FACTORS IN THE DETERMINATION OF SODIUM, CALCIUM IRON AND COPPER IN SOME ANIMAL PRODUCTS

BY

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DECLARATION

I hereby declare that this thesis has been written by me and that it is a record of my own research work. It has not been presented in any previous application for a higher degree. All quotations are indicated and the sources of information are specifically acknowledged by means of references.

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DEDICATION

It is with gratitude to God that I dedicate this work to my darling wife, Dolapo and our beloved children Ibukunoluwa and Oreoluwa.

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ABSTRACT

The levels of the essential minerals, Calcium, Iron, Copper and Sodium in some canned mackerels, beefs and sardines and crayfish samples have been estimated after wet digestion using an atomic absorption spectrophotometer and a flame photometer. The effects of heating time and temperature during wet digestion on the levels of minerals obtained were also studied, and the influence of sample size on the amounts of calcium and iron elucidated. The levels of minerals found are generally in agreement with those quoted in the literature for similar samples.

For Calcium and Sodium, the maximum mineral contents obtained for each sample decrease with increase in digestion temperature in the order 80 > 100 > 1120°C, and generally for calcium with increase in sample size. The mackerels and crayfish show a decrease of the maximum iron content with increase in temperature, while the beefs and sardines tend to show an increase in iron content obtained. For copper there is also a general decrease of maximum content with increase in temperature except for the mackerels and *one* of the beef samples. The maximum iron contents also tend to decrease with increase in sample size.

It would appear that the use of the same digest solution to determine several minerals, as is often done is vulnerable to errors, and more specific digestion conditions should be stated for each mineral.

TABLE OF CONTENTS

		Page
Title page	.. ..	i
Declaration	.. ..	ii
Dedication	.. ..	iii
Acknowledgements	.. ..	iv
Abstract	.. ..	v
Table of Contents	.. ..	vii
List of Tables	.. ..	x
List of Figures	.. ..	xi
CHAPTER I: INTRODUCTION		
1.1 Research Aims and Objectives	.. .. ..	1
1.2 Elemental Composition of the animal body	..	2
1.3 Meat Processing and effect on minerals	..	4
1.4 Mineral Elements Studied	.. ..	5
1.4.1 Sodium	.. ..	5
1.4.2 Calcium	.. ..	7
1.4.3 Iron	.. ..	9
1.4.4 Copper	.. ..	10
1.5 Destruction of Organic Matter	.. .. ..	11
1.5.1 Wet-digestion	.. ..	11
1.5.2 Dry-Ashing	.. ..	13
1.6 Instrumentation in Atomic Absorption and Flame Emission Techniques	.. ..	14

Table of Contents (cont'd) Page

CHAPTER II: EXPERIMENTAL

2.1	Apparatus	20
2.2	Reagents	20
2.3	Sample Collection and Coding	21
2.4	Moisture Determination	23
2.5	Preparation of Solutions for Calibration ..	26
2.5.1	Preparation of Lanthanum Solutions	26
2.5.2	Calcium Standards	26
2.5.3	Iron Standards	27
2.5.4	Copper Standards	28
2.5.5	Sodium Standards	28
2.6	Digestion Procedure	29
2.6.1	Calibration of the hot plates..	29
2.6.3	The digestion	30
2.7	Treatment of worked-up solutions instumental settings and measurements.	32

CHAPTER III: RESULTS AND DISCUSSIONS:

3.1	Introduction	37
3.2	Moisture contents of the samples	37
3.3	Estimation of Percentage Relative Deviations of Analysis	40
3.4	Calcium Contents	42
3.5	Iron contents	50
3.6	Copper contents	61
3.7	Sodium Contents	67
3.8	Calcium hydrogen or thophosphate and haemoglobin samples	74

Table of Contents (cont'd) Page

CHAPTER IV: SUMMARY AND CONCLUSION

4.1 Summary and Conclusion 77

4.2 Suggestions for further studies 79

References 80

Appendix I Variation of Calcium 87
Contents with heating Time and Temperature

Appendix II: Variation of Iron contents , with heating Time and Temperature 91

Appendix III: Variation of Copper Contents with heating Time and Temperature .. 95

Appendix IV: Variation of Sodium contents with heating Time and Temperature 98

LIST OF TABLES

Table	Title	Page
1.1	Major Elements in Animal Body	3
2.1	Identification Codes and Description of the samples	22
2.2	Sample Sizes and Volumes of Acid Mixture Used	33
2.3	Instrumental Parameters Used During Analysis	35
3.1	Moisture contents of the samples	38
3.2	Estimated Percentage Relative Standard Deviations at all temperatures	41
3.3	Maximum Calcium Contents of the samples at various temperatures	43
3.4	Maximum Iron Contents of the samples at various temperatures	51
3.5	Maximum Copper contents of the samples at various temperatures	62
3.6	Maximum Sodium Contents of the samples at various temperatures	68

LIST OF FIGURES

		Page
1.1	Relationship between emission and absorption of energy by a valence electron	15
1.2	Block diagram of an Atomic Absorption Spectrophotometer	17
1.3	Block diagram of a Flame Photometer	18
3.1	Variation of Maximum Calcium contents of 0.25g samples with Temperature	46
3.2	Variation of concentration of calcium with heating time during digestion (samples A & D)	47
3.3	Variation of concentration of calcium with heating Time during digestion (samples J & K)	48
3.4	Variation of Maximum Calcium content with sample size	49
3.5	Variation of maximum Iron contents of 0.25g samples with Temperature	56
3.6	Variation of concentration of Iron with heating time during digestion (Samples A & J)	57
3.7	Variation of concentration of Iron with heating Time during digestion (Samples D & K)	58
3.8	Variation of maximum Iron content with sample size	59
3.9	Variation of Maximum Copper Content of 0.25g samples with temperature	64
3.10	Variation of concentration of copper with heating Time during digestion (Samples B & J)	65
3.11	Variation of concentration of Copper with heating Time during digestion (Samples F & K)	66

List of Figures (cont'd) Page

3.12 Variation of maximum sodium contents of 0.25g samples with Temperature 71

3.13 Variation of concentration of sodium with heating Time during digestion (Samples A & D) 72

3.14 Variation of concentration of sodium with Heating Time during digestion (samples H & K) 73

CHAPTER I

I N T R O D U C T I O N

1.1 RESEARCH AIM AND OBJECTIVES

This study is aimed at estimating the levels of some essential minerals such as calcium, iron, copper and sodium in some processed meats and investigating some of the factors during wet digestion which influence the levels of the minerals obtained.

Although various methods are available for the determination of inorganic nutrients in animal products, many of them, particularly at the organic matrix destruction stage, lack specificity and are vulnerable to errors. For instance we consider that instructions that digest mixtures be heated initially at moderate heat, with a later increase, or until the evolution of white fumes of sulphuric acid has subsided (1 - 8) are inadequate. Furthermore, efforts which have been directed in recent years towards reducing the deficiencies of the conventional digestion procedures have led to new methods, which show no advantage in precision or accuracy over the conventional methods.

In this study therefore, we intend to examine the effects of heating time, temperature and sample size on the levels of the minerals determined. Ten processed

meat products consisting of three, four, and two brands of canned mackerels, corned beef and sardines respectively and dried crayfish purchased locally will be analyzed. It is intended also to compare the results obtained with those carried out on plant materials in this laboratory.

1.2 ELEMENTAL COMPOSITION OF THE ANIMAL BODY.

The animal body normally contains about thirty six elements of which about twenty have been detected (9, 10). An additional 30 - 35 elements have been detected in mammalian and avian tissues but their presence is probably coincidental with their presence in the animal's environment during its lifetime. Elements in the animal body are grouped into two classes: major and trace elements. The major elements are shown in table 1.1.

TRACE ELEMENTS:

From the view point of the food analyst, the term 'trace element' refers to the inorganic elements (mostly metals), which may be present in foods in amounts usually well below 50 ppm and have some toxioological or nutritional significance (1, 9). Calvery (11), broadly classified trace elements into three categories:

TABLE 1.1

MAJOR ELEMENTS IN ANIMAL BODY (9)

ELEMENT	% of Body Weight	
Oxygen	65.0	96.0%
Carbon	18.0	
Hydrogen	10.0	
Nitrogen	3.0	
Calcium	1.5	3.4%
Phosphorus	1.0	
Potassium	0.35	
Sulphur	0.25	
Sodium	0.15	
Chlorine	0.15	
Magnesium	0.05	

the essential nutritive elements such as Co, Cu, Fe, I, Mn, Zn; the non-nutritive, non-toxic elements such as Al, B, Cr, Ni, Sn - which are not known to produce harmful effects when present in quantities not exceeding 100 ppm; the non-nutritive toxic elements such as Sb, As, Cd, F. Pb, Hg, Se, which are known to have deleterious effects even when the diet contains less than 100 ppm.

A more comprehensive but slightly different list of trace elements in the animal tissue is given by Forrest et al (9), and Lawrie (11), thus:

the essential microelements, such as Co, Fe, Mn, Mo, Se, Zn; microelements with the evidence of their essentiality still lacking, such as Ba, Br, Cd, Cr, Cu, F, I, Sr;

elements without known metabolic functions such as As, B, Pb, Li, Rb, Ag, Ti, V, Si, Ni.

1.3 MEAT PROCESSING AND EFFECT ON MINERALS.

Generally nutrients are destroyed when foods are processed, largely because they are sensitive to the pH of the solvent, to oxygen, light, heat or combinations of these. Also losses of 0 - 3% have been experienced for mineral salts (which are relatively stable), during cooking (12, 13).

Curing Effects:

Meat curing involves smoking, and addition of a number of chemicals as 'meat ingredients' which act as flavouring and preservation reagents. Prominent among such chemicals are sodium salts, particularly sodium chloride and sodium nitrite, spices, sugar and water (1, 9, 12). It would be expected that the sodium contents (and possibly those of some other elements), in processed meats will be much higher than the raw meats because the effects of the added sodium salts during processing would more than compensate for any volatilisation losses during processing (9, 13, 14).

1.4 MINERAL ELEMENTS STUDIED.

Four mineral elements known to be normal constituents of all animal body (9, 12 - 16), namely sodium, calcium, iron and copper, have been used as the basis of this investigation. Their levels in animal tissues, their widely accepted methods of analysis as well as interference effects during their analysis are outlined below:-

1.4.1 SODIUM.

Sodium is a major element in the animal body - constituting some 0.15% of the total body weight (table 1.1). Also sodium constitutes the largest cation

concentration in the animal blood (15).

Flame emission spectrometry is the preferred means for the determination of sodium in biological materials (4, 15, 17, 18, 51). This is achieved by aspirating the sample solution (after wet-digestion treatment), into the flame photometer. At its 589.0nm resonance line, Na is detectable in a concentration of 0.0001 µg/ml (17 - 19). With atomic absorption spectrometry, it has a detection limit of 0.005 µg/ml.

The sodium line at 589nm encounters very little spectral interference. However, with fairly large amounts of calcium present (e.g. 6,300 ppm in cements), the use of filters may prove inadequate. Some form of monochromator is preferrable, and a slit setting giving a spectral band with less than 0.5nm. Also employment of low temperature of an air-fuel flame virtually eliminates spectral interference of foreign elements, particularly due to calcium whose spectral interference becomes significant in high temperature flames (17 - 19). Other interferences on the flame photometer reading include:

flame background emission - which requires proper correction of the background reading;

self-absorption - which can be eliminated by using very low concentration ranges (e.g. 0 - 1 or 0 - 5 ppm),

for the calibration curves;

ionization and cation-enhancement effects - which can be overcome by mixing about 50% by volume of nitrogen with the oxygen of an oxygen - gas flame, and by adding a large amount of an easily ionizable element (but devoid of spectral interference), to both standards and samples (17, 20).

1.4.2 CALCIUM.

More than 99% of all the calcium in the animal body is in the skeleton where it exists mainly as hydroxyapatite, $[Ca_{10}(PO_4)_6(OH)_2]$. Hydroxyapatite is the major mass of the bone mineral and is the only calcium and phosphorus salt stable under physiological conditions. The remaining calcium (less than 1%), in soft tissues and body fluids exists as protein-bond, soluble complexes and ionized form. Normal plasma contains 100mg/100ml calcium of which 50% is ionized (15, 21, 22). Also, according to Kimizuka and Koketsu (23), Lecithin may be the substance in cell membrane which binds calcium.

Although the emission spectroscopy is more sensitive, atomic absorption spectrometry is the method of choice for the analysis of calcium in biological materials since interferences from constituents in the biological

matrix are more easily eliminated (19). The worked up solution is aspirated after wet digestion. Calcium has a detection limit of 0.0009 µg/ml by flame emission, and 0.003 ug/ml by atomic absorption spectrometry (15, 19, 24). Elements such as phosphorus berylium, silicon, titanium and zirconium interfere with calcium measurements by the atomic absorption spectrometry by depressing the response in the air-acetylene flame. Addition of a releasing agent such as about 0.2 - 1% ($^{m}/V$) Lanthanum or strontium solution to both samples and standards eliminates this interference: Also, addition of about0.5% ($^{m}/V$) EDTA (which preferentially combines with calcium), eliminates this interference as well as the depression in response due to protein in biological samples (24, 25, 26, 27). The use of higher temperature nitrous oxide-acetylene flame is a more effective method of overcoming this response depression, but as calcium is appreciably ionized in this flame, an ionization buffer such as 0.01% ($^{m}/V$) KCl must be added to the solutions.

Instrumental instability which is peculiar with atomic absorption spectrophotometric measurement of calcium often results in variation of analyses. It can be eliminated by allowing sufficient "warm-up" time (about ½ hr.), for both the hollow cathode lamp

and the burner. Instrumental instability should further be monitored by rereading a standard solution after every five determinations (19).

1.4.3 IRON.

Practically all the iron in the animal body occurs in pigments as protein heme-complexes [heme ≡ proto-porphyrin]. These include haemoglobin - the pigment of the blood containing some 75% of the total iron in the animal body; the pigment of the muscles - myoglobin contains 3% while other heme-enzymes contain 0.2%. Non-heme iron-bearing proteins in the body include Ferritin and Homosiderin, containing 20%, and siderophilin which contains 0.1% (9, 15).

A widely used method of analysis is by the atomic absorption spectrometry. Grove (19), claimed a detection limit of 0.01 ug/ml by this method. Limits by flame emission are generally higher than the biological iron concentration being measured. Determination by atomic absorption is done on the wet-digested worked up solution (see chapter 2). The most sensitive wavelength is 248.33nm. (15). A few interference effects experienced with iron such as depression of response by perchloric, nitric and

citric acids, and nickel can be eliminated by using a highly oxidising flame (i.e. a hot fuel-lean flame). (24, 25)

1.4.4 COPPER.

Copper exists in the animal body as homocyanins - an oxygen carrying protein. Copper is also an integral part of enzyme cytochrome (15, 28, 29). Also, Holmberg and Laurell (15) showed the evidence of copper in blood plasma as copper-protein complex, which they named "ceruloplasmin".

Wet digestion with subsequent analysis by atomic absorption spectrometry is recommended. With the 324.76nm resonance line, the detection limits for copper in aqueous solutions are 0.1 µg/ml by flame emission and 0.005 µg/ml by atomic absorption spectrometry. A lean flame is recommended (15, 19, 25). Only large excesses of transition metals in the presence of mineral acids depress the response. The calibration solutions should be matched to the concentrations of any of these elements present as major components in the sample solutions (25).

1.5 DESTRUCTION OF ORGANIC MATTER - AS A NECESSARY PRELIMINARY TO MINERAL DETERMINATION.

There are two major methods of destroying the organic matter of foodstuffs namely 'wet (acid) digestion' and 'dry-ashing'.

1.5.1 WET (ACID) DIGESTION (1 - 4, 8, 30 - 34, 52)

This involves the use of oxidizing reagents (acids), to destroy the organic matter. During wet digestion, there are little or no volatilisation losses of metallic elements and phosphorus. In addition, the procedure is fairly rapid and easily controlable; liquid conditions are maintained throughout and the oxidation is carried out by oxidising agents in solution: Finally, a larger number of nutrient elements can be determined in one digest solution. These advantages make wet digestion more reliable and hence the preferred method for trace metal analysis in biological materials.

Some problems often encountered during wet digestion include:-

possibility of the digest mixture charring during heating;

large sample weights (during analysis of trace elements), require large volumes of acid for complete oxidation - which may result in a high reagent blank;

alkaline earth metals when present in appreciable amounts give insoluble sulphates which absorb a considerable proportion of trace metals, particularly lead.

In view of these problems associated with wet digestion, it would be necessary to work on as small a scale as possible, stop the digestion immediately charring occurs, and, in order to avoid explosion, do not digest materials high in fat or oily substances (particularly animal samples), or containing hydroxy groups with perchloric acid.

