IMPROVING THE UTILISATION AND QUALITY
OF LOW VALUE FISH (BRACHYDEUTERUS
AURITUS AND DACTYLOPTERUS VOLITANS) BY
PROCESSING

A thesis submitted for the degree of Doctor of Philosophy

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September 2005
ABSTRACT

Two pelagic underutilised fish species in Ghana, the Burrito (Brachydeuterus auritus) and the flying gurnard (Dactylopterus volitans) were selected for study. Proximate composition analysis indicated high protein content (18-22%), an excellent amino acid profile with high levels of lysine (7-8%) as well as poly unsaturated fatty acids predominantly 20:5ω3 and 22:6ω3 (omega 3 fatty acids), which constituted between 44 and 51% of the total fatty acids. Sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis of the myofibrillar extracts showed that both fish species may be distinguished from each other by protein bands with molecular weights estimated to be 18,400 and 14,300 daltons. Rheological methods, differential scanning calorimetry (DSC), phase contrast microscopy and FT-Raman spectroscopy indicated a positive synergistic interaction between flying gurnard proteins and cowpea flour resulting in greater than expected elastic modulus values. The networks were compatible and stabilized by hydrophobic and hydrogen bonds as well as disulfide bonds. Optimum gels were formed with flying gurnard fish and cowpea flour mixed in the ratio of 5:5 (w/w) and Burrito fish mince to cowpea flour ratio (2:8 w/w). Interactions of the fish minces with cassava starch resulted in phase separation and weak non-covalent interactions. Infant weaning food formulations including flying gurnard mince and other food ingredients showed good nutritional and low viscous properties which compared favourably with commercial infant foods. The shelf life of the products was enhanced with the exclusion of oxygen in vacuum-packed foil packaging. High bloom (275 g) gelatin was produced from flying gurnard fish skin and compared favourably with gelatins from other sources. Using a low cost single screw food extruder built for developing countries, fish based extruded snack pellets incorporating minced burrito:
cassava starch, were successfully developed at a barrel temperature of 120 °C. Extruded snack products stored in aluminium foil pouches were better protected in terms of chemical changes in free fatty acids and TBARS formation, than those packaged in polyethylene pouches. This development of fish-based extruded products will lead to new products of high nutritional quality and acceptability as shown by chemical and sensory evaluation results.
OBJECTIVES

- To characterize the selected low value fish species by their physicochemical properties including chemical and biochemical properties.

- To study the interaction of the fish mince of the selected species with cowpea flour and cassava starch.

- To design and fabricate a low cost extruder which would serve as a prototype for SME in Ghana.

- To develop value-added products as infant food and extruded dried products from the fish species incorporating local food crops.

- To develop gelatin from the fish skin and characterize it by its physicochemical and rheological properties.

- To monitor the quality and biochemical changes of the extruded products and infant weaning foods.
ABBREVIATIONS

AFO BV  Agrotechnology and Food Innovations B.V. The Netherlands
BSA  Bovine serum albumin
CECAF  FAO-Fishery Committee for the Eastern Central Atlantic
DHA  22:6ω3 docosahexaenoic acid
DHSS  Department of Health and Social Security
DSC  Differential scanning calorimetry
EEZ  Exclusive economic zone
EPA  20:5ω3 eicosapentaenoic acid
FFA  Free fatty acids
FRI  Food Research Institute, Ghana
HPLC  High performance liquid chromatography
INCO-DEV  International Cooperation with Developing Countries
MDA  Malondialdehyde
MTBE  Methyl tertiary-butyl ether
NOSB  National Organic Standards Board
NPN  Non-protein nitrogen
PITC  Phenylisothiocyanate
PUFA  Poly unsaturated fatty acids
PV  Peroxide value
RDA  Recommended Daily allowance
SDS-PAGE  Sodium dodecyl sulphate polyacrylamide gel electrophoresis
SME  Small and medium scale enterprises
t  tonne, sometimes known as metric ton
TAP  Technical Advisory Panel
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Name</th>
</tr>
</thead>
<tbody>
<tr>
<td>TBARS</td>
<td>Thiobarbituric acid reactive substances</td>
</tr>
<tr>
<td>TEA</td>
<td>Triethanoalamine</td>
</tr>
<tr>
<td>TEP</td>
<td>Tetraethoxypropane</td>
</tr>
</tbody>
</table>
ACKNOWLEDGEMENTS

The author acknowledges with gratitude the financial support provided by the European Union (EU) for this project (INCO-DEV Project No. INCO-DEV ICA4-CT-2001-10032). I am extremely grateful to Prof. N. K. Howell of the University of Surrey, Guildford for not only securing me the project and funds under the INCO-DEV programme, but also for her immense supervision and inspiration. My thanks also go to the research team of the Agrotechnology and Food Innovations B. V. of the Netherlands for designing the extruder used in this work. My thanks also go to Dr. Farah Badii, Dr. Sue Saeed, Hajo and last but not the least Rabia for their expert assistance in carrying out some of the essential components of this work. I would also like to express my sincere gratitude to my co-supervisor Dr. P. N-T. Johnson of the Food Research Institute, for his immense assistance.

Last but not the least, the supporting staff of the Test Kitchen of the Food Research Institute, Ghana also deserves my gratitude for their assistance towards this project.
# Table of contents

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abstract</td>
<td>i</td>
</tr>
<tr>
<td>Objectives</td>
<td>iii</td>
</tr>
<tr>
<td>Abbreviations</td>
<td>iv</td>
</tr>
<tr>
<td>Acknowledgements</td>
<td>vi</td>
</tr>
<tr>
<td>Table of contents</td>
<td>vii</td>
</tr>
<tr>
<td>List of Figures</td>
<td>xiii</td>
</tr>
<tr>
<td>List of Tables</td>
<td>xvii</td>
</tr>
</tbody>
</table>

## CHAPTER 1 GENERAL INTRODUCTION

1.1 Summary background and objectives
   1.1.1 Objectives of Study
2. Fish handling and processing in Ghana
3. Importance of fish in Ghana
4. Biological characteristics of the selected species
   1.4.1 The bigeye grunt (burrito) (*Brachydeuterus auritus*)
   1.4.2 The flying gurnard (*Dactylopterus volitans* Linnaeus)
5. Fish muscle structure and composition
   1.5.1 Physical composition of fish muscles
   1.5.2 Chemical composition of fish muscles
   1.5.3 Characteristics of pelagic species muscles
6. Proteins
   1.6.1 Primary structure
   1.6.2 Secondary structure
   1.6.3 Tertiary structure
   1.6.4 Quaternary structure
7. Fish proteins
   1.7.1 Sarcoplasmic proteins
   1.7.2 Connective tissue proteins
   1.7.3 Myofibrillar proteins
   1.7.4 Factors affecting the physical properties of fish protein gels
8. Fish oils and fatty acids
9. Minerals in fish
1.10 Vitamins in fish
1.11 Cowpea and cowpea proteins
  1.11.1 Importance cowpea
  1.11.2 Cowpea proteins
1.12 Protein-protein and other macromolecules interactions
  1.12.1 Protein-protein
  1.12.2 Protein-starch interactions
1.13 Product development
  1.13.1 Infant foods
  1.13.2 Snack foods
1.14 Shelf life and packaging of fish and fish products

CHAPTER 2 MATERIAL AND METHODS

2.1 Materials

2.2 Methodology
  2.2.1 Moisture content
  2.2.2 Protein determination
  2.2.3 Fat content
  2.2.4 Ash content
  2.2.5 Phosphorus determination
  2.2.6 Calcium content
  2.2.7 Iron content
  2.2.8 Fatty acid analysis
  2.2.9 Thiobarbituric acid reactive substances (TBARS)
  2.2.10 Peroxide value (PV).
  2.2.11 Biochemical characterization
    2.2.11.1 Extraction of myofibril proteins
    2.2.11.2 Protein content of extract (Bradford Protein Assay method)
    2.2.11.3 Amino acid analysis
    2.2.11.4 SDS-Polyacrylamide gel electrophoresis (SDS-Page)
Table of contents

2.3 Protein interaction studies 54
  2.3.1 Principle of oscillatory rheometry 54
  2.3.2 Differential scanning calorimetry (DSC) 56
  2.3.3 FT-Raman spectroscopy 60
  2.3.4 Phase contrast microscopy 62
2.4 Statistical analysis 64

CHAPTER 3 PROXIMATE AND BIOCHEMICAL CHARACTERISATION OF THE BURRITO (BRACHYDEUTERUS AURITUS) AND THE FLYING GURNARD (DACTYLOPTERUS VOLITANS) 65

3.1 Introduction 65
3.2 Materials and methods 65
  3.2.1 Materials 65
  3.2.2 Methods 65
3.3 Results and discussion 66
3.4 Conclusion 72

CHAPTER 4 RHEOLOGICAL, DSC, FT-RAMAN SPECTROSCOPY AND PHASE MICROSCOPY STUDIES OF FISH MINCE, COWPEA FLOUR AND CASSAVA STARCH MIXTURES 73

4.1 Introduction 73
4.2 Materials and Methods 74
  4.2.1 Small-deformation oscillatory measurements. 74
  4.2.2 DSC Measurements 74
  4.2.3 FT-Raman spectroscopy 75
  4.2.4 Phase contrast microscopy 76
4.3. Results and discussions 76
  4.3.1 Rheological studies 76
    4.3.1.1 Effect of cowpea flour and cassava starch on the gelation profiles of flying gurnard fish minces 78
    4.3.1.2 Effect of cowpea flour and cassava starch on the gelation profiles of the burrito fish mince 85
4.3.2 DSC Studies

4.3.2.1 Effect of cowpea flour and cassava starch on the DSC thermograms of the flying gurnard minces

4.3.2.2 Effect of cowpea flour and cassava starch on the DSC thermograms of the burrito fish minces

4.3.3 FT-Raman spectroscopy

4.3.3.1 Comparison of flying gurnard mince and mixed mince with cowpea flour

4.3.3.2 Comparison of flying gurnard mince and mixed mince with cassava starch

4.3.3.3 Comparison of burrito fish minces and mixed mince with cowpea flour

4.3.3.4 Comparison of burrito mince and mixed mince with cassava starch

4.3.4 Phase contrast microscopy studies

4.3.4.4 Effect of adding cowpea flour and cassava starch to flying gurnard mince

4.3.4.2 Effect of adding cowpea flour and cassava starch to burrito fish mince

4.5 Conclusion

CHAPTER 5 PROCESSING OF THE SELECTED FISH SPECIES INTO INFANT WEANING FOODS

5.1 Introduction

5.2 Materials and Methods

5.2.1 Preparation of weaning food

5.2.2 Proximate and chemical analysis

5.2.3 Amino acid analysis

5.2.4 Rheological studies

5.2.5 Shelf life studies

5.2.6 Chemical analyses

5.3 Results and discussions
<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.4 Shelf life of developed food formulations</td>
<td>131</td>
</tr>
<tr>
<td>5.5 Conclusion</td>
<td>134</td>
</tr>
<tr>
<td>CHAPTER 6 FLYING GURNARD (DACTYLOPTERUS VOLITANS, L) SKIN GELATIN AND GELATION PROPERTIES</td>
<td>135</td>
</tr>
<tr>
<td>6.1 Introduction</td>
<td>135</td>
</tr>
<tr>
<td>6.2 Materials and Methods</td>
<td>139</td>
</tr>
<tr>
<td>6.2.1 Materials</td>
<td>139</td>
</tr>
<tr>
<td>6.2.2 Methods</td>
<td>139</td>
</tr>
<tr>
<td>6.2.2.1 Gelatin preparation</td>
<td>139</td>
</tr>
<tr>
<td>6.2.2.2 Proximate composition</td>
<td>140</td>
</tr>
<tr>
<td>6.2.2.3 Bloom strength</td>
<td>140</td>
</tr>
<tr>
<td>6.2.2.4 Amino acid analysis</td>
<td>141</td>
</tr>
<tr>
<td>6.2.3 Interaction studies with cassava starch</td>
<td>142</td>
</tr>
<tr>
<td>6.2.3.1 Rheological measurements</td>
<td>142</td>
</tr>
<tr>
<td>6.2.3.2 DSC measurements</td>
<td>142</td>
</tr>
<tr>
<td>6.3 Results and Discussions</td>
<td>143</td>
</tr>
<tr>
<td>6.3.1 Proximate composition</td>
<td>143</td>
</tr>
<tr>
<td>6.3.2 Bloom strength</td>
<td>144</td>
</tr>
<tr>
<td>6.3.3 Amino acid analysis</td>
<td>146</td>
</tr>
<tr>
<td>6.3.4 Interaction studies with cassava starch</td>
<td>150</td>
</tr>
<tr>
<td>6.3.4.1 Rheological analysis</td>
<td>150</td>
</tr>
<tr>
<td>6.3.4.2 DSC studies</td>
<td>155</td>
</tr>
<tr>
<td>Conclusion</td>
<td>159</td>
</tr>
<tr>
<td>CHAPTER 7 EXTRUSION AND MANUFACTURE OF SNACK PRODUCTS FROM BURRITO (BRACHYDEUTERUS AURITUS) MINCE AND COWPEA FLOUR BLENDS</td>
<td>160</td>
</tr>
<tr>
<td>7.1 Introduction</td>
<td>160</td>
</tr>
<tr>
<td>7.1.1 Snack food</td>
<td>160</td>
</tr>
<tr>
<td>7.1.2 Extrusion process</td>
<td>161</td>
</tr>
<tr>
<td>7.2 Methods</td>
<td>162</td>
</tr>
<tr>
<td>Section</td>
<td></td>
</tr>
<tr>
<td>-----------------</td>
<td></td>
</tr>
<tr>
<td>7.2.1 Fabrication of the single screw extruder</td>
<td>162</td>
</tr>
<tr>
<td>7.2.2 Extrusion process</td>
<td>164</td>
</tr>
<tr>
<td>7.2.3 Preparation of mixtures for extrusion</td>
<td>164</td>
</tr>
<tr>
<td>7.2.4 Die swell</td>
<td>166</td>
</tr>
<tr>
<td>7.2.5 Expansion ratio</td>
<td>167</td>
</tr>
<tr>
<td>7.2.6 Proximate and chemical analyses</td>
<td>168</td>
</tr>
<tr>
<td>7.2.7 Sensory evaluation</td>
<td>168</td>
</tr>
<tr>
<td>7.2.8 Shelf life studies</td>
<td>168</td>
</tr>
<tr>
<td>7.2.9 Chemical analyses</td>
<td>169</td>
</tr>
<tr>
<td>7.2.10 Sensory evaluation</td>
<td>169</td>
</tr>
<tr>
<td>7.2.11 Colour determination</td>
<td>170</td>
</tr>
<tr>
<td>7.2.12 Statistical analyses of data</td>
<td>170</td>
</tr>
<tr>
<td>7.3 Results and discussion</td>
<td>170</td>
</tr>
<tr>
<td>7.3.1 Effect of barrel temperature on extruded products</td>
<td>170</td>
</tr>
<tr>
<td>7.3.2 Proximate and chemical composition</td>
<td>174</td>
</tr>
<tr>
<td>7.3.3 Shelf life studies</td>
<td>177</td>
</tr>
<tr>
<td>7.4 Conclusion</td>
<td>182</td>
</tr>
</tbody>
</table>

**CHAPTER 8 GENERAL DISCUSSION AND CONCLUSION**

8.1 Proximate and biochemical characterisation | 184 |
8.2 Rheological, DSC, FT-Raman spectroscopy and phase contrast microscopy studies of mixtures | 186 |
8.3 Processing of flying gurnard fish into infant weaning foods | 190 |
8.4 Flying gurnard (*Dactylopterus volitans* L) skin gelatin and gelation properties | 191 |
8.5 Extrusion and manufacture of snack products from burrito (*Brachydeuterus auritus*) mince and cowpea flour blends | 192 |
8.6 Conclusion | 192 |
8.7 Further work | 195 |

**REFERENCES**

**APPENDICES**

xii
# LIST OF FIGURES

## CHAPTER 1 GENERAL INTRODUCTION

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.1</td>
<td>The bigeye grunt/burrito, <em>Brachydeuterus auritus</em> (Valenciennes. 1831)</td>
<td>8</td>
</tr>
<tr>
<td>1.2</td>
<td>The flying gurnard (<em>Dactylopterus volitans</em>)</td>
<td>10</td>
</tr>
<tr>
<td>1.3</td>
<td>Primary structure of a protein</td>
<td>14</td>
</tr>
<tr>
<td>1.4</td>
<td>Secondary structure of a protein</td>
<td>15</td>
</tr>
<tr>
<td>1.5</td>
<td>Tertiary structure of protein</td>
<td>16</td>
</tr>
<tr>
<td>1.6</td>
<td>Quaternary structure of protein</td>
<td>17</td>
</tr>
<tr>
<td>1.7</td>
<td>Successive stages in the formation of collagen</td>
<td>21</td>
</tr>
<tr>
<td>1.8</td>
<td>Fish muscle cells and component myofibrils showing actin and myosin filaments</td>
<td>22</td>
</tr>
<tr>
<td>1.9</td>
<td>Molecular forces involved in protein interactions</td>
<td>37</td>
</tr>
</tbody>
</table>

## CHAPTER 2 MATERIALS AND METHODS

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.1</td>
<td>Stress/strain signals from an oscillatory rheometer</td>
<td>54</td>
</tr>
<tr>
<td>2.2</td>
<td>The relationship between the loss modulus (<em>G''</em>), storage modulus (<em>G'</em>) and complex modulus (<em>G</em>')</td>
<td>55</td>
</tr>
<tr>
<td>2.3</td>
<td>Schematic diagram of a rheometer</td>
<td>55</td>
</tr>
<tr>
<td>2.4</td>
<td>A typical DSC thermogram showing a possible baseline construction and the following parameters: (a) peak maximum temperature, (b) extrapolated onset temperature, and (c) heat capacity</td>
<td>59</td>
</tr>
</tbody>
</table>

## CHAPTER 3 PROXIMATE AND BIOCHEMICAL CHARACTERISATION OF THE BURRITO (*BRACHYDEUTERUS AURITUS*) AND THE FLYING GURNARD (*DACTYLOPTERUS VOLITANS*)

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.1</td>
<td>SDS Page patterns for the flying gurnard and burrito</td>
<td>68</td>
</tr>
<tr>
<td>Figure 4.1</td>
<td>A dynamic temperature sweep of homogenised burrito muscle</td>
<td>77</td>
</tr>
<tr>
<td>Figure 4.2</td>
<td>A dynamic temperature sweep of homogenised flying gurnard muscle</td>
<td>77</td>
</tr>
<tr>
<td>Figure 4.3a</td>
<td>Gelation profile of flying gurnard mince with varying proportions (w/w) of cowpea flour during heating</td>
<td>79</td>
</tr>
<tr>
<td>Figure 4.3b</td>
<td>Gelation profile of flying gurnard mince with varying proportion of cowpea flour (w/w) during cooling</td>
<td>79</td>
</tr>
<tr>
<td>Figure 4.4a</td>
<td>Gelation profile of flying gurnard mince with varying proportion of cassava starch (w/w) during heating</td>
<td>82</td>
</tr>
<tr>
<td>Figure 4.4b</td>
<td>Gelation profile of flying gurnard mince with varying proportion of cassava starch (w/w) during cooling</td>
<td>83</td>
</tr>
<tr>
<td>Figure 4.5a</td>
<td>Gelation profile of burrito fish mince with varying proportion of cowpea flour (w/w) during heating</td>
<td>85</td>
</tr>
<tr>
<td>Figure 4.5b</td>
<td>Gelation profile of burrito fish mince with varying proportion of cowpea flour (w/w) during cooling</td>
<td>86</td>
</tr>
<tr>
<td>Figure 4.6a</td>
<td>Gelation profile of burrito fish mince with varying concentration of cassava starch (w/w) during heating</td>
<td>88</td>
</tr>
<tr>
<td>Figure 4.6b</td>
<td>Gelation profile of burrito fish mince with varying proportion of cassava starch (w/w) during cooling</td>
<td>88</td>
</tr>
<tr>
<td>Figure 4.7</td>
<td>DSC of flying gurnard muscle thermogram indicating the transitions and heat flow</td>
<td>91</td>
</tr>
<tr>
<td>Figure 4.8</td>
<td>DSC of burrito muscle thermogram indicating the transitions and heat flow</td>
<td>91</td>
</tr>
<tr>
<td>Figure 4.9</td>
<td>Raman spectra (A) 1700-650 cm⁻¹ and (B) 3500-2700 cm⁻¹ for flying gurnard mince and cowpea flour in the ratio (a) 10:0 (b) 8:2 (c) 5:5 (d) 2:8 after subtracting the spectra of the respective proportion of cowpea flour from each mixture</td>
<td>99</td>
</tr>
<tr>
<td>Figure 4.10</td>
<td>Raman spectra (A) 1700-650 cm⁻¹ and (B) 3500-2700 cm⁻¹ for flying gurnard mince and cassava starch in the ratio (a) 10:0 (b) 8:2 (c) 5:5 (d) 2:8 after subtracting the spectra of the respective proportion of cassava starch from each mixture</td>
<td>104</td>
</tr>
</tbody>
</table>
Table of contents

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.11</td>
<td>Raman spectra (A) 1700-650 cm(^{-1}) and (B) 3500-2700 cm(^{-1}) for burrito fish mince and cowpea flour in the ratio (a) 10:0 (b) 8:2 (c) 5:5 (d) 2:8 after subtracting the spectra of the respective proportion of cowpea flour from each mixture</td>
</tr>
<tr>
<td>4.12</td>
<td>Raman spectra (A) 1700-650 cm(^{-1}) and (B) 3500-2700 cm(^{-1}) for burrito fish mince and cassava starch in the ratio (a) 10:0 (b) 8:2 (c) 5:5 (d) 2:8 after subtracting the spectra of the respective proportion of cassava starch from each mixture</td>
</tr>
<tr>
<td>4.13</td>
<td>Phase contrast micrographs of mixtures of flying gurnard fish mince and cowpea flour (a) 8% flying gurnard mince and 2% cowpea flour, (b) 5% flying gurnard mince and 5% cowpea flour (c) 2% fish gurnard and 8% cowpea flour</td>
</tr>
<tr>
<td>4.14</td>
<td>Phase contrast micrographs of mixtures of flying gurnard fish mince and cassava starch (a) 8% flying gurnard mince and 2% cassava starch, (b) 5% flying gurnard mince and 5% cassava starch (c) 2% flying gurnard fish mince and 8% cassava starch</td>
</tr>
<tr>
<td>4.15</td>
<td>Phase contrast micrographs of mixtures of burrito fish mince and cowpea flour (a) 8% burrito fish mince and 2% cowpea flour, (b) 5% burrito fish mince and 5% cowpea flour (c) 2% burrito fish mince and 8% cowpea flour</td>
</tr>
<tr>
<td>4.16</td>
<td>Phase contrast micrographs of mixtures of burrito fish mince and cowpea flour (a) 8% burrito fish mince and 2% cassava starch, (b) 5% burrito fish mince and 5% cassava starch (c) 2% burrito fish mince and 8% cassava starch</td>
</tr>
</tbody>
</table>

**CHAPTER 5 PROCESSING OF FLYING GURNARD FISH (DACTYLOPTERUS VOLITANS) INTO INFANT WEANING FOODS**

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.1</td>
<td>A dynamic temperature sweep of infant recipe 2 with the inclusion of flying gurnard mince</td>
</tr>
<tr>
<td>5.2</td>
<td>A dynamic temperature sweep of infant recipe 3 with the inclusion of flying gurnard mince.</td>
</tr>
<tr>
<td>5.3</td>
<td>A dynamic temperature sweep of infant recipe 4 with the inclusion of flying gurnard mince</td>
</tr>
<tr>
<td>5.4</td>
<td>A dynamic temperature sweep of infant recipe 5 with the inclusion of flying gurnard mince</td>
</tr>
<tr>
<td>5.5</td>
<td>FFA (%) changes in dried infant food formulation during storage (Recipe 4)</td>
</tr>
</tbody>
</table>
Chapter 6 Flying Gurnard (Dactylopterus Volitans, L) Skin Gelatin and Gelation Properties

Figure 6.1 Comparison of the bloom strength of flying fish gelatin and gelatine from pig’s skin and catfish

Figure 6.2 Amino acid (no. proline and hydroxyproline residues in 1000 residues) content of flying gurnard, Horse Mackerel and commercial gelatins

Figure 6.3 Hydrophobic amino acid content (Number of residues of ala + val + ileu + ileu + pro + phe + met in 1000 residues) of flying gurnard fish, Horse mackerel and commercial gelatins

Figure 6.4 A typical gelation of profile of flying gurnard fish skin gelatin with varying proportion of cassava starch (w/w) during a dynamic temperature ramp

Figure 6.5 Gelation temperature of flying gurnard fish gelatin and cassava starch (w/w) during dynamic temperature sweep

Figure 6.6 Storage modulus (G’) at gelation temperature of flying gurnard fish gelatin and cassava starch (w/w) during dynamic temperature sweep

Figure 6.7 Typical DSC thermograms for flying gurnard fish gelatin scanned from 0 to 90°C cooled and rescanned

Figure 6.8 DSC thermograms for mixtures of flying gurnard fish gelatin and cassava starch at various concentrations (w/w)

Chapter 7 Extrusion and Manufacture of Snack Products from Burrito (Brachydeuterus Auritus) Mince and Cowpea Flour Blends

Figure 7.1 Extruder components before assembling (a and b)

Figure 7.2 Low cost single extruder in final stages of completion

Figure 7.3 Process flow chart for extruding the fish snack products
Table of contents

Figure 7.4 Effect of temperature of the third section of the barrel on die swell on control mixture 60% (70:30 mince/cowpea) and 40% cassava starch (values are means of 20 samples ±sd) 171

Figure 7.5 Effect of the temperature of the third segment of the barrel on expansion ratio after deep frying and Torbed drying on control mixture 60% (70:30 mince/cowpea) and 40% cassava starch (values are means of 20 samples ±sd) 171

Figure 7.6 Effect of temperature of the third segment of the barrel on bulk density on control mixture 60% (70:30 mince/cowpea) and 40% cassava starch (w/w) (values are means of 20 samples ±sd) 172

Figure 7.7 Effect of fish/cowpea: cassava starch on die swell of extruded snack pellets at the third segment of the barrel temperature of 120 °C (values are means of 20 samples ±sd) 172

Figure 7.8 Effect of fish/cowpea: cassava flour on expansion ratio of extruded snack pellets at the third section of the barrel temperature of 120 °C (values are means of 20 samples ±sd) 173

Figure 7.9 Unfried extruded pellets 177

Figure 7.10 Fried extruded pellets 177

Figure 7.11 FFA (%) changes of extruded fish products 180

Figure 7.12 TBARS changes of extruded fish products 181

List of Tables

CHAPTER 1 GENERAL INTRODUCTION

Table 1.1 Annual landings of the burrito fish 9
Table 1.2 Annual landings of the flying gurnard fish 11
Table 1.3 Essential amino acids composition of fish proteins compared to other proteins 19
Table 1.4 Mineral content of some fish compared with lean beef 30
Table 1.5 Vitamin content of cod, herring and lean beef 31
Table 1.6 Proximate composition of some important grain legumes 32
CHAPTER 3 PROXIMATE AND BIOCHEMICAL CHARACTERISATION OF THE BURRITO (BRACHYDEUTERUS AURITUS) AND THE FLYING GURNARD (DACTYLOPTERUS VOLITANS)

Table 3.1 Proximate and chemical compositions of fresh burrito (Brachydeuterus auritus) and the flying gurnard (Dactylopterus volitans) 67

Table 3.2 Protein concentration of protein extracts of burrito and the flying gurnard (mg/ml) 67

Table 3.3 Amino acid compositions of flying gurnard and burrito 69

Table 3.4 Fatty acid profile of the flying gurnard and the burrito (%) 70

Table 3.5 TBARS and PV of the flying gurnard and the burrito 70

CHAPTER 4 RHEOLOGICAL, DSC, FT-RAMAN SPECTROSCOPY AND PHASE MICROSCOPY STUDIES OF FISH MINCE, COWPEA FLOUR AND CASSAVA STARCH MIXTURES

Table 4.1 G' and G" (Pa) of flying gurnard and burrito mince after cooling from 90 °C to 20 °C 78

Table 4.2 Storage modulus (G' Pa) and loss modulus (G" Pa) of flying gurnard mince and cowpea flour during a dynamic temperature sweep at 90 °C and 20 °C 80

Table 4.3 Storage modulus (G' Pa) and loss modulus (G" Pa) of flying gurnard mince and cassava starch during a dynamic temperature sweep at 90 °C and 20 °C 83

Table 4.4 Storage modulus (G' Pa) and loss modulus (G" Pa) of burrito fish mince and cowpea flour during a dynamic temperature sweep at 90 °C and 20 °C 87

Table 4.5 Storage modulus (G' Pa) and loss modulus (G" Pa) of burrito fish mince and cassava starch during a dynamic temperature sweep at 90 °C and 20 °C 89

Table 4.6 Transition temperatures and enthalpy change for the burrito and the flying gurnard 92

Table 4.7 Transition temperatures and enthalpy change for the flying gurnard mince / cowpea flour 93
Table 4.8 Transition temperatures and enthalpy change for the flying
gurnard mince / cassava starch 94

Table 4.9 Transition temperatures and enthalpy change for the
burrito mince / cowpea flour 95

Table 4.10 Transition temperatures and enthalpy change
for the burrito mince / cassava starch 97

Table 4.11 Relative peak intensity of Raman spectra in the
regions 3200-700 cm$^{-1}$ for flying gurnard mince and varying addition of cowpea flour 100

Table 4.12 Relative peak intensity of Raman spectra in the
regions 3200-700 cm$^{-1}$ for flying gurnard mince and varying addition of cassava starch 105

Table 4.13 Relative peak intensity of Raman spectra in the
regions 3200-700 cm$^{-1}$ for burrito mince and varying addition of cowpea flour 110

Table 4.14 Relative peak intensity of Raman spectra in the regions
3200-700 cm$^{-1}$ for burrito mince and varying addition of
cassava starch 114

CHAPTER 5 PROCESSING OF FLYING GURNARD FISH
(DACTYLOPTERUS VOLITANS) INTO INFANT WEANING FOODS

Table 5.1 Composition of recipe/ingredient per 100 g for infants
between 6-9 months (wet weight basis) 122

Table 5.2 Proximate and chemical composition of the recipes 125

Table 5.3 Proximate compositions for the formulated recipes and
commercial infant food 126

Table 5.4 Amino acid composition of the formulations
(mg/100 g dry weight) 127

Table 5.5 G' and G' (Pa) of flying gurnard mince and recipe 2-5 128

CHAPTER 6 FLYING GURNARD (DACTYLOPTERUS VOLITANS.
L) SKIN GELATIN AND GELATION PROPERTIES

Table 6.1 Proximate composition of gelatin derived from skins of flying
gurnard as compared to that of horse mackerel and commercial
gelatins 144
## Table of contents

| Table 6.2 | Amino acid content for flying gurnard gelatin as compared to that of horse mackerel and commercial gelatin | 147 |
| Table 6.3 | Storage modulus (G' Pa) and loss modulus (G'' Pa) of flying gurnard gelatin and cassava starch during a dynamic temperature sweep at 90 °C and 20 °C | 152 |
| Table 6.4 | Transition temperatures and enthalpy change for gelatin from flying gurnard and commercial gelatin | 156 |
| Table 6.5 | Transition temperatures and enthalpy change for gelatin from flying gurnard / cassava starch | 158 |

### CHAPTER 7 EXTRUSION AND MANUFACTURE OF SNACK PRODUCTS FROM BURRITO (BRACHYDEUTERUS AURITUS) MINCE AND COWPEA FLOUR BLENDS

| Table 7.1 | Processing conditions of the single screw extruder and temperature profile in each section of the barrel | 166 |
| Table 7.2 | Proximate and chemical composition of extruded snack products of 70:30 (fish mince: cowpea flour) with varying concentration of cassava starch | 175 |
| Table 7.3 | Mean* scores for sensory evaluation of extruded snack products of (75:25) fish mince: cowpea flour with varying concentration of cassava starch | 176 |
| Table 7.4 | Mean sensory scores for colour and odour of extruded fish products (unfried) during eight months of storage* | 178 |
| Table 7.5 | Mean sensory scores for colour and odour of extruded fish products (fried) during eight months of storage * | 178 |
| Table 7.6 | Colour (L, a, b) values of extruded fish products (unfried) during eight months of storage | 179 |
| Table 7.7 | Colour (L, a, b) values of extruded fish products (fried) during eight months of storage | 179 |
CHAPTER ONE
Chapter 1 General Introduction

1.0 GENERAL INTRODUCTION

1.1 Summary background and objectives

The promotion and utilization of underutilized or low-priced fish species such as small pelagics and other by-catch for human consumption have in recent times intensified globally to meet the ever-increasing demand for protein. The nutritional value of these fish is as high as that of any of the more desirable species in terms of protein quality and other nutrients like fats, notably the omega-3 polyunsaturated fatty acids, minerals and vitamins (Nettleton, 1985; Pigott and Tucker, 1990). During bumper harvests in the fish industry in Ghana, large quantities of fish species which are undervalued and hence underutilized are harvested. Moreover, traditional processing techniques such as salting, drying and smoking which to a large extent are inadequate to processing the large mass of the catch are applied to the known and commercial species for economic reasons.

With protein as the major nutrient in these species as in other fish species, the inclusion of these species in product development could contribute towards the nutritional value, texture as well as flavour of such products. Asiedu et al. (1994) indicated that the addition of fish protein in the form of dried pelagic fishmeal increased the overall nutritional level of corn flour. James (1984) observed that mixtures of maize and fish protein concentrate had a higher chemical score (calculated based on the essential amino acid composition) than the amino acid composition of egg protein.

In most food processing, gelled products are formed following some form of heat treatment. These developments are brought about by the interactions of the fish proteins within the fish and the other macromolecules from the ingredients that may occur both under physiological conditions and during the processing of the food (Friedman, 1996).
Chapter 1 General Introduction

Basically, the formation of gels from pelagic species often present problems due to the intrinsic characteristics of their muscles and more so when unrefined muscles are used (Leinot and Cheftel, 1990; Niwa et al., 1988; Chung and Lee, 1990). Interactions of globular proteins-protein and other macromolecules have been followed by a number of methods including rheological measurements, differential scanning calorimetry, phase contrast microscopy, electron microscopy (Howell and Lawrie, 1985; Howell and Taylor, 1991) and relatively more advanced techniques such as FT-Raman spectroscopy (Howell and Li Chan, 1996; Howell et al., 1999a). Studies of the interactions of the unrefined proteins from these selected species and other macromolecules can provide valuable information on their use and new protein applications in food formulations (Chung and Lee, 1990; Gómez-Guillén et al., 1996). Technological and research efforts would also be required to develop value-added products from these resources in the form of infant food, snacks or other convenience type of food products.

Plant protein products, such as cowpea (a legume) proteins, are gaining interest as ingredients in food systems throughout many parts of the world in the production of value-added foods such as cowpea flour, extruded snack items, cowpea rice and infant foods. A number of reports claim that inclusion of legumes in the daily diet has many beneficial physiological effects in controlling and preventing various metabolic diseases such as diabetes mellitus, coronary heart disease and colon cancer (Shehata et al., 1988; Simpson et al., 1981). The consensus of recent opinion on healthy eating habits favours an increase in the proportion of legume-based polymeric plant carbohydrates including starch in the diet. They are generally good sources of slow release carbohydrates (viz. dietary fibre) and are rich in proteins (18–25%). On the other hand, legumes have been
reported to contain adequate amounts of lysine, but are deficient in S-containing amino acids (methionine, cystine and cysteine) (Farzana and Khalil, 1999).

Fish processing in general leads to an enormous amount of waste accounting for approximately 75% of the total fish weight (Shahidi, 1994) after filleting. About 30% of the waste is in the form of bones and skins (Gómez-Guillén et al., 2002). The fish skins and bones can be processed into gelatin, thus contributing to solving the problem of waste disposal in addition to creating a value-added product. To aid post-harvest processing of these species in Ghana, the innovative design and fabrication of a low cost extruder for small and medium scale enterprises (SME) was undertaken in the INCO-DEV project to expand and complement the traditional techniques.

Two fish species, which in the glut season are undervalued and not patronized by most people in Ghana, are the burrito (*Brachydeuterus auritus*) and the flying gurnard (*Dactylopterus volitans*) (Linnaeus, 1758). This may be due to, among other factors, their dark flesh, bony nature and consumer prejudices against these species.

### 1.1.1 Objectives of Study

The objectives of the study:

1. To characterize the selected low value fish species by their physicochemical properties including chemical and biochemical properties.

2. To study the interaction of the fish mince of the selected species with cowpea flour and cassava starch.
Chapter 1 General Introduction

3. To design and fabricate a low cost extruder which would serve as a prototype for SME in Ghana.

4. To develop value-added products as infant food and extruded dried products from the fish species incorporating local food crops.

5. To develop gelatin from the fish skin and characterize it by its physicochemical and rheological properties.

6. To monitor the quality and biochemical changes of the extruded products and infant weaning foods.
1.2 Fish handling and processing in Ghana

The fish industry in Ghana consists of marine and inland fisheries. With a coastline of 563 km and an exclusive economic zone (EEZ) of 200 nautical miles, the marine fisheries provide about 80% and inland fisheries 20% of the total fish catch in Ghana (Anon. 2001). Although fish is caught all year round, most of the catches are produced in the months of July to September. In the artisanal fishery industry through which most of the catches are made, pelagics, which are of low commercial value, are not chilled in spite of the high ambient temperatures. Under these conditions autolysis is accelerated in the viscera releasing bacteria and enzymes, which invade the flesh (Sikorski et al., 1990). Other post harvest handling and processing practices lead to poor quality products resulting in economic as well as nutritional losses (Hodari-Okae et al., 1996a).

Sun drying, salting and drying and smoking are the main processing techniques generally employed in the artisanal fish industry. During the major fishing season, when about 40% of the total annual catch is landed, these techniques are wholly inadequate to meet the large quantities of fish landed (Mensah, 1991) resulting in post harvest losses. These losses do not only affect the protein availability for human consumption but are also an economic loss to the artisanal fisher folk (Mensah, 1991).

Though these traditional processes have certain advantages in supplying desirable products at low cost to the local markets, these traditional processes unfortunately result in products with poor functional and nutritional qualities. Some protein and other nitrogenous substances including free amino acids may be lost during salting.
Aitken and Connell (1979) observed that salting and drying could preserve much of the nutritional value of fresh fish but excessive heat treatment tended to impair the nutritional value of fish proteins. Hoffman et al., (1977) reported that traditionally prepared hot smoked fish suffered appreciable protein damage from the loss of lysine availability. As much as 71% loss of lysine was reported for heavily smoked salmon. Obileye and Spinelli (1978) observed a minimal loss in lysine availability by gradual removal of moisture from Tilapia.

Opstvedt (1988) in a review on views on the effects of smoking and drying on the protein quality of fish muscle, concluded that at drying temperatures of less than 100 °C losses are moderate. This view was subsequently shared by Ma'ruf et al., (1990). He also concluded that, although the results are not uniform, normal cooking procedures at temperatures up to 100 °C have little effect. However frying, especially in oxidised oil, has been shown to reduce the digestibility of fish protein (Motohiro, 1988).

The typical tropical climate with humid conditions has been noted to affect traditionally processed fish (Wood, 1981; Hodari-Okae et al., 1996b). Heavy infestation of unsalted dried fish by blowflies may cause up to 30% loss of the product (Osuji, 1976; Wood, 1981; Hodari-Okae et al., 1996b). In certain instances, dried fish containing about 21% salt were heavily infested by insects of different species (Indriati et al., 1985). The sensory properties of dried products may deteriorate during storage due to oxidation of lipids (Bligh et al., 1988) and excessive heating during smoking could lead to losses in free amino acids and lysine residues in proteins (Opstvedt, 1988). In salting, fish proteins could be denatured resulting in decreased protein extractability (Tülsner, 1978).
1.3 Importance of fish in Ghana

Fish and fishery products account for the major animal protein intake in the diet of the majority of Ghanaians, as these are the cheapest animal protein available. Fish provides about 60% of the country's protein requirement (Mensah, 1991). Of the fish consumed in Ghana, 75-80% is smoked whilst the remaining is either salted and sun-dried or consumed fresh. The industry provides the main income for the artisanal fishermen and their dependants, including the many fish mammies involved in fish processing and marketing (Mensah, 1991).

