

UNIVERSITI PUTRA MALAYSIA

EVALUATION OF NUTRITIONAL QUALITY OF COMPLEMENTARY FOODS FORMULATED FROM BLENDS OF NIGERIAN YELLOW MAIZE, SOYBEAN, AND CRAYFISH

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FPSK(p) 2022 41



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Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Doctor of Philosophy

January 2022

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DEDICATION

This thesis is dedicated to:

ALLAH (SWT) for everything about me, my late parents, my supporting wife and children and those individuals who have the courage to change themselves instead of others.



Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Doctor of Philosophy

EVALUATION OF NUTRITIONAL QUALITY OF COMPLEMENTARY FOODS FORMULATED FROM BLENDS OF NIGERIAN YELLOW MAIZE, SOYBEAN, AND CRAYFISH

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January 2022

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In order to develop adequate complementary foods (CFs) to improve infant and young child feeding, the inclusion of animal source foods (ASFs) into plant-based foods (PBFs) is paramount. Unfortunately, the incorporation of ASFs into PBFs to formulate adequate CFs was infrequent in the developing countries, especially Nigeria. Yet, few CFs that were formulated by this strategy lacked adequate studies. The current study investigated the nutritional quality of a CF formulated from the combination of yellow maize, soybean, and crayfish flours. Treated dried yellow maize, soybean, and crayfish were used to formulate maize flour (MF, 100:0% w/w), maize + soybean flour (MSF, 72:28% w/w) and maize + soybean + crayfish (MSCF, 80:10:10% w/w). Individual food ingredients, MF, MSF, and fortified wheat milk flour (FWMF) were evaluated for nutrients composition, using food compositional analysis method. Food ingredients, and MF, MSF, FWMF were modified into dried, rectangular-shaped diets for a fourteen-day feeding trial on eight groups of healthy male Sprague Dawley rats. Each group comprising four rats was fed, with *ad libitum* feeding and drinking, on one of the diets; maize (MD), soybean, crayfish, maize + soybean (MSD), maize + soybean + crayfish (MSCD), fortified wheat milk (FWMD), protein-free, and standardized laboratory chow. During the last four days of the trial, daily body weight, amount of feed intake was recorded, and total faeces were collected from each diet-fed group to evaluate for the biological quality of the CFs. On the 14th day of the trial, blood was drawn and organs harvested from rats of each group to assess the physiological changes in the serum and blood compositions, and in the relative organ weight. Optimal CF was identified by nutrient profiling technique. Statistical analysis was conducted with multiple analyses of variance and Tukey's honestly significant difference test ($P \le 0.05$).

Analyses' outcomes revealed a significantly higher ($P \le 0.05$) protein energy percent (17.59 PE%) and ash content (2.72%) in MSCF than other CFs, and higher contents of iron (3.43 mg/100 g), zinc (1.40 mg/100 g) and calcium (141.47 mg/100 g) in MSCF

than MF and MSF. Due to crayfish supplementation, sulphur amino acids content in MSCF was about 132% higher than MSF's, astaxanthin was detected only in MSCF whilst absented in other CFs. The phytate and total tannin contents in MSCF were insignificantly lower ($P \le 0.05$) compared with other CFs. The body weight gain (23.75 g) in MSCD-fed was significantly higher ($P \le 0.05$) than other diet-fed groups, whilst the amount of feed intake (79.50 g) was nominally higher in MSCD relative to other groups. There were nominal higher values of protein efficiency ratio (2.59), feed efficiency ratio (0.30), net protein ratio (3.37), and true digestibility (91.50%) in MSCD compared with other diet-fed groups. The value of protein digestibility corrected amino acid score (70%) in MSCD was significantly higher than those of MD and MSD but lower than FWMD's. There were no signs of illness, infection, and organ damage observed among the rats. MSCF was discovered to have possessed optimal nutritional quality compared with other CFs. The current study demonstrated that crayfish could be utilized in a dietary modification to produce an adequate CF that potentiates improve growth performance and positive health outcomes in animals. Conclusively, MSCF may serve as a better alternative to MF, MSF and FWMF, which upon consumption may help to achieve a sustainable healthy growth and development in children in Nigeria and other poor-resource communities of the world.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