Acid mixtures most commonly used include: Nitric Acid + Perchloric Acid; Nitric Acid + Perchloric Acid + sulphuric Acid; and Nitric Acid + Sulphuric Acid. The acid mixture of choice will depend on the sample as well as the nutrient element to be estimated. However, Gorsuch (8), obtained highest recoveries for copper and iron from biological samples with the nitric acid and sulphuric acid mixture. Also, the use of hydrogen peroxide-sulphuric acid mixture has been recommended due to its safety and simplicity (34); however hydrogen peroxide freely gives off oxygen, and hence should not be used near inflammable substances (4).

1.5.2 DRY-ASHING (1 - 4, 6, 8, 30, 31, 34)

The ash of a foodstuff is the inorganic residue remaining after the organic matter has been burnt away.

The general procedure involves weighing out about 1g of the dry sample into a silica or platinum crucible, which has been previously ignited and cooled before weighing. The crucible and the contents are then ignited first gently and then at 500°C in a muffle furnace for 3hrs. It is then transferred into a desicator, cooled and weighed. The mineral constituents are dissolved by boiling a known weight of the ash in water (for water soluble ash), or in hydrochloric acid followed by nitric acid (for the acid soluble ash).

The method is good for trace metal analysis; but is known to have several disadvantages. For instance low recoveries of specific elements have been reported by many workers (3, 8, 31 - 33), due to the volatilisation losses of elements such as mercury, selemium (8), and chlorides (30); combination or absorption of the element with ash oonstituents on the silica wall of the crucible e.g. copper, zinc, iron, manganese, lead, silver and cadmium (8, 33), and incomplete extraction of the ash. On the other hand, unexpected increases for specific elements such as iron, aluminium and boron, attributable

to contamination from the lining of the muffle furnace have been reported (4). These difficulties can be avoided by adding an ash aid (e.g. MgO or $Mg(NO_3)_2$, Na_2CO_3 or H_2SO_4), and by using a suitable acid for the mineral extraction.

1.6 INSTRUMENTATION IN ATOMIC ABSORPTION AND FLAME EMISSION TECHNIQUES.

1.6.1 INTRODUCTION.

Prior to 1940, spectroscopic applications for the analysis of trace metals in biological materials were limited mainly to flame and arc spectrographic methods. Many workers such as Sheldom and Ramage (19, 35), used this method to estimate many elements in tissues. Various investigations and developments since then have led to the use of various spectroscopic methods for analysing elements in biological materials.

1.6.2 ATOMIC SPECTROSCOPY. (17, 19, 24, 36 - 41)

This consists of 'Flame Emission', 'Atomic Absorption' and 'Fluorescence' spectroscopy. Analytical techniques employing the first two have proved to be the most adaptable for elemental analysis of biological materials - possessing the ability for multiple trace analyses in the same volume and with good specificity, precision and accuracy.

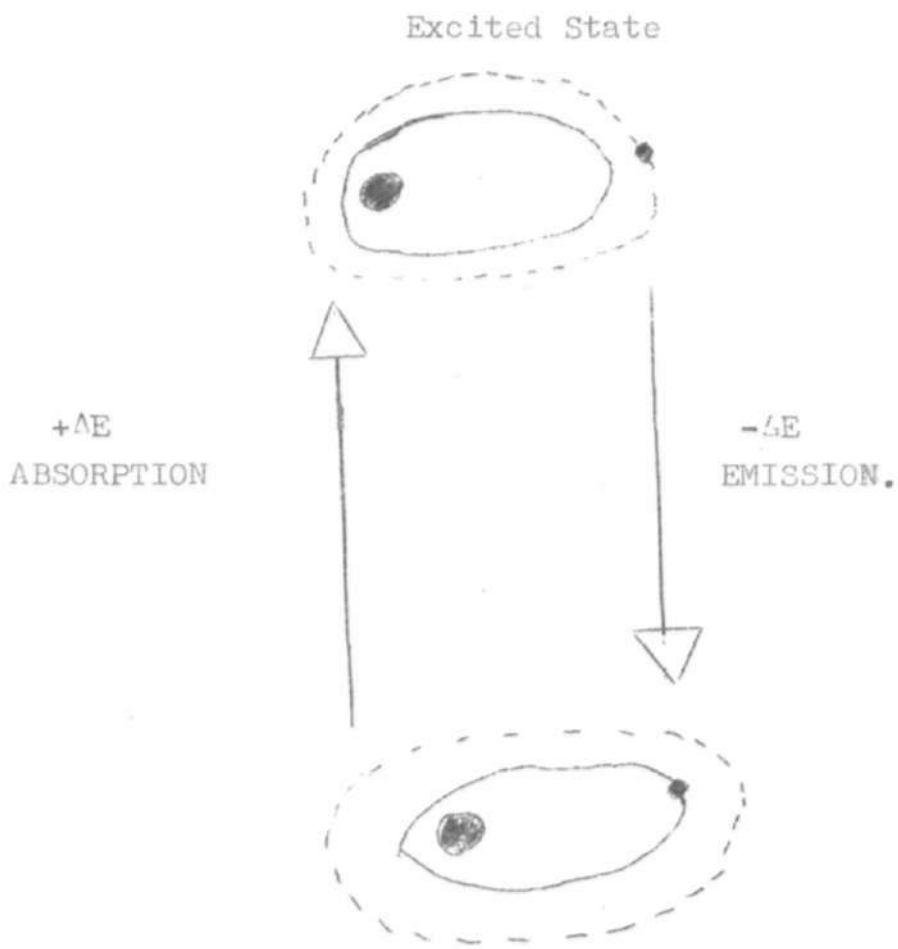


Fig. 1.1 Relationship between emission and absorption of energy by a valence electron.

In absorption spectorscopy an electron of an atom in its ground state absorbs a quantum of energy ($+\Delta E$), and undergoes transition to an excited state. Emission occurs when this quantum of energy is released ($-\Delta E$), and the electron returns to the ground state (see Fig.1.1). The 'Flame photometer' is the analytical tool in emission spectroscopy, while the 'Atomic Absorption Spectrophotometer (AAS), is the analytical tool in absorption spectroscopy. (see figs.1.2 and 1.3).

1.6.3 PRINCIPLE OF A.A.S. (24, 36 - 41).

The hollow cathode lamp (see fig. 1.2). emits radiation characteristic of the cathode material (an element), usually a single element (analyte). Ground state analyte atoms produced thermally in the atom reservoir absorb resonance radiation from the lamp, reducing the intensity of the incident beam - which then results in electronic output proportional to the absorption by the analyte atoms.

1.6.4 PRINCIPLE OF THE FLAME PHOTOMETER (17, 19, 37-42) (See Fig. 1.3)

When atoms of an element are heated in a flame some of the atoms become excited. On reverting to the ground state, they emit this energy as

AN ATOMIC ABSORPTION SPECTROPHOTOMETER (A.A.S.)

The essential components of the A.A.S. are shown in figure 1.2.

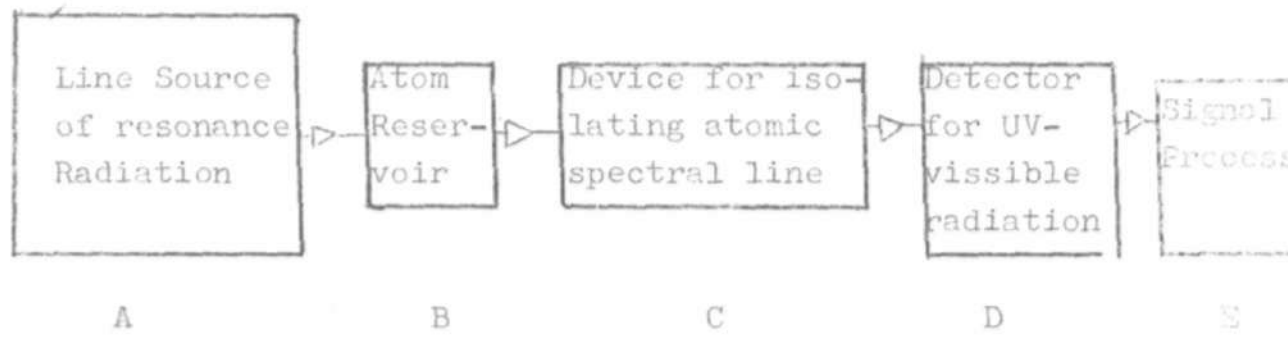


Fig. 1.2: Block diagram of an Atomic Absorption spectrophotometer.

In most commercial instruments.

A = Hollow cathode lamp

B = Flame or electrothermal device

C = Grating monochromator.

D = Photomultiplier

E = Meter or Digital readout.

THE FLAME PHOTEMETER

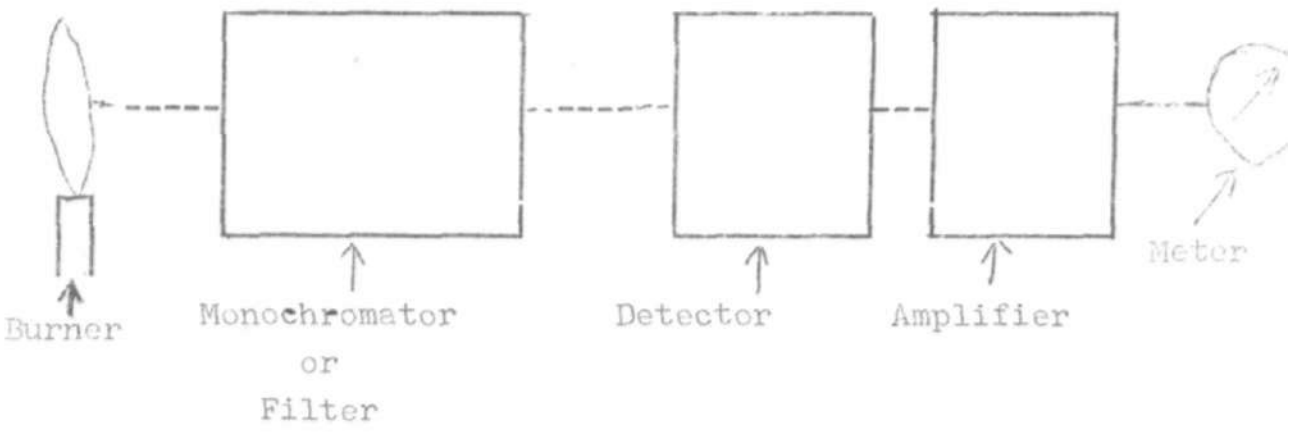


Fig. 1.3: Block diagram of a Flame Photometer.

electromagnetic radiation with a wavelength dependent on the energy change involved. For each element, the intensity of the characteristic radiation is a measure of the number of atoms of the element in the flame at any instant - which is the basis of quantitative flame photometry.

In A.A.S, radiation sources used are specific for each element. This overcomes the problem of overlap of spectral bands frequently encountered in flame photometry. Partly because of this and partly because inter element effects have been a major problem in flame photometry, A.A.S. is generally preferred to flame photometry.

CHAPTER II

E X P E R I M E N T A L

2.1 APPARATUS:

B & T Thermostatic Hotplates;
Metler Balances [H8 and H15] ; Flame Photometer [EEL UNIGALVO: 200] ; Atomic Absorption Spectrophotometer - Pye Unicam SP 1900 ;
Thermometers 0 - 150°C and 0 - 250°C ;
Nickel crucibles; Watchclock; Grinder; Oven [Gallenkamp B.S.] ; Washbottles; Desicator; Pipette - washer and filter papers 9cm and 12.5cm Whatman 541.
Glassware; Conical flasks (50 cm^3); Filter-funnels, Volumetric flasks 25, 50, 250, 500, 1000 and 2,000 cm^3 ; Measuring cylinders, Beakers; sample bottles; Burettes 50cm^3 , Pipettes 5, 10, and 25cm^3 .

2.2 REAGENTS**

Concentrated sulphuric Acid 98% $^m/_m$;
Nitric Acid 70% $^m/_m$;
Hydrochloric Acid 36% $^m/_m$;
Calcium carbonate; Copper sulphate pentahydrate; Ferrous Ammonium sulphate; Sodium chloride; Lanthanum oxide; Lanthanum - chloride, Liquid paraffin, Haemoglobin; Calcium hydrogen orthophosphate.

**Unless otherwise stated all reagents were of analytical reagent grade.

2.3 SAMPLE COLLECTION AND CODING.

Ten different processed animal products have been chosen for the purpose of this investigation. Of these nine are canned while the remaining one is just dried. All of them were purchased at various supermarkets and the open market located in Samaru, Zaria. The samples, sample codes, constituents and country of origin are shown in table 2.1 below.

2.3.1 SAMPLE PREPARATION. (1, 2, 6)

To obtain a homogenous sample which is suitable for analytical work, meat, sausages and other animal products are normally first minced (using a mincer or grinder). Then the minced sample is then mixed thoroughly using a pestle and morter. The process is usually repeated several times depending on the coarseness of the original sample. The mixture is then transfered into a screw-capped jar (or bottle), and then stored in a refrigerator.

In this analysis, for the mackerels (A, B, C), and the sardines (H, J), which are canned in tomato sauce and edible oils respectively, the solid fish pieces were first carefully removed and wiped dry

TABLE 2.1

IDENTIFICATION CODES AND DESCRIPTION OF THE SAMPLES.

NAME OF SAMPLE (BRAND)	IDENTIFICATION CODE	CONTENTS	COUNTRY OF ORIGIN
SONA MACKEREL	A	Mackerel in Tomato Sauce.	Japan
GEISHA MACKEREL	B	Mackerel in Tomato Sauce.	Japan
POWER KING MACKEREL	C	Mackerel in Tomato Sauce.	Japan
KONDOR CORNED BEEF	D	Beef, Salt, Spices, KNO_3.	France
POWER KING CANNED BEEF	E	Beef, soy protein, starch, salt, sugar, spices, $NaNO_2$.	Brazil.
PLUMROSE LUNCHEON MEAT	F	Beef, spices, $NaNO_2$.	Denmark
CELEBRITY LUNCHEON MEAT	G	Pork, Beef, water, salt, spices, $NANO_2$.	Denmark
QUEEN OF THE COAST SARDINE	H	Sardines, edible oil, salt.	Spain
SONASHAH SARDINE	J	Sardines, edible oil, salt.	Spain
CRAYFISH (DRIED)	K	Dried crayfish.	Zaria, Nigeria.

before grinding to homogenize them. For the beef samples (D, E, F, G), the solid fat on their surface was carefully removed before homogenization.

Each of the samples was first ground with a mortar and pestle, and then transfered into the grinder where they were further ground to make them finer and more homogenous. Each was again transfered into the mortar and ground further (1, 2, 6). The bulk material of each sample was then spread on large watch-glasses and then heated in the oven at 105°C first for 24 hrs. After this, they were removed, ground with the pestle and mortar and reheated in the oven to dryness for an additional 12 hrs at 105°C. They were then finally ground more homogenously with the blender, transfered separately into screw-capped bottles, labelled and stored under refrigeration.

Dry storage of the bulk samples for subsequent analysis is preferred because it facilitates stabilization and easier handling of the samples (2).

2.4 MOISTURE DETERMINATION (1, 4, 6, 9, 10, 13, 43, 44)

2.4.1 INTRODUCTION.

Moisture accounts for 45 - 70% of the finished processed meat products. Most of the moisture originates from the lean meat ingredients. However, the processor adds

additional water to many products as part of the formulation (10). Methods which are often employed for moisture determination in foods include:- indirect distillation (or drying) methods (1, 4, 6, 13), direct distillation methods (1, 6); chemical methods (1, 6, 45 - 47) and the more modern techniques such as: nuclear magnetic resonance, gas chromatography and infra-red absorption (1, 48).

The indirect distillation (or drying) method, which was employed during this work involves calculating the percentage weight loss of water due to evaporation. Drying methods are frequently employed because they are fast and usually give reasonably accurate results. However, the following drawbacks exist:

it is sometimes difficult to remove all the moisture present by drying (e.g. in cereals where only the 'free', and not the 'bound' water is lost at 100°C);

some foods are liable to decompose at certain temperatures e.g. sugar and fruit products can be dried only at 70°C ;

volatile matter other than water may be lost (e.g. volatile oil is also lost at 100°C): Generally, the internal mechanically ventillated ovens give more consistent results and an increased rate of drying.

2.4.2 PROCEDURE: (1, 4, 6, 13)

A nickel crucible of about 3½ - 5cm in diameter was cleaned and then dried in the oven at 105°C for 1hr. The dish was then transfered into a desiccator, allowed to cool and weighed. About 5g of the freshly mixed sample was accurately weighed into the dish. The dish with the content was transfered into an oven at 105°C and heated first for 24 hrs; it was then transfered into the desiccator, cooled and weighed. The dish with the content was again returned to the oven, heated for 3 hrs., removed, cooled and weighed. This was continued until a constant weight was obtained for each sample.