With limited resources, depleting fish stocks and growing population, there is the need to improve utilization of the artisanal fish catches through product development of low value species (Whittle and Wood, 1992). Such products would not only add value to the fish but would be able to attract export markets as well. In this study, the development of two products, which were thought to be of commercial importance, were investigated; these were extruded snack products and infant weaning foods, which generally lack adequate protein (FAO, 1991). The products in this study, incorporated fish mince, cowpea flour, cassava starch and other food ingredients.

1.4 Biological characteristics of the selected species

1.4.1 The bigeye grunt (burrito) (Brachydeuterus auritus)

The big eye grunt (Figure 1.1), *Brachydeuterus auritus* (Valenciennes, 1831), is an important marine fish in Ghanaian coastal waters and belongs to the family Pomadysidae. It inhabits the coastal waters off the West African coast from Mauritania (26°N) to the south of Angola (17°S), and is one of the most important by-catch fish species within the sub-region. It is also found in the Mediterranean to the northern
regions of Morocco (Fisher and Bianchi, 1984). Its distribution varies generally between depth contours of 10 to 100 m but is most commonly found in inshore areas between 30 – 80 m. It is also an important fish species in the CECAF (FAO-Fishery Committee for the Eastern Central Atlantic) region.

Within Ghanaian coastal waters the big eye grunt, which is popularly called burrito, is the smallest of the family Pomadysidae, yet it is one of the most important in terms of abundance and quality. It could be used for local consumption and marketed either smoked or dried for protein supplement in poultry feeds. These small pelagic species grow up to 30 cm but are commonly caught at lengths of 18-23 cm in Ghana coastal waters. Fischer and Bianchi (1984) described the big-eye grunt as characterised by an oblong and compressed body with a large head. The snout is shorter than the eye diameter. It is coloured olive brown on its back with a distinctive dark blotch on the
upper corner of the opercular bone. It is distinguished from other members of its family by having less than 51 scales on its lateral line and a deeply notched dorsal fin with not more than 13 rays. The grunts are known as such because of their ability to produce sound vibrations by rubbing of their pharyngeal bones thus amplifying sounds by resonance on their swim-bladders (Fischer and Bianchi, 1984).

Table 1.1 Annual landings of the burrito fish

<table>
<thead>
<tr>
<th>Year</th>
<th>Weight (metric ton) t</th>
<th>Year</th>
<th>Weight (metric ton) t</th>
</tr>
</thead>
<tbody>
<tr>
<td>1980</td>
<td>10,047</td>
<td>1991</td>
<td>11,589</td>
</tr>
<tr>
<td>1981</td>
<td>5,338</td>
<td>1992</td>
<td>10,848</td>
</tr>
<tr>
<td>1982</td>
<td>5,835</td>
<td>1993</td>
<td>17,985</td>
</tr>
<tr>
<td>1983</td>
<td>9,533</td>
<td>1994</td>
<td>16,171</td>
</tr>
<tr>
<td>1984</td>
<td>17,268</td>
<td>1995</td>
<td>12,489</td>
</tr>
<tr>
<td>1985</td>
<td>13,806</td>
<td>1996</td>
<td>12,113</td>
</tr>
<tr>
<td>1986</td>
<td>20,825</td>
<td>1997</td>
<td>17,388</td>
</tr>
<tr>
<td>1987</td>
<td>16,627</td>
<td>1998</td>
<td>9,773</td>
</tr>
<tr>
<td>1988</td>
<td>11,114</td>
<td>1999</td>
<td>9,976</td>
</tr>
<tr>
<td>1989</td>
<td>9,743</td>
<td>2000</td>
<td>8,865</td>
</tr>
<tr>
<td>1990</td>
<td>19,997</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Source: Anon. 2001

Other scientific names (synonyms) still in use are *Otoperca aurita* (Val. 1831) with local vernacular names including ‘Moi, ‘Boeboe’, and ‘Ebie’. In the 1970s to 1980s, *Brachydeuterus auritus* accounted for over 5 percent of the artisanal fish caught in the country (Anon. 2001). As shown on Table 1.1, from the early 1980s catches appeared to decline to as low as 5,000 t, probably due to inaccurate data collection since data collection had generally focused on the main commercially important fish species but
again rose to approximately 20,000 t, in 1986. Since then, 1990s catches have fluctuated around a mean of 11,000 – 17,000 t with peaks in 1990 and 1994.

1.4.2 The flying gurnard (*Dactylopterus volitans* Linnaeus)

The flying gurnard (*Dactylopterus volitans*. Linnaeus, 1758) (other names *Cephalacanthus volitans* (local) Pampansre) has a depressed body with a reddish upper dark portion, the lower part of the body is coloured red and head is made up of shells (Figure 1.2). The skin of the fish is rough with 2 pectoral fins. Length of the fish is up to 45 cm commonly between 25-30 cm. The species is a common bottom dwelling fish inhabiting soft-sandy bottoms of depths ranging between 30-65 m.

![Figure 1.2 The flying gurnard (*Dactylopterus volitans*) Source: Fischer and Bianchi (1984)](image)

Table 1.2 shows the yearly landings from industrial and inshore vessels of the flying gurnard for the years 1988 to 2000. The fish is mainly caught in the month of June to August. The flying gurnard is quite abundant in Ghanaian waters but due to its very low market value it is regarded as of no statistical importance and the available data
Chapter 1 General Introduction

appear to depict the low quantities landed, hence the declining landed weights over the years as captured in Table 1.2 (Anon., 2001).

Table 1.2 Annual landings of the flying gurnard fish

<table>
<thead>
<tr>
<th>Year</th>
<th>Weight (metric ton) t</th>
<th>Year</th>
<th>Weight (metric ton) t</th>
</tr>
</thead>
<tbody>
<tr>
<td>1988</td>
<td>65</td>
<td>1995</td>
<td>1</td>
</tr>
<tr>
<td>1989</td>
<td>181</td>
<td>1996</td>
<td>68</td>
</tr>
<tr>
<td>1990</td>
<td>21</td>
<td>1997</td>
<td>68</td>
</tr>
<tr>
<td>1991</td>
<td>2</td>
<td>1998</td>
<td>0.4</td>
</tr>
<tr>
<td>1992</td>
<td>5</td>
<td>1999</td>
<td>0.1</td>
</tr>
<tr>
<td>1993</td>
<td>4</td>
<td>2000</td>
<td>0.1</td>
</tr>
<tr>
<td>1994</td>
<td>1</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>


1.5 Fish muscle structure and composition

1.5.1 Physical composition of fish muscles

Fish muscle (somatic muscle) is the dominant tissue in fish and constitutes more than 60% of the live fish weight (Love, 1980). It is also the tissue that is consumed as human food. Physically two main types of muscles can be identified; dark and white (Love, 1970, 1980). Based on structure, the two types of muscle are similar in that both are arranged into fibrils, but the dark muscle fibres are thinner than the white ones (Braekkan, 1959) and they also have different patterns of innervation (Bone, 1966).

1.5.2 Chemical composition of fish muscles

The two types of muscle are chemically very different (Love, 1970, 1980). Being more vascular than white muscle, dark muscle contains higher concentrations of haemoglobin, myoglobin and cytochrome C. (Love, 1988; Shimizu and Matsura, 1971;
Matsui et al., 1975). These three factors contribute to the difference in appearance between the two types of muscles. Having more mitochondria than white muscle, dark muscle is therefore better supplied with the enzymes and metabolic systems associated with these sub-cellular particles. Due to the above properties, dark muscles have the ability to function aerobically as well as anaerobically, while the energy metabolism of white muscle is almost entirely dependent on anaerobic glycolysis. Dark muscle usually can sustain propulsion on a continuous basis, but the white is liable to early exhaustion. Hence white muscle can be used primarily only intermittently when chasing prey or attempting to avoid predators. Within fish species, white flesh has more protein than dark flesh (Suzuki, 1981).

Dark muscle is characterised by high concentrations of lipid compared to white muscle. In addition, despite its dependence on anaerobic glycolysis for providing energy, white muscle usually contains less glycogen than dark muscle, even in the resting state. The differences in these reflect first in metabolic differences between the two types of tissue, and then in the modes of life of those fish, which have small or large proportions of dark muscle.

1.5.3 Characteristics of pelagic species muscles

Pelagic species, due to their composition and structure can be fatty or non-fatty. Fatty species tend to have high proportions of dark muscles, which are associated with high and seasonally variable lipid contents. Pelagic fish are more active than demersal ones and have a higher ratio of dark to white muscle. In addition to this, the relative amounts of dark and white muscle vary along the length of the fish. There is more dark muscle in regions associated with higher activity such as the tail and certain fins (Love, 1970,
Chapter 1 General Introduction

1980, 1988). Most species have the dark muscle as a subcutaneous layer in the region of the lateral line. It is thickest just under the lateral line and tapers off dorsally and ventrally. The deposits of lipid, which are used by pelagic fish for providing energy, are situated not only in the dark muscle but also in depot cells elsewhere.

1.6 Proteins

Proteins are complex macromolecules consisting of basic amino acids joined by peptide bonds. The amino acids consist of a basic amino group (\(-\text{NH}_2\)), an acidic carboxyl group (\(-\text{COOH}\)), and an organic \(R\) group (or side chain), which is unique to each amino acid and vary in chemical complexity to give different chemical properties and structures (DeMan, 1990). The general formula is usually represented by CHRNHCOOH. The sequence of the amino acids determines the primary structure of each protein. Subsequent interactions within this primary structure give rise to secondary, tertiary and quaternary structures (Branden and Tooze, 1991).

1.6.1. Primary structure

The primary structure of a protein is formed when a peptide bond joins the carboxyl group (\(-\text{COOH}\)) of one amino acid to the amino group (\(-\text{NH}_2\)) of another in a linear sequence (Figure 1.3) releasing water (H\(_2\)O). The chain length and sequence of the polypeptide determines its three dimensional structure in solution, and its function (Holme and Hazel, 1998).
1.6.2 Secondary structure

In the secondary structure, the polypeptide chains fold up in a variety of ways via two main types of chemical interactions to form a three-dimensional helical structure. (Figure 1.4) These are: (i). hydrogen bonds between the oxygen of one peptide bond and the nitrogen of another. (ii) interaction between the side chains of amino acids. Depending on the nature of the side chains, different regions of the chain may fold into α-helix or β-pleated sheet. In the α-helix the peptide backbone of the protein adapts a spiral form (Nakai and Modler, 1996). The α-helical structures are predominantly amphiphilic that is half of the helical surface is hydrophilic and the other half is hydrophobic. Generally in native proteins, the hydrophobic surface of the α-helix faces the interior of the protein and is engaged in hydrophobic interactions with other non-polar groups which are said to contribute to the stability of the folded form of the protein (Damodaran, 1996). The β-sheet structure is an extended structure in which the C=O and N-H groups are oriented perpendicular to the direction of the backbone.
1.6.3. Tertiary structure

The tertiary structure is a compact three-dimensional folded unit formed by the spatial arrangement of the entire polypeptide chain of secondary structure segments (Figure 1.5). Since the folding of the chains is determined by the interactions between amino acid side chains and its environment, the sequence of amino acids and their positions largely affect the nature of the structure (Holme and Hazel, 1998). The tertiary structure is effectively the sum of all possible non-covalent interactions within a protein and between the solvent. The tertiary formation affects a relocation of the non-polar residues to the interior thereby reducing the interfacial area between the non-polar groups of the protein and the surrounding solvent. However, not all the hydrophobic groups are buried as in globular proteins.
Lee and Richards (1971) reported that about 40-50% of the water accessible protein surface is occupied by non-polar residues. Some polar residues which are inevitably buried in the interior of the structure may be hydrogen bonded to each other or involved in electrostatic interaction with oppositely charged residues thereby contributing to the stability of the proteins. The relative spatial distribution of the hydrophilic and the hydrophobic residues also influences several physico-chemical properties such as morphology and catalytic functions (Damodaran, 1996).

1.6.4 Quaternary structure

The quaternary structure refers to the spatial arrangement of the subunits (Nakai and Modler, 1996) via interactions between the three-dimensional structures (Figure 1.5) to form a large, complex protein referred to as the oligomeric structure (Figure 1.6). The subunits may be identical polypeptide chains or composed of units from different origins (Zubay et al., 1995; Berg and Tymoczko, 2002).
Figure 1.6 Quaternary structure of protein (Adapted from Microsoft® Encarta® Encyclopedia 2002).

Typical protein aggregation is human haemoglobin, which contains 4 polypeptide chains ($\alpha_2 \beta_2$) held non-covalently, in a specific conformation. Other food proteins such as those present in legumes and oilseeds are oligomeric with several subunits.

1.7 Fish Proteins

Fish proteins, like all animal sources of protein, are taken to be the component proteins of the contractile muscle. However, the anatomical, physiological and biochemical properties of fish species give rise to distinctive muscle composition in terms of physical and chemical properties. Muscle tissue contains hundreds of different proteins together with other nitrogen-containing molecules referred to as non-protein nitrogen (NPN). The NPN compounds include peptides, amino acids, amines, amine oxides, guanidine compounds, quaternary ammonium compounds, purines and urea. The protein and NPN are usually classified as the crude protein, and ranges from less than 8
Chapter 1 General Introduction

to more than 25% of fresh fish weight (Iwasaki and Harada, 1985; Nettleton, 1985; Wheaton and Lawson, 1985).

Fish proteins are of high quality comparable to those of meat, milk and egg (Table 1.3). In this respect, fish appears to contain a high-class protein comparable to those derived from other animal sources (FAO, 1962; Friedman, 1996). On a quantitative basis, edible portions of fresh water fish contain approximately 14-25% of protein, while marine fish contains about 9-26% (Love, 1980). Thus qualitatively and quantitatively fish can in a significant measure, supplement low protein high cereal diet consumed among the low-income population. Fish and fish products have been found to improve such diets (Cameron and Hofvander, 1983). Cereal proteins are rather low in lysine and methionine, which are high in fish (Friedman, 1996). Fish can thus provide an excellent supplementary source of proteins to infant whose diet is often made of starchy gruels. Walker (1990) has noted that these diets are often bulky but very low in nutrient and energy density. On the basis of their solubilities in water and strong salt solutions, fish proteins can be divided into three main groups namely; sarcoplasmic, myofibillar and connective tissue proteins.
Table 1.3 Essential amino acids composition of fish proteins compared to other Proteins

<table>
<thead>
<tr>
<th>Amino Acid</th>
<th>Fish (mg/g total N)</th>
<th>Beef</th>
<th>Milk</th>
<th>Egg</th>
<th>Wheat</th>
</tr>
</thead>
<tbody>
<tr>
<td>Histidine</td>
<td>180</td>
<td>230</td>
<td>190</td>
<td>150</td>
<td>130</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>330</td>
<td>320</td>
<td>350</td>
<td>350</td>
<td>210</td>
</tr>
<tr>
<td>Leucine</td>
<td>530</td>
<td>500</td>
<td>640</td>
<td>520</td>
<td>420</td>
</tr>
<tr>
<td>Lysine</td>
<td>610</td>
<td>570</td>
<td>510</td>
<td>390</td>
<td>150</td>
</tr>
<tr>
<td>Methionine</td>
<td>180</td>
<td>170</td>
<td>180</td>
<td>200</td>
<td>100</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>260</td>
<td>280</td>
<td>340</td>
<td>320</td>
<td>280</td>
</tr>
<tr>
<td>Threonine</td>
<td>300</td>
<td>290</td>
<td>310</td>
<td>320</td>
<td>170</td>
</tr>
<tr>
<td>Tryptophan</td>
<td>70</td>
<td>80</td>
<td>90</td>
<td>110</td>
<td>70</td>
</tr>
<tr>
<td>Valine</td>
<td>360</td>
<td>330</td>
<td>460</td>
<td>470</td>
<td>280</td>
</tr>
</tbody>
</table>

Source: Garrow and James (1993).

1.7.1 Sarcoplasmic proteins

Sarcoplasmic proteins which are either soluble in water or in dilute buffers, constitute between 20 - 35% of the protein content of the muscle (Connell 1968; Sikorski et. al., 1976; Shenouda, 1980; Matsumoto, 1980). They are of relatively low molecular weight and do not contribute to the water holding capacity or gel-forming properties of the muscle (Smith, 1991). Myoglobin associated with oxygen transport is found in this fraction. The sarcoplasmic proteins appear to be separately involved in initiating oxidative or Maillard type reactions, which have a detrimental effect on the organoleptic properties of processed fishery products (Opstvedt, 1988).

Sarcoplasmic proteins exhibit a greater consistent degree of differences between species than do any other components or groups of compounds. However, similarities exist between related species. The content of sarcoplasmic protein is usually higher in
pelagic fish than in demersal fish (Suzuki, 1981). Sarcoplasmic proteins are separated in electrophoresis or isoelectric focussing, which give a unique set of ‘fingerprints for individual species (Laird et al., 1982, Mackie, 1980).

1.7.2 Connective tissue proteins

Connective tissue proteins comprise about 2-3% of the total proteins in most finfishes. In elasmobranches, the amount of these proteins can reach as high as 10% of the total proteins. These proteins of which collagen is the principal component were regarded as of little technological significance (Love, 1970, 1980). However, recent global developments in the quest for alternative sources of gelatin other than bovine or porcine sources, fish species have generated quite a significant interest in the study of fish collagen as a primary source of gelatin (Choi and Regenstein, 2000). This is principally attributed to the periodic outbreaks of bovine spongiform encephalopathy (BSE) coupled with religious prohibitions. Collagen is a white fibrous connective tissue (Voet et al., 1999) found in tendons skin, bone, the vascular system and the sheaths surrounding muscles. Collagen forms one-third or more of the total protein of mammals but the amount in fish is generally much less (Sikorski and Borderias, 1994). The basic molecular unit of collagen is a triple-helical rod (tropocollagen triple helix). This consists of three α-chains arranged in a left-handed axis, with the whole structure wound into a right-handed super helix. These are arranged in bundles, which make up the connective tissue matrix (Figure 1.7).
Figure 1.7 Successive stages in the formation of collagen. (a-d) free amino acids are joined in a peptide chain, which twists itself into a left-handed helix. (e) Three of these intertwine to form a right-handed super helix. (f) This is the tropocollagen molecule (g-h). Molecules line up in a staggered fashion to overlap by one-quarter length to form a fibril. Source: Gross, (1961).

1.7.3 Myofibrillar proteins

Of the total protein content of the fish muscle (Figure 1.8), the myofibrillar proteins constitute about 65 - 75%. These proteins are those that constitute the contractile apparatus or myofibril within the muscle cell (Connell, 1954; Buttkus, 1966; Seki and Watanabe, 1984; Chen et al., 1988; Sano et al., 1989).
Figure 1.8 Fish muscle cells and component myofibrils showing actin and myosin filaments. Source: Bechtel (1986).

The myofibrillar proteins consist of myosin, actin, tropomyosin and other minor proteins such as C protein, M protein, α-actinin and the troponins T, I and C (Bechtel, 1986; Asghar et al., 1985).

The structural proteins form about 70-80% of the total proteins of fish muscle. Actin and myosin probably account for over 90% of the structural proteins, with tropomyosin comprising most of the remainder. As in the case with meat muscle, it is the structural-
proteins that give fish muscle its characteristic physical properties (Sikorski et. al., 1976; Shenouda, 1980; Matsumoto, 1980).

The most important property of fish muscle is the ability of the myofibrillar proteins to form a three dimensional network, or gel, upon heating (Niwa, 1985; Doi, 1993). This property among other variables depends on the fish species, type of muscle and its freshness quality (Sikorski et. al., 1976; Shenouda, 1980; Matsumoto, 1980).

The distribution of salt soluble myofibrillar proteins (actin and myosin) and water-soluble sarcoplasmic protein in fish muscle tissue varies from species to species (Shenouda, 1980; Matsumoto, 1980; Shimizu, 1985; Martinez et al., 1990). Fish muscle contains considerably less stroma (the main protein found in the connective tissue) than mammalian muscle, allowing the fish muscle tissue to form a more uniform gel matrix than mammalian muscle tissue upon comminution with salt (Montejano et al., 1983). During the post-rigor stage, actin and myosin in fish muscle form an actomyosin complex, which becomes important to fish processing. The salt solubilisation of myofibrillar protein with a proper amount of water results in the formation of sol, which subsequently turns to an elastic gel upon heating (Roussel and Cheftel, 1988). This unique functional behaviour of fish myofibrillar protein enables the development of various textured products from either fish mince or surimi (Yasui et al., 1980; Lin and Lanier, 1981; Rodger et al., 1984; Lanier, 1986).
Factors affecting the physical properties of fish protein gels

The physical properties of fish protein gels of specific fish species may be affected by the following (Asghar et al., 1985; Roussel and Cheftel, 1990; Jiménez Colmenero et al., 1994; Gómez-Guillén et al., 1997):

- Condition of the raw fish
- The refining process
- Sol formation by comminution
- Gel formation by heat setting

The quality of freshness, age and seasonality determine the condition of the raw fish. Fish is a very perishable commodity and undergoes various stages of post-mortem biochemical changes from the point of catch to processing as noted by a number of authors (Amu and Disney, 1973; Sikorski et al., 1990; FAO, 1995; Davis, 1995; Abbey, 1996). The post-mortem biochemical changes are proteolytic degradation of the muscle tissue due to proteases. This results in the breakdown of the myofibrillar protein with a gradual loss of gel-forming ability and a decrease in the level of extractable actomyosin (Kawashima et al., 1973). The proteolytic activity varies from species to species and specific part of the fish body (Davis, 1995), as well as temperature and pH (Geist and Crawford, 1974).

The muscle tissue of fish responds to seasonal changes due to spawning and feeding. Throughout the spawning period, fish muscle loses gel-forming ability as a result of increased proteolytic action on the muscle tissue mainly by acid proteases such as cathepsin D (Haard, 1994). This is accompanied by a decrease in the myofibrillar protein level and moisture retention in the tissue (Haard, 1994).
Chapter 1 General Introduction

In fish mince preparation, the refining process involves washing and straining. During washing, the sarcoplasmic protein is removed thereby increasing the myofibrillar protein concentration and further straining also removes most of the connective tissue (stroma) (Suzuki, 1981; Sonu, 1986).

Comminution ("chopping") breaks the muscle tissue into small fragments, which increases the surface area exposed. During comminution with salt (mainly NaCl) and water, myofibrillar protein is solubilised and forms a viscous sol (Rizvi, 1981; Hamn, 1986; Yamamoto et al., 1991). The sodium and chloride ions bind to the acidic and basic amino residues breaking the intermolecular ionic bonds of the protein molecules with water molecules also replaced. The sol so formed may be readily converted to a gel through setting, with or without heat. The mechanism of the setting phenomenon has been extensively studied by Niwa (1975); Shimizu et al. (1981) and Montejano et al. (1984). The gel is made up of water, which is immobilised within a 3-dimensional-protein matrix. The strength of the gel depends on the degree of water binding within the matrix and it is expressed by an inverse relationship (Ziegler and Foegeding, 1990; Okada, 1963; Lee, 1991). Tightly bound water molecules produce stronger gels. However, gel strength can be improved with water binding agents, such as starch, non-fish proteins and powdered cellulose with the added ability to expand during thermal transition (gelatinisation) (Niwa et al., 1988; Chung and Lee, 1990).

Sols undergo gelation where randomly arranged peptide chains, irreversibly turn to an ordered state called "setting" by three different means. These are cold-setting, partial-heat setting and full-heat setting. The on-set of cold setting is either at refrigeration temperature (0-4 °C) or room temperature (22 °C). Cold setting requires a long time
and as heat is not involved no thermal denaturation is observed. However, setting at refrigeration temperature takes longer than at ambient temperature and gels so formed are firmer than those formed at ambient. Gel strength during cold setting is time dependent and the type of bond involved in the gel network is basically hydrogen bonding (Okada, 1963). Due to the absence of denaturation the gels appear to be glassy and translucent as compared to heat set gels.

The application of heat in partial heat setting is achieved in the temperature range of 40-50 °C. This often results in a mild protein denaturation. Based on fluorimetry, chemical modification and solubilisation studies, hydrophobic bonds are predominantly observed to be involved in the gel network (Niwa, 1975; Niwa et al., 1981, 1988) and to a lesser extent, disulfide (S-S) and hydrogen bonds (Niwa, 1975).

During a full-heat setting, which is at 80-95 °C for a period of time sufficient enough to cook the gel, the protein undergoes complete denaturation. The gel network is stabilised by hydrophobic and disulfide bonds as well as hydrogen bonds (Niwa et al., 1986). Compared to partial-heat gels the protein networks of cooked gels are less uniform.

Water is known to be the major chemical constituent of all fish flesh. However, fatty pelagic fish have significantly lower water contents than non-pelagic species. The sum of the lipid and water concentrations in fatty fish is approximately 80% (Love, 1970). Starvation, which occurs in many fish species during spawning, depletes the energy reserves of the tissues and consequently increases the water content of the flesh. Water serves as a solvent for a host of organic and inorganic solutes in muscles and other
tissues hence providing the environment for biochemical and many reactions. In addition, water has a great impact on the conformation and reactivity of proteins. Protein hydration influences the rheological properties and juiciness of muscle foods. The state of water in the fish flesh depends upon various interactions of water molecules with different solutes and especially with proteins. The hydrophilic amino acid residues are involved in H-bonding with water molecules and structures. The hydrophobic groups in the proteins and lipids induce around themselves layers of highly ordered water structures thus acting as structure makers. In fish meat, only a part of the aqueous medium can be regarded as bulk water. The rest of the water is involved in water-protein-lipid-solute interactions.

1.8 Fish oils and fatty acids

Fatty pelagics contain lipids in all tissues, which are concentrated mostly in the subcutaneous fatty layer and in the muscle tissue. Fatty acid composition varies between and within species (Leu et al., 1981), but generally the edible portion in the form of white and dark muscle account for more than 50% of the body components (Ackman and Eaton, 1971). Seasonal variations of fat in the edible muscle of some pelagic species were observed by Ackman (1982) and Ackman and Eaton (1971). It was observed that the dark muscle have a relatively higher level of total lipid (9-18%), than the light muscle (2.25-10%) in spring. Jangaard et al. (1967) and Orlick et al. (1991) observed very little variation in total lipid of cod muscle with season.

The current concern and interest in the health and nutritional properties of polyunsaturated fatty acids (PUFA), particularly of the ω3 series, have created the need for detailed composition of all the lipids of pelagic fish (Nettleton, 1985; 1987;
Chapter 1 General Introduction

Somogyi and Hötzel, 1990). The lipids in fatty fish species are most commonly present in the form of phospholipids and triglycerides. Phospholipids are particularly rich sources of ω3 PUFA, and the consumption of any fish, even non-pelagic ones, can make a useful contribution in nutritional terms. Pelagic fish are of importance in the preparation of isolates for dietary supplements or pharmaceutical purposes in reducing the risk of coronary disease (Kinsella, 1988). These effects have been attributed to PUFA of 20:5ω3-eicosapentaenoic acid (EPA) and 22:6ω3-docosahexaenoic acid (DHA) (Barlow et al., 1990; Newman et al., 1993). Studies by Phillipson et al. (1985) showed that fish oils in diets decreased plasma cholesterol and triglyceride in patients with elevated blood lipid levels. Considerable evidence shows that n-3 polyunsaturated fatty acids have a place in the dietary requirements of the normal human with intakes of about 1 g per day being sufficient (Bjerve et al., 1992; Newman et al., 1993). The role of fish oils in infant nutrition has been extensively studied (Cockburn, 1997; Koletzko, 1992; Agostoni et al., 1995) indicating that infants might not be able to make the essential long chain polyunsaturated fatty acids. Cockburn (1997) suggested that during the first year of life approximately 50% increase in brain weight occurs in the cerebral cortex and 60% of this increase is due to the accretion of phospholipids in neural membranes and myelin sheath.

The greater proportions of the more PUFA (those with 5 and 6 double bonds) come from fish that live in the Southern Hemisphere (Ackman, 1980). However, a disadvantage associated with this observation is the greater reactivity of southern fish meals compared with that of the meals, which originate in the north. The instability of the residual lipids in the southern fish species renders the associated meals more liable (in the absence of stabilisation with antioxidants or by other means) to damage by
spontaneous over-heating. There is some evidence that fish lipases are relatively heat stable and when fatty fish are converted into fish meal and oil the lipases will continue to hydrolyse the oil even after processing, unless they are removed rapidly and efficiently by centrifugation (Banks, 1967).

Post-mortem lipid degradation proceeds mainly due to enzymatic hydrolysis. Both lipid classes are hydrolysed by the endogenous enzymes at rates that are dependent on species, nutritional and maturation status, tissue and temperature (Ackman, 1980; Hardy, 1980; Nakano et al., 1992). The major products are fatty acids, glycerol and various amine and phosphoric acid derivatives (Lovern and Olley, 1962; Wu and Toyomizu, 1974). However, studies have proved that unsaturated lipids in close association with proteins, enzymes, nucleotides and amino acids induce polymerisation of the proteins, enzymes and nucleotides and cause destruction of amino acids (Roubal, 1970; Jarenbäck and Liljemark, 1975). Hseih and Kinsella et al. (1989) reported high levels of cellular unsaturated lipids in fish, which deteriorated very fast by peroxidation leading to the development of objectionable odours and flavours.

Many reports are available on the fat and fatty acid profiles of a number of fish and shellfish (Gooch et al., 1987; Ackman, 1982), but these at best cover species found outside the Ghanaian coastal waters and do not cover the burrito (Brachydeuterus auritus) and the flying gurnard (Dactylopterus volitans).

From these reports it is apparent that the PUFA of many fish lipids are dominated by EPA and DHA including pelagic species as the burrito (Brachydeuterus auritus) and the flying gurnard (Dactylopterus volitans).
1.9 Minerals in Fish

Fish like other living organisms contain most of the 90 naturally occurring elements (Lall, 1995). The mineral content of fish depends on the type of fish. It is evident from the comparative data in Table 1.4 that fish contains less than one half of the amount of iron in lean meat due to its lower concentration of myoglobin.

Table 1.4 Mineral content of some fish compared with lean beef

<table>
<thead>
<tr>
<th>Mineral</th>
<th>Grilled Cod</th>
<th>Grilled herring</th>
<th>Grilled beef</th>
</tr>
</thead>
<tbody>
<tr>
<td>Iron (mg/100 g)</td>
<td>0.40</td>
<td>1</td>
<td>3.50</td>
</tr>
<tr>
<td>Iodine (µg/100 g)</td>
<td>11</td>
<td>32</td>
<td>6</td>
</tr>
<tr>
<td>Selenium (µg/100 g)</td>
<td>25</td>
<td>41</td>
<td>3</td>
</tr>
<tr>
<td>Calcium (mg/100 g)</td>
<td>10</td>
<td>33</td>
<td>7</td>
</tr>
<tr>
<td>Phosphorus (mg/100 g)</td>
<td>200</td>
<td>240</td>
<td>230</td>
</tr>
</tbody>
</table>

Source: British Nutrition Foundation (1993)

The important functions of these minerals include the formation of skeletal structure, maintenance of colloidal systems, regulation of acid-base equilibrium and components of hormones, enzymes and activators (Lall, 1995). Invariably reports on the mineral composition of fish and fishery products are limited to selected minerals and cover mostly commercially important species. Selected species have been covered in North America (Exler, 1987; Gordon and Martin, 1982; Gordon and Roberts, 1977). There are also reports from Finland (Nuurtamo et al., 1980), Norway (Julshamn et al., 1978) and the United Kingdom (Lovern, 1984). The main minerals of fish include calcium,
Chapter 1 General Introduction

phosphorus and iodine, with small amounts of magnesium, sodium, lead and fluoride (Lall, 1995).

Calcium is involved in bone formation as well as in a number of regulatory functions (Karp, 1996). Phosphorus is directly involved in energy producing cellular reactions (Karp, 1996). About 99% and 80-85% of calcium and phosphorus respectively are found in bones as calcium phosphate (Ca$_3$(PO$_4$)$_2$) and hydroxyapatite (Ca$_{10}$(PO$_4$)$_6$(OH)$_2$) and the rest in extracellular fluids and intracellular structures and cell membranes.

1.10 Vitamins in fish

The liver of many fish species contains large amounts of vitamins A and D with very substantial variations within species (Billis et al., 1935). Many reviews have been published on the vitamin composition of fish and shellfish (Love, 1970; Sidwell et al., 1978; Gordon and Martin, 1982). A comparison of cod, herring and lean beef (Table 1.5) shows that oil-rich fish are a better source of fat-soluble vitamin A (Retinol) and D than white fish or red meat.

Table 1.5 Vitamin content of cod, herring and lean beef

<table>
<thead>
<tr>
<th>Vitamin</th>
<th>Grilled Cod</th>
<th>Grilled Herring</th>
<th>Grilled Beef</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamin A (Retinol) μg/100 g</td>
<td>2</td>
<td>34</td>
<td>Trace</td>
</tr>
<tr>
<td>Vitamin D (μg/100 g)</td>
<td>Trace</td>
<td>25</td>
<td>Trace</td>
</tr>
<tr>
<td>Vitamin E (mg/100 g)</td>
<td>0.44</td>
<td>0.3</td>
<td>0.29</td>
</tr>
<tr>
<td>Thiamin (mg/100 g)</td>
<td>0.08</td>
<td>Trace</td>
<td>Trace</td>
</tr>
<tr>
<td>Riboflavin (mg/100g)</td>
<td>0.06</td>
<td>0.18</td>
<td>0.36</td>
</tr>
<tr>
<td>Niacin (mg/100 g)</td>
<td>1.9</td>
<td>4</td>
<td>6.4</td>
</tr>
<tr>
<td>Vitamin B$_6$ (mg/100 g)</td>
<td>0.41</td>
<td>0.39</td>
<td>0.33</td>
</tr>
<tr>
<td>Vitamin B$_{12}$ (μg/100 g)</td>
<td>2</td>
<td>8</td>
<td>2</td>
</tr>
<tr>
<td>Folate μg/100 g)</td>
<td>10</td>
<td>7</td>
<td>17</td>
</tr>
<tr>
<td>Pantothenic Acid (mg/100 g)</td>
<td>0.25</td>
<td>0.6</td>
<td>0.9</td>
</tr>
</tbody>
</table>

Source: British Nutrition Foundation (1993)
Chapter 1 General Introduction

1.11 Cowpea and Cowpea Proteins

1.11.1 Importance of Cowpea

Cowpea (*Vigna unguiculata* [L.] Walp) referred to as beans, is a food legume with superior nutritional attributes (Aykroyd *et al.*, 1982; Deshpande and Damodaran, 1990; Davis *et al.*, 1991; Ehlers and Hall, 1997) and paste prepared from cowpea forms the basis of many popular African dishes such as akara and moinmoin (Dovlo *et al.*, 1976). It has been found to contribute up to 80% of the total dietary protein intake in some parts of West Africa (Sefa-Dedeh *et al.*, 2000). Cowpea is a highly nutritious crop with a dry seed protein content of about 25% (Table 1.6) and protein digestibility higher than that of other legumes (Afoakwa *et al.*, 2002; Olongbobo and Fetuga, 1983). It has been reported that cowpea contains good quality proteins with additional advantages of fibre, flavour and functional properties (Prinyawiwatkul, 1996). Nell *et al.* (1992) also reported that the average protein content of 150 different cowpea cultivars was 28.4±1.8% and for that matter were valuable protein sources.

Table 1.6 Proximate composition of some important grain legumes

<table>
<thead>
<tr>
<th>Nutrient (g/100g)</th>
<th>Chickpea</th>
<th>Cowpea</th>
<th>Lentil</th>
<th>Greenpea</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture</td>
<td>7.3</td>
<td>9.4</td>
<td>9.3</td>
<td>7.8</td>
</tr>
<tr>
<td>Crude Protein</td>
<td>24</td>
<td>24.7</td>
<td>26.1</td>
<td>24.9</td>
</tr>
<tr>
<td>Crude Fat</td>
<td>5.2</td>
<td>4.8</td>
<td>3.2</td>
<td>1.5</td>
</tr>
<tr>
<td>Ash</td>
<td>3.6</td>
<td>4.2</td>
<td>2.8</td>
<td>3.6</td>
</tr>
</tbody>
</table>


Cowpeas are used extensively to fortify cereal-based weaning foods in Ghana (Plahar and Annan, 1998). Foods containing considerable amounts of protein such as cowpea
maybe utilized either as they are or by preparing their protein concentrates and isolates. Protein concentrates or isolates may constitute one of the nutritious additives to other foods.

Many varieties of cowpea seeds exist and are known by their different sizes, shapes and especially seed colour which can be either white, red, brown, black, cream or mottled (Sefa-Dedeh et al., 2001; Afoakwa et al., 2002).

Cowpea is cultivated extensively, mostly in the savannah areas of West Africa, and in Nigeria and Ghana. Efforts aimed at breeding and improvement of cowpea cultivars for specific characteristics such as early maturity, high yield, desired seed quality and resistance to insects and diseases have been reported (Singh and Mare, 1985). However, studies are being conducted to find the best treatment to remove non-nutritional lectins, protease inhibitors, and other factors to lower cost and acceptability of the final processed meal (Tuan and Phillips, 1992).

1.1.2 Cowpea proteins

Cowpea flours, pastes and their respective protein concentrates, isolates and fractions have been studied extensively (Ragab et al., 2004; Rangel et al., 2003; Sefa-Dedeh and Stanley, 1979). Cowpea proteins have also been characterized (Freitas et al., 2004; Rangel et al., 2003; Sefa-Dedeh and Stanley, 1979). However, the information available on the protein isolates is focussed more on their application in nutrition and chemical evaluation than those biochemical properties that bear upon the transformation (reaction with other proteins and macromolecules) to maintain increase usage and post harvest stability. Cowpea protein on the basis of solubility could be fractionated into albumin
Chapter 1 General Introduction

(71.4%), globulin (11.1%), prolamin (2.20%) and glutelin (11.0%). Fleming et al. (1975) have reported that protein concentration, especially the globulin fraction, is an interaction between proteins, carbohydrates and lipids. This interaction is responsible for the gelation capacity of the legumes and oilseed proteins.

Studies on soya proteins also a legume indicated the globulins are made up conglycinin (7S) and glycinin (11S), which together make up of about 70% of the proteins, (Fukushima, 1991). These proteins play an important role in several food systems because of their functional properties (Puppo et al., 1995). The other fraction is made up of 2S and 15S globulins. The 7S β-conglycinin, which is a trimeric glycoprotein with molecular weight of 141,000-170,000 Da, is composed of six fractions. Each fraction is made up of three subunits, which are associated by hydrophobic interactions (Thanh and Shibasaki, 1977). Glycinin (11S) with a molecular weight ranging between 35,000 and 37,000 Da is made up of twelve units packed in two hexagonal rings (Moreira et al., 1979; Nielsen, 1985). Heat gelation studies of the proteins have shown the dissociation and association of the subunits (Damodaran and Kinsella, 1981; German et al., 1982; Kang et al., 1991). However, the studies suggest that the 11S globulins undergo only small conformational changes (Mori et al., 1982; Nakamura et al., 1984). Interaction studies of the proteins, which consist of heterogeneous complex quaternary structures of high molecular weight, have been reported (Mori et al., 1982; Nakamura et al., 1984; Damodaran, 1988). Studies by Utsumi and Kinsella (1985) and Nagano et al. (1994) on the gelation of 7S and 11S globulins and soya isolate proteins indicate that hydrogen bonding occurs in 7S globulins, electrostatic interactions and disulphide bonds in 11S globulins and both hydrogen bonding and hydrophobic interactions in soya isolate gels. Other studies by Kamata et al., (1991) indicated that
Chapter 1 General Introduction

the 7S globulin gels were able to reform after shearing on standing whereas the 11S globulin gels did not. Earlier studies by, Damodaran (1988) suggested that 11S globulins undergo partial refolding to some extent during the cooling part of the thermal gelation process, hence their failure to form a gel.