PENILAIAN KUALITI NUTRISI MAKANAN KOMPLEMENTARI DIRUMUS DARIPADA ADUNAN JAGUNG KUNING NIGERIA, KACANG SOYA DAN UDANG KRAI

Oleh

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Bagi memajukan makanan komplementari (CF) yang mencukupi untuk meningkatkan pemakanan bayi dan kanak-kanak kecil, rangkuman makanan sumber haiwan (ASF) dengan makanan berasaskan tumbuhan (PBF) adalah amat penting. Malangnya, inkorporasi ASF ke dalam PBF bagi merumuskan CF yang mencukupi adalah jarang di negara membangun, terutama Nigeria. Namun, sedikit CF yang dirumus melalui strategi tersebut diselidiki akibat kekurangan kajian yang memadai. Kajian ini menyelidiki kualiti nutrisi CF yang dirumus daripada kombinasi jagung kuning, kacang soya, dan tepung udang krai. Jagung kuning kering, kacang soya, dan udang krai yang diolah telah digunakan untuk merumus tepung jagung (MF, 100:0% w/w), jagung + tepung kacang soya (MSF, 72:28% w/w) dan jagung + kacang soya + udang krai (MSCF, 80:10:10% w/w). Bahan makanan individu, MF, MSF, dan tepung susu jagung diperkaya (FWMF) telah dinilai bagi komposisi nutrien, menggunakan kaedah analisis komposisi. Bahan makanan, dan MF, MSF, FWMF telah diubah suai kepada diet kering berbentuk segi empat tepat, untuk percubaan pemakanan empat belas hari ke atas lapan kumpulan tikus Sprague Dawley jantan yang sihat. Setiap kumpulan yang merangkumi empat tikus telah diberi makan, dengan makanan dan minuman ad libitum, ke atas salah satu diet; jagung (MD), kacang soya, udang krai, jagung + kacang soya (MSD), jagung + kacang soya + udang krai (MSCD), susu gandum diperkaya (FWMD), bebas protein dan makanan makmal yang standard. Semasa empat hari terakhir percubaan, berat badan harian, jumlah pengambilan makanan telah direkodkan, dan kesemua najis telah dikumpul daripada setiap kumpulan yang diberikan makanan diet bagi menilai kualiti biologikal CF. Pada hari keempat belas percubaan, darah telah diambil dan organ yang diperoleh daripada tikus bagi setiap kumpulan telah dinilai perubahan fisiologikal dalam komposisi serum dan darah, dan dalam berat organ relatif. CF optimal telah dikenal pasti melalui teknik pemprofilan nutrien. Analisis statistikal telah dijalankan dengan analisis berbilang varians dan ujian perbezaan signifikan sejujurnya Tukey ($P \le 0.05$).