CALCULATIONS:

Moisture contents of the samples were estimated using the formula below:-

$$\text{Moisture (or Water) \%} = \frac{\text{Loss in wt on drying (g)}}{\text{Initial Sample Wt (g)}} \times 100$$

Also,

$$\text{Total Solid \% (or Dry Matter)} = \frac{\text{Oven dry wt (g)}}{\text{Initial sample wt (g)}} \times 100$$

2.5 PREPARATION OF SOLUTIONS FOR CALIBRATION. (2, 4, 17, 24, 25, 40, 41).

2.5.1 5000 ppm (0.5 % $^{m}/V$) LANTHANUM SOLUTIONS:

(i) From Lanthanum Chloride $[LaCl_3.7H_2O]$

13.368g of lanthanum chloride was dissolved in distilled water containing $2cm^3$ of 2M hydrochloric acid solution in a 1 dm^3 volumetric flask. It was then made to the mark with distilled water - giving a 5000 ppm lanthanum solution.

(ii) From Lanthanum Oxide $[La_2O_3]$.

5.864g of lanthanum oxide was dissolved in $35.0cm^3$ of concentrated hydrochloric acid, transferred into a 1 dm^3 volumetric flask and made to mark with distilled water. This gave a 0.5% ($^{m}/V$) or 5000 ppm lanthanum solution.

2.5.2 CALCIUM STANDARDS.

The calcium stock solution was prepared by dissolving 2.4973g of dry calcium carbonate in $100cm^3$ 3M hydrochloric acid solution contained in 1 dm^3 volumetric flask. The flask was heated on a steam bath to drive off carbon-dioxide. It was then cooled and made up to the mark with distilled water. This gave a 1000 ppm solution in calcium.

A 100 ppm (Ca) solution was prepared by diluting 10 cm^3 of the stock solution to 100 cm^3. Working standards were made from this by diluting 0, 5, 10, 15, 20, 25, 30, 40 and 50 cm^3 of it to 100 cm^3 to give 0, 5, 10, 15, 20, 25, 30, 40 and 50 ppm calcium solutions respectively. The working standards were used to obtain the standard (calibration) curve of the calcium with the A.A.S.

2.5.3 IRON STANDARDS.

A 1,000 ppm stock solution was first prepared by dissolving 7.022g of Ferrous Ammonium sulphate $[(NH_4)_2SO_4.FeSO_4.6H_2O]$, in 100 cm^3 distilled water in a 1 dm^3 volumetric flask. 50 cm^3 of 1:5 sulphuric acid was added and the solution was made up to 1 dm^3 with distilled water.

Working standards were prepared by first preparing an intermediate standard of 100 ppm (Fe), by diluting 10 cm^3 of the stock solution to 100 cm^3 in a volumetric flask. From the 100 ppm (Fe), solution, 0, 0.5, 1.0, 2.0, 4.0, 6.0, 8.0, and 10cm^3 was transferred to each of eight 100 cm^3 volumetric flask and then made to the mark, giving 0, 0,5, 1.0, 2.0, 4.0, 6.0, 8.0 and 10.0) ppm iron solutions respectively. These final solutions were used to obtain the standard (calibration) curves for iron with the A.A.S.

2.5.4 COPPER STANDARDS:

A 1,000 ppm stock solution was first prepared by dissolving 1.962 g of hydrated copper sulphate [$CuSO_4.5H_2O$], in 50 cm^3 3M hydrochloric acid solution in a 500 cm^3 volumetric flask. It was then made to the mark with distilled water - giving a 1000 ppm copper solution.

Intermediate standards were prepared by first diluting 10cm^3 of the stock solution (1000 ppm), to 100 cm^3 (≡ 100 ppm - Cu), and again 10 cm^3 of the 100 ppm - Cu - solution was diluted to 100cm^3 to give a 10 ppm - Cu - solution.

Working standards were prepared by diluting 1, 2, 4, 6, 8, 10 cm^3 of the 10 ppm - Cu - solution each to 100 cm^3, giving 0.1, 0.2, 0.4, 0.6, 0.8 and 1.0 ppm - Cu - solutions respectively. These were used to obtain the standard (calibration), curves for copper with the A.A.S.

2.5.5 SODIUM STANDARDS.

The sodium chloride [NaCl], to be used was first dried for 2 hours in an oven at 105°C. It was then allowed to cool in a desiccator. The stock solution [1,000 ppm], was prepared by dissolving 2.544g of the dried sodium chloride in 100 cm^3 of 3M hydrochloric acid

solution in a one dm^3 volumetric flask. This solution was then made to level with distilled water.

An intermediate standard of 100 ppm (Na), was prepared by diluting 10 cm^3 of the stock [1000 ppm], solution to the mark with distilled water in a 100 cm^3 volumetric flask. Then 1, 2, 3, 4, 5, 6, 8 and 10 cm^3 each of the intermediate standard was diluted to 100cm^3 to give 1, 2, 3, 4, 5, 6, 8 and 10 ppm Na , respectively. These standard solutions were used in calibrating the flame photometer for the sodium measurements in the samples.

2.6 DIGESTION PROCEDURE (1 - 3, 5, 6, 8, 30, 32, 34, 52).

2.6.1 CALIBRATION OF THE HOT PLATES.

The hot plates used for heating the digest mixtures were calibrated for the chosen temperatures - 80°C, 100°C and 120°C respectively. This was done by placing a 50 cm^3 conical flask (the same type and size used for all the digestions), containing some liquid paraffin on the hot plates. Thermometers were set inside the liquid paraffin but not allowed to touch the base. The hot plates were connected, started and the thermostat regulator marked for 80 ± 2°C, 100 ± 2°C and 120 ± 2°C. The above set up was alwa-

made to accompany all the digestions done, so as to detect and correct for any slight temperature variations during digestions.

2.6.2 THE DIGESTION:

The wet (acid) digestion method was used for the digestion of all the samples studied. Based on the recommendations of several workers in the lietrature (1, 2, 3, 5, 6, 8, 30, 34, 52), the nitric acid/sulphuric acid mixture was employed for wet digestion of all the samples; Temperatures used were 80 ± 2°C, 100 ± 2°C and 120 ± 2°C., sample sizes used were 0.25g, 0.50g and 0.75g.

PROCEDURE:

0.25g sample was weighed separately into three 50 cm^3 conical flasks. 5.0 cm^3 of nitric acid and 0.5 cm^3 of sulphuric acid were added to each flask. The flasks were heated at 80 ± 2°C for 10 min. (on a calibrated hotplate), inside a fume cupboard, and then allowed to cool. The procedure was repeated for the sample for heating periods of 20, 30, 40, 50 and 60 min. The same procedure was carried out for each sample at 100 ± 2°C and 120 ± 2°C. All the ten samples were subjected to this treatment.

Three samples, one from each of the first three groups namely C (a mackerel), F (a beef), and H(a sardine), were also investigated using 0.5g and 0.75g samples. The corresponding volumes of the acid mixture used for them are as shown in table 2.3 below.

DIGESTION OF HAEMOGLOBIN AND CALCIUM HYDROGEN ORTHOPHOSPHATE ($CaHPO_4$).

It was proposed that some pure chemicals containing two of the mineral elements, namely calcium and iron (in the same chemical form as expected in animal tissues), be digested in a similar way as the samples. This would allow a comparison of the trends in the mineral levels obtained with those of the analysed animal samples. Thus, calculated amounts of haemoglobin [0.2g] and calcium hydrogen orthophosphate [0.01g] containing iron and calcium respectively in about the same concentrations as the analysed meat products were subjected to the same digestion procedure as the actual processed samples. The levels of iron and calcium in their solutions were similarly estimated.

2.7 TREATMENT OF WORKED UP SOLUTIONS, INSTRUMENTAL SETTINGS AND MEASUREMENTS.

2.7.1 PREPARATION OF WORKED UP SOLUTIONS.

For all the digestions carried out the following procedure was used to treat the digest mixture for each sample for analysis: After removing it from the hotplate, the digest mixture was allowed to cool. It was then filtered into a 50 cm^3 volumetric flask with 9 cm or 12.5 cm Whatman 541 filter-paper. 10 cm^3 of the 5000 ppm Lanthanum solution was added, and then made to the mark with distilled water, (thus giving a final concentration of 1000 ppm Lanthanum in each sample). It was then transfered into a labelled sample bottle and covered for the analysis.

Dilutions For Sodium Measurements:

Except for sample D(Kondor Corned beef), which was diluted 62½ times, for the other 0.25g sample series, the solution was diluted 25 times while for the 0.5g series for the estimation of standard deviation, the solution was diluted 50 times.

TABLE 2.2

SAMPLE SIZES AND VOLUMES OF ACID MIXTURE USED.

Sample Size (g)	Volume of Acids (cm^3)	
	HNO_3	H_2SO_4
0.25	5.0	0.5
0.50	10.0	1.0
0.75	15.0	1.5

2.7.2 INSTRUMENTAL SETTINGS AND MEASUREMENTS:

The 'Flame Photometer' was used for the estimation of sodium, while the A.A.S. was used for the estimation of calcium, iron and copper in the worked up sample solutions. The instrumental parameters used are shown in table 2.3.

2.7.3 MEASUREMENTS:

(i) Sodium With the Flame Photometer:

The sodium standard solutions [0 - 5 or 0 - 10 ppm], were used in calibrating the instrument for each set of readings. Usually, the instrument was allowed to warm up for about 10 min. before measurements were made. The concentrations of the solutions (in ppm), were obtained by extrapolation from the calibration curves. The readings (in ppm), were converted to mg/100g as shown below:(p.36).

(ii) Calcium, Iron and Copper With the A.A.S.

After following the setting in table 2.3 for each element, the hollow cathode lamp and burner were allowed to warm up for 10 - 30min. The instrument was first flushed with distilled water before aspirating the standard solutions for the calibration curve. The sample solutions were then aspirated for the estimation of calcium, iron and copper. Usually, a

TABLE 2.3

INSTRUMENTAL PARAMETERS USED DURING ANALYSIS.

(i) Flame Photometer (For Na)

Flame : Air - gas mixture.

Pressure : 10 psi

Filter : Na - filter (589 nm).

(ii) Atomic Absorption Spectrophotometer (A.A.S)*

(For Ca, Fe and Cu).

ELEMENT	WAVELENGTH (nm)	LAMP CURRENT:(mA)	FLAME FLOW RATE(L/min)	
			ACETYLENE	AIR
Calcium	422.67	8 - 10	0.9 - 1.1	5
Iron	248.33	12 - 15	0.8 - 1.0	5
Copper	324.75	4 - 5	0.8 - 1.0	5

*For all the elements:

Lamp source = Hollow cathode lamp of the respective element;

Flame type = Air-Acetylene mixture - fuel lean.

standard solution was re-aspirated to ascertain instrumental stability. Also, readings for the standards of each element were taken each time the A.A.S. was used to allow for any instrumental fluctuations.

Calculation:

The mineral concentration (in mg/100g) for each sample was calculated using the following expression.

Metal Concentration (mg/100g)

$$= \frac{100 \times [\text{Vol. to which diluted}]\ cm^3 \times [\text{ppm}]}{\text{Sample Wt(g)} \times 1{,}000}$$

$$\equiv \frac{[\text{Vol. to which diluted}]\ cm^3 \times [\text{ppm}]}{10 \times \text{Sample Wt (g)}} .$$

CHAPTER III

RESULTS AND DISCUSSION

3.1 INTRODUCTION.

The ten samples analysed have been grouped into four categories:-

(i) The Mackerels - comprising samples A, B, and C;

(ii) The Beefs - comprising samples D, E, F and G;

(iii) The Sardines - comprising samples H and J, and

(iv) Crayfish comprising sample K.

For the variation of temperature and length of heating, all the ten samples were analysed using 0.25g sample weights, and taking an average of three close determinations for each reading. However, for the variation of the sample size, one sample was chosen from three of the above groups and these were analysed only for their calcium and iron contents.

3.2 MOISTURE CONTENTS OF THE SAMPLES.

The moisture contents of the samples determined as described in section 2.4 are shown in table 3.1.

The moisture contents of the analysed canned samples range from 51.3 to 66.7 (%). As can be seen from table 3.1 the moisture contents of the sardines are very simil-r

TABLE 3.1

MOISTURE CONTENTS OF THE SAMPLES.

SAMPLE	Wet Wt. (g)	Dry Wt. (g)	Wt. of Moisture (g)	Moisture (%)
A	4.2	1.4	2.8	66.7
B	5.7	2.2	3.5	61.4
C	6.4	2.3	4.1	64.1
D	11.7	4.4	7.3	62.4
E	6.1	2.1	4.0	65.6
F	7.6	3.7	3.9	51.3
G	9.5	4.0	5.5	57.9
H	7.5	2.8	4.7	62.7
J	5.2	1.9	3.3	63.5
K	5.4	5.0	0.4	7.4

(62.7 and 63.5%), while the widest variation is obtained for the beefs (51.3 - 65.6%); the range for the mackerels being (61.4 - 66.7%): The very low value of the moisture content of the crayfish is not unexpected; this being because the sample was obtained already fairly dried from the market. Generally, the moisture contents of the mackerels are highest, closely followed by the sardines and lastly the beefs with the exception of sample E (Power King Canned beef), which has a moisture content comparable to those of the mackerels.

For the canned samples, the values of the moisture contents obtained compare favourably with those cited in the literature; for Mackerel: 60 - 67% (1, 4, 6, 16); Corned beef and Luncheon meat: 52 - 58.5% (1, 4, 6, 16) and Sardine 50 - 65% (1, 6, 16). The higher values obtained for some of the samples might be due to additional water added to the materials during processing as part of their formula for preservation and additives (6, 13, 15). Also, in addition to the excess water resulting from industrial processing of the canned samples, incompletely drained volatile oils in which they were preserved (particularly the beefs and the sardines), might contribute to the measured moisture (or 'total volatile') contents of these samples.

3.3 ESTIMATION OF PERCENTAGE RELATIVE STANDARD DEVIATIONS OF ANALYSIS.

In order to estimate how much spread would be expected using the various variations of digestion procedure, trial runs of ten determinations for each temperature and element were measured using sample F. As can be seen from table 3.2, the values of the percentage relative standard deviations obtained for all the metal contents at all temperatures are reasonably low. Thus, results obtained by using these procedures are likely to be reliable and reproducible.

TABLE 3.2

ESTIMATED PERCENTAGE RELATIVE STANDARD DEVIATIONS* AT ALL TEMPERATURES USING SAMPLE F.

ELEMENT	80 ± 2°C	100 ± 2°C	120 ± 2°C
Ca	1.8	1.0	1.0
Fe	3.3	3.4	2.0
Cu	3.5	3.0	4.1
Na	1.4	1.6	1.6

*Each result was calculated from ten determinations.

3.4 CALCIUM CONTENTS.

The maximum contents of calcium obtained at the various temperatures are shown in table 3.3. Table 3.3 also shows the variation of maximum calcium content with sample size for samples C, F and H. The variation of calcium content obtained with heating time at the three temperatures studied is shown in appendix I. Figures 3.1 and 3.4 show the variation of the maximum calcium value with temperature and sample size respectively for representatives of the various groups. The variation of the amount of calcium with heating time for representatives of the groups is shown in figures 3.2 and 3.3.

The amounts of calcium obtained varied from group to group. The highest calcium contents were obtained for the sardines -H and J with a range of 434 - 1070 mg/100g, while the calcium contents obtained for the beefs are in the range 120 - 332mg/100g. The values for the mackerels are intermediate between the two at 434 - 920 mg/100g. The calcium contents obtained for the crayfish are the lowest with a range of 60 - 72 mg/100g. The values obtained for the beefs and crayfish are within the range (30 - 300 mg/100g), (4), expected for animal tissues excluding the bones, while the values for the mackerels and sardines,

TABLE 3.3

MAXIMUM CALCIUM CONTENTS (mg/100g) OF THE SAMPLES* AT VARIOUS TEMPERATURES.

SAMPLE	80 ± 2°C	100 ± 2°C	120 ± 2°C
A	920	888	808
B	840	840	828
C	610	590	610
C (0.50g)	600	510	485
C (0.75g)	634	484	434
D	230	220	208
E	126	120	124
F	332	220	230
F (0.50g)	260	236	236
F (0.75g)	302	212	166
G	320	284	254
H	960	912	868
H (0.50g)	1070	975	830
H (0.75g)	880	834	720
J	745	575	434
K	72	62	60

*Unless otherwise stated the sample size for each determination was 0.25g.

analysed with the bones, are as would be expected outside this range. The values of calcium contents obtained for the sardines are fairly in agreement with the value of 409 mg/100g quoted by McCance and Widdowson (16) for canned sardine. These values however, differ significantly from the 33 mg/100g (uncanned sample excluding bone) for sardine quoted by Charley (14) and 30mg to 300 mg/100g quoted by Allen et al (4) for animal tissues. It has also been observed that the calcium contents of samples with bones (i.e. mackerels and sardines), are two to five times higher than those without bones (c.f. the beefs) - as is the case in this work. The levels of calcium observed for the samples might also be influenced by added salts and other ingredients during industrial processing.

It is observed that generally the maximum calcium values for all the samples decrease as the temperature is raised in the order 80°C > 100°C > 120°C (fig. 3.1). Except in two cases where there is an initial increase at 80°C, the maximum value also tend to decrease with increase in sample size at a given temperature (fig. 3.4). The decrease with temperature is probably due to volatilisation losses and is similar to the observation for plant materials (53). The decrease with increase in sample size is possibly due to enhanced precipitation

as the insoluble sulphate together with the losses due to volatilisation.