Interactions between 7S and 11S globulins of the proteins have been reported. Elucidation of the mechanisms associated with interactions suggest, that the basic subunits of the 11S globulins interact with the β-7S globulins in the gels via electrostatic interactions (German et al., 1982; Damodaran and Kinsella, 1981; Utsumi and Kinsella, 1985). In addition, the association of the basic units is by disulphide bonds. Utsumi and Kinsella (1985) suggested that with the 7S globulins gel the three subunits are associated by similar types of bonds. Nakamura et al., (1986) in other studies associated non-covalent interactions of 7S globulin with itself and those between 7S and 11S globulins. However, mainly hydrophobic and covalent disulphide bondings are involved in the interaction of 11S globulins with itself. Studies have also shown that gel formation and strength of gels are dependent on protein concentration and relative proportions of globulins present (Nakamura et al., 1986; Puppo et al., 1995).

1.12 Protein-protein and other macromolecules interactions

1.12.1 Protein-protein

The protein-protein interactions that occur both under physiological conditions and during the processing of food involve complex molecular aggregation. The processing, texture, storage and appearance of foods are greatly influenced by these interactions as well as in the formation of flavours. Intermolecular forces, which are generally
associated with protein-protein interactions, are derived from the intramolecular forces within a protein (Figure 1.9).

These forces are involved in the association of the basic amino acid units in the formation of the protein macromolecular polymer. In a linear sequence, the amino acids form the primary structural unit with subsequent folding and bonding into α-helix and β-sheet secondary structures of polypeptide chains.

These forces are involved in the association of the basic amino acid units in the formation of the protein macromolecular polymer. In a linear sequence, the amino acids form the primary structural unit with subsequent folding and bonding into α-helix and β-sheet secondary structures of polypeptide chains. These chains undergo extensive coiling or folding into tertiary and quaternary structures (Brendan and Tooze, 1991).

The molecular interactions involved include covalent disulphide, non-covalent electrostatic van der Waals, hydrogen, ionic and hydrophobic bonds (Howell, 1992). The covalent bonds include peptide bonds of the primary structure of the protein and disulphide bonds found between two Cys residues in the tertiary structure (Creighton, 1983).

Though the peptide bonds are not affected during protein denaturation, the covalent disulphide bonds are affected as in heat gelation or foaming.
However, studies made on protein-protein interactions vary significantly in the types of protein used, test conditions and methodology and may not be relatively compared. The present studies have therefore focussed on the functional properties of the proteins namely, the effect of heat on viscosity and gelation (Howell, 1992).

\[ \text{Protein-Protein Interactions} \]

Figure 1.9 Molecular forces involved in protein interactions (Howell, 1992).

Rha and Pradipasena (1986) proposed that, rheology can offer measurements in fundamental units to describe protein behaviour and as such a measure of the viscosity or resistance to flow can be employed to monitor interactions during heating. Howell (1995) described three main phenomena, which could be involved in these interactions of proteins with other proteins leading to textural changes. These are (i) synergistic interactions and compatible gel formation and stable forms (ii) precipitation through predominantly electrostatic interactions and (iii) phase separation.

Howell and Lawrie (1987) observed that the viscosity values of egg albumin, porcine and bovine plasma proteins mixtures were lower than the additive values calculated for each component in the mixture. They attributed this observation to negative interaction.
However, positive synergistic interaction occurred at high temperatures of heating (73-79 °C) for some of the mixtures.

A number of studies on the interaction of plasma proteins and other proteins have been reported and possible mechanisms of interactions had been proposed. These include plasma proteins with egg albumin proteins (Clark and Lee-Tuffnell, 1986; Howell and Lawrie, 1986), whey protein interactions (Monahan et al., 1995) and interaction of α-lactalbumin with -κ-casein (Paulsson et al., 1986).

Few studies have been reported on the interactions of cowpea proteins with muscle proteins. However, quite a number of such studies have been carried out on the interaction of soya proteins with muscle proteins. Notably the studies involved interaction with the myofibrillar proteins and in particular, myosin, which is a major component of the muscle protein. Howell (1992) in an extensive review, made reference to an increase in the specific viscosity of a mixture of 7S globulins and rabbit myosin at 75-100 °C. This was accompanied by a decrease in the sedimentation coefficient and a change in the composition of the soya protein component of the aggregate. Howell (1992) attributed the variations in temperatures at which interaction occurs to the use of different myosin species, differences in the pH and the method of preparation of the soya proteins. However, the methods used could not elucidate the mechanism of the interaction. Other methods indicating interaction were observed at different temperatures (Peng and Nielson, 1986; Peng et al., 1982). In addition the mode of interaction has been attributed to globulins dissociating into acidic and basic subunits and the basic subunits subsequently interacting with the myosin heavy chains via disulphide bond (Peng et al., 1982).
1.12.2 Protein-starch interactions

Many food systems are composed of protein and starch as the main constituents. These food products have been formed from the interaction between proteins and starch, leading to individual texture and flavour characteristics for each product and has many industrial applications as a thickener, colloidal stabilizer, gelling agent, bulking agent, water retention agent and adhesive.

Starch is a major polysaccharide in plants and is in the form of granules that exist naturally within the plant cells. Starch is semicrystalline in nature with varying levels of crystallinity. The crystallinity is exclusively associated with the amylopectin component, while the amorphous regions mainly represent amylose (Zobel, 1988a, 1998b). Amylose is a linear polymer composed of glucopyranose units linked through $\alpha$-D-(1-4) glycosidic linkages, while the amylopectin is a branched polymer with one of the highest molecular weights known among naturally occurring polymers (Karim et al., 2000). When starch molecules are heated in excess water, the crystalline structure is disrupted and water molecules become linked by hydrogen bonding to the exposed hydroxyl groups of amylose and amylopectin, which causes an increase in granule swelling and solubility.

Studies on protein-starch interactions tend to suggest that the molecular forces in this complex formation are in part due to charges on the protein and the starch colloids (Takeuchi, 1969). More and Cater (1974), in their work on food proteins and hydrolysed starch, indicated that several non-covalent factors are involved in protein-starch interactions as well as hydrogen bonding.
Protein-starch interactions could be two types, depending on temperature. Takeuchi (1969) and Dable (1971) observed that at low temperatures, the interaction involved is ionic and therefore pH dependent (Dable et al., 1975). At high temperatures the proteins denature and undergo much cross-linking through disulphide bond formation network. Starch at high temperatures gelatinises with a disruption of the granules and formation of a matrix of amylose holding the amyllopectin in a network (Remsen and Clark, 1978). Obviously in the studies reported the type of protein, species and experimental conditions affected the interactions of proteins and other macromolecules to a large extent.

1.13 Product development

One of the objectives of this project was the potential use of the under-utilised fish species in food products through the development of value-added products. For this purpose two products incorporating fish mince were thought to be very important. These were extruded snack products and infant food with an adequate amount and quality of essential nutrients from the fish proteins.

1.13.1 Infant foods

Infant foods, commonly known as weaning foods, are semi-solid or solid foods that are introduced to infants to make a transition from breast milk to an adult diet. This transition period naturally is associated with the rapid growth and development of the infant and hence the requirement of adequate diet in terms of essential food nutrients is of utmost importance. Most traditional infant foods in Ghana and indeed in many developing countries are based on cereals, which comprised mostly starch (Clark and Laing, 1991). Since it is a natural property of starchy granules to swell on boiling in
water to gelatinise on cooling, the gruel increases in viscosity forming a thick paste of low solid concentration (Mosha and Svanberg, 1990). Consequently, these foods are bulky but low in nutrient density. This consumption of viscous, high bulk, low energy density weaning foods is a major contributor to the incidence of infant malnutrition within developing countries (WHO, 1998). Sometimes sugar, salt and milk are added but rarely are fish or palm oil added (Walker and Pavitt, 1989; Walker, 1990). In addition, the commercially available, formulated foods usually contain a large proportion of milk, which generally makes them too expensive for less privileged populations.

Maize flour is used mostly in infant foods in Ghana. The chemical composition of maize varies considerably due to the numerous varieties available (Johnson, 1991). On average, the protein content is about 10% and 80% is carbohydrate (Kent, 1984). Approximately, the crude fibre content is about 3.5% whilst the mineral matter and fat contents are 4.5% and 2% respectively (Kent, 1984). Howling (1980) reported 74:26 as the amylopectin: amylose ratio of maize starch.

Cowpea like soya contains adequate quantities of essential amino acids as earlier reviewed in this report. Complementation of cereals such as maize, rice or wheat with cowpea protein therefore improves the protein quality (Rangel et al., 2003; Erdman and Fordyce, 1989). The use of soya in infant foods relates to its use in formula milk and cowpea with comparable properties such as overall positive nutritional profile, low cost, increasing availability, excellent functional properties and innovative product development could also be used as such (Rangel et al., 2003; Erdman and Fordyce, 1989). Cowpea flours, pastes and their respective protein concentrates, isolates and
fractions can potentially be employed to complement soy flours and proteins as functional ingredients in food systems (Ragab et al., 2004; Rangel et al., 2003; Sefa-Dedeh and Stanley, 1979). The appropriateness of cowpea and soya products for infants has been reviewed by Torun (1981). Recent studies by Obatolu (2003) on the growth pattern of infants fed with a mixture of extruded malted maize and cowpea attributed the improved nutritional status of infants to the formulated mixture diet.

EgounleTy and Aworh (2003) reported that soaking, cooking, dehulling and fermentation reduced trypsin inhibitors, phytic acid, stachyose (the most flatulent oligosaccharide in soybean, cowpea and groundbean). Thus well processed cowpea protein products could be comparable with milk/casein in protein quality and antinutritive factors such as trypsin inhibitors, are removed during the processing.

1.13.2 Snack foods
Snack foods are generally very light meals that come in various shapes and forms. These range from potato chips, nuts, instant hot pot snacks/cup soups and confectionary items. Snack products are also known to have in the last 25 years become important food items in the Western World (Wang, 1997). This report, however, focussed on the dry snack products generally based on starchy raw materials such as the flours of maize, barley, cassava, rice and legumes (Pagani, 1986). They are generally small crispy and ready to eat. These products have a relatively low bulk density. This low bulk density and the crispness are both partly caused by the dry foamy structure of the product matrix brought about by the processing technique (Lai et al., 1989). Most dry snack products are produced by expanded extrusion cooking by the superheating of water under elevated pressure conditions in the extruder (Smith, 1992). Compared to other
processing techniques, extruded snack products have the advantages of convenience, prolonged storage, and increase flavour and texture properties (Laarhoven, 1988). Influence of extrusion-cooking conditions have been generally studied and modelled (Gomez and Aguilera, 1984; Owusu-Ansah et al., 1984; Bhattacharya and Hanna, 1987).

In this study, cassava flour, which provided the starch as a constituent of the snack product, was used. Cassava with an average yield of about 7.8 t/ha is grown in almost all the regions of Ghana (Anon. 1991). Cassava produced in Ghana is either cooked fresh for "Ampesi" or "fufu" or processed into other products.

1.14 Shelf life and packaging of fish and fish products

The storage stability of fish and fish products is greatly influenced by the fish components susceptibility to oxidation (Smith et al., 1990; Smith and Hole, 1991; Davis et al., 1993) and hydrolysis (Olley and Lovern, 1960; Bligh and Scott, 1966). These are the lipids, which form unacceptable odours and flavours during storage. However, the susceptibility of muscle tissues to lipid oxidation differs among species (Wilson et al., 1976; Shamberger et al., 1977; Siu and Draper, 1978; Rhee an Ziprin, 1987) and may also differ among muscles of the same species (Rhee et al., 1986).

The general mechanism of lipid oxidation is well documented (German and Kinsella, 1983; Kanner et al., 1987; German and Kinsella, 1985), and factors such as temperature, light and oxygen play very important roles. Lipid oxidation is a very important event leading to the deterioration of foods containing highly unsaturated fats. Kinsella et al., (1978) reported high levels of cellular unsaturated lipids in fish, which readily deteriorated
via peroxidation, resulting in the development of objectionable odours and flavours in rancid products (Ke and Ackman, 1975). Lipid oxidation can also lead to other nutritional damage including losses of essential fatty acids, fat-soluble vitamins and essential amino acids (Ericksson, 1987), and the development of toxicity (Ames, 1983; Pearson et al., 1983). Other problems usually associated with storage are browning. In studies relating to the storage of salted dried trout at ambient temperatures (25-30 °C), extensive browning was observed with a potential loss of nutritional value (Lubis and Buckle, 1990; Maruf et al., 1990; Smith et al., 1990; Smith and Hole, 1991; Davis et al., 1993). The browning may have been brought about by enzymic and non-enzymic reactions. Browning of fish muscle has been thought to arise from a two-stage reaction involving oxidised lipids (Pokorny et al., 1974). Pokorny et al., (1974) described the first stage, as the interaction of decomposed products of hydroperoxides, especially carbonyl compounds, with free amino groups of proteins and nitrogenous groups of phospholipids to form light coloured fluorescent intermediates as Schiff bases. Melanoidins, the brown macromolecular products are formed in the second stage as a result of the transformation of these Schiff bases.

Lipid hydrolysis can occur with heating or the action of enzymes (Olley and Lovern, 1960; Bligh and Scott, 1966; Audley et al., 1978). Hwang and Regenstein, (1989, 1993) observed that lipid hydrolysis occurred in fish muscle during storage at refrigerated or frozen temperatures which supported the possibility that lipid hydrolysis could affect the storage of fish.

Free fatty acids (FFA) are among the hydrolytic products from fish lipids and in their interaction with fish proteins, denaturation of the proteins may occur (Jaranback and
Lijemark, 1975; Oshima et al., 1984). The FFA produced may also cause nutritional deterioration and are thought to be a useful index of degradation of nutritional components (Ke and Ackman, 1975).

Lipid oxidation has been monitored by various methods. Smith et al. (1990) and Hanson and Normington (1985) showed that determination of fluorescence and soluble colour gave suitable indicators of extensive rancidity. Lipid oxidation can also be monitored by measuring the formation of thiobarbituric acid-reactive substances (TBARS) (McDonald and Hultin, 1987; Vyncke, 1970; Smith and Whittle, 1984; Tarladgis et al., 1960). Other methods include changes in fatty acid composition (Lowry and Tinsley, 1973) peroxide value and production of carbonyl compounds (Lawrence, 1965; Keller and Kinsella, 1973).

The use and properties of packaging materials have been elucidated by Fellows (1998). Aluminium foils are generally used to provide a barrier to the passage of light, water, gases and odours to the food content. Other advantages of the foils include a good appearance, dead-folding and the ability to reflect radiant energy. Polyethylene as one of the plastic materials in packaging is also a good barrier to water but has relatively high permeability to water vapour and gases such as oxygen depending on its density. High-density polyethylene films with thickness of about 350-1000 μm has a moisture vapour transmission rate of 6.4 ml m⁻² per 24 h and an oxygen transmission rate of 500-2,000 ml for the same period. With low-density films, the moisture vapour transmission rate and the oxygen transmission rate are higher in the region of 3,000 and 8,000 ml respectively. However, lamination of polyethylene films with two or more films and vacuum conditions improved the barrier properties of the film among others (Cannon et al., 1995).
In a general summary, the nutritional value of underutilised fish species may be compared favourably with other desirable species and species such as burrito (*Brachydeuterus auritus*) and the flying gurnard (*Dactylopterus volitans*) are no exceptions and may have potential uses for product development and for human nutrition. The investigations therefore carried out in this report after the review, were based on the assumption that:

(a) The biochemical and proximate properties of burrito (*Brachydeuterus auritus*) and the flying gurnard (*Dactylopterus volitans*) may compare favourably with other known commercial fish species.

(b) The interaction of their component proteins with other macromolecules such as cowpea flour and cassava starch would lead to the development of new value added products (infant food and extruded snack) with desirable nutrient and sensory properties, and subsequent storage stability. Thus: (i) protein-energy malnutrition can be reduced by developing complementary infant foods of good quality in the right proportion with locally available ingredients (ii) value-added products including extruded snack food with locally developed processing equipment and production of gelatin from the fish waste would not only increase the range of processing techniques in the local food industry but improve the socioeconomic potentials of the stakeholders involved.
CHAPTER TWO
Chapter 2 Materials and methods

2 MATERIALS AND METHODS

2.1 Materials

Quantities of freshly landed burrito (*Brachydeuterus auritus*) and the flying gurnard (*Dactylopterus volitans* L.) were procured from fishmongers at the canoe landing beach of Tema in Ghana. The fish were either held in ice at 0 °C or frozen at -30 °C until analyzed.

2.2 Methodology

Proximate and chemical characterization was carried out at the Food Research Institute, Ghana. The protein profiling by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) was performed on myofibril proteins extracted from the muscles of frozen and thawed fish samples at The University of Surrey, Guildford, United Kingdom. Determination of the fatty acid profiles, rheological and differential scanning calorimetry as well as FT-Raman spectroscopy of fish and mixtures of either cowpea flour or cassava starch were also performed at the University of Surrey.

2.2.1 Moisture content

Moisture was determined by the AOAC (1990) method. Accurately weighed uniformly blended samples (1-2 g) were introduced into preweighed aluminum dishes (75 mm diam., 25 mm deep) and dried in a vacuum oven at 70 °C and 100 mm Hg for a period of 9 - 12 h. The moisture content was determined as the loss in weight of samples after drying in triplicate.
2.2.2 Protein determination

Protein was determined in triplicate by the Kjedahl method using a Kjeltec Auto System (AOAC, 1990).

2.2.3 Fat content

Fat was obtained by cold extraction with chloroform and methanol extraction in triplicate (Bligh and Dyer, 1959). The solvent was evaporated with a rotary evaporator. The extracted crude fat was weighed, and proportion of fat in the samples determined.

2.2.4 Ash content

Accurately weighed samples (1-2 g) were placed into conditioned porcelain crucible following the AOAC (1990) method 942.05. The crucibles were placed in a preheated muffle furnace for 2 days at 550 °C. The ash content in triplicate was expressed as a proportion of the original sample weight.

2.2.5 Phosphorus determination

Phosphorus was determined by a photometric method (AOAC, 1990) at 400 nm in a Uvikon Double Beam spectrophotometer, against a 0.5 mg standard set at 100% T. Phosphorus was determined in triplicate from a standard curve of 0.5, 0.8, 1.0 and 1.5 mg P standard solutions prepared from a working solution of 0.1 mg P/mL. The working solution was obtained from a 50 mL stock solution (8.788 g KH₂PO₄ in 1 L) diluted to 1 L. From the standard curve, % P = mg P in aliquot/ (g sample in aliquot x 10).
2.2.6 Calcium content

Calcium was determined in triplicate, titrimetrically by the method of AOAC (1990) and was reported as % calcium (wet weight).

2.2.7 Iron content

Iron was determined in triplicate by the spectrophotometric method measured at 500 nm (AOAC, 1990)

2.2.8 Fatty acid analysis

Fat obtained by cold extraction with chloroform and methanol extraction (Bligh and Dyer 1959) from minced fish, was esterified as outlined: 200 μl of the oil was added to 2 ml of diluted MTBE (sodium methlate 4 ml; MTBE 6 ml) and left in a dark enclosure after 1 min of vortexing. Following the method of Saeed and Howell (1999), the methylesters formed were injected into a Varian Gas Chromatograph Series 3600, using argon as carrier gas and a hydrogen flame ionization detector. A reference profile was obtained by running individual standard fatty acids from Sigma. The analyses were carried out in triplicate.

2.2.9 Thiobarbituric acid reactive substances (TBARS)

Rancidity of the fish species was assessed by the TBARS according to Saeed and Howell (1999). The TBARS method measures the level of aldehyde particularly malondialdehyde (MDA) which is formed as result of oxidation of polyunsaturated fatty acids (PUFA). Thiobarbituric acid reacts with MDA to form a red chromogen. The TBARS were measured by a reversed phase HPLC at 532 nm. A standard curve was prepared from the
readings of a serial solution of tetraethoxypropane (TEP) of 0, 0.2, 0.4, 0.6, 0.8, and 1.0 µl/ml MDA.

2.2.10. Peroxide value (PV)

Peroxide value is used to measure the primary oxidation products of lipids (hydroperoxides). The PV was measured (Saeed and Howell, 1999) as the absorbance at 500 nm using a Kontron Uvikon 860 spectrophotometer after the sample had been prepared as outlined: Ten to 15 µl of extracted fish oil was dissolved in 1000 µl of hexane and 5 ml absolute ethanol was added. Subsequently, 100 µl ferrous chloride was added and vortex for 3 min with 100 µl of 30% ammonium thiocyanate. The PV was calculated as follows from a standard curve using a serial dilution of Ferric chloride solution:

\[
\frac{\{\text{Abs} (S) - \text{Abs} (B) - C\}}{m} = \text{PV} \\
\text{55.84} \times \text{Sample mass}
\]

Abs S = absorbance of sample
Abs B = absorbance of Blank
C = Intercept of standard curve
m = Slope of the standard curve

All determinations were carried out in triplicate.

2.2.11 Biochemical characterization

2.2.11.1 Extraction of myofibril proteins

In studying the amino acid profile of the fish species, their myofibrillar proteins were extracted from the fish muscles. Two grams of the fish muscle was homogenised with 25 ml of solution A (50 mM phosphate buffer pH 7.5) made up 1.25 g of sodium di-
hydrogen orthophosphate and 5.96 g of di-sodium hydrogen orthophosphate in a litre of water). The mixture was centrifuged at 5000 \( \times \) g for 10 min. The supernatant was kept as the water-soluble proteins. The pellet was resuspended in 30 ml of solution B (50 mM phosphate buffer + 0.8M NaCl pH 7.5) made up of 1.25 g of sodium di-hydrogen orthophosphate, 5.96 g of di-sodium hydrogen orthophosphate and 46.75 g NaCl in a litre of water. The mixture was homogenised at 6000 rpm for 3 min. This was washed out with 20 ml of the same solution and kept in a cold room for 2 h after which it was centrifuged at 5000 \( \times \) g for 20 min. The supernatant was kept as the salt soluble proteins and together with the water soluble proteins were used for the analyses of protein content, amino acid and the electrophoretic profile of proteins from the fish species.

### 2.2.11.2 Protein content of extract (Bradford Protein Assay method)

**Principle**

This assay method uses a reagent kit containing a pre-prepared Brilliant blue G-250, phosphoric acid, methanol, water and solubilising agents. The reagent functions on the basis that an absorbance shift occurs when Coomassie Brilliant Blue G-250 binds to proteins in acidic solution from 465 to 595 nm and is essentially the same as the Bradford method (Bradford, 1976).

**Method**

The protein content of both the water soluble and salt soluble protein extracts of the fish species were determined by the Bradford Protein Assay method. Standards were prepared from the protein solution of bovine serum albumin (BSA). A 0.1 ml sample of unknown protein solution was added to 3 ml of Bradford reagent reagent and vortexed immediately. The absorbance of the sample was read between 5-60 min at 595 nm in a
Chapter 2 Materials and methods

Uvikon double beam spectrophotometer. The concentration was read from the standard curve.

2.2.11.3 Amino acid analysis

Method

Samples were hydrolysed to yield amino acids which were measured in triplicate by HPLC according to Bidlingmeyer et al. (1987) and Badii and Howell (2002) using the Waters Pico-Tag workstation (Water Model 712 WISP) (Waters, Watford, Herts. U.K.) Samples were hydrolysed with 6 N HCl (20 mL) at 110 °C for 24 h to yield free amino acids. The hydrolysed samples and amino acid standards (20 μL) were then treated as outlined below. All samples were derivatized with phenylisothiocyanate (PITC) according to the Waters Pico-Tag method.

Deivatization of Amino Acids with PITC.

Drying solution (20 μL), containing methanol:water: Triethanoalamine (TEA), (2:2:1) was added to each vacuum dried standard and sample in tubes and vacuum-dried again. The derivatization reagent was freshly made by mixing 50 μL PITC (kept at -20 °C, under nitrogen, to prevent degradation), 350 μL methanol (HPLC grade), 50 μL TEA, and 50 μL Milli-Q water. This PITC reagent (20 μL) was added to each tube, vortex mixed, sealed, and left at room temperature for 20 min. The reagent was then evaporated under vacuum. The derivatized samples were vacuum-dried and dissolved in 100 μL of sample buffer (eluent A, prepared by dissolving 19.0 g of sodium acetate trihydrate in 1 L Milli-Q water, followed by addition of 0.5 mL TEA, adjusted to pH 6.4 and filtered. To 940 mL of this solution was added 60 mL acetonitrile). The samples were analysed in triplicate by reverse-phase HPLC together with the amino acids
Chapter 2 Materials and methods

standards according to the Waters Pico-Tag Amino Acid Analysis Manual, Waters Chromatography Division, 1986.

2.2.11.4 SDS-Polyacrylamide gel electrophoresis (SDS-PAGE)

Principle

The application of electrophoretic techniques for the identification of fish species is well established (Mackie, 1972; Laird et al., 1982). Scobie and Mackie (1988) described an electrophoretic procedure suitable for cooked and raw fish species identification. The technique has proved useful for the estimation of protein molecular mass, purity assessment, and the structure of subunits. The proteins are extracted in SDS and analysed by SDS-PAGE. Separation of protein components is achieved on the basis of their molecular size. This however could not give well defined separation but Scobie and Mackie (1988) demonstrated that by markedly increasing the concentration of acrylamide in the separation gel, the resolution of the molecular weights is improved particularly in the weight range of 67 000 to 14 000. Significant methodological developments have recently taken place in protein science, namely, improvements in gel electrophoresis and its combination with mass spectrometry (Henzel et al., 1993; Shevchenko et al., 1996). These advances render the characterisation of proteins at the macromolecular level more accessible. The complex pattern of proteins produced using SDS-PAGE indicates that each sample analysed had a characteristic protein distribution, which could be potentially valuable as a fingerprint of fish species (Civera and Parisi, 1991; Mackie et al., 2000; Rehbein et al., 1999; Scobbie and Mackie, 1988). The SDS-PAGE technique allowed discrimination among closely related Gadidae species, such as Gadus morhua, Merlangius merlangus, Pollachius virens and Melanogrammus aeglefinus (Rehbein et al., 1999).


**Method**

SDS-Page of the myosin extracts of each species was undertaken by the method of Hames and Rickwood (1990) with a Phast Gel electrophoresis unit.

### 2.3 Protein interaction studies

#### 2.3.1 Principle of oscillatory rheometry

**Principle**

Rheology is the study of flow and deformation of material (Barnes *et al.*, 1989; Rao, 1999). This involves the application of an oscillatory stress to a sample, usually in a sinusoidal fashion and measuring the resultant strain (Figure 2.1) (Barnes *et al.*, 1989).

For a Hookean solid (not exhibiting flow properties) the stress and strain waves would be in-phase ($\delta=0$). In contrast, for a purely viscous liquid the stress would be exactly 90° out-of-phase with strain ($\delta=90°$). For viscoelastic gels the behaviour lies somewhere in between. The ratio of shear stress to shear strain can be written as the sum of two components, one in-phase with the strain and the other 90° out-of-phase (Barnes *et al.*, 1989).

![Figure 2.1 Stress/strain signals from an oscillatory rheometer (Barnes *et al.*, 1989)](image-url)
A complex number co-ordinate system is often used for mathematical clarity and easy manipulation. The y-axis is the imaginary axis and the real part of the number is represented on the x-axis (Figure 2.2).

![Diagram of complex number system](image)

**Figure 2.2 The relationship between the loss modulus (G'"), storage modulus (G') and complex modulus (G*).**

The real term (G') is in-phase and is a measure of elastic energy stored, and the imaginary term (G'")) is the out-of-phase component, which is a measure of the energy dissipated due to viscous behaviour during dynamic visco-elastic measurements (Whorlow, 1992).

![Diagram of rheometer](image)

**Figure 2.3 Schematic diagram of a rheometer (Whorlow 1992)**

In this thermal gelation process, temperature sweeps are used to probe changes in the sample structure as a function of temperature. As the temperature is raised and the
protein molecules unfold and associate, the structure begins to form and $G'$ increases. Aggregation processes continue as the temperature is elevated and more structure is formed in the sample with increases in $G'$. Figure 2.3 depicts a schematic representation of an oscillatory rheometer used in the studies.

**Method**

In a preliminary rheometric study, a quantity of fish muscle was homogenised in water to obtain 100% and 90% of the fish muscle and subjected to small-deformation oscillatory measurements with a Rheometrics controlled stress rheometer and a 40 mm diameter parallel plate geometry gap of 3 mm and set at a frequency of 1 radian per second, to obtain sufficient data without compromising the measurements of entanglements. A dynamic temperature sweep was performed between 20 °C and 90 °C at a rate of 1 °C /min and then cooled to 20 °C. The applied stress was 1 Pa to keep the oscillatory strain at about 1%, sufficiently low to ensure the measurements were within the linear viscoelastic region (Hamann, 1991). The sample was surrounded by silicone oil to prevent evaporation of solvent during the sweep. The viscoelastic properties of the gels measured include the storage modulus ($G'$) and loss modulus ($G''$) as a function of time within the linear viscoelastic range of the gels (Mitchell, 1980).

### 2.3.2 Differential scanning calorimetry (DSC)

**Principle**

DSC is a thermoanalytical technique to probe changes in physical or chemical properties of materials as a function of temperature. The technique of studying the thermal transitions of protein systems by DSC has been reviewed by Privalov (1980) and Biliaderis (1983). The DSC technique allows continuous monitoring of the physico-chemical changes of the systems. This tends to elucidate the mechanism of any
possible intermolecular interactions on the unfolding and denaturation of proteins. Subsequently information on protein functionality and the effects of processing conditions in food systems is obtained.

Either an absorption or liberation of energy accompanies the change in state of a system or material as a result of melting, crystalline transition or chemical reaction though in certain situations the process could be initiated by the application of heat as in melting. The basic operating principle of the DSC is based on the above facts. The technique of DSC requires the sample under investigation and an inert reference to be maintained at the same temperature whilst the temperature of both is gradually increased usually at a linear rate. The thermally induced changes in the sample are then recorded as a differential heat flow displayed normally as a peak on a thermogram. The heat flow is then integrated with respect to time or temperature to give the enthalpy change associated with the process. The DSC technique may also be employed to measure heat capacity, sample purity, reaction kinetics, transition temperatures and phase diagram information.

The DSC technique is applied widely in chemical studies, biological systems and in the area of food research especially in the study of protein behaviour in food systems. Wright and Wilding (1984) applied DSC to investigate the effect of orthophosphate, pyrophosphate and tripolyphosphate ions on the denaturation of myosin. In an earlier study with DSC, Wright *et al.* (1977) obtained thermograms for whole muscle showing thermal denaturation at different transitions of individual proteins or groups of proteins present in the muscle. DSC has been used to study the thermal behaviour of bovine achilles tendon collagen in the solid state and in deionised water (Finch and Ledward,
1972). Quinn et al. (1980) examined the changes in the thermal stability of meat proteins during processing into sausage batter with the aid of DSC.

Differences in denaturation temperatures, overall thermal transitions and half bandwidths were observed for several proteins from plant sources by differential scanning calorimetry (Arntfield and Murray, 1981).

Interactions of proteins with proteins can be shown in a number of ways as changes in the temperature of denaturation, the size or shape of the DSC thermogram or the rate of denaturation (Wright and Wilding, 1984). An increase in the denaturation temperature of a complex mixture of soya bean trypsin inhibitor and ovomucoid was observed and attributed to kinetic factors (Laskowski and Sealock, 1971). The thermal denaturation behaviour of metmyoglobin was altered in an interaction with bovine serum (Finch and Ledward, 1972). DSC studies on interactions of β-lactoglobulin and both α-lactalbumin and κ-casein showed a higher transition temperature and a change in the shape of the thermogram (Wright and Wilding, 1984).

DSC studies on protein interactions with polysaccharides are limited in spite of the fact that protein interactions with polysaccharides or carbohydrates are important in the context of textural properties of foods. Imeson et al. (1977) reported that there was a slight destabilisation of the protein molecule and decrease of the denaturation temperature of myosin and bovine serum albumin in the presence of anionic polysaccharides, such as pectate, alginate and carboxymethyl cellulose. This was associated with the broadening of the transition peak. These observations were related to the existence of a fairly weak electrostatic interaction between the two
macromolecular species (Imeson et al., 1977). The effect of various sugars on the thermal stability of egg-white proteins has also been reported (Donavan et al., 1975; Donavan and Mapes, 1976), as well as the baking process in angel cakes (Donavan, 1977). This practical application of DSC in the study of food processing is significant in the assessment and control of ingredient quality and the study of ingredient interactions in understanding the principles involved in the processing operation.

A typical DSC thermogram is depicted in Figure 2.4, the important parameter shown is the temperature at which a transition occurs, which is termed the denaturation temperature \( T_m \).

![Figure 2.4 A typical DSC thermogram showing a possible baseline construction and the following parameters: (a) peak maximum temperature, (b) extrapolated onset temperature, and (c) heat capacity. (Wright and Wilding, 1984).](image-url)
Method

A Setaram Micro DSC VII was used with distilled water as the reference. Eight hundred milligrams of samples as prepared for the rheological studies were placed in a pre-weighed DSC cell. An equal weight of distilled water was also introduced into the reference cell to obtain a flat baseline. A temperature scan of 10 °C to 90 °C and heating rate at 0.5 °C/min were maintained throughout the study. Heat absorbed or released by the transformation of the samples resulted in an endothermic or exothermic peak as a function of temperature. The temperature attained when half of the sample is transformed is referred to as the transition temperature ($T_m$) and was measured at the tip of the peak. Peak areas showing transition temperatures and enthalpies ($\Delta H$) were calculated automatically by integrating the area under the peak by the DSC.

2.3.3 FT-Raman spectroscopy.

Principle

Raman spectroscopy is one of the branches of vibrational spectroscopy which is based on the interaction of a laser radiation with molecular vibrations in order to obtain information about the material (Scotter, 1997). The energy generated by the laser beam is accumulated within the molecules which increases the amplitude of the molecular vibration. This exciting incident beam raises the energy of the molecules of the material to a state which is proportional to the frequency of the molecular vibration. As a result the re-emitted light is scattered with a changed frequency or shift of wavelength from the incident laser. That portion of the light, which undergoes a change in wavelength or shift, is known as Raman scattered light. The physical origin of Raman scattering lies in inelastic collisions between the molecules composing the liquid and photons, which are the particles of light composing the light beam. Since the collisions are inelastic then the total energy is conserved during the scattering process and the
energy gained or lost by the photon must equal an energy change within the molecule. It follows that by measuring the energy gained or lost by the photon one can probe changes in molecular energy which are referred to as transitions between molecular energy levels (Carey, 1982).

The spectrum of a material is obtained by plotting the intensity (quantity of photons) of this signal for the different variations that its frequency undergoes. Each peak of the spectrum is generated by a molecular vibration at a particular frequency and its intensity is linearly proportional to the concentration of the molecular bond (Celedón and Aguilera, 2000). The width of the peaks is due to the vibration frequency and is affected by the microenvironment and chemical changes around the atoms (Schrader, 1995). These observations allow typical bands to be assigned to bonds in molecules and to probe structural changes in these molecules as a function of interaction or processing such as heat denaturation, gelation and emulsification (Painter, 1984).

FT-Raman spectroscopy is one of the more direct spectroscopy that could be used to examine protein-protein interactions non-invasively in more detail. The advantage of Raman spectroscopy over other analytical techniques is that it can be used for aqueous solutions, crystals, fibres, gels, and films without destruction of the samples. Li-Chan et al. (1994) have reviewed a number of studies on protein structure and food systems where Raman spectroscopy has been applied. Studies on proteins and their components have been the main applications of Raman spectroscopy and assignments bands were carried out by comparing the protein spectrum with that obtained by a mixture of the constituent amino acids to aid in interpretation of the spectra (Frushour and Koenig, 1975; Yu, 1977; Carey, 1982; Careche et. al., 1999; Matsumoto et al., 1992; Jiang et
al., 1989; Howell et al., 1999; Badii and Howell 2002). Other applications of Raman spectroscopy are in the fields of lipid biochemistry (Carmona et al., 1987), food systems (Thygesen et al., 2003; Badii and Howell, 2002) medical research (Ozaki, 1988).

Method

Raman spectra of samples were taken in 7-mL glass containers (FBG-Anchor, Cricklewood, London) on a Perkin-Elmer System 2000 FT-Raman spectrophotometer with excitation from a Nd:Y AG laser at 1064 nm. Frequency calibration of the instrument was performed using the sulfur line at 217 cm\(^{-1}\). The spectra in triplicate were an average of 64 scans which were baseline corrected, smoothed, and normalized to the intensity of the phenylalanine band at 1004 cm\(^{-1}\) (Howell and Li-Chan, 1996; Howell et al., 1999). The recorded spectra were analyzed using Grams 32 (Galactic Industries Corp., Salem, NH). Assignments of the bands in the spectra to protein vibrational modes were made according to Careche et al. (1999), Matsumoto et al. (1992), Jiang et al. (1989) and Badii and Howell (2002).

2.3.4 Phase contrast microscopy

Principle

The application of microscopy in biochemical analysis is well known (Wilson and Walker, 1995). Microscopy serves two independent functions of enlargement (magnification) and improved resolution (the rendering of two objects as separate entities). Light microscopes employ optical lenses to focus sequentially the image of objects, whereas electron microscopes use electromagnetic lenses. Light and electron microscopes may work either in a transmission or scanning mode depending on whether the light or electron beam passes through the specimen and is diffracted or whether it is
deflected by the specimen surface. Light microscopes in phase contrast mode are often used to improve image contrast of unstained materials. Changes in the phase of emergent light may be caused either by diffraction or by changes in the refractive index of material within the specimen, or even by differences in thickness of the specimen. At their point of focus the converging light rays show interference, resulting in either increases or decreases in the amplitude of the resultant wave (constructive or destructive interference, respectively), which the eye detects as differences in brightness.

Phase contrast as a result of separation occurs above certain polymer concentrations if the interaction between two segments of two polymers is repulsive and the two polymers collect separately in two opposite phases. Phase separation mechanisms had been proposed by Patterson (1982), Piculell and Lindman (1992) and Piculell et al. (1994).

Two types of phase separation are most commonly encountered in mixtures of two polymers. One case is where the two equilibrium phases have similar overall concentrations of polymers, but where each phase is enriched in one of the polymeric components (Patterson, 1982). The other type, in contrast to the first, is where one of the two phases is enriched in both of the polymeric components. An example is that of oppositely charged copolymers of acrylamide (Frugier and Audebert, 1994). On the basis of the partitioning of the polymers between the separating phases, these two types of phase separation are referred to as segregative and associative respectively (Piculell and Lindman, 1992). In an associative phase separation both polymers are enriched in
one of the separating phases, whereas, in a segregative phase separation, the two polymers are enriched in separate phases.

Method

Drops of the samples prepared for rheological and DSC studies were placed on acetone cleaned microscope slides and covered with a cover slip to prevent dehydration. The slides were viewed under a Leitz microscope fitted with a binocular eyepiece and a phase contrast annulus matching an objective of a different magnification. The microscope was attached to a Wild MPS 05 system comprising a camera and an exposure meter set on camera factor 0.32. The sensor was set for integrated metering of objects, which are uniformly distributed.

2.4 Statistical Analysis

A computer programme was used for statistical calculations (MINITAB 10.2 Inc. State College, PA. USA Inc). For most of the data, the difference of means between pairs was determined by means of confidence intervals using $t$-tests. The level of significance was set at $P<0.05$. 
CHAPTER THREE
3 PROXIMATE AND BIOCHEMICAL CHARACTERISATION OF THE BURRITO (BRACHYDEUTERUS AURITUS) AND THE FLYING GURNARD (DACTYLOPTERUS VOLITANS)

3.1 Introduction

Though underutilized pelagic species have no particularly desirable characteristics as yet, the nutritional value of these fish is considered to be as high as that of any of the more desirable species in terms of protein quality, fats and minerals. This study is to characterise proximate composition and biochemical properties of the selected underutilized fish species to evaluate their potential for use in food processing and nutritional supplementation.