Dapatan analisis menunjukkan peratus energi protein secara signifikan adalah lebih tinggi $(P \le 0.05)$ (17.59 PE%) dan kandungan abu (2.72%) dalam MSCF berbanding dengan CF yang lain, dan kandungan zat besi (3.43 mg/100 g), zink (1.40 mg/100 g) dan kalsium (141.47 mg/100 g) yang lebih tinggi dalam MSCF berbanding dengan MF dan MSF. Disebabkan penambahan udang krai, kandungan asid amino sulfur dalam MSCF adalah lebih kurang 132% lebih tinggi daripada MSF, astaksantin telah dikesan hanya dalam MSCF manakala tidak dikesan dalam CF lain. Kandungan fitat dan keseluruhan tanin dalam MSCF secara signifikan adalah lebih rendah ($P \le 0.05$) berbanding dengan CF lain. Tambahan berat badan (23.75 g) dalam makanan MSCD secara signifikan adalah lebih tinggi ($P \le 0.05$) daripada kumpulan makanan diet lain, manakala jumlah pengambilan makanan (79.50 g) secara nominal adalah lebih tinggi dalam MSCD berbanding dengan kumpulan lain. Terdapat nisbah kecekapan protein secara nominal vang lebih tinggi (2.59), nisbah kecekapan makanan (0.30), nisbah protein net (3.37), dan kebolehhadaman yang sebenar (91.50%) dalam MSCD berbanding dengan kumpulan makanan diet lain. Nilai skor asid amino diperbetul kebolehhadaman protein (70%) dalam MSCD secara signifikan adalah lebih tinggi daripada MD dan MSD tetapi lebih rendah daripada FWMD. Tidak terdapat tanda penyakit, infeksi, dan kerosakan organ yang dikesan pada tikus. MSCF telah dikesan mempunyai kualiti nutrisi optimal berbanding dengan CF lain. Kajian ini mengutarakan bahawa udang krai dapat digunakan dalam pengubahsuaian dietari bagi menghasilkan CF yang mencukupi yang berpotensi untuk meningkatkan prestasi pertumbuhan dan hasil kesihatan yang positif pada haiwan. Kesimpulannya, MSCF berupaya sebagai alternatif yang lebih baik daripada MF, MSF dan FWMF, yang jika dikonsumpsi dapat membantu mencapai pertumbuhan dan perkembangan yang sihat dan mampan dalam kalangan kanak-kanak di Nigeria dan komuniti miskin sumber lain di dunia.

ACKNOWLEDGEMENTS

First, my profound gratitude goes to Almighty God for giving me the understanding that through Him, everything is possible. Am grateful to the Nigerian government for providing the fund for my study in Malaysia.

My deep and sincere gratitude goes to my supervisor, Professor Dr. Amin Ismail for his guidance, understanding, patience, and most importantly, his encouragement in times of new ideas and difficulties. His mentorship, knowledge, and commitment inspired and motivated me during a tough time in my Ph.D. pursuit. I also would like to acknowledge my thesis committee members, including Associate Professor Dr. Norhaizan Mohd Esa and Associate Professor DR. Zulfitri Azuan Mat Daud, for their academic encouragement, support, and guidance throughout my study, Thank you very much for your intellectual comments, suggestions, and feedback on my thesis writing. I would also like to thank the staff of the Nutrition Science Laboratory, most especially Mr. Syed Hasbullah, who kindly supported me throughout my laboratory work in Malaysia.

I sincerely appreciate and thank the Nigerian government through Tertiary Education Trust Fund (TETFUND), for supporting my studies in UPM. I thank the Nigeria Police Academy (POLAC), Wudil, who approved my study leave.

Lastly, I am immensely grateful to my wife and children for patiently staying with me during the Ph.D. pursuit in Malaysia.

This thesis was submitted to the Senate of the Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Doctor of Philosophy. The members of the Supervisory Committee were as follows:

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Declaration by Members of Supervisory Committee

This is to confirm that:

- the research conducted and the writing of this thesis was under our supervision;
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L	Procedure for calculating protein digestibility corrected amino acid score (PDCAAS) of a diet with two components.	139	
М	Vitamin A standard curve	140	
N	Bands on thin-layer plates from CRF and CFs extracts, and $\mbox{AST}_{\rm std}$ solution	141	

LIST OF ABBREVIATIONS

	ABS	Acrylonitrile butadiene styrene
	CAN	Acetonitrile
	AEU	Animal Experimental Unit
	AF	As fed
	ALB	Albumin
	ALP	Alkaline phosphatase
	ALT	Alanine aminotransferase
	ASF	Animal source food
	AABA	α–Aminobutyric acid
	AAS	Amino acid score
	AST	Aspartate amino transferase
	AST _{TC}	Astaxanthin as a total carotenoid
	BASO	Basophils
	BCG	Bromocresol-green
	ВНТ	Butylated hydroxyl toluene
	BUKHAN	Bayero university kano herbarium access number
	BWG	Body weight gain
	BWL	Body weight loss
	Ca	Calcium
	CF	Complementary Food
	СНО	Carbohydrate
	CHOE	Carbohydrate energy
	СР	Crude protein

CRD	Crayfish-diet
CRF	Crayfish-only flour
CSB	Corn-soy-blend
DC	Diet consumed
DCP	Dietary crude protein
DF	Digestibility factor
DM	Dry matter
DNA	Deoxyribonucleic acid
EAA	Essential amino acid
EBF	Exclusive breastfeeding
EDTA	Ethylene-diamine tetra acetic acid
ELISA	Enzyme-linked immunosorbent assay
EOS	Eosinophils
FER	Feed efficiency ratio
FN	Fecal nitrogen
FWM	Fortified wheat milk
FWMD	Fortified wheat milk diet
FWMF	Fortified wheat milk flour
GCN2	General control nonderepssible
GNLC	Grains and Legumes Nutrition Council
Hb	Hemoglobin
HCL	Hydrochloric acid
HDL	High-density lipoprotein
HDLC	High-density lipoprotein cholesterol

	HNO ₃	Nitric acid
	HPLC	High-performance liquid chromatography
	HRP	Horseradish peroxidase
	IACUC	Institutional animal care and use committee
	ICP-MS	Inductively coupled plasma mass spectrometer
	IOMFND	Institute of medicine food and nutrition board
	IOMNA	Institute of medicine of the national academies
	LDH	Lactate dehydrogenase
	LDL	Low-density lipoprotein
	LDLC	Low-density lipoprotein cholesterol
	LYMPH	Lymphocytes
	MAD	Minimum adequate diet
	МСНС	Mean corpuscular hemoglobin concentration
	MCV	Mean cell volume
	MD	Maize-only diet
	MDD	Micronutrient deficiency diseases
	MDH	Malate dehydrogenase
	MF	Maize-flour
	MONO	Monocytes
	mRNAs	Messanger ribonucleic acids
	MSCD	Composition of maize, soybean, and crayfish diet
	MSCF	Composition of maize, soybean, and crayfish flour
	MSD	Composition of maize and soybean diet
	MSF	Composition of maize and soybean flour

	MT	Million ton
	mTORC1	Mammalian target of the rapamycin complex1
	NA	Not available
	NAD+	Nicotinamide adenyl dinucleotide oxidized
	NADH	Nicotinamide adenyl dinucleotide reduced
	NAS	National Academy of Sciences
	NB	No band
	ND	Not detected
	NEUT	Neutrophiles
	NH4OA	Ammonium acetate
	NI	Nitrogen intake
	NPR	Net protein ratio
	PBF	Plant-based food
	PC	Protein consumed
	PCRM	Physicians Committee for responsible medicine
	PCV	Packed cell volume
	PDCAAS	Protein digestibility corrected amino acid score
	РЕ	Protein-energy
	РЕМ	Protein-energy malnutrition
	PER	Protein efficiency ratio
	PFD	Protein free diet
	PFF	Protein free flour
	Р	phosphorous
	PL	Parameter Logistic

PLT	Platelets
RBC	Red blood cell
RDA	Recommended dietary allowances
RDI	Recommended daily intakes
RF	Relative factor
RNI	Recommended nutrient intakes
RP	Reverse-phase
SAA	Sulphur amino acid
SBD	soybean diet
SBF	Soybean flour
SLC	Standardized laboratory chow
SSA	Sub-Saharan Africa
TAE	Tannin acid equivalent
TC	Total carotenoid
TCHOL	Total cholesterol
TD	True digestibility
TEA	Tri-ethylamine
TEAA	Total essential amino acid
TG	Triglyceride
THF	Tetrahydrofuran
TLC	Thin-layer chromatography
TMB	3,3,5,5-tetramethyl benzidine
TP	Total protein
UEASF	Unconventional edible animal source foods

USDAFNS United state department of agriculture food and nutrition services

UV Ultraviolet

WBC White blood cell

WG Weight gain



CHAPTER 1

INTRODUCTION

1.1 Study Background

A scientific study proved that breast milk is the perfect food for infants during the first six months of life because it contains all the required nutrients and immunological factors to maintain optimal health and growth (Ijarotimi & Keshinro, 2013). At the age of six months and above, when children are undergoing rapid growth, physiological maturation, and development, breast milk alone is insufficient to provide essential nutrition, especially for energy, protein, and micronutrients such as zinc, iron, and vitamin A. Therefore, it is necessary to complement breast milk with other nutritious foods as the children grow older (Ijarotimi & Keshinro, 2013; Ukegbu & Anyinka, 2012). World Health Organization (WHO) and United Nations International Children's Emergency Funds (UNICEF) recommended the timely introduction of adequate CFs to infants and young children at the age of 6 months (White et al., 2017).

Complementary foods (CFs) are homemade or commercially prepared foods, semi-solid or soft foods that progress to solid foods, and typical family foods introduced to infants and older children between the ages of 6–23 months. CFs are given in addition to breast milk to provide necessary nutrients and calories that are no longer sufficient in breast milk (United States Department of Agriculture Food Nutrition Services (USDA-FNS), 2009; Abiose et al., 2015). Good CFs should be rich in energy, protein, vitamins, and minerals; they should be clean, safe, soft, and easy for the child to eat; they should not be too spicy or salty; the ingredients must be locally available and affordable; and easy to prepare (National Food and Nutrition Commission, Government of the Republic of Zambia, 2007). Poor nutrition during infancy may increase the risk of growth faltering and micronutrient deficiencies, which may adversely affect the health and mental development of infants and children. Hence, improved CFs during the complementary feeding period are essential for average child growth and cognitive development (Ijarotimi & Keshinro, 2013).

In most developed countries, nutrient-fortified cereals are the first CFs introduced to infants, followed by fruits, vegetables, and meat products (Akinola et al., 2014). Contrary to this, the available fortified commercial formulas are too expensive and inaccessible to most families in developing countries, especially in Nigeria, in which over 40.1% of the population is extremely poor, with earnings of less than \$1/day (National Bureau of Statistics, 2020). Therefore, it is essential to introduce homemade CFs for children's nutritional needs that can be ready-prepared, available, and affordable to substitute for expensive, commercially adequate types (Abeshu et al., 2016). Although various CFs were developed and served children in various localities in developing countries such as Nigeria, their nutritional outcomes were below expectations (Ijarotimi & Keshinro, 2013), and many children have not benefitted from minimum complementary feeding practices (White et al., 2017).

Like other developing countries, CFs in Nigeria are usually produced from staple cereals (Shiriki et al., 2015; Abeshu et al., 2016). The cereals contain low-quality protein and high antinutritional factors such as phytates, oxalate, and tannins. The cereals' nutritional quality is sometimes inferior compared to commercial CFs (Solomon, 2005). Therefore, a combination of cereals with inexpensive plant protein sources such as legumes can be used to improve the nutritional inadequacy of cereals as a source of CFs (Ijarotimi & Keshinro, 2013; Abiose et al., 2015). In Nigeria, several cereal-legume combinations were introduced and effective (Onofiok & Nnanyelugo, 1998). For example, Fashakin & Ogunsola (1982) formulated peanut-ogi, a mixture of corn gruel and peanut; Akinrele & Edwards (1971) formulated soya-ogi, a mixture of corn gruel and soybean. Other valuable combinations included ogi and melon protein, corn gruel and melon seed, cowpea-ogi, corn gruel, and cowpea. Despite the combination of different sources, it was shown that plant-based CFs generally provided insufficient amount of nutrients to meet recommended nutrient intakes for 6-23 months old children during complementary feeding (WHO, 1998; Dewey & Brown, 2003; Pan American Health Organization (PAHO) & WHO, 2003).

In the follow-up to the recommendation that CFs can be developed from low-cost, locally available, and nutritious foodstuffs as a strategy against food insecurity, malnutrition, and diseases (Solomon & Owolawashe, 2006; Kunyanga et al., 2012), the Nigeria Food-Based Dietary Guidelines recommended sustainable food-based approaches to encourage dietary diversification through the production and consumption of both macro-and micro-nutrient rich foods, including traditional foods found in different parts of the country (Solomon, 2005). One of the recommendations was that starchy staple roots, tubers, and cereals in combination with legumes, vegetables, fruits, and possibly animal source foods (ASFs) could be used to develop low-cost, nutritious CFs for infants and children (Solomon, 2005).

Studies have shown that plant-based foods (PBFs) generally provide insufficient amounts of nutrients to meet the recommended nutrient intakes for children during complementary feeding except when supplemented with ASFs (PAHO & WHO, 2003). Concerning increasing attention to ASFs, especially locally available and underutilized ones, it is important to highlight their effects on infant nutrition, growth, and development. Furthermore, the strategy of supplementing PBF with ASF, especially underutilized, local, and readily available alternatives such as crayfish in the formulation of adequate CF, is uncommon in Nigeria. Besides that, the knowledge of the nutritional quality of the formulated compositions is also inadequate. This study was designed to contribute to the improvement of the nutritional quality of traditional plant-based CFs, by harnessing the nutritive value of crayfish as an animal source protein to supplement and improve low and poorly bioavailable nutrients from a combination of maize and soybean flour. A good CF thus formulated from the combination of yellow maize, soybean, and crayfish may serve as a better alternative for common traditional plantbased CFs, and costly, fortified commercial CFs. Thus, findings from this study focused on the following issues; (1) can CF formulated from a local blend of yellow maize, soybean, and crayfish flours offers higher nutritional value for infants and children compared to existing traditional CF, and (2) can the supposed blend substitute for commercial infant formulas such as Nestle Cerelac sold in Nigeria markets?

1.2 Problem Statements

CFs are available in commercial and traditional forms, but owning to poverty, only 7% of children aged between 6-23 months consumed commercial CFs in Nigeria (National Population Commission [Nigeria] (NPC) & International Classification of Functioning Disability and Health (ICF), 2019). Children, in Nigeria, commonly consume plant-based traditional CFs (Agbemafle et al., 2020), such that about 78%-90% of children aged between 6-23 months were mostly fed with food made from grain products (NPC & ICF, 2019). These plant-based CFs are mainly from cereals (e.g., corn, millet, and guinea corn) (Umerah et al., 2020), and occasionally the cereal may be supplemented with such local legumes as soybean or peanut to improve the protein content of cereal-legume blends (Mekuria et al., 2021). However, PBFs are generally of low protein and micronutrient density, high in antinutritional factors such as phytate and tannin that may hinder the absorption of available nutrients, and consequently contribute to growth faltering and undernutrition in children (Oladiran & Emmambux, 2020).

As plant-based CFs cannot sufficiently supply the recommended nutrient intake of some key nutrients (iron, zinc, and vitamin B_6), the inclusion of ASF in CFs formulation can help meet the gap in some cases (Oladiran & Emmambux, 2020). ASFs do not only increase the nutrient density of CFs but also improve the bioavailability of micronutrients (Agbemafle et al., 2020). Unfortunately, the commonly recommended ASFs, such as egg, meat, fish or poultry, etc. are expensive for low-income households, but using crayfish offers a promising alternative. Crayfish (Procambarus clarkii) is an animal polypeptide with a relatively cheap source of protein and is readily available all year round across Nigerian markets due to its annual production of approximately 12,000 metric tons (Iwuchukwu et al., 2017; Okoye et al., 2019). Crayfish was reported to have a high content of essential amino acids and protein efficiency that is favorable compared to casein (Fasuan et al., 2017). Supplementation with crayfish is initiated on its amino acids complementation with maize and soybean composite. Maize protein is deficient in lysine and tryptophan but has fair amounts of methionine and cystine (Oladiran & Emmambux, 2020). Conversely, the protein of soybean is a relatively rich source of lysine and tryptophan but has a limiting amino acid in methionine (Food and Agriculture Organisation (FAO), 2016). Crayfish, being an ASF, is rich in methionine and therefore can complement diets formulated with soybean as a component (Smith et al., 2021). Moreover, crayfish have zero or very low content of antinutritional factors, easily digestible fiber, and low carbohydrate content (Okoye et al., 2019).

Based on the foregoing, supplementation of yellow maize (*Zea mays*) and soybean (*Glycine max*) flours blended with crayfish flour may produce an adequate CF having a higher nutritional quality than maize-only, maize + soybean flours blend CFs, and that adequate CF thus formed may compare favorably with commercial CF available in Nigeria.

1.3 Research Questions

- 1. Does CF formulate from a combination of maize, soybean, and crayfish flour improve the nutritional quality of traditional CFs prepared from maize-only flour and a combination of maize and soybean flour?
- 2. Does CF formulate from a combination of maize, soybean, and crayfish flour compared favorably with the more expensive commercial infant formula (fortified wheat-milk flour, Nestle Cerelac) sold in Nigerian markets?

1.4 Research Hypotheses

- 1. CF formulated from a combination of maize, soybean, and crayfish should have improved nutritional quality over traditional CFs formulated from maize–only and a combination of maize and soya bean.
- 2. CF formulated from a combination of maize, soybean, and crayfish should compare favorably with the more expensive commercial infant formulas (Nestle Cerelac) sold in Nigerian markets.

1.5 Objective of the Study

The main objective of this study was to evaluate the nutritional quality of CFs formulated at a 16% protein level from combination of yellow maize, soybean, and crayfish flours, which was achieved by the following specific objectives:

- 1. To determine the nutrient composition of formulated CFs in respect of proximate composition; calcium, iron, and zinc; essential amino acids; phytates and tannins; lutein and astaxanthin contents.
- 2. To evaluate the protein quality of the formulated diets, and investigate their effects on body weight gain, feed efficiency ratio, and protein efficiency ratio in Sprague Dawley rats.
- 3. To assess the physiological responses in the serum and blood compositions, and the relative organ weight of rats fed with the formulated diets.
- 4. To characterize and rank the formulated CFs for identifying the CF with the optimal nutritional characteristics.

1.6 Conceptual Framework

Based on the literature review, a conceptual framework was produced to facilitate doing the current study. From the existing literature, the nutritional quality of food such as CF is influenced by its nutrient composition, protein quality, and available caloric energy (World Health Organization (WHO), 1998). The protein quality of a CF is affected by its amino acid profile. The available caloric energy of a CF is dependent on its moisture, carbohydrate, lipid, and protein contents. The growth, hematological, and serum biochemical status of a healthy diet-fed rat are directly dependent on the nutritional quality of the diet, which in turn is affected by its nutrient bioavailability. In over all, the nutrient bioavailability of the diet is affected by the level of antinutritional factors, crude fiber, and true digestibility. The diagram showing the relationship of this study is presented in **Figure 1.1**.



Figure 1.1 : Conceptual framework of the study

Fe = Iron; Zn = Zinc; Ca = Calcium; TD = True digestibility; IDV = Independent variable; ID = Dependent variable.

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