The trends in the calcium contents as the heating time is increased for most of the samples are fairly similar at the different temperatures (figs. 3.2 & 3.3) At $80^{o}C$, for all the samples with the exception of the sardines and Power-king mackerel, the calcium contents obtained increase with the heating time until a peak amount is obtained followed by a decrease in calcium contents determined with subsequent heating. For the mackerels (A & B only), heating time for maximum calcium content is between 40 - 50 minutes, for the beefs it is 20 - 30 minutes while 20 minutes of heating is required for the crayfish. Maximum calcium contents were obtained for the sardines and Power King mackerel during the first ten minutes of heating at this temperature ($80^{o}C$). At $100^{o}C$, only the beefs show a gradual rise in the calcium content up to a heating time of 20 - 30 minutes followed by decreasing levels with further heating (fig. 3.2 & 3.3). All the other samples show decreasing calcium levels with further heating. At $120^{o}C$, maximum calcium contents were obtained during the first ten minutes of heating after which the levels decreased on prolonged heating.

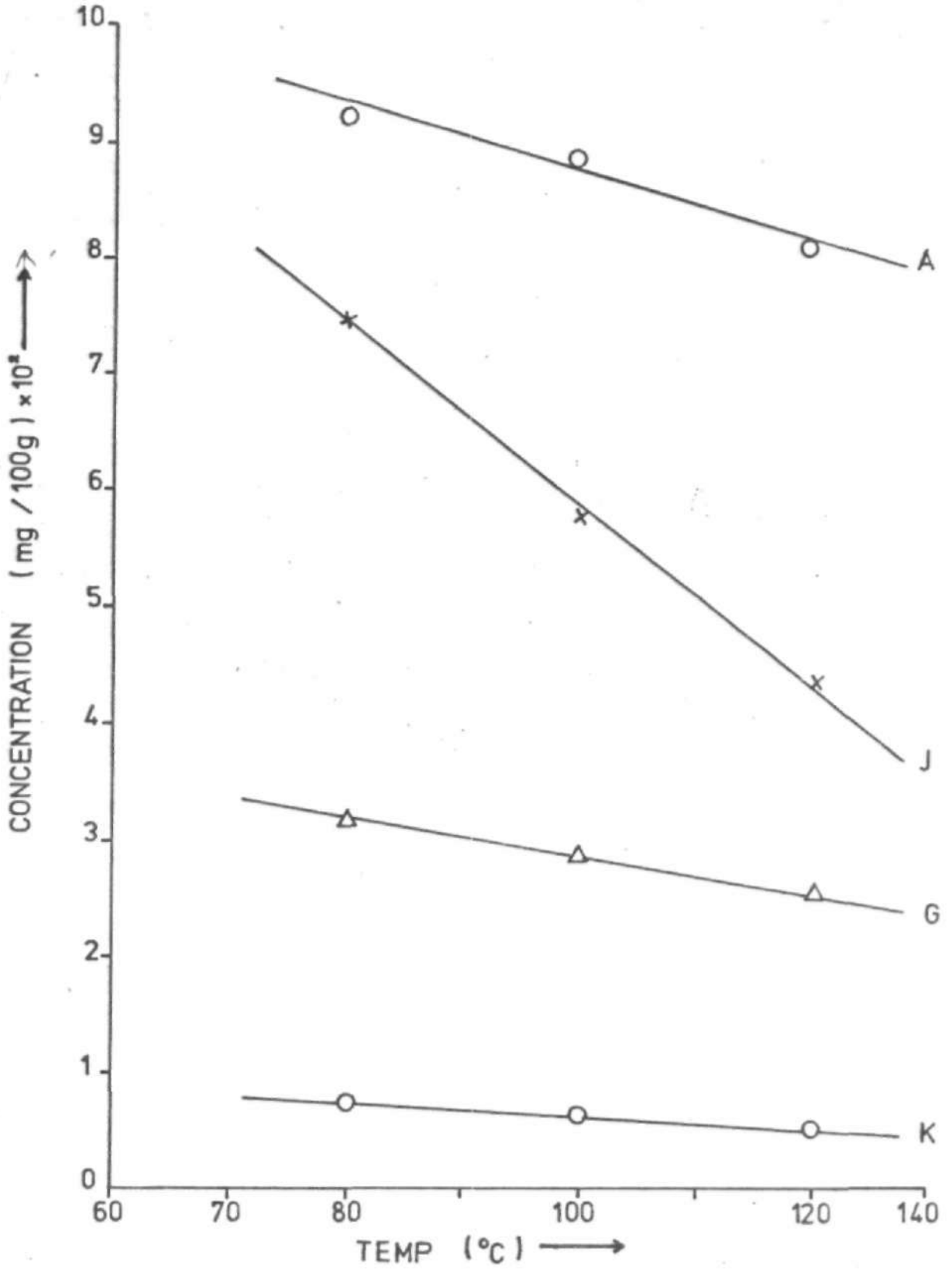


Fig. 3.1: Variation of maximum calcium contents of 0·25g samples with temperature.

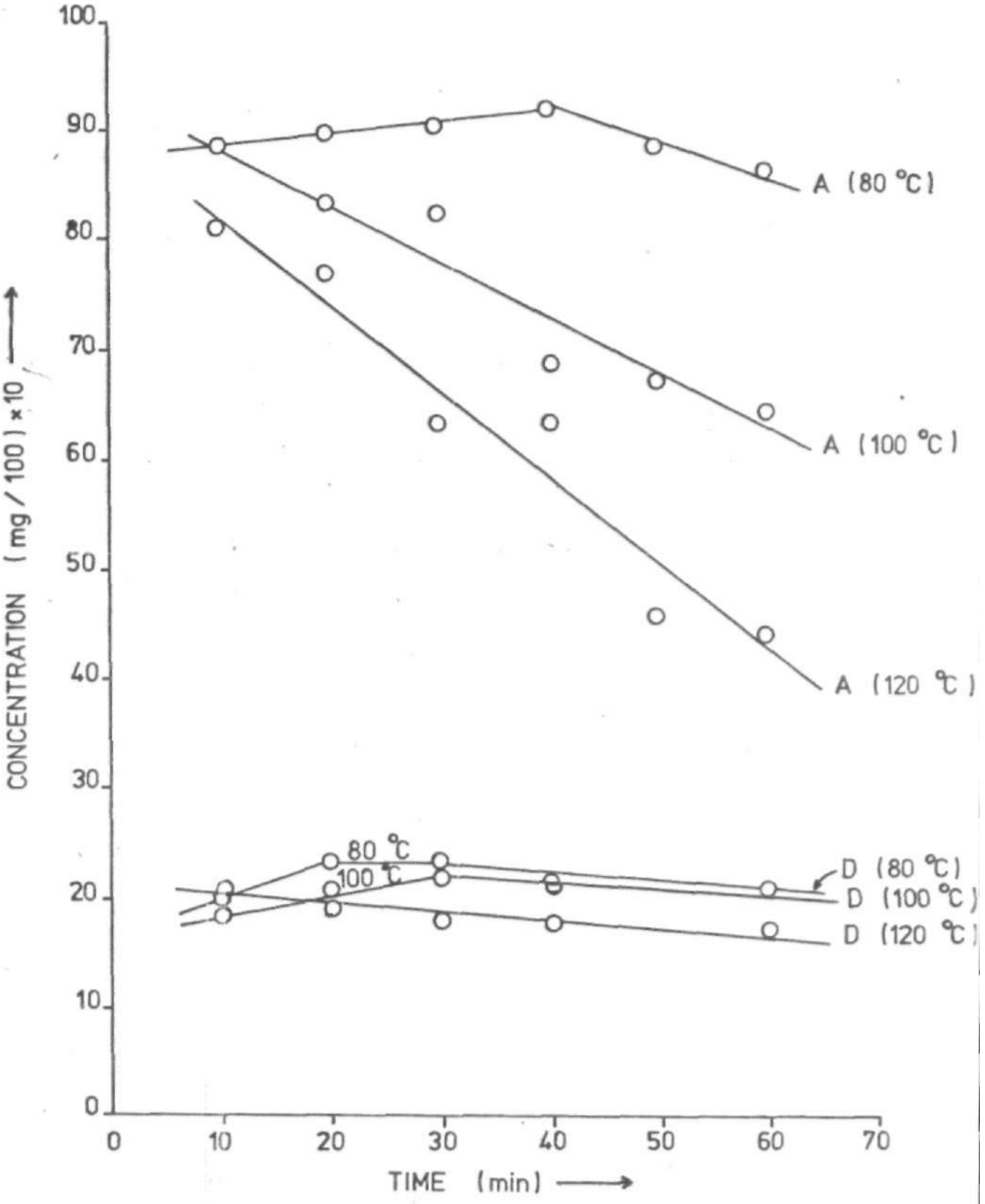


Fig. 3.2 : Variation of concentration of calcium with heating time during digestion (samples A & D)

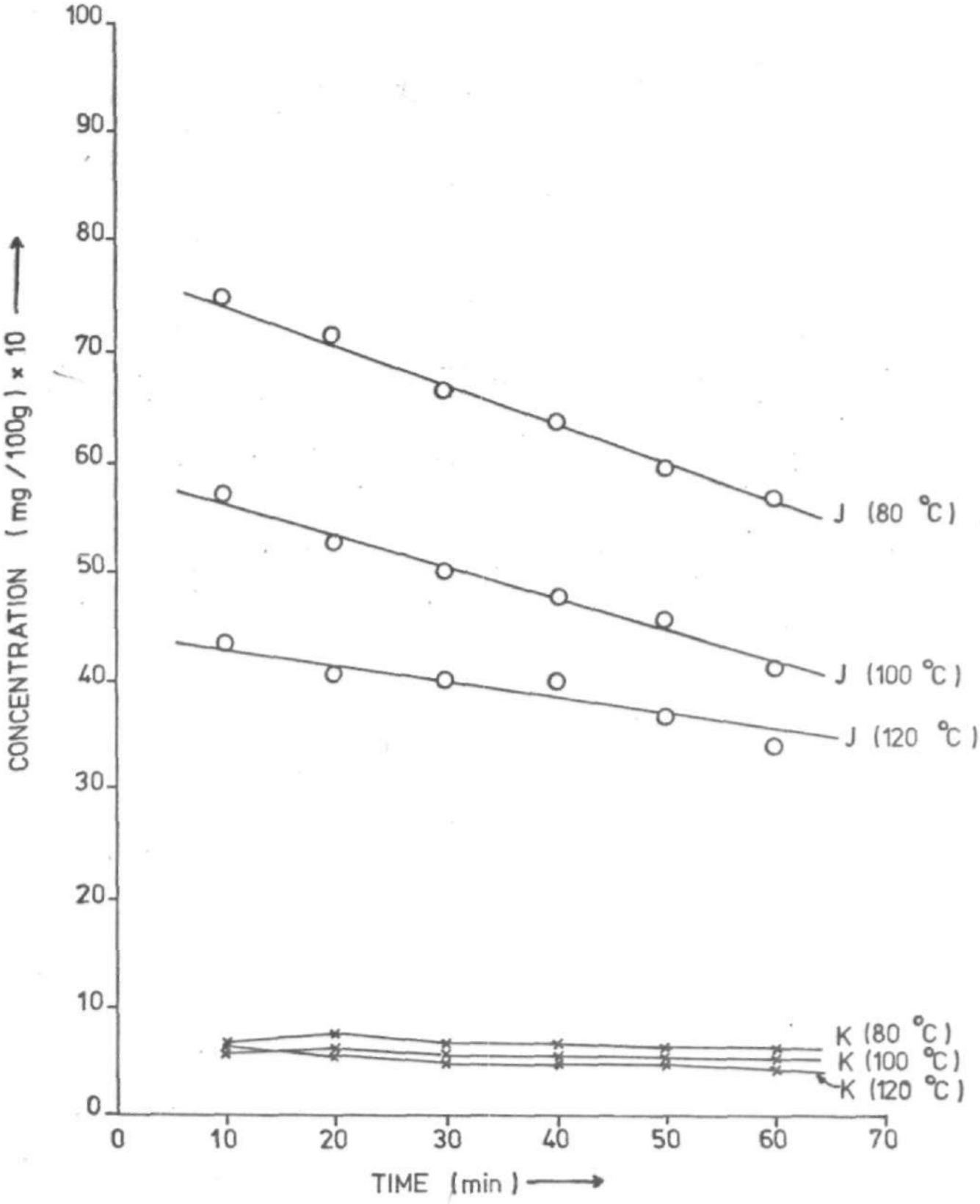


Fig.3·3: Variation of concentration of calcium with heating time during digestion (samples J & K)

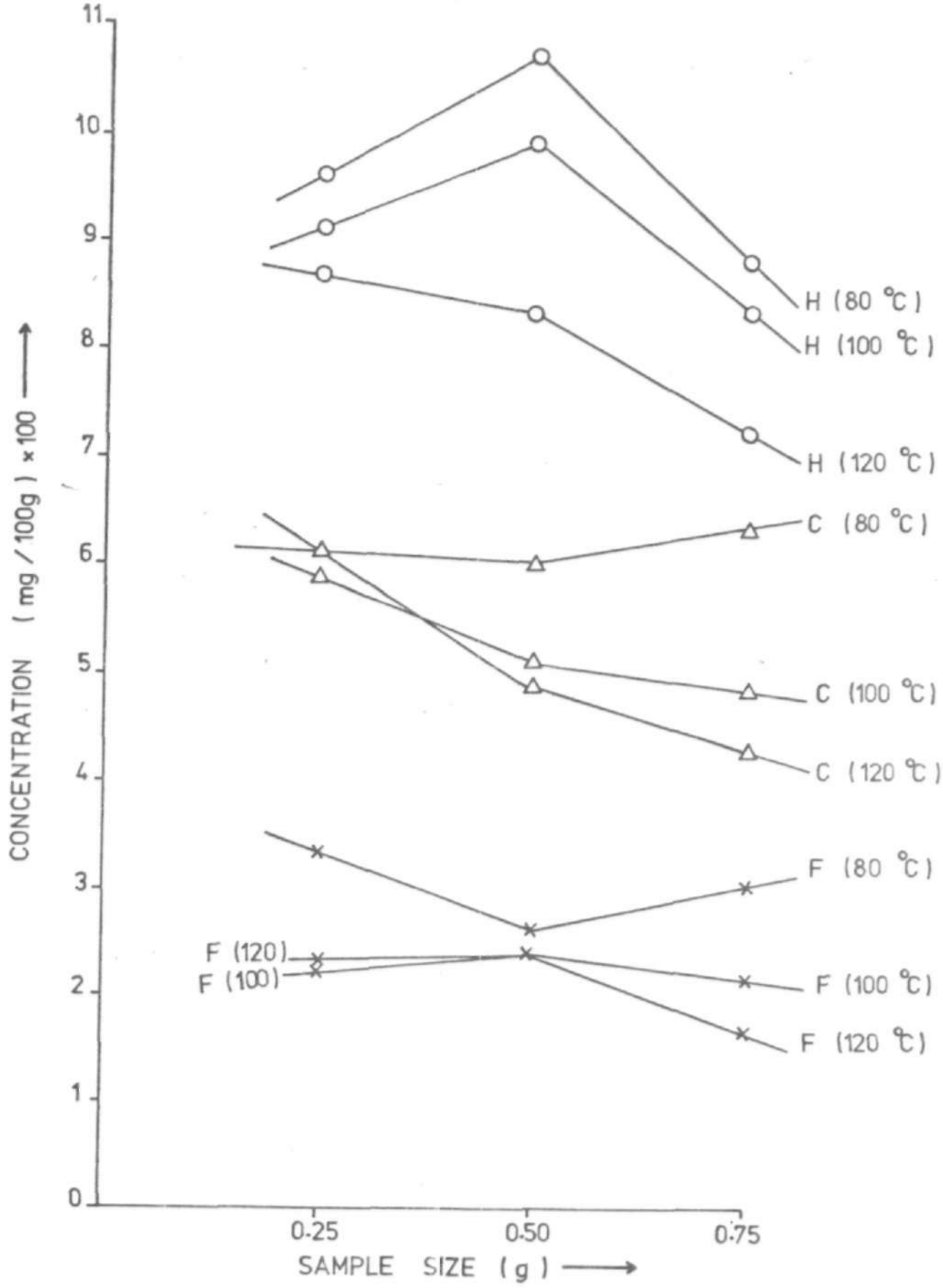


Fig. 3.4: Variation of maximum calcium content with sample size.

In all cases the lowest values were obtained for the longest heating time i.e. 60 minutes. It is also observed that heating times required for peak calcium contents at these temperatures is in the order 80°C > 100°C > 120°C. The maximum amounts of calcium obtained at these temperatures follow the same order except in a few cases where those at 80°C and 100°C are essentially the same. These observations would tend to support the suggestion that losses due to volatilisation are important for calcium in these samples, and agree also with the observations made for plant materials in this laboratory (53).

3.5 IRON CONTENTS.

Table 3.4 gives the maximum contents of iron (mg/100g) obtained for all the samples at the three temperatures. The variation of maximum iron content with temperature is illustrated in fig. 3.5 for samples A, D, E, J and K, drawn from the four groups. The variation of the amount of iron determined with heating time during wet digestion at the three temperatures, and with varying sample size (samples C, F & H), is shown in appendix II, and illustrated in Figures 3.6 and 3.7, and 3.8 (for samples A and J, D and K, C, F and H respectively).

TABLE 3.4

MAXIMUM IRON CONTENTS (mg/100g) OF THE *SAMPLES AT VARIOUS TEMPERATURES.

SAMPLE	80 ± 2°C	100 ± 2°C	120 ± 2°C
A	97	85	58
B	134	134	134
C	42	32	30
C (0.50g)	28	26	25
C (0.75g)	33.6	29.4	26.8
D	70	64	62
E	28	28	32
F	104	102	100
F (0.50g)	84	83	71
F (0.75g)	49.6	44.2	46.2
G	40	40	48
H	46	56	56
H (0.50g)	47	53	57
H (0.75g)	48.2	43.6	47.0
J	24	26	36
K	20	18	18

*Unless otherwise stated the sample size for each determination was 0.25g

The maximum contents (mg/100g) in all the samples have a range of 18 - 134 (mg/100g), with the crayfish (K) having the lowest value while Geisha mackerel (B), has the highest value. Maximum iron contents obtained for samples C (26 - 42), E(28 - 32), G(40 - 48); J(24 - 36 and K(18 - 20) mg/100g, at the various temperatures agree with the range (10 - 40) mg/100g quoted by Allen et al (4), for animal tissues. Values obtained for the other samples are higher (Table 3.4). McCance & Widdowson (16) quoted the following iron contents (uncanned samples): Corned beef (4 mg/100g), Sardine (4 mg/100g) and mackerel (1.2 mg/100g). Generally the concentration of iron in any of the above analysed samples is possibly a result by three factors:- concentration of iron naturally present in the food sample, the extent of contamination during industrial processing as well as during mechanical grinding while preparing sample for analysis, and the extent of contamination by the can in which the product is stored. Thus, the enhanced levels of iron found in the analysed samples (particularly the canned ones), could probably be attributed to the effect of corrosion on them (10, 12, 15). Furthermore, since this latter effect is also a function of other factors such as the

length of storage and the preservative ingredients of the samples, it is not surprising that significant variations are found even among canned products in the same group.

Except for sample B(Geisha), in which the maximum iron contents at the three temperatures have the same value, for the other mackerels (A and C), maximum iron levels decrease with increasing temperature (table 3.4 and fig. 3.5). A similar trend is observed for the crayfish (K) and D(Kondor Corned beef), though the rate of decrease in the latter cases is not as pronounced as for the mackerels. Maximum amounts of iron obtained for the sardines (H and J) increase as the temperature is raised (fig. 3.5). Two of the beefs (E and G), have the same maximum contents at 80°C and 100°C but with a rise at 120°C. These variations in the observed trends of maximum iron contents might be due to varying degree of contribution to the total iron from processing and containing cans. Where such contributions are high, volatilisation losses with increasing temperature might be significant as such additional iron content might not be tightly bound to the biological matrix, and hence the decreasing iron levels with increasing temperature (as in the case of the mackerels).

The opposite situation probably holds for the sardines and two of the beefs (E and G), where the maximum iron contents increase with temperature, (fig. 3.5). This latter situation (i.e. increasing iron levels obtained with increasing heating temperature), has been observed for plant samples analysed in this laboratory (53).

For all the samples analysed, the variation of iron contents with heating time seems to follow a fairly similar trend at 80°C and 100°C. As can be seen from appendix II and figures 3.6 and 3.7, at these temperatures, the iron contents obtained increase gradually with heating time until a maximum value after which there is a gradual decrease on prolonged heating. The heating times at which maximum amounts of iron are obtained differ for the two temperatures as well as for the different groups of samples. (i.e. mackerels, beefs, sardines and crayfish). Within each group, however, the heating periods for the maximum values of iron are closer but also differ for the two temperatures in some cases. At 80°C, maximum iron contents are obtained for A and B within 40 - 50 minutes heating time while that for C is between 20 - 30 minutes. At 100°C, maximum iron contents are obtained for all the mackerels after heating for about 20 minutes (i.e. peak values obtained faster at 100°C). For the beefs

(samples D, E, F & G), at 80°C maximum iron contents are obtained after heating periods ranging from 20 to 40 minutes. At 100°C, maximum contents are obtained after heating for 20 minutes except for sample D, with a peak time of 30 minutes. The peak period for all the other samples heated at 100°C is 20 minutes. At 120°C, the variation in iron content with the length of heating follows essentially the same pattern (fig. 3.6 and 3.7) for all the samples except for the sardines (samples H, J). For the sardines maximum contents are obtained after heating for 20 minutes, followed by a gradual decrease in the iron contents obtained. For all the other samples (i.e. mackerels, beefs and crayfish), the iron content decreases with increase in heating time, implying a peak value after 10 minutes or in a shorter time. It would appear that there are defined lengths of heating for peak contents at each temperature for the samples. These periods vary more among the samples at the lower temperature of 80°C. The heating periods for maximum iron contents at this temperature are longest for each sample or group. The gradual decrease in the value obtained after the peak value for the samples at each temperature may be attributed to volatilisation losses of iron on prolonged heating. The observed variations in the iron levels with heating time agree with those obtained for plant samples in this laboratory (53).

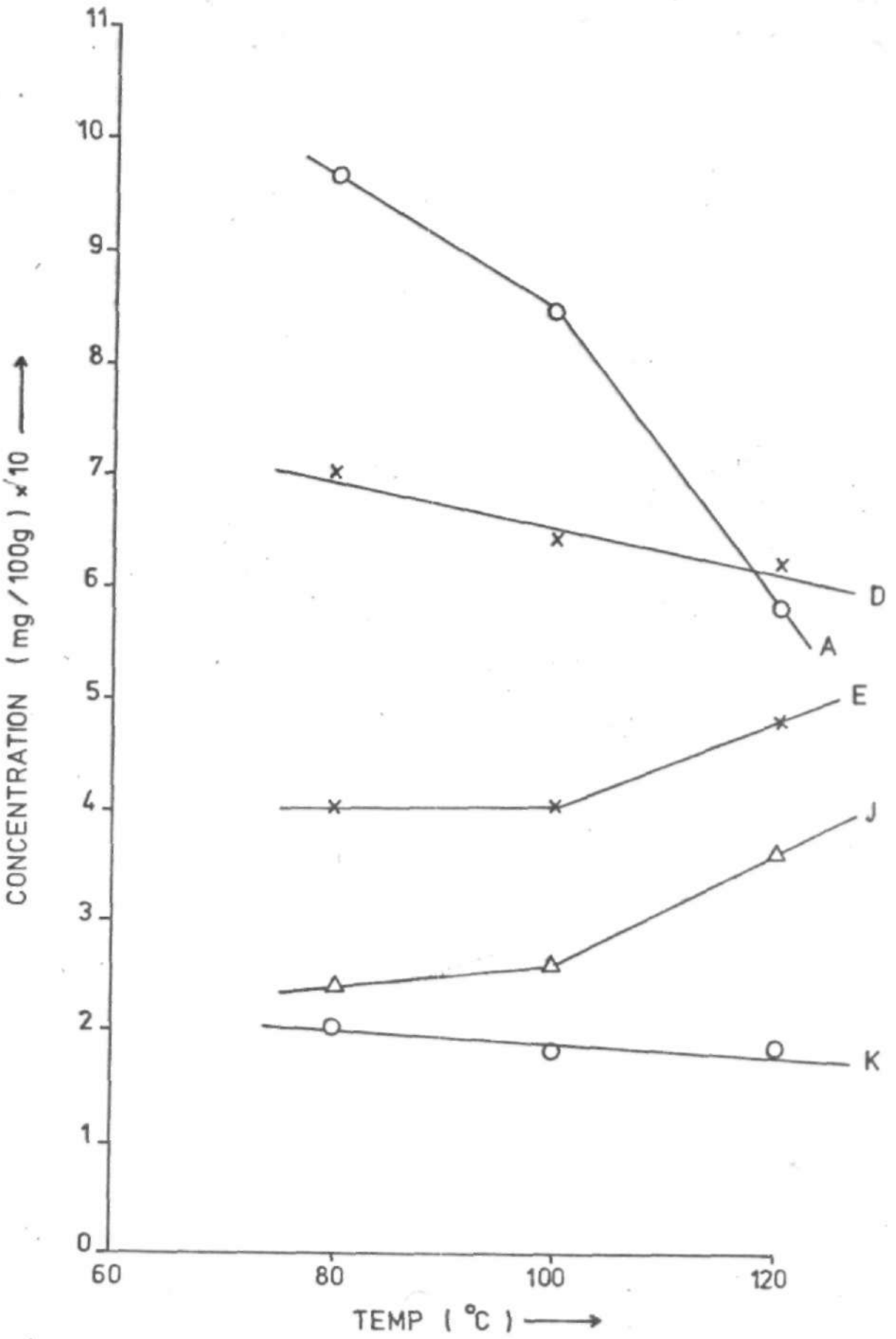


Fig. 3.5 : Variation of maximum Iron contents of 0·25g samples with temperature.

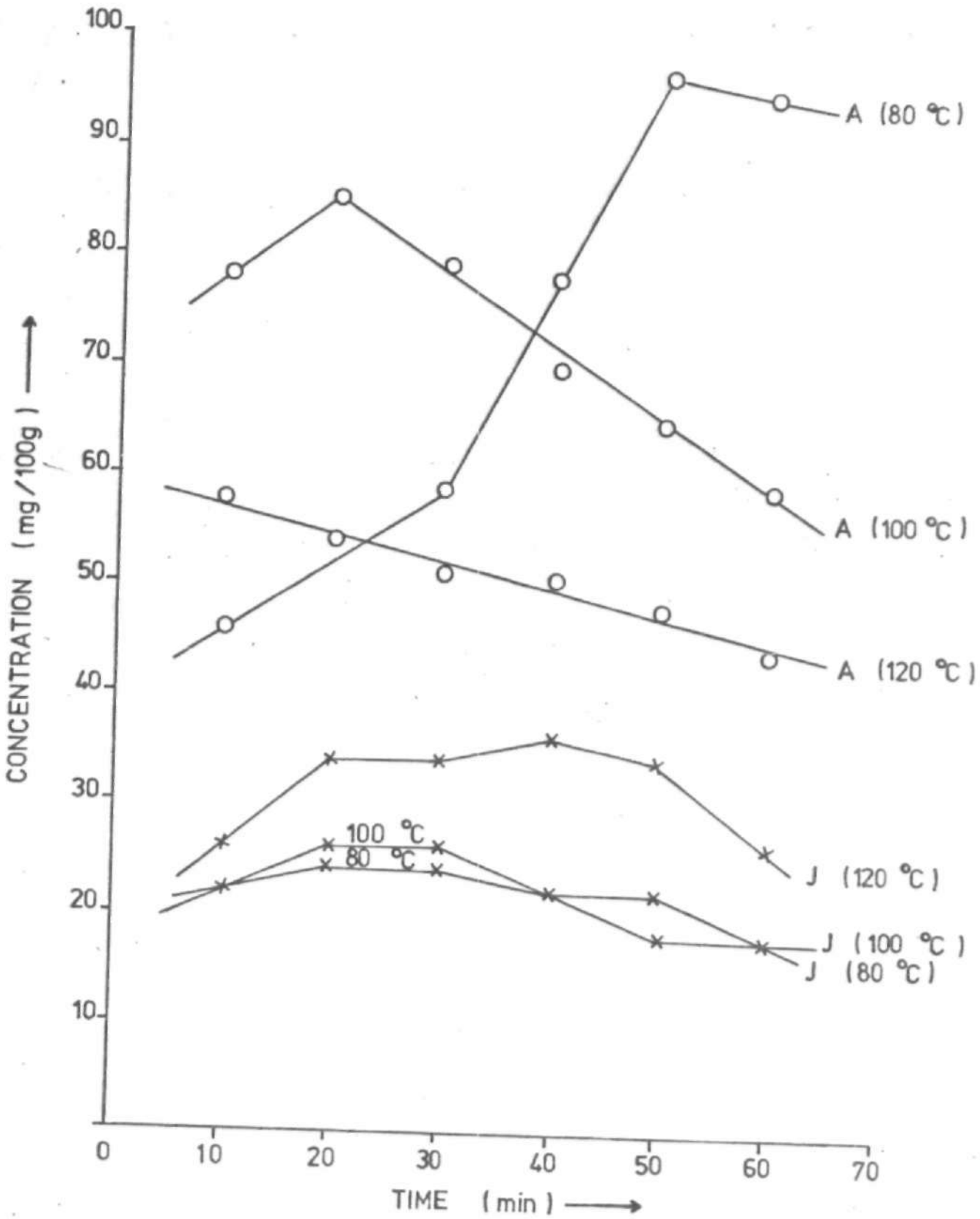


Fig. 3.6: Variation of concentration of Iron with heating time during digestion (samples A & J)

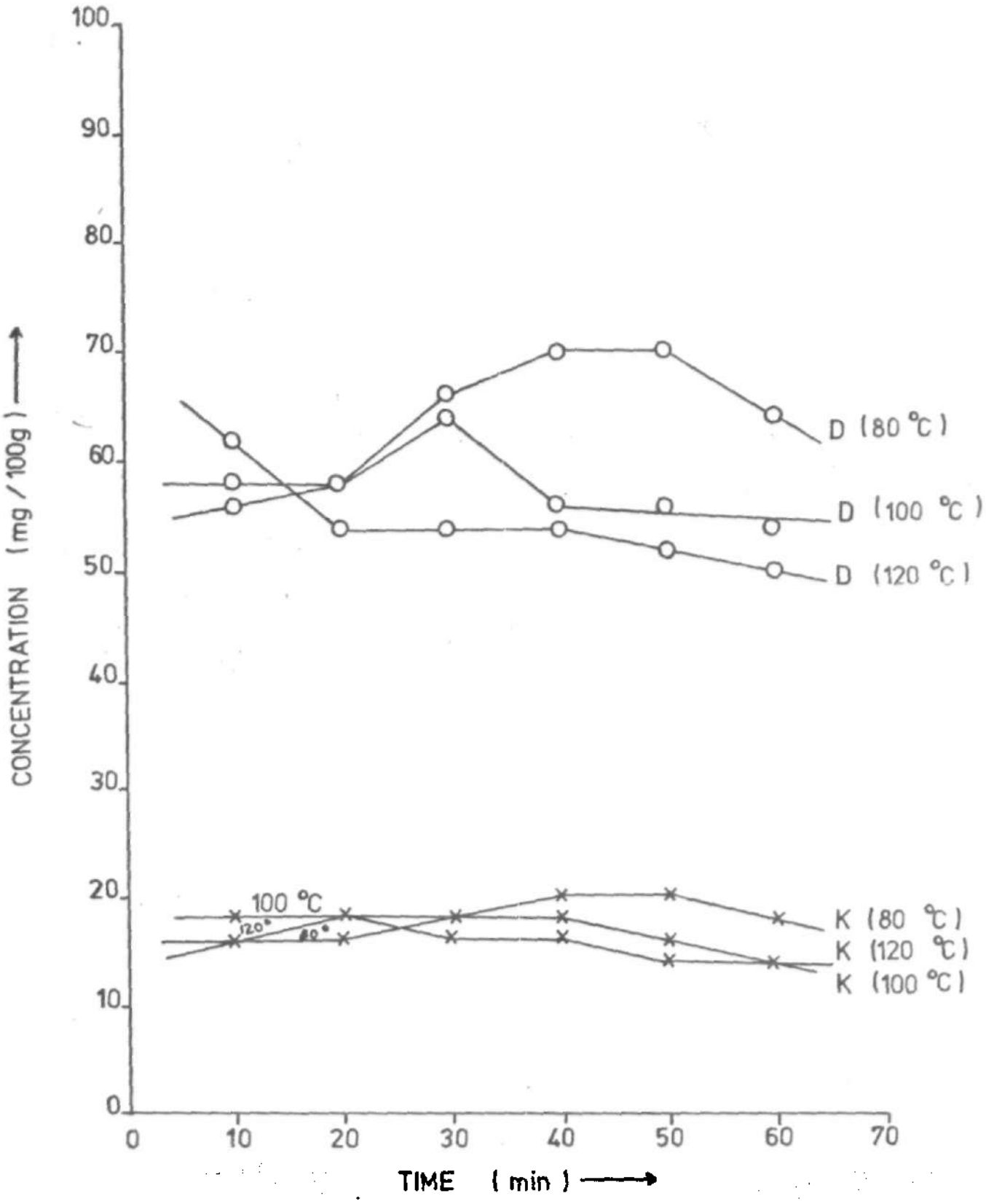


Fig 3 7 Variation of concentration of Iron with heating time during digestion (samples D & K)