3.2 Materials and Methods

3.2.1 Materials

Freshly harvested burrito (Brachydeuterus auritus) and the flying gurnard (Dactylopterus volitans) were obtained from the fishing harbour in Tema, Ghana. They were immediately held in ice at 0°C and thereafter frozen at –30°C until analyzed.

3.2.2 Methods

Protein, moisture, fat, ash, phosphorus, calcium and iron were determined by the methods of AOAC (1990).

The myofibrillar proteins were extracted from the fish as described in section 2.2.9.1. The protein content of both the water soluble and salt soluble protein extracts of the fish species were determined by the Bradford Protein Assay method (Chapter 2).
Chapter 3 Proximate and biochemical characterisation of the burrito (*Brachydeuterus auritus*) and the flying gurnard (*Dactylopterus volitans*)

SDS-PAGE of the myosin extracts of the species was by the method of Hames and Rickwood (1990) with a Phast Gel electrophoresis unit as outlined in Chapter 2).

Fat was obtained by cold extraction with chloroform and methanol from minced fish (Bligh and Dyer, 1959) and was esterified as described by the method of Saeed and Howell (1999). Rancidity of the fish species was assessed by the TBARS and PV as described by Saeed and Howell (1999).

3.3 Results and discussion

Table 3.1 shows that the protein contents of the burrito (*Brachydeuterus auritus*) and the flying gurnard (*Dactylopterus volitans*), which ranged from 18 to 23% are above the levels of 16±3% reported for pelagic fish by Windsor and Barlow (1981). This suggests that the two species are good sources of protein and may be used in fish protein concentrate production or in food supplements (Windsor and Barlow, 1981). The ash contents for the species were within the range of 0.5 to 1.8% of wet weight for most other fish species (Sidwell, 1981). The low fat contents of the burrito (0.6%) and the flying gurnard (0.7%) were below the range of 1-8% reported for other pelagic species (King and Poulter, 1985) and may not be suitable for fish oil production (Urdahl, 1992). The low fat contents of the species, as suggested by Talabi *et al.* (1980) is an indication of the fact that most of the lipids are present as phospholipids which is a rich source of PUFA. In relatively species of higher fat percentage (1-3.8%) Talabi *et al.* (1980) reported comparatively, lower percentage of PUFA. The calcium contents of the burrito (296 mg/100 g) and the flying gurnard (185 mg/100 g) though quite high, were also below the values of 580 mg/100 g of calcium reported for other fish species (Sidwell, 1981). The phosphorus contents of the species were within the average values
of fillets, which ranged from 113-to 350-mg/100 g (Sidwell, 1981; Teeny et al., 1984).

Though, the iron content of the species analysed were significantly different (P<0.05) from each other with the burrito (*Brachydeuterus auritus*) having a value of 4 mg/100 g and the flying gurnard (*Dactylopterus volitans*) 1 mg/100 g, they are within a wider range of 0.8 to 373 reported for many other pelagics (Sidwell, 1981; Teeny et al., 1984).

Table 3.1 Proximate and chemical compositions of fresh burrito (*Brachydeuterus auritus*) and the flying gurnard (*Dactylopterus volitans*)

<table>
<thead>
<tr>
<th>Parameter Evaluated</th>
<th>Burrito</th>
<th>Flying gurnard</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture</td>
<td>80.8±3.5</td>
<td>74.0±2.5</td>
</tr>
<tr>
<td>% (wet weight basis)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Protein</td>
<td>18±4.3</td>
<td>22.3±3.4</td>
</tr>
<tr>
<td>% (N x 6.25)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fat %</td>
<td>0.6±0.1</td>
<td>0.7±0.1</td>
</tr>
<tr>
<td>Ash %</td>
<td>2±0.8</td>
<td>3.3±0.7</td>
</tr>
<tr>
<td>Calcium (mg/100 g)</td>
<td>296.0±8</td>
<td>185.0±11</td>
</tr>
<tr>
<td>Iron (mg/100 g)</td>
<td>4.1±0.4</td>
<td>1.0±0.5</td>
</tr>
<tr>
<td>Phosphorus (mg/100 g)</td>
<td>254.0±17</td>
<td>215.6±12</td>
</tr>
</tbody>
</table>

* Values are means of three determinations ± standard deviation

Table 3.2 Protein concentration of protein extracts of burrito and the flying gurnard (mg/ml)

<table>
<thead>
<tr>
<th>Fish species</th>
<th>Water soluble proteins</th>
<th>Salt soluble proteins</th>
</tr>
</thead>
<tbody>
<tr>
<td>Burrito</td>
<td>4.6±1.2</td>
<td>6.3±1.3</td>
</tr>
<tr>
<td>Flying gurnard</td>
<td>3.7±1.4</td>
<td>8.9±1.5</td>
</tr>
</tbody>
</table>

* Values are means of three determinations ± standard deviation
Chapter 3 Proximate and biochemical characterisation of the burrito (*Brachydeuterus auritus*) and the flying gurnard (*Dactylopterus volitans*).

![Figure 3.1 SDS-Page patterns for the flying gurnard and burrito.](image)

(1 and 7) standard, (2) flying gurnard-WS, (3) burrito-WS, (4) burrito-SS, (5) flying gurnard-SS, (6) horse mackerel-SS.

- **WS** – Water soluble protein
- **SS** – Salt soluble protein

The electrophoretogram (Figure 3.1) shows reduced intensity of proteins in the myosin heavy chain bands (97,400-200,000 daltons) of the water-soluble proteins of the flying gurnard (lane 2) and burrito (lane 3) from their corresponding salt soluble proteins (lane 4 and 5). However, there are remarkable features from the individual species as well as that of horse mackerel (lane 6), around the protein zones whose molecular weights are estimated to be 18,400 and 14,300 daltons by the standard in lane 1 and 7. These proteins are considered to be species specific (Scobbie and Mackie, 1988) and could enable easy identification of the species.
Chapter 3 Proximate and biochemical characterisation of the burrito (*Brachydeuterus auritus*) and the flying gurnard (*Dactylopterus volitans*).

### Table 3.3 Amino acid compositions of flying gurnard and burrito*

<table>
<thead>
<tr>
<th>Amino acids</th>
<th>Burrito Salt soluble proteins %</th>
<th>Flying gurnard Salt soluble proteins %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asp</td>
<td>6.6 ± 0.21</td>
<td>6.05 ± 0.45</td>
</tr>
<tr>
<td>Glu</td>
<td>10.39 ± 0.22</td>
<td>9.9 ± 0.22</td>
</tr>
<tr>
<td>h. pro</td>
<td>1.7 ± 0.05</td>
<td>1.39 ± 0.11</td>
</tr>
<tr>
<td>Ser</td>
<td>4.63 ± 0.09</td>
<td>4.76 ± 0.13</td>
</tr>
<tr>
<td>Gly</td>
<td>7.11 ± 0.05</td>
<td>6.98 ± 0.22</td>
</tr>
<tr>
<td>His</td>
<td>0.78 ± 0.05</td>
<td>0.71 ± 0.07</td>
</tr>
<tr>
<td>Arg</td>
<td>6.24 ± 0.1</td>
<td>5.59 ± 0.04</td>
</tr>
<tr>
<td>Thr</td>
<td>4.6 ± 0.007</td>
<td>4.88 ± 0.08</td>
</tr>
<tr>
<td>Ala</td>
<td>11.8 ± 0.26</td>
<td>12.07 ± 0.17</td>
</tr>
<tr>
<td>Pro</td>
<td>2.99 ± 0.26</td>
<td>3.46 ± 0.39</td>
</tr>
<tr>
<td>Tyr</td>
<td>4.56 ± 0.34</td>
<td>4.48 ± 0.05</td>
</tr>
<tr>
<td>Val</td>
<td>5.77 ± 0.1</td>
<td>5.9 ± 0.03</td>
</tr>
<tr>
<td>Met</td>
<td>2.97 ± 0.1</td>
<td>3.21 ± 0.34</td>
</tr>
<tr>
<td>Cys</td>
<td>1.14 ± 0.09</td>
<td>1.0 ± 0.06</td>
</tr>
<tr>
<td>I leu</td>
<td>3.96 ± 0.05</td>
<td>4.31 ± 0.02</td>
</tr>
<tr>
<td>Leu</td>
<td>8.04 ± 0.1</td>
<td>8.27 ± 0.1</td>
</tr>
<tr>
<td>Phe</td>
<td>3.37 ± 0.06</td>
<td>3.34 ± 0.007</td>
</tr>
<tr>
<td>Trp</td>
<td>6.0 ± 0.33</td>
<td>5.34 ± 0.09</td>
</tr>
<tr>
<td>Lys</td>
<td>7.32 ± 0.14</td>
<td>8.31 ± 0.64</td>
</tr>
</tbody>
</table>

* Values are means of three determinations ± standard deviation

The major amino acids (Table 3.3) present in both species are glutamine (9-10%) and alanine (11-12%). The fish species are also good sources of lysine (7-8%). Their percentage sulphur amino acid, methionine (2-3%), compares favourably to other species (Garrow and James, 1993; Batista *et al.*, 2001; Iwasaki and Harada, 1985)).
Chapter 3 Proximate and biochemical characterisation of the burrito (*Brachydeuterus auritus*) and the flying gurnard (*Dactylopterus volitans*)

Table 3.4 Fatty acid profile of the flying gurnard and the burrito*

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>Flying gurnard (%)</th>
<th>Burrito (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C 14:0</td>
<td>1.8 ±0.6</td>
<td>2.2 ±0.9</td>
</tr>
<tr>
<td>C 15:0</td>
<td>1.4 ± 0.2</td>
<td>0.2 ± 0.1</td>
</tr>
<tr>
<td>C 16:0</td>
<td>7.1 ± 3.7</td>
<td>10.3 ± 3.7</td>
</tr>
<tr>
<td>C 17:0</td>
<td>2.4 ± 0.8</td>
<td>1.6 ± 0.2</td>
</tr>
<tr>
<td>C 18:0</td>
<td>4.7 ± 2.6</td>
<td>6.3 ± 2.9</td>
</tr>
<tr>
<td>C 20:0</td>
<td>1.5 ± 0.9</td>
<td>0.9 ± 0.1</td>
</tr>
<tr>
<td>C 22:0</td>
<td>1.3 ± 0.7</td>
<td>2.1 ± 1.3</td>
</tr>
<tr>
<td>Σ Saturated</td>
<td>20.2±1.4</td>
<td>23.6±1.4</td>
</tr>
<tr>
<td>C 14:1</td>
<td>0.1 ± 0.0</td>
<td>0.01 ±0.01</td>
</tr>
<tr>
<td>C 16:1ω7</td>
<td>3.6 ± 1.6</td>
<td>4.2 ± 0.4</td>
</tr>
<tr>
<td>C 17:1ω8</td>
<td>1.1 ± 0.1</td>
<td>0.2 ± 0.1</td>
</tr>
<tr>
<td>C 18:1ω9</td>
<td>5.6 ± 2.7</td>
<td>6.2 ± 1.5</td>
</tr>
<tr>
<td>C 20:1ω9</td>
<td>1.6 ± 1.2</td>
<td>3.3 ± 1.1</td>
</tr>
<tr>
<td>C 22:1ω11+ω13</td>
<td>0.6 ± 0.2</td>
<td>1.1 ± 0.2</td>
</tr>
<tr>
<td>Σ Mono unsaturated</td>
<td>12.6±1.1</td>
<td>15.0±0.6</td>
</tr>
<tr>
<td>C 18:2ω6</td>
<td>1.6 ± 0.3</td>
<td>1.1 ± 0.4</td>
</tr>
<tr>
<td>C 18:3ω3</td>
<td>0.7 ± 0.1</td>
<td>0.3 ± 0.1</td>
</tr>
<tr>
<td>C 20:2ω6</td>
<td>1.6 ± 0.6</td>
<td>3.8 ± 1.3</td>
</tr>
<tr>
<td>C 20:3ω6</td>
<td>0.6 ± 0.3</td>
<td>1.7 ± 0.6</td>
</tr>
<tr>
<td>C 20:4ω6</td>
<td>3.7 ± 1.4</td>
<td>4.0 ± 1.8</td>
</tr>
<tr>
<td>C 20:4ω3</td>
<td>1.8 ± 0.3</td>
<td>2.9 ± 1.1</td>
</tr>
<tr>
<td>C 20:5ω3</td>
<td>8.3 ± 1.6</td>
<td>6.8 ± 1.6</td>
</tr>
<tr>
<td>C 21:5ω3</td>
<td>3.3 ± 1.2</td>
<td>3.7 ± 1.9</td>
</tr>
<tr>
<td>C 22:4ω6</td>
<td>2.4 ± 0.7</td>
<td>3.9 ± 0.9</td>
</tr>
<tr>
<td>C 22:5ω6</td>
<td>2.7 ±0.1</td>
<td>1.1 ± 0.5</td>
</tr>
<tr>
<td>C 22:5ω3</td>
<td>4.6 ± 0.6</td>
<td>2.7 ± 0.9</td>
</tr>
<tr>
<td>C 22:6ω3</td>
<td>32.3 ± 1.1</td>
<td>28.2 ± 1.7</td>
</tr>
<tr>
<td>Σ Poly unsaturated</td>
<td>63.6±0.7</td>
<td>60.2±1.1</td>
</tr>
<tr>
<td>Total ω3 content</td>
<td>51±3.7</td>
<td>44.5±2.7</td>
</tr>
</tbody>
</table>

* Values are means of three determinations ± standard deviation

Table 3.5. TBARS and PV values of the flying gurnard and the burrito

<table>
<thead>
<tr>
<th>Indicator</th>
<th>Flying gurnard</th>
<th>Burrito</th>
</tr>
</thead>
<tbody>
<tr>
<td>TBARS (mgMDA/kg)</td>
<td>1.1± 0.5</td>
<td>1.2± 0.3</td>
</tr>
<tr>
<td>PV (mBq/kg fish)</td>
<td>2.5± 0.8</td>
<td>2.7± 0.9</td>
</tr>
</tbody>
</table>

* Values are means of three determinations ± standard deviation
Chapter 3 Proximate and biochemical characterisation of the burrito (*Brachydeuterus auritus*) and the flying gurnard (*Dactylopterus volitans*)

The overall profiles of the essential amino acids of the two species appear to suggest that the species have a high class protein comparable to that of the mammalian meat which contains high levels of lysine and histidine ([FAO, 1962; Garrow and James, 1993; Friedman, 1996](#)). The fish species may be good sources of protein supplement in infants' diets.

From the fatty acids profiles of the fish species (Table 3.4), differences were observed in their qualitative and quantitative compositions though both showed predominance of saturated (C16:0) palmitic acid (7% for flying gurnard and 10% for burrito). The fatty acid (C 18:1ω9) oleic (flying gurnard was 5.6% and 6.2% for the burrito fish) was also the highest mono unsaturated acid in both species. However, the total percentage values of both the saturated and monounsaturated fatty acids respectively, for the burrito fish (saturated fatty acid – 23.6%; monounsaturated fatty acid – 15.1%) were higher than those of the flying gurnard (saturated fatty acid – 20.2%; monounsaturated fatty acid – 12.6%). The differences may be partly attributed to the variations in the species. However, the percentage values were similar to results reported by [Pozo et al. (1992)](#) for other pelagic species. Of particular importance is the predominance of the high percentage of PUFA in both the flying gurnard (63.6%) and burrito (60.2%) fish species. Other PUFA was the omega-3 fatty acids for flying gurnard (51%) and the burrito (44.5%) as shown on Table 3.4. The predominant ones were the EPA and DHA fatty acids. Similar observations were made by [Pozo et al. (1992)](#) and [Batista et al. (2001)](#) in their studies on pelagic fish. The abundance of omega-3-fatty acids, suggests an additional advantage for the use of the fish species in the formulation of infant foods as they help in the healthy growth and development of the brain, the nervous system and functioning of the eye (Cockburn 1997).
Chapter 3 Proximate and biochemical characterisation of the burrito (*Brachydeuterus auritus*) and the flying gurnard (*Dactylopterus volitans*)

The TBARS values of the flying gurnard (1.1 mgMDA/kg) and burrito (1.2 mgMDA/kg) are within the acceptable limits of 3 mgMDA/kg reported by Sinhuber and Yu (1958) and Huss (1988). The PV values of flying gurnard (2.5 mEq/kg fish) and burrito (2.7 mEq/kg fish) were lower than those reported by Gormley *et al.*, (2002) for other species and were below the possible limit of 10-30 mEq/kg fish (FAO, 1995).

The low values of the TBARS and the PV (Table 3.4) are indications of the level of freshness of the fish species and that they may not have undergone any major deterioration in terms of lipid oxidation and its associated reactions. The low levels of these indices are also unlikely to affect the sensory properties of the fish species.

3.4 Conclusion

Characterisation of the burrito and the flying gurnard showed that both species are of high nutritional significance in either human food supplements or formulations, as they tend to have a high protein content, good general amino profile and abundance of polyunsaturated fatty acids.
CHAPTER FOUR
4 RHEOLOGICAL, DSC, FT-RAMAN SPECTROSCOPY AND PHASE MICROSCOPY STUDIES OF FISH MINCE, COWPEA FLOUR AND CASSAVA STARCH MIXTURES

4.1 Introduction

The inclusion of fish and other sources of protein in product development contribute towards the nutritional value; texture as well as flavour of products such as gels which are formed following heat treatment. These developments are brought about by the interactions of the fish proteins within the fish and the other macromolecules that may occur during the processing of the food (Friedman, 1996). Studies on interactions of proteins and other macromolecules have shown that these can lead to synergism, phase separation or precipitation, depending on their structure and physico-chemical properties which influence the properties of the food (Howell and Lawrie, 1985; Howell et al., 1995; Comfort and Howell, 2002). More often such studies were done with isolates of the proteins and the macromolecules involved which were more likely to form gels. However, the formation of gels from pelagic species often present problems due to the intrinsic characteristics of their muscles and more so when unrefined muscles are used (Leinot and Cheftel, 1990; Niwa et al., 1988; Chung and Lee, 1990). In view of the heterogeneity of macromolecules, studies of the interactions of these unrefined fish muscles (mince) and other macromolecules like cowpea flour and starch would provide valuable information to their use and new protein applications in food formulations (Chung and Lee, 1990; Gómez-Guillén et al., 1996). Rheology, differential scanning calorimetry (DSC) and phase contrast microscopy have been used to investigate protein-protein or other macromolecule interactions (Howell and Lawrie, 1985; Howell et al., 1995; Comfort and Howell, 2002). With the advent of more direct spectroscopy, FT-Raman spectroscopy is being employed to investigate such
interactions non-invasively in more detail (Howell and Li Chan, 1996; Howell et al., 1999).

4.2 Materials and Methods

4.2.1 Small-deformation oscillatory measurements.

Minced fish muscles (0.6 g fish and 3 ml water made into a paste) were subjected to small-deformation oscillatory measurements with a Rheometrics controlled stress rheometer and a parallel plate geometry with a gap of 2 mm set at a frequency of 1 radian per second. A dynamic temperature sweep was performed between 20 °C and 90 °C at a rate of 1 °C/min and then cooled to 20 °C. The viscoelastic properties of the gels measured include the storage modulus (G') and loss modulus (G'') as a function of time within the linear viscoelastic range of the gels (Mitchell, 1980) as outlined in Chapter 2.

4.2.2 Differential scanning calorimetry (DSC) Measurements

A Setaram Micro DSC VII was used with distilled water as the reference. Eight hundred milligrams of sample (fish mince: flour) of 10:0; 8:2; 5:5; 2:8; 0:10, % in water were placed in a pre-weighed DSC cell. An equal weight of distilled water was also introduced into the reference cell to obtain a flat base line. Water was used as a reference. Prior to scanning, the samples were held in place in the DSC at 10 °C for 10 min. A temperature scan of 10 °C to 90 °C and heating rate at 1.2 °C/min were maintained through out the study. Nitrogen was used to flush the chamber in between scans. With base lines determined by the reference cell, peak areas showing transitions were determined and transition temperatures and enthalpies calculated automatically by the DSC.
4.2.3 FT-Raman spectroscopy

Sample preparation: Fish mince and either cowpea flour or cassava starch were mixed together in the ratio of (fish mince : flour/starch) 10:0; 8:2; 5:5; 2:8; 0:10, % in water and heated at 90° C for 30 min to form gels in stainless steel tubes (50 mm long and 15 mm diameter). The gels were stored overnight and dried in a freeze dryer to obtain fine powdery particles so as to minimize background noise during the spectral analyses. Raman spectra of samples were taken in 7-mL glass containers (FBG-Anchor, Cricklewood, London) on a Perkin-Elmer System 2000 FT-Raman spectrophotometer with excitation from a Nd:Y AG laser at 1064 nm. Frequency calibration of the instrument was performed using the sulphur line at 217 cm⁻¹. The spectra in duplicate were an average of 64 scans which were baseline corrected, smoothed, and normalized to the intensity of the phenylalanine band at 1004 cm⁻¹ (Howell and Li-Chan, 1996; Tu, 1986). The recorded spectra were analyzed using Grams 32 (Galactic Industries Corp., Salem, NH). Assignments of the bands in the spectra to protein vibrational modes were according to Howell and Li-Chan (1996); Li-Chan et al. (1994) and Careche et al. (1999). The interaction of the fish minces with either cowpea flour or cassava starch was investigated by subtraction of the spectra of the respective proportion of either cowpea flour or cassava starch as the case may be from the mince mixtures. This was done as a means of focusing on the protein in the Raman spectrum (Piot et al., 2000). The difference in the spectra thus obtained were related to the changes in the structures of the proteins of the fish minces as consequences of interactions of the proteins with the flours.
4.2.4 Phase contrast microscopy

Drops of the fish mince and either cowpea flour or cassava starch samples prepared for rheological and DSC studies were placed on acetone cleaned microscope slides and covered with a cover slip to prevent dehydration. The slides were viewed under a Leitz microscope fitted with a binocular eyepiece and a phase contrast annulus matching an objective of a different magnification. The microscope was attached to a Wild MPS 05 system comprising a camera and an exposure meter set on camera factor 0.32. The sensor was set for integrated metering of objects, which are uniformly distributed.

4.3. Results and discussions

4.3.1 Rheological studies

The results of the viscoelasticity measurements of the muscle homogenates of the fish species are shown in Figures 4.1 and 4.2.

The gelation profile of the minces (10% w/w) of the species as a function of temperature followed the same pattern for all the muscle homogenates. During heating from 20 °C to 90 °C, there was a decrease in storage modulus with a rise in temperature up to 45 °C after which the storage modulus \( (G') \) increased considerably until it dipped down slightly towards the 90 °C. Though there were slight increases in the loss modulus \( (G'') \) these were not as high as those of the storage modulus \( (G') \). Subsequent cooling from 90 °C to 20 °C resulted in a more marked increase in both the storage and loss modulus. However, the values of the \( G' \) and \( G'' \) (Table 4.1) of the burrito were significantly (\( P<0.05 \)) lower than those of the flying gurnard.
Chapter 4 Rheological, DSC, FT-Raman spectroscopy and phase microscopy studies of fish mince, cowpea flour and cassava starch mixtures

Fig. 4.1 A dynamic temperature sweep of homogenised Burrito muscle

Fig. 4.2 A dynamic temperature sweep of homogenised Flying gurnard muscle
Chapter 4 Rheological, DSC, FT-Raman spectroscopy and phase microscopy studies of fish mince, cowpea flour and cassava starch mixtures

Table 4.1 G' and G'' (Pa) of flying gurnard and burrito mince after cooling from 90 °C to 20 °C

<table>
<thead>
<tr>
<th>Fish species</th>
<th>G' (Pa) Temp 90 °C</th>
<th>G'' (Pa) Temp 20 °C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flying gurnard</td>
<td>39,400 ± 316</td>
<td>7,170 ± 201</td>
</tr>
<tr>
<td>Burrito</td>
<td>12,000 ± 480</td>
<td>2,330 ± 512</td>
</tr>
</tbody>
</table>

The increase in $G'$ of the fish protein minces during heating may be an indication of gel formation and as described by Wu et al. (1985), a degree of unfolding of the protein molecules occurs leading to protein-protein association via hydrophobic interactions. The marked increase in both the storage and loss modulus during the cooling period (90 °C - 20 °C) was an indication of the development of rigidity of the gels. Increase in gel rigidity during cooling may be attributed to the reorientation and rearrangement of denatured polypeptide. Montejano et al., (1983) attributed this to formation of stable network by cross-linked protein aggregation through hydrophobic interactions.

4.3.1.1 Effect of cowpea flour and cassava starch on the gelation profiles of the fish minces.

The gelation profiles of the fish minces after addition of either cowpea flour or cassava starch are shown in Figures 4.3 (a –d). In all the compositions for the flying gurnard mince and the cowpea flour, the trends in $G'$ (storage modulus) were identical over a temperature sweep of 20 °C – 90 °C. There were increases in the storage modulus of the samples as the temperature was increased with the mixture containing a mince to cowpea flour ratio of 5:5 attaining a significantly higher (P<0.05) $G'$ (1369 Pa) as shown
Chapter 4 Rheological, DSC, FT-Raman spectroscopy and phase microscopy studies of fish mince, cowpea flour and cassava starch mixtures

Figure 4.3a Gelation profile of flying gurnard mince with varying proportions (w/w) of cowpea flour during heating

Figure 4.3b Gelation profile of flying gurnard mince with varying proportion of cowpea flour (w/w) during cooling
on Table 4.2, than the other mixtures. On cooling, however, the curves showed an increase in their elastic modulus, as $G'$ of each mixture increased sharply, to indicate the development of rigidity of the gels with the exception of the cowpea alone (0:10) which on contrast showed a decrease in the $G'$ value and hence may not have formed a gel.

Table 4.2  Storage modulus ($G'$ Pa) and loss modulus ($G''$ Pa) of flying gurnard mince and cowpea flour during a dynamic temperature sweep at 90 °C and 20 °C*

<table>
<thead>
<tr>
<th>Composition</th>
<th>Flying gurnard mince: cowpea flour</th>
<th>90 °C</th>
<th>20 °C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$G'$ (Pa)</td>
<td>$G''$ (Pa)</td>
<td>$G'$ (Pa)</td>
</tr>
<tr>
<td>10:0</td>
<td>18±3.8</td>
<td>2.45±0.9</td>
<td>116.64±19</td>
</tr>
<tr>
<td>8:2</td>
<td>206.08±56</td>
<td>48.6±4.24</td>
<td>245.26±25</td>
</tr>
<tr>
<td>5:5</td>
<td>1369±209</td>
<td>318.5±79</td>
<td>1608.9±121</td>
</tr>
<tr>
<td>2:8</td>
<td>275.4±47</td>
<td>67.4±14.8</td>
<td>615.4±51</td>
</tr>
<tr>
<td>0:10</td>
<td>429.5±65</td>
<td>95.9±13.2</td>
<td>113.4±51</td>
</tr>
</tbody>
</table>

* Values are means of three determinations ± standard deviation

The low $G'$ value (18 Pa) of the flying gurnard mince during the temperature sweep could be attributed to the intrinsic characteristics of the unrefined minces of the fish (Ishikawa, 1978; Leinot and Cheftel, 1990) and lower concentrations (in comparison with homogenized fish flesh $G'$ value (39400 Pa on Table 4.1) which affected the myofibrillar proteins to gel (Niwa, 1985; Doi, 1993). This property among other variables depends on the fish species, type of muscle and its freshness quality (Sikorski et. al., 1976; Shenouda, 1980; Matsumoto, 1980). With an increase in temperature during the process, the proteins of the fish mince would denature and gel, hence the
significant ($P<0.05$) value of the $G'$ (116 Pa) attained. Similar observations have been reported by a number of researchers (Montejano et al., 1983; Gómez-Guillén et al., 1997). This gel network is stabilised by hydrophobic and disulfide bonds as well as hydrogen bonds (Niwa et al., 1986). As described by Wu et al., (1985), a degree of unfolding of the protein molecules occurs leading to protein-protein association via hydrophobic interactions. On cooling, however, the increase in the $G'$ value indicated the development of rigidity of the gel. The development of rigidity in gels during cooling is consistent with the observations by Clark et al. (1982). They also reported increases in $G'$ value of BSA/agar gels during cooling. In protein gels, increase in gel rigidity during cooling is attributed to the reorientation and rearrangement of denatured polypeptide, described by Montejano et al. (1983) as a formation of stable network by cross-linked protein aggregation.

As shown in Table 4.2, addition of cowpea flour resulted in some form of interactions with the proteins of the fish mince as noted for the increase in the $G'$ value. The mode of interaction may be attributed to the globulins dissociating into acidic and basic subunits and the basic subunits subsequently interacting with the myosin heavy chains of the fish mince via disulphide bond (Peng et al., 1982). The interaction may be described as a positive synergistic interaction and the gels formed are compatible and stable (Nakamura et al., 1986; Puppo et al., 1995). The interaction as observed may be dependent on the type of proteins and the relative proportions of the components present. As noted, there was a relative decrease in $G'$ value when the cowpea flour was increased in the fish mince to cowpea ratio of 2:8 (615 Pa) from that of the 5:5 ratio mixture. There was obviously a reduction in the fish mince protein as well hence a reduction of the available myosin components and the significantly ($P<0.05$) low $G'$
value (114.3 Pa) obtained for the cowpea flour alone. Cowpea flour protein is known to have poor gelling properties and this could be related to the 11S globulins. Studies by Kamata et al. (1991) indicated that the 7S globulins gels were able to reform after shearing on standing whereas the 11S globulins gels did not. Previously, Damodaran (1988) suggested that 11S globulins undergo partial refolding to some extent during the cooling part of the thermal gelation process, hence their failure to form a gel. The observation in this study suggested that, gelation is not only a function of protein quantity but may also be related to the type of protein as well as to the nonprotein components and protein solubility. Sathe and Salunkhe (1981) made similar observations in their study on the great northern bean.

The gelation profiles of the flying gurnard mince and cassava starch are depicted by Figure 4.4 (a-b)
Chapter 4 Rheological, DSC, FT-Raman spectroscopy and phase microscopy studies of fish mince, cowpea flour and cassava starch mixtures

Figure 4.4b Gelation profile of flying gurnard mince with varying proportion of cassava starch (w/w) during cooling

The gelation profiles of the mixtures showed similar trends. The mixtures with high amounts of starch content showed marked shifts after 79 °C recording drops in the $G'$ and $G''$ values before 90 °C during the heating.

Table 4.3 Storage modulus ($G'$ Pa) and loss modulus ($G''$ Pa) of flying gurnard mince and cassava starch during a dynamic temperature sweep at 90 °C and 20 °C*

<table>
<thead>
<tr>
<th>Composition</th>
<th>90 °C</th>
<th>20 °C</th>
<th>G/G cross over point (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$G'$ (Pa)</td>
<td>$G''$ (Pa)</td>
<td>$G'$ (Pa)</td>
</tr>
<tr>
<td>Flying gurnard mince: Cassava starch</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10:0</td>
<td>18±3.8</td>
<td>2.45±0.9</td>
<td>116.64±19.3</td>
</tr>
<tr>
<td>8:2</td>
<td>38.68±6</td>
<td>6.94±1.0</td>
<td>133±8.3</td>
</tr>
<tr>
<td>5:5</td>
<td>126±23.</td>
<td>24.7±6.9</td>
<td>265.29±21.9</td>
</tr>
<tr>
<td>2:8</td>
<td>83.32±7.5</td>
<td>22.38±4.8</td>
<td>126±13.9</td>
</tr>
<tr>
<td>0:10</td>
<td>209.29±45</td>
<td>65.59±8.2</td>
<td>562.94±54.7</td>
</tr>
</tbody>
</table>

* Values are means of three determinations ± standard deviation
As shown in Table 4.3, significant (P<0.05) increases in G' values were also observed for mixtures with high contents of starch with the highest recorded for the sample with no fish mince (0:10) during cooling. Instances of cross over points (G'/G'') were also observed at a temperature range of 55 to 59 °C during the heat gelation process of some of the mixtures. The increases in G' value of 18 Pa to 126 Pa as the starch content increased to the same fish mince content (5:5) may be regarded as some form of interaction with the fish proteins and the starch molecules. Similar observations were made by Gómez-Guillén et al. (1996). As suggested by Takeuchi (1969), the molecular forces in such complex formation are in part due to charges on the protein as they denature and undergo much cross-linking through disulphide bond formation network and the starch colloids. Starch at high temperatures gelatinises with a disruption of the granules and formation of a matrix of amylose holding the amylopectin in a network (Remsen and Clark, 1978). More and Cater (1974), also indicated that several non-covalent factors could be involved in protein-starch interactions as well as hydrogen bonding. It may be assumed that gels are formed as a result of these interactions; however, the gels formed may not be stable as a decrease in the storage modulus with further increase in temperature was observed. This was an indication that the gel structure was destroyed as temperature was increased (Tsai et al., 1997). This destruction has been attributed to the melting of the crystalline region remaining in the swollen starch granule, which deforms and loosens the particles (Eliasson, 1986). Again, the increase in the storage modulus of the mixtures could be attributed largely to the degree of granular swelling of the starch during gelatinisation within the system (Eliasson, 1986) and intergranule contact (as the starch content increased) that might have formed a three-dimensional network of the swollen granules (Wong and Lelievre, 1981). The increase in viscosity during the cooling period could be attributed to the
various constituents present and in particular the swollen granules and fragments to associate or retrograde as a result of molecular interactions (hydrogen bonding) (Hoover, 2000). During retrogradation, amylose forms double helical associations of 40–70 glucose units (Jane and Robyt, 1984) whereas amylopectin crystallization occurs by association of the outermost short branches (Ring et al., 1987).

4.3.1.2 Effect of cowpea flour and cassava starch on the gelation profiles of the Burrito fish mince.

Figures 4.5 (a-b) shows the gelation profiles of the burrito fish mince and cowpea flour.

Figure 4.5a Gelation profile of burrito fish mince with varying proportion of cowpea flour (w/w) during heating
Remarkable variations were noted in the gelation profiles of the mixture as the cowpea flour was increased and G' values were noted to increase significantly (P<0.05) only after 70 °C. However, the increase in the burrito fish mince alone (10:0) was marginal. During cooling, the trend was not reversed but a lower G' value was observed for the 8:2 (mince: cowpea) than all the other mixtures with the 2:8 (fish: cowpea) ratio being the highest. As shown in Table 4.4, the mixtures appear to exhibit a type of interaction which involved aggregation at a rather lower content of fish but this aggregation tends to decrease as the fish mince is increased. This is contrary to normal expectations in which increasing the protein of the mixtures by increasing fish mince content leads to increase in the G' value if a positive interaction is thought to occur at lower fish mince content. The G' value (140 Pa) of the mixture with high content of mince fish ratio (8:2) to cowpea flour was significantly (P<0.05) lower than the G' value (1041 Pa) as the fish mince decreased in the 2:8 ratio mixture in view of the assertion that cowpea flour has poor gelling properties (Kamata et al., 1991; Damodaran, 1988). This could mean that small amounts of fish mince were sufficient enough to interact with the
cowpea protein as exhibited by the low $G'$ value in the mixture without fish mince (0:10).

Table 4.4 Storage modulus ($G'$ Pa) and loss modulus ($G''$ Pa) of burrito fish mince and cowpea flour during a dynamic temperature sweep at 90 °C and 20 °C*

<table>
<thead>
<tr>
<th>Composition burrito fish mince: Cowpea flour</th>
<th>90 °C</th>
<th>20 °C</th>
<th>G/G cross over point (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$G'$ (Pa)</td>
<td>$G''$ (Pa)</td>
<td>$G'$ (Pa)</td>
</tr>
<tr>
<td>10:0</td>
<td>38.1±4.3</td>
<td>3.74±0.6</td>
<td>140.21±22.6</td>
</tr>
<tr>
<td>8:2</td>
<td>167.3±13.7</td>
<td>116.3±17</td>
<td>228.7±12.5</td>
</tr>
<tr>
<td>5:5</td>
<td>2384±24</td>
<td>144.6±25</td>
<td>360.14±61.9</td>
</tr>
<tr>
<td>2:8</td>
<td>699±47.3</td>
<td>191.9±15.5</td>
<td>1041±23.8</td>
</tr>
<tr>
<td>0:10</td>
<td>429.5±65.6</td>
<td>95.9±13.2</td>
<td>113.4±51.9</td>
</tr>
</tbody>
</table>

*Values are means of three determinations ± standard deviation

It may be necessary to consider the intrinsic characteristics of the unrefined minces of the burrito fish mince (Ishikawa 1978; Leinot and Cheftel, 1990) which could affect the myofibrillar proteins to gel (Niwa, 1985; Doi, 1993). The observation in this study confirmed that, gelation is not only a function of protein quantity, but may also be related to the type of protein as well as to the nonprotein components and protein solubility as indicated by Sathe and Salunkhe (1981)

Figures 4.6 (a-b) show the gelation profiles of the burrito fish and varying proportions of cassava starch as a function of temperature. Identical trends were observed for all the mixtures with increasing $G'$ value as the starch component was increased in the mixtures. Though there were sharp rises in $G'$ values as temperature increased the $G'$...
values dropped to almost the same values across at 90 °C (Table 4.5) with the samples containing fish mince and cassava starch.

Figure 4.6a Gelation profile of burrito fish mince with varying concentration of cassava starch (w/w) during heating

Figure 4.6b Gelation profile of burrito fish mince with varying proportion of cassava starch (w/w) during cooling
### Table 4.5 Storage modulus (G' Pa) and loss modulus (G'' Pa) of burrito fish mince and cassava starch during a dynamic temperature sweep at 90 °C and 20 °C*

<table>
<thead>
<tr>
<th>Composition</th>
<th>90 °C</th>
<th>20 °C</th>
<th>G'/G'' cross over point (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>burrito fish mince: cassava starch</td>
<td>G' (Pa)</td>
<td>G'' (Pa)</td>
<td>G' (Pa)</td>
</tr>
<tr>
<td>10:0</td>
<td>38.1±4.3</td>
<td>3.74±0.6</td>
<td>140.21±22.6</td>
</tr>
<tr>
<td>8:2</td>
<td>75.47±8.1</td>
<td>8.6±1.7</td>
<td>228.7±12.5</td>
</tr>
<tr>
<td>5:5</td>
<td>152.14±19.8</td>
<td>24.67±5.2</td>
<td>411.09±41.9</td>
</tr>
<tr>
<td>2:8</td>
<td>120.37±47.1</td>
<td>24.32±5.5</td>
<td>203.78±23.8</td>
</tr>
<tr>
<td>0:10</td>
<td>209.29±45.5</td>
<td>65.59±8.2</td>
<td>562.94±54.7</td>
</tr>
</tbody>
</table>

*Values are means of three determinations ± standard deviation

However, the mixture with the fish mince to starch ratio (5:5) recorded a significantly higher increase G' value (411 Pa) following that of the cassava starch alone (562.9 Pa) after cooling (Table 4.5). This interaction has been attributed to molecular forces, due to charges on the protein (Gómez-Guillén, et al., 1996; Takeuchi, 1969; More and Cater, 1974; Remsen and Clark, 1978). The gels formed may not be stable, as a decrease in the storage modulus with a further increase in temperature, destroyed the gel structure (Tsai et al., 1997; Eliasson, 1986; Eliasson, 1986). The increase in the storage modulus of the mixtures is largely due to the degree of granular swelling of the starch during gelatinisation (Eliasson, 1986) and intergranule contact (Wong and Lelievre, 1981; Hoover, 2000).
4.3.2 DSC Studies

The thermograms generated for the minces of each fish species from the DSC studies are shown in Figures 4.7 and 4.8. All the thermograms were endothermic with each having four transitions. Table 4.6 shows the calculated enthalpy and transition temperatures for the fish minces. The transitions observed in the thermograms with maximum transition temperatures could be assigned to the denaturation of specific constituents of the protein (Privalov, 1980; Biliaderis, 1983) namely myosin - Peak 1 ($T_m$, 40.92 °C, for the burrito and $T_m$, 40.04 °C, for the flying gurnard), water soluble sarcoplasmic proteins- Peaks 2 and 3 ($T_m$ 50 - 60.86 °C for the burrito and $T_m$ 48.37-52.22 °C for the flying gurnard) and lastly actin-Peak 4 ($T_m$ 66.92 °C for the burrito and $T_m$ 60.17 °C for the flying gurnard). Apart from peak one for each species, there were significant differences ($P<0.05$) in the enthalpy change ($\Delta H$) in the subsequent peaks assigned to the water-soluble sarcoplasmic proteins and actin among the species. The variations in the thermograms may be specific for the muscles of each fish species and may be used as a ‘fingerprint’ for the species. Similar deductions have been made by a number of workers (Stabursvik and Martins, 1980). It may also reflect differences in the relative amounts of the protein components, the basic structure of these proteins or the state of rigor of the muscles of each specific fish species. (Wright and Wilding, 1984)
Chapter 4 Rheological, DSC, FT-Raman spectroscopy and phase microscopy studies of fish mince, cowpea flour and cassava starch mixtures

Figure 4.7. Differential scanning calorimetry of flying gurnard muscle thermogram indicating the transitions and heat flow.

Figure 4.8. Differential scanning calorimetry of burrito muscle thermogram indicating the transitions and heat flow.
Chapter 4 Rheological, DSC, FT-Raman spectroscopy and phase microscopy studies of fish mince, cowpea flour and cassava starch mixtures

Table 4.6 Transition temperatures and enthalpy change for the burrito and the flying gurnard

<table>
<thead>
<tr>
<th>Fish species</th>
<th>Transitions</th>
<th>Onset Temp ((T_o) ^\circ C)</th>
<th>Peak Temperature ((T_m) ^\circ C)</th>
<th>Enthalpy/J/G (wet weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Burrito</td>
<td>1</td>
<td>35.20</td>
<td>40.92</td>
<td>0.28</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>47.73</td>
<td>50.56</td>
<td>0.04</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>55.23</td>
<td>60.86</td>
<td>0.16</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>64.52</td>
<td>66.92</td>
<td>0.03</td>
</tr>
<tr>
<td>Flying gurnard</td>
<td>1</td>
<td>35.57</td>
<td>40.04</td>
<td>0.25</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>46.75</td>
<td>48.37</td>
<td>0.01</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>50.18</td>
<td>52.22</td>
<td>0.03</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>57.38</td>
<td>60.17</td>
<td>0.08</td>
</tr>
</tbody>
</table>

4.3.2.1 Effect of cowpea flour and cassava starch on the DSC thermograms of the flying gurnard minces

The change in transitions generated as result of adding cowpea flour to flying gurnard mince in varying composition are shown in Table 4.7. Addition of cowpea flour led to changes of the shapes of the thermograms from the initial thermogram of the flying gurnard mince (10:0) with the disappearance of three peaks and appearance of another peak. As shown in Table 4.6, the peaks namely, the water-soluble sarcoplasmic proteins-Peaks 2 and 3 \((T_m 48.37-52.22 ^\circ C)\) and actin-Peak 4 \((T_m 60.17 ^\circ C)\) were affected. The new transition appeared at 72-75 \(^\circ C\). This peak remained visible even in the mixture without flying fish mince (0:10) and was deemed to have been as a result of the denaturation of the globular proteins of the cowpea (Arntfield and Murray, 1981). Data from the DSC (Table 4.7) show changes in peak denaturation temperatures \((T_m)\) and / or the enthalpy \((\Delta H)\) values. These may be indicative of progressive interaction of the fish proteins with the cowpea molecules.
Table 4.7 Transition temperatures and enthalpy change for the flying gurnard mince / cowpea flour

<table>
<thead>
<tr>
<th>Composition mince: flour</th>
<th>Transitions</th>
<th>Onset Temp ($T_o$) °C</th>
<th>Peak Temperature ($T_m$) °C</th>
<th>Enthalpy/ J/G (wet weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10:0</td>
<td>1</td>
<td>35.57</td>
<td>40.04</td>
<td>0.25</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>46.75</td>
<td>48.37</td>
<td>0.01</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>50.18</td>
<td>52.22</td>
<td>0.03</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>57.38</td>
<td>60.17</td>
<td>0.08</td>
</tr>
<tr>
<td>8:2</td>
<td>1</td>
<td>36.21</td>
<td>37.32</td>
<td>0.01</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>74.25</td>
<td>76.03</td>
<td>0.01</td>
</tr>
<tr>
<td>5:5</td>
<td>1</td>
<td>30.22</td>
<td>36.57</td>
<td>0.85</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>73.06</td>
<td>76.21</td>
<td>0.17</td>
</tr>
<tr>
<td>2:8</td>
<td>1</td>
<td>72.59</td>
<td>76.08</td>
<td>0.28</td>
</tr>
<tr>
<td>0:10</td>
<td>1</td>
<td>41.10</td>
<td>46.93</td>
<td>0.71</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>68.42</td>
<td>76.56</td>
<td>0.06</td>
</tr>
</tbody>
</table>

A significant increase (P<0.05) in enthalpy ($\Delta H$) value (0.85 J/g) was noticed in the mixture ratio of flying gurnard mince to cowpea flour (5:5). Similar changes in peak denaturation temperatures ($T_m$) and / or the enthalpy ($\Delta H$) have been attributed to proteins-proteins interactions (Wright and Wilding, 1984; Laskowski and Sealock, 1971; Finch and Ledward, 1972). These results may confirm the observations made by the rheological studies in this report and probably the fish mince and cowpea flour ratio of 5:5 may indicate that possibility.

Table 4.8 shows the transitions generated by adding cassava starch to flying gurnard mince in varying composition.
Table 4.8 Transition temperatures and enthalpy change for the flying gurnard mince / cassava flour

<table>
<thead>
<tr>
<th>Composition</th>
<th>Transitions</th>
<th>Onset Temp $\left(T_0\right)$ °C</th>
<th>Peak Temperature $\left(T_m\right)$ °C</th>
<th>Enthalpy/ J/G (wet weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10:0</td>
<td>1</td>
<td>35.57</td>
<td>40.04</td>
<td>0.25</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>46.75</td>
<td>48.37</td>
<td>0.01</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>50.18</td>
<td>52.22</td>
<td>0.03</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>57.38</td>
<td>60.17</td>
<td>0.08</td>
</tr>
<tr>
<td>8:2</td>
<td>1</td>
<td>67.04</td>
<td>70.44</td>
<td>1.01</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>33.26</td>
<td>33.70</td>
<td>0.03</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>65.79</td>
<td>70.05</td>
<td>0.36</td>
</tr>
<tr>
<td>5:5</td>
<td>1</td>
<td>65.57</td>
<td>69.82</td>
<td>0.56</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>37.62</td>
<td>39.53</td>
<td>0.02</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>65.79</td>
<td>70.05</td>
<td>0.36</td>
</tr>
<tr>
<td>2:8</td>
<td>1</td>
<td>63.82</td>
<td>69.47</td>
<td>0.67</td>
</tr>
<tr>
<td>0:10</td>
<td>1</td>
<td>63.82</td>
<td>69.47</td>
<td>0.67</td>
</tr>
</tbody>
</table>

Table 4.8 shows that with the addition of cassava starch, in the first instance of the 2% cassava, there were significant changes in transitions with the known peaks of (Privalov, 1980; Biliaderis, 1983) myosin - Peak 1 ($T_m$, 40.04 °C), water soluble sarcoplasmic proteins- Peaks 2 and 3 ($T_m$, 48.37-52.22 °C) and actin-Peak 4 ($T_m$, 60.17 °C) disappearing and a new peak at 70.44 °C was apparently formed. This peak however was observed in the other mixtures as well, including the mixture without the fish mince (0:10). The reappearance of additional two peaks (33-39 °C) in only the mixture of (5:5) fish mince to cassava ratio, could have been as result of disruptions of the myosin rather than any strong interaction with the cassava starch. However some form of interactions could not be ruled out as a result of slight destabilisation of the protein molecule and decrease of the denaturation temperature (Imeson et al., 1977). These interactions may be attributed to a fairly weak electrostatic interaction between the two macromolecules contained in the mixtures (Imeson et al., 1977). The new peak at 70.44 °C could be
attributed to starch gelatinization as typical DSC thermograms, similar to this study have
been reported by other researchers (Biliaderis et al., 1986; Normand and Marshall, 1989), where a single large endothermic transition was observed.

4.3.2.2 Effect of cowpea flour and cassava starch on the DSC thermograms of
the burrito fish minces

Table 4.9 shows the transitions generated by adding cowpea flour to burrito fish mince in varying composition.

Table 4.9 Transition temperatures and enthalpy change for the burrito mince / cowpea flour

<table>
<thead>
<tr>
<th>Composition mince: flour</th>
<th>Transitions</th>
<th>Onset Temp $T_o$ °C</th>
<th>Peak Temp $T_m$ °C</th>
<th>Enthalpy/ J/G (wet weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10:0</td>
<td>1</td>
<td>35.20</td>
<td>40.92</td>
<td>0.28</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>47.73</td>
<td>50.56</td>
<td>0.04</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>55.23</td>
<td>60.86</td>
<td>0.16</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>64.52</td>
<td>66.92</td>
<td>0.03</td>
</tr>
<tr>
<td>8:2</td>
<td>1</td>
<td>37.21</td>
<td>40.89</td>
<td>0.04</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>45.01</td>
<td>47.80</td>
<td>0.03</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>73.52</td>
<td>76.66</td>
<td>0.05</td>
</tr>
<tr>
<td>5:5</td>
<td>1</td>
<td>41.13</td>
<td>44.12</td>
<td>0.01</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>73.24</td>
<td>76.54</td>
<td>0.13</td>
</tr>
<tr>
<td>2:8</td>
<td>1</td>
<td>42.61</td>
<td>48.63</td>
<td>0.02</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>72.88</td>
<td>76.36</td>
<td>0.51</td>
</tr>
<tr>
<td>0:10</td>
<td>1</td>
<td>41.10</td>
<td>46.93</td>
<td>0.71</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>68.42</td>
<td>76.56</td>
<td>0.06</td>
</tr>
</tbody>
</table>

From the data presented in Table 4.9, the addition of cowpea flour generally affected the initial thermogram obtained for the burrito mince alone (10:0). The peaks observed for the mixtures showed decreases in the denaturation temperatures especially the water-soluble sarcoplasmic proteins- Peaks 2 and 3 ($T_m$ 50 – 60.86 °C) with the disappearance
of myosin - Peak 1 ($T_m$, 40.92 °C), as the cowpea flour concentration was increased. These peaks were also accompanied by decreases in the enthalpy ($\Delta H$) values (0.04 to 0.02 J/g).

These changes could be ascribed to some weak interaction though as the denaturation temperatures of the myosin peaks remained significantly ($P<0.05$) unchanged (40 to 44 °C) when detected in the mixtures. The transition that appeared at 72-75 °C with the mixtures was as a result of the denaturation of the globular proteins of the cowpea (Arntfield and Murray, 1981).

Table 4.10 gives details of the calculated transition temperatures and enthalpy associated by adding cassava starch to burrito fish mince in varying amounts.

It is evident that the addition of cassava starch did not change remarkably the thermogram generated by the burrito mince alone (10:0) except for the non-detection of the water soluble sarcoplasmic proteins - Peaks 2 and 3 ($T_m$ 50 – 60.86 °C) and the detection of the peak ascribed to starch gelatinization (Biliaderis et al., 1986; Normand and Marshall, 1989) as the starch content was increased in the mixture. This phenomenon which may not be a strong interaction could be due to weak electrostatic interaction between the macromolecules contained in the mixtures (Imeson et al., 1977).
Table 4.10 Transition temperatures and enthalpy change for the burrito mince / cassava Flour

<table>
<thead>
<tr>
<th>Composition mince: flour</th>
<th>Transitions</th>
<th>Onset Temp ($T_o$) °C</th>
<th>Peak Temperature ($T_m$) °C</th>
<th>Enthalpy/ J/G (wet weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10:0</td>
<td>1</td>
<td>35.20</td>
<td>40.92</td>
<td>0.28</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>47.73</td>
<td>50.56</td>
<td>0.04</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>55.23</td>
<td>60.86</td>
<td>0.16</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>64.52</td>
<td>66.92</td>
<td>0.03</td>
</tr>
<tr>
<td>8:2</td>
<td>1</td>
<td>37.17</td>
<td>39.66</td>
<td>0.01</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>48.24</td>
<td>52.15</td>
<td>0.01</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>67.38</td>
<td>71.33</td>
<td>0.15</td>
</tr>
<tr>
<td>5:5</td>
<td>1</td>
<td>43.63</td>
<td>48.97</td>
<td>0.05</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>66.84</td>
<td>70.95</td>
<td>0.40</td>
</tr>
<tr>
<td>2:8</td>
<td>1</td>
<td>42.20</td>
<td>46.41</td>
<td>0.01</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>66.16</td>
<td>70.27</td>
<td>0.60</td>
</tr>
<tr>
<td>0:10</td>
<td>1</td>
<td>63.82</td>
<td>69.47</td>
<td>0.67</td>
</tr>
</tbody>
</table>
4.3.3 FT-Raman spectroscopy

4.3.3.1 Comparison of flying gurnard mince and mixed mince with cowpea flour

The spectra for the flying fish mince and mixtures of the mince and cowpea flour (Figure 4.9 and Table 4.11) showed distinct peaks at 760 cm\(^{-1}\) which is due to indole-ring vibrations of tryptophan (Trp) residues (Ogawa et al., 1999; Li Chan, 1996; Tu, 1982) with buried residues giving a more intense band than residues exposed to a polar environment (Ogawa et al., 1999; Li Chan, 1996; Tu, 1982). This band increased in intensity becoming broader as the cowpea flour content increased. The band is known to be sensitive to the environment polarity of Trp residues and increases in the intensity on heat-induced gelation as a result of intermolecular hydrophobic interactions and disulfide exchange reactions. The increase in the area of the band suggested that more residues were buried in the heat-induced gel (Ogawa et al., 1999; Li Chan, 1996; Tu, 1982). These observations were also seen in the increased intensity of the doublet bands at 1340 and 1360 cm\(^{-1}\) (Miura et al., 1988), as the ratio of the cowpea flour was increased in the samples. However, only one peak (1340 cm\(^{-1}\)) was observed in this case (Figure 4.9).

Frushour and Koenig (1975) reported similar observations in β-lactoglobulin and attributed that to CH deformation bands of aliphatic side chain.

The intensity ratio \(I_{855}/I_{830}\) of the tyrosine doublet near 855 cm\(^{-1}\) and 830 cm\(^{-1}\) which is used to determine the number of buried and exposed Tyr residues and the state of the hydrogen bonding of the phenolic OH group (Tu, 1986) tended to decrease (0.85 - 0.3) as the cowpea content increased. This could be assumed to relate to the case of hydrogen bonding between the flying fish proteins to a negative acceptor of the molecules in the cowpea flour (Van Dael et al., 1987).
Figure 4.9 Raman spectra (A) 1700-650 cm\(^{-1}\) and (B) 3500-2700 cm\(^{-1}\) for flying gurnard mince and cowpea flour in the ratio (a) 10:0 (b) 8:2 (c) 5:5 (d) 2:8 after subtracting the spectra of the respective proportion of cowpea flour from each mixture.
Chapter 4 Rheological, DSC, FT-Raman spectroscopy and phase microscopy studies of fish mince, cowpea flour and cassava starch mixtures

Table 4.11. Relative peak intensity of Raman spectra in the regions 3200-700 cm\(^{-1}\) for flying gurnard mince and varying addition of cowpea flour\(^1\)

<table>
<thead>
<tr>
<th>Peak assignment (wavenumber ± 2 cm(^{-1}))</th>
<th>Relative peak intensity 10% Flying gurnard mince</th>
<th>8% Flying gurnard mince + 2% cowpea flour</th>
<th>5% Flying gurnard mince + 8% cowpea flour</th>
<th>2% Flying gurnard mince + 8% cowpea flour</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trp (760)</td>
<td>0.38 ± 0.08</td>
<td>0.25 ± 0.04</td>
<td>0.3 ± 0.08</td>
<td>0.15 ± 0.08</td>
</tr>
<tr>
<td>Tyr (830, 855)</td>
<td>0.34 ± 0.11 / 0.40 ± 0.08</td>
<td>0.36 ± 0.11 / 0.41 ± 0.19</td>
<td>0.31 ± 0.06 / 0.91 ± 0.17</td>
<td>0.09 ± 0.1 / 0.11</td>
</tr>
<tr>
<td>Helix C-C stretch, CH(_3) symmetric stretch (937)</td>
<td>0.26 ± 0.04</td>
<td>0.18 ± 0.07</td>
<td>1.03 ± 0.31</td>
<td>0.90 ± 0.12</td>
</tr>
<tr>
<td>Phe, ring band (1004)</td>
<td>1 ± 0.06</td>
<td>1 ± 0.05</td>
<td>1 ± 0.08</td>
<td>1 ± 0.01</td>
</tr>
<tr>
<td>Isopropyl anti symmetric stretch CH stretch back bone (1128)</td>
<td>0.37 ± 0.04</td>
<td>0.31 ± 0.06 (1125)</td>
<td>0.84 ± 0.16 (1154)</td>
<td>0.21 ± 0.09 (1120)</td>
</tr>
<tr>
<td>CH(_3) anti symmetric (aliphatic) CH(_3) rock (aromatic) (1160)</td>
<td>0.21 ± 0.04</td>
<td>0.06 ± 0.02 (1125)</td>
<td>0.21 ± 0.11 (1164)</td>
<td></td>
</tr>
<tr>
<td>β-sheet type (1239)</td>
<td>0.46 ± 0.05</td>
<td>0.29 ± 0.10</td>
<td>0.37 ± 0.03</td>
<td>0.14 ± 0.12</td>
</tr>
<tr>
<td>Amide III (1264)</td>
<td>0.68 ± 0.10</td>
<td>0.42 ± 0.18 (1260)</td>
<td>0.5 ± 0.02 (1261)</td>
<td>0.21 ± 0.11 (1261)</td>
</tr>
<tr>
<td>Amide III (1320)</td>
<td>0.57 ± 0.03</td>
<td>0.09 ± 0.01</td>
<td>0.68 ± 0.06</td>
<td>0.14 ± 0.17</td>
</tr>
<tr>
<td>H band doublet from trp (1340)</td>
<td>0.53 ± 0.10</td>
<td>0.31 ± 0.34</td>
<td>1.14 ± 0.30</td>
<td></td>
</tr>
<tr>
<td>Aliphatic groups, CH bend (1451)</td>
<td>2.61 ± 1.97</td>
<td>2.7 ± 0.24</td>
<td>1.83 ± 0.23</td>
<td>0.18 ± 0.02</td>
</tr>
<tr>
<td>Trp (1554)</td>
<td>0.16 ± 0.05</td>
<td>0.22 ± 0.02</td>
<td>0.18 ± 0.12</td>
<td>0.18 ± 0.06</td>
</tr>
<tr>
<td>Amide I (1660)</td>
<td>2.43 ± 0.16</td>
<td>1.48 ± 0.13</td>
<td>0.62 ± 0.07</td>
<td>0.11 ± 0.03</td>
</tr>
<tr>
<td>CH stretch, aliphatic (2937)</td>
<td>10.56 ± 0.39</td>
<td>8.54 ± 0.64 (2940)</td>
<td>7.86 ± 1.87</td>
<td></td>
</tr>
<tr>
<td>Shoulder (2875)</td>
<td>6.64 ± 0.31</td>
<td>3.81 ± 0.21</td>
<td>5.29 ± 0.87</td>
<td>0.46 ± 0.03</td>
</tr>
<tr>
<td>Shoulder (2976) (2969)</td>
<td>6.21 ± 0.24</td>
<td>4.31 ± 0.82</td>
<td>4.60 ± 1.42</td>
<td>0</td>
</tr>
<tr>
<td>CH stretch, aromatic (3063)</td>
<td>0.50 ± 0.06</td>
<td>0.41 ± 0.07</td>
<td>0.26 ± 0.04</td>
<td>0.07</td>
</tr>
<tr>
<td>NH stretch (backbone) (3200)</td>
<td>0.21 ± 0.07</td>
<td>0.19 ± 0.07</td>
<td>0.30 ± 0.12</td>
<td>0.11 ± 0.10</td>
</tr>
</tbody>
</table>

\(^1\)Figures in parenthesis next to peak intensity values refer to shift wavenumbers (± 2 cm\(^{-1}\)). The spectra relative intensity values were averaged from 64 scans, baseline corrected, smoothed and normalized to the intensity of the phenylalanine band at 1004 cm\(^{-1}\).

The involvement of tyrosine residues in the gelation of α-lactalbumin was indicated by a decrease in the intensity ratio suggesting the role of phenolic hydroxyl groups as strong hydrogen bond donors or an increase in how well buried the tyrosine residues are within
the gel network (Nonaka et al., 1993). Similar observations were reported in the case of alkaline-induced aggregation of β-lactoglobulin (Frushour and Koenig, 1975). High ratio intensity could be attributed to the tyrosine residue being in an extremely strong hydrogen bond as a proton acceptor (Honzaiko and Williams, 1982).

The increasing vibrational peak or strong intensity around 937-940 cm$^{-1}$ which is ascribed to a skeletal C-C stretching and proportional to the α-helix content (Frushour and Koenig, 1975; Howell and Li-Chan, 1996) in the samples as the cowpea flour increased was consistent with the observations in the studies by Ikeda and Li Chan (2003) who attributed this to the high protein concentration which in this case was contributed by the cowpea flour. However, Howell and Li-Chan (1996), noted that this band intensity decreased on heating and could be a result of the low α-helix in the native β-lactoglobulin used in the study (Belloque and Smith, 1998). The addition of cowpea flour to the fish mince in this study may have increased the protein content relatively and thus offered an improved heat-resistance of the α-helix with perhaps contributions from non-aggregated and hence renatured molecules after cooling (Ikeda and Li Chan, 2003).

The phenylalanine band (1004 cm$^{-1}$) is reported to be insensitive to conformation or microenvironment hence the sharp bands observed for all the samples (Figure 4.9), was used as an internal reference for normalization of the spectra of the samples (Howell and Li-Chan, 1996; Tu, 1986).

The band (1200-1300 cm$^{-1}$) assigned to an amide III mode region increased with increasing cowpea flour in the samples. This region resulted mainly from C-N stretching
and N-H in plane bending vibrations of the peptide bond (Howell and Li-Chan, 1996; Tu, 1986).

As expected the β-sheets and disordered structures between 1230-1240 and 1245-1270 cm\(^{-1}\) respectively were observed at 1239-1242 and 1245-1264 cm\(^{-1}\) for the samples with increasing intensity as the cowpea flour content increased. The apparent unchanged position of the Amide III position within the samples was consistent with observations of β-sheet structures in native β-lactoglobulin, thus after heat induced gelation, a significant amount of β-sheet structures remained in the gels (Belloque and Smith, 1998). As explained by Ikeda and Li-Chan (2003) the peak position of at 1242 cm\(^{-1}\) suggested the predominant contributions from β-sheets structures.

The amide band I (1665-1672 cm\(^{-1}\)) which corresponds to the secondary structure of polypeptide chains is ascribed to in-plane peptide C=O stretching vibrations and N-H bending vibrations (Howell and Li-Chan, 1996; Tu, 1986) was observed to decrease in intensity as the cowpea flour increased but significant shifts from 1660 to 1668. The peak position depended on the state of hydrogen bonding and the backbone conformation (Howell and Li-Chan, 1996; Tu, 1986). As reported by Carew et al. (1983) α-helix bands of globular proteins showed a peak around 1660 cm\(^{-1}\), while β-sheet and disordered structures bands have a peak around 1679 cm\(^{-1}\). Consistent with the observations made by Carew et al. (1983), the peak shift of α-helix from 1660 to 1668 cm\(^{-1}\) (β-sheet and disordered structures bands) with increasing content of cowpea flour could be explained as a result of heat induced gelation of the mixtures.
The band in the CH stretching region at 2937 cm\(^{-1}\) assigned to aliphatic amino acid residues did not shift significantly within the samples. However, there was a gradual smoothing of the shoulders at 2876 and 2969 cm\(^{-1}\) as the cowpea flour content increased. The peak intensity and position at the CH stretching region 3063 cm\(^{-1}\) (aromatic groups) remained largely unaffected with increasing cowpea flour within the samples. These observations indicate that the proteins protected as deduced from Li-Chan (1996), who noted that shifts in this region to higher wavenumbers may have suggested sensitivity of the band to the polarity of the microenvironment and to protein denaturation.

The results of the spectra as analysed above suggested some form of interactions between the proteins of the flying gurnard mince and cowpea notwithstanding the unrefined nature of the proteins involved in the mixtures. These interactions could have been brought about by intermolecular hydrophobic interactions and disulfide exchange reactions as well as hydrogen bonding.

### 4.3.3.2 Comparison of flying gurnard mince and mixed mince with cassava starch

Table 4.12 and Figure 4.10 show the Raman spectra for flying gurnard mince and mixtures of flying gurnard mince and cassava starch flour. Mixtures of flying gurnard mince and cassava starch showed increased intensity at 760 cm\(^{-1}\), which is attributed to indole -ring vibrations of tryptophan (Trp) residues (Ogawa et al., 1999; Li Chan, 1996;Tu, 1982) from the fish mince (10:0). The band intensity of the mixtures remained nearly constant but the peak was not well resolved in the 2:8 mince: starch ratio as it broadened with increase in the starch content. Further increases in the starch content did not affect the peak intensity.
Figure 4.10 Raman spectra (A) 1700-650 cm\(^{-1}\) and (B) 3500-2700 cm\(^{-1}\) for flying gurnard mince and cassava starch in the ratio (a) 10:0 (b) 8:2 (c) 5:5 (d) 2:8 after subtracting the spectra of the respective proportion of cassava starch from each mixture.
### Table 4.12 Relative peak intensity of Raman spectra in the regions 3200-700 cm⁻¹ for flying gurnard mince and varying addition of cassava starch

<table>
<thead>
<tr>
<th>Peak assignment (wavenumber ± 2 cm⁻¹)</th>
<th>10% Flying gurnard mince</th>
<th>8% Flying gurnard mince + 2% cassava starch</th>
<th>5% Flying gurnard mince + 8% cassava starch</th>
<th>2% Flying gurnard mince + 8% cassava starch</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trp (760)</td>
<td>0.38 ± 0.08</td>
<td>0.67 ± 0.18</td>
<td>0.57 ± 0.17</td>
<td>0.60 ± 0.14</td>
</tr>
<tr>
<td>Tyr (830, 855)</td>
<td>0.34 ± 0.11 / 0.40 ± 0.08</td>
<td>0.83 ± 0.70 / 1.65 ± 0.52</td>
<td>0.80 ± 0.60 / 2.19 ± 0.27</td>
<td>0.4 ± 0.14 / 1.9 ± 0.67</td>
</tr>
<tr>
<td>Helix C-C stretch, CH₃ symmetric stretch (937)</td>
<td>0.26 ± 0.04</td>
<td>1.43 ± 0.54</td>
<td>2.36 ± 0.36</td>
<td>2.48 ± 1.5</td>
</tr>
<tr>
<td>β-sheet type structure 990</td>
<td>0.09 ± 0.01</td>
<td>0.12 ± 0.01</td>
<td>0.18 ± 0.08</td>
<td>0.46 ± 0.10</td>
</tr>
<tr>
<td>Phe, ring band (1004)</td>
<td>1 ± 0.06</td>
<td>1 ± 0.03</td>
<td>1 ± 0.01</td>
<td>1 ± 0.02</td>
</tr>
<tr>
<td>Isopropyl anti symmetric stretch CH stretch backbone (1128)</td>
<td>0.37 ± 0.04</td>
<td>1.19 ± 0.37</td>
<td>1.55 ± 0.43</td>
<td>2.84 ± 0.02</td>
</tr>
<tr>
<td>CH₃ anti symmetric stretch CH₃ rock (aromatic) (1160)</td>
<td>0.21 ± 0.04</td>
<td>0.57 ± 0.17</td>
<td>1.32 ± 0.24</td>
<td>2.15 ± 0.68</td>
</tr>
<tr>
<td>β-sheet type (1239)</td>
<td>0.46 ± 0.05</td>
<td>0.76 ± 0.23</td>
<td>0.27 ± 0.06</td>
<td>0.66 ± 0.1</td>
</tr>
<tr>
<td>Amide III (1264)</td>
<td>0.68 ± 0.10</td>
<td>1.42 ± 0.57</td>
<td>1.34 ± 0.39</td>
<td>1.37 ± 0.85</td>
</tr>
<tr>
<td>Amide III (1320)</td>
<td>0.57 ± 0.03</td>
<td>0.95 ± 0.07</td>
<td>0.62 ± 0.12</td>
<td>0.75 ± 0.19</td>
</tr>
<tr>
<td>H band doublet from trp (1340)</td>
<td>0.53 ± 0.10</td>
<td>1.23 ± 0.65</td>
<td>2.08 ± 0.06</td>
<td>2.64 ± 0.13</td>
</tr>
<tr>
<td>Aliphatic groups, CH bend (1451)</td>
<td>2.61 ± 1.97</td>
<td>5.3 ± 1.45</td>
<td>3.7 ± 0.17</td>
<td>2.80 ± 0.94</td>
</tr>
<tr>
<td>Trp (1554)</td>
<td>0.16 ± 0.05</td>
<td>0.29 ± 0.02</td>
<td>0.16 ± 0.03</td>
<td>0.65 ± 0.32</td>
</tr>
<tr>
<td>Amide I (1660)</td>
<td>2.43 ± 0.16</td>
<td>3.33 ± 0.96</td>
<td>1.88 ± 0.09</td>
<td>0.88 ± 0.57</td>
</tr>
<tr>
<td>CH stretch, aliphatic (2937)</td>
<td>10.56 ± 0.39</td>
<td>14.17 ± 2.51</td>
<td>16.25 ± 1.61</td>
<td>13.06 ± 1.85</td>
</tr>
<tr>
<td>Shoulder (2875)</td>
<td>6.64 ± 0.31</td>
<td>6.95 ± 2.20</td>
<td>5.64 ± 1.39</td>
<td>4.22 ± 1.92</td>
</tr>
<tr>
<td>Shoulder (2976)</td>
<td>6.21 ± 0.24</td>
<td>4.66 ± 1.43</td>
<td>9.19 ± 0.87</td>
<td>8.8 ± 2.39</td>
</tr>
<tr>
<td>CH stretch, aromatic (3063)</td>
<td>0.50 ± 0.06</td>
<td>0.35 ± 0.10</td>
<td>0.42 ± 0.25</td>
<td>0.68 ± 0.43</td>
</tr>
<tr>
<td>NH stretch (backbone) (3200)</td>
<td>0.21 ± 0.07</td>
<td>0.19 ± 0.12</td>
<td>0.33 ± 0.09</td>
<td>0.75 ± 0.33</td>
</tr>
</tbody>
</table>

1-Figures in parenthesis next to peak intensity values refer to shift wavenumbers (± 2 cm⁻¹). The spectra relative intensity values were averaged from 64 scans, baseline corrected, smoothed and normalized to the intensity of the phenylalanine band at 1004 cm⁻¹.

The band being sensitive to the environment polarity of Trp residues, increased in intensity on heat-induced gelation as a result of intermolecular hydrophobic interactions and...
disulfide exchange reactions. However, without further increase in the peak intensity, it may be suggested that no more residues were buried in the heat-induced gel (Ogawa et al., 1999; Li Chan, 1996; Tu, 1982). Thus partial or limited intermolecular hydrophobic interactions and disulfide exchange reactions may have occurred.

The doublet bands (1340 and 1360 cm\(^{-1}\)) also attributed to Trp residues revealed only a single peak at 1340 cm\(^{-1}\), which however increased in intensity, as the ratio of the cassava starch increased in the samples (Figure 4.10). This peak could be the one observed by Ikeda and Li Chan (2003) centered around 1344 cm\(^{-1}\) which was associated with heat induced gels leading to the formation of particulate gels (Miura et al., 1988). Frushour and Koenig (1975) reported similar observations in β-lactoglobulin and attributed that to CH deformation bands of aliphatic and aromatic side chains.

The intensity ratio \(I_{855}/I_{830}\) of the tyrosine doublet near 855 cm\(^{-1}\) and 830 cm\(^{-1}\) of the mixtures decreased (0.85 - 0.2) as the starch content increased. This relates to hydrogen bonding between the flying gurnard proteins to a negative acceptor of the molecules in the cassava starch (Van Dael et al., 1987). This decrease in the intensity ratio suggested the role of the phenolic hydroxyl groups as strong hydrogen bond donors and state of the buried tyrosine residues within the gel network (Nonaka, et al., 1993; Frushour and Koenig, 1975). The vibrational peak around 937-940 cm\(^{-1}\) increased gradually with increasing starch content which is ascribed to a skeletal C-C stretching and is proportional to the α-helix content (Frushour and Koenig, 1975; Howell and Li-Chan, 1996). Though Ikeda and Li Chan (2003) attributed this increase to the presence of a high protein concentration, this may not hold for the starch. However, the starch may offer protection to the protein in heat-induced gelation thus increasing the peak intensity.
Perhaps contributions from non-aggregated and hence renatured molecules after cooling (Ikeda and Li Chan, 2003) may also have been involved.

The band (1200-1300 cm\(^{-1}\)) assigned to an amide III mode region increased with increased cassava starch but the intensity of the peaks were not affected significantly with an increase in starch content within the mixtures. The \(\beta\)-sheets and disordered structures between 1230-1240 and 1245-1270 cm\(^{-1}\) respectively were observed for 8:2 (1239 cm\(^{-1}\)), 5:5 (1207) cm\(^{-1}\) and were absent in 2:8 mince:starch ratios of the mixture. The wavenumber shift and subsequent loss of vibrational peaks with increase in starch may suggest that \(\beta\)-sheet structures may not have remained in the heat induced gels (Belloque and Smith, 1998). Frushour and Koenig (1975) observed a decrease in this peak in alkaline denaturation of \(\beta\)-lactoglobulin.

The amide band I (1665-1672 cm\(^{-1}\)) was observed to decrease in intensity as the starch increased with no shifts in wavenumber (1660 cm\(^{-1}\)). The maintenance of the peak positions indicate that induced gelation of the mixtures may not involve hydrogen bonding or backbone conformation (Carew et al., 1983; Howell and Li-Chan, 1996; Tu, 1986).

The band in the CH stretching band at 2937 cm\(^{-1}\) did not shift significantly within the samples. The results of the spectra as analysed above suggested that some form of interactions between the proteins of the flying gurnard mince and cowpea notwithstanding the unrefined nature of the proteins involved in the mixtures. These interactions may be brought about by partial intermolecular hydrophobic interactions and disulfide exchange.
Chapter 4 Rheological, DSC, FT-Raman spectroscopy and phase microscopy studies of fish mince, cowpea flour and cassava starch mixtures

reactions with little or no hydrogen bonding. Gels formed may have been of particulate nature.

4.3.3.3 Comparison of burrito fish minces and mixed mince with cowpea flour

The spectra for the burrito mince and mixtures of the mince and cowpea flour are shown in Figure 4.11 and detailed in Table 4.13. The indole -ring vibrations of tryptophan (Trp) residues peak at 760 cm\(^{-1}\) (Ogawa et al., 1999; Li Chan, 1996; Tu, 1982) band intensity did not show any remarkable change. This indicates that intermolecular hydrophobic interactions and disulfide exchange reactions may not be intense and the residues either buried or exposed would be partially involved in the heat induced gel (Ogawa et al., 1999; Li Chan, 1996; Tu, 1982). However, increased intensity of the doublet bands at (Trp) 1340 and 1360 cm\(^{-1}\) (Miura et al., 1988), as the ratio of the cowpea flour was increased in the samples, was observed as a single peak (1340 cm\(^{-1}\)). The intensity ratio \(I_{855}/I_{830}\) of the tyrosine doublet (855 cm\(^{-1}\) and 830 cm\(^{-1}\)) decreased steadily (1.16-0.36) as the cowpea content was increased. This shows that hydrogen bonding of the phenolic OH group (Tu, 1986) could be a strong hydrogen bond donor or indicates well-buried tyrosine residues within the gel network (Nonaka, et al., 1993; Frushour and Koenig, 1975).

The increasing vibrational skeletal C-C stretching peak (937-940 cm\(^{-1}\)) in the samples as the cowpea flour increased could be related to the high protein concentration contributed by the cowpea flour (Frushour and Koenig, 1975; Howell and Li-Chan, 1996; Ikeda and Li Chan, 2003).
Figure 4.11 Raman spectra (A) 1700-650 cm\(^{-1}\) and (B) 3500-2700 cm\(^{-1}\) for burrito fish mince and cowpea flour in the ratio (a) 10:0 (b) 8:2 (c) 5:5 (d) 2:8 after subtracting the spectra of the respective proportion of cowpea flour from each mixture.
Table 4.13 Relative peak intensity of Raman spectra in the regions 3200-700 cm\(^{-1}\) for burrito mince and varying addition of cowpea flour

<table>
<thead>
<tr>
<th>Peak assignment (wavenumber ± 2 cm(^{-1}))</th>
<th>Relative peak intensity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10% Burrito mince</td>
</tr>
<tr>
<td>Trp (760)</td>
<td>0.40 ± 0.11</td>
</tr>
<tr>
<td>Tyr (830, 855)</td>
<td>0.36 ± 0.15 / 0.31</td>
</tr>
<tr>
<td>Helix C-C stretch, CH(_3)</td>
<td>0.57 ± 0.05</td>
</tr>
<tr>
<td>structure 990</td>
<td>0.33 ± 0.21</td>
</tr>
<tr>
<td>Phe, ring band (1004)</td>
<td>1 ± 0.05</td>
</tr>
<tr>
<td>Amide III (1264)</td>
<td>0.38 ± 0.05</td>
</tr>
<tr>
<td>CH stretch back bone (1128)</td>
<td></td>
</tr>
<tr>
<td>CH(_3) anti symmetric (aliphatic)</td>
<td>0.21 ± 0.03</td>
</tr>
<tr>
<td>CH(_3) rock (aromatic) (1160)</td>
<td>0.59 ± 0.06</td>
</tr>
<tr>
<td>Amide III (1320)</td>
<td>0.56 ± 0.16</td>
</tr>
<tr>
<td>H band doublet from trp (1340)</td>
<td>0.68 ± 0.48</td>
</tr>
<tr>
<td>CH(_3) groups, CH bond (1451)</td>
<td>2.31 ± 0.39</td>
</tr>
<tr>
<td>Trp (1554)</td>
<td>0.17 ± 0.05</td>
</tr>
<tr>
<td>Amide I (1660)</td>
<td>1.83 ± 0.23</td>
</tr>
<tr>
<td>CH stretch, aliphatic (2937)</td>
<td>5.24 ± 1.85</td>
</tr>
<tr>
<td>Shoulder (2875)</td>
<td>1.68 ± 0.03</td>
</tr>
<tr>
<td>Shoulder (2976)</td>
<td>4.06 ± 0.34</td>
</tr>
<tr>
<td>CH stretch, aromatic (3063)</td>
<td>0.59 ± 0.12</td>
</tr>
<tr>
<td>NH stretch (backbone) (3200)</td>
<td>0.30 ± 0.03</td>
</tr>
</tbody>
</table>

1-Figures in parenthesis next to peak intensity values refer to shift wavenumbers (± 2 cm\(^{-1}\)). The spectra relative intensity values were averaged from 64 scans, baseline corrected, smoothed and normalized to the intensity of the phenylalanine band at 1004 cm\(^{-1}\).
The intensity of the amide III (1200-1300 cm\(^{-1}\)) mode region though varied was not significantly different with increasing cowpea flour in the samples. The \(\beta\)-sheets and disordered structures between 1230-1240 and 1245-1270 cm\(^{-1}\) respectively were observed at 1241 and 1245-1264 cm\(^{-1}\) for the samples with a stable intensity that changed with the 2:8 fish mince to cowpea flour ratio. The Amide III position within the samples was unchanged. This indicates that a significant amount of \(\beta\)-sheet structures remained in the gels (Belloque and Smith, 1998; Ikeda and Li-Chan, 2003).

The amide band I (1665-1672 cm\(^{-1}\)) (Howell and Li-Chan, 1996; Tu, 1986) was observed to decrease moderately in intensity as the cowpea flour increased with significant shifts from 1660 to 1669 cm\(^{-1}\) (Howell and Li-Chan, 1996; Tu, 1986), indicating a peak shift of \(\alpha\)-helix from 1660 to 1668 cm\(^{-1}\) (\(\beta\)-sheet and disordered structures bands) as a result of heat-induced gelation of the mixtures (Carew et al., 1983).

The CH stretching band at 2937 cm\(^{-1}\) (Li-Chan, 1996) did not shift significantly within the samples though well resolved peaks of the shoulders at 2876 and 2969 cm\(^{-1}\) as the cowpea flour content increased could not be identified. The peak intensity and position at the CH stretching region 3063 cm\(^{-1}\) (aromatic groups) remained largely unaffected with increasing cowpea flour within the samples (Li-Chan, 1996). The results of the spectra as analysed above suggested some interactions between the proteins of the flying gurnard mince and cowpea notwithstanding the unrefined nature of the proteins involved in the mixtures. These interactions could have been brought about by partial intermolecular hydrophobic interactions and disulfide exchange reactions as well as strong hydrogen bonding.
4.3.3.4 Comparison of burrito mince and mixed mince with cassava starch

Raman spectra for burrito fish mince and mixtures of burrito fish mince and cassava starch flour is shown in Figure 4.12 and a tabular representation of the spectra is on Table 4.14. The spectra revealed that mixtures of burrito fish mince and cassava starch showed increased intensity at 760 cm\(^{-1}\) (Trp residues) (Ogawa et al., 1999; Li Chan, 1996; Tu, 1982) indicating intermolecular hydrophobic interactions and disulfide exchange reactions (Ogawa et al., 1999; Li Chan, 1996; Tu, 1982). The Trp residues doublet bands at 1340 and 1360 cm\(^{-1}\) (Ogawa et al., 1999; Li Chan, 1996) registered a single peak at 1340 cm\(^{-1}\), which maintained significant increases in intensity, as the ratio of the cassava starch increased in the samples (Figure 4.12). Ikeda and Li Chan (2003) suggested that such a peak centered around 1344 cm\(^{-1}\) was associated with heat induced gels may indicate the formation of particulate gels (Miura et al., 1988; Frushour and Koenig 1975). There was a significant loss in the intensity ratio \(I_{855}/I_{830}\) of the tyrosine doublet near 855 cm\(^{-1}\) and 830 cm\(^{-1}\) of the mixtures, which decreased (1.16 - 0.2) as the starch content increased. However, this intensity remained unchanged with further increment of the cassava starch. Though phenolic hydroxyl could be in a hydrogen bonding as a strong hydrogen bond donor (Van Dael et al., 1987), the bond might be partial since further increment in the starch content did not affect the peak intensity. This may affect the number of buried tyrosine residues within the gel network (Nonaka et al., 1993; Frushour and Koenig, 1975).

The peak around 937-940 cm\(^{-1}\) (Frushour and Koenig, 1975; Howell and Li-Chan, 1996) increased with well resolved peaks as the starch content was increased. Contributions from non-aggregated and hence renatured molecules after cooling (Ikeda and Li Chan, 2003) may have also been involved in the formation of the peak.
Figure 4.12 Raman spectra (A) 1700-650 cm\(^{-1}\) and (B) 3500-2700 cm\(^{-1}\) for burrito fish mince and cassava starch in the ratio (a) 10:0 (b) 8:2 (c) 5:5 (d) 2:8 after subtracting the spectra of the respective proportion of cassava starch from each mixture.
### Table 4.14: Relative peak intensity of Raman spectra in the regions 3200-700 cm\(^{-1}\) for burrito mince and varying addition of cassava starch\(^1\)

<table>
<thead>
<tr>
<th>Peak assignment (wavenumber ± 2 cm(^{-1}))</th>
<th>Relative peak intensity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10% Burrito mince</td>
</tr>
<tr>
<td><strong>Trp (760)</strong></td>
<td>0.40 ± 0.11</td>
</tr>
<tr>
<td><strong>Tyr (830, 855)</strong></td>
<td>0.36 ± 0.15/0.31</td>
</tr>
<tr>
<td></td>
<td>0.18</td>
</tr>
<tr>
<td><strong>Helix C-C stretch, CH(_3) symmetric</strong></td>
<td>0.57 ± 0.03</td>
</tr>
<tr>
<td><strong>(\beta)-sheet type</strong></td>
<td>0.33 ± 0.21</td>
</tr>
<tr>
<td><strong>Structure 990</strong></td>
<td>1 ± 0.05</td>
</tr>
<tr>
<td><strong>Phe, ring band (1004)</strong></td>
<td>0.38 ± 0.05</td>
</tr>
<tr>
<td><strong>IISopropyl anti symmetric stretch</strong></td>
<td></td>
</tr>
<tr>
<td><strong>CH(_3) stretch backbone (1128)</strong></td>
<td>0.21 ± 0.03</td>
</tr>
<tr>
<td><strong>CH(_3) anti symmetric</strong></td>
<td></td>
</tr>
<tr>
<td><strong>(aliphatic) CH(_3) rock (1156)</strong></td>
<td>0.73 ± 0.38</td>
</tr>
<tr>
<td><strong>(aromatic)</strong></td>
<td></td>
</tr>
<tr>
<td><strong>H(_3)N stretch (1160)</strong></td>
<td></td>
</tr>
<tr>
<td><strong>(\beta)-sheet type (1239)</strong></td>
<td>0.59 ± 0.06</td>
</tr>
<tr>
<td><strong>Amide III (1264)</strong></td>
<td>0.56 ± 0.16</td>
</tr>
<tr>
<td><strong>Amide III (1320)</strong></td>
<td>0.67 ± 0.19</td>
</tr>
<tr>
<td><strong>H band doublet from trp (1340)</strong></td>
<td>0.68 ± 0.48</td>
</tr>
<tr>
<td><strong>Amide groups, CH rock (1451)</strong></td>
<td>2.31 ± 0.03</td>
</tr>
<tr>
<td><strong>Trp (1554)</strong></td>
<td>0.17 ± 0.05</td>
</tr>
<tr>
<td><strong>Amide I (1600)</strong></td>
<td>1.83 ± 0.23</td>
</tr>
<tr>
<td><strong>CH(_3) stretch, aliphatic</strong></td>
<td>5.24 ± 1.85</td>
</tr>
<tr>
<td><strong>Shoulder (2888)</strong></td>
<td>1.35 ± 0.03</td>
</tr>
<tr>
<td><strong>Shoulder (2976)</strong></td>
<td>4.06 ± 0.34</td>
</tr>
<tr>
<td><strong>CH(_3) stretch, aromatic</strong></td>
<td>0.59 ± 0.12</td>
</tr>
<tr>
<td><strong>NH stretch (backbone)</strong></td>
<td>0.30 ± 0.03</td>
</tr>
</tbody>
</table>

\(^1\)Figures in parenthesis next to peak intensity values refer to shift wavenumbers (± 2 cm\(^{-1}\)). The spectra relative intensity values were averaged from 64 scans, baseline corrected, smoothed and normalized to the intensity of the phenylalanine band at 1004 cm\(^{-1}\).
The amide III mode region (1200-1300 cm\(^{-1}\)) increased with increased cassava starch. The \(\beta\)-sheets and disordered structures between 1230-1240 and 1245-1270 cm\(^{-1}\) respectively were observed for 8:2 (1241 cm\(^{-1}\)), 5:5 (1236 cm\(^{-1}\) and 1231 cm\(^{-1}\) in 2:8 mince: starch ratios of the mixture. The wavenumber shift and subsequent increase in peak intensity with increase in starch suggest that \(\beta\)-sheet structures remained in the heat-induced gels (Belloque and Smith, 1998; Frushour and Koenig, 1975).

The amide band I (1665-1672 cm\(^{-1}\)) was observed to decrease in a not well-defined pattern in intensity as the starch increased with one shift in wavenumber (1665 cm\(^{-1}\)). The heat-induced gelation of the mixtures may not involve hydrogen bonding (Carew et al., 1983; Howell and Li-Chan, 1996; Tu, 1986).

The band in the CH stretching region at 2937 cm\(^{-1}\) did not shift significantly within the samples. The results of the spectra as analysed above suggested some form of interactions between the proteins of the burrito fish mince and cowpea notwithstanding the unrefined nature of the proteins involved in the mixtures. These interactions could have been brought about by partial intermolecular hydrophobic interactions and disulfide exchange reactions with little or no hydrogen bonding. Gels formed may have been of particulate nature with no or few amounts of \(\beta\)-sheet structures in the heat induced gels.
4.3.4 Phase contrast microscopy studies

4.3.4.1 Effect of adding cowpea flour and cassava starch to flying fish mince

The phase contrast micrographs of flying fish mince and cowpea flour are shown in Figure 4.13 (a-c).

Figure 4.13 (a-c) Phase contrast micrographs of mixtures of flying gurnard fish mince and cowpea flour (a) 8% flying gurnard mince and 2% cowpea flour, (b) 5% flying gurnard mince and 5% cowpea flour (c) 2% fish gurnard and 8% cowpea flour.

From the flying gurnard fish mince rich mixture (a- 8% flying gurnard mince and 2% cowpea flour) through the central point (b) with a balanced composition (5% flying gurnard mince and 5% cowpea flour) to the cowpea flour rich mixture (c) of 2% fish gurnard and 8% cowpea flour, the transformations that appear across the mixtures as the cowpea flour content increased was that of gradual dominance in phase colour (brown network of the mince and the light blue colour of the cowpea flour) depending on which particular component was higher in the composition of the mixture. However the matrix was observed to be continuous with little or no agglomerates of either of the components as they appeared to be evenly distributed in the medium (b). This could suggest a possible form of a positive synergistic interaction and the continuous (smooth) nature of the matrix could favour a compatible and stable network (Nakamura et al., 1986; Puppo et al., 1995). These deductions appear to be consistent with the results of the observed rheological and other studies carried out in this report on the behaviours of flying fish mince and cowpea flour.
Figure 4.14 shows the phase contrast micrographs of samples obtained after the mixing of flying gurnard fish mince and cassava flour.

![Phase contrast micrographs of mixtures of flying gurnard fish mince and cassava starch.](image)

**Figure 4.14 Phase contrast micrographs of mixtures of flying gurnard fish mince and cassava starch (a) 8% flying gurnard mince and 2% cassava starch, (b) 5% flying gurnard mince and 5% cassava starch (c) 2% flying gurnard fish mince and 8% cassava starch.**

The phase transformations within the progression of increasing starch content show (a) a large cluster of brown fish mince aggregates in the mince rich mixture (8% flying gurnard fish mince and 2% cassava starch), which changed to a strand-like phase dispersed within a two equilibrium phases of (b) 5% flying fish mince and 5% cassava starch. Increasing starch content (c - 2% fish mince and 8% cassava starch equal component) reduced the size of the few clusters formed within the matrix of predominately continuous network of gelatinised starch. These phase transitions may be attributed to the induced weak electrostatic interaction between the biopolymers (More and Cater 1974; Takeuchi, 1969; Remsen and Clark, 1978). Though phase-separated systems were recognised in the micrographs, the dispersion of the fish mince strands within the starch may be described as an interaction by co-operative binding in a synergistic gelation (Williams et al., 1991) and the matrix may not be stable. This assertion and interpretations confirmed the observations made in this section by the
preceding studies to elucidate the interactions of the flying gurnard mince with starch in this report.

4.3.4.2. Effect of adding cowpea flour and cassava starch to burrito fish mince

The phase contrast micrographs of samples obtained after the mixing of the burrito fish mince and cowpea flour are shown in Figure 4.15.

![Figure 4.15 Phase contrast micrographs of mixtures of burrito fish mince and cowpea flour](image)

(a) 8% burrito fish mince and 2% cowpea flour, (b) 5% burrito fish mince and 5% cowpea flour, (c) 2% burrito fish mince and 8% cowpea flour

The medium (a) with the rich phase of the mince (8% burrito fish mince and 2% cowpea flour) shows scattered small agglomerates (dark bluish spots) of the burrito mince which more or less were diffused in an equilibrium mixture (b) of 5% burrito fish mince and 5% cowpea flour. The cowpea flour agglomerates appearing like white spheres were not visibly altered and tended to form spherical pores which were more visible in the cowpea rich mixture (2% burrito fish mince and 8% cowpea flour). This behaviour of the agglomerates of each component may constitute a non interactive effect in the mixture and hence could favour self-association and incompatibility of the phases (Samant et al., 1993; Tolstoguzov, 1991). Perhaps the tendency for this nature of interaction may be due to differences in molecular weight and structure (Comfort and Howell, 2002). This could also be coupled with the fact that cowpea flour protein has poor gelling properties (Kamata et al., 1991; Damodaran, 1988). The observations from the micrographs appear to be consistent with the results of the rheological, DSC and
FT-Raman spectroscopic studies, indicating the incompatibility of the phases of these two macromolecules of the burrito fish species and the cowpea flour.

Figure 4.16 depicts the transitional phases of mixing of the burrito fish mince and cassava flour under the phase contrast microscope.

Figure 4.16 Phase contrast micrographs of mixtures of burrito fish mince and cowpea flour (a) 8% burrito fish mince and 2% cassava starch, (b) 5% burrito fish mince and 5% cassava starch (c) 2% burrito fish mince and 8% cassava starch

The burrito minces (dark patches) in the mince rich medium (a) of 8% burrito fish mince and 2% cassava starch appeared to exhibit similar characteristics with its interaction with cowpea flour. Thus phase-separated systems were recognised in the micrographs of the subsequent systems namely: (b) 5% burrito fish mince and 5% cassava starch (c) 2% burrito fish mince and 8% cassava starch. It could be inferred that the phase systems may likely constitute a non-interactive effect of components culminating in self-association and incompatibility of the phases (Samant et al., 1993; Tolstoguzov, 1991).

4.4 Conclusion

Compatible and stable gel formation between minces of flying gurnard and cowpea flour was observed through positive synergistic interaction. Rheological studies indicated that from all the combinations tested, flying gurnard fish mince and cowpea flour
mixed in the ratio of 5:5 (w/w) formed the strongest gel. The transition temperatures for protein unfolding and denaturation as monitored by DSC, also supported the transitions observed in small deformation rheology, which led to increases in $G'$ and $G''$ values. Phase contrast microscopy indicated that the gels produced were phase separated. This gel network was stabilised by hydrophobic, disulfide bonds and hydrogen bonds as shown by FT-Raman spectroscopy, which further elucidated the role of buried and exposed residues and the state of the hydrogen bonding of the phenolic OH groups of the globulins within the gel network.

On the contrary a lower mince to cowpea flour ratio (2:8 w/w) of burrito mince could form the best gel albeit described as weak, among the various mince and cowpea mixtures.

Interactions of the fish minces with cassava starch through fairly weak electrostatic interaction was observed between the two macromolecules contained in the mixtures. This form of interaction was described as weak and the gels formed may not be stable. The molecular forces in such complex formation were in part due to charges on the protein as they denature and undergo much cross-linking through a combination of disulphide and several non-covalent bonds to form a network and a matrix of amylose and amylopectin from the heat disruption of the starch granule.
CHAPTER FIVE
Chapter 5 Processing of flying gurnard fish (*Dactylopterus volitans. L*) into infant weaning foods

CHAPTER 5 PROCESSING OF FLYING GURNARD FISH (*Dactylopterus volitans. L*) INTO INFANT WEANING FOODS

5.1 Introduction

Infant foods, commonly known as weaning foods, are semi-solid or solid foods that are introduced to infants to make a transition from breast milk to an adult diet especially when breast milk or formula alone can no longer meet the baby’s growing nutritional requirements. Their nutritional content is important to the growth and development of children, particularly in developing countries such as Ghana. Infant foods in Ghana generally tend to be based on cereals, which comprise mostly starch (Clark and Laing, 1991), hence these foods tend to be bulky but low in nutrient density (Mosha and Svanberg, 1990). In this study attempts were made to include the flying gurnard in the formulation of infant weaning foods with other food components to increase the protein content as well as other nutrients which may be needed during weaning of infants.

5.2 Materials and Methods

5.2.1 Preparation of weaning food

Five recipes were formulated with the aid of “Microdiet” dietary analysis software package (Downlee Systems Ltd, 2000 UK) as outlined in Table 5.1. The formulations were done with reference to the DHSS 1979 RDA for infants between 6-9 months, to give a total components weight of 100 g and an overall nutrient energy of 20% protein, 40% fat and 40% carbohydrate for each recipe. Food ingredients used included the following flying gurnard mince; cowpea flour, cassava flour, margarine (hard vegetable oil only); milk (dried skimmed) and among others whole raw eggs. The recipes were cooked in a microwave oven at 80 °C for 1.5 min to ensure that the food was pasteurised with
minimum denaturation of the proteins. The samples were freeze dried for storage or reconstitution for further work.

Table 5.1 Composition of ingredient /recipe (g/100 g) for infants between 6-9 months with reference to DHSS 1979 RDA (wet weight basis)

<table>
<thead>
<tr>
<th>Component</th>
<th>Recipe 1</th>
<th>Recipe 2</th>
<th>Recipe 3</th>
<th>Recipe 4</th>
<th>Recipe 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flying gurnard</td>
<td>9</td>
<td>7</td>
<td>7</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>Cowpea flour</td>
<td>8</td>
<td>5</td>
<td>5</td>
<td>6</td>
<td>5</td>
</tr>
<tr>
<td>Cassava flour</td>
<td>4</td>
<td>4</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Water</td>
<td>70</td>
<td>76</td>
<td>73</td>
<td>70</td>
<td>72</td>
</tr>
<tr>
<td>Margarine (hard vegetable oil only)</td>
<td>5</td>
<td>-</td>
<td>3</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Milk (dried skimmed)</td>
<td>4</td>
<td>-</td>
<td>-</td>
<td>4</td>
<td>6</td>
</tr>
<tr>
<td>Cod liver oil</td>
<td>-</td>
<td>2</td>
<td>-</td>
<td>4</td>
<td>-</td>
</tr>
<tr>
<td>Onions raw</td>
<td>-</td>
<td>1</td>
<td>1</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Eggs whole raw</td>
<td>5</td>
<td>6</td>
<td>3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Corn oil</td>
<td>-</td>
<td>--</td>
<td>-</td>
<td>-</td>
<td>4</td>
</tr>
</tbody>
</table>

5.2.2 Proximate and chemical analysis

Protein, moisture, fat and ash phosphorus, calcium and iron were determined by the methods of AOAC (1990), outlined in Chapter 2.

5.2.3 Amino acid analysis

Formulated infant food was hydrolysed to yield amino acids which were measured in triplicate by HPLC according to Bidlingmeyer et al., (1987) and Badii and Howell (2002). Samples were hydrolysed with 6 N HCL (20 mL) at 110 °C for 24 h. The
hydrolysed samples and amino acid standards (20 µL) were then treated as outlined below. All samples were derivatized with phenylisothiocyanate (PITC) according to the Waters Pico-Tag method.

Deivatization of Amino Acids with PITC. Drying solution (20 µL), containing methanol:water:TEA (2:2:1) was added to each vacuum dried standard and sample in tubes and vacuum-dried again. The derivatization reagent was freshly made by mixing 50 µL PITC (kept at -20 °C, under nitrogen, to prevent degradation), 350 µL methanol (HPLC grade), 50 µL TEA, and 50 µL Milli-Q water. This PITC reagent (20 µL) was added to each tube, vortex mixed, sealed, and left at room temperature for 20 min. The reagent was then evaporated under vacuum. The derivatized samples were vacuum-dried and dissolved in 100 µL of sample buffer (eluent A, prepared by dissolving 19.0 g of sodium acetate trihydrate in 1 L Milli-Q water, followed by addition of 0.5 mL TEA, adjusted to pH 6.4 and filtered. To 940 mL of this solution was added 60 mL acetonitrile). The samples were analysed in triplicate by reverse-phase HPLC together with the amino acids standards according to the Waters Pico-Tag Amino Acid Analysis Manual, Waters Chromatography Division, 1986.

5.2.4 Rheological studies

Rheological studies were carried on the samples before they were freeze dried as outlined under small deformation oscillatory measurements with a Rheometrics controlled stress rheometer (section 2). The viscoelastic properties of the gels measured included the storage modulus (G') and loss modulus (G'') as a function of time within the linear viscoelastic range of the gels (Mitchell 1980) as outlined in Chapter 2.
5.2.5 Shelf studies

A six-month storage period of recipe 4 of the dried recipes was carried out at the FRI at normal temperature (27 ± 2 °C) and R.H. 75%. Samples were taken at monthly intervals. Approximately, 80 g of the product was placed in the packaging pouches and sealed under the following conditions.

1. Vacuum packaged in aluminium foil pouch (24 cm x 14 cm) with a gauge of 36.93 mil.
2. Vacuum packaged in polyethylene pouch (24 cm x 14 cm) with a gauge of 49.25 mil.
3. Non-vacuum packaged in polyethylene pouch (24 cm x 14 cm) with a gauge of 49.25 mil.

5.2.6 Chemical analyses

Changes in chemical properties of the products during storage were monitored by measuring the free fatty acid (FFA) as percentage oleic acid (i.e. the cm$^3$ 0.25M NaOH used in the titration corresponds to % oleic acid) (AOAC 1990). TBARS were measured as follows (Saeed and Howell 1999): A standard curve was prepared from tetraethoxypropane (TEP) solution to give dilutions containing the equivalent of 0, 0.2, 0.4, 0.6, 0.8, 1.0 μg/ml malonaldehyde. Samples (1 g) were homogenised with 5 ml of distilled water. The standard solutions and samples were treated with 100 μL of BHT, 500 μL HCL and 500 μL of thiobarbituric acid. The mixture was vortexed thoroughly and placed in a water bath at 80 °C for 30 minutes. The mixture was allowed to cool for 20 minutes, then 2 ml butanol was added and vortexed well to extract the pink chromogen. The extract was evaporated to dryness using nitrogen at 37 °C. The residue was resuspended with 1 ml of 50% water and 50% acetonitrile. An aliquot (100 μL) was injected into the HPLC (Spectra System AS3000, from Spectra-Physics,
Chapter 5 Processing of flying gurnard fish (Dactylopterus volitans, L) into infant weaning foods

Hemel Hempstead, UK) for analysis. A Kromasil KR100 5C18 column, Hichrom Ltd, Reading, Berks, UK) was used and the sample eluted with an isocratic solvent (50% TEA, 0.1% acetonitrile and 50% water).

5.3 Results and discussions

Table 5.2 shows the proximate and chemical results of the recipes. The results indicate that the recipes formulated by the Microdiet programme contained 21-25% protein, 2-4% ash and 4.4-17% fat. These results with reference to the protein content compared favorably to the expected values from DHSS 1979 RDA for infants between 6-9 months. However, the fat content (4.4%) of recipe 1 and 3 were significantly below the values of the other recipes and indeed did not meet the recommendation as regards to the DHSS 1979 RDA. However, Brown et al. (1998) have concluded that infants age 6–8 months who are consuming an average amount of breast milk per day (674 g/day) do not need dietary supplementation of fatty acids in order to achieve 30% of the daily energy intake except where the breast milk has a low fat concentration. As this may be the case in developing country like Ghana, infants would require an additional 10-24% of energy from fat. As shown by the result of the proximate composition of the recipes, this requirement could be adequately met with the rest of the formulations (15-17%).

Table 5.2 Proximate and composition of the recipes

<table>
<thead>
<tr>
<th>Recipe</th>
<th>% Moisture</th>
<th>% Ash</th>
<th>% Protein</th>
<th>% Fat</th>
<th>Fe (mg/100 g)</th>
<th>Ca (mg/100 g)</th>
<th>P (mg/100 g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10.1±2.1</td>
<td>3.8±1.9</td>
<td>24.7±2.7</td>
<td>4.4±0.1</td>
<td>8.3±2.1</td>
<td>417±23.4</td>
<td>44±6.7</td>
</tr>
<tr>
<td>2</td>
<td>7.5±1.4</td>
<td>2.3±1.2</td>
<td>22.4±2.4</td>
<td>15.5±3.2</td>
<td>8.9±2.4</td>
<td>620±16.9</td>
<td>384±28.1</td>
</tr>
<tr>
<td>3</td>
<td>7.7±1.6</td>
<td>2.7±1.1</td>
<td>22.8±1.9</td>
<td>4.6±0.9</td>
<td>12.5±3.1</td>
<td>518±19.6</td>
<td>156±21.7</td>
</tr>
<tr>
<td>4</td>
<td>8.4±1.1</td>
<td>2.9±1.1</td>
<td>21.9±1.5</td>
<td>17.0±3.1</td>
<td>9.3±1.7</td>
<td>579±31.3</td>
<td>107±9.5</td>
</tr>
<tr>
<td>5</td>
<td>10±2.0</td>
<td>3.4±1.4</td>
<td>22.8±1.3</td>
<td>16.5±2.7</td>
<td>5.8±1.1</td>
<td>618±41.7</td>
<td>33±5.7</td>
</tr>
</tbody>
</table>

* Values are means of three determinations ± standard deviation
The mineral content of the recipes are adequate in such diets and meet the nutritional needs of infants. The calcium content (400-620 mg/100 g) compare favourably with the recommended intake of 500-600 mg/100 g (DHSS 1979 RDA; FAO/WHO 1962). Calcium is essentially important in bone formation as well as in a number of regulatory functions (Karp 1996) in infants as well as adults. As well as strengthening the skeleton, these minerals act as a reserve supply for other needs such as muscle contraction, nerve function, enzyme activity and blood-clotting (Karp, 1996). Calcium deficiency in young children results in impaired growth and rickets. Though there is no recommended intake of phosphorus, it is expected that diets containing the mineral would be of good value since phosphorus is involved in energy producing cellular reactions (Karp, 1996) in body metabolism. The iron content (5-12 mg/100 g) is well within the FAO/WHO and USA figure of 8 mg or more/day. The deficiency of such an important mineral in infant nutrition could lead to anaemia a common symptom of iron deficiency. The results show that the infant foods formulated may be important sources of micronutrients for the developing infant where breast milk becomes insufficient to meet the needs of the growing child. Iron, zinc, phosphorus, magnesium, and calcium have been identified as deficient nutrients and must be supplemented by weaning foods (Brown et al., 1998).

Table 5.3 Proximate compositions for the formulated recipes and commercial infant food

<table>
<thead>
<tr>
<th>Parameter</th>
<th>g/100 g dry weight of recipes 2-5</th>
<th>g/100 g dry commercial infant food (Cerelac™)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture</td>
<td>7.5-10.</td>
<td>2.5</td>
</tr>
<tr>
<td>Protein</td>
<td>21.9-22.8</td>
<td>15</td>
</tr>
<tr>
<td>Fat</td>
<td>4.6-17</td>
<td>9.0</td>
</tr>
<tr>
<td>Ash</td>
<td>2.3-3.4</td>
<td>2.6</td>
</tr>
</tbody>
</table>
Chapter 5 Processing of flying gurnard fish (*Dactylopterus volitans* L) into infant weaning foods

The range of the proximate compositions for the formulated recipes were comparable to that of a commercial food “Cerelac™” (Nestlé S.A. Switzerland) which is available as a suitable complimentary food for infants from 6 months, as shown in Table 5.3.

The results of the amino acid composition of recipes 2, 4 and 5 of the formulations are shown in Table 5.4. Generally there were no significant variations in most of the amino acid values across the recipes.

<table>
<thead>
<tr>
<th>Amino Acids</th>
<th>Recipe 2</th>
<th>Recipe 4</th>
<th>Recipe 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asp</td>
<td>63.4 ± 3.9</td>
<td>58.63 ± 3.1</td>
<td>55.56 ± 2.6</td>
</tr>
<tr>
<td>Glu</td>
<td>79.39 ± 4.4</td>
<td>73.07 ± 5.7</td>
<td>69.96 ± 5.1</td>
</tr>
<tr>
<td>h. pro</td>
<td>5.37 ± 1.2</td>
<td>6.73 ± 1.8</td>
<td>5.42 ± 1.7</td>
</tr>
<tr>
<td>Ser</td>
<td>4.38 ± 1.9</td>
<td>4.21 ± 1.5</td>
<td>4.15 ± 1.3</td>
</tr>
<tr>
<td>Gly</td>
<td>18.34 ± 2.7</td>
<td>17.87 ± 3.3</td>
<td>17.73 ± 3.2</td>
</tr>
<tr>
<td>His</td>
<td>22.71 ± 2.0</td>
<td>21.82 ± 2.8</td>
<td>21.78 ± 2.4</td>
</tr>
<tr>
<td>Arg</td>
<td>28.55 ± 4.2</td>
<td>27.17 ± 2.2</td>
<td>27.10 ± 1.6</td>
</tr>
<tr>
<td>Thr</td>
<td>21.90 ± 1.3</td>
<td>20.85 ± 1.8</td>
<td>19.82 ± 2.9</td>
</tr>
<tr>
<td>Ala</td>
<td>23.66 ± 2.6</td>
<td>21.34 ± 2.1</td>
<td>20.97 ± 1.5</td>
</tr>
<tr>
<td>Pro</td>
<td>16.45 ± 2.4</td>
<td>18.36 ± 2.6</td>
<td>18.49 ± 2.5</td>
</tr>
<tr>
<td>Tyr</td>
<td>15.14 ± 1.3</td>
<td>11.42 ± 2.4</td>
<td>11.03 ± 2.9</td>
</tr>
<tr>
<td>Val</td>
<td>25.81 ± 2.6</td>
<td>23.12 ± 3.6</td>
<td>22.43 ± 3.0</td>
</tr>
<tr>
<td>Met</td>
<td>14.23 ± 3.2</td>
<td>14.39 ± 2.0</td>
<td>12.97 ± 2.4</td>
</tr>
<tr>
<td>Cys</td>
<td>30.00 ± 1.4</td>
<td>26.82 ± 1.7</td>
<td>26.46 ± 2.3</td>
</tr>
<tr>
<td>Ileu</td>
<td>17.99 ± 3.2</td>
<td>16.68 ± 1.9</td>
<td>15.47 ± 3.4</td>
</tr>
<tr>
<td>Leu</td>
<td>42.62 ± 3.7</td>
<td>39.68 ± 1.7</td>
<td>37.32 ± 5.3</td>
</tr>
<tr>
<td>Phe</td>
<td>28.06 ± 3.3</td>
<td>28.10 ± 3.6</td>
<td>27.23 ± 3.8</td>
</tr>
<tr>
<td>Trp</td>
<td>21.47 ± 2.7</td>
<td>19.87 ± 3.8</td>
<td>20.08 ± 3.7</td>
</tr>
<tr>
<td>Lys</td>
<td>41.66 ± 2.6</td>
<td>39.71 ± 3.3</td>
<td>39.20 ± 2.5</td>
</tr>
</tbody>
</table>

*Values are means of three determinations ± standard deviation*
Chapter 5 Processing of flying gurnard fish (*Dactylopterus volitans. L*) into infant weaning foods

The overall profile of the amino acid content of the formulations show the presence of both essential amino acids that cannot be synthesized in the body and a supply of non-essential amino acids which provide nitrogen for protein synthesis.

The results of the viscoelasticity measurements of the recipes are shown in Figures 5.1-5.3. The result for recipe 1 was not recorded because after cooking and cooling, the product was found to have solidified and not suitable for such measurements. This was probably due to the combined effect of ingredients, with the cowpea flour playing a dominant role since it was the recipe with the highest amount of cowpea flour that was the most solid. The gelation profile of the recipes as a function of temperature followed quite distinctive patterns from each other. This may be attributed to the variations in the components as well as the quantities added.

| Table 5.5 G' and G'' (Pa) of flying gurnard mince and recipe 2-5 |
|------------------|------------------|------------------|------------------|------------------|
| Fish species/Recipe | Onset of heating | After cooling |
|                   | G'            | G''           | G'            | G''           |
| Flying gurnard     | 11600 ± 419   | 3330 ± 153    | 39400 ± 316   | 7170 ± 201    |
| Recipe 2           | 28.8 ± 3.5    | 3.84 ± 0.9    | 42.4 ± 4.9    | 6.77 ± 1.2    |
| Recipe 3           | 14.9 ± 1.9    | 1.51 ± 0.3    | 37.5 ± 3.5    | 5.75 ± 1.6    |
| Recipe 4           | 144 ± 5.9     | 41.5 ± 2.1    | 153 ± 8.2     | 32.3 ± 4.1    |
| Recipe 5           | 129 ± 8.1     | 31.5 ± 2.9    | 122 ± 11.1    | 32.6 ± 2.8    |
Chapter 5 Processing of flying gurnard fish (*Dactylopterus volitans* L) into infant weaning foods

**Fig 5.1** A dynamic temperature sweep of infant recipe 1 with the inclusion of Flying gurnard mince.

**Fig 5.2** A dynamic temperature sweep of infant recipe 3 with the inclusion of Flying gurnard mince.
Chapter 5 Processing of flying gurnard fish (*Dactylopterus volitans, L.*) into infant weaning foods

Fig 5.3 A dynamic temperature sweep of infant recipe 4 with the inclusion of Flying gurnard mince

Fig 5.4 A dynamic temperature sweep of infant recipe 5 with the inclusion of Flying gurnard mince
However G’ and G” (Table 5.3) attained for each recipe after cooling was significantly (P<0.05) lower than the values attained for the flying fish mince alone. The G’ and G” of Recipe 4 and 5 were significantly higher (P<0.05) than the respective values for Recipe 2 and 3 after the cooling.

A further inspection of curves reveals that the differences between the onset of heating and at the end of cooling for the G’ and G” for recipe 2 and 3 was quite noticeable those of recipe 4 and 5 were quite negligible. The values for the G’ and G” for recipe 4 and 5 respectively appear to be equal to those obtained at the onset of the heating to those at the end of the cooling. The G” for recipe 4 and 5 virtually followed the same trend and curve during heating and cooling respectively.

The rheological properties of the four formulations were comparable to values reported for Cerelac™ (10-25 Pa) and fall within the viscosity range of < 500 Pa after heating and cooling. The viscosity of the recipes was important as infant foods serve as a gradual transition from fluid to solid foods.

5.4 Shelf life of developed food formulations

Figure 5.5 graphically depicts the FFA values for the stored recipe 4. There were significant (P<0.05) increases of FFA from their initial values. However, the samples in the vacuum packaged aluminium foils did not show rapid increases in FFA compared to those in the polyethylene pouches, which were transparent. FFA increased from the onset of the studies attaining only a value of 2.5% on the fifth month with these products. Figure 5.6 showed the trend in the TBARS values observed over the period. However, subsequent results could not give a clear trend for the products as depicted by Figure 5.6.
Figure 5.5 Free fatty acid (%) changes in dried infant food formulation during storage (Recipe 4)

Figure 5.6 TBARS changes in infant food formulation during storage (Recipe 4)
The unclear trend of the TBARS values which measure the amount of MDA, a byproduct of lipid oxidation can be explained by the fact that the MDA can interact with other components such as amines, nucleosides and nucleic acid, proteins, amino acids, phospholipids, or other aldehydes that are end products of lipid oxidation and thus its presence for measurements may vary greatly (Auburg, 1993).

The chemical development associated with infant food formulations showed the products susceptibility to light exposure in the polyethylene pouches. Though the light brownish colour of the formulation was not visibly changed, such increases in TBARS may tend to affect the flavour of the products. The peroxidising lipids (and reducing sugars) tend to decrease the nutritional value of products by reacting with one or more of the essential amino acids present (Lubis and Buckle, 1990; Ma’ruf et al., 1990; Smith et al., 1990; Smith and Hole, 1991; Davis et al., 1993). Fish body oils are known to be very susceptible to oxidation by atmospheric oxygen and when dried, such fats have little protection against oxidation (Wolfe, 1975). However, products may develop distinctive “oxidised” flavours, but these are not necessarily unacceptable (Waterman, 1976). It has been claimed that in addition to modifying flavour, some compounds formed by peroxidising lipids are toxic (Sanders, 1987), and that lipid peroxides can destroy vitamins A and E (Olcott, 1962). This degradation of fats could lead to decrease in consumer appeal and safety for the products because the presence of free fatty acids indicates rancidity (Hwang and Regenstein 1993). The initial increases in TBARS values in the formulated infant foods are an indication that breakdown of hydroperoxides to form carbonyl compounds may have occurred (Lovern 1962; Ma’ruf et al., 1990).
Chapter 5 Processing of flying gurnard fish (*Dactylopterus volitans, L*) into infant weaning foods

5.5 Conclusion

Flying gurnard fish mince may be used to develop infant food as shown by the results in this study. The formulations as developed through the recipes 2 to 5 showed good nutritional composition, which compared favourably with commercial infant food such as cerelac. The rheological properties of the formulations appeared to fall within a viscosity range below 500 Pa, which was considered adequate for consistency. Shelf life of the products may be enhanced with the exclusion of oxygen in vacuum-packed foils. However, the addition of naturally occurring antioxidants such as vitamin C, citric acid and α-tocopherol could help stabilising the lipids in the formulations (Huang *et al.*, 1995; Badii and Howell, 2002).
CHAPTER SIX
6 FLYING GURNARD (DACTYLOPTERUS VOLITANS. L) SKIN GELATIN AND GELATION PROPERTIES

6.1 Introduction

Gelatin is a water-soluble protein that is widely used in the food, pharmaceutical and photographic industries. It is used to increase the viscosity of aqueous systems and to form aqueous gels. Gelatin has been extensively researched and continues to attract attention because of its importance in the advancement in colloid chemistry. The use of gelatin in the food industry and as in temperature-dependent gel desserts has an estimated annual global demand of 200,000 metric tonnes (Choi and Regenstein, 2000).

Gelatin is derived by hydrolytic degradation of collagen; the principal component of white fibrous connective tissue (Voet et al., 1999) found in tendons skin, bone, the vascular system the sheaths surrounding muscles. Collagen forms one-third or more of the total protein of mammals but the amount in fish is generally much less (Sikorski and Borderias, 1994). The basic molecular unit of collagen is a triple-helical rod (tropocollagen triple helix). This consists of three $\alpha$-chains arranged in a left-handed axis, with the whole structure wound into a right-handed super helix. These are arranged in bundles, which make up the connective tissue matrix. The hydrolytic degradation of the collagen is brought about through acid or alkaline hydrolysis initially. This process is irreversible and leads to the breakdown of covalent bonds and conversion of the regular structure of the collagen to that of gelatin and gelatin fragments. Subsequently, following a thermal denaturation at $40^\circ$C the triple helical structure of collagen is destroyed to form one, two or three random chain gelatin molecules (Flory and Waver, 1960). This process leads to the disruption of the hydrogen and electrostatic bonds of the triple helix structure, which pass into solution as random coils in an amorphous form known as gelatin (Veis, 1965). The functional
properties of gelatin are related to their chemical characteristics; molecular weight, number of each kind of amino acid residues, number of polypeptide chains and on the position of the breaks. The gel strength, viscosity, setting behaviour and melting point of gelatin depend on their molecular weight distribution and the amino acid composition (Johnston-Banks, 1990). The imino acids, proline and hydroxyproline are important in the denaturation of gelatin subunits during gelling (Johnston-Banks, 1990). Gelatin with high levels of imino acids tends to have higher gel strength and melting point. The molecular weight distribution is also important in determining the gelling behaviour of gelatin. In general the quality of the gelatin depends on the composition of the raw material and factors including the species, breed, age, manner of feeding the animal, storage condition of raw materials and to some extent the manufacturing processes (Hinterwaldner, 1977). In commercial terms, the value of gelatin is basically placed on gel strength and to a large extent the viscosity (Wainewright, 1977), but a number of physical and chemical tests may be carried out on gelatin and its products. Gelatin with low viscosity is associated with a short and brittle gel whilst that of a high viscosity forms a tougher and stronger gel. In commerce, high viscosity gelatin attracts a premium price. Gelatin is classified into three kinds, low bloom (150 and below), medium (150-220) and high bloom (over 220-300) (Johnston-Bank, 1990).

Gelatin is not a nutritionally complete protein since it has no tryptophan and is deficient in isoleucine, threonine and methionine (Potter and Hotchkiss, 1998). Other sulphur containing amino acids such as cysteine and cystine are deficient or absent as well. As a gel, gelatin has some unique functional properties that are similar, but not identical in a number of gels. The long molecular strands and partially stacked triple helixes in gelatin offer strength and flexibility not found in alginate, cornstarch or carrageenan.
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Chapter 6. Flying gurnard (Dactylopterus volitans L.) Skin gelatin and gelation properties

(Walstra, 1996). These vegetable based gels lack the 'melt-in-the mouth' and elastic properties of gelatin (Cole, 2000).

Various methods employed in the production of gelatin provide distinct differences in the properties of gelatin. A neutral or slightly acid pH medium after pre-treatment of the raw materials with cold alkaline could be used in the extraction process. The extraction process could also be done by briefly soaking the material in an acidic medium before the process at a moderate temperature and pH 4 (Ward and Courts, 1977). Commercially and traditionally both acid and alkaline hydrolysis routes are employed, and most commercial samples are extracted from either bovine or porcine sources. Periodic outbreaks of Bovine spongiform encephalopathy (BSE) coupled with religious prohibitions have created a demand for alternative sources of gelatin (Choi and Regenstein, 2000). Fish skins and possibly the bones are being considered alternative sources and processed into gelatin, thus creating a value-added product rather than their being discarded and creating waste disposal problems. Fish processing in general leads to an enormous amount of waste accounting for approximately 75% of the total fish weight (Shahidi, 1994) after filleting. About 30% of the waste is in the form of bones and skins (Gómez-Guillén et al., 2002).

Studies on properties of fish skin gelatins (Choi and Regenstein, 2000; Fernández-Diaz, et al., 2001; Gómez-Guillén and Montero, 2001; Gudmundsson, 2002; Gudmundsson and Hafsteinsson, 1997) showed that their properties differ from those of mammalian gelatins and vary between species. The main differences in properties as observed between the mammalian and fish gelatins are that fish gelatins have low melting gelling temperatures (Leuenberger, 1991). In addition, gelatin from low temperature fish
species contain a lower amount of proline and hydroxyproline; lower hydrogen bonds and tend to have lower melting points than the species from tropical environment (Arnesen and Gildberg, 2002). However these properties may be modified by the use of gel enhancers like salts, glycerol and variations of pH. By the addition of MgSO₄, the setting time and melting points of gelatin from megrin tilapia were increased (Sarabia et al., 2002). Viscoelastic properties were affected in varying conditions of ionic strength and pH. In addition sugars and glycerol also increased the strength of these gels (Naftalian and Symons, 1974)

The limited studies conducted on warm water fish gelatin showed that they have better functional properties than gelatin from cold-water fish species (Gilsenan and Ross-Murphy, 2000; Grossman and Bergman, 1992). This has been attributed to their higher content of imino acids. The high imino acid content in gelatins from mammalian and warm water fish are considered to be related to a lower critical concentration and higher melting point. In addition gelatin with a molecular weight of 300 KDa and above is known to have high bloom value (Arnesen and Gildberg, 2002).

During bumper harvest in the fish industry in Ghana, large quantities of fish species which are undervalued and hence underutilised are harvested. It is therefore proposed that such species could be processed into value added products to enhance its efficient utilisation. The processing of such fish species like the flying gurnard (Dactylopterus volitans)(Linnaeus, 1758) (other names Cephalocanthus volitans (local) Pampansre) can lead to an enormous amounts of waste from the skin which appears to be a thick and rough tissue. Thus the skins and possibly the bones can be processed into gelatin, to
produce a value-added product to the fish, which could be used in food applications after the muscle has been removed for product development.

The objectives of this study were to extract and characterise the gelatin from the skin of the flying gurnard and examine the effect on its properties with cassava starch.

6.2 Materials and Methods

6.2.1 Materials
Fish skin was removed from frozen and thawed flying gurnard obtained from Ghana.

6.2.2 Methods

6.2.2.1 Gelatin preparation
Gelatin was prepared from the flying gurnard skins by the method of Gudmundsson and Hafsteinsson (1997) and Badii and Howell (2005). Skin of fish was separated and cleaned, washed several times with tap water and was dried in between two pieces of Whatman filter paper. Dried clean skins were cut into 2x2 cm. A litre of sodium hydroxide solution (1.5 g NaOH in 1000 ml-distilled water) was added into 140 g of fish skins, shaken well and slowly stirred at room temperature for 40 minutes. Skins were separated and rinsed with water. This procedure was repeated three times.

Skins were then treated, in the same way, with 1000-ml sulphuric acid solution (1.5 ml H₂SO₄ in 1000 ml-distilled water) and rinsed with water. This procedure was also repeated three times. Finally the skins were treated with 1000-ml citric acid solution (7 g citric acid in 1000 ml distilled water) with subsequent rinsing that was also repeated three times. The fish skin was then extracted with distilled water in a 45 °C water bath over night (about 18 h) without stirring. The mixture was filtered through a Buchner
funnel with Whatman paper No 4. The resultant filtrate was evaporated in a rotary evaporator at 45 °C to reduce the volume to 1/10. The concentrated solution, which contained gelatin, was freeze-dried.

6.2.2.2 Proximate composition

The moisture, protein, crude fat and ash, were determined according to the methods outlined in Chapter 2. However, a conversion factor of 5.4 was used for calculating the protein content from the Kjeldahl nitrogen content since collagen, the main protein in skin, contains approximately 18.7% nitrogen (Eastoe and Eastoe, 1952).

6.2.2.3 Bloom Strength

Bloom strength of the flying gurnard gelatin was determined according to the method described by Stable micro system, which was in accordance with the British Standard Method for Sampling and Testing Gelatin (BS 757:1975). Five grams of gelatin was placed into cold water to make 5% w/v solution, stirred with a glass rod, covered and allowed to stand at room temperature (not more than 22 °C) for 3 h. After this time, the mixture was heated in a water bath at 60 °C on a magnetic heater stirrer for 15 min to dissolve the gelatin completely. Gelatin solution (150 ml) was immediately poured into standard bloom jars over which a covering was placed after 2 min. Bloom jars were kept in the cold room (4 °C) over night and tested by Stable Microsystems TA-XT2 texture analyser.

After a trigger force of 4 g was attained the probe then proceeded to penetrate into the gel to a depth of 4 mm. At this depth the maximum force reading (the resistance to
penetration) was obtained and translated as the bloom strength (g) of the gel. The bloom strength was compared to that of other gelatins.

6.2.2.4 Amino acid analysis

Flying gurnard gelatin was hydrolysed to yield amino acids, which were measured in triplicate by HPLC according to Bidlingmeyer et al., (1987) and Badii and Howell (2002). Samples were hydrolysed with 6 N HCl (20 mL) at 110 °C for 24 h. The hydrolysed samples and amino acid standards (20 μL) were then treated as outlined below. All samples were derivatized with phenylisothiocyanate (PITC) according to the Waters Pico-Tag method.

Derivatization of Amino Acids with PITC.

Drying solution (20 μL), containing methanol:water:TEA (2:2:1) was added to each vacuum dried standard and sample in tubes and vacuum-dried again. The derivatization reagent was freshly made by mixing 50 μL PITC (kept at -20 °C, under nitrogen, to prevent degradation), 350 μL methanol (HPLC grade), 50 μL TEA, and 50 μL Milli-Q water. This PITC reagent (20 μL) was added to each tube, vortex mixed, sealed, and left at room temperature for 20 min. The reagent was then evaporated under vacuum. The derivatized samples were vacuum-dried and dissolved in 100 μL of sample buffer (eluent A, prepared by dissolving 19.0 g of sodium acetate trihydrate in 1 L Milli-Q water, followed by addition of 0.5 mL TEA, adjusted to pH 6.4 and filtered. To 940 mL of this solution was added 60 mL acetonitrile). The samples were analysed in triplicate by reverse-phase HPLC together with the amino acids standards according to the Waters Pico-Tag Amino Acid Analysis Manual, Waters Chromatography Division, 1986.
6.2.3 Interaction studies with cassava starch

Flying gurnard gelatin and cassava starch mixtures of different ratio concentrations (w/v) of 0:10; 2:8; 5:5; and 10:0 were subjected to rheological, and DSC studies to evaluate the components interactions which could be employed in food preparations where gelatin may be required in addition to starch.

6.2.3.1 Rheological measurements.

A 5% gelatin (w/v) with cassava starch (0:10; 2:8; 5:5; 10:0) was subjected to small-deformation oscillatory measurements with a Rheometrics controlled stress rheometer and a 40 mm parallel plate geometry gap of 0.3 mm set at a frequency of 1 radian per second, to obtain sufficient data without compromising the measurements of entanglements. A dynamic temperature sweep was performed between 20 °C and 2 °C at a rate of 1 °C/min and then heated to 20 °C. The applied stress was 1 Pa to keep the oscillatory strain at about 1%, sufficiently low to ensure the measurements were within the linear viscoelastic region (Hamann, 1991). The sample was surrounded by silicone oil to prevent evaporation of solvent during the sweep. The viscoelastic properties of the gels measured include the storage modulus (G') and loss modulus (G'') as a function of time within the linear viscoelastic range of the gels (Mitchell, 1980). The cross-over point G'/G'' at which gel formation occurred (Gudmundsson, 2002) and the gelation temperature were noted.

6.2.3.2 Differential Scanning Calorimetry (DSC) Measurements

A Setaram Micro DSC VII was used with distilled water as the reference. Eight hundred milligrams of gelatin samples as prepared for the rheological studies were placed in a pre-weighed DSC cell. An equal weight of distilled water was also
Chapter 6. Flying gurnard (*Dactylopterus volitans, L*) Skin gelatin and gelation properties

introduced into the reference cell to obtain a flat base line. A temperature scan of 2 °C to 90 °C and heating rate at 0.5 °C/min were maintained throughout the study. Heat absorbed or released by the transformation of the samples resulted in an endothermic or exothermic peak as a function of temperature. The temperature attained when half of the sample is transformed is referred to as the transition temperature \( T_m \) and was measured at the tip of the peak. Peak areas showing transitions temperatures and enthalpies \( (\Delta H) \) were calculated automatically by integrating the area under the peak by the DSC. Each sample was scanned in triplicate.

6.3 Results and Discussions

6.3.1 Proximate composition

The results of the proximate composition of the gelatin from the flying gurnard as compared to that of the horse mackerel which is also marine species and commercial gelatins are shown on Table 6.1.

The proximate composition of the flying gurnard skin gelatin was 9.7% moisture, 89.6% crude protein, 0.9% crude ash and 0.21% crude lipid and that of horse mackerel and commercial gelatins were 12.4, 11.4% moisture; 88, 89% crude protein; 0.25, 0.1% crude ash and 0.1, 0.1% crude lipid respectively. These values compared favourably with the results obtained for the flying gurnard gelatin and meet the standards reported by NOSB (2002).
Chapter 6. Flying gurnard (*Dactylopterus volitans. L*) Skin gelatin and gelation properties

Table 6.1 Proximate composition of gelatin derived from skins of flying gurnard as compared to that of Horse mackerel and commercial gelatins*

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Flying gurnard</th>
<th>Horse mackerel</th>
<th>Commercial</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture</td>
<td>9.7±1.4</td>
<td>12.4±0.3</td>
<td>11.4±0.3</td>
</tr>
<tr>
<td>Crude Fat</td>
<td>0.2±0.05</td>
<td>0.1±0.03</td>
<td>0.1±0.02</td>
</tr>
<tr>
<td>Ash</td>
<td>0.9±0.2</td>
<td>0.25±0.01</td>
<td>0.1±0.01</td>
</tr>
<tr>
<td>Protein</td>
<td>89.6±1.9</td>
<td>88.0±2.7</td>
<td>89.0±1.7</td>
</tr>
</tbody>
</table>

* Values are means of three determinations ± s.d.

The proximate compositions of the gelatins show that they consist mainly of protein and water. The other components as ash, crude fat and other impurity contents are important for the quality of gelatin. The fish skin gelatin was generally lower in ash content than the recommended maximum of 2.6% (Jones, 1977) and can be accepted in food applications. Therefore, gelatin from flying gurnard skin has a potential to serve as an alternative source of non-bovine gelatin and for food applications.

6.3.2 Bloom Strength

The results of bloom strength of the gelatin of the flying gurnard fish skin as compared to that of cat fish and commercial gelatin are in Figure 6.1.
Chapter 6. Flying gurnard (*Dactylopterus volitans. L*) Skin gelatin and gelation properties

Figure 6.1 Comparison of the bloom strength of flying gurnard gelatin and gelatin from pig's skin and catfish

The bloom gel strength (275 g) of the flying gurnard fish skin was significantly (P<0.05) higher than that of the catfish (150 g) and compared favourably to that of the commercial gelatin (290 g). Though the value obtained falls within the high bloom strength (220-300), higher values for other tropical fish species have been reported. Badii and Howell (2003) reported a value of 280 g for tilapia (tropical species). Bloom gel strengths of 81–229 g, were respectively, reported for skin gelatins from young and adult Nile perch (Muyonga *et al.*, 2004). These variations in bloom strength to some degree could have been brought about by certain factors, which largely affect the gel strength of particular fish species. As reported by Hinterwaldner (1977) and Muyonga *et al.* (2004), quality of gelatin depended on the species, breed, age, environment and to some extent the production process. Again in certain instances, 6.67% concentration of
Chapter 6. Flying gurnard (*Dactylopterus volitans. L*) Skin gelatin and gelation properties

The gelatin in water was used to determine bloom strength (Gudmundsson and Hafsteisson, 1997; Arnesen and Gildberg, 2002). Different concentrations of acid and alkali in the extraction method yielded varied gel strengths for cod gelatin (Gudmundsson and Hafsteisson, 1997).

### 6.3.3 Amino acid analysis

The percentage amino acid content for flying gurnard gelatin as compared to that of horse mackerel and commercial gelatin are presented in Table 6.2.

Although the content of most of the amino acids in the three gelatins were not different from each other, the alanine content of the flying gurnard gelatin (22.5%) was significantly (P<0.05) higher than those of the horse mackerel (12.2%) and the commercial gelatin (13.09%). Significant (P<0.05) differences were also noted with the phenylalanine content of the flying gurnard gelatin (2%) and those of the horse mackerel (0.7%) and commercial gelatin (0.54%) respectively. Tryptophan was also found to be significantly (P<0.05) higher in the flying gurnard skin gel (4.09%) than those of the horse mackerel (2.06%) and the commercial gel. Glycine the most dominant amino acid in the flying gurnard gelatin (28.4%) was found to be similar in content as compared to those of the horse mackerel (31.6%) and the commercial gelatin (31.13). Though the amino acid composition is mainly dependent on the source of species (Eastoe and Leach, 1977) the values obtained for the flying gurnard skin compared favourably with levels reported for gelatins especially those from warm water fish (Badii and Howell, 2003; Muyonga et al., 2004).
Chapter 6. Flying gurnard (Dactylopterus volitans. L) Skin gelatin and gelation properties

Table 6.2 Amino acid content for flying gurnard gelatin as compared to that of horse mackerel and commercial gelatin*

<table>
<thead>
<tr>
<th>Amino acids</th>
<th>% Amino acid in flying gurnard gelatin</th>
<th>% Amino acid in horse mackerel gelatin</th>
<th>% Amino acid in commercial gelatin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asp</td>
<td>3.57±0.43</td>
<td>3.44±0.36</td>
<td>3.57±0.06</td>
</tr>
<tr>
<td>Glu</td>
<td>6.30±0.72</td>
<td>5.98±0.17</td>
<td>6.20±0.09</td>
</tr>
<tr>
<td>h. pro</td>
<td>7.04±0.49</td>
<td>7.38±0.14</td>
<td>8.46±0.08</td>
</tr>
<tr>
<td>Ser</td>
<td>2.93±0.2</td>
<td>3.79±0.06</td>
<td>2.86±0.01</td>
</tr>
<tr>
<td>Gly</td>
<td>28.43±2.21</td>
<td>31.61±0.85</td>
<td>31.13±0.06</td>
</tr>
<tr>
<td>His</td>
<td>0.76±0.02</td>
<td>0.97±0.01</td>
<td>0.85±0.04</td>
</tr>
<tr>
<td>Arg</td>
<td>4.28±0.37</td>
<td>4.62±0.05</td>
<td>4.34±0.06</td>
</tr>
<tr>
<td>Thr</td>
<td>2.58±0.28</td>
<td>3.17±0.08</td>
<td>3.05±0.1</td>
</tr>
<tr>
<td>Ala</td>
<td>22.57±2.13</td>
<td>12.15±0.17</td>
<td>13.09±0.5</td>
</tr>
<tr>
<td>Pro</td>
<td>14.70±0.85</td>
<td>12.55±0.8</td>
<td>12.62±0.7</td>
</tr>
<tr>
<td>Tyr</td>
<td>0.31±0.00</td>
<td>0.65±0.04</td>
<td>0.85±0.02</td>
</tr>
<tr>
<td>Val</td>
<td>1.99±0.20</td>
<td>1.96±0.03</td>
<td>1.92±0.02</td>
</tr>
<tr>
<td>Met</td>
<td>1.45±0.06</td>
<td>1.20±0.01</td>
<td>1.01±0.07</td>
</tr>
<tr>
<td>Cys</td>
<td>0.25±0.08</td>
<td>0.14±0.00</td>
<td>0.14±0.01</td>
</tr>
<tr>
<td>I leu</td>
<td>0.87±0.09</td>
<td>0.95±0.02</td>
<td>0.86±0.01</td>
</tr>
<tr>
<td>Leu</td>
<td>1.97±0.15</td>
<td>2.42±0.03</td>
<td>2.14±0.03</td>
</tr>
<tr>
<td>Phe</td>
<td>1.95±0.20</td>
<td>0.66±0.00</td>
<td>0.54±0.01</td>
</tr>
<tr>
<td>Trp</td>
<td>4.09±0.37</td>
<td>2.06±0.03</td>
<td>1.81±0.01</td>
</tr>
<tr>
<td>Lys</td>
<td>3.64±0.21</td>
<td>4.26±0.18</td>
<td>4.53±0.01</td>
</tr>
</tbody>
</table>

* Values are means of three determinations ± s.d.
Proline and hydroxyproline residues referred to as the imino acid content of the gels were expressed as the number of the residues in 1000 residues as presented in Figure 6.2.

![Figure 6.2 Imino acid (no. proline and hydroxy proline residues in 1000 residues) content of flying gurnard, horse mackerel and commercial](image)

There were significant (P<0.05) differences between the imino acid content of the flying gel (217/1000 residues) and those of the horse mackerel (199/1000 residues), but not with the commercial gelatin (210/1000 residues). The imino acid content is important in the renaturation of gelatin subunits during gelling (Johnston-Banks, 1990) as the turns of the helix and the stability of the triple helix structure of the gelatin depend largely on these acids. Regions rich in proline and hydroxyproline are likely to be involved in the formation of nucleation zones (Ledward, 1986). In these regions, hydroxyproline plays a key role in the stabilization of triple helical structures by establishment of water bridges through its hydroxyl group (Burjandze, 1979; Ledward, 1986; Mizuno et al., 2003). These regions are rich in non-polar sequences Gly-Pro-Y,
where the third position is normally occupied by hydroxyproline or by alanine (Ala). Thus, in general, a gelatin preparation with high proline, hydroxyproline, and alanine content shows better viscoelastic properties than others with a lower content in these amino acids, (Gómez-Guillén et al., 2002; Sarabia, et al., 2002).

As a result, gelatin with high levels of imino acids tends to have higher gel strength and melting point, however, as noted earlier, bloom strength may be affected by the species, breed, age, environment and to some extent the production process (Hinterwaldner, 1977; Muvonga et al., 2004). Notwithstanding this, gelatin with low imino acid content have a higher critical concentration and lower melting point as compared to those containing higher amount of proline and hydroxyproline residues (Arnesen and Gildberg, 2002). Thus, good viscoelastic properties of the gelatin prepared from the flying gurnard can be predicted from the foregoing analyses of its amino acid composition. The hydrophobic amino acids for the flying gel as compared to those of the horse mackerel and the commercial gelatins are depicted in Figure 6.3.

Hydrophobic amino acids were also significantly (P<0.05) higher for the flying gurnard gelatin (458.8/1000) than those of the horse mackerel (318.9/1000) and the commercial gelatin (321/1000). High amounts of hydrophobic amino acids contributed to high bloom values although extraction conditions may affect the composition of these acids more than the imino acid content (Montero and Gómez-Guillén, 2000).
Chapter 6. Flying gurnard (Dactylopterus volitans. L) Skin gelatin and gelation properties

6.3.4 Interaction studies with cassava starch

6.3.4.1 Rheological Analysis

A typical gelation profile of the gelatin with the cassava flour as a function of temperature is shown in Figure 6.4. The profiles for the various compositions followed similar trends. All the gel mixtures tended to show marked shifts after 79 °C recording drops in the G' and G'' values before 90 °C during the heating. Higher temperatures tended to weaken the structure of the gels as shown by the decrease in both the G' and G'' values. The storage modulus and loss modulus of the mixtures as shown on Table 6.3 show that at 90 °C there was a drop of viscosity and loss of rigidity of gelatin : starch (10:0) but upon cooling 20° C high values of G' (2530.35 Pa) and G'' (261.47 Pa) were obtained. Similar observations were made for the other mixtures with exception of the 5:5 gelatin: starch mixture. This particular mixture, on the contrary, recorded
significant (P<0.05) decrease of $G'$ (22.23 Pa) and $G''$ (5.40 Pa) to $G'$ (16.07) and $G''$ (1.74 Pa) respectively.

Gelatin is influenced by heat treatment and forms gels at lower temperatures (Ferry, 1948) and the viscosity decreases with temperature. The increase in the storage modulus of the mixtures could be attributed largely to the degree of granular swelling of the starch during gelatinisation within the system (Eliasson, 1986) and intergranule contact that might have formed a three-dimensional network of the swollen granules (Wong and Lelievre, 1981). The decrease in the storage modulus with further increase in temperature was an indication that the gel structure was destroyed during prolonged heating (Tsai et al., 1997). This destruction has been attributed to the melting of the crystalline region remaining in the swollen starch granule, which deforms and loosens
cooling period may be attributed to the various constituents present and in particular the swollen granules and fragments to associate or retrograde as a result of molecular interactions (hydrogen bonding) (Hoover, 2001). During retrogradation, amylose forms double helical associations of 40–70 glucose units (Jane and Robyt, 1984) whereas amylopectin crystallization occurs by association of the outermost short branches (Ring et al., 1987).

Table 6.3 Storage modulus (G’ Pa) and loss modulus (G” Pa) of flying gurnard gelatin and cassava starch during a dynamic temperature sweep at 90 °C and 20 °C

<table>
<thead>
<tr>
<th>Composition</th>
<th>90 °C</th>
<th>20 °C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gelatin: Cassava starch</td>
<td>G’</td>
<td>G”</td>
</tr>
<tr>
<td>10:0</td>
<td>0.01±0.00</td>
<td>0.01±0.00</td>
</tr>
<tr>
<td>8:2</td>
<td>0.05±0.02</td>
<td>0.17±0.04</td>
</tr>
<tr>
<td>5:5</td>
<td>22.23±1</td>
<td>5.40±0.18</td>
</tr>
<tr>
<td>2:8</td>
<td>101.81±13</td>
<td>30.44±8.09</td>
</tr>
<tr>
<td>0:10</td>
<td>246.09±22</td>
<td>84.26±6.50</td>
</tr>
</tbody>
</table>

* Values are means of three determinations ± s.d.

However, the gelation points as shown on Figure 6.5 of the 5:5 (75 °C), 2:5 (72 °C), 0:10 (66.4 °C) gelatin to starch component were significantly (P<0.05) higher than 10:0 (19 °C) and 8:2 (15.5 °C) compositions. The storage modulus G’ (Figure 6 6) observed at the gelation temperatures (G’/G”) showed significantly (P<0.05) lower values as compared to that of the 10:0 gelatin : starch component. These reflected a lower degree of internal structure for the mixtures at the point of gelation. However the gels formed at 20 °C were much stronger and may indicate compatability.
Chapter 6. Flying gurnard (Dactylopterus volitans L.) Skin gelatin and gelation properties

Figure 6.5 Gelation temperature of flying gurnard gelatin and cassava starch (w/w) %

Flying gurnard gelatin and cassava starch (w/w) %

Figure 6.6 Storage modulus ($G'$) at gelation temperature of flying gurnard gelatin and cassava starch (w/w) during dynamic temperature sweep

Flying gurnard gelatin and cassava starch (w/w) %
The changes in the gelation temperatures suggest some interactions between the gelatin and the starch granule, attributed to charges on the proteins and starch (Takeuchi, 1969). With increasing starch concentration, the cross-link density in the gel increased, yielding higher values of $G'$. The overall phenomenon may be explained in terms of the different gelation points of the components and may be ascribed by associations between the gelatin and the starch granules. In such mixed systems two networks may have been formed; first a gelatin - starch network, and the other a sol-gel transition which corresponded to the predominant component. This could be observed more closely from the $G'$ and $G''$ values when individual fish and starch components were run independently (Table 6.3). A number of studies have elucidated this mechanism of interaction and the main effect of the starch component is to raise the modulus of the gelatin phase. This property could be employed in food applications (Walker, 1983; Stanley, 1990; White et al., 1993). Previously, Takeuchi, (1969), demonstrated that the concentrations of gelatin and starch in the individual phases were in good agreement with the main propositions of the thermodynamic approach to polymer incompatibility.
6.3.4.2 Differential scanning calorimetry (DSC)

The thermograms generated by the flying gurnard gelatin gel is shown in Figure 6.7.

![Graph showing DSC thermograms](image)

Figure 6.7 Typical DSC thermograms for flying gurnard gelatin scanned from 0 to 90 °C cooled and rescanned

The samples were heat reversible without any significant changes from the initial transition temperatures \( T_m \) and enthalpies \( \Delta H \) on a second scan. The trend observed in the gelation profiles are similar to those reported by Badii and Howell (2003) for horse mackerel gelatin solutions.

Table 6.4 shows the transition temperatures and enthalpy change for gelatin from flying gurnard and commercial gelatin (fish skin). The flying gurnard gelatin and that of the commercial gel had identical onset temperature at which denaturation of the gelatins commenced.
As Table 6.4 shows a common feature for all the enthalpy values were that they were endothermic. However, the flying gurnard gelatin was denatured at a significantly higher (P<0.05) $T_m$ (18.89 °C) and enthalpy change ($\Delta H$) of 2.95 J/g than the commercial gelatin $T_m$ (14.87 °C) and enthalpy change ($\Delta H$) of 0.51 J/g. (Table 6.4). The enthalpy reflected the amount of energy required to denature the samples and is related to the number of junction zones involved in the gelation of each sample. These junction zones are formed by the partial formation of the ordered triple helices as found in the more stabilised parent collagen as well as disordered regions (Johnston-Banks, 1990). Similar observations were made by Michon et al. (1997), and Badii and Howell, (2003). However the formation of these helical junction zones in gelatin gels depended on the number of junctions and their mean size per chain, which varies in a complex way with the concentration and temperature (Michon et al., 1997). These observations tended to give an insight in to the stability of the flying gurnard gelatin which offers an important role in the context of the gelling properties of the gelatin.

Figure 6.8 shows the thermograms of the gelatin with varying composition of cassava starch. As expected all the thermograms were endothermic.
Table 6.5 shows the transition temperatures, transition peaks and enthalpy values of the gelatin and the cassava flour. The DSC thermograms for the mixture showed additional transition peaks with varying degrees from the initial single peak ($T_m$ value 18.89 °C) of the gelatin and cassava starch (10:0) concentration. These peaks recorded significantly lower (P<0.05) enthalpy values ($\Delta H$) change from that of the gelatin without starch (2.95 J/g). The starch with no gelatin (0:10) however recorded a single transition ($T_m$ values 69.47 °C). The thermograms for the ratio of 8:2 and 5:5, gelatin and starch mixtures recorded three thermal transitions ($T_m$ values of 19.02 °C; 50.66 °C; 69.69 °C) and ($T_m$ values 29.74 °C; 44.44 °C; 70.07 °C) respectively. However the 2:8 (gelatin: starch mixture), recorded two transitions ($T_m$ values of 53.83 °C; 69.71 °C).
Chapter 6. Flying gurnard (*Dactylopterus volitans* L) skin gelatin and gelation properties

Table 6.5. Transition temperatures and enthalpy change for gelatin from flying gurnard / cassava starch

<table>
<thead>
<tr>
<th>Composition gelatin: starch</th>
<th>Transitions</th>
<th>Onset Temp $(T_o)$ °C</th>
<th>Peak Temp $(T_m)$ °C</th>
<th>Temperature</th>
<th>Enthalpy/ J/G $(ΔH)$</th>
</tr>
</thead>
<tbody>
<tr>
<td>10:0</td>
<td>1</td>
<td>8.98</td>
<td>18.89</td>
<td></td>
<td>2.9509</td>
</tr>
<tr>
<td>8:2</td>
<td>1</td>
<td>12.70</td>
<td>19.02</td>
<td></td>
<td>0.1707</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>43.31</td>
<td>50.66</td>
<td></td>
<td>0.0270</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>65.01</td>
<td>69.69</td>
<td></td>
<td>0.9740</td>
</tr>
<tr>
<td>5:5</td>
<td>1</td>
<td>27.41</td>
<td>29.74</td>
<td></td>
<td>0.0053</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>51.35</td>
<td>44.44</td>
<td></td>
<td>0.0007</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>65.35</td>
<td>70.07</td>
<td></td>
<td>0.3130</td>
</tr>
<tr>
<td>2:8</td>
<td>1</td>
<td>48.17</td>
<td>53.83</td>
<td></td>
<td>0.0005</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>65.61</td>
<td>69.71</td>
<td></td>
<td>0.5234</td>
</tr>
<tr>
<td>0:10</td>
<td>1</td>
<td>63.82</td>
<td>69.47</td>
<td></td>
<td>0.6713</td>
</tr>
</tbody>
</table>

Changes of more than 50% decrease in the enthalpy values $(ΔH)$ occurring at the first two transitions of the gelatin and starch mixtures over a range of 40 to 55 °C; as noted above were consistent with the existence of fairly weak electrostatic interactions between the gelatin and the starch molecules as reported by Wright *et al.* (1984) The third transition over the temperature range of 65 to 71 °C was attributed to the starch as observed by DSC thermograms for starch in excess of water hence the single endotherm for the starch without gelatin (0:10) (Biliaderis *et al.*, 1980). These results tend to offer support to the type of interactions observed in the rheological studies in this report. The mixtures which show interaction with the starch may enhance the potential of using a mixture of gelatin and cassava starch in food applications where such properties exhibited by the mixtures are required.
Chapter 6. Flying gurnard (*Dactylopterus volitans. L.*) skin gelatin and gelation properties

6.4 Conclusion

Gelation properties of the flying gurnard gelatin especially the high bloom strength as shown indicates that the gelatin could be used to replace directly, other gelatins in some food applications without significantly altering the texture. The changes in gelation temperatures with mixtures of cassava starch were as a result of fairly weak electrostatic interactions and confer textural changes as well and since melting point also determines the sensory quality of some food products in which gelatin is used (Choi and Regenstein, 2000), substitution of mammalian gelatins with fish gelatins and starch would be an added advantage in food applications.
CHAPTER SEVEN
7 EXTRUSION AND MANUFACTURE OF SNACK PRODUCTS FROM BURRITO (*BRACHYDEUTERUS AURITUS*) MINCE AND COWPEA FLOUR BLENDS

7.1 Introduction

The artisanal fish industry in Ghana produces a lot of known commercial fish species. However, during the glut season, one particular fish species which is undervalued and attracts very low prices is the burrito (*Brachydeuterus auritus*) or the big eye grunt. Despite the fact that the nutritional value of this fish is as high as that of any of the more desirable species in terms of protein quality and other nutrients like fats it still remains underutilised. This may be due to, among other factors, its dark muscle colour and consumer prejudices against this species. Technological and research efforts are therefore required to develop value-added products from this resource in the form of snacks or convenience type of food products.

7.1.1 Snack food

Snack foods are generally very light meals that come in various shapes and forms. These range from potato chips, nuts, instant hot pot snacks/cup soups and confectionary items. Snack products are also known to have, in the last 25 years, become important food item in the Western World and America (Beasley, 1994; Wang, 1997). The dry snack products are generally based on starchy raw materials such as the flours of maize, barley, cassava, rice and legumes (Pagani, 1986). They are generally small crispy and ready to eat. These products have a relatively low bulk density and crispness that are both partly caused by the dry foamy structure of the product matrix brought about by the processing technique (Lai *et al.*, 1989). Most dry snack products are produced by expanded extrusion cooking, by the superheating of water under elevated pressure conditions in an extruder (Smith, 1992). Compared to other processing techniques,
extruded snack products have the advantages of convenience, prolonged storage, an
enhanced flavour and texture properties (Laarhoven, 1988). Influence of extrusion-
cooking conditions have been generally studied and modelled (Gomez and Aguilera,
1984; Owusu-Ansah et al., 1984; Bhattacharya and Hanna, 1987).

7.1.2 Extrusion process
Extrusion is a process by which materials are forced through a specially designed barrel
containing a narrow fitting screw. In the mid-1930s, forming extruders were used to
mix semolina flour and water to form pasta products. Later extrusion technology,
which combines transport, mixing and shaping operations, was used to produce the first
ready-to-eat breakfast cereal from pre-cooked, oat-flour dough (Hui, 1992). During
extrusion the material whose properties such as moisture content, physical state,
chemical components are very important, undergoes a temperature, shear and pressure
treatment. The material thus becomes plasticised and could be shaped into a desired
form at the barrel end through a die (Hui, 1992). There are a wide variety of extruders
(Frame, 1994; Kokoni et al., 1992; Mercier et al., 1989), but basically they are either
single screw or twin screw-operating machines (Blenford, 1986).

The role of the die has been acknowledged to affect the texture of the extrudates.
Expansion ratios were noted to increase with small dies on single screw extruders.
However, Mercier et al., (1989) observed that using a twin-screw extruder under
constant extrusion conditions, the expansion ratio decreased whilst the bulk density
increased at increasing die sizes. Expansion ratios also increased with small diameter
dies and short length barrel sizes (Sokhey et al., 1997). Increasing barrel temperatures
had also led to higher expansion rates (Bhattacharya, 1997; Bhattacharya and
Chapter 7 Extrusion and manufacture of snack products from burrito (Brachydeuterus auritus) mince and cowpea flour blends

Choudhury, 1994) with lowering of moisture contents of mixtures (Mohamed, 1990). Mixture components such as NaCl (2-2.5%) (Mohamed, 1990), decreasing gluten content (Akdogan, 1996) and also increasing amounts of starch with low protein content (Gogoi et al., 1996), have been reported to lead to significant high expansion rates.

After extrusion, the pellets expand directly after leaving the die (die swell) and require no further processing other than drying (Frame 1994). The swelling has been attributed to the vaporisation of water into steam during this rapid pressure drop. This results in stretching and expansion of the starch matrix thereby rendering the products very low density and light textured (Hsieh et al., 1990). Final products are usually expected to have a moisture content of between 15-30% (Wang, 1997; Sokhey et al., 1997; Hsieh et al., 1990; Bhattacharya, 1997; Gogoi et al., 1996; Bhattacharya and Choudhury, 1994; Frame, 1994; Mohamed, 1990). The products so formed are dried and could be expanded by hot air or deep-frying.

7.2 Methods

7.2.1 Fabrication of the single screw extruder

A number of extrusion studies have indicated the possibility of using a single screw extruder for food snack products from fish muscle and rice flour blends (Clayton and Miscourides, 1992), hence the design and fabrication of a low cost extruder under the project. The extruder was designed by AFO BV of the Netherlands under this project. It consists mainly of a single screw and a barrel length of 700 mm with three sections. The components were fabricated and assembled at the Food Research Institute, Ghana. Figures
Chapter 7 Extrusion and manufacture of snack products from burrito (*Brachydeuterus auritus*) mince and cowpea flour blends

7.1 (a and b) and 7.2 depicting the various fabricated components as they were being assembled for the extruder.

![Extruder components before assembling](image)

**Figure 7.1 (a and b) Extruder components before assembling**

![Low cost single extruder in final stages of completion](image)

**Figure 7.3 Low cost single extruder in final stages of completion**

The machine had an estimated delivery of 10 kg/h and was made mainly with mild steel. The screw had pitch of 45 mm. The machine was powered by a 7-10 kW electric
Chapter 7 Extrusion and manufacture of snack products from burrito (Brachydeuterus auritus) mince and cowpea flour blends

motor. The machine also had electrical heating with a temperature controller and a pressure-sensing device.

7.2.2 Extrusion process

The process combines the unit operations such as mixing, conveying and cooking under the conditions of high shear and compression. As stated earlier, product expansion and textural properties are affected by the screw configuration and mixture components. This study was undertaken to test and obtain proper control of the operating parameters of the extruder in producing the desired products’ attributes.

7.2.3 Preparation of mixtures for extrusion

Fish mince and cowpea flour in the ratio of 70:30 was used and cassava starch was added to aid in gelatination. The starch also formed the base material for the mixtures. Based on the total wet weight of the fish and cowpea flour ratio, varying weights of starch were added and mixed in a BEAR varimixer until a homogeneous dough was obtained as outlined in Figure 3. The amount of starch added was 40, 50, 60, 70, 80 and 90% of the weight of the fish/cassava mixture. During the mixing 2% of NaCl (w/w) was added. Water was added where necessary to bring the moisture content to about 22%. Consequently, a number of recipes with the cassava starch were formulated. The mixtures were extruded with the single screw extruder which was fitted with a die through which the mass was pelleted. Product expansion and textural attributes are affected by many factors including screw configuration (Choudhury et al., 1998), rotation velocity and temperature (Guha et al., 1997). Unlu and Faller (2002) observed that, feed materials, processing parameters and residence time (De Ruyck, 1997) also affected the properties of the extruded products. These parameters can be
Chapter 7 Extrusion and manufacture of snack products from burrito (*Brachydeuterus auritus*) mince and cowpea flour blends

adjusted during the process to improve the quality of the final product (Bhattacharya, 1997). Among the variables studied, those that most prominently control expansion volume are the barrel temperature and moisture content of the raw material (Mercier and Eeillet, 1975; Owusu-Ansah et al., 1983, 1984; Chinnaswamy and Hanna, 1988a, b).

As a preliminary stage, the temperature of the third section of the barrel was varied from 40 – 160 °C for a control mixture of 60/40 mince and cowpea flour/cassava starch to test and determine the optimum barrel temperature for a preferred product formation.

![Process flow chart for extruding the fish snack products](image)

*Figure 7.3 Process flow chart for extruding the fish snack products*
However, the temperature of the die section of the barrel was maintained at 50 °C. The processing conditions and other important parameters maintained at constant rates were as shown in Table 1.

### Table 7.1 Processing conditions of the single screw extruder and temperature profile in each section of the barrel

<table>
<thead>
<tr>
<th>Barrel Section</th>
<th>Screw speed (Rpm)</th>
<th>Motor power</th>
<th>Barrel Temp °C.</th>
<th>Die Pressure (Bar)</th>
<th>Throughput (g/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Section 1</td>
<td>90</td>
<td>12</td>
<td>20</td>
<td>-</td>
<td>95</td>
</tr>
<tr>
<td>Section 2</td>
<td>90</td>
<td>12</td>
<td>40</td>
<td>-</td>
<td>95</td>
</tr>
<tr>
<td>Sections 3</td>
<td>90</td>
<td>12</td>
<td>40-160</td>
<td>-</td>
<td>95</td>
</tr>
<tr>
<td>Die Section</td>
<td>90</td>
<td>12</td>
<td>50</td>
<td>16</td>
<td>95</td>
</tr>
</tbody>
</table>

The die pressure, though maintained constant was also important for the shape of the pellet (smoothness and die swell). In this study a radial die, with a diameter of 4 mm which formed flat circular pellets was used. The pellets produced were further dried to moisture content of 11% in an oven with air temperature of 45 °C. Subsequently, the following physico-chemical characteristics were assessed on the pellets: die swell, expansion ratio, bulk density before and after expansion, moisture content before and after expansion, fat/oil content. Organoleptic evaluation of the expanded samples was also carried out.

#### 7.2.4 Die Swell

Die swell was calculated from the thickness of the extruded pellet measured with a Vernier calliper in reference to the size of the die slit (4 mm) (Frame, 1994).
7.2.5 Expansion ratio

The expansion ratio (radial expansion) was calculated by measuring the diameter of the pellet with a Vernier calliper before and after expansion at five different places and the mean diameter taken (Bhattacharya and Choudhury, 1994). The radial expansion was calculated by dividing the cross-sectional area of the pellet by the cross-sectional area of the die (Mercier et al., 1989; Sokhey et al., 1997).

Radial expansion = \( \frac{\pi r_A^2}{\pi r_B^2} \),

Where \( r_A \) = radius of pellet and \( r_B \) = radius of die.

7.2.5.1 Expansion by deepfrying

Expansion measurements were carried out after the pellets had been deep-fried in peanut oil at 190 °C for 30 seconds.

7.2.5.2 Expansion by turbulent hot air (Torbed)

This was to expand the snack pellets fat free to minimize the relatively high fat content associated with fried snack products. The Torbed air equipment was operated at a high air temperature of 280 °C. The dryer consisted of a cylindrical vessel in which the hot air was circulated. The snack pellets were introduced into this vessel and were kept in the hot air stream by centrifugal forces for 30 seconds. Expansion measurements were taken after the samples were allowed to cool to ambient temperature.

7.2.5.3 Bulk Density Determination

The bulk density is defined as the weight of 1 litre of the pure sample and it is a good measure of expansion ratio as well as an indication for texture (Hseih et al., 1990). Bulk
density was determined by using a JEL bulk density meter. The number of hammer hits was set to one hundred (Sokhey et al., 1997).

7.2.6 Proximate and chemical analyses
Protein, moisture, fat, ash, phosphorus, calcium and iron were determined by the methods of AOAC (1990) on the extruded products.

7.2.7 Sensory evaluation of developed products
Sixteen trained panellists of the Food Research Institute (FRI) were asked to evaluate the products organoleptically. Dried extruded pellets prepared from each of the six products were deep-fried before being served to the panellists. Characteristics assessed were appearance, flavour, taste and over-all acceptability. The characteristics were given a 9-point hedonic scale as 9 - Like extremely, 1 - dislike extremely (Appendix A). Scores were analysed statistically.

7.2.8 Shelf studies on extruded snacks
An eight-month storage period of the extruded snack pellets was carried out at the FRI at normal temperature (27 ± 2 °C) and R.H. 75%. The extruded pellets were either fried or unfried for the studies since such snack products are usually presented fried. Samples were taken at monthly intervals.

Approximately, 80 g of the product was placed in the packaging pouches and sealed under the following conditions.

1. Vacuum packaged in aluminium foil pouch (24 cm x 14 cm) with a gauge of 36.93 mil.
2. Vacuum packaged in polyethylene pouch (24 cm x 14 cm) with a gauge of 49.25 mil.

3. Non-vacuum packaged in polyethylene pouch (24 cm x 14 cm) with a gauge of 49.25 mil.

7.2.9 Chemical analyses

Changes in chemical properties of the products during storage were monitored by measuring the FFA as percentage oleic acid (AOAC, 1990).

TBARS of the snack products were determined according to Saeed and Howell (1999), as outlined in Chapter 2. A standard curve was prepared from the readings of a serial solution of tetrachloropropane (TEP) of 0, 0.2, 0.4, 0.6, 0.8, and 1.0 μl/ml MA. Samples (1 g) were homogenised with 5 ml of distilled water. The standard solutions and samples were treated with 100 μL of BHT, 500 μL HCL and 500 μL of thiobarbituric acid. The mixture was vortexed thoroughly and placed in a water bath at 80 °C for 30 minutes. The mixture was allowed to cool for 20 minutes, then 2 ml butanol was added and vortexed well to extract the pink chromogen. The extract was dehydrated using nitrogen at 37 °C. The residue was resuspended with 1 ml of 50% water and 50% acetonitrile. An aliquot (100 μL) was injected into the HPLC (Spectra System AS3000, from Spectra-Physics, Hemel Hempstead, UK) for analysis. A Kromasil KR100 5C18 column, Hichrom Ltd, Reading, Berks, UK) was used and the sample eluted with an isocratic solvent (50% TEA, 0.1% acetonitrile and 50% water).

7.2.10 Sensory evaluation of stored products

Samples of the extruded fried and unfried pellets were periodically removed from their respective sealed pouches and served to 16 panellists. The panellists were asked to
assess the appearance particularly colour and odour of the products. The characteristics were given a 5-point hedonic scale as 5 - white, 1 - brown for colour and 5-neutral, 1- mushy/rancid for odour (Appendix B). Scores were then statistically analysed.

7.2.11 Colour determination

The colour (L, a, b value) was determined by Minolta Colour Meter (Model R-200).

7.2.12 Statistical analysis of data

Two-way analysis of variance was used to analyse the data from sensory evaluations, using the computer program Statgraphics (STSC Inc., Rockville, MD). The difference of means between pairs was determined by means of confidence intervals using Duncan’s Multiple Range Test. The level of significance was set at P<0.05.

7.3 Results and Discussions

7.3.1 Effect of barrel temperature on extruded products

Figure 7.4 shows the die swell of the control mixture of 60% of mince fish: cowpea flour (70:30) and 40% cassava starch at varying temperatures of the third segment of the barrel. Die swell increased with increasing extruder temperature up to a maximum at 120 °C. Further increases of temperature did not affect the die swell. Increases in the temperature of the third segment of the barrel also resulted in increases in the expansion ratios of the products of the control mixture after either deep-frying or Torbed air frying (Figure 7.5). However, increases in expansion ratios of the products from the Torbed were higher than those that were deep-fried.
Chapter 7 Extrusion and manufacture of snack products from burrito (*Brachydeuterus auritus*) mince and cowpea flour blends

Figure 7.4 Effect of temperature of the third section of the barrel on die swell on control mixture 60% (70:30 mince/cowpea) and 40% cassava starch (values are means of 20 samples ± sd)

Figure 7.5 Effect of the temperature of the third segment of the barrel on expansion ratio after deep frying and Torbed drying on control mixture 60% (70:30 mince/cowpea) and 40% cassava starch (values are means of 20 samples ± sd)
Chapter 7 Extrusion and manufacture of snack products from burrito (Brachydeuterus auritus) mince and cowpea flour blends

Figure 7.6 Effect of temperature of the third segment of the barrel on bulk density on control mixture 60% (70:30 mince/cowpea) and 40% cassava starch (w/w) (values are means of 20 samples ± sd).

Figure 7.7 Effect of fish/cowpea : cassava starch on die swell of extruded snack pellets at the third segment of the barrel temperature of 120 °C (values are means of 20 samples ± sd)
Expansion ratios of products obtained at higher extruder temperatures (above 120 °C) were negligible (P>0.05), either deep fried or Torbed air fried. Bulk densities were significantly (P<0.05) lower for products of higher temperatures from 100° C as recorded in Figure 7.6.

As observed from Figure 7.7, at a temperature of 120 °C of the third segment of the barrel, die swell of the extruded products increased with increasing amounts of cassava flour. Expansion ratio also increased generally with increased cassava flour (Figure 7.8). However, there were significant (P<0.05) decreases in bulk density with increasing cassava flour in the mixture.

Increasing the temperature of the third section of the barrel and lowering of moisture contents led to higher expansion rates. Similar observations were made by Bhattacharya (1997); Bhattacharya and Choudhury (1994) and Mohamed (1990). Frame (1994) explained that at high temperatures the proteins denature and undergo much cross-
linking through disulphide bond formation network. Akdogan (1996) and Gogoi et al. (1996) reported that decreasing gluten content (lowering fish mince) and also increasing amounts of starch, resulting in low protein content led to significant high expansion rates. As mentioned earlier on, starch at high temperatures gelatinises with a disruption of the granules and formation of a matrix of amylose holding the amylopectin in a network (Remsen et al., 1978). This could also explain the increase in storage modulus as the temperature of the third section of the barrel increased. The increasing die swell was attributed to the vaporisation of water with increasing barrel temperature into steam and the rapid pressure drop as the product was released from the die. This results in stretching and expansion of the starch matrix thereby rendering the products very low in density and light textured (Hsieh et al., 1990) hence the decreasing bulk density.

Based on the above observations a maximum temperature of 120 °C was maintained for the third section of the barrel during the extrusion process. Different products were produced by varying the amount of cassava starch added to the fish mince/ cowpea flour (70:30) blend.

### 7.3.2 Proximate and chemical composition

The proximate and chemical composition of the extruded snack pellets are shown in Table 7.2. The protein content of the products was much lower than the aggregate values of the fish mince and the cowpea flour. This obviously could be attributed mainly to the inclusion of the cassava flour, which basically did not contain any appreciable amounts of proteins. However, the effect of the heat processing may have also affected the proteins to some degree (Hoffman et al., 1977; Gumbmann et al., 1983; Raghunath et al., 1995). The heat and the low moisture level employed in the
extrusion can produce irreversible chemical modification of amino acids, bioavailability or both that can affect the nutritional quality of the products (Gujska and Khan, 1990; Campos and Arêas, 1993). On the other hand, the heat processing can improve the nutrition, quality, and safety of the products by inactivation of antinutritional factors such as inhibitors of digestive enzymes and hemagglutinins in the cowpea flour (Gujska and Khan, 1991; Friedman et al., 1991; Liener 1994).

Table 7.2  Proximate and chemical composition of extruded snack products of 70:30 (fish mince: cowpea flour) with varying proportions of cassava starch*

<table>
<thead>
<tr>
<th></th>
<th></th>
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<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture(%)</td>
<td>11.1±2.6a</td>
<td>10.8±1.7a</td>
<td>11.7±1.4a</td>
<td>11.6±1.5a</td>
<td>11.5±1.7a</td>
<td>11.3±1.7a</td>
<td></td>
</tr>
<tr>
<td>Protein (%)</td>
<td>14.3±4.8b</td>
<td>12.8±2.3c</td>
<td>11.7±1.9c</td>
<td>10.7±1.7c</td>
<td>8.9±1.2d</td>
<td>6.1±1.9e</td>
<td></td>
</tr>
<tr>
<td>(Nx6.25)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fat (%)</td>
<td>5.1±1.5e</td>
<td>4.5±1.7f</td>
<td>3.4±1.3g</td>
<td>2.7±1.4h</td>
<td>1.7±0.9m</td>
<td>1.1±1.1m</td>
<td></td>
</tr>
<tr>
<td>Ash (%)</td>
<td>6.6±0.9n</td>
<td>6.3±1.1n</td>
<td>6.2±1.3n</td>
<td>4.7±1.2p</td>
<td>2.0±1.6q</td>
<td>2.1±1.2q</td>
<td></td>
</tr>
<tr>
<td>Calcium (mg/100g)</td>
<td>131±6r</td>
<td>126±3s</td>
<td>124±3s</td>
<td>121±3s</td>
<td>60±3t</td>
<td>59±4t</td>
<td></td>
</tr>
<tr>
<td>Iron (mg/100g)</td>
<td>2.0±0.8u</td>
<td>2.1±0.4u</td>
<td>2.0±0.7u</td>
<td>1.7±0.9u</td>
<td>1.0±0.3v</td>
<td>1.0±1.1v</td>
<td></td>
</tr>
<tr>
<td>Phosphorus (mg/100g)</td>
<td>434±13w</td>
<td>430±10w</td>
<td>426±11w</td>
<td>418±7x</td>
<td>345±5y</td>
<td>241±9z</td>
<td></td>
</tr>
</tbody>
</table>

*Values are means of four determinations. Means with same letter in a row are not significantly different (P<0.05) level.

Though there are slight differences in some of the nutrient components within the products with increase in the proportion of the cassava starch, these values are similar to previously published reports on extruded snack products of different blends of...
Chapter 7 Extrusion and manufacture of snack products from burrito (*Brachydeuterus auritus*) mince and cowpea flour blends

ingredients (Arèas and Lawrie, 1984; Campos and Arèas, 1993). Nutritional analysis on such products reported by Cardoso-Santiago and Arèas, (2001) showed that the products had good nutrient profiles.

Table 7.3 shows the result of the organoleptic evaluation of the fried products. As shown, the taste panellists detected no significant (P<0.05) difference in colour, taste or overall acceptability between the extruded products. However, the ratio of fish mince/cowpea flour to cassava flour, significantly (P<0.05) affected the panellists rating for flavour, with the samples containing lower cassava flour levels being ranked lower than the others (Table 7.3). The level of cassava flour did not significantly (P<0.05) affect the overall acceptability of the products.

<table>
<thead>
<tr>
<th>Product Composition (fish mince / cowpea flour)</th>
<th>Appearance</th>
<th>Flavour</th>
<th>Taste</th>
<th>Overall acceptability</th>
</tr>
</thead>
<tbody>
<tr>
<td>cassava flour</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A (60:40)</td>
<td>7.8±1.4a</td>
<td>5.5±1.2b</td>
<td>7.6±0.3d</td>
<td>7.8±1.1e</td>
</tr>
<tr>
<td>B (50:50)</td>
<td>7.8±1.7a</td>
<td>5.7±0.9b</td>
<td>7.0±1.7d</td>
<td>7.1±1.4e</td>
</tr>
<tr>
<td>C (40:60)</td>
<td>6.5±1.7a</td>
<td>6.0±1.3b</td>
<td>7.4±1.5d</td>
<td>7.3±1.9e</td>
</tr>
<tr>
<td>D (30:70)</td>
<td>7.4±1.8a</td>
<td>7.1±1.3c</td>
<td>7.4±1.5d</td>
<td>7.3±1.9e</td>
</tr>
<tr>
<td>E (20:80)</td>
<td>6.4±1.3a</td>
<td>7.3±1.0c</td>
<td>7.1±1.2d</td>
<td>7.0±1.1e</td>
</tr>
<tr>
<td>F (10:90)</td>
<td>7.2±1.1a</td>
<td>7.4±1.3c</td>
<td>7.3±0.9d</td>
<td>7.6±1.7e</td>
</tr>
</tbody>
</table>

*Means for sensory scores are for 16 panellists ± sd.

*Means with same letter in a column are not significantly different (P<0.05) level

1Hedonic scale: 1= dislike extremely; 9= like extremely
Figures 7.9 and 7.10 show the unfried and fried extruded products, respectively. As expected after frying, the extruded pellets expanded (puffed) and changed colour from the brownish appearance to a yellow tinge.

![Figure 7.9 Unfried extruded pellets](image1)

![Figure 7.10 Fried extruded Pellets](image2)

### 7.3.3 Shelf life studies

Tables 7.4 and 7.5 show the sensory evaluation results on the extruded products during storage. The unfried extruded products did not show any marked changes in physical appearance hence there were no obvious change in colour and odour. However, the fried products, which had a yellow colour after frying, acquired a strong rancid smell in the unvacuumed pouch during the storage after two months.
### Table 7.4 Mean sensory scores for Colour and Odour of extruded fish products (unfried) during eight months of storage *

<table>
<thead>
<tr>
<th>Months of storage</th>
<th>Vacuum packaged in aluminium foil pouch</th>
<th>Vacuum packaged in polyethylene pouch</th>
<th>Non-vacuum packaged in polyethylene pouch</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Colour</td>
<td>Odour</td>
<td>Colour</td>
</tr>
<tr>
<td>0</td>
<td>4.36±0.15a</td>
<td>4.68±0.2b</td>
<td>4.36±0.3a</td>
</tr>
<tr>
<td>4</td>
<td>4.36±1.1a</td>
<td>4.68±0.4b</td>
<td>4.36±0.4a</td>
</tr>
<tr>
<td>6</td>
<td>4.36±0.9a</td>
<td>4.68±0.3b</td>
<td>4.36±0.6a</td>
</tr>
<tr>
<td>8</td>
<td>4.36±0.3a</td>
<td>4.68±0.6b</td>
<td>4.36±0.3a</td>
</tr>
</tbody>
</table>

*Mean scores from 16 panellists ± sd. Means with identical letters under a particular attribute are not significantly different (P<0.05) level. Colour scale: 1 = white; 5 = brown. Odour scale: 1 = fishy, rancid; 5 = neutral.

### Table 7.5 Mean sensory scores for Colour and Odour of extruded fish products (fried) during eight months of storage *

<table>
<thead>
<tr>
<th>Months of Storage</th>
<th>Vacuum packaged in aluminium foil pouch</th>
<th>Vacuum packaged in polyethylene pouch</th>
<th>Non-vacuum packaged in polyethylene pouch</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Colour</td>
<td>Odour</td>
<td>Colour</td>
</tr>
<tr>
<td>0</td>
<td>3.2±0.6a</td>
<td>4.1±0.6b</td>
<td>3.1±0.5a</td>
</tr>
<tr>
<td>4</td>
<td>3.1±0.7a</td>
<td>4.1±0.6b</td>
<td>3.3±0.7a</td>
</tr>
<tr>
<td>6</td>
<td>3.2±0.5a</td>
<td>4.0±0.4b</td>
<td>3.1±0.4a</td>
</tr>
<tr>
<td>8</td>
<td>3.3±0.5a</td>
<td>3.9±0.3b</td>
<td>3.2±0.3a</td>
</tr>
</tbody>
</table>

*Mean scores from 16 panellists ± sd. Means with identical letters under a particular attribute are not significantly different (P<0.05) level. Colour scale: 1 = white; 5 = brown. Odour scale: 1 = fishy, rancid; 5 = neutral.
Chapter 7 Extrusion and manufacture of snack products from burrito (*Brachydeuterus auritus*) mince and cowpea flour blends

Table 7.6 Colour (L, a, b) values of extruded fish products (unfried) during eight months of storage

<table>
<thead>
<tr>
<th>Months of Storage</th>
<th>Vacuum packaged in</th>
<th>Vacuum packaged in</th>
<th>Non-vacuum packaged in</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>aluminium foil pouch</td>
<td>polyethylene pouch</td>
<td></td>
</tr>
<tr>
<td></td>
<td>L</td>
<td>a</td>
<td>b</td>
</tr>
<tr>
<td>0</td>
<td>63.3</td>
<td>-0.3</td>
<td>17.7</td>
</tr>
<tr>
<td>4</td>
<td>63.3</td>
<td>-0.3</td>
<td>17.7</td>
</tr>
<tr>
<td>6</td>
<td>63.3</td>
<td>-0.3</td>
<td>17.7</td>
</tr>
<tr>
<td>8</td>
<td>63.3</td>
<td>-0.3</td>
<td>17.7</td>
</tr>
</tbody>
</table>

Table 7.7 Colour (L, a, b) values of extruded fish products (fried) during eight months of storage

<table>
<thead>
<tr>
<th>Months of Storage</th>
<th>Vacuum packaged in</th>
<th>Vacuum packaged in</th>
<th>Non-vacuum packaged in</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>aluminium foil pouch</td>
<td>polyethylene pouch</td>
<td></td>
</tr>
<tr>
<td></td>
<td>L</td>
<td>a</td>
<td>b</td>
</tr>
<tr>
<td>0</td>
<td>62.9</td>
<td>-0.2</td>
<td>15.7</td>
</tr>
<tr>
<td>4</td>
<td>62.9</td>
<td>-0.2</td>
<td>15.7</td>
</tr>
<tr>
<td>6</td>
<td>62.9</td>
<td>-0.2</td>
<td>15.7</td>
</tr>
<tr>
<td>8</td>
<td>62.9</td>
<td>-0.2</td>
<td>15.7</td>
</tr>
</tbody>
</table>
Figure 7.11 graphically depicts the FFA values for the extruded products. There were apparent increases of FFA in all the products from their initial values. However, as could be observed for the unfried products in the entire vacuum packaged aluminium foils the FFA values did not show rapid increases as compared to either the unfried or fried products in the transparent polyethylene pouches.

Figure 7.12 shows the trend in the TBARS values observed over the period. There were initial minimal increases in TBARS values however; these could not give a clear trend for most of the products, especially the non-vacuum fried products.
The strong rancid smell of the fried extruded products in the non-vacuumed polyethylene pouches which had a yellow colour after frying could be an indication that the process of lipid oxidation leading to the development of the rancid smell was highly accelerated by heat application in the frying process (Ma’ruf et al., 1990; Kaitaranta, 1992). The slow increases in the FFA values of the products in the vacuumed aluminium foils as compared to the rapid increases in the fried products in the polyethylene pouches, were indications that extensive lipid hydrolysis of the lipid has occurred in these polyethylene pouches.

This could be attributed generally to the frying and exposure to sunlight of the products of the transparent polyethylene pouches, as well as the presence of oxygen in the non-vacuum packaged products (Hardy, 1980). Fish body oils particular omega 3-polyunsaturated fatty acids are known to be very susceptible to oxidation by atmospheric oxygen and when dried, such fats have little protection against oxidation.
Chapter 7 Extrusion and manufacture of snack products from burrito (Brachydeuterus auritus) mince and cowpea flour blends (Wolfe, 1975). However, products may develop distinctive ‘oxidised’ flavours, but these are not necessarily unacceptable (Waterman, 1976). It has been claimed that in addition to modifying flavour, some compounds formed by peroxidising lipids are toxic (Sanders, 1987), and that lipid peroxides can destroy vitamins A and E (Olcott, 1962). This degradation of fats could lead to decrease in consumer appeal for the products, because the presence of free fatty acids indicates rancidity (Hwang and Regenstein, 1993).

The unclear trend of the TBARS values is explained by the fact that the MDA may be interacting with other end products of lipid oxidation such as amines, nucleosides and nucleic acid, proteins, amino acids, phospholipids, or other aldehydes hence its concentration may vary greatly (Auborg, 1993).

7.4 Conclusion

The design and fabrication of the low cost extruder, which serve as a prototype for production by SME in Ghana, would expand the process of food extrusion as one other means of food postharvest processes. Fish based extruded snack pellets incorporating minced fish:cowpea (70:30) with varying amounts of cassava starch were successfully developed on the extruder at a temperature of 120 °C of the third section of the barrel thus adding value to basic raw food products such as underutilised fish species and locally available crops like cowpea and cassava. The extruded products were a successful attempt at the development and introduction of new products with respect to quality and acceptability as shown by the chemical and sensory evaluation results.

The products stored in aluminium pouches were more protected in terms of chemical changes in free fatty acids and to some extent TBARS formation than those packaged in
Chapter 7 Extrusion and manufacture of snack products from burrito (*Brachydeuterus auritus*) mince and cowpea flour blends

the polyethylene pouches. There were therefore fewer changes in the quality characteristics of these products over the eight-month storage period.

Overall, the studies have demonstrated the potential use of the species in the development of new value-added product. The techniques employed could offer opportunities to entrepreneurs to expand the locally available processing techniques in fish utilization.
Fish and fishery products account for the greatest percentage of animal protein intake in the diet of the majority of Ghanaians, as fish is the cheapest animal protein source available. Fish provides about 60% of the country’s protein requirement. With limited resources, depleting popular fish species’ stocks and growing population, there is the need to improve utilization of the artisanal fish catches through product development of low value species. Two such fish species are the burrito (*Brachydeuterus auritus*) and the flying gurnard (*Dactylopterus volitans*). The main objective of this study was to investigate the physicochemical and biochemical properties of these two fish species and to develop value-added products such as infant food, gelatin and extruded snack products. A low-cost extruder designed by the Netherlands’ partner in the INCO-DEV project was also manufactured at the Food Research Institute, Accra, to serve as prototype processing equipment for SME in Ghana, to make added-value products from underutilized fish species.

### 8.1 Proximate and biochemical characterisation of the burrito (*Brachydeuterus auritus*) and the flying gurnard (*Dactylopterus volitans*)

The protein content of the burrito and the flying gurnard ranged from 18 to 23 %, with the flying gurnard fish protein content being higher than that of the burrito thus indicating that both species, are good sources of protein and may be used in fish protein concentrate production or in food supplements. The low fat contents of the burrito (0.6%) and the flying gurnard (0.7%) were considered to be too low for fish oil production. The calcium contents of the burrito (296 mg/100 g) and the flying gurnard (185 mg/100 g) and other minerals were not as high as those of other fish but may be
augmented from other food components in infant food or other food product formulation.

Protein characterisation by SDS-polyacrylamide gel electrophoresis enabled easy identification of burrito and flying gurnard species from other species; with distinct differences around the protein zones with molecular weights estimated to be 18,400 and 14,300 daltons. Amino acid analysis indicated that major amino acids present in both species were glutamine (burrito 10.4% and the flying gurnard 9.9%) and alanine (burrito 11.8% and the flying gurnard 12.1%). The fish species were also good sources of lysine (burrito 7.3% and the flying gurnard 8.3%). Their percentage sulphur amino acid, methionine (2-3%), compared favourably with other fish species. The overall profiles of the essential amino acids of the two species suggested that the species have a high class protein comparable to that of the mammalian meat and may be good sources of protein for supplementation in infants’ diets. The fatty acids profiles of the fish species showed the predominance of palmitic (C 16:0), oleic (C 18:1ω9), C 20:4ω6 and docosahexaenoic (C 22:6ω3) acids. The PUFA were the omega-3 fatty acids namely EPA and DHA. The use of these fish species in the formulation of infant foods has the added benefit of the presence of EPA and DHA as these fatty acids help in the healthy growth and development of the brain, the nervous system and functioning of the eye.
8.2 Rheological, DSC, FT-Raman spectroscopy and phase microscopy studies of fish mince, cowpea flour and cassava starch mixtures

Interactions of proteins of the minces of the fish species and other macromolecules like cowpea flour and cassava starch were investigated to provide information on their use and new protein applications in food formulations.

Rheological studies of the flying gurnard fish mince and cowpea flour mixtures indicated $G'$ (elastic modulus) and $G''$ (viscous modulus) values higher than expected. A ratio of (5:5) fish mince to cowpea was the best combination with the highest $G'$ (1609 Pa) indicating positive synergistic interaction. It is proposed that the gel was produced by cowpea globulins dissociating into acidic and basic subunits with the basic subunits subsequently interacting with the myosin heavy chains of the fish mince via disulphide bonds.

Interactions of flying gurnard fish mince with cassava starch may, in part, be due to charges on the protein as they denatured and underwent cross-linking through disulphide bond formation network with the starch colloids. However, the gels formed may not be stable as a decrease in the storage modulus with further increases in temperature was observed.

Remarkable variations were noted in the gelation profiles of the burrito fish mince mixture as the cowpea flour was increased and $G'$ values were noted to increase significantly only after 70 °C. The highest $G'$ value (157 Pa) was attained in the burrito fish mince: cowpea (2:10) mixture ratio. The mixtures exhibited a type of interaction which involved aggregation at a rather lower content of burrito fish.
However, the interaction of the burrito fish mince and cassava starch, showed that the mixture with the fish mince to starch ratio (5:5) had a significant high increase G' value (411 Pa). It is reported that phase separation occurs with the maximum concentration of the biopolymers in each fraction resulting in greater than expected G' values (Howell and Friedli, 1996).

Flying gurnard fish mince and cowpea flour mixtures showed changes in peak denaturation temperatures ($T_m$) and / or the enthalpy ($\Delta H$) values of the water soluble sarcoplasmic proteins- Peaks 2 and 3 ($T_m$ 48.37-52.22°C) and actin-Peak 4 ($T_m$ 60.17°C), with the appearance of another peak. The new transition appeared at 72-75 °C probably as a result of the denaturation of the globular proteins of the cowpea. These changes indicated a progressive interaction of the flying gurnard fish proteins with the cowpea flour. These results confirmed the observations made by the rheological studies in this report and probably the fish mince and cowpea flour ratio of 5:5 may indicate that possibility. DSC studies showed that the addition of cassava starch to flying gurnard fish mince led to significant changes in transitions peaks of myosin- Peak 1 ($T_m$ 40.04°C), water soluble sarcoplasmic proteins- Peaks 2 and 3 ($T_m$ 48.37-52.22°C) and actin-Peak 4 ($T_m$ 60.17°C) disappearing and a new peak at 70.44 °C was apparently formed.

DSC thermograms generated by adding cowpea flour to burrito fish mince in varying ratios showed decreases in the denaturation temperatures especially the water soluble sarcoplasmic proteins- Peaks 2 and 3 ($T_m$ 50 – 60.86 °C) with the disappearance of myosin - Peak 1 ($T_m$ 40.92 °C), as the cowpea flour was increased. These peaks were also accompanied by negligible decreases in the enthalpy ($\Delta H$) values (0.04 to 0.02 J/g). These forms of interaction may be weak. Cassava starch added to burrito fish
mince in varying amounts did not change the DSC thermograms substantially. This phenomenon may be due to weak electrostatic interaction between the macromolecules in the mixtures.

In elucidating further the interactions of the fish proteins with either cowpea flour or cassava starch, FT-Raman spectroscopy spectra for the flying gurnard fish mince and mixtures of the mince and cowpea flour showed that peaks at 760 cm\(^{-1}\) which was due to indole-ring vibrations of tryptophan (Trp) residues with buried residues, increased in intensity, becoming broader as the cowpea flour increased. In addition, the intensity of tryptophan doublet bands at 1340 and 1360 cm\(^{-1}\), also increased as the ratio of the cowpea flour was increased in the samples, indicating hydrophobic interactions.

The intensity ratio \(I_{855}/I_{830}\) of the tyrosine doublet near 855 cm\(^{-1}\) and 830 cm\(^{-1}\) which determined the number of buried and exposed Tyr residues and the state of the hydrogen bonding of the phenolic OH group decreased (0.85 - 0.3) as the cowpea content increased. This demonstrated hydrogen bonding between the flying gurnard fish proteins to a negative acceptor of the molecules in the cowpea flour suggesting the role of phenolic hydroxyl groups as strong hydrogen bond donors or an increase in how well buried the tyrosine residues are within the gel network.

The spectra of the mixtures of flying gurnard fish mince and cassava starch showed broadening of the intensity of the peak at 760 cm\(^{-1}\) and a decrease in the intensity ratio \(I_{855}/I_{830}\) of the tyrosine doublet of the mixtures as the starch ratio was increased. Thus partial or limited intermolecular hydrophobic interactions and disulfide exchange reactions may have occurred. The spectra for the mixtures of the mince and cowpea flour showed
that the indole–ring vibrations of tryptophan (Trp) residues peak at 760 cm$^{-1}$ band intensity did not show any remarkable change. This could mean that intermolecular hydrophobic interactions and disulfide exchange reactions may not be intense. However, increased intensity of the doublet bands at (Trp) 1340 and 1360 cm$^{-1}$ as the ratio of the cowpea flour was increased in the samples was observed at a single peak (1340 cm$^{-1}$).

The intensity ratio $I_{855}/I_{830}$ of the tyrosine doublet (855 cm$^{-1}$ and 830 cm$^{-1}$) decreased steadily (1.16-0.36) as the cowpea content was increased.

Raman spectra for burrito fish mince and cassava starch showed increased intensity at 760 cm$^{-1}$ (Trp) residues. There was a significant loss in the intensity ratio $I_{855}/I_{830}$ of the tyrosine of the mixtures which decreased (1.16 - 0.2) as the starch content increased. The results of the Raman spectroscopy suggest weak interactions between the proteins of the burrito fish mince and the cassava starch.

Phase contrast micrographs of flying gurnard fish mince and cowpea flour suggested positive interaction as a continuous (smooth) matrix was observed. This was consistent with the results observed in the rheological, DSC and FT-Raman studies undertaken. The phase contrast micrographs of the samples obtained after the mixing of flying gurnard fish mince and cassava flour showed strand-like phases of flying gurnard fish dispersed within two equilibrium phases of 5% flying fish mince and 5% cassava starch. These phase transitions were attributed to the induced weak electrostatic interaction between the biopolymers and described as co-operative binding in synergistic gelation. These assertions and interpretations confirmed the observations made by rheological, DSC and FT-Raman studies carried out in this report. The phase contrast micrographs of samples obtained after the mixing of the burrito fish mince and
Chapter 8 General Discussion and Conclusions

cassava showed agglomerates of each component constituting a non-interactive effect in the mixture which favoured self-association and incompatibility of the phases. The micrograph of mixtures of burrito fish mince and cassava flour exhibited characteristics similar to those seen in its interaction with cowpea flour. Thus, burrito mixtures with cassava or cowpea resulted in phase-separated systems through self-association and incompatibility of the system.

Although, fish protein-cassava starch interaction do not result in strong gel formation, starch contribution in foods is important for several reasons. In infant foods and dietetic products a low viscosity is a requirement for palatability and ease of eating by infants and patients; starch provides an energy source and finally starch contributes to sensory properties (appearance, mouthfeel and texture) of the product. Increased gel strength results in decreased flavour/aroma release. Starch can also form inclusion complexes with some hydrophobic aroma components and phase separated inclusions have been used as simulating fat particles in low fat products.

8.3 Processing of the selected fish species into infant weaning foods

Infant food recipes were developed with aid of “Microdiet” dietary analysis software package (Downlee Systems Ltd, 2000 UK) with reference to the DHSS 1979 RDA for infants between 6-9 months.

It is important that the infant food developed, in a developing country like Ghana adequately addresses the nutritional requirements for a weaning food in addition to natural breast feeding during the weaning period. The proximate composition of the recipes, were adequate in such diets and should meet the nutritional needs of infants. As important sources of micronutrients for the developing infant where breast milk is
insufficient, the recipes should meet the needs of the growing child. The overall profile of the amino acid content of the formulations showed adequate amounts of both essential amino acids that cannot be synthesised in the body, and a supply of non-essential amino acids which provide nitrogen for protein synthesis.

The viscosity of the recipes was important as infant foods serve as a gradual transition from fluid to solid foods. The recipes provided feeds that were close to commercial ones. Adequate packaging and storage precaution would be needed to ensure that nutrients are well protected as results of the storage studies showed product susceptibility to light exposure with the production of FFA and TBARS that would affect the flavour of the products as well as nutritional value and safety.

8.4 Flying gurnard fish skin gelatin and gelation properties

Proximate values and high bloom value (275 g) of the gelatin produced from flying gurnard skins compared favourably with standards, indicating that the gelatin would be useful in food applications and has potential as an alternative source of non-bovine gelatin. The percentage amino acid content for flying fish gelatin as compared to that of horse mackerel were similar and the most dominant amino acid in the flying gurnard fish skin was glycine. Interaction studies with cassava starch was important, in view of starch contribution in most local foods. Starch contributes substantially to sensory properties of the product and an ability to either raise or lower the elastic modulus of the gelatin phase could be employed in food applications.
Chapter 8 General Discussion and Conclusions

8.5 Fabrication of a low cost single screw extruder and its use in the extrusion and manufacture of snack from Burrito mince and cowpea flour blends

The low-cost extruder, designed by ATO, the Netherlands partner in the INCO-DEV project, was successfully made in Ghana. Extruded products were made initially on the prototype in the Netherlands; extruded snack pellets indicated proximate and chemical composition reflecting good nutrient profiles. The use of vacuum packaged aluminium foils suppressed the formation of the FFA and TBARS values observed over a 6 month storage period of either the unfried or fried extruded fish products.

8.6 Conclusions

Characterization of the burrito and the flying gurnard showed that both species are of high nutritional significance in either human food supplements or formulations, as they contained a high protein content, good general amino acid profile and abundance of polyunsaturated fatty acids.

Compatible and stable gel formation between minces of flying gurnard and cowpea flour was possible through positive synergistic interaction. This gel network was stabilised by hydrophobic and disulphide bonds as well as hydrogen bonds. Mixtures of flying gurnard fish mince and cowpea flour in the ratio of 5:5 (w/w) formed strong gels. On the contrary, a lower mince to cowpea flour ratio (2:8 w/w) of burrito mince gave the best gel in the series of mixtures, although it was weak in comparison with the flying gurnard-cowpea combinations.

Detailed information on protein denaturation and thermodynamic parameters can be obtained by DSC, which also indicated interactions in the mixtures.
Chapter 8 General Discussion and Conclusions

The presence of continuous networks as seen in flying fish-cowpea mixtures and phase separated gels in fish-starch mixtures were indicated by phase contrast microscopy.

Evidence of the presence of hydrophobic interactions, hydrogen bonding and disulphide bonding were confirmed by FT-Raman spectroscopy.

This study has shown that a combination of techniques such as rheology, DSC, microscopy and spectroscopy may be applied successfully to follow biopolymer interactions in food systems to obtain detailed, insightful results.

This study has provided some understanding of the functionality of the biopolymers which would be valuable in terms of innovative product development.

Flying fish mince was used successfully to develop infant food as shown by the results in this study. The formulations as developed through the recipes 2 to 5 showed good nutritional composition which compared favourably with commercial infant food such as cerelac. The rheological properties of the formulations were within the viscosity range of $< 500$ Pa which was considered adequate for consistency. Shelf life of the products was enhanced with the exclusion of oxygen in vacuum packed foils.

Gelatin was developed from flying gurnard fish skin. The properties of the flying fish gelatin especially the high bloom strength as shown by this study may be used to replace directly, other gelatins in some food applications. Developing gelatin from the flying fish skin would reduce waste by-products, that would otherwise be discarded and
produce a value added product that could be of economic benefit to the fish industry in Ghana.

The design and fabrication of the low cost extruder serves as a model for SMEs in Ghana, to expand food extrusion as a postharvest process. Fish based extruded snack pellets incorporating minced fish:cowpea ratio of 70:30 (w/w) with varying amounts of cassava starch were successfully developed on the extruder at a temperature of 120°C of the third section of the barrel, thus adding value to basic raw food materials such as underutilised fish species and locally available crops like cowpea and cassava. The extruded products were a successful attempt at the development and introduction of new products with respect to quality and acceptability as shown by the chemical and sensory evaluation.

The products stored in aluminium pouches were more protected in terms of chemical changes in FFA and to some extent TBARS formation than those packaged in the polyethylene pouches. There were therefore fewer changes in the quality characteristics of these products over the eight month storage period.

Overall, the studies have demonstrated the potential use of underutilised fish species in the development of new value-added product. The techniques employed offer opportunities to entrepreneurs to expand the locally available processing techniques in fish utilization.
8.7 Further work

- The addition of naturally occurring antioxidants such as vitamin C, citric acid and α-tocopherol could be undertaken to stabilise the lipids in the formulations.

- Work should continue to assess the nutritional value of the developed foods as well as their clinical properties. Efforts should also be made to transfer these formulations to household women.
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9. REFERENCES


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APPENDICES

APPENDIX A Sensory evaluation score sheet for developed fish products

Name:
Product

Please evaluate the sample and check much you like or dislike it in terms of appearance, odour, taste and overall acceptability.

Please answer the question below.

<table>
<thead>
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<th>No.</th>
<th>APPEARANCE</th>
<th>ODOUR</th>
<th>TASTE</th>
<th>OVERALL ACCEPTABILITY</th>
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</thead>
<tbody>
<tr>
<td>9</td>
<td>Like extremely</td>
<td></td>
<td></td>
<td></td>
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<td>8</td>
<td>Like very much</td>
<td></td>
<td></td>
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<tr>
<td>7</td>
<td>Like moderately</td>
<td></td>
<td></td>
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<tr>
<td>6</td>
<td>Like slightly</td>
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<td>5</td>
<td>Neither like or dislike</td>
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<td>Dislike slightly</td>
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<td></td>
</tr>
<tr>
<td>1</td>
<td>Dislike extremely</td>
<td></td>
<td></td>
<td></td>
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Comments:
APPENDIX B Sensory evaluation score sheet for developed fish products during storage

Name:
Product
Please evaluate the sample and check the colour and odour intensity within the marked portions of the line.

Colour:

<table>
<thead>
<tr>
<th></th>
<th>White</th>
<th>Brown</th>
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</thead>
<tbody>
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<td>5</td>
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</table>

Odour:

<table>
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<tr>
<th></th>
<th>Fish/ rancid</th>
<th>Neutral</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td></td>
<td>5</td>
</tr>
</tbody>
</table>

Comments: