation method of Kampen and Zijlstra (1961). In addition, at the end of the first feeding trial (using normal rats) the liver, spleen and heart of each rat were removed and their iron contents determined. The total iron contents of the storage organs of each group of three rats as well as the iron contents of the diets were determined, after ashing at 550°C in a muffle furnace for 24 hr., by reaction with either potassium thiocyanate or dd-dipyridyl (Pearson, 1970).

RESULTS

Protein Quality - The amino acid scores of the IM beef immediately after processing and after various durations of storage at 38°C are summarized in Table 2. It is evident that the chemical score of each amino acid was lower after cook-soak equilibration in glycerol than after conventional cooking in water. Apart from alanine and histidine, there appeared to be slight increases in the amino acid scores during the first six weeks of storage at 38°C. With further storage, however, all amino acids in IM beef decreased in concentraion; the chemical scores after 12 weeks of storage being only 46-58% except for lysine and theonine which were less affected.

The chemical reactivity of the amino acids in IM meat during storage at 38°C leading to general losses in concentration was equally well detected by determinations of FDNB-reactive ϵ-lysine and protein solubility in 3%-SDS-plus-1%-β-mercaptoethanol as Fig. 1 shows. These chemical para-

meters of protein quality were very highly significantly correlated ($P<0.001$) and parallel the bioassay results with rats. Fig. 2 shows the protein efficiency ratio (PER) and net protein utilization (NPU) values for the IM beef immediately after processing and after 3,12 and 24 weeks storage at 38°C. The nutritive value of the freshly processed beef was similar to that of freshly cooked beef but with storage progressive depriciation value occurred in IM beef despite consistently high apparent digestibilities of over 97% which were similar to that of the cooked beef (97.3%).

Table 2: Chemical Scores*of the Protein Quality of IM Beef Relative to Freshly Cooked Beef

Amino Acid	0.85 a_w Beef Stored at 38°C				
	0 Weeks	3 Weeks	6 Weeks	9 Weeks	12 Weeks
Aspartic acid	78.59	85.25	70.24	50.91	49.34
Threonine	76.10	100.03	78.68	60.52	72.93
Serine	64.39	69.25	55.44	49.45	50.66
Glutamic acid	62.66	75.94	65.40	53.33	54.48
Proline	69.65	72.80	68.58	50.69	52.17
Glycine	62.99	67.54	66.75	51.69	57.03
Alamine	73.43	70.56	69.16	57.11	58.19
Cystine	53.07	78.83	86.93	49.07	43.50
Valine	70.50	74.70	75.89	49.21	51.26
Methionine	85.27	91.18	83.78	63.23	55.46
Iso-Leucine	75.42	77.85	77.35	58.30	52.28
Leucine	76.23	81.23	92.62	58.62	57.85
Tyrosine	68.68	80.32	71.55	54.17	46.78
Phenylalanine	77.20	89.61	81.02	55.89	49.84
Lysine	79.10	93.34	84.83	69.26	67.80
Histidine	62.66	57.44	56.49	52.12	46.32

*Chemical Score = $\frac{\text{Concentration of Amino acid in IM Beef x 100}}{\text{Concentration of Amino acid in Freshly Cooked Beef}}$

Iron Availability - Table 3 summarizes the efficiency of conversion of the iron in IM beef at various storage durations into haemoglobin and body reserves in normal iron-replete rapidly growing weaning rats. Comparison of the performance of these rats on the various IM meat diets with their performance on the cooked meat and casein + $FeSO_4$ control diets shows that irrespective of the nature of the iron source the haemoglobin levels in all rats were always within the normal range (Wintrobe, 1956) without the depletion of body iron reserves as was the case in rats fed on the basal iron-free diet (Table 3). In fact, in all except the 3-week sample, in which the diet was inadvertently made up with 50g NaCl instead of 50g glucose, the iron in the IM beef samples was slightly more available in the rats than iron in the freshly cooked beef and compared favourably with $FeSO_4$.

Table 4 shows the efficiency of the iron in the IM meats and cooked beef and casein + $FeSO_4$ for regeneration of haemoglobin in anaemic rats. Irrespective of growth, the blood haemoglobin levels of the anaemic rats increased slightly in all diets except the basal iron-free control diet. As in the first feeding trial, the iron in the IM beef samples compared well with $FeSO_4$ and was more available than the iron in freshly cooked beef.

DISCUSSION

Results show that after the desorption processing in glycerol the protein of the IM meat is similar to that of ordinary cooked meat. In fact, the NPU values for the freshly processed IM beef appeared slightly better than that of freshly cooked beef (Fig. 2) suggesting that the glycerol in the IM meats may be improving their quality by a protein sparing effect. However, with storage at 38°C the IM meats deteriorated rapidly, yeilding, even after only 3 weeks, a product with significantly decreased available lysine, PER and NPU (Fig. 1 and 2).

It is generally recognized that in meat, a high quality protein food, lysine is not the limiting amino acid but it is seen from the present results that both PER and NPU are highly correlated with the available lysine values (cf. Figs. 1 and 2). If lysine was the only amino acid involved in the crosslinking reactions, it would be anticipated that the PER and NPU values would remain relatively constant during storage, at least until the available lysine concentration was decreased to such a level that it became the limiting amino acid in the system. This is obviously not the case indicating that the protein reactions leading to crosslinking are of a more general nature so that several, or even all, the amino acids become less available during storage.

This observation would agree with the amino acid analyses (Table 2) which showed a decrease in the concentration of all amino acids (determined after hydrolysis) in stored, compared to freshly processed, IM beef samples. Thus, if prolonged storage of IM meats at tropical temperatures is to be achieved, it is necessary to find means to inhibit these reactions so as to minimize nutritional losses.

It is evident from Table 3 and 4 that the iron in the IM meats remained highly available compared with freshly cooked beef. In fact, in both feeding trials, the iron availability of both freshly processed and stored IM meats is apparently better than in fresh meat cooked in water at the same heating regime as the IM meat. This effect was more marked in the second feeding trial where the iron availability was significantly higher ($P<0.01$) in the IM meats. However, the iron availability for ordinary cooked beef found with anaemic rats in this study (28.1%) was lower than 45% found by Mahoney, van Orden and Hendricks (1974) for ground beef although their value of 51% for casein + $FeSO_4$ control diet compares with 56% obtained for same in the present study.

As regards iron availability in IM meats during storage at tropical temperatures results indicate that the gross protein changes (Obanu et al., 1975a, b, 1976) and haemoglobin degradation (Obanu and Ledward, 1975) reported to occur in stored IM meats do not affect the availability and efficiency of the iron both in normal iron-replete rats and in anaemic rats. These results may be of more general applicability as they appear to demonstrate that the availability of the iron in meat is not due to its attachment to meat proteins but rather to its presence in the haematin complex.

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Fig. 1. Effect of Storage at 38°C on the Available Lysine and Solubility in SDS/B-mercaptoethanol of the Proteins in Intermediate Moisture Beef.

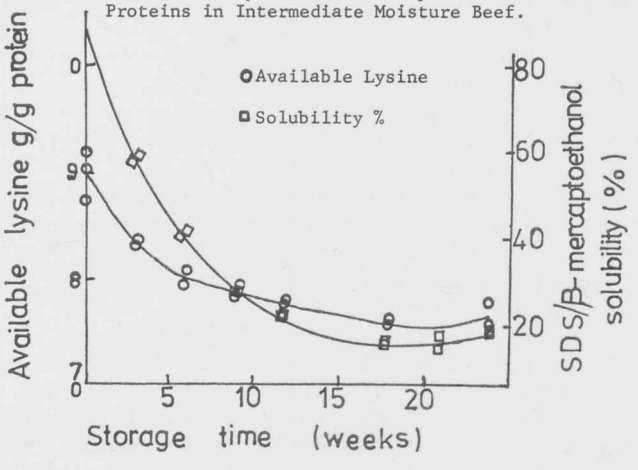
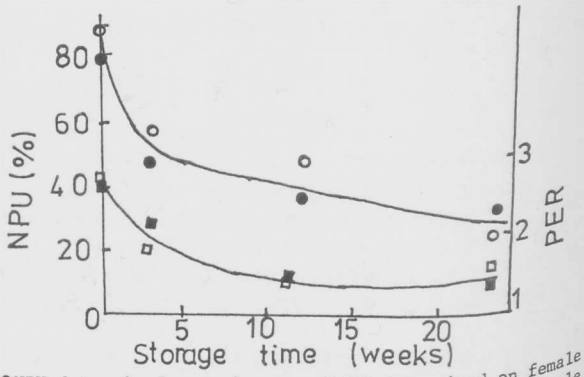


Fig. 2. Effect of Storage at 38°C on the Nutritional Quality of the Proteins of Intermediate Moisture Beef.



○NPU determined on male rats, ●NPU determined on female rats, □PER determined on male rats, ■PER determined on female rats. For the freshly cooked meat the NPU values were 75.4 and 65.1% for the males and females respectively and the PER values were 3.2 and 2.8 for the male and females.

Table 3. Efficiency of Conversion of the Iron in IM(0.85 a_w) Meat into Haemoglobin by Normal Rats

Weeks of Storage of IM Beef at 38°C	Rat Group	Dietary Iron Intake (mg/Rat)	Iron in Blood Haemoglobin (Bb)** (mg Fe/Rat)			Iron in Storage organs (mg Fe/Rat)			Total Iron Gain (mg/Rat)	Proportion of Dietary Iron in Hb+ Stores (%)
			Initial	Final	Increase	Initial*	Final	Increase		
0	I	2.142	1.95	3.32	1.37	1.76	2.04	0.28	1.65	76.9
	II	1.984	1.78	3.14	1.36	1.76	1.73	-0.03	1.33	66.9
3***	I	1.371	2.03	2.69	0.66	1.76	1.50	-0.26	0.41	29.8
	II	1.167	1.64	2.45	0.81	1.76	1.65	-0.11	0.70	60.3
6	I	1.979	1.87	3.24	1.37	1.76	1.70	-0.06	1.31	66.2
	II	1.777	1.78	3.18	1.40	1.76	1.87	0.11	1.51	85.0
9	I	1.946	1.97	3.30	1.33	1.76	1.82	0.06	1.39	71.3
	II	2.069	1.61	3.32	1.71	1.76	1.88	1.12	1.83	88.45
Cooked Beef	I	1.946	1.91	3.16	1.25	1.76	1.95	0.19	1.44	74.1
	II	2.031	1.77	2.96	1.19	1.76	1.68	-0.08	1.11	54.6
Casein Diet	I	3.126	2.13	4.13	2.00	1.76	2.10	0.34	2.34	75.0
	II	2.900	1.64	3.77	2.13	1.76	2.25	0.49	2.62	90.3
Protein & Iron-free	I	0.066	1.92	1.82	-0.10	1.76	0.77	-0.99	-1.09	0
	II	0.075	1.75	1.67	-0.06	1.76	0.68	-1.08	-1.13	0

* Initail iron content of storage organs is the mean value for six rats. All other values given are mean values for 3 rats caged together.

** Iron in haemoglobin (mg/Rat) = 6.7% Body weight x 3.35 (Anderson et al., 1972).

*** The 3-week IM beef diet was inadvertently made up with 50g sodium chloride instead of 50g glucose making the diet less palatable.

Table 4: Efficiency of Conversion of the Iron inIM (0.85 a_w) Meat into Haemoglobin by Anaemic Rats

Weeks of Storage of IM Beef at at 38°C	Rat Group	Dietary Iron Intake (mg/Rat)	Iron in Blood* Haemoglobin (mg Fe/Rat)		Increase in Haemoglobin Iron (Mg/Rat)	Availability** of Dietary Iron (%)
			Initial	Final		
0	I	2.07	2.11	3.00	0.89	42.7
	II	1.97	2.27	2.97	0.70	35.8
3	I	2.34	2.12	3.17	1.05	44.6
	II	1.69	2.37	3.01	0.64	37.8
12	I	2.17	2.39	3.29	0.90	41.9
	II	1.99	2.57	3.22	0.65	32.6
24	I	2.29	2.46	3.57	1.11	48.3
	II	2.28	2.64	3.54	0.90	39.4
Freshly Cooked Beef	I	2.19	2.48	3.04	0.56	25.5
	II	1.82	2.39	2.95	0.56	30.7
Casein/$FeSO_4$	I	2.16	2.29	3.51	1.22	56.9
	II	2.18	2.71	3.92	1.21	55.2

All values given are mean values for 3 rats caged together. Group I = Male; II = Female

* Iron in Haemoglobin (mg/rat) = 6.7% Body weight x 3.35.

** Availability of dietary iron (%) = Increase in Hb-Fe x 100/Dietary Iron Intake