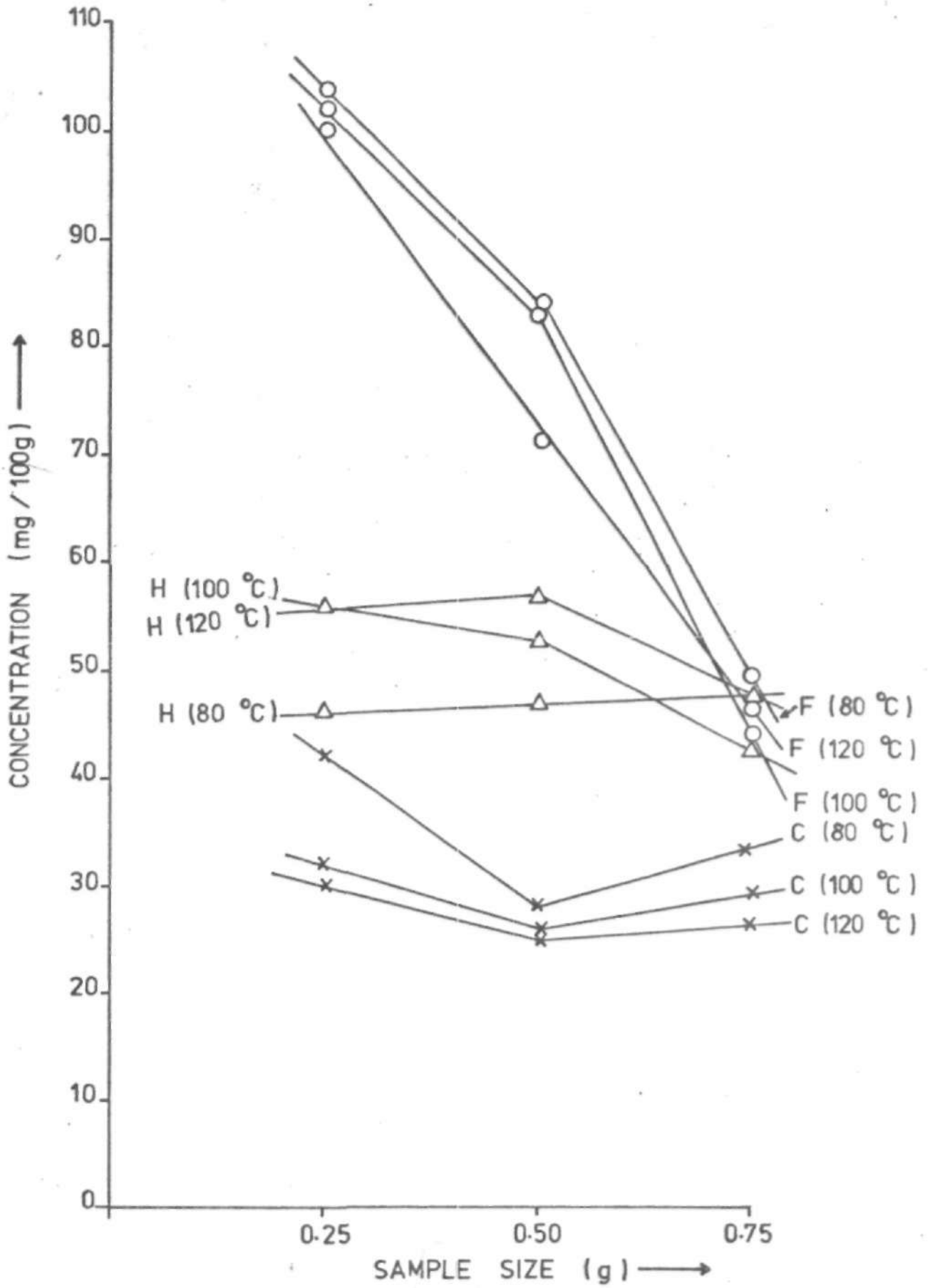


Fig. 3.8: Variation of maximum Iron content with sample size.

As can be seen from fig. 3.8 and appendix II, except for the sardine (H) at 80°C, and 120°C (only up to 0.5g), generally there is a decrease in maximum iron content obtained with increase in sample size. The rate of decrease is most pronounced for sample F(a beef). For sample C, the largest decrease was obtained at 0.5g sample size. The increased volumes of the nitric acid as the sample size was increased (see table 2.2), might have possibly led to increased depression in the response to iron by the A.A.S. (24, 25, 36). Precipitation as insoluble sulphate might also have increased with increase in sample size.

3.6 COPPER CONTENTS.

Table 3.5 shows the maximum copper contents (mg/100g) of all the samples at the three temperatures. The variation of maximum copper content with temperature is illustrated in fig. 3.9 (for samples B, F, J and K), while the variation of copper contents with heating time during wet digestion at the three temperatures is reported in appendix III and illustrated in Fig. 3.10 and 3.11 for samples B, J, and F, K, respectively. The values of copper contents of the samples at the three temperatures range from 1.0 to 9.4 mg/100g. These values are in agreement with the copper contents of animal tissues of 1 - 10 mg/100g quoted by Allen et al (4).

From appendix III and fig. 3.9, it is seen that highest copper contents were obtained for the mackerels (4 - 9.4 mg/100g), followed by those obtained for the crayfish (3 - 4.8 mg/100g), while values obtained for the beefs and sardines range from 1.0 to 3.4 mg/100g. Trends in the maximum copper contents obtained at the three temperatures differ slightly for the various groups. For the sardines and the crayfish, there are slight decreases in the maximum copper content in going from 80°C to 100°C, after which the values become constant.

3.6 COPPER CONTENTS.

TABLE 3.5

MAXIMUM COPPER CONTENTS (mg/100g) OF THE SAMPLES* AT VARIOUS TEMPERATURES

SAMPLE	80 ± 2°C	100 ± 2°C	120 ± 2°C
A	7.4	5.8	8.8
B	9.0	9.4	9.0
C	4.4	6.0	4.8
D	3.0	3.0	3.4
E	2.0	1.2	0.8
F	3.0	3.0	2.6
G	1.8	1.4	1.4
H	2.2	1.8	1.8
J	3.4	2.6	2.6
K	4.8	3.6	3.6

* 0.25g sample size used.

Except for sample D, the beefs tend to demonstrate some decrease in the maximum copper contents as the heating temperature is raised. These observations tend to suggest the possibility of slight volatilisation losses with an initial rise in heating temperature, followed by a levelling up of this effect at the higher temperatures. Except for sample A, the makerels show a rise in the maximum copper content as the temperature is raised from 80°C to 100°C followed by a small decrease at 120°C. The latter decrease might also be due to volatilisation losses, particularly in sample C.

As can be seen from figures 3.10 and 3.11, and appendix III, variations in the copper contents with heating time at the three temperatures are not as pronounced as for calcium, iron and sodium. This is particularly evident at 100°C and 120°C where, in some cases, there is hardly any variation at all. However, the samples still demonstrate some peak values with increase in the lengths of heating, the heating lengths being as follows; at 80°C, 40 - 60 minutes for the mackerels; 10 - 50 minutes for the beefs and 30 minutes for the sardines and the crayfish; at 100°C, 20 - 50 minutes for the mackerels and the beefs, 10 - 20 minutes for the sardines and the crayfish; at 100°C, 30 minutes for the

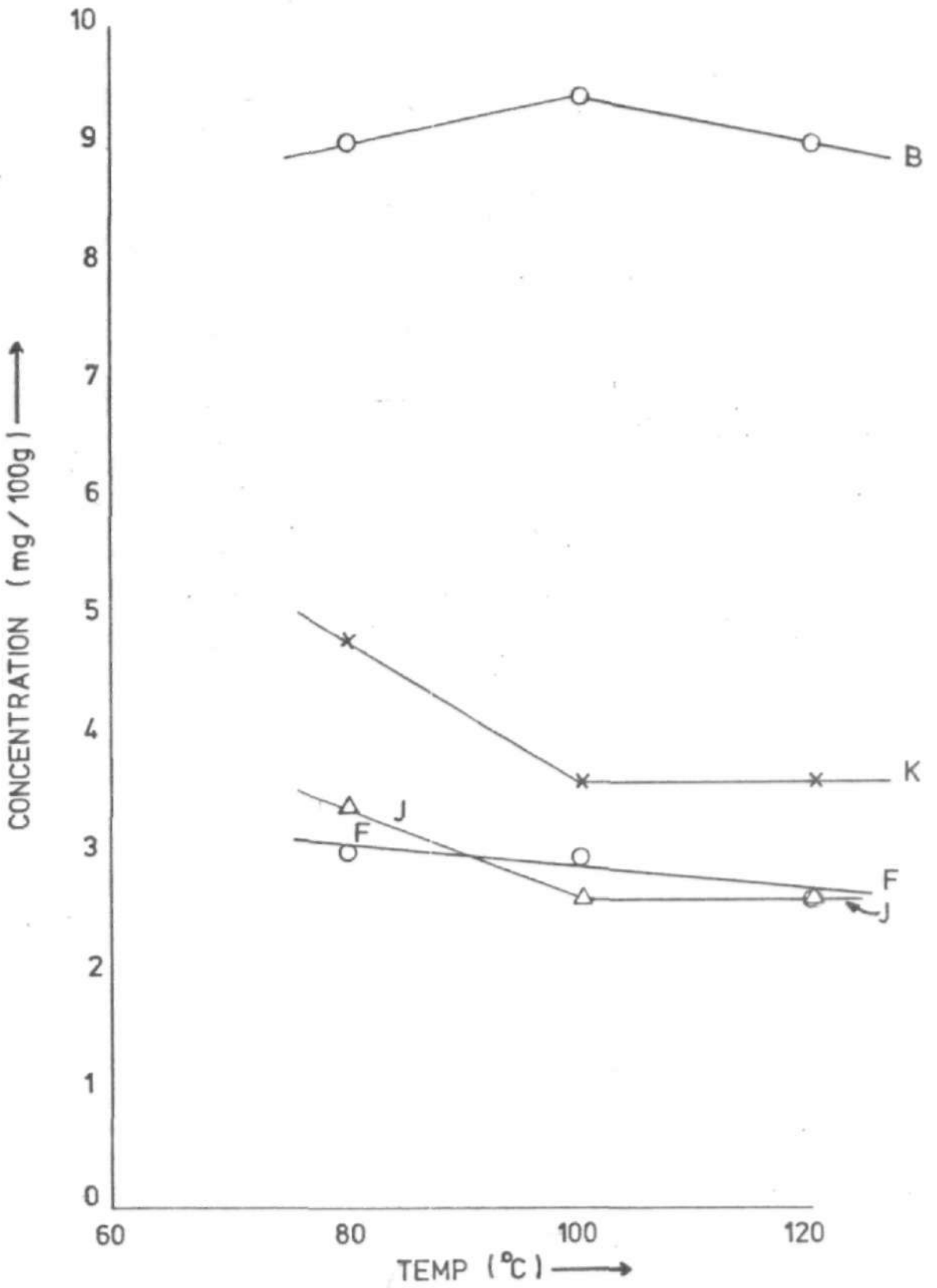


Fig. 3. 9 : Variation of maximum copper contents of 0.25g samples with temperature.

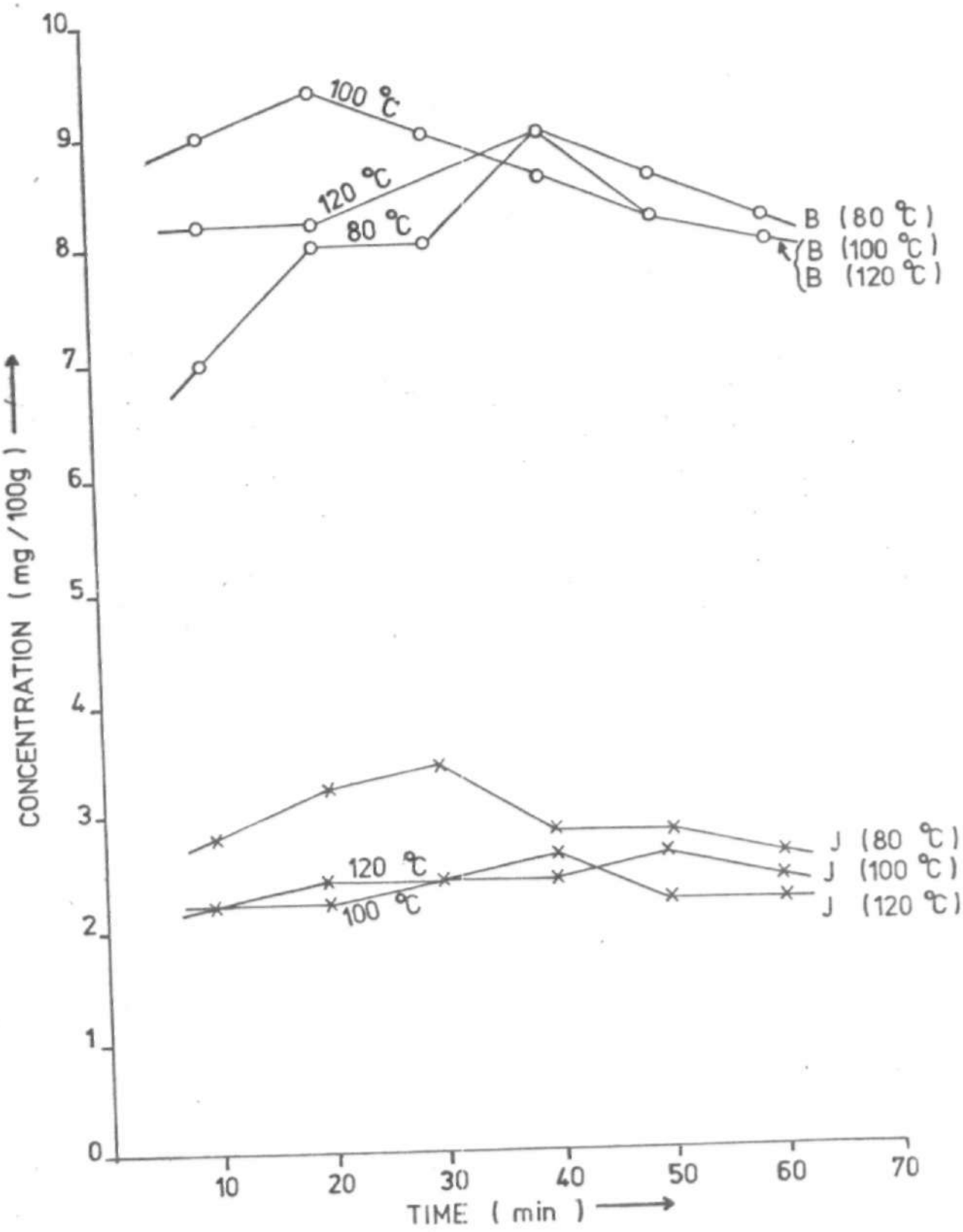


Fig. 3.10 : Variation of concention of copper with heating time during digestion (samples B & J)

mackerels, 10 - 30 minutes for the beefs, 10 - 30 minutes for the sardines and 30 minutes for the crayfish. Results obtained on plant materials in this laboratory showed that volatisation losses are not important as there were rather insignificant variation in the copper levels with varying heating time and temperature (53).

3.7 SODIUM CONTENTS.

The maximum sodium contents of all analysed samples at the three temperatures are shown in table 3.6 and illustrated for samples A, F, H and K from the four groups in figure 3.12. The variation of sodium contents of the samples with heating time during digestion at the three temperatures is shown in appendix IV and illustrated for samples A, D, and K, H in figures 3.13 and 3.14 respectively.

The sodium contents of the analysed samples show a range of 630 - 3,200 mg/100g. The beefs show the highest sodium contents followed by the crayfish while the sardines have the lowest concentrations. A sodium content of 785 mg/100g quoted by McCance and Widdowson (16), for tinned sardines agrees with the values obtained for the sardines in this work (table 3.6 & appendix IV). A range of 200 - 1,000 mg/100g has been

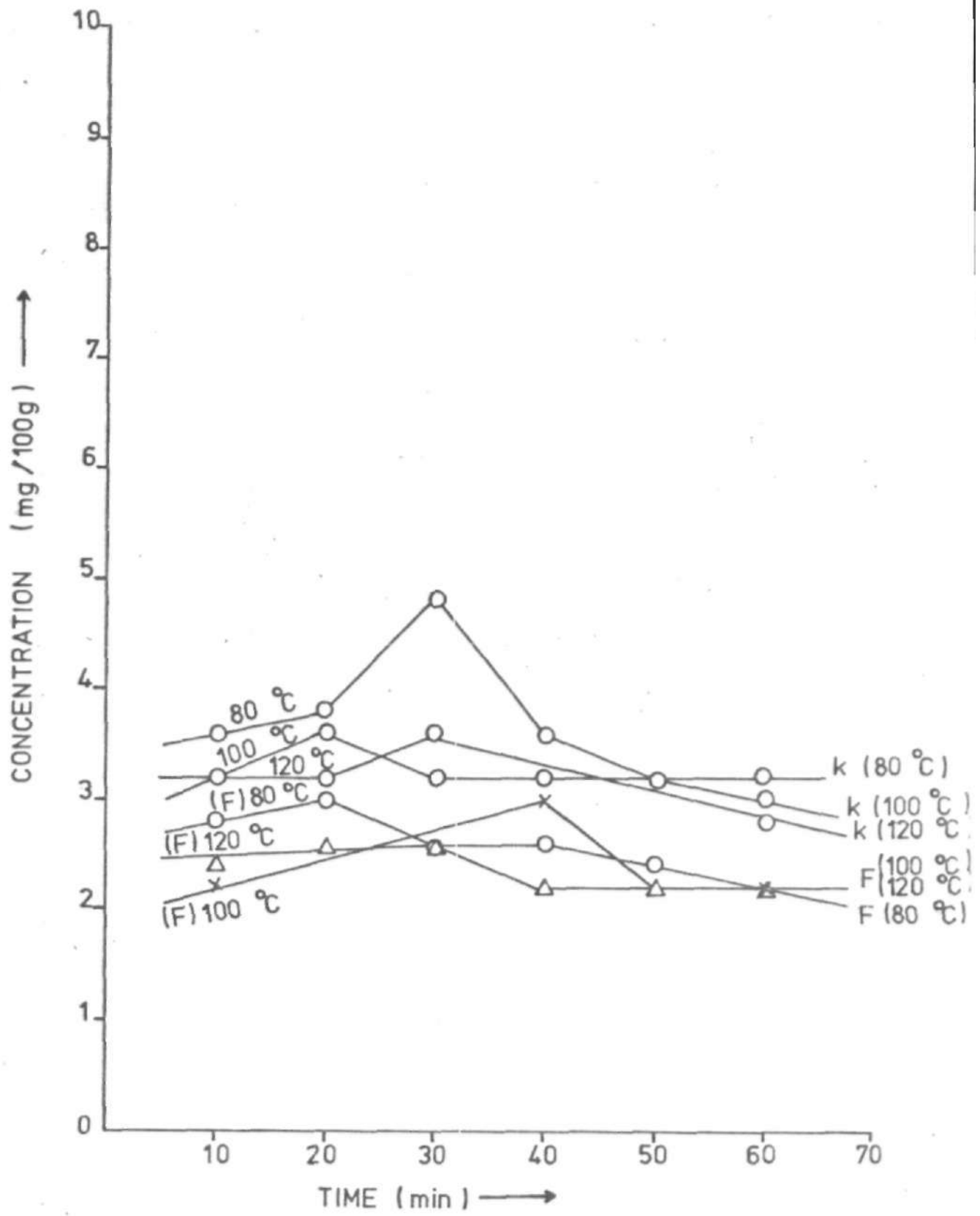


Fig. 3.11 : Variation of concentration of copper with heating time during digestion (samples F & K)

TABLE 3.6

MAXIMUM SODIUM CONTENTS (mg/100g) OF THE SAMPLES* AT VARIOUS TEMPERATURES (X 100).

SAMPLE	80 ± 2°C	100 ± 2°C	120 ± 2°C
A	12.3	11.9	11.3
B	24.5	23.8	22.8
C	8.8	7.9	7.6
D	27.5	27.5	26.9
E	30.1	29.0	28.8
F	30.2	29.2	27.8
G	32.0	30.9	31.5
H	10.4	9.5	9.0
J	8.7	8.4	7.0
K	26.4	25.0	21.5

* 0.25g sample size used.

quoted by Allen et al (4), and McCance and Widdowson (16), for sodium contents in animal tissues without the added salts. Some values obtained for the analysed samples fall outside this range. This is due mainly to the added sodium salts, particularly sodium chloride (table 2.1) as preservative ingredients during industrial processing. The magnitude of the difference in the obtained sodium contents would depend on both the quantity and the number of sodium salts added to each sample (9, 12, 14).

From table 3.6 and figure 3.12, it can be seen that, as is the case with calcium, the maximum sodium contents for all the samples decrease as the temperature is raised in the order $80^{o}C > 100^{o}C > 120^{o}C$. The decrease is probably due to volatilisation losses resulting from the increased heating temperature. This may also imply that sodium is not much tightly bound to the organic matrix and can be easily dislodged from the matrix at low temperatures. This observation differs from that obtained for plant materials in this laboratory (53).

Appendix IV, figures 3.13 and 3.14 show that the variation of sodium contents with temperature and heating time is similar to that obtained for calcium

(Appendix I; figures 3.2 and 3.3). At 80°C, all the samples show an increase in sodium content with increase in heating time up to a peak value after which further heating results in lower sodium contents. The heating times for maximum sodium values at this temperature are 40 minutes for the mackerels; 20 - 30 minutes for the beefs; 30 - 50 minutes for the sardines and 30 minutes for the crayfish. At 100°C, the trends are similar but peak values were obtained within shorter heating times of 10 minutes for the mackerels; 20 - 30 minutes for the beefs, sardines and crayfish. At 120°C, only the sardines show a small increase in content up to 20 - 30 minutes; all other samples gave maximum sodium contents within 10 minutes of heating or in a shorter time. The significant decrease in the sodium contents with prolonged heating time particularly at high temperatures probably implies volatilisation losses under these conditions. These observations agree with those obtained for the plant materials analysed in this laboratory (53).

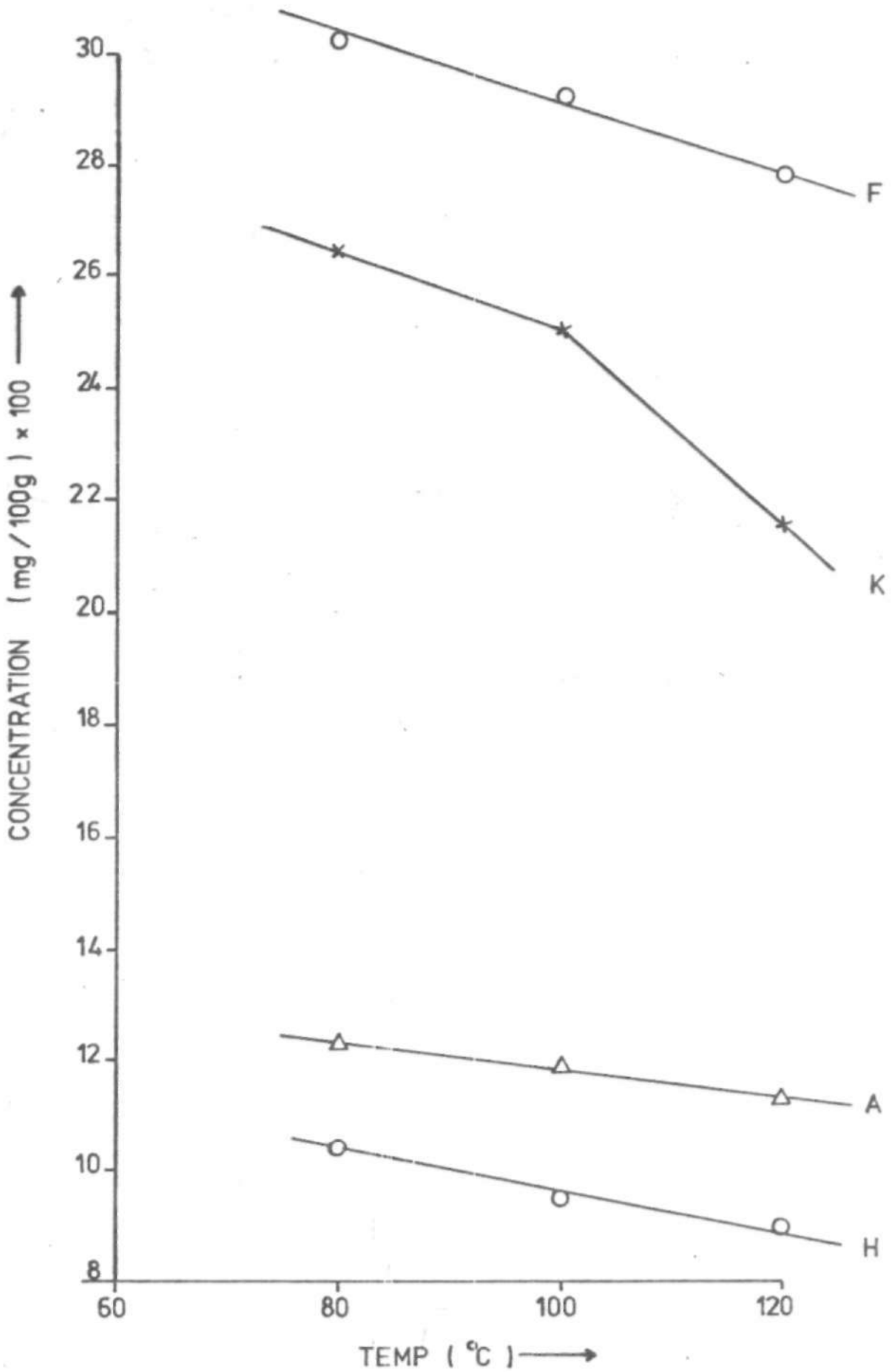


Fig. 3.12: Variation of maximum sodium contents of 0.25g samples

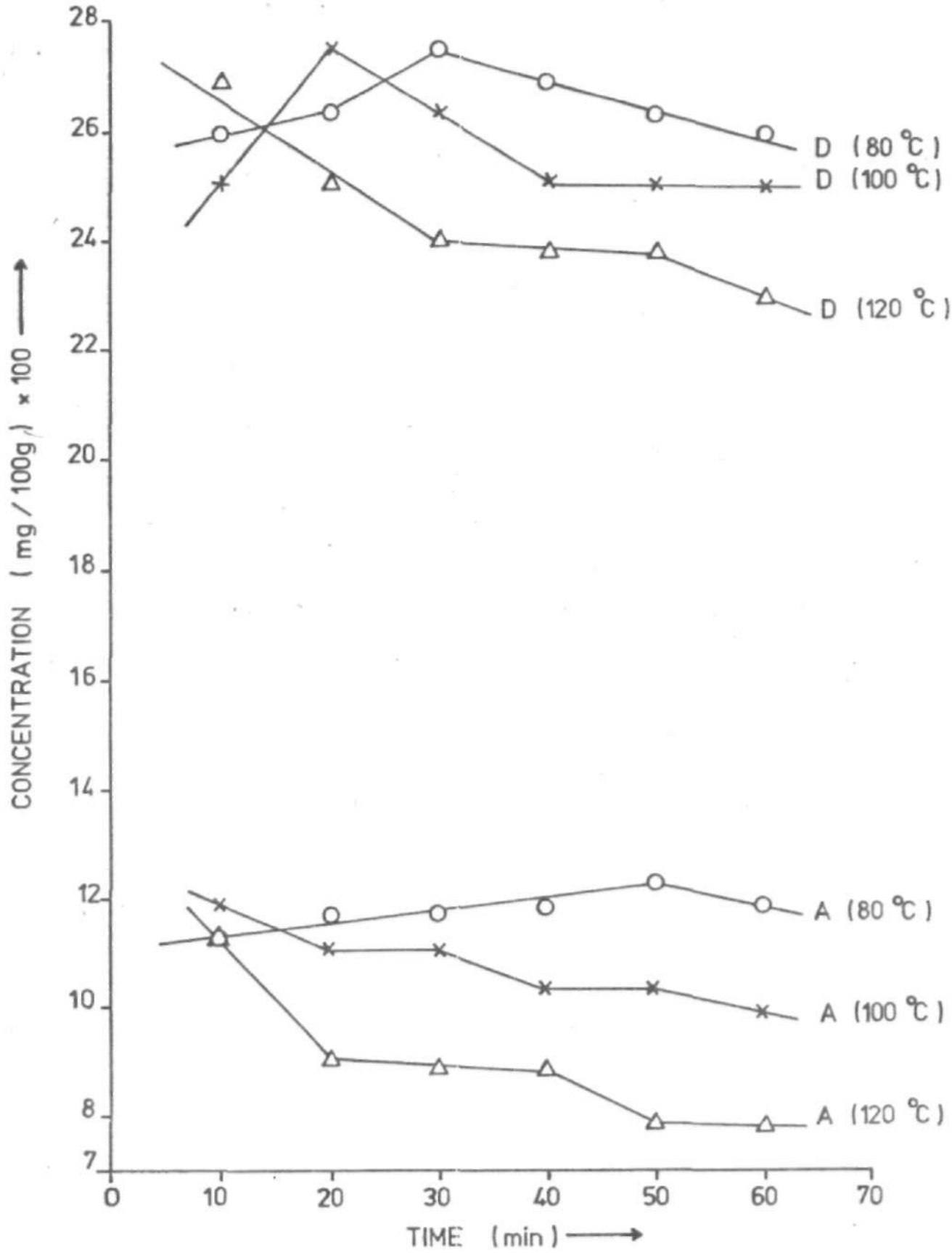


Fig. 3.13: Variation of concentration of sodium with heating time during digestion samples (A & D).

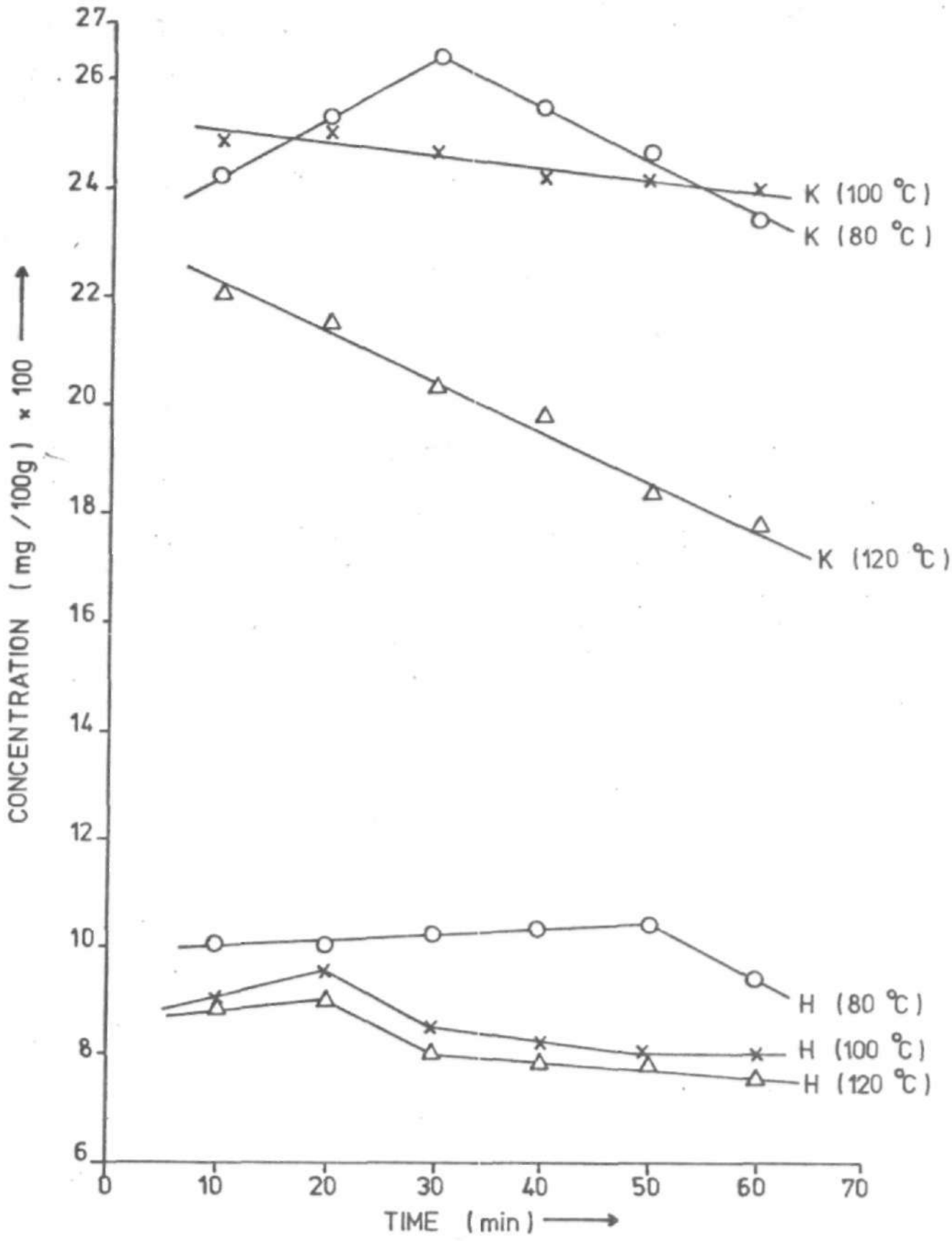


Fig. 3.14: Variation of concentration of sodium with heating time during digestion (samples K & H)

3.8 CALCIUM HYDROGEN ORTHOPHOSPHATE AND HAEMOGLOBIN SAMPLES.

Table 3.7 shows the percentage recoveries obtained when known samples of haemogolobin and calcium hydrogen orthophosphate which contain iron and calcium respectively in about the same form as would be expected in the animal samples were subjected to the same type of treatment and analysis as the latter samples. This analysis was made to enable a comparison of the various factors examined in this study with their effects on compounds of known iron and calcium contents. According to Gorsuch (8), one of the major setbacks suffered by such recovery experiments is that it is hardly possible to get the elements in exactly the same chemical form as they are in the organic material, but the experiments could give some indication of the efficiency of the methods. As can be seen from table 3.7, the trends in percentage recoveries are similar to those obtained for iron and calcium at the various temperatures and heating times employed for the analysis of the animal products. Thus, the highest percentage recovery was obtained for iron after 20 minutes of heating at 80^{o}C while maximum values were obtained at 100^{o}C after 20 minutes and after 10 minutes of heating at 120^{o}C. For calcium, maximum percentage recoveries were obtained at 80^{o}C after

TABLE 3.7

PERCENT RECOVERIES OF IRON AND CALCIUM FROM HAEMOGOLOBIN AND CALCIUM HYDROGEN ORTHOPHOSPHATE RESPECTIVELY - TREATED AS SAMPLES.

(i) IRON IN HAEMOGOLOBIN (12.0 ppm).

TIME (min.)	% at 80°C	% at 100°C	% at 120°C
10	80.8	77.5	77.3
20	82.5	80.8	75.8
30	78.5	78.3	75.8
40	77.5	78.3	75.8
50	76.7	75.8	75.0
60	73.3	73.3	75.0

(ii) CALCIUM IN CALCIUM HYDROGEN ORTHOPHOSPHATE (58.92 ppm)

10	84.7	92.7	98.0
20	90.1	89.7	94.5
30	89.3	88.0	91.1
40	88.6	88.0	91.1
50	87.6	88.0	87.6
60	87.0	88.0	87.6

20 minutes and at 100°C and 120°C after 10 minutes. The highest percentage calcium recovry (98%) was obtained at 120°C on heating for 10 minutes.

Again it is observed that the levels of minerals obtained tend to decrease on prolonged heating as was the case for the animal samples. For iron in the haemoglobin it would appear that volatilisation losses were quite important as the highest recovery was obtained at 80°C with decreased maxima at 100°C and 120°C in that order. The fact that the highest percentage recovery for calcium was obtained at 120°C would probably suggest that the high temperature of 120°C is required for completion of the digestion reaction. The reduction of percentage recovery of calcium with prolonged heating is also indicative of losses due to volatilisation. The volatile substances from the animal products are probably compounds of calcium and some other components of the samples not present in calcium hydrogen orthophosphate.

CHAPTER IV

SUMMARY AND CONCLUSION

4.1 SUMMARY AND CONCLUSION.

Several of the specified procedures in the literature for sample dissolution by mixed-acid digestion are often silent on the exact conditions of temperature and heating length that should be used. Changes such as evolution of brown fumes from nitric acid or white fumes of sulphuric acid, (which could be difficult to distinguish in a mixture of acids) are often used as indicators to the completion of digestion reactions. Although the chemistry of the reactions involved in wet digestion is still not well understood, it would appear from this study that the reactions go to completion after a time interval which depends on the material being digested, and on the digestion temperature (55). The lengths of heating during the wet digestion required to obtain peak values for the minerals in this study are sufficiently consistent with the variation in digestion temperature and tend to agree with the above observation. Thus, in general, the time required to obtain peak values for the minerals are related to the temperature in the order $80^{\circ} > 100^{\circ} > 120^{\circ}C$.

For all the elements in all the samples (except for very few cases for iron and copper), the average and maximum mineral contents obtained are lowest at 120°C. This trend is most pronounced for calcium and sodium for which the maximum and average mineral contents follow the order 120° < 100 < 80°C in all cases. In the literature losses of calcium have been reported in the presence of sulphuric acid due to the formation of insoluble sulphates, these being known to increase with increase in sample size. Our study tends to agree with this observation. However, the analysis of the meat products taken together with that for the calcium hydrogen orthophosphate also tends to suggest that volatilisation losses are important for calcium. Possibly formation of calcium compounds not easily atomized may also contribute to low mineral recoveries. The maximum iron contents also tend to decrease with increase in sample size, possibly due also to the formation of insoluble sulphates.

Although many authors (1 - 6, 8, 31, 52, 54) have advocated the use of nitric-sulphuric acid mixture for the wet digestion of animal products, it would appear from the analysis of the haemoglobin sample that this mixture is not very efficient for the determination of iron in complexed forms. Probably,

with this mixture the digestion reaction does not even proceed to completion in addition to losses due to volatilisation and formation of insoluble compounds in some cases.

It seems fairly obvious from this study that there is a need to work out a balance between digestion temperatures high enough to ensure completion of digestion reaction, and at the same time minimise losses due to volatilisation. The appropriate sample size needs be used, and sample sizes of up to 1.0g and above quite often found in the literature for similar samples would probably lead to an underestimation of the desired mineral by nitric acid - sulphuric acid digestion.

4.2 SUGGESTIONS FOR FURTHER STUDIES:

It is suggested that further studies be carried out on more samples to examine the effects of the above and other factors on the levels of minerals obtained after wet digestion. The study should be extended to other essential and toxic minerals, with a view to working out more specific conditions for wet digestion of animal and other biological substances.

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APPENDIX I

VARIATION OF CALCIUM CONTENTS (mg/100g) WITH HEATING TIME AND TEMPERATURE.

SAMPLE	HEATING TIME(min)	80°C	100°C	120°C
A	10	888	888	808
	20	896	832	768
	30	904	824	632
	40	920	688	632
	50	888	672	456
	60	864	640	440
B				
	10	724	840	828
	20	750	840	808
	30	760	826	710
	40	780	820	692
	50	840	816	680
	60	816	810	680
C(0.25g)				
	10	610	590	610
	20	590	590	552
	30	590	590	550
	40	590	584	550
	50	580	580	550
	60	570	564	540
C(0.5g)				
	10	600	510	485
	20	580	460	415
	30	540	450	415
	40	505	445	415
	50	505	433	390
	60	477	405	370

APPENDIX I (Cont'd)

SAMPLE	HEATING TIME(min.)	80°C	100°C	120°C
C(0.75)				
	10	633.7	483.6	433.6
	20	591.0	460.2	340.2
	30	527.0	383.5	310.2
	40	527.0	340.2	310.2
	50	460.2	283.5	266.8
	60	460.2	283.5	233.5
D				
	10	200	184	208
	20	230	204	190
	30	230	220	176
	40	216	210	176
	50	210	208	170
	60	208	208	170
E				
	10	107	109	124
	20	120	114	120
	30	126	120	120
	40	112	114	113
	50	112	114	113
	60	110	113	111
F(0.25g)				
	10	270	188	210
	20	332	200	230
	30	332	210	230
	40	210	220	220
	50	200	220	220
	60	200	200	200

Appendix I (cont'd)

SAMPLE	HEATING TIME(Min.)	80°C	100°C	120°C
F(0.50g)				
	10	248	225	236
	20	260	236	218
	30	250	225	218
	40	248	218	215
	50	236	218	215
	60	236	215	212
F(0.75g)				
	10	289.4	201.0	166.2
	20	301.5	212.1	144.7
	30	254.6	186.7	132.7
	40	219.8	186.7	132.7
	50	201.0	144.7	120.6
	60	151.4	120.6	120,6
G				
	10	272	284	254
	20	272	272	236
	30	300	256	220
	40	320	248	220
	50	300	248	220
	60	296	216	188
H(0.25g)				
	10	960	912	868
	20	924	888	816
	30	924	864	792
	40	924	864	792
	50	912	840	756
	60	876	840	720

Appendix I (cont'd)

SAMPLE	HEATING TIME(min.)	80°C	100°C	120°C
H(0.50g)				
	10	1010	975	830
	20	1070	935	810
	30	1070	890	670
	40	975	770	610
	50	930	740	610
	60	900	660	590
H(0.75g)				
	10	880.4	833.8	720.4
	20	867.1	667.0	700.4
	30	773.7	567.0	667.0
	40	773.7	501.0	433.6
	50	720.4	501.0	400.2
	60	600.3	467.0	400.2
J				
	10	745	575	434
	20	715	525	404
	30	668	500	400
	40	632	475	400
	50	595	455	365
	60	566	410	336
K				
	10	66	58	60
	20	72	62	56
	30	66	58	50
	40	64	58	50
	50	62	56	50
	60	60	54	48

APPENDIX II

VARIATION OF IRON CONTENTS (mg/100g) WITH HEATING TIME AND TEMPERATURE.

SAMPLE	HEATING TIME (min.)	80°C	100°C	120°C
A				
	10	46	78	58
	20	52	85	54
	30	59	79	51
	40	78	70	51
	50	97	65	44
	60	95	59	44
B				
	10	128	128	134
	20	134	134	114
	30	134	134	108
	40	134	128	102
	50	134	120	102
	60	128	118	102
C(0.25g)				
	10	34	30	28
	20	38	32	28
	30	42	28	30
	40	36	26	30
	50	32	26	26
	60	32	26	26
C(0.5)g)				
	10	25	25	25
	20	28	26	22
	30	27	25	22
	40	27	25	21
	50	25	22	21
	60	23	20	20

Appendix II (cont'd)

SAMPLE	HEATING TIME(min.)	80°C	100°C	120°C
C(0.75g)				
	10	26.8	26.8	22.8
	20	29.4	29.4	26.8
	30	33.6	22.8	20.2
	40	26.8	20.2	13.4
	50	26.8	18.8	12.0
	60	22.8	16.0	12.0
D				
	10	58	56	62
	20	58	58	54
	30	66	64	54
	40	70	56	54
	50	70	56	52
	60	64	54	50
E				
	10	26	26	32
	20	28	28	30
	30	26	26	30
	40	26	26	28
	50	22	26	28
	60	22	26	26
F(0.25g)				
	10	88	90	100
	20	94	102	96
	30	96	100	94
	40	104	88	94
	50	100	88	94
	60	94	88	88

Appendix II (cont'd)

SAMPLE	HEATING TIME(min.)	80°C	100°C	120°C
F(0.50g)				
	10	75	78	71
	20	84	83	61
	30	78	74	53
	40	78	74	52
	50	78	71	52
	60	75	71	45
F(0.75g)				
	10	46.2	42.9	46.2
	20	49.6	44.2	45.6
	30	41.5	44.2	41.5
	40	37.5	40.2	40.2
	50	35.5	36.2	40.2
	60	27.5	33.5	33.5
G				
	10	32	32	48
	20	36	40	32
	30	40	36	32
	40	40	36	28
	50	36	32	28
	60	32	32	24
H(0.25g)				
	10	40	42	46
	20	42	56	56
	30	46	50	52
	40	44	46	46
	50	42	40	44
	60	40	40	40

Appendix II (cont'd)

SAMPLE	HEATING TUNE(min.)	80°C	100°C	120°C
H(0.50g)				
	10	41	51	55
	20	44	53	57
	30	46	51	56
	40	47	48	53
	50	47	48	53
	60	45	47	50
H(0.75g)				
	10	40.2	40.2	42.8
	20	43.8	43.6	47.0
	30	47.0	41.6	41.6
	40	48.2	37.6	37.6
	50	41.6	34.8	34.8
	60	36.2	34.8	30.8
J				
	10	22	22	26
	20	24	26	34
	30	24	26	34
	40	22	22	36
	50	22	18	34
	60	18	18	26
K				
	10	16	16	18
	20	16	18	18
	30	18	18	16
	40	20	18	16
	50	20	16	14
	60	18	14	14

APPENDIX III

VARIATION OF COPPER CONTENTS (mg/100g)
WITH HEATING TIME AND TEMPERATURE.

SAMPLE	HEATING TIME(min.)	80°C	100°C	120°C
A				
	10	3.3	5.0	5.0
	20	3.3	5.0	6.4
	30	5.0	5.0	8.8
	40	6.4	5.0	7.4
	50	6.4	5.8	7.4
	60	7.4	5.0	6.4
B				
	10	7.0	9.0	8.2
	20	8.0	9.4	8.2
	30	8.0	9.0	8.6
	40	9.0	8.6	9.0
	50	8.6	8.2	8.2
	60	8.2	8.0	8.0
C				
	10	4.0	4.4	4.0
	20	4.0	4.4	4.4
	30	4.2	4.8	4.8
	40	4.2	5.6	4.4
	50	4.4	6.0	4.0
	60	3.8	4.0	4.0
D				
	10	1.8	2.0	2.8
	20	2.4	2.4	3.4
	30	2.4	2.4	2.2
	40	3.0	2.4	2.0
	50	2.4	3.0	2.0
	60	2.4	2.8	2.0

Appendix III (cont'd)

SAMPLE	HEATING TIME(min.)	80°C	100°C	120°C
E				
	10	2.0	1.2	0.8
	20	1.6	1.2	0.8
	30	1.6	0.8	0.8
	40	1.2	0.8	0.8
	50	1.2	0.6	0.6
	60	0.8	0.6	0.6
F				
	10	2.8	2.2	2.4
	20	3.0	2.6	2.6
	30	2.6	2.6	2.6
	40	2.6	3.0	2.2
	50	2.4	2.2	2.2
	60	2.2	2.2	2.2
G				
	10	1.2	1.2	1.2
	20	1.2	1.2	1.2
	30	1.4	1.4	1.4
	40	1.8	1.4	1.4
	50	1.4	1.2	1.2
	60	1.4	1.0	1.0
H				
	10	2.0	1.8	1.8
	20	2.0	1.6	1.2
	30	2.2	1.6	1.0
	40	2.0	1.6	1.0
	50	2.0	1.4	1.0
	60	1.8	1.4	0.8

Appendix III (cont'd)

SAMPLE	HEATING TIME(min.)	80°C	100°C	120°C
J				
	10	2.8	2.2	2.2
	20	3.2	2.2	2.4
	30	3.4	2.4	2.4
	40	2.8	2.4	2.6
	50	2.8	2.6	2.2
	60	2.6	2.4	2.2
K				
	10	3.6	3.2	3.2
	20	3.8	3.6	3.2
	30	4.8	3.2	3.6
	40	3.6	3.2	3.2
	50	3.2	3.2	3.2
	60	3.2	3.0	2.8

APPENDIX IV

VARIATION OF SODIUM CONTENTS (mg/100g) WITH HEATING TIME AND TEMPERATURE (X 100)

SAMPLE	HEATING TIME(min,)	80°C	100°C	120°C
A				
	10	11.3	11.9	11.3
	20	11.7	11.0	9.1
	30	11.7	11.0	8.9
	40	11.8	10.3	8.9
	50	12.3	10.3	7.9
	60	11.8	9.9	7.8
B				
	10	20.3	23.8	22.8
	20	21.0	23.5	22.8
	30	22.5	23.5	22.0
	40	24.5	23.4	22.0
	50	23.1	23.4	21.8
	60	23.0	22.3	21.5
C				
	10	7.9	7.9	7.6
	20	8.0	7.8	7.3
	30	8.0	7.8	7.3
	40	8.8	7.6	7.3
	50	7.8	7.6	7.2
	60	7.6	7.6	7.0
D				
	10	25.9	25.0	26.9
	20	26.3	27.5	25.0
	30	27.5	26.3	24.0
	40	26.9	25.0	23.8
	50	26.3	25.0	23.8
	60	25.9	25.0	23.0

Appendix IV (cont'd)

SAMPLE	HEATING TIME(min.)	80°C	100°C	120°C
E				
	10	29.5	28.5	28.8
	20	29.5	29.0	27.5
	30	30.1	28.6	27.4
	40	28.6	28.6	27.2
	50	28.6	28.4	27.0
	60	28.4	28.4	26.7
F				
	10	29.5	28.0	27.8
	20	30.2	28.0	27.6
	30	30.0	29.2	27.0
	40	30.0	28.0	27.0
	50	29.5	28.0	27.0
	60	29.2	27.0	26.5
G				
	10	30.7	29.7	31.5
	20	32.0	30.9	31.0
	30	31.4	30.0	30.5
	40	30.5	30.0	30.5
	50	30.2	29.9	29.2
	60	29.5	29.0	28.0
H				
	10	10.0	9.0	8.9
	20	10.0	9.5	9.0
	30	10.2	8.5	8.0
	40	10.3	8.2	7.8
	50	10.4	8.0	7.8
	60	9.4	8.0	7.6

Appendix IV (cont'd)

SAMPLE	HEATING TIME(min.)	80°C	100°C	120°C
J				
	10	8.2	7.8	6.7
	20	8.2	7.8	6.7
	30	8.7	8.4	7.0
	40	7.9	7.7	6.8
	50	7.9	6.7	6.3
	60	7.8	6.6	6.3
K				
	10	24.2	24.9	22.0
	20	25.3	25.0	21.5
	30	26.4	24.7	20.3
	40	25.5	24.2	19.8
	50	24.7	24.2	18.3
	60	23.5	24.0	17